Imperial College London

Modelling the impact of iKIRs in adaptive immune responses

Laura Mora Bitria

Imperial College London

Department of Medicine

Submitted in part fulfilment of the requirements for the degree of Doctor of Philosophy of the Imperial College of London, June 2023

Abstract

Inhibitory killer cell immunoglobulin-like receptors (iKIRs) are a family of inhibitory receptors that are expressed by natural killer cells and late-stage differentiated T cells. iKIRs are well-known for their function during innate responses but there is evidence that they also regulate T cell-mediated immunity. Importantly, recent immunogenetic studies indicate that iKIRs enhance T cell-mediated control in chronic viral infections in humans. Whether iKIRs play an analogous role in other contexts is currently unknown. This thesis aims to tackle that question by combining immunogenetic analysis, mathematical modelling and iKIR expression data analysis in the context of type 1 diabetes (T1D). In autoimmunity, an enhanced T cell response might be considered detrimental and so iKIRs might have an opposite effect and exacerbate self-reactive T cell responses. The immunogenetic analysis of two T1D cohorts confirms this hypothesis and reveals a large detrimental effect of iKIR genes in T1D. Specifically, the protection conferred by some HLA class II genotypes is weakened in individuals carrying a high number of iKIR genes together with their ligand genes. Mathematical modelling shows that an iKIR-mediated increase of $CD8^+$ and $CD4^+$ T cell survival can explain the effect of iKIR genes in individuals carrying protective HLA class II genotypes. Finally, the iKIR expressing $CD4^+$ and $CD8^+$ T cell populations are relatively small and not expanded in blood in T1D patients, suggesting that crosstalk between autoreactive T cells and other iKIR expressing immune populations is more likely to explain the observed iKIR effect. Overall, this thesis expands the iKIR modulatory role of T cells during antiviral responses to autoimmune responses, hinting at a core and widespread function of iKIRs in adaptive human immunity.

Statement of Originality

I hereby certify that the results, analyses, and literature review of this thesis are my own work unless otherwise stated throughout this thesis and/or listed below. Experimental data used in this thesis has also been appropriately acknowledged throughout and/or listed below.

1. In Chapter 3, the imputation of HLA and KIR genotypes presented was performed previously by Dr. Johnny Bisrat Debebe. The list of HLA drivers in was derived by Prof Becca Asquith and curated by both Prof Becca Asquith and myself.

2. In Chapter 5, blood samples from T1D patients were provided by Linda Hadcocks in Prof Derek Macallan group (St George's University) and Susan Wong (University of Cardiff). Blood samples from healthy controls were collected at Imperial College London by Ms Charandeep Kaur. Flow cytometry data was generated by Kelly Miners and Kristin Ladell at University of Cardiff.

Copyright Declaration

The copyright of this thesis rests with the author. Unless otherwise indicated, its contents are licensed under a Creative Commons Attribution-Non Commercial 4.0 International Licence (CC BY-NC).

Under this licence, you may copy and redistribute the material in any medium or format. You may also create and distribute modified versions of the work. This is on the condition that: you credit the author and do not use it, or any derivative works, for a commercial purpose. When reusing or sharing this work, ensure you make the licence terms clear to others by naming the licence and linking to the licence text. Where a work has been adapted, you should indicate that the work has been changed and describe those changes. Please seek permission from the copyright holder for uses of this work that are not included in this licence or permitted under UK Copyright Law.

Acknowledgements

The work described in this thesis was completed through collaboration between the Imperial College London (London, UK) and Bayer AG (Leverkusen, Germany) under the Imperial College Partner Research Institution scheme. The research presented here has received funding from the Horizon 2020 programme of the European Union (2018-2023) under grant agreement 764698 (QuanTII).

This work would not have been possible without the patience and invaluable feedback of my supervisors Prof Becca Asquith and Dr Christoph Niederalt. Thank you for taking the time to teach me and to guide me through the course of this PhD. I would also like to thank current and past members of Asquith group, specially Danai, Jonas, Charandeep and Ada, for great discussions and useful advice. I am also thankful to my colleagues at Bayer: Flavia, Dirke, Annika and Rebekka thanks for your support – in person and virtually – during my time in Germany.

Special thanks also go to Dr Jan Schlender for giving me the opportunity work at Bayer. To the QUANTII cohort, thanks for your companionship and encouragement, specially during corona times. I would also like to thank Dr Kristin Ladell and Mrs Kelly Miners for producing and sharing the immunophenotype data. Thanks also to Linda Hadcocks and Dr Susan Wong for sharing the T1D samples.

Lastly, I would like to acknowledge friends and family for their immense support during the last few years. To Sara, John and Ignacio for making Cologne feeling more like home. To everybody back at home, Cris, Mar, Gerard, Edu, David and Eloi, for making the pandemic less disruptive. To my friends Aina, Meritxell and Laura for keep visiting me wherever I go. To my partner, Javier, for always being there. And to my father, to my mother and to Ferran for all the unconditional support.

A la meva familia

Contents

1 Regulation of the immune response **22**

1.1 The immune system 22

1.1.1 The sequence of immune defences 23

1.1.2 Immune inhibitory receptors 25

1.2 Killer-cell Immunoglobulin-like Receptors 26

1.2.1 The diverse KIR gene family 26

1.2.2 KIR evolution 28

1.2.3 KIR expression and function 30

1.3 Clinical relevance of KIR diversity 30

1.3.1 KIR disease associations linked to innate NK cell responses 31

1.3.2 KIR-disease associations linked to T cell responses 32

1.3.3 iKIRs modulating adaptive T cell responses: beyond chronic viral infections 36

1.4 Thesis scope 36

2 Modelling adaptive immune responses **38**

2.1 Cohorts 39

2.1.1 The UK-GRID case-control cohort 39

2.1.2 HBDI family cohort 39

2.1.3 Kallionpaa single-cell RNA sequencing dataset . . . 40
2.1.4 Twins UK cohort . . . 41
2.1.5 Cardiff cohort . . . 41
2.2 Genotype imputation . . . 42
2.3 Logistic Regression . . . 42
2.3.1 Effect modification of KIR genotype . . . 44
2.4 Family-based analysis . . . 46
2.4.1 Transmission/Disequilibrium test . . . 47
2.5 (Multiple) Hypothesis testing . . . 50
2.5.1 Approximate permutation test using Monte Carlo . . . 51
2.5.2 Multiple testing procedures . . . 52
2.5.3 Multiple testing procedures for dependent tests . . . 55

3 Immunogenetic analysis of a T1D case control cohort 57

3.1 Genetics of T1D . . . 58
3.2 KIR gene association studies in T1D . . . 59
3.3 The UK-GRID cohort . . . 60
3.3.1 Genotype imputation accuracy . . . 60
3.3.2 Functional iKIR genes, iKIR Count and iKIR score . . . 61
3.4 iKIR score modulates protective *DR*∼*DQ* haplotypes . . . 64
3.5 Disentangling the iKIR score effect on HLA-mediated protection: effect on *DQ6* dominant protection . . . 66
3.5.1 iKIR score modifies the *DQ6* protective association . . . 66
3.5.2 *DQ6* protection decreases as a function of iKIR score . . . 68

3.5.3 iKIR count or iKIR score . . . 68
3.5.4 HLA-I genes or iKIR score? . . . 68
3.5.5 Do all iKIRs contribute to iKIR modification? . . . 70
3.5.6 Do activating KIRs affect *DQ6* protection? . . . 71
3.5.7 Does the iKIR effect on *DQ6* depend on *DR3* and *DR4* detrimental haplotypes? . . . 72
3.6 HLA drivers in T1D . . . 73
3.7 Impact of HLA-I genes . . . 75
3.8 iKIR score modifies protective HLA-II drivers . . . 76
3.9 Replication: a family-based study . . . 78
3.10 Discussion . . . 79

4 Mathematical modelling of the iKIR effect during autoreactive T cell responses 81

4.1 HLA-II-mediated resistance to T1D . . . 82
4.1.1 Islet specific Tregs deacrease as a function of HLA-II risk . . . 83
4.1.2 HLA-II mediated T1D risk has no effect on overall T_{reg} frequencies in healthy individuals . . . 83
4.2 KIR effect on HLA-II associations . . . 87
4.3 Mathematical model of the transition from health to T1D . . . 87
4.3.1 Parameter estimation . . . 88
4.3.2 Assumptions . . . 89
4.3.3 Simulations . . . 91
4.4 Results T1D model . . . 92
4.4.1 T_{reg} levels correlate with protection . . . 93

4.4.2 Impact of iKIRs on HLA-II associations with T1D . . . 94
4.5 Discussion . . . 95

5 iKIR expression in T1D **100**

5.1 KIRs during innate NK cell-mediated responses . . . 100
5.2 KIRs during T cell responses . . . 101
5.2.1 Direct pathways . . . 101
5.2.2 Indirect pathways . . . 104
5.3 Frequency of T cells expressing iKIR transcripts in T1D patients . . . 107
5.3.1 scRNAseq analysis . . . 107
5.3.2 Expression of KIR transcripts in memory T cells and NK cells . . . 109
5.4 KIR protein expression in T1D . . . 110
5.4.1 Gating strategy . . . 110
5.4.2 KIR expression in $CD4^+$ and $CD8^+$ T cell subsets . . . 112
5.4.3 KIR expression in NK cell subsets . . . 114
5.5 Discussion . . . 114

6 Final conclusions **117**

6.1 Limitations . . . 119
6.2 Next steps . . . 121

Bibliography **124**

Appendices **169**

A Immunogenetic analysis of a T1D case control cohort **170**

A.1 iKIR score modifies the *DQ6* protective association 171

A.2 HLA drivers in T1D 171

A.3 Detrimental genotypes are not modified by iKIR score 173

B iKIR expression in T1D **175**

B.1 Expression of KIR transcripts in T and NK cells 175

B.2 Multi-colour antibody panel 178

B.3 Compensation 178

B.4 Gating strategy 179

B.5 KIR expression in $CD4^+$ and $CD8^+$ T cell subsets 180

B.6 KIR expression in NK cell subsets 180

List of Tables

1.1 Functional iKIR gene modulation of HLA-I associations in chronic viral infections 35

2.1 2x2 contingency table Disease status vs Genotype. 44

2.2 Transmitted and non-transmitted alleles among case-parent trios 49

2.3 Error matrix for statistical classification 51

2.4 Classification of possible outcome scenarios in a family of m tests 53

3.1 iKIR gene associations reported in the literature since 2003 to date 60

3.2 Definitions of functional iKIR genes 61

3.3 iKIR score effect on *DQ6* is independent of *DR3* and *DR4* detrimental genotypes 72

3.4 iKIR score interaction terms with protective HLA-II drivers in a subcohort without HLA-I drivers 77

3.5 Odds of observing an iKIR modification across *DQA1*01:02*, *DQB1*03:01*, *DQA1*02:01* and $DQA1*01:02^{D}QB1*06:02 in an independent cohort$ 79

4.1 Description of state variables included in the system of differential equations described by (4.2). 87

4.2 Parameters of both model 1 and model 2 90

5.1 Percentage of cells expressing KIR transcripts within NK cell and memory T cell susbsets 110

5.2 Median number of KIR^{+} T cells between cases and controls 113

5.3 Median number of KIR$^+$ NK cells between cases and controls 114

A.1 *DQ6* protection in T1D is enhanced amongst individuals with a low iKIR score . 171

A.2 The HLA-I and HLA-II genotypes most closely identified with outcome in the GRID cohort 172

B.1 Percentage of cells expressing KIR transcripts within immune subsets annotated with Monaco reference 176

B.2 Percentage of cells expressing KIR transcripts within immune subsets annotated with HPCA reference 177

B.3 Flow cytometry panel to assess iKIR expression in CD4$^+$, CD8$^+$ and NK cell populations 178

B.4 Multiple linear regression model to identify predictors of iKIR expression on T cells. 180

B.5 Multiple linear regression model to identify predictors of iKIR expression on NK cells. 180

List of Figures

1.1 Sources of Killer cell immunoglobulin-like receptors (KIR) diversity 29

1.2 KIR-HLA interactions during innate and adaptive immune responses 33

2.1 Interaction 44

2.2 Population stratification confounds case-control studies of unrelated individuals 47

2.3 Transmission/Disequilibrium test 49

3.1 Loci associated with T1D risk 59

3.2 Definition of functional iKIR genes considered in this analysis 61

3.3 Ribbon diagram of the α_1 and α_2 helix of the HLA molecule 62

3.4 Sequence logos of HLA epitopes recognized by iKIRs 63

3.5 Distribution of iKIR count and iKIR score in the UK-GRID cohort 64

3.6 Impact of iKIR score on 17 protective *DRB1∼.DQB1* phased haplotypes 65

3.7 *DQ6*-associated protection is enhanced amongst individuals with low number of functional iKIR 67

3.8 iKIR score effect is not attributable to specific HLA-I alleles 70

3.9 All functional iKIRs contribute to the iKIR score effect 71

3.10 Forest plot with all HLA drivers associated with T1D 74

3.11 Correlation between *B*57:01* and *DQA1*02:01* genotypes masks the iKIR effect on *DQA1*02:01*-mediated protection 76

3.12 Impact of iKIR score on 9 protective HLA-II genotypes 77

3.13 Simulation-based power analysis in the GRID cohort 78

4.1 Islet-specific Treg frequency as a function of HLA-II risk in healthy individuals . 83

4.2 The TwinsUK dataset 84

4.3 HLA-II polygenic risk score as a predictor of $CD4^+$ immunophenotypes 86

4.4 Minimal model of the T cell mediated destruction of pancreatic β cells 88

4.5 Cell population levels over time in 3 *in silico* individuals 92

4.6 β-cell mass remaining as a function of T_{regs} levels 93

4.7 T_{reg} levels represent HLA-II risk 93

4.8 iKIR effect as a function of T_{reg} levels 95

5.1 The indirect and direct pathways that affect T cell lifespan 103

5.2 QC of scRNAseq data 108

5.3 KIR gene expression within memory $CD8^+$ T cells and NK cells is similar between seropositive children and healthy controls 109

5.4 Gating strategy for enumeration of $iKIR^+$ $CD8^+$ T cell subsets 111

5.5 Gating strategy for enumeration of $iKIR^+$ NK cells 112

5.6 Correlation between manual and automated gating 112

5.7 Percentage of T cells expressing different iKIR in T1D patients and healthy controls 113

5.8 Percentage of NK cells expressing different iKIR in T1D patients and healthy controls 114

A.1 Functional iKIR do not consistently modify detrimental genotypes 174

B.1 Gating hierarchy 179

B.2 Gating strategy for enumeration of iKIR+ $CD4^+$ memory subsets 180

Chapter 1

Regulation of the immune response

1.1 The immune system

Pathogens have a massive impact on hosts' lives. The COVID-19 pandemic is only another example of the constant threat that pathogens represent for many aspects of human life. Many lines of defence are put into place to cope with and minimise the effects of new pathogens and strains alien to the body. As demonstrated again by the pandemic, one of the most effective approaches to deal with pathogens' threat is the administration of vaccines to harness the host's *immune system*.

The immune system is a tightly regulated network of molecules and cells. This network has the mission of detecting and eliminating pathogens and aberrant cells. Most living organisms have an *innate* immune system, while jawed vertebrates have evolved an additional *adaptive* immune system. The difference between innate and adaptive systems is their speed of response and specificity. The innate response is broadly constitutive and represents an early attempt to clear the infection before the host becomes sick. On the other hand, the adaptive response is induced by the innate response and will try to eliminate the pathogen through a specific mechanism.

Most of the time, in the face of an infection, a coordinated immune response between the two systems is induced, clearing the pathogen and reestablishing homeostasis in the host. But on particular occasions, things go awry, and the immune response results in immunopathology

and/or in immune-related diseases like autoimmunity, chronic viral infections or the spread of cancer. It is still largely unknown how the innate and adaptive systems orchestrate protective immune responses and how that interplay can lead to autoimmune diseases. In this Thesis, I study the role of a set of innate receptors in the regulation of adaptive immune responses in humans and their role in autoimmunity.

1.1.1 The sequence of immune defences

Upon exposure to a pathogen, the innate response is the first attempt of the immune system to clear an infection. Innate defences are germ-line encoded, constitutive or rapidly induced and widely expressed in the mechanical barriers such as the skin or mucosa. One of the first innate responses is the activation of the complement system, a set of proteins that lyse pathogen membranes and also act as a marking strategy for phagocytosis by dendritic cells (DCs) and macrophages. Complement activation and phagocytosis trigger inflammation, which facilitates the recruitment of other immune defences, like granulocytes, innate lymphoid cells such as natural killer (NK) cells and also cells from the adaptive immune system.

Phagocytes, like macrophages or dendritic cells (DCs), can also directly bind conserved molecules on the pathogen surface (known as pathogen-associated molecular patterns or PAMPs) through pattern recognition receptors (PRRs). Although there are several PRRs families, with several receptors in each family, the set of genes that encode PRRs is still vastly outnumbered by the great diversity of pathogens and pathogen-derived peptides. The adaptive immune system addresses this problem by generating an open-ended repertoire of recognition molecules.

The adaptive immune response is recruited if the innate mechanisms fail to clear the infection. The mechanism of this response activates after PRR recognition and phagocytosis. Succinctly explained, DCs migrate from the site of infection to the draining lymph nodes, where they present antigens to adaptive immune cells through the presentation molecules of the major histocompatibility complex (MHC) or Human Leucocyte Antigens (HLA) in humans. Similarly to PAMPs, antigens are pathogen-derived small peptides (9-12 amino acids) processed upon DC phagocytosis. Together with antigen presentation, signalling via cytokines and other co-stimulatory molecules results in DC-dependent activation of the adaptive immune cells.

The main adaptive immune cells are B and T cells. These cells, produced in the bone marrow and thymus respectively, are initially inactive (*naive* state). Each particular B and T cell

clone can be activated through the presentation of a specific MHC-antigen complex. B and T cells activation trigger its differentiation into effector cells and their *clonal expansion*. These effector cells migrate to the inflammation sites where they exert their function. Effector B cells become plasma cells and execute *humoral responses*. T cells, on the other hand, are divided into two types, $CD8^+$ (cytotoxic T cells) and $CD4^+$ (helper T cells). $CD8^+$ T cells are the main cytotoxic cells of the adaptive response, eliminating infected cells via the target recognition of peptide-MHC complexes on their surface. Alternatively, $CD4^+$ T cells secrete cytokines and regulate the activity of other immune system cells[1] including B cells and $CD8^+$ T cells.

The reason behind the specificity of the adaptive immune response lies in the variability of the recognition receptors on the adaptive cell membrane. For example, the adult body typically harbours 10^{11} T cells, with over 10^8 different somatically-generated T-cell receptors (TCRs) (Qi et al., 2014). However, the maximum number of possible TCRs is estimated to be around 10^{20} (Mora and Walczak, 2016). This great somatic diversity of TCRs lowers the chances of the pathogen evading the immune system action, even at the expense of a delayed response. The delay in this response is due to the limited number of cell clones specific to a particular antigen and, to make matters worse, most antigen-specific T cells will die shortly after the infection. However, a few of those responding T cells will survive for long periods in the form of *memory cells*, promoting a swift response in subsequent encounters with the same pathogen. The accumulation of memory cells, activated in previous immune responses, effectively acts as a Darwinian evolutionary process that helps increase the host immune efficiency against common infectious agents (Burnet et al., 1959)[2].

While an open-ended antigen recognition repertoire can potentially target the entire pathogenic landscape, it is also a power to be reckoned with. The ability to detect a wide selection of molecular motifs harbours the risk of making the immune system respond to native elements of the host that are similar to foreign peptides[3]. Not surprisingly, several control mechanisms are in place to ensure effective immune responses while sparing self-damage. Besides control of the adaptive response by the innate one (Iwasaki and Medzhitov, 2015), there are two tolerance mechanisms in place, known as central and peripheral tolerance respectively. Central tolerance

[1] Some $CD4^+$ T cells can also display a cytotoxic phenotype (e.g. Cachot et al. (2021)).

[2] B cells follow an analogous process, although with an additional layer of complexity due to somatic hypermutation of the rearranged B cell receptors.

[3] In fact, it is well-established that adaptive immune receptors display variable degrees of cross-reactivity between different antigens (Yu et al., 2015), resulting in healthy individuals carrying auto-reactive T and B cells, i.e. immune cells that are specific to elements of the host body (one example are insulin-specific T cells, present both in diabetic and healthy humans (Culina et al., 2018)).

removes autoreactive lymphocytes during their development whereas peripheral tolerance is executed by dedicated $CD4^+$ T cells that regulate the immune response (regulatory T cells, T_{regs}) but also through different signalling pathways activated by immune inhibitory receptors (Daëron et al., 2008; Rumpret et al., 2020).

1.1.2 Immune inhibitory receptors

Immune inhibitory receptors constitute a wide array of inhibitory molecules that dampen and fine-tune the level of activation of the adaptive immune cells. Although mainly studied in T cells, PRRs also display inhibitory isoforms indicating that the innate response against PAMPs is also dampened by inhibitory receptors (Rumpret et al., 2021). Immune inhibitory receptors are also known as immune checkpoint receptors, since under physiological conditions, they activate immune checkpoints that restrain the immune response.

Immune checkpoints are inhibitory signalling pathways that counteract TCR and other activating signals on T cells. Inappropriate signalling of immune checkpoints can be detrimental to the maintenance of self-tolerance and the effective clearance of pathogens and tumours. For example, cancer cells can escape the antitumoral response of activated T cells by expressing ligands that trigger immune checkpoint suppressive function. Similarly, sustained inhibitory signalling can lead to T cell exhaustion, a dysfunctional metabolic state associated with worse outcomes in viral infections and cancer (Wherry and Kurachi, 2015). Immune checkpoint receptors have therefore become a popular target for immunotherapy; for example, monoclonal antibodies specific for the programmed cell death protein 1 (PD-1) or the cytotoxic T-lymphocyte protein 4 (CTLA-4), have been approved as a treatment for some types of cancer (Marin-Acevedo et al., 2021).

The diversity of inhibitory receptors extends beyond PD-1 and CTLA-4 on T cells (Vivier and Daëron, 1997). This catalogue of inhibitory receptors fine-tunes immune responses in slightly different ways. For example, these receptors prevent the activation of other receptors or dampen their signal after activation. The effect of these inhibitory receptors also depends on the presence of its ligand and the receptor expression, i.e. some are constitutive, but others are inducible. Given the plethora of available receptors and their expression/ligand-dependent activity, the immune response can be finely adjusted to the specific context of the cell. In the following sections, I describe the *killer cell immunoglobulin-like receptors*, the set of inhibitory

receptors[4] that are the focus of this Thesis.

1.2 Killer-cell Immunoglobulin-like Receptors

Parts of section 1.2 and section 1.3 have been previously published in (Mora-Bitria and Asquith, 2023).

Killer cell immunoglobulin-like receptors (KIRs) are a family of glycoproteic transmembrane *paired receptors*[5]. KIRs are expressed in NK cells and a subset of terminally differentiated T cells (Moretta et al., 1990; Ugolini and Vivier, 2000). The ligands for KIRs include the highly polymorphic and polygenic HLA class I (HLA-I) molecules and non-polymorphic ligands such as non-classical HLA molecules. For NK cells, activating and inhibitory KIR-HLA interactions regulate both functional maturation and NK cell activation. In the case of T cells, KIR engagement has been shown to increase T cell survival, with some studies hinting at an impact on its function as well (Anfossi et al., 2001).

1.2.1 The diverse KIR gene family

KIRs are encoded by a gene family located on chromosome 19[6]. KIR genes were generated through a series of duplications, deletions and point mutations and so are highly homologous 10-15kb long genes separated by only ∼1kb. The human genome can contain up to 14 different KIR loci with 9 genetic motifs according to a recent classification (Roe et al., 2020a). Although over a thousand KIR alleles have been reported to date (1,617 different alleles are reported in the IPD-KIR database (Robinson et al., 2012)), any two KIR alleles from any two loci share 85-98% sequence identity (Roe et al., 2017).

KIRs can be categorised according to their number of extracellular immunoglobulin-like domains and their function (Marsh et al., 2003). According to the former, *KIR2D* genes encode receptors with two immunoglobulin domains, whereas *KIR3D* genes encode receptors with three. According to their function, KIRs can be activating KIRs (aKIRs) or inhibitory (iKIRs).

[4] A recent and more complete review about the function of inhibitory receptors can be found in Rumpret et al. (2020).

[5] Paired receptors are receptor families with both activating and inhibitory isoforms.

[6] In particular, KIRs are encoded within the leucocyte receptor complex (LRC) region: a gene-dense region containing other immune families of inhibitory receptors like *Siglec* and the leukocyte Ig-like receptor family (*LILR*) (Daëron et al., 2008).

aKIRs are characterised by a short cytoplasmatic tail – for example, KIR2DS2, with S for short – with a charged lysine that allows association with the adapter molecule DAP12. DAP12 contains an immunoreceptor tyrosine-based activation (ITAM) motif and transduces activating signals (Lanier et al., 1998). In contrast, iKIRs contain an immunoreceptor tyrosine-based inhibitory motif (ITIM) in their long cytoplasmatic tail – for example, KIR2DL2, with L for long – and directly transduce inhibitory signals[7] (Burshtyn et al., 1996).

Individuals vary in the number of activating and inhibitory KIR genes they carry in their genomes. This population's KIR copy number variation is driven by the KIR genes' high recombination rates; the high degrees of homology between alleles and transposon-rich elements facilitate the generation of dozens of structural variants or haplotypes (Traherne et al., 2010; Ordonez et al., 2011; Rajalingam et al., 2004). KIR haplotypes have been broadly grouped according to their gene content into group A and group B haplotypes (Figure 1.1 **A**). Group A haplotypes contain several iKIR genes, namely *KIR2DL1*, *KIR2DL2/L3* and *KIR3DL1*, plus the framework genes *KIR3DL2*, *KIR3DL3* and *KIR2DL4*. KIR A haplotypes also contain an additional activating KIR, *KIR2DS4*, but most alleles are non-functional due to an exonic deletion (Graef et al., 2009; Maxwell et al., 2002). Group B haplotypes show more structural diversity than group A haplotypes and contain more aKIRs (Pyo et al., 2010; Uhrberg et al., 1997).

A second level of diversity is allelic polymorphism[8] (Figure 1.1 **B**). The full functional implications of this allelic variation are still unknown, but allelic variation has been shown to impact surface protein levels. For example, *KIR3DL1* alleles have been grouped into "high expressers" and "low expressers" whilst the KIR3DL1*004 molecule is not expressed on the cell surface and is considered a null allele (Boudreau et al., 2014; Gardiner et al., 2001; Pando et al., 2003; Yawata et al., 2006). The binding strength of KIRs to their ligands is also determined by allelic variation; for example, KIRs encoded by *KIR2DL2* alleles at the *KIR2DL2/L3* locus are typically stronger binders than those encoded by the *KIR2DL3* alleles (Moesta et al., 2008). Although KIR A haplotypes are invariant in terms of gene content, they have retained high levels of polymorphism (Pyo et al., 2010). Conversely, KIR B haplotypes display high structural diversity but little allelic polymorphism. The clinical relevance of KIR allelic variation is demonstrated by disease associations such as the association between the *KIR3DL1*004* allele

[7] An exception to this rule is KIR2DL4 which displays features of both activating and inhibitory isoforms; despite having an ITIM motif in its long cytoplasmatic tail, KIR2DL4 contains a transmembrane activation motif and is considered an aKIR (Rajagopalan and Long, 2012; Faure and Long, 2002).

[8] The highest number of alleles has been reported for *KIR3DL1* and *KIR3DL3* genes, which display 184 and 248 alleles, respectively (Maccari et al., 2020).

and slower AIDS progression (Martin et al., 2007) or *KIR3DL2*107* and early onset of allergic pathologies (Gao et al., 2022).

A third level of diversity is conferred by the KIR ligands (Figure 1.1 **C**). Each KIR binds a subset of ligands with similar, if not higher, levels of polygenism and polymorphism, the HLA-I molecules[9]. Although not all KIR ligands have been identified – especially those for aKIRs – it is well established that iKIRs bind HLA molecules in broad allele groups. For example, KIR2DL1 recognizes HLA molecules carrying the C2 motif – Asparagine at position 77 and lysine at position 80 of the α-1 α-helix – while KIR2DL2/L3 recognize molecules encoded by *HLA-C1* allele group, defined by serine at position 77 and asparagine at position 80 of said helix (Biassoni et al., 1997; Valés-Gómez et al., 1998). On the other hand, KIR3DL1 binds HLA molecules carrying the Bw4 epitope, a sequence motif determined by amino acids 77-83 on the α-1 α-helix (Gumperz et al., 1997). In addition, KIRs and their HLA ligands are encoded on different chromosomes, making their inheritance independent. This fact gives rise to a high combinatorial diversity: individuals vary in the number of *KIR-HLA* gene pairs they carry as they may miss the genes encoding either a KIR receptor, its HLA ligand, or both. Therefore, in addition to KIR and HLA allelic variation, functional KIR polymorphism is ultimately amplified by the combinations inherited from these two unlinked loci[10].

1.2.2 KIR evolution

KIR diversification from the ancestral *KIR3DL* lineage is relatively recent; KIR genes have duplicated and mutated only over the last 30-40 million years in simians (Martin et al., 2000). KIR genes are rapidly evolving: most MHC-I specific KIRs are absent in the genome of humans' closest relatives, the chimpanzees (Abi-Rached et al., 2010; Khakoo et al., 2000). Similarly, few KIR genes are found in other mammals, and those identified have diverged significantly in terms of sequence and function or are non-functional[11] (Futas and Horin, 2013; Takahashi et al., 2004;

[9]The high variability of the HLA-I molecules is demonstrated by the 25,228 HLA-I alleles reported in the IPD-HLA database as of April 2023.

[10]Another layer of complexity in the KIR region is gene expression. Splicing isoforms have been documented (Bruijnesteijn et al., 2018) and might have functional consequences. Additionally, KIR expression is stochastic, so within an individual it is possible to find different NK cell populations expressing different combinations of KIRs. There is evidence of clonal and persistent NK cell subpopulations during infections (Béziat et al., 2013; Rückert et al., 2022). Since long-term persistence and clonality are characteristics associated with adaptive immunity, it is speculated that NK cells represent a subsystem of proto-Darwinian immunity (Müller et al., 2018).

[11]A distantly-related KIR lineage, *KIR3DX*, has also diversified in cattle (Guethlein et al., 2007; Dobromylskyj and Ellis, 2007).

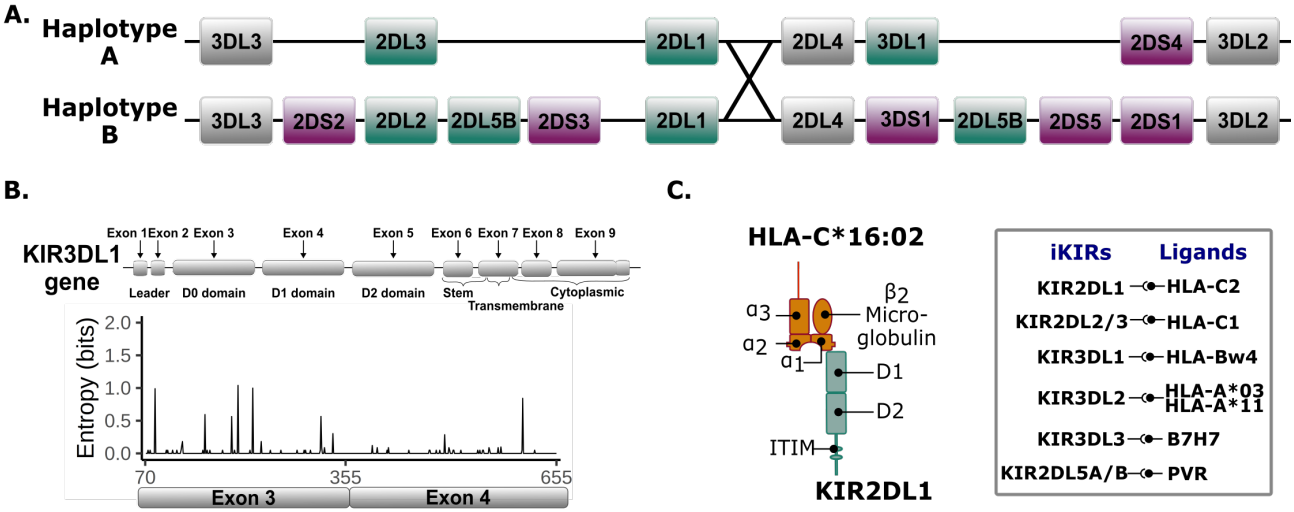


Figure 1.1: Sources of Killer cell immunoglobulin-like receptors (KIR) diversity. **A** Diverse KIR content in different haplotypes. Diagram with two examples of KIR A and B haplotypes. Inhibitory KIRs are shown in green and activating KIRs in purple. Framework genes are shown in grey. Cross indicates a recombination hotspot. **B** KIR sequence variability. Entropy (in bits) of nucleotide positions from multiple sequence alignment of the 181 *KIR3DL1* alleles with full coding sequence reported in the IPD-KIR database (Robinson et al., 2019). Higher entropy indicates higher variability at a given position (maximum is 2 bits). **C** Independent segregation of the human leucocyte antigen (HLA) and KIR genes amplifies functional KIR polymorphism. Some KIRs bind HLA-I molecules in broad allele groups, e.g. KIR2DL1 binds HLA-C2 molecules like HLA-C*16:02. Note that the inhibitory KIRs KIR3DL3 and KIR2DL5A/B do not bind HLA molecules but a B7 receptor and the polio virus receptor (PVR) respectively (Bhatt et al., 2021; Husain et al., 2019).

Sambrook et al., 2006; Hoelsbrekken et al., 2003). Nevertheless, other mammalian species have expanded different receptors with analogous KIR function (Futas et al., 2020; Schwartz and Hammond, 2018; Barten et al., 2001). In particular, rodents have diversified the genes coding for Ly49 receptors (Anderson et al., 2001). KIR and Ly49 receptors are structurally unrelated – immunoglobulin-like vs C-type lectin-like glycoproteins, respectively – and there are significant differences in tissue distribution. Ly49 receptors are expressed on neuronal soma, axons and dendrites, playing a role in neurite branching, synapse formation and neuronal survival (Zohar et al., 2008). No similar roles have been described for KIRs. However, KIR and Ly49 share other similarities: they both bind MHC molecules and are expressed in NK cells.

The different evolutionary paths of NK cell receptor diversification underscore the well-established role of KIRs and analogous families in immune defence and reproduction (Welsh and Waggoner, 2013; Hiby et al., 2004). In humans, KIR A haplotypes are better at fighting infections but are associated with preeclampsia (Hiby et al., 2004) whereas KIR B haplotypes promote higher birth weights (Hiby et al., 2014)[12]. These two competing survivalist functions constrain the diversification of KIR genes and the genes encoding their ligands – HLA-I genes –, promoting

[12] Although present at different frequencies, KIR A and B haplotype groups are found in all human populations studied to date (Hollenbach et al., 2012), suggesting that both haplotypes are actively maintained by balancing selection.

ongoing coevolution[13].

1.2.3 KIR expression and function

NK cells are the main KIR-expressing population (Cooley et al., 2007), although KIRs can also be detected in a subset of terminally differentiated T cells (Mingari et al., 1996; Young et al., 2001).

KIR expression is determined by KIR genotype and minimally influenced by HLA-I genotype (Andersson et al., 2009). However, the stochastic epigenetic regulation at the KIR gene promoter level results in a clonally distributed expression of KIRs: each cell will express a different set of one, two or more KIRs (Björkström et al., 2010) although T cells typically express only one KIR (Björkström et al., 2012). Additionally, NK cells progressively acquire KIRs as they develop and differentiate to establish the NK cell repertoire (Björkström et al., 2010).

KIR expression is considered stable in NK cells (Uhrberg, 2005; Le Garff-Tavernier et al., 2010), which contrasts with T cells, where KIR^+ T cells accumulate with age (Anfossi et al., 2001), as they are expressed after T cell development in a subset of antigen-experienced cells (Vely et al., 2001; Mingari et al., 1996; Young et al., 2001). There is also evidence that iKIRs are transiently upregulated upon TCR-dependent T cell activation (Huard and Karlsson, 2000; Zhang et al., 2023).

1.3 Clinical relevance of KIR diversity

Even when T cells can express both aKIRs and iKIRs (Björkström et al., 2012), until now, most KIR gene disease associations are attributed to NK cell activity during the innate immune response. The rationale behind this relationship is that NK cells are the first lymphocyte responders during viral infections (Welsh and Waggoner, 2013) and the most prevalent lymphocyte subset in the decidua during early pregnancy stages, where they actively participate in placentation (Wallace et al., 2012). However, given that T cells express KIRs, variation in KIR genotypes could, in principle, also explain variation in antigen-specific responses between individuals during the adaptive immune response and the possible autoimmune conditions resulting from the erroneous activation of autoantigen-specific T cells (Coppieters et al., 2012).

[13]For a recent review see Pollock et al. (2022).

In the following sections, I review the current evidence on KIR-HLA diversity influencing disease outcomes. I classify those associations in those that are NK cell-mediated – innate responses modulated by KIRs – and those that are T cell-mediated –adaptive responses modulated by KIRs.

1.3.1 KIR disease associations linked to innate NK cell responses

The relevance of KIRs in immune responses stems from the seminal studies showing that NK cells specifically targeted tumor cells lacking HLA-I expression but were inhibited when the same tumors expressed HLA (Ljunggren and Kärre, 1990). This observation was originally named the "missing-self" response. KIRs were not known at that time but these experiments predicted the existence of inhibitory receptors on NK cells. After the discovery of the underlying mechanism of the missing-self response (Moretta et al., 1990), Valiante and Parham (1997) hypothesized that, in the context of hematopoietic Stem Cell Transplants (HSCT), KIR-HLA mismatches between donor KIR genes and recipient HLA-I genes would promote graft versus host disease (GvHD) due to donor NK cell alloreactivity. Indeed, the first KIR disease association was reported in an study following individuals undergoing HSCT (Ruggeri et al., 1999). Unexpectedly, KIR-HLA mismatch was associated with lower relapse rates and higher survival (Ruggeri et al., 1999, 2002) and so it seems that unlike donor T cells, donor NK cells do not promote GvHD. However, this association seems strongly dependent on the conditioning regime, graft treatment and the type of disease since other studies have failed to show a significant protective effect of KIR-HLA mismatched donor grafts (Hsu, 2010). KIRs and KIR-HLA combinations have also been associated with malignancies other than leukaemia and interestingly with response to immune checkpoint blockade: inhibitory KIR-HLA gene combinations are associated with increased overall survival in lung cancer patients treated with αPD-1 (Roe et al., 2022) whereas presence of the activating *KIR3DS1* gene is associated with resistance to αPD-1 therapy also in lung cancer (Trefny et al., 2019).

Another classic KIR-HLA disease association is found in Human Immunodeficiency Virus 1 (HIV-1) infection. In the presence of its ligand gene, *HLA-Bw4-80I*, the aKIR allele at the *KIR3DL1/S1* locus, *KIR3DS1*, is associated with low setpoint HIV-1 viral load, slower progression to AIDS, high $CD4^+$ cell count, and reduced occurrence of opportunistic infections in different HIV-1 infected cohorts (Barbour et al., 2007; Boelen et al., 2018; Martin et al., 2002; Pelak et al., 2011; Qi et al., 2006). *KIR3DS1+HLA-Bw4-80I+* genotype is also associated with

increased odds of seronegativity in HIV-exposed individuals (Boulet et al., 2008; Jennes et al., 2013). Other examples of KIR gene pairs affecting clinical outcome include associations with spontaneous clearance of hepatitis C virus infection (HCV) (Khakoo et al., 2004; Thöns et al., 2017).

There are also reported associations between KIR genes and reproductive success, with inhibitory KIR-HLA maternal-fetal interactions increasing the risk of preeclampsia and miscarriage (Hiby et al., 2004; Huhn et al., 2018), although counterintuitively, the risk of acute atherosis in preeclampsia is also associated with aKIR interactions (Johnsen et al., 2018). The cellular mechanism underlying the KIR associations with reproductive success is attributed to decidual NK cells (Smith et al., 2009). Mouse studies show that NK cells participate in trophoblast invasion and remodellation of spiral arteries in the endometrium (Ashkar et al., 2000; Sojka, 2020). NK cell inhibition by iKIRs might hamper trophoblast infiltration, resulting in insufficient blood supply to the placenta, leading to complications like preeclampsia or low birth weight (Male and Moffett, 2023; Hiby et al., 2014).

Finally, KIR genes have also been associated with autoimmune disease risk. The *KIR2DL2+HLA-C2+* compound genotype increases psoriasis risk whereas *KIR3DL1+HLA-Bw4+* is protective against multiple sclerosis (Ahn et al., 2021; Hollenbach et al., 2016) and late-onset type 1 diabetes (T1D) (Traherne et al., 2016). However, both beneficial and detrimental roles of NK cells have been reported in autoimmune diseases like type 1 diabetes, multiple sclerosis or rheumatoid arthritis (Kucuksezer et al., 2021; Marca et al., 2018; Beliën et al., 2022). Therefore, the mechanism underlying these KIR associations is less clear.

1.3.2 KIR-disease associations linked to T cell responses

In addition to their role in innate responses (Figure 1.2 **A**), NK cells have two mechanisms to regulate adaptive T cell responses, either through cytokine secretion or via direct contact with T cells or other immune cells like DCs (Pallmer and Oxenius, 2016). Therefore, KIR-HLA interactions on NK cells might *indirectly* affect the T cell response (Figure 1.2 **B**). Furthermore, *in vitro* T cell KIR ligation increases T cell survival (Boelen et al., 2018) so KIRs can *directly* impact human adaptive T cell immunity (Figure 1.2 **C**). Whilst there are multiple pathways whereby KIRs could affect T cell responses, whether these interactions have any impact on the course of disease in humans has not yet been elucidated.

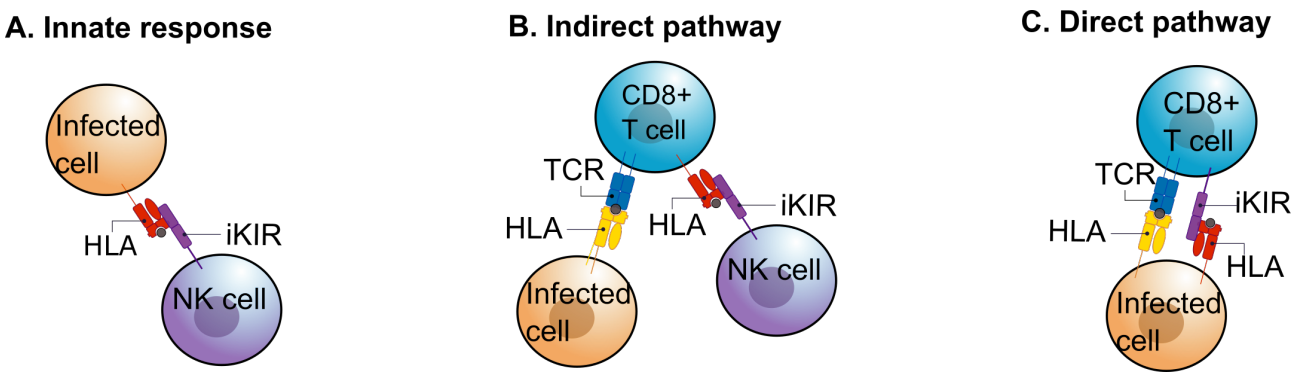


Figure 1.2: KIR-HLA interactions during innate and adaptive immune responses. **A** KIR-HLA interactions modulate innate NK-cell responses (i.e. missing self-responses). NK cell innate response is typically the mechanism attributed to KIR-HLA associations. **B** and **C** KIR-HLA interactions affect T-cell survival. **B** Indirect pathway: NK cells can affect $CD8^+$ T-cell mediated responses. This immunoregulatory role might be modulated by KIR-HLA interactions. **C** Direct pathway: iKIR expression on antigen-specific $CD8^+$ T cells reduces activated induced cell death and increases T-cell lifespan upon ligation of the cognate KIR ligand. Figure reproduced from Cook et al. (2013) with permission of the rights holder, Elsevier.

Recent immunogenetic analysis from the Asquith lab aimed to determine the effect of these KIR-mediated T cell modulation in the context of three persistent viral infections (Boelen et al., 2018; Seich al Basatena et al., 2011). These two studies focus on the subset of classical HLA-I disease associations attributable to T cells, in contrast to KIR-HLA association studies, which address the impact of KIR modulation of NK cells. The HLA-I disease associations indicate that $CD8^+$ T cells are clinically relevant[14]. If iKIR-HLA interactions modulate $CD8^+$ T cell responses, then functional iKIR genes (iKIR genes together with their HLA ligand genes) would modify – i.e. enhance or weaken – these T cell-mediated HLA-I disease associations.

The studied iKIR-HLA ligand interaction is a three-way (gene-gene-gene) interaction: iKIR gene – iKIR ligand gene – disease risk HLA allele. Three-way gene interactions are not investigated in GWAS studies due to multiple comparisons resulting in a combinatorial explosion which renders them impractical (Benjamini and Hochberg, 1995); three-way gene interactions are thus invisible to hypothesis-free GWAS approaches and would have been missed by GWAS studies to date. To detect the iKIR-HLA ligand interactions, it is necessary to perform hypothesis-driven – candidate gene studies – in moderate to large cohorts.

To investigate the clinical relevance of iKIR-T cell interactions, Boelen et al. (2018); Seich al Basatena et al. (2011) analyzed three independent cohorts of individuals living with persistent viral infections: Hepatitis C Virus (HCV), Human Immunodeficiency Virus 1 (HIV-1), and Human T-lymphotropic virus 1 (HTLV-1). During these studies, the strength of the HLA associations with clinical outcomes was assessed in patient cohorts stratified into iKIR gene

[14]HLA-I molecules present antigen to $CD8^+$ T cells and so restrict $CD8^+$ responses. In contrast, HLA class II (HLA-II) molecules present antigen to $CD4^+$ T cells and therefore restrict $CD4^+$ responses.

carriers and non-carriers. In Seich al Basatena et al. (2011), the iKIR gene *KIR2DL2* was found to enhance both protective and detrimental HLA-I associations. This enhancement was more prominent in the presence of genes encoding the high-affinity ligand of KIR2DL2 – HLA alleles carrying the C1 motif – suggesting that iKIR ligation is important for the observed *KIR2DL2* impact on the T-cell response. Together, these observations showed for the first time that the iKIR genotype has a significant impact on HLA-I associations, and suggested that iKIRs affect antiviral HLA-restricted T cell responses. No effects were detected for similar iKIR genes like *KIR2DL1* or *KIR2DL3*. However, the absence of significant results for these iKIR genes may be explained by a cohort size effect due to the low frequency of individuals with functional *KIR2DL1* and *KIR2DL3* compared to that for the *KIR2DL2* gene.

Evidence of a similar effect for *KIR3DL1* can be found in a study investigating the role of *KIR3DS1* in HIV-1 control (Pelak et al., 2011). In that study, *KIR3DL1* in the presence of its ligand gene, *HLA-Bw4*, enhanced the known protection conferred by *KIR3DS1+HLA-Bw4-80I+* compound genotype (Martin et al., 2002). The authors attributed this effect to KIR3DS1-KIR3DL1 interactions on NK cells. Interestingly, a *HLA-Bw4-80I* allele, *HLA-B*57*, is associated with low setpoint viral load and slow progression to disease in multiple HIV-1 cohorts (Carrington et al., 1999; Kaslow et al., 1996); preferential presentation of conserved epitopes to $CD8^+$ T cells is thought to explain this protective association (Borghans et al., 2007). Given that *HLA-B*57* is prevalent in *KIR3DS1+HLA-Bw4-80I+* individuals, *KIR3DL1* in the presence of its ligand gene, *HLA-Bw4* might enhance the well-known protective association of *HLA-B*57* with low viral load. The potential *KIR3DL1*-mediated enhancement of *B*57* protection in this study is reminiscent of the effect observed in HCV and HTLV-1 with *KIR2DL2* gene (Seich al Basatena et al., 2011), suggesting that different iKIR genes similarly modulate T cell responses.

To test this hypothesis, in a follow-up study Boelen et al. (2018) investigated the effect of multiple iKIR genes, namely *KIR2DL1*, *KIR2DL2/L3* and *KIR3DL1*. A weighted count of the number of iKIR-ligand gene pairs – the iKIR score – present in each individual was calculated. Again, the cohorts were split into individuals with a high inhibitory score and individuals with a low inhibitory score and the strength and significance of HLA associations were assessed in each subcohort (Table 1.1). All HLA-I associations in this HTLV-1 cohort were strengthened in individuals carrying a high inhibitory score and were greatly weakened and nonsignificant in individuals with a low inhibitory score. In HCV infection a similar result was found: the protective *HLA-B*57* association was enhanced in individuals with a high iKIR score. Finally,

in HIV-1 infection, both the protective *HLA-B*57* and detrimental *HLA-B*35Px* associations were enhanced in individuals with a high iKIR score; this was replicated in two independent cohorts (Boelen et al., 2018). This extensive immunogenetic analysis of three different chronic viral infections validated earlier preliminary findings that iKIR genes, in the presence of their ligand genes, modulate HLA-I associations.

Cohort	Viral infection	Outcome metric	iKIR score threshold	HLA association	HLA association in iKIR lo		HLA association in iKIR hi		N	
					Effect	P value	Effect	P value	HLA+	HLA−
Pooled IAVI and IAVI partners	HIV-1	Log viral load	2	*B*57*	Coeff= -0.33	0.08	Coeff= -0.47	0.0002***	91	750
Pooled IAVI and IAVI partners	HIV-1	Log viral load	2	*B*35Px*	Coeff= -0.12	0.4	Coeff= 0.31	0.003**	153	688
US	HIV-1	Time to low CD4 count (<200 cells/mm3)	2	*B*57*	HR=0.29	0.25	HR=0.25	0.004**	44	446
US	HIV-1	Time to low CD4 count (<200 cells/mm3)	2	*B*35Px*	HR=1.54	0.22	HR=2.57	0.001**	64	426
Kagoshima	HTLV-1	Odds of HAM/TSP	1.5	*B*54*	OR=1.29	0.7	OR=5.33	0.0007***	82	310
Kagoshima	HTLV-1	Odds of HAM/TSP	1.5	*A*02:07*	OR=0.81	0.8	OR=0.10	0.003**	30	362
Kagoshima	HTLV-1	Odds of HAM/TSP	1.5	*C*08*	OR=1.09	0.9	OR=0.32	0.02*	56	336
HCV-seropositives	HCV	Odds of spontaneous clearance of HCV	2.5	*B*57*	OR=1.55	0.3	OR=2.04	0.03*	84	697

Table 1.1: Functional iKIR gene modulation of HLA-I associations in chronic viral infections. The effect of iKIR score (weighted sum of functional iKIR genes) on HLA-I associations was assessed in different chronic viral infection cohorts. IAVI and IAVI partners cohorts were pooled to increase cohort size. Each cohort was stratified into individuals with high and low iKIR score according to a set cut-off (chosen to give balanced groups). The risk of the HLA-I alleles significantly associated with clinical outcome was re-assessed in each stratum i.e in individuals with a high iKIR score (denoted iKIR hi) and in individuals with a low iKIR score (denoted iKIR lo). The measure of association (Effect) varies according to the outcome metric: coefficient of regression (Coeff) for log viral load, hazard ratio (HR) for time to low $CD4^+$ count and odds ratio (OR) for odds of disease or odds of spontaneous clearance. The effect sizes for each HLA association in each iKIR stratum are reported alongside p-values and cohort sizes. Analysis with groups based on the count of functional iKIR genes rather than iKIR score gave very similar results (the two numbers are highly correlated). Additional analyses considering each iKIR independently, using non-pooled cohorts and with different iKIR score cutoffs are reported in the original studies (Boelen et al., 2018; Seich al Basatena et al., 2011). $P < 0.001$ ***; $P < 0.01$ **; $P < 0.05$ *; $P < 0.1$ ·; P values are two-tailed. Table reproduced from Mora-Bitria and Asquith (2023) licensed under CC BY.

1.3.3 iKIRs modulating adaptive T cell responses: beyond chronic viral infections

We have seen that iKIR genes are clinically relevant in three chronic viral infections. The observed iKIR enhancement of detrimental and protective HLA-I associations suggests that iKIRs modulate T cell responses. In Boelen et al. (2018), mathematical modelling showed that the immunogenetic findings could be explained if iKIR ligation increases T cell survival. This hypothesis has just been validated experimentally; Zhang et al. (2023) reports an unexpectedly large effect of functional iKIR genes on $CD8^+$ T cell lifespan in humans *in vivo*.

Whether this iKIR-mediated modulation impacts disease outcome in other contexts has not been explored. An increased T cell survival is expected to be beneficial during antiviral responses. In autoimmunity though, where an enhanced T cell response might be considered detrimental, iKIRs might have the opposite effect.

1.4 Thesis scope

In this Thesis, I aim to investigate whether iKIR-HLA interactions modulate HLA-restricted autoreactive responses.

Several KIR and KIR-HLA gene associations with autoimmune diseases like type 1 diabetes (T1D), rheumatoid arthritis (RA), multiple sclerosis (MS) or Systemic Lupus Erythematosus (SLE) have been reported. However, these studies analyse small cohorts and report both detrimental and protective iKIR associations (Pollock et al., 2022). Here, I take a different approach and build on previous evidence on the effect of the number functional iKIR genes on HLA associaitons in persistent viral infections (Boelen et al., 2018). This approach focusing on the count of functional iKIR genes rather than on discrete KIR-HLA compound genotypes hints at a pan-iKIR immunoregulatory mechanism where the degree of iKIR engagement has consequences for T cell longevity, and in turn affects the outcome of adaptive responses.

The study of functional iKIR genes in autoimmune disease will assess whether the iKIR-dependent modulation of adaptive T cell immunity has broader implications on human health. Further, this study holds other potential rewards like revealing additional genetic risk factors in autoimmunity and a novel therapeutic target for the treatment of autoimmune disease.

Objectives:

1. Investigate the role of iKIR genes in autoimmunity to address the following hypothesis: functional iKIR genes have a detrimental effect on HLA associations in T1D.

2. Implement mathematical models to find possible mechanisms for the iKIR modulation of HLA associations in T1D. The working hypothesis is that iKIR engagement increases T cell lifespan.

3. Analyze protein and RNA expression data to investigate which pathway, the direct or indirect, is more likely to be responsible for the observed iKIR effect in T1D.

Chapter 2 outlines the datasets and statistical methods used in this Thesis.

In Chapter 3, I address objective 1. It contains the immunogenetic analysis of a large T1D case-control dataset. The findings are validated in an independent dataset.

In Chapter 4, I present the results from objective 2. It contains a plausible explanation of the counter-intuitive iKIR effects observed in objective 1.

Chapter 5 focuses on the results from objective 3. It contains the analysis of flow cytometry and RNA seq data of PBMCs.

Finally, in Chapter 6, I summarize the findings of this Thesis and their implications, discuss the limitations of the study and propose next steps.

Thesis-related publications

Mora-Bitria, L and Asquith B. Innate receptors modulating adaptive T cell responses: KIR-HLA interactions and T cell-mediated control of chronic viral infections. *Immunogenetics*, 2023. doi: 10.1007/s00251-023-01293-w.

Mora-Bitria, et. al. Inhibitory KIRs decrease HLA class II-mediated protection in Type 1 Diabetes. *In preparation.*

Chapter 2

Modelling adaptive immune responses

The growing volume of high-throughput and high-quality genomic data has allowed unbiased data-driven research methods to uncover hundreds of variants associated with disease[1]. Although the search for causal genes has obviously been productive, genomics' promise to explain the mechanisms behind complex diseases has not been fully realised. Each of these risk variants confers relatively small increments in disease risk. Further, their cumulative sum typically explains only a small proportion of the overall disease genetic variation, i.e. disease heritability[2]. This is known as the "missing heritability problem" and emphasizes that big data by itself is not always useful to identify the molecular and/or cellular mechanisms underlying complex diseases.

In this Thesis, I use mathematical models and an hypothesis-driven approach to study the role of iKIR genes in autoimmunity. Mathematical models can be divided into phenomenological and mechanistic models. Phenomenological models, also known as empirical models, statistical models or data-driven models, aim to describe relationships between the observed variables. As a consequence, statistical models are also named black box models because they are not derived from first principles. Mechanistic models try to represent explicitly the different parts and processes of a system. Both types of models are considered in this work but statistical models will be the main focus. Despite mechanistic models being superior in theory, the knowledge required to create such models is typically not available in practise. Therefore, I mainly use statistical models to test and validate hypotheses. I also implement a mechanistic model to

[1] Almost 60k distinct SNP associations are reported in NHGRI-EBI GWAS Catalog (Sollis et al., 2023).
[2] Heritability of a trait is the amount of phenotypic variation explained by genetic variation in a population.

find plausible explanations for observed KIR effects on disease risk.

2.1 Cohorts

This section introduces the cohorts and datasets analyzed throughout the Thesis.

2.1.1 The UK-GRID case-control cohort

The primary cohort is a UK-based cohort of 13,452 individuals, with 6,783 T1D cases from the UK Genetic Resource Investigating Diabetes (UK-GRID) (Genetics of Type 1 Diabetes in Finland et al., 2007), aged between 6 months and 16 years.

Clinical case definition: UK-GRID case collection included subjects diagnosed with T1D (insulin-dependent) that were under 17 years of age. T1D patients over 16 years of age were excluded. Additional exclusion criteria include insulin-treated diabetes which is secondary to other pathology, Cystic fibrosis, Thalassemia and Haemophilia.

UK-GRID cases were matched with 6,669 controls from the British 1958 Birth Cohort (B58C) (Power and Elliott, 2006). All subjects were genotyped on the Illumina Infinium high density Immunochip array (Illumina Inc., USA) (Cortes and Brown, 2010). HLA and KIR genotypes were imputed using SNP data (see Genotype imputation). Individuals with missing information (sex or HLA/KIR genotype) were discarded, leaving a total of 11,961 individuals (6,219 cases and 5,742 controls).

2.1.2 HBDI family cohort

An independent T1D dataset analysed in this Thesis consists of 402 US families from the Human Biological Data Interchange (HBDI), a repository founded for the purposes of diabetes research. The HBDI family cohort contains children affected with T1D and their first-degree relatives. All families have at least 2 affected children and parents are not affected. All 1,721 individuals in this repository have been previously KIR and HLA genotyped (Noble et al., 1996; Erlich et al., 2008; Traherne et al., 2016). Pedigree information was available for all individuals.

2.1.3 Kallionpaa single-cell RNA sequencing dataset

The Kallionpaa dataset contains single-cell RNA sequencing (scRNA-seq) data of peripheral blood mononuclear cells (PBMCs) of 4 children postive for islet auto-antibodies and 4 matched controls (Kallionpää et al., 2019). The PBMC samples were collected at multiple timepoints (12, 18 and 24 months of age) from seven children who developed β-cell autoimmunity (case subjects) at a young age. Samples from matched controls were also collected. Of those, 8 samples (4 cases and 4 controls) were sequenced at single cell resolution using Chromium Single Cell Gene Expression platform from 10X Genomics (Single Cell 3' Solution v2 reagents).

Single cell technologies like those from 10X Genomics can partition single cells into wells or, in this case, into droplets. In addition to the single cell, each droplet contains a bead coated with poly(dT) – deoxythymidines – capture sequences, enabling the capture of poly-adenylated mRNA molecules. In addition to the poly(dT) sequence, the capture sequences contain a cell barcode – short nucleotide sequence common to all capture sequences in a single bead – and a unique molecular identifier (UMI). UMIs are also short nucleotide sequences but they are random: each capture sequence in a bead contains a random UMI sequence and so it is very unlikely to find two capture sequences with the same UMI in a bead. Therefore, before any PCR amplification step, each transcript in a cell will be barcoded by a unique barcode, the UMI. The barcoding step (cell bardoding + UMI barcoding) takes place at the beginning of library preparation, before any PCR amplification step. UMIs are particularly useful when starting with low amounts of material; stochastic amplification in the first rounds of PCR due to low input cDNA plus inherent polymerase amplification biases lead to erroneous transcript quantification during the bioinformatic analysis step. UMIs solve these issues as they will enable more accurate quantification of the number of initial transcripts – avoiding PCR bias, e.g. allowing bioinformatic identification of PCR duplicates – and better discrimination of PCR/sequencing errors.

After library preparation, indexed libraries were sequenced with Illumina HiSeq 3000 (one sample per lane) using paired-end sequencing. I downloaded the raw FASTQ files from the scRNAseq experiment from the European Genome-Phenome Archive (EGA).

2.1.4 Twins UK cohort

The UK Adult Twin Registry, in short TwinsUK Registry, was established in 1992 to investigate osteoarthritis and osteoporosis genetics in women but as of now includes 12,000 twins from UK, both male and female, aged between 18 and 103 years. The study participants have been studied for a wide range of clinical and behavioral traits, and more recently longitudinal phenotypes were included to study healthy aging (Moayyeri et al., 2013). In this Thesis, I focus on a set of 497 female twins in the TwinsUK registry, which will be referred as the *TwinsUK cohort*. These individuals were recently immunophenotyped for thousands of traits (Roederer et al., 2015). Of those, 457 have been genotyped on the HumanHap300 and the HumanHap610Q. Access to QCed SNP data was provided by TwinsUK registry. All individuals were healthy at the time of blood collection for immunophenotyping (Roederer et al., 2015).

2.1.5 Cardiff cohort

The Cardiff cohort contains KIR immunophenotype data from 10 T1D patients and 10 matched controls (matching factors: age and gender). Healthy controls were recruited at Imperial College London by the Asquith lab. The healthy cohort age ranged between 22-65 with 60% females. Blood samples from healthy individuals were collected and processed by Charandeep Kaur. Blood samples from T1D patients with long-standing disease (N=3) were provided by Linda Hadcocks (University of St George's). Additionally, 5 blood samples from T1D patients with recent diagnosis (within 1 year of diagnosis) and 2 samples from patients with long standing disease were provided by Susan Wong (University of Cardiff). The T1D cohort age ranged between 18-58 with 70% females.

All samples were stained and analysed on the flow cytometer in the same experiment by Kelly Miners and Kristin Ladell (University of Cardiff). The multi-colour antibody panel (see section B.2) was designed to assess the expression of KIR2DL1, KIR2DL2/L3 and KIR3DL1 in the different $CD4^+$ and $CD8^+$ memory populations as well as in NK cells.

2.2 Genotype imputation

Genotype imputation refers to the process of predicting –or imputing– a genotype at a locus or at multiple loci that have not been directly assayed. Imputation methods use a reference panel where SNP genotype data and the genotype of interest are available for a set of individuals. Then, a statistical model is fitted to the reference panel to associate the SNP variation to the target genotype. Using this statistical model, one can then impute the genotype or genotypes of interest for individuals for whom only the relevant SNP genotypes are available.

HLA 4-digit resolution alleles at classical HLA loci (*HLA-A*, *B*, *C*, *DRB1*, *DQA1*, *DQB1* and *DPB1*) for the UK-GRID cohort were previously imputed to two field resolution with HIBAG v1.14.018 (Zheng et al., 2014).

KIR genotypes in the UK-GRID cohort were also previously imputed using KIR*IMP imputation software (Vukcevic et al., 2015). For KIR genotype imputation of the TwinsUK cohort, I used the github repository bjohnnyd/hla-kir-imputation that contains a Snakemake (Mölder et al., 2021) workflow to process SNP data for KIR*IMP. The workflow has 3 main steps. First, it converts genomic coordinates to GRCh37 coordinates (build 37) so SNP positions match those of the KIR*IMP SNP panel. This step can be skipped if SNP positions are already in GRCh37 coordinates. Secondly, the KIR SNPs should be encoded as in the KIR*IMP reference panel so strand reversal is performed (if needed). Lastly, haplotypes at the KIR region are estimated using SHAPEIT (Delaneau et al., 2014). KIR imputation is then performed using KIR*IMP software wtih the overlapping SNPs between the KIR*IMP panel and the SNPs in the dataset being imputed. I kept imputations at a probability threshold of 0.5 which has been reported to ensure 92% accuracy at KIR loci (Vukcevic et al., 2015).

2.3 Logistic Regression

In this Thesis, I investigate the effect of KIR genotype on disease outcome. The response or dependent variable is dichotomous: individual is healthy or has disease. Logistic regression models are typically used to analyse binary data.

Consider the following response variable Y_i with only two possible values:

$$y_i = \begin{cases} 1, & \text{if individual carries the disease} \\ 0, & \text{if individual is healthy} \end{cases}$$

Each realization of Y_i can take values of one and zero with a given probability p or 1-p, respectively. Now consider that we have multiple individuals that can be classified into k groups based on the value of an explanatory covariate, e.g. presence of a risk genotype:

$$x_i = \begin{cases} 1, & \text{if individual carries the risk genotype} \\ 0, & \text{if individual does not have the risk genotype} \end{cases}$$

We can then group the data points and define:

$$y_i = \text{number of individuals in genotype group } i$$

If the n_i observations in each genotype group i are independent and the probability of having the disease p_i is the same, we can represent the number of diseased and healthy individuals as a set of n_i Bernoulli trials using a binomial distribution with parameters p_i and n_i:

$$Y_i \sim B(n_i, p_i)$$

Logit Transformation

To test hypotheses, one can estimate p_i based on x_i. However, we cannot represent the probability p_i using a linear function of the covariate x_i because a linear predictor of the form $\eta = X\beta$ with estimated parameters β and a matrix of independent variables X can take any real value whereas p_i takes values between 0 and 1. To solve this issue, the probability p is transformed to odds by dividing the probability p by its complement $1 - p$, which removes the ceiling restriction:

$$odds_i = \frac{p_i}{1 - p_i}$$

Then, the odds are transformed to log-odds to remove the floor restriction:

$$logit(p_i) = log\frac{p_i}{1 - p_i}$$

This is known as the logit transformation and provides a link between the linear predictor η and the binomial distribution of the random variable Y_i by mapping the probabilities to any real value between $-\infty$ and $+\infty$.

The data can be aggregated in a 2 x 2 contigency table:

where y_i now is the number of diseased individuals in each group and fit the following logistic

	$Genotype(1)$	$Genotype(0)$
$Disease(+)$	a	b
$Disease(-)$	c	d

Table 2.1: 2x2 contingency table Disease status vs Genotype.

regression model:

$$logit(p) = log(\frac{p}{1-p}) = x_i^{'}\beta \tag{2.1}$$

Where $x_i^{'}$ is a vector of covariates and β a vector of regression coefficients. Parameters are obtained by maximizing the likelihood given the data.

2.3.1 Effect modification of KIR genotype

The biologic mechanism of interest is the KIR regulation of the T cell response, probably through an increase in lifespan. To estimate the magnitude and significance of this effect for human health, the starting point are HLA associations with disease, as these represent good evidence that T cells are implicated in disease etiopathogenesis. Then, if KIR genes impact the T cell response, we expect that the effect size of HLA associations changes depending on the KIR genotype (Figure 2.1 **A**). This KIR modulatory effect on HLA associations can be assessed using two different approaches: via stratification (or subgroup) analysis or using interaction terms in the logistic regression model.

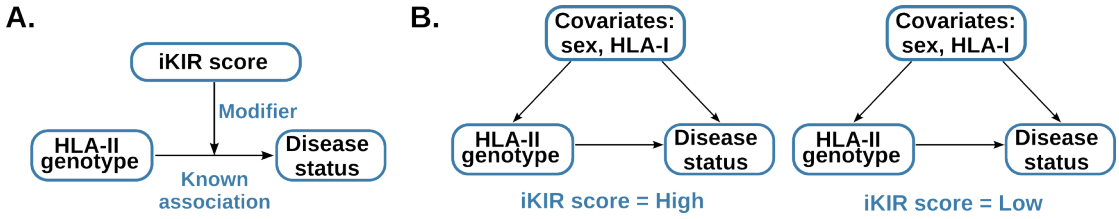


Figure 2.1: Effect modification of iKIR score on HLA risk. **A** Diagram representing the effect modification of interest. **B** Causal diagram in iKIR stratified populations.

Stratification

To assess the effect of KIR genotype on HLA associations we can classify individuals into those carrying a high number of KIRs (KIRhi) and those carrying a low number of KIRs (KIRlo). Then, the beta coefficient for the HLA genotype of interest can be estimated separately in each KIR stratum, which results in two logistic regression models (Figure 2.1 **B**). The advantage of this approach is that the interpretation of the results is straightforward and any covariates, not only HLA but also gender for example, in the model are allowed to differ by iKIR genotype

category. However this approach has less power as the datasets are halved (or reduced to the size of the strata) and one cannot directly calculate a p-value for the difference observed between the two strata. Nevertheless, we can calculate a p-value using Monte Carlo methods (see Approximate permutation test using Monte Carlo). The two stratified models are as follows:

$$logit(p) = \begin{cases} \beta_0^{iKIRhi} + \beta_{HLA}^{iKIRhi} HLA_i, & \text{if iKIR score} = 1 \\ \beta_0^{iKIRlo} + \beta_{HLA}^{iKIRlo} HLA_i, & \text{if iKIR score} = 0 \end{cases}$$

Interaction

Alternatively, we can consider a joint model for the two iKIR strata. The system of two regression models above can be collapsed into one regression model by considering two HLA genotype covariates, one for each iKIR strata, instead of one:

$$HLA_i^{Hi} = \begin{cases} HLA_i, & \text{if iKIR score} = 1 \\ 0, & \text{if iKIR score} = 0 \end{cases}$$

and:

$$HLA_i^{Lo} = \begin{cases} 0, & \text{if iKIR score} = 1 \\ HLA_i, & \text{if iKIR score} = 0 \end{cases}$$

The new explicit interaction model has three covariates HLA_i^{Hi}, HLA_i^{Lo} and iKIR score:

$$logit(p) = \beta_0 + \beta_{Lo} HLA_i^{Lo} + \beta_{Hi} HLA_i^{Hi} + \beta_{iKIR} iKIRscore$$

The β estimates for the HLA_i^{Lo} and HLA_i^{Hi} explanatory variables are the same as in the system of two regression models, i.e., β_{HLA}^{iKIRhi} and β_{HLA}^{iKIRlo}. Albeit having a joint model with covariates for each stratum, we still do not have a direct estimate of the difference between iKIR high and iKIR low and we need to take additional steps to calculate a p-value. Additionally, both the explicit interaction model and the stratification approach cannot deal with continuous effect modifiers, e.g. when iKIR score is a continuous variable with real values between 0 and 4, and therefore are restricted to discrete modifiers.

To overcome these issues, we can include an interaction term between the HLA genotype and the iKIR score (as a discrete or as a continuous variable):

$$logit(p) = \beta_0 + \beta_1 HLA_i + \beta_2 iKIR_i + \beta_3 : HLA_i iKIR_i$$

With this approach we directly get and estimate for the interaction between HLA and iKIR

score and the associated p-value.

2.4 Family-based analysis

To validate the results obtained from the analysis of the case-control UK-GRID cohort, I use the HBDI family cohort. Due to the relatedness between the individuals in this dataset, common statistical tools used for the analysis of cohorts of unrelated individuals cannot be used. In this section, I review family-based association methods.

Family-based studies are in principle superior to case-controls as confounding is better controlled than in unrelated case-control studies. In such population-based studies, a significantly higher frequency of a specific genetic variant in cases compared to controls suggests that this variant plays a functional role in the development of the disease. However, because of the non-relatedness between individuals in the study, information about the inheritance of the variant is lacking and any significant findings can be influenced by non-genetic factors like population stratification (Figure 2.2). Population stratification occurs when there is improper matching between cases and controls of the sample under study, which results in an uneven representation of the genetic subgroups or ancestries. In this scenario, differences in gene frequency between these sub-populations may appear as differences in gene frequencies between individuals with disease and those disease-free. In contrast, family-based designs are typically protected from population stratification or genetic *admixture*; the control sample set is chosen from the same families where the case sample set is selected and so appropiate matching can be ensured.

Furthermore, family-based studies offer additional advantages in minimizing the impact of other confounding factors. Family members typically share similar environmental and dietary exposures, making it more likely that phenotypic differences between them can be attributed to genetic variations rather than non-genetic uncontrolled factors.

One of the first approaches to leverage the advantages of family-based design was the haplotype relative risk (HRR). Falk and Rubinstein (Falk and Rubinstein, 1987) postulated that in trios (two parents and one affected child), those genes not transmitted to the affected children could be used as a well-matched pseudo-control genotypes. The frequency of a given genotype in affected children and in pseudo-controls can then be compared using standard statistical methods like χ^2 test. However, χ^2 test assumes independence between the observations and

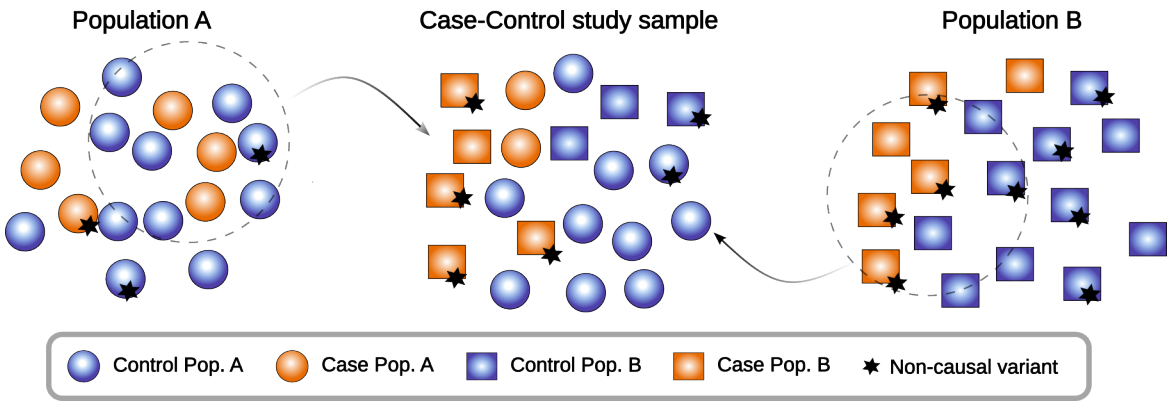


Figure 2.2: Population stratification confounds case-control studies of unrelated individuals. Population stratification confounds case-control studies of unrelated individuals. Note that the variant denoted by a black star is more frequent in population B (squares) than in population A (circles). Nevertheless, it is present at similar frequencies in cases and controls in both populations and therefore is unlikely to be a causal variant of the disease present in cases (orange squares and orange circles). In the study sample, there is an uneven sampling of cases (over-represented by population B) and controls (over-represented by population A) and a spurious disease association is detected.

this assumption does not hold in the presence of recombination between the variant of interest and the causal locus (Ott, 1989).

2.4.1 Transmission/Disequilibrium test

Spielman et al. (1993) introduced the transmission disequilibrium test (TDT) as a valid method for assessing linkage in the presence of association[3]. The TDT requires genotypes from simplex families, i.e. trios comprising both parents and one affected child. Similar to the HRR, the TDT compares transmitted and non-transmitted alleles. However, it employs a matched approach where each pair of alleles from each parent is treated as a matched pair. Each allele in the pair is categorized as transmitted or non-transmitted to the affected child, as illustrated Table 2.2, and so complete genotype information from both progenitors is required.

Mendel's first law of segregation states that the odds of transmitting the maternal allele instead of the paternal allele to offspring are 1:1. Therefore, in the absence of an association between the variant of interest and the causal locus, each allele at the locus of interest will be transmitted to affected children with an equal probability. Since Mendelian segregation can only be observed at heterozygous loci, only families with at least one heterozygote parent for the locus of interest are considered informative. As a result, family-based association studies are typically more expensive compared to population studies[4].

[3]It can also be used to test for association in the presence of linkage.

[4]To achieve similar statistical power, family studies require a larger number of genotypes compared to case-control cohorts. Specifically, in case-control studies with a balanced number of cases (N) and controls (N)

In this framework, where the transmission and non-transmission of alleles from progenitors are negatively correlated, a χ^2 test of independence is not appropriate. Instead, the valid non-parametric test is the McNemar χ^2 test for matched pairs. This test allows us to evaluate whether the expected proportions of transmitted and non-transmitted alleles are 0.5, indicating equal probabilities of transmission ($E(b/(c+b)) = E(c/(c+b)) = 0.5$, as shown in Table 2.2).

The McNemar test (or TDT) can only detect linkage if the variant is associated with the disease and vice versa. Therefore, rejecting the null hypothesis implies the presence of both linkage and association between the variant and the disease locus. This is more clearly illustrated in Figure 2.3. If there is no linkage between the variant and the causal locus (they are really distant on the chromosome and therefore the probability of recombination between homologous chromatides is $\sigma = 0.5$), then the variant will be transmitted together with the causal variant with probability 0.5: scenarios in Figure 2.3 **A** and Figure 2.3 **B** are equally likely and so are those in Figure 2.3 **C** and Figure 2.3 **D**.

Alternatively, even if the locus of interest and the causal locus are linked and the alleles at these loci tend to be inherited *en bloc* ($\sigma < 0.5$), when there is no association, the causal allele can occur with the variant of interest in the same haplotype (Figure 2.3 **A**) with equal probability as the neutral allele (Figure 2.3 **C**). In other words, both haplotype phases are equally likely. Finally, in the case of neither association nor linkage, then scenarios in Figure 2.3 **B** and Figure 2.3 **D** are equally likely. Therefore, under these 3 scenarios, the variant of interest will segregate independently of the causal variant to offspring, and the transmission disequilibrium test (TDT) will not reject the null hypothesis.

We apply the TDT to test the effect of iKIR score on HLA genotypes. Each HBDI family is analysed as a trio for each affected sibling. If multiple affected children are available only one child is randomly selected. Similar to the case-control setting, trios are stratified into "iKIR high" or "iKIR low" based on the iKIR score of the affected child (iKIR high: iKIRscore>threshold, iKIR low: iKIRscore≤threshold). Then for both strata and each driver allele, the number of transmissions of the protective allele from heterozygous parents to affected children and the number of non-transmissions i.e. the transmissions of the protective allele to a non-person is counted following Spielman et al. (1993); Ott (1989) (Table 2.2).

The difference in the ln[OR], i.e. difference of the log of the ratio of transmitted to non-

one needs to genotype 2N individuals whereas in family studies one typically needs needs (3+x)N times more genotyping, where x is the number of additional informative trios to achieve similar power to the case-control setting (Cordell and Clayton, 2005).

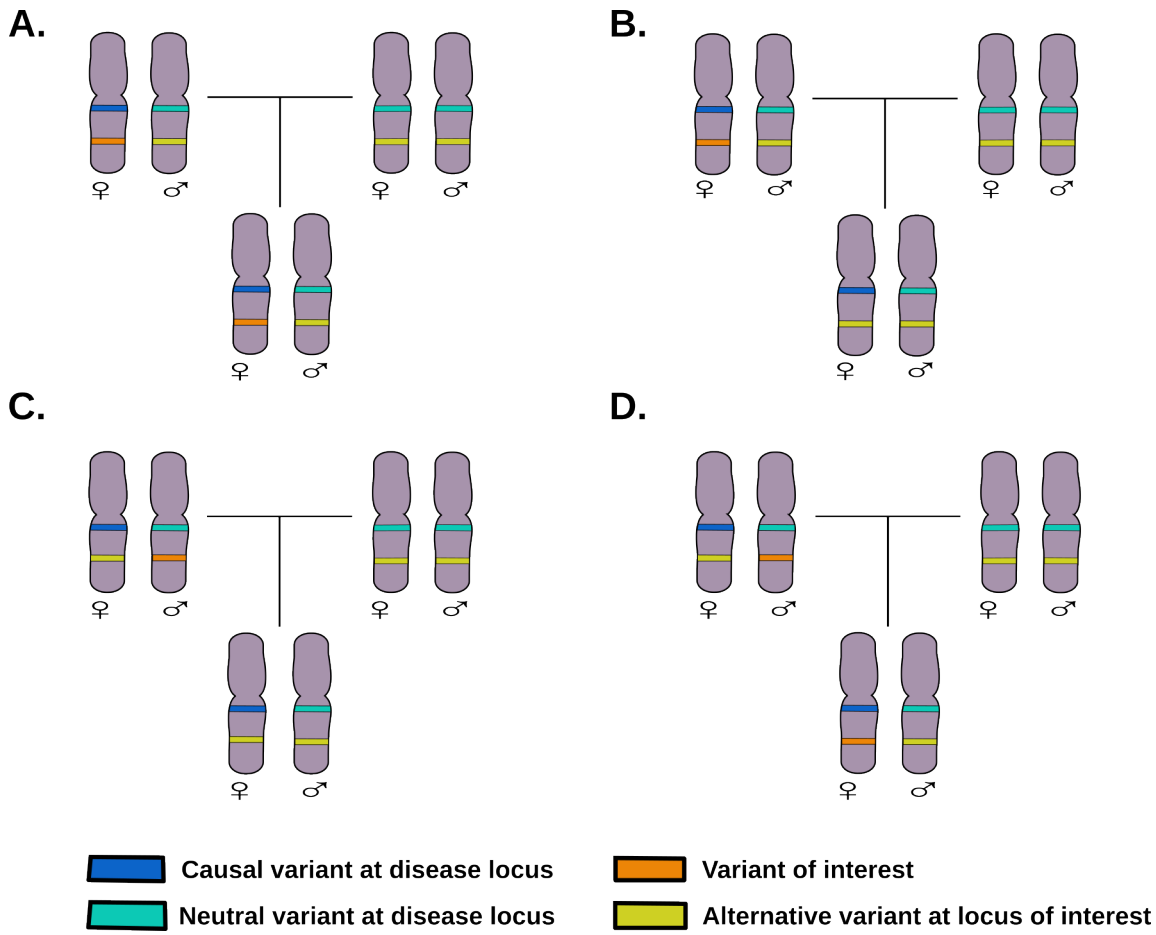


Figure 2.3: TDT tests for linkage and association. Transmission scenarios given a progenitor that carries the causal variant (darkblue, penetrant in offspring) and is heterozygote at the locus of interest (orange and yellow alleles). **A** The variant of interest (orange) and the causal variant (darkblue) are on the same chromosome and are passed on to offspring without recombination. **B** The variant of interest and the causal variant are on the same chromosome but due to recombination, they are not jointly transmitted. **C** The variant of interest and the causal variant are on different chromosomes and there is no recombination. **D** The variant of interest and the causal variant are on different chromosomes and there is recombination (no linkage). Figure reproduced from (Balding et al., 2007), with permission of the rights holder, John Wiley & Sons.

	Non-transmitted allele		
Transmitted allele	M_1	M_2	Total
M_1	a	b	a+b
M_2	c	d	c+d
Total	a+c	b+d	2n

Table 2.2: Transmitted and non-transmitted alleles among case-parent trios. M_1 is the variant of interest and M_2 all other alleles at the same locus. M is the locus of interest, M_1 is the variant of interest and M_2 all other alleles at that locus.

transmitted genes between children with a high iKIR score and children with a low iKIR score is used as the test statistic (Keenan et al., 2010):

$$\ln[b/c]_{KIR^{high}} - \ln[b/c]_{KIR^{low}} \tag{2.2}$$

Spielman et al. (1993) calculated the distribution of the transmission ratio under the null hypotheses of interest analytically. Since the goal here is to test the effect of iKIR score on multiple HLA genotypes whose transmission could not be guaranteed to be independent of each other (because of linkage between HLA loci) an analytical approach is not available and the null distribution is estimated using Monte Carlo methods (see Approximate permutation test using Monte Carlo).

2.5 (Multiple) Hypothesis testing

Throughout the Thesis I apply the statistical inference method of hypothesis testing to draw conclusions about the effect of KIR genes in T cell mediated responses. A statistical hypothesis makes an statement about the distribution of a random variable X. In hypothesis testing, our objective is to examine whether there is sufficient statistical evidence to reject the null hypothesis (H_0) in favor of a proposed alternative hypothesis (H_1). An hypothesis test allows us to reach a statistical decision, i.e. to reject or fail to reject the H_0 hypothesis we apply an hypothesis test. Test statistics summarize the observed values X to one value and can be used in an hypothesis test to reject H_0 in favor of the H_1. A valid test statistic is one that takes different values under the null hypothesis and the alternative hypothesis. For some test statistics the distribution of the statistic under the null hypothesis can be calculated exactly. One example is the t-student test for the difference of the means from two populations. The probability that the test statistic would be as or more extreme than the observed values, where extreme is interpreted relative to the null hypothesis H_0, is the p-value for the test. Therefore, the p-value can take values between 0 and 1 and the smaller the p-value the stronger the evidence against the H_0. The significance value of the test statistic (α), that is the value at which we decide to reject the null hypothesis in favour of the alternative is typically set to $\alpha = 0.05$. When making a decision about an hypothesis test, i.e. to reject or not reject H_0, we might be correct or wrong and we usually do not know which of these scenarios took place. However, we can estimate the probability of these two types of errors (Table 2.3). A Type I error is a false positive and occurs when we reject H_0 despite being true. A Type II error

is a false negative and occurs when we fail to reject H_0 despite being false. The maximum probability of a Type I error is the significance level of the test. That is why significance levels are low and usually set to $\alpha = 0.1$ or $\alpha = 0.05$.

	Decision	
Truth	Fail to reject H_0	Reject H_0
H_0 True	Correct (TN)	Type I error (FP)
H_1 True	Type II error (FN)	Correct (TP)

Table 2.3: Error matrix for statistical classification. TN=true negative, FP=false positive, FN=false negative, TP=true positive.

2.5.1 Approximate permutation test using Monte Carlo

Under some null hypotheses, a theoretical distribution of test statistics like the Student's t-distribution is not available. In these cases, an empirical distribution of test statistic can be estimated using resampling methods. The H_0 hypothesis of interest here is that the number of iKIR genes does not affect risk of disease. Individuals are divided into iKIR high and iKIR low strata and the HLA risk is estimated within each group by the $ln[OR]$. As we have seen for the family based analysis, the test statistic is therefore the absolute difference in $ln[OR]$ between the two groups:

$$statistic = |ln[OR]_{iKIRhigh} - ln[OR]_{iKIRlow}|$$

We can use a permutation test, also known as exact test or randomization test, to calculate the distribution of the statistic under the null hypothesis. This is achieved by calculating the test statistic under all possible rearrangements (without replacement) of the labels of the observed values, in this case the iKIR status (high or low labels).

To calculate an empirical p-value, the following steps are performed. First, the iKIR labels (high and low) in the original dataset are shuffled without replacement. Then, the test statistic is calculated based on this permuted dataset and recorded. These two steps are repeated for all possible permutations of the dataset. The sampling distribution of the test statistic under H_0 is represented by the distribution of the statistic obtained from the permuted datasets. The p-value is the proportion of permuted statistics that are as or more extreme than the observed statistic in the real dataset (without shuffling the labels).

Given the size of the datasets analysed in this Thesis, the exact permutation test is intractable. To solve this issue, I use Monte Carlo methods implemented in the R function `sample()` with the option `replacement=FALSE` to estimate the null distribution of the statistic from a subset of all possible permutations.

When testing the effect of iKIR score on multiple HLA genotypes, a permutation test is also needed to calculate the test statistic. Here, the statistic is the weighted mean of the ln[OR] difference between iKIR high and iKIR low. The weights are the frequencies of the HLA genotypes being tested:

$$statistic = \frac{\sum_{i=1}^{n} w_i \left| ln[OR]^i_{iKIRhigh} - ln[OR]^i_{iKIRlow} \right|}{\sum_{i=1}^{n} w_i}$$

where i is the ith genotype, n the total number of driver genotypes and w_i the frequency of the ith genotype. Again, using monte carlo methods I sample a subset of all possible permutations. Again, the p-value is the count of permuted statistics that are as or more extreme than the observed statistic divided by the number of permutations performed.

2.5.2 Multiple testing procedures

We have seen that if H_0 is true, at a significance level $\alpha = 0.05$, 5% of the tests we do, e.g. in 100 different samples, will still come up as significant by chance. These significant tests will be false positives or type I errors.

Therefore, performing multiple tests on a given dataset increases the chances of rejecting a null hypothesis. This is a consequence of the underlying logic of hypothesis testing, which involves the rejection of the null hypothesis when a rare event is observed. As the number of (independent) tests increases, the probability of obtaining a statistically significant result by chance also increases as follows:

$$\text{P(Making an error)} = \alpha$$

$$\text{P(Not making an error)} = 1 - \alpha$$

$$\text{P(Not making an error in m tests)} = (1 - \alpha)^m$$

$$\text{P(Making at least 1 error in m tests)} = 1 - (1 - \alpha)^m$$

Thus, the chance of making at least 1 error is closer to 100% as m gets bigger[5]. This issue commonly known as the inflation of the α level or the multiple testing problem.

Following Table 2.3 we can summarise the outcome of a family of m tests in the same 2x2 contingency table:

	Decision		
Truth	Fail to reject H_0	Reject H_0	Total
H_0 True	U	V	m_0
H_1 True	T	S	m_1
Total	m-R	R	m

Table 2.4: Classification of possible outcome scenarios in a family of m tests.

To control the number of false positives when conducting m hypothesis tests, we need to apply a multiple testing procedure. Multiple testing corrections typically aim at controlling either the **family-wise error rate** or the **false discovery rate**.

The family-wise error rate (FWER) is the probability of making at least 1 Type I error and can be expressed as:

$$FWER = 1 - P(V = 0)$$

Where $P(V = 0)$ is as before the probability of not making an error in m tests, $(1 - \alpha)^m$. An equivalent expression of FWER is :

$$FWER = P(V > 0)$$

The false discovery rate (FDR) is the expected proportion of false positives (FP):

[5]For example, consider a case where we want to test the effect of 20 different SNPs on disease risk at $\alpha = 0.05$. The probability of observing a significant result just by chance under the complete null hypothesis is 0.64.

$$FDR = E(FP)$$

where

$$FP = \begin{cases} V/R, & \text{if R} > 0 \\ 0, & \text{if R} = 0 \end{cases}$$

Whether we want to control the FWER or FDR depends on the nature of our experiment. FWER is more strict than FDR and therefore should be used when false positives are to be avoided. FDR will lead to a higher number of rejections and is typically used in genomics where a decent number of significant findings out of thousands tests are expected. The aim there is to detect those significant results at the expense of having few false positives.

A multiple testing correction method is said to have strong control of the FWER if the $FWER \leq \alpha$ is true for any configuration of true m_0 and false m_1 null hypotheses. On the other hand, a method that only controls the FWER in the weak sense means that $FWER \leq \alpha$ is only true if the global null hypothesis is true, i.e. $m_0 = m$.

A classic multiple testing procedure to control FWER is the Sidak equation. Sidak equation allows control of the FWER at level α. So if we want $\alpha_{FWER} = 0.05$, we can use the equation in subsection 2.5.2 to find the adjusted significance α_{Sidak} per test to ensure the type I error rate in a family of m tests stays at α_{FWER}:

$$\begin{aligned} \text{P(Making at least 1 error in m tests)} = \alpha_{FWER} &= 1 - (1-\alpha)^m \\ \alpha_{FWER} - 1 &= -(1-\alpha)^m \\ 1 - \alpha_{FWER} &= (1-\alpha)^m \\ (1 - \alpha_{FWER})^{\frac{1}{m}} &= 1 - \alpha \\ \alpha_{Sidak} = \alpha &= 1 - (1 - \alpha_{FWER})^{\frac{1}{m}} \end{aligned}$$

The Sidak equation allows for strong control of the FWER. The adjusted p-values are given by:

$$p_{adj}^{Sidak} = 1 - (1 - p_{unadj})^m$$

A related correction and the most used one is the Bonferroni correction, which is an approximation of the Sidak equation:

$$\alpha_{Bonf} = \alpha/m$$

And the Bonferroni adjusted p-values are:

$$p_i^{adj} = min\{m \times p_i^{unadj}, 1\}$$

An hypothesis $H_{0,i}$ is rejected if the $p_i, unadj \leq \frac{\alpha}{m}$. We can also show that Bonferroni correction strongly controls for FWER for a set of null hypothesis I, where $|I| = m_0$, and using Boole's inequality:

$$FWER = P(V \geq 1) = P(\bigcup_{i \in I} V_i) \leq \sum_{i \in I} P(V_i) \leq \sum_{i \in I} \frac{\alpha}{m} = m_0 \frac{\alpha}{m}$$

Although Bonferroni and Sidak are approximately the same, Boole's inequality makes Bonferroni is slightly more pessimistic and will give a smaller significance level α_{Bonf} than α_{Sidak}.

Both Sidak and Bonferroni methods are single-step multiple test corrections that set a new more conservative significance level, α_{Bonf} or α_{Sidak}. Other variations of the Sidak-Bonferroni correction include the step-down Holm correction, the step-up Hochberg correction and the step-up Hommel correction, which apply the Bonferroni correction to the ordered p-values. These step-wise approaches are uniformly more powerful than the Sidak or Bonferroni single-step approaches, meaning that they are at least as powerful as the single-step procedures but still control FWER at level α.

Nevertheless, all multiple testing corrections described above become more conservative as the number of tests m increases. Further, both single-step and step-wise Sidak-Bonferroni adjustments do not take into account the distribution of tests and they will lead to erroneous rejections when the tests m are correlated.

2.5.3 Multiple testing procedures for dependent tests

The Sidak-Bonferroni family of multiple leads to conservative adjustments of the $FWER_\alpha$ when tests are correlated. This means that the Bonferroni adjusted significance level is very strict, which might impact on the power of the test and increase the probability of false negative results. Therefore, a multiple testing procedure that accounts for the joint distribution of the tests will be more powerful. Typically, the joint null distribution is not known but as we have seen we can apply non-parametric methods like a permutation test to estimate the null distribution.

Maximum statistic (maxT)

The $maxT$ method is a multiple testing procedure that takes into account the dependency of the tests. The goal is to estimate the joint distribution of the maximum test statistics under the null by permuting the labels of the dependent variable and thus preserving the dependency structure between the explanatory variables (Westfall and Young, 1993).

The probability of type I error can also be defined as the complement of the cumulative distribution function evaluated a t, where t is the critical value:

$$P(T > t) = 1 - F_T(t) = \alpha$$

Given a set of null hypothesis H, the probability that the ith test statistic t_i is bigger than the critical when the null hypothesis is true is given by:

$$P(t_i > t) = 1 - F_i(t)$$

Since we have $|H|$ hypotheses, the probability of type I error above will be higher than α. We can adjust the critical value t so that the probability of rejecting one or more true null hypothesis among the family of hypothesis remains at the desired level α.

The family-wise null hypothesis is a joint null hypothesis where there is no effect for any test, or $H_0 = \cap_i H_{0,i}$. If we assume that the $|H|$ tests are independent, the probability of that happening is:

$$P(t_i \leq t) = \prod_i P(t_i \leq t) = \prod_i F_i(t)$$

And the probability of any significant result given that the joint null hypothesis is:

$$P(V > 0) = \prod_i P(t_i > t) = 1 - \prod_i F_i(t) = 1 - F_i(t)^H$$

Under the null hypothesis, the probability that any test t_i is greater than t is equivalent to the probability that the maximum test statistic t_{max} in the family exceeds t. Therefore, one can control the FWER using the t_{max} distribution under the null and then set the family-wise significance level α_{FWER} using that distribution.

When unknown, the distribution of maximum statistics under the null hypothesis can be approximated using re-sampling procedures the Monte Carlo permutation test (see Approximate permutation test using Monte Carlo).

Finally, an equivalent approach to the maxT is the **minP**, where minimum p-value in a family of tests is the statistic used to estimate the joint null distribution under the null (by permutation). The 5% quantile of the distribution of minimum P-values represents the family-wise significance threshold (i.e. adjusted significance level).

Chapter 3

Immunogenetic analysis of a T1D case control cohort

We have seen that there is accumulating experimental evidence that iKIRs regulate T cell-mediated immunity. We have also seen that this pathway has clinical implications: T cell-mediated control is enhanced by iKIRs in the presence of their ligands in chronic viral infections. Here I explore the hypothesis that in the context of autoimmunity, where an enhanced T cell response might be considered detrimental, iKIRs have an opposite effect and exacerbate self-reactive responses.

Type 1 diabetes (T1D) is taken as a paradigmatic example of autoimmunity. T1D is characterized by insulin-deficiency due to the T cell-mediated destruction of insulin-producing β-cells in the pancreatic islets. Monozygotic concordance rates range between 42% and 53%[1] whereas dizygotic concordance rates range from 7% to 11% (Hyttinen et al., 2003; Kyvik et al., 1995). Therefore, between 70% and 84% of phenotypic variation in T1D is heritable[2]. Variation in the HLA genes explains up to 50% of this genetic risk in T1D (Clayton, 2009). Together with histological evidence of autoreactive T cells in the pancreatic islets (Bender et al., 2020), strong HLA associations with T1D indicate that T cells have a major role in T1D etiopathogenesis. To investigate if iKIR genes affect the T cell response in this context we ask whether HLA associations are modified by functional iKIR genes. The results indicate that iKIR genes, in the

[1] Concordance rates increase up to 70% if monzygotic twins are followed for longer (Redondo et al., 2008).

[2] Falconer's formula can be used to estimate heritability (H^2) by comparing correlation estimates in monzygotic vs dizygotic twins, $H^2 = 2(r_{MZ} - r_{DZ})$.

presence of genes encoding their ligands, have a consistent and significant effect on protective HLA class II (HLA-II) associations.

3.1 Genetics of T1D

The exact aetiology of T1D remains elusive but given that concordance in monozygotic twins is less than 100%, environmental triggers are thought to initiate the break in peripheral tolerance in genetically susceptible individuals. Although the association between HLA and T1D was discovered 50 years ago (Nerup et al., 1974; Singal and Blajchman, 1973), how variation in this highly polymorphic region alters risk is not completely understood. Within the HLA region, the closely linked classical class II *HLA-DRB1*, *HLA-DQB1* and *HLA-DQA1* genes display the strongest associations (Risch, 1987; Noble et al., 1996). In particular, *DRB1*03:01∼DQA1*05:01∼DQB1*02:01* and *DRB1*04:01/02/04/05∼DQA1*03:01∼DQB1*03:02* haplotypes (also known as *DR3* and DR4 respectively) are associated with the highest T1D susceptibility whereas *DRB1*15:01∼ DQA1*01:02∼DQB1*06:02* is associated with dominant protection (Erlich et al., 2008). Recent evidence shows that HLA-II variation correlates with TCR epitope binding region variants that typically recognize insulin peptide B:9-23 (García et al., 2021), the primary autoantigen in T1D, thus providing a plausible mechanism for HLA associations. Other associations include some HLA-I alleles (Howson et al., 2011; Nejentsev et al., 2007), the insulin gene *INS* (Bell et al., 1984), the gene encoding for the lymphoid protein phosphatase, *PTPN22* (Bottini et al., 2004), and the Cytotoxic T-lymphocyte-associated protein 4 (*CTLA-4*) gene (Nisticò et al., 1996) to name a few among an increasing list of implicated genes (Robertson et al., 2021) (see Figure 3.1).

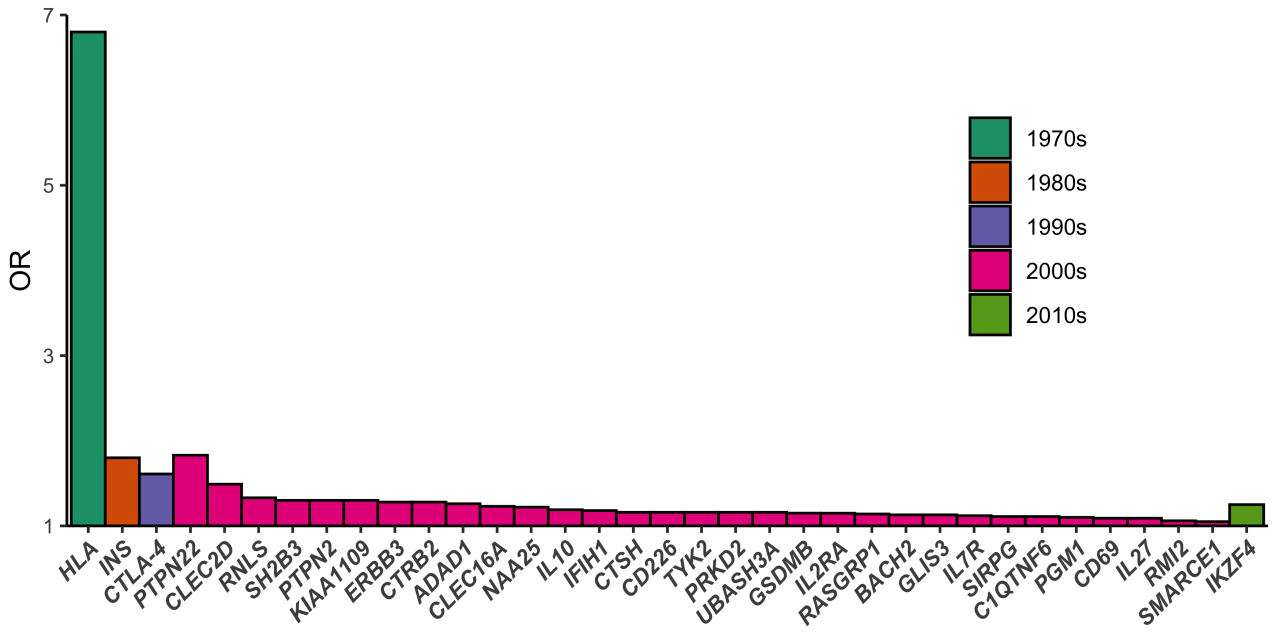


Figure 3.1: Loci associated with T1D risk. The odds ratio (OR) for variants in other loci as reported in the literature. Summary statistics for Type 1 diabetes trait were downloaded from NHGRI-EBI GWAS Catalog (Sollis et al., 2023). Only variants reported in at least two studies are shown. Associations are grouped and colour-coded by decade when the association was first reported (not necessarily fine-mapped) and then within each decade, associations are ranked by size of OR. Gene names refer to most likely candidate in the region. Figure is after a similar figure in (Rich, 2017).

3.2 KIR gene association studies in T1D

Evidence for a main effect of iKIR genes or functional iKIR genes in T1D is weak. Since 2003, 15 small candidate gene studies, have reported KIR gene associations with T1D risk, but none has been consistently reproduced (see Table 3.1) and, in a recent meta-analysis, no associations survived correction for multiple comparisons (Liu et al., 2017). For example, in a multiplex family study, KIR A haplotype (containing several iKIR genes) had a detrimental effect in a subset of T1D patients (Traherne et al., 2016), which is consistent with experimental evidence in NOD mouse showing that expression of the KIR3DL1 impairs T_{reg} function and promotes T1D (Qin et al., 2011). Surprisingly, no association was found for *KIR3DL1* gene in an earlier and much larger case-control study (Pontikos et al., 2014).

The impact of iKIRs on HLA associations has not been studied. A few studies though have investigated iKIR and functional iKIR associations in case-control cohorts where individuals were stratified by HLA-II risk groups. However, none of these associations was significant (Middleton et al., 2006; Shastry et al., 2008; van der Slik et al., 2003; Smigoc Schweiger et al., 2014). However, the sample size in these studies was very small and the fact that some HLA-I genes, which are iKIR ligands, are significantly associated with T1D was not considered.

Finally, if we look at GWAS studies in the NHGRI-EBI GWAS catalog (Sollis et al., 2023), none of the SNPs associated with T1D maps to the KIR region.

Study	Population	Cases	Controls	*KIR2DL1*	*KIR2DL2*	*KIR2DL3*	*KIR3DL1*
van der Slik et al. (2003)	Dutch	149	207	0.43	1.34	0.95	0.95
Nikitina-Zake et al. (2004)	Latvian	98	100	0.67	10	0.71	0.98
Middleton et al. (2006)	Finnish	137	101	0.14	0.76	0.78	0.95
Santin et al. (2006)	Basque	76	71	0.56	0.68	0.85	0.93
Park et al. (2006)	S. Korean	139	132	0.1	1.59	1.05	1.05
Mogami et al. (2007)	Japanese	204	240	0.55	0.83	1.06	0.37
Jobim et al. (2010)	S. Brazilian	248	250	0.54	0.81	1.14	0.49
Zhi et al. (2011)	C. Han	259	262	0.54	0.82	0.24	0.64
Mehers et al. (2011)	British	551	168	0.1	0.97	1.7	1.37
Pontikos et al. (2014)	British	6744	5362				1.25
Sanjeevi et al. (2016)	E. Indian	135	98	0.74	3.26	0.58	0.65
Osman et al. (2016)	S. Arabian	106	148	0.71	1.07	1.33	2.8

Table 3.1: iKIR gene associations reported in the literature since 2003 to date. Odds ratios (OR) are reported for the iKIRs considered in this Thesis. A value above 1 indicates indicates T1D risk and below 1 protection. Colored cells indicate nominal significance (not corrected for multiple comparisons). aKIR gene, functional KIR gene and haplotype associations investigated in these and other studies are not reported (e.g. KIR A haplotype association with increased T1D risk in Traherne et al. (2016)). Shastry et al. (2008) is not included in the table because the sample under study was the same as (Nikitina-Zake et al., 2004). Similarly, (van der Slik et al., 2007) studied KIR-HLA associatins but not KIR gene associations alone and so is not reported in the table.

The findings reported to date seem to indicate that KIRs on their own are unlikely to have a significant effect on T1D risk. Nevertheless, the focus of this Thesis is different. Contrary to the aforementioned KIR studies, a main effect of functional iKIR genes in T1D risk, which would be attributable to the NK cell-mediated response, is not the primary focus here. Instead, in the next sections I investigate whether functional iKIR genes modulate HLA associations using a large (N=11,961) T1D case-control dataset.

3.3 The UK-GRID cohort

3.3.1 Genotype imputation accuracy

The UK-GRID cohort was previously genotyped using imputation (see Genotype imputation). To assess the accuracy of HLA imputations, imputed genotypes were compared with high-resolution experimentally typed HLA genotypes available in a subset of UK-GRID samples. For the UK-GRID cohort, KIR copy number accuracy was previously assessed using copy number data on a subset of individuals directly typed by qPCR at *KIR3DL1/S1* locus (Pontikos et al., 2014). Prediction accuracy ranged from 86.68% to 98.06% (Figure 3.2 **A**). KIR copy number accuracy was also very high, with sensitivity and specificity estimates above 97% and 93% respectively (Figure 3.2 **B**). Individuals with blank calls at any of the classical HLA loci or the

iKIR genes are discarded (see Genotype imputation). Additionally, individuals with different genotype at *KIR3DL1ex4* and *KIR3DLex9* are discarded[3].

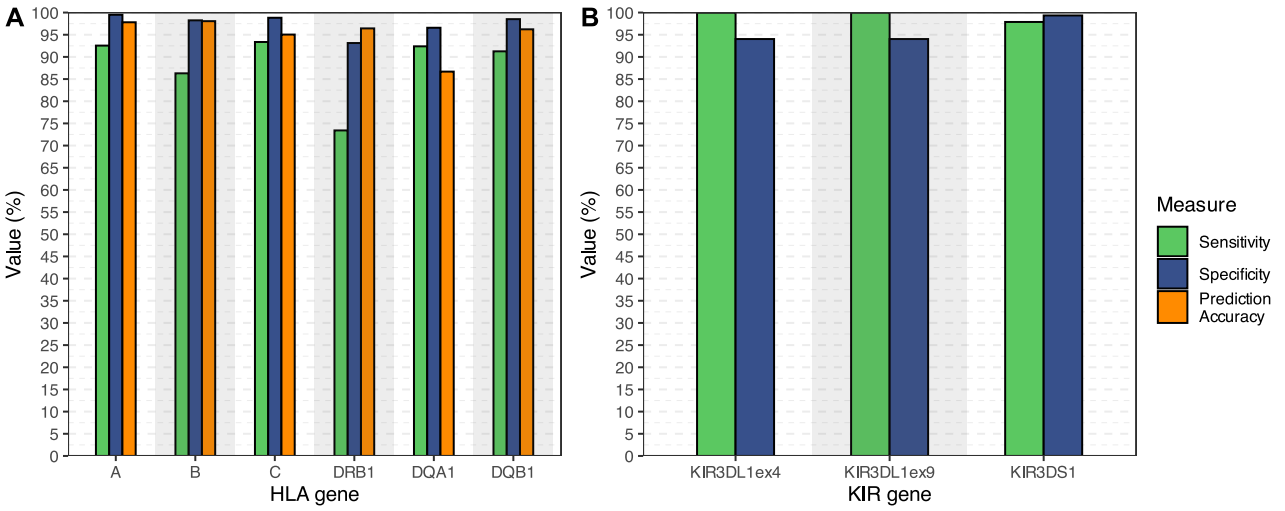


Figure 3.2: HLA and KIR imputation accuracy. **A** Imputation accuracy measures at each HLA loci. Prediction accuracy is computed as the number of correctly imputed alleles divided by the number of experimentally typed chromosomes. **B** Sensitivity and specificity values for copy number imputations at *KIR3DL1/S1* locus. *KIR3DL1* copy number was measured using two assay methods, one targeting *KIR3DL1* exon 4 (*KIR3DL1ex4*) and the other assay targeting exon 9 (*KIR3DL1ex9*).

3.3.2 Functional iKIR genes, iKIR Count and iKIR score

An individual is positive for a functional iKIR gene if they carry both the gene for the iKIR and the gene encoding the cognate HLA ligand for that particular iKIR. Polymorphisms both at KIR and HLA genes determine the binding specificity and affinity of KIR-HLA pairs (Gwozdowicz et al., 2019; Yawata et al., 2006; Moesta et al., 2008; Winter et al., 1998). Definitions of functional iKIRs genes are presented in Table 3.2:

Functional *KIR2DL1*	*KIR2DL1* & *HLA-C2*	Moretta et al. (1993)
Strong functional *KIR2DL2*	*KIR2DL2* & *HLA-C1*	Moesta et al. (2008)
Weak functional *KIR2DL2*	*KIR2DL2* & *HLA-C2*	Moesta et al. (2008)
Functional *KIR2DL3*	*KIR2DL3* & *HLA-C1*	Moesta et al. (2008)
Functional *KIR3DL1*	*KIR3DL1* & *HLA-Bw4*	Gumperz et al. (1995)

Table 3.2: Definitions of functional iKIR genes.

HLA-C1, *-C2* and *-Bw4* in Table 3.2 denote alleles postive for different conserved HLA-I epitopes, namely, C1, C2 and Bw4 respectively. These epitopes are recognized by iKIRs.

[3] *KIR3DL1* variants with altered or deleted regions are reported to be negative for either exon 4 or exon 9 (Jiang et al., 2012). Nevertheless, virtually all individuals have the same genotype at these two *KIR3DL1* gene positions.

To classify HLA-I alleles from the UK-GRID individuals into the 3 different epitope groups, we downloaded the sequences of all HLA-I alleles stored in the IPD database (Robinson et al., 2019). Residues 76-83 in the α_1 helix domain (exon 2) – which define KIR specificity – were used to classify the alleles (Figure 3.3). To determine the start of exon 2, we queried the information on the length of the signal peptide (exon 1). Leader peptide length is conserved and is typically 24 amino acids long. All alleles in the UK-GRID cohort have leader peptides of that length.

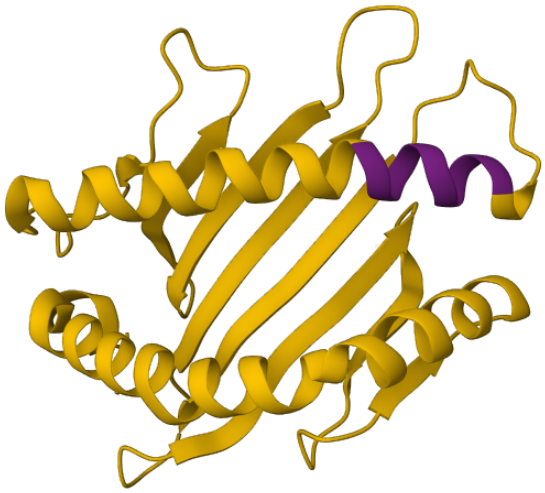


Figure 3.3: Ribbon diagram of the α_1 and α_2 helix of the HLA molecule. Residues 77-83 at the α_1 helix that determine HLA epitopes defining KIR specificity are shown in purple. Surface residues within this motif (not shown) are in direct contact with KIRs. Additional residues (not highlighted in purple) also interact with KIR. Note that the α_3 domain and the β_2 microblobulin ($\beta_2 m$) are not shown. PDB ID: 5VGE (Kaur et al., 2017).

The Bw4 motif can be found in a subset of *HLA-B* and *HLA-A* alleles. The Bw4 motif can be further subdivided into Bw4-80I and Bw4-80T motifs depending on the amino acid present at position 80 of the α_1 helix (Figure 3.4 **A**). An exception to this rule is A*25:01; despite carrying a Bw4-80I motif, it does not bind KIR3DL1 (Stern et al., 2008). There is evidence that this 80I/T polymorphism modulates the strength of the KIR3DL1 interaction (Boudreau et al., 2016).

Two dimorphic amino acids at positions 77 and 80 delineate HLA-C allotypes into either C1 or C2 groups (Biassoni et al., 1997) (Figure 3.4 **B**). The presence of a conserved valine at position 76 is also necessary to form HLA-C epitopes and distinguishes C1 and C2 groups from the Bw6 group, which is present in *HLA-B* alleles. In fact, Bw6 motif is identical to the C1 motif at key amino acids 77-83 but lacks other C1 specific amino acids including the conserved valine at

position 76 (Moesta et al., 2008; Gwozdowicz et al., 2019). Therefore, *HLA-B* alleles carrying the Bw6 epitope do not interact with KIRs.

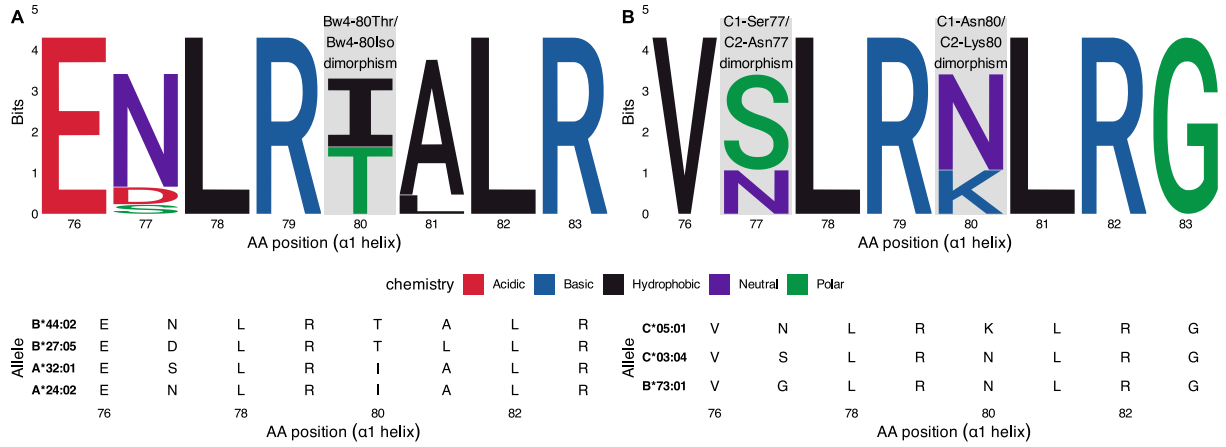


Figure 3.4: Sequence logos of HLA epitopes recognized by iKIRs. The allele amino acid sequences (76-83 aa sequence) from UK-GRID individuals containing Bw4 or C1/C2 epitopes were used to calculate the information content at each amino acid position of the KIR specific HLA epitope. **A** Sequence logo for the Bw4 sequence motif with I/T dimorphism at position 80. Below the logo, 4 representative alleles with different variations of the Bw480I and Bw80T motifs found in the UK-GRID allele pool. *A*25:01* alleles present in the UK-GRID allele pool were excluded from this analysis. **B** Sequence logo showing the two dimorphic amino acids at positions 77 and 80 that differentiate C1 from C2 allotypes. Note that some *HLA-B* alleles carry a C1 motif. These include the alleles coding for B*73 and B*49 allotypes (Barber et al., 1996; Abi-Rached et al., 2010; Moesta et al., 2008).

Once the presence or absence of KIR specific HLA epitopes is ascertained for each UK-GRID individual, we can compute the presence or absence of functional iKIR genes using the definitions in Table 3.2. The sum of the functional KIR variables is denoted as iKIR Count and varies between 0 and 4 (Figure 3.5 **A**). Additionally, following Boelen et al. (2018), a weighted functional iKIR gene count is computed to reflect the different strengths of inhibitory signal:

iKIR score = (1 if Func *2DL1*) + (1 if Strong Func *2DL2* or 0.5 if weak Func *2DL2*) + (0.75 if Func *2DL3*) + (1 if Func *3DL1*)

A large iKIR score reflects someone with a large number of strong iKIR-ligand interactions. iKIR score varies between 0 and 3.75 (Figure 3.5 **B**) and it is highly correlated with iKIR count (r=0.995).

iKIR score is protective in the UK-GRID cohort (ln[OR]= -0.21, P=2.7×10^{-25}). However, in a model including iKIR ligands (*HLA-Bw4*, *HLA-C1* and *HLA-C2*) as covariates, the iKIR score association is lost (ln[OR]=-0.009, P=0.83) and *HLA-Bw4* is strongly protective (ln[OR]=-0.45, P=6.9.1 $\times$ 10^{-16}). This finding is not unexpected; some HLA-I alleles have been associated with T1D, including *HLA-Bw4-80I* alleles like the protective *B*57:01* allele or the detrimental *A*24:02* allele (Noble and Valdes, 2011). Since 95% of *HLA-Bw4+* individuals are

also *KIR3DL1+*, we wondered wether the *HLA-Bw4* protective effect could be attributable to KIR3DL1:Bw4 interactions. To rule out this possibility, the effect of iKIR score was assessed in *HLA-Bw4+* and *HLA-Bw4−* strata. Again, iKIR score was not a signifcant predictor. In summary, the iKIR score association with T1D is probably driven by KIR ligands alone, which are known genetic risk factors in T1D (Nejentsev et al., 2007).

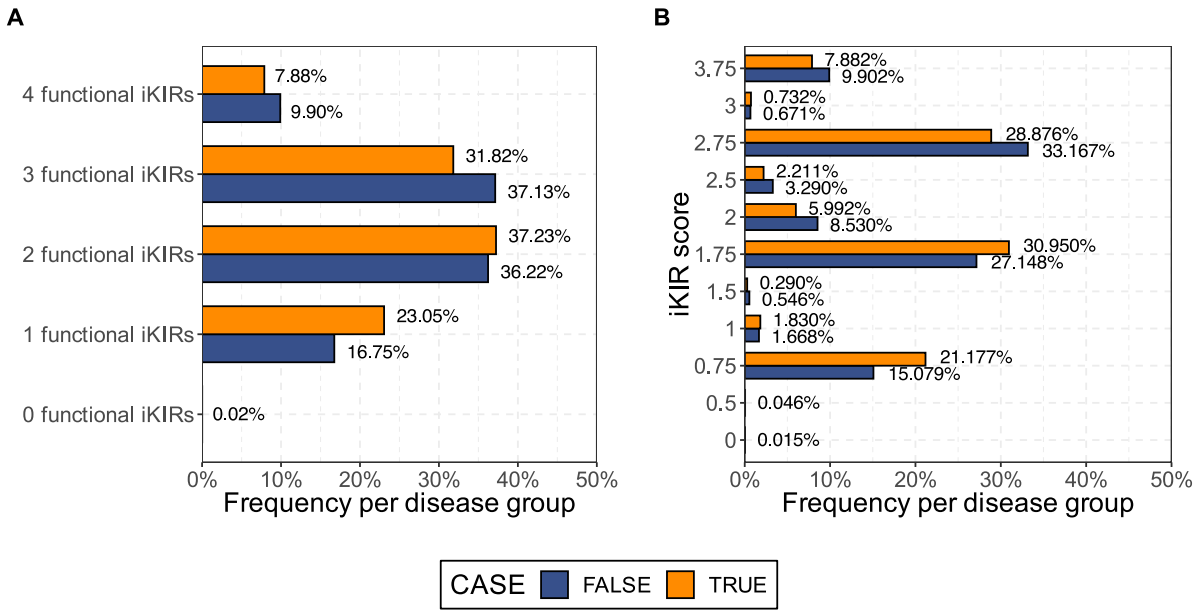


Figure 3.5: Distribution of iKIR count and iKIR score in the UK-GRID cohort. **A** Count of functional iKIRs can take values between 0 and 4. Only one T1D case has 0 functional iKIRs. **B** iKIR score distribution in the UK-GRID cohort. Although there are more iKIR score groups, the majority of individuals still fall in 4 different functional iKIR score categories.

3.4 iKIR score modulates protective *DR∼DQ* haplotypes

Specific amino acids at the *DRB1* and *DQB1* (e.g. Dβ57) loci independently explain most of the HLA-II risk (Hu et al., 2015; Lenz et al., 2015; Todd et al., 1987). Because multiple alleles at multiple HLA-II loci are associated with T1D and there is linkage disequilibrium between them, most analysis of HLA-II focus on phased haplotypes. Therefore, we estimated *DR∼DQ* haplotypes and then assessed the effect of functional iKIRs on the each haplotype by stratification (see Effect modification of KIR genotype). We found a consistent pattern of iKIR score modulation of protective haplotypes, where the presence of a high number of functional iKIRs (iKIR high) weakened the protective effect of HLA II haplotypes in all but one instance (Figure 3.6 **A**).

Then, we tested the hypothesis of seeing such a risk difference between iKIR high and iKIR low strata across so many different haplotypes. Figure 3.6 **B** shows the distribution of test

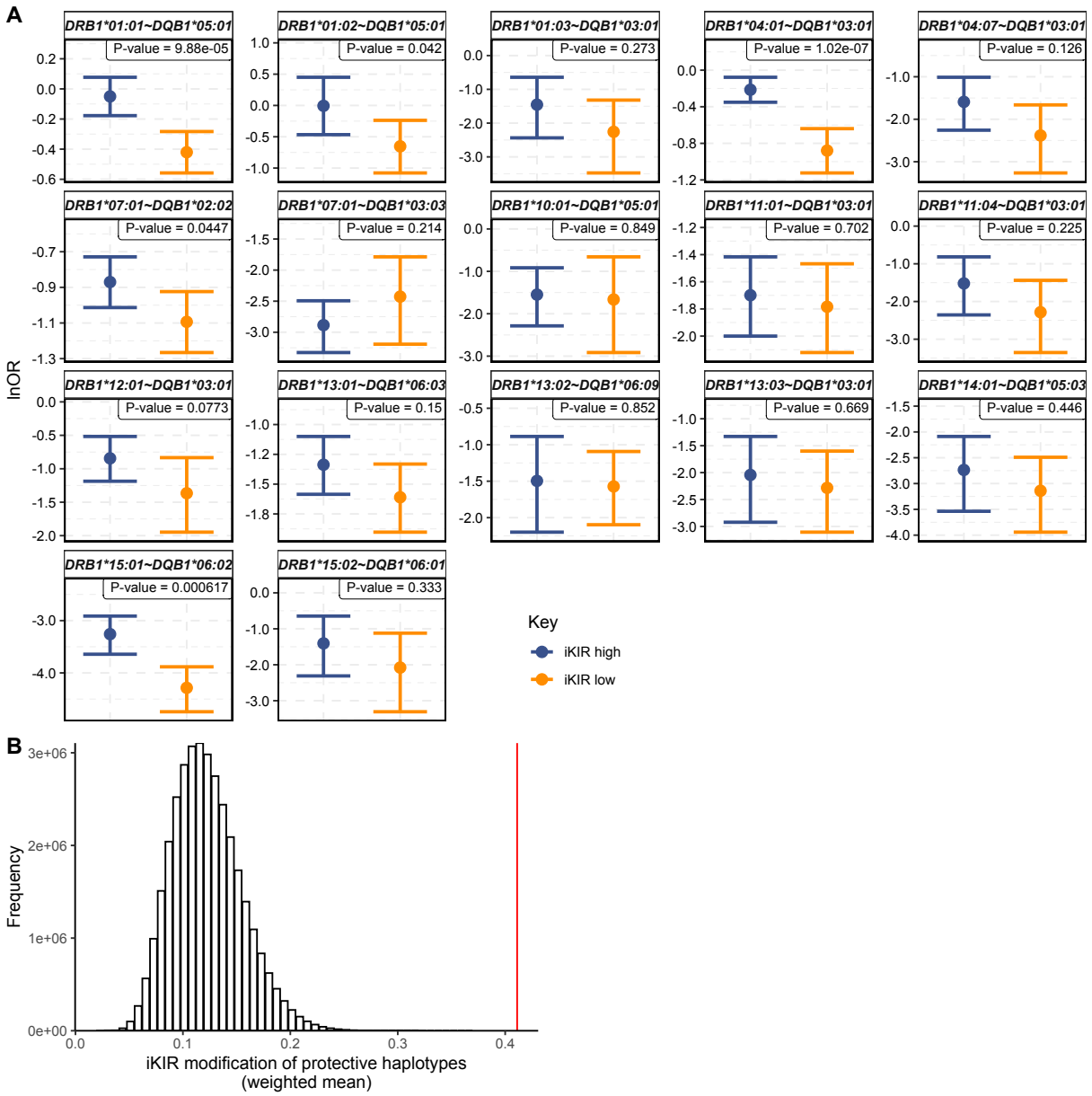


Figure 3.6: Impact of iKIR score on 17 protective *DRB1∼DQB1* haplotypes. **A** The protective effect of class II *DRB1∼DQB1* haplotypes is enhanced in the group of individuals with an iKIR score equal to 1.75 or lower (iKIR score threshold = 1.75) with the exception of *DRB1*07:01∼DQB1*03:03*. The number in the top right box corresponds to the odds of seeing this difference by chance (10^8 permutations). The dot is the log of the odds ratio (ln[OR]) and the bars the 95% confidence intervals obtained from the regression model in iKIR high (blue) and in iKIR low (orange) strata. **B** The observed value of the test statistic, indicated by the red arrow, lies far above the distribution (histogram) of the same test statistic under the null hypothesis that the iKIR score has no impact on the protective haplotypes.

statistic under the null hypothesis[4] and the red vertical line indicates the value of the observed test statistic. The chance of observing this effect by chance in so many haplotypes was very low (P=1×10^{-8}), suggesting functional iKIRs modify HLA-II mediated protection.

Although the iKIR score effect is pervasive among protective haplotypes the effect varied from one haplotype to the other. Also, the protection conferred by one frequent haplotype,

[4]The test statistic is a weighted mean of the absolute difference between $iKIR_{high}$ and $iKIR_{low}$ ln[OR]s across all 17 haplotypes. Weights are given by the haplotype frequencies.

*DRB1*07:01∼DQB1*03:03*, was not weakened by iKIR score, and if anything it was enhanced. These results suggest that protective haplotypes in Figure 3.6 might not be T1D genetic drivers but passengers marking true causal variants in LD. Additionally, we have seen that KIR ligands are strongly associated with T1D and so LD between *DR∼DQ* haplotypes and HLA-I alleles, could be mistaken for an iKIR score effect on HLA-II associations.

To move the study forward and address these issues, we next focused on one protective haplotype, *DRB1*15:01∼DQB1*06:02*.

3.5 Disentangling the iKIR score effect on HLA-mediated protection: effect on *DQ6* dominant protection

To investigate whether the negative iKIR score effect on *DR∼DQ* haplotypes was explained by iKIR score alone and not by other confounders, we first focused on the dominant protective genotype *DRB1*15:01^DQA1*01:02^DQB1*06:02*. The *DRB1*15:01^DQA1*01:02^DQB1*06:02* compound genotype has repeatedly been described to confer protection from T1D (Todd et al., 1987; Pugliese et al., 2016) and this protective effect is reproduced in the UK-GRID cohort (ln[OR]= -3.75, P=2.1×10^{-155}). There is some evidence that the protection associated with the *DRB1*15:01^DQA1*01:02^DQB1*06:02* genotype maps to *DQA1*01:02^DQB1*06:02* (henceforth *DQ6*), which encodes the *DQ6* molecule, rather than *DRB1*15:01^DQB1*06:02* (henceforth *DR15*) (Hoover and Marta, 1997; Erlich et al., 1991). In this cohort, virtually everyone who carries *DQ6* also carries *DR15* (99.2% of *DQ6+* individuals are *DR15+*) so it is difficult to fine map the protective genotype. When both *DQ6* and *DR15* are included simultaneously in a regression analysis, *DQ6* retains significance (ln[OR]= -2.86, P=6.1×10^{-3}) whereas *DR15* becomes non-significant (ln[OR]= -0.9, P= 0.4) in line with the literature (Hoover and Marta, 1997; Erlich et al., 1991). The following analysis focuses on *DQ6* genotype but results focusing instead on *DR15* (either as a phased haplotype or as a compound genotype) are virtually identical.

3.5.1 iKIR score modifies the *DQ6* protective association

On stratifying the cohort into individuals with a high iKIR score (> 1.75) and individuals with a low iKIR score (≤ 1.75) we found that the protective effect of *DQ6* varied significantly between

the strata, as we had seen for class I effects in the context of virus infection, but as expected the effect was reversed, with the strongest class II protection seen in individuals with a low iKIR score (Figure 3.7 **A**). The odds of seeing this by chance were found, by permutation test, to be P=5.6×10^{-4}. The iKIR score threshold selected to categorise someone as having a "high" iKIR score (threshold=1.75) was chosen to give a balanced stratification but we investigated whether this result, that the protective effect of *DQ6* was stronger amongst people with a low iKIR score than amongst people with a high iKIR score, was dependent on the choice of threshold. The small number of cases having this protective genotype makes exploration of more extreme thresholds problematic as the number of individuals in one stratum may be quite sparse. Nevertheless, we explored 4 different thresholds: 1.5, 1.75, 2.0 and 2.5. In every case the result was replicated (Figure 3.7 **B**, Table A.1). These observations are not independent, but they do demonstrate that our result is robust to the choice of threshold.

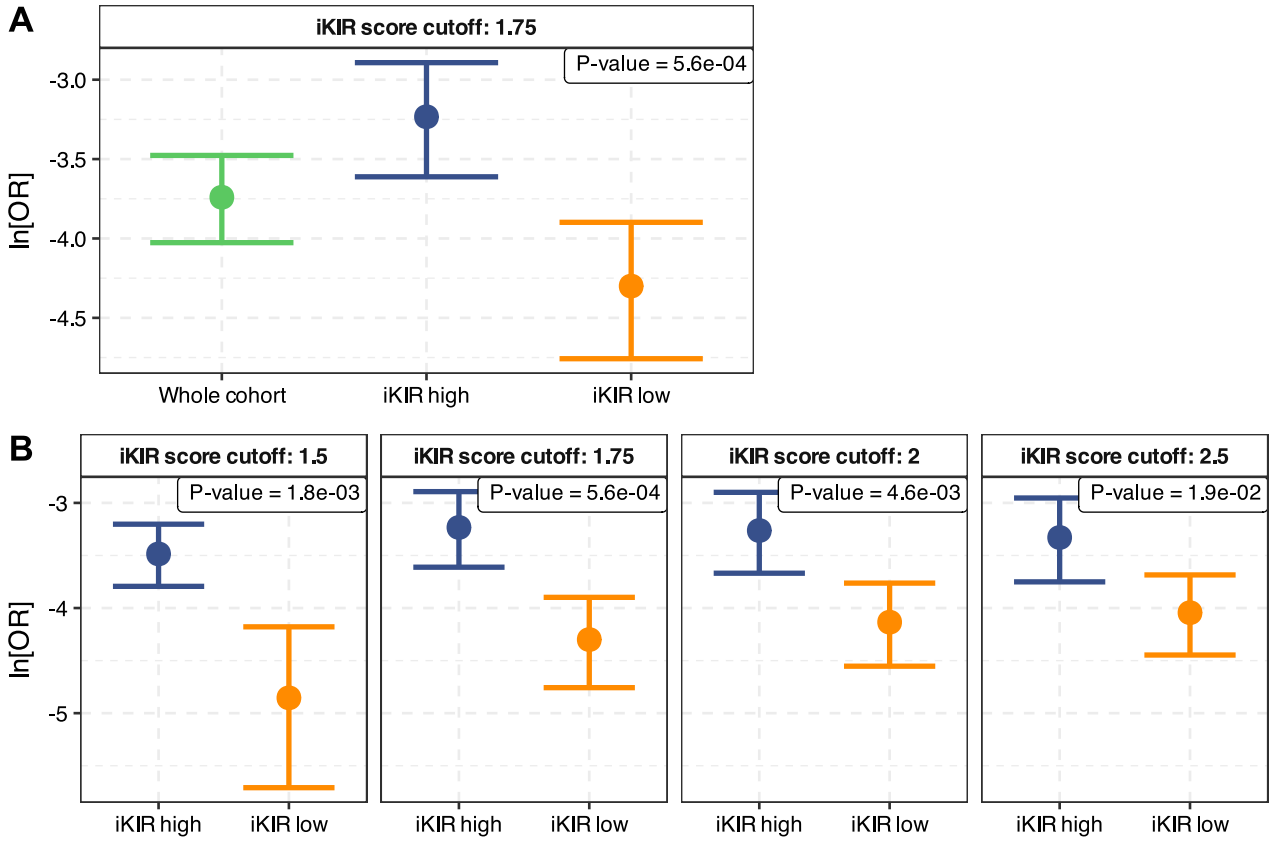


Figure 3.7: *DQ6*-associated protection is enhanced amongst individuals with low number of functional iKIR. **A** The protective effect of *DQ6* is enhanced in the group of individuals with an iKIR score equal to 1.75 or lower (iKIR score threshold = 1.75). The number in the top right box corresponds to the odds of seeing this enhancement by chance [5]. **B** The same analysis was repeated using different iKIR score thresholds to define "high" and "low" (1.5, 1.75, 2.0 and 2.5, indicated in grey box above the relevant plot). The observed effect is robust to the exact choice of threshold. In all cases the dot is the natural log of the odds ratio, ln[OR], and the bars the 95% confidence intervals obtained from the logistic regression; green: whole cohort, blue: high iKIR score strata, orange: low iKIR score strata. ln[OR]<0 indicates protection, with greater protection associated with a lower ln[OR].

3.5.2 *DQ6* protection decreases as a function of iKIR score

If iKIR score is a meaningful measure, we might expect a "dose effect" i.e. the impact on the protective class II effect depends on the value of the iKIR score. To investigate this, rather than stratifying the cohort we modelled the whole cohort and included iKIR score in the model as a continuous variable interacting with *DQ6* (i.e. $OUTCOME \sim DQ6 + DQ6 : iKIR_score + GENDER$). The interaction term was significant (P=5.5×10^{-6}, model AIC= 14172); this is stronger than if we included stratified iKIR score as an interaction term (P=3×10^{-4}, model AIC=14177). Instead of a binary stratification, we also classified individuals in high, intermediate and low iKIR score strata and we found the same picture, the ln[OR] decreased in a dose-dependent manner as the iKIR score decreased. In short, with both regression by interaction and by stratification we reached the same conclusions: *DQ6* protection decreases as a function of iKIR score.

3.5.3 iKIR count or iKIR score

We also assessed whether iKIR score was a better predictor of impact than iKIR count. The iKIR score has a marginally stronger effect than the iKIR count on the protective effect of *DQ6* (P=6.57×10^{-7} for iKIR score, P=1.1×10^{-6} for iKIR count); in backwards stepwise regression (starting from a full model with both interaction terms) iKIR count is removed from the model and the model with iKIR Count has a higher AIC than a model with iKIR score (difference=10). However, both terms are very similar and the difference in coefficient (for standardised variables) is very small (0.69 for the score 0.68 for the count) making it difficult to reach firm conclusions but on balance the effect seems to be better predicted by the iKIR score.

3.5.4 HLA-I genes or iKIR score?

An individual's iKIR score will depend on their HLA-I genotype since the iKIR ligands are HLA-I molecules. Some HLA-I alleles are associated with protection from or susceptibility to T1D (Nejentsev et al., 2007; Noble et al., 2010). Therefore, our observation that *DQ6* protection is enhanced in individuals with a low iKIR score could be due to an interaction between HLA-I alleles and *DQ6* and have nothing to do with iKIR genes. To investigate

whether our observations were attributable to the class I ligands effects rather than the iKIR genes, we repeated all the analysis including iKIR ligand status (*Bw4*, *C1*, *C2*) as covariates. In line with the literature, iKIR ligands were significantly associated with outcome but iKIR score still significantly modified the *DQ6* protective effect when iKIR ligands were included as covariates.

To further investigate whether the observed modification of *DQ6* was attributable to the effects of class I ligands rather than the iKIR genes, we constructed a score, ligand_no_kir, to reflect presence of the HLA ligand but absence of the matching iKIR. Although this score in itself was associated with significant protection (ln OR = -0.37 P =0.0006) in line with the published literature that HLA-I molecules are associated with T1D risk, it had no impact on the protective effect of *DQ6* (P-value$_{\text{interaction}}$=0.73) when included as an interaction term ($OUTCOME \sim DQ6 \times ligand_no_iKIR + GENDER$), suggesting that the effect we had observed for iKIR score (functional iKIRs) requires presence of the gene encoding the iKIR not just the one encoding the ligand. However, there was little variation in this ligand count since most individuals have at least one functional iKIR in our cohort. This could have reduced the power of the test resulting in the non-significant result. We also developed the "ligand count" measure which quantified the number of HLA ligands. This is a more problematic measure as it is significantly correlated with the iKIR score (corr=0.85, P$< 2 \times 10^{-16}$) making it difficult to disentangle the effects. However, we found that iKIR score has a stronger effect than ligand count on *DQ6* (P=6.6×10^{-7} for iKIR score, P=1.5×10^{-4} for ligand count); in backwards stepwise regression (starting from a full model with both interaction terms) ligand count is removed from the model and the model with ligand count has a considerably lower AIC (difference=48). Upon standardising the variables for comparability, we also found iKIR score had a higher coefficient than the ligand count (0.69 for iKIR score 0.52 for ligand count). Additionally, in a model including both interaction terms, iKIR score remained significant (P= 6.7×10^{-4}) whilst HLA ligand count became non-significant (P=0.43).

Finally, we assessed whether the iKIR score effect on *DQ6* was attributable to specific HLA-I drivers in our cohort associated with this haplotype. A detrimental Bw4 or a protective Bw6 allele in linkage disequilibrium with *DQ6* could potentially explain the iKIR score effect on *DQ6*: for example, people with the detrimental Bw4 allele would tend to have a higher iKIR score than those without and this detrimental additive effect would reduce the protective effect of *DQ6* in the iKIR high strata. If a particular *HLA-B* allele was responsible for the iKIR effect, then on removing individuals with this allele the iKIR score effect will disappear. We

tested this hypothesis by discarding all individuals with a given allele and then performing regression analysis in the resulting subcohort. We repeated this process for all HLA-I alleles, one allele at a time. For all the HLA-I allele negative subcohorts, the $DQ6 : iKIR_score$ effect interaction term remained significant (Figure 3.8).

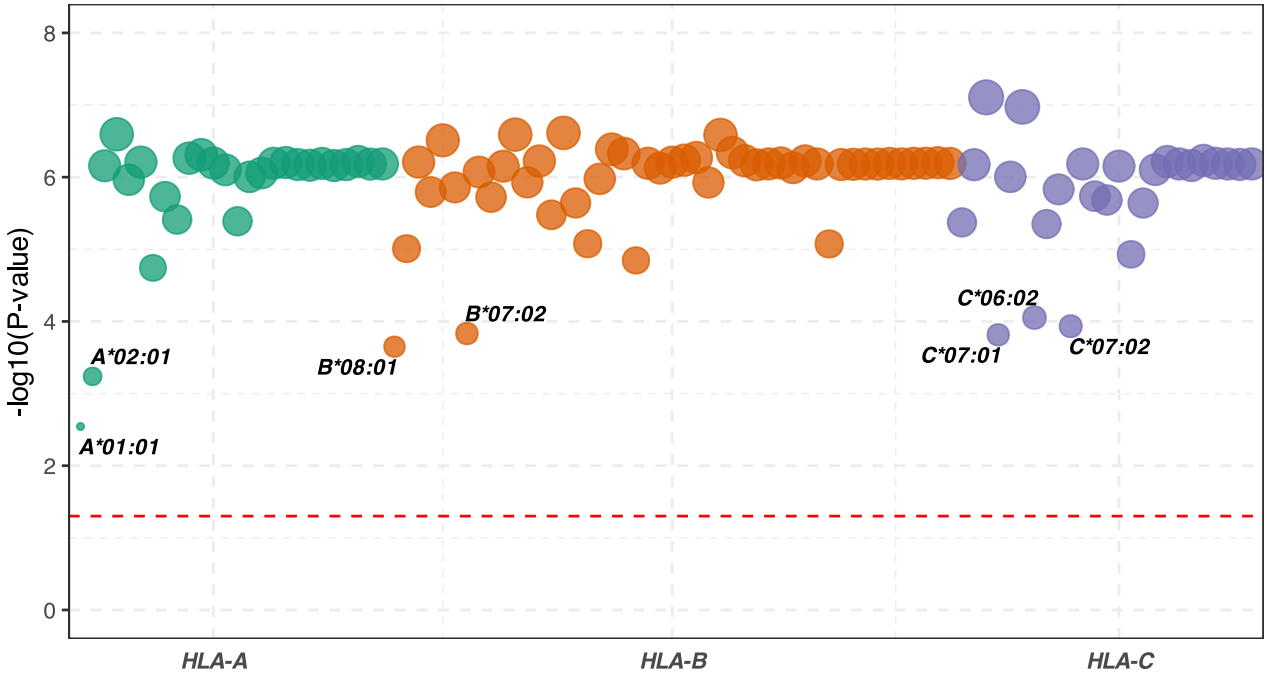


Figure 3.8: iKIR score effect is not attributable to specific HLA-I alleles. Manhattan plot of the interaction term p-values ($DQ6 : iKIR_score$) against each allele that was excluded from the cohort. As expected, frequent alleles, e.g. *A*02:01*, or alleles correlated with *DQ6*, e.g. *B*07:02*, had the biggest impact on effect size but nevertheless the iKIR score effect remained significant. Dashed red line indicates nominal significance (significance level set to $\alpha = 0.05$).

Overall, this set of results indicate that the observed functional iKIR modification of *DQ6* is not driven by HLA-I genes alone and suggests that the effect of functional iKIR on this protective genotype is due to the iKIR-ligand interaction.

3.5.5 Do all iKIRs contribute to iKIR modification?

In a logistic regression model, the $DQ6 : Bw4$ interaction term is significant (P=1.4×10^{-5}), although iKIR score as an interaction term is more significant (P=6.6×10^{-7}). Since 95% of Bw4 carriers carry the corresponding iKIR gene, *KIR3DL1*, we hypothesized that the $DQ6 : Bw4$ interaction was reflecting the effect of *KIR3DL1-Bw4* gene pair on *DQ6* and that perhaps the iKIR score effect was only driven by functional *KIR3DL1* gene. Therefore, we investigated the contribution of the rest of iKIR-ligand pairs to the iKIR score by excluding non-functional

KIR3DL1 individuals from the cohort (so that the cohort was homogeneous for functional *KIR3DL1*) and then repeating the stratification analysis on the resulting subcohort. Albeit non-significant in 3 out of 5 thresholds tested, the iKIR score effect showed the expected trend (Figure 3.9). Similarly, $DQ6 : iKIR_score$ interaction term remains significant in this subcohort (ln[OR]= 0.66, P=7.4×10^{-3}) suggesting that *KIR2DL1*/*KIR2DL2*/*KIR2DL3* also contribute to the modulatory effect along with *KIR3DL1*.

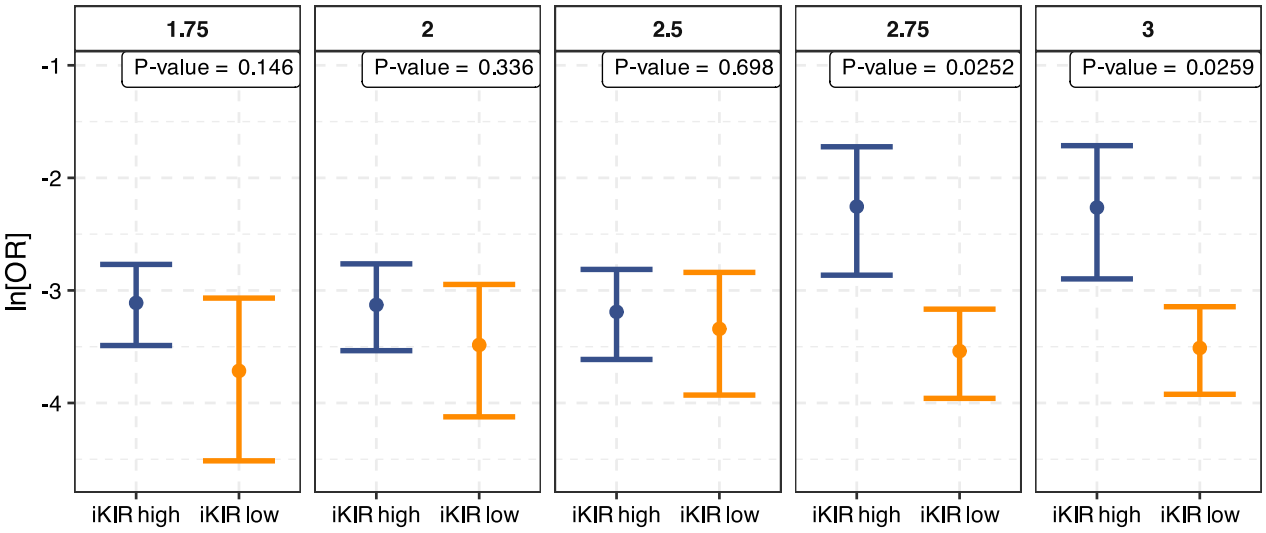


Figure 3.9: All functional iKIRs contribute to the iKIR score effect. In a subcohort in which all individuals carry functional *KIR3DL1* we still observe an enhanced protection of *DQ6* in individuals with low iKIR score albeit the effect is non-significant for iKIR score cutoffs, 1.75, 2 and 2.5. The number on the top right box corresponds to the odds of seeing this difference by chance (10^5 permutations).

3.5.6 Do activating KIRs affect *DQ6* protection?

Some studies have reported an association between activating KIRs (aKIRs) and increased risk of T1D (Shastry et al., 2008; Zhi et al., 2011). We investigated whether the observed iKIR effect could be explained by activating KIR genes. We computed a measure similar to the iKIR count but for the functional activating KIR genes, the aKIR Count, including all activating KIRs with known HLA ligands namely *KIR2DS1*, *KIR2DS2*, *KIR2DS4* and *KIR3DS1* (Pollock et al., 2022); lack of information about the strength of signaling of these ligand-aKIR interactions precluded development of a score for activating KIR genes. Although the iKIR score is highly correlated with aKIR count (corr=0.6, P$< 2.2 \times 10^{-16}$), in a model including both metrics interacting with *DQ6*, iKIR score remained significant whereas aKIR count did not.

3.5.7 Does the iKIR effect on *DQ6* depend on *DR3* and *DR4* detrimental haplotypes?

The most significant detrimental genotypes in T1D are *DR3* (*DRB1*03:01^DQB1*02:01*) and *DR4* (*DRB1*04:01/02/04/05^DQB1*03:02*) (Noble and Valdes, 2011). We investigated whether the iKIR score effect on *DQ6* varied when these haplotypes were included as covariates. We consistently found that, whilst the *DR3* and *DR4* genotypes were highly detrimental they had little or no impact on the interaction between iKIR score and *DQ6*; that is both the coefficient of the interaction (for standardised score) and the p-value were very similar with or without inclusion of the detrimental haplotypes (Table 3.3). This indicates that the impact of iKIR on *DQ6* is independent of the strong detrimental genes of the *DR3* and *DR4* genotypes.

Covariates	Coefficient of interaction	P-value of interaction
GENDER + DR3	+0.62	4.9×10^{-6}
GENDER + DR4	+0.65	2.1×10^{-6}
GENDER + DR3 + DR4	+0.61	8.9×10^{-6}

Table 3.3: iKIR score effect on *DQ6* is independent of *DR3* and *DR4* detrimental genotypes. *DR3* (defined here to be *DRB1*03:01^DQB1*02:01* in cis or in trans) and *DR4* (defined to be *DRB1*04:01/02/04/05^DQB1*03:02* in cis or in trans) detrimental genotypes were included as covariates and standardised iKIR score was included as an interaction term for comparison.

In summary, the extensive analysis presented above on *DQ6* suggests that:

1. *DR*∼*DQ* haplotypes might not be drivers of protection. We saw that *DQA1*01:02^DQB1*06:01* genotype was likely the driver of dominant protection instead of the *DRB1*15:01*∼*DQB1*06:01* haplotype.

2. iKIR score modifies *DQ6* in a dose dependent manner. This effect cannot be explained by detrimental HLA genotypes in LD nor by functional activating KIRs.

3. Disentangling the effect of the iKIR score from the effect of HLA-I T1D risk is difficult but using different approaches (including iKIR ligand genes as covariates, ligand_score effect, ligand_count effect, discarding carriers of HLA-I alleles), our analyses indicate that there is content in the iKIR score.

3.6 HLA drivers in T1D

One of our concerns was that some of the *DRB1*∼*DQB1* haplotypes may not be protective themselves but that they were neutral passengers marking the true, causal driver genotypes. Therefore, we aimed to understand whether the *DRB1* and *DQB1* genes of the protective haplotype themselves or other class I or class II alleles best marked the protective effect (and if the latter whether these protective alleles were also modulated by iKIR score). 10 HLA-I alleles and 21 HLA-II genotypes (single genes, two-gene compound genotypes or three-gene compound genotypes, see Figure 3.10) in our cohort were independently and significantly associated with T1D; this comprehensive list was derived by Prof Becca Asquith. As anticipated, none of the 17 protective *DRB1*∼*DQB1* haplotypes studied in Figure 3.6 were the drivers of protection. Instead, 15 protective HLA-II genotypes were better associated with outcome (Table A.2). Henceforth we will focus on these 15 protective genotypes.

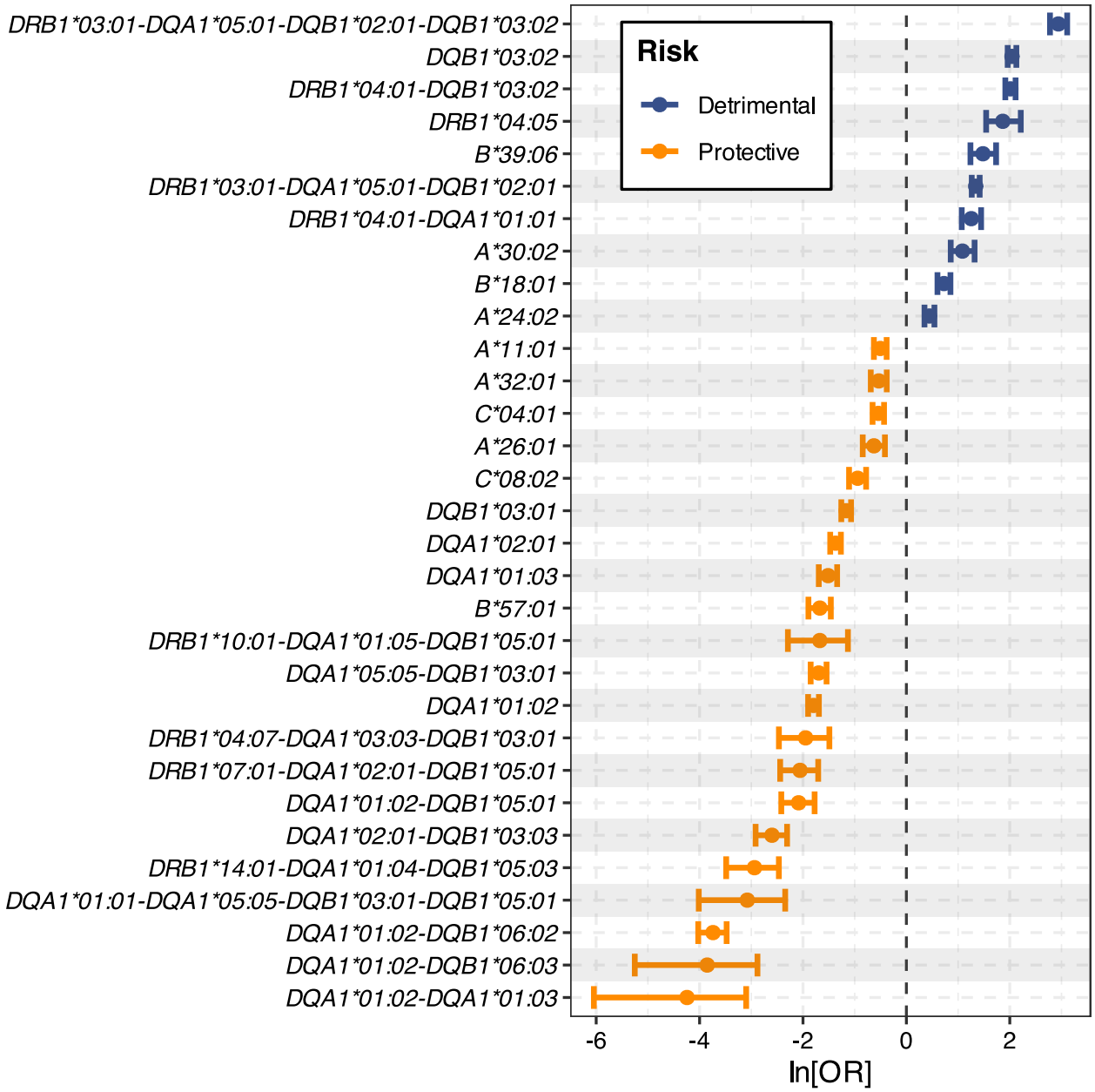


Figure 3.10: Forest plot with all HLA drivers associated with T1D. We show all HLA-I and HLA-II driver genotypes identified in the UK-GRID cohort. The coefficients for each genotype were obtained from a logistic regression model in which the only other covariate was gender. The coefficients will depend both on the genetic risk of the whole cohort (i.e. the background that the risk/protection is measured relative to) and the other genotypes correlated with the genotype of interest. Note, although *DRB1*03:01^DQA1*05:01^DQB1*02:01^DQB1*03:02* appears to be a compound of the detrimental genotype *DRB1*03:01^DQA1*05:01^DQB1*02:01* and the detrimental genotype *DQB1*03:02* it was retained in the list of independent drivers as it retained direction and significance of effect (albeit considerably weakened) in multiple regression when both *DRB1*03:01^DQA1*05:01^DQB1*02:01* and *DQB1*03:02* were included simultaneously with it. We cannot rule out the possibility that these genotypes mark unsequenced variants that are even more closely identified with outcome, but we can say they are the HLA-I and HLA-II alleles most closely associated with outcome.

3.7 Impact of HLA-I genes

Having established the true drivers of risk in the UK-GRID cohort, the other concern raised in the *DQ6* analysis was that correlations between the protective HLA-II genotypes of interest and HLA-I genotypes (which are also iKIR ligands) could be mistaken for iKIR modification. This LD could explain why iKIR score effect on *DQ6* in a subcohort of functional KIR3DL1+ individuals is significantly weakened (see Figure 3.9). Similarly, it could explain why some HLA-II genotypes are not strongly modified by iKIR score. Risk HLA-I alleles, which are in turn iKIR ligands, will fall into the different iKIR strata depending on the choice of threshold and their independent effect on T1D risk could be mistaken by iKIR modification (or lack thereof).

A clear example is observed in *DQA1*02:01* genotype identified in section 3.6. *DQA1*02:01* is a frequent genotype (N=2,212) and is strongly associated with protection (ln[OR]=-1.37, P=8×10^{-149}). However, it is not modified by iKIR score in the expected direction (Figure 3.11 **A**). *DQA1*02:01* genotype is significantly correlated with *B*57:01* (corr=0.3, P=1.3×10^{-248}). *B*57:01* is a protective driver gene in the UK-GRID cohort (see Figure 3.10), in line with previous literature (Noble et al., 2010). It also carries a Bw480I motif and therefore *B*57:01* gene encodes a ligand for one of the iKIRs, KIR3DL1. Therefore, upon stratification by iKIR score, *B*57:01* will be enriched in the iKIR-high stratum. Since *DQA1*02:01* genotype is associated with *B*57:01* then individuals carrying both *DQA1*02:01* and *B*57:01* genotypes will also be more likely to appear in the high iKIR stratum. Any additive effects on T1D risk from both *DQA1*02:01* and *B*57:01* would then risk either being misinterpreted as iKIR modification of the *DQA1*02:01* or masking an iKIR modification. The latter effect can be seen in this example (Figure 3.11 **B**); when discarding *B*57:01* positive individuals, iKIR score modifies *DQA1*02:01* in the expected direction, i.e. the same as for *DQ6* genotype.

To remove both confounding and masking due to correlations with HLA-I driver genotypes, all individuals who were positive for any of the class I driver alleles were removed from the cohort, leaving a reduced cohort of size N=5,420. Simply removing class I drivers is the cleanest approach to dealing with the problem. In many immunogenetics analyses this is not an option due to the reduction in cohort size; in this case we are fortunate in starting with a very large cohort which allows removal of the class I drivers.

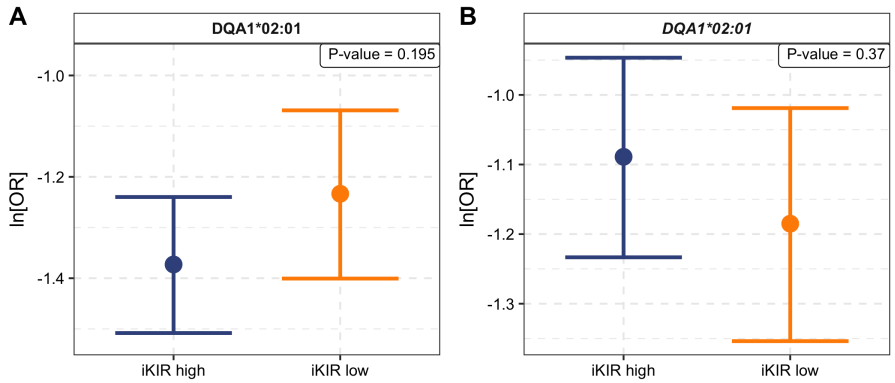


Figure 3.11: Correlation between *B*57:01* and *DQA1*02:01* genotypes masks the iKIR effect on *DQA1*02:01*-mediated protection. **A** The protective effect of *DQA1*02:01* is enhanced in individuals with an iKIR score higher than 1.75. **B** In a *B*57:01*− subcohort, now the protective effect of *DQA1*02:01* is enhanced in the expected stratum, i.e. in individuals with a low iKIR score.

3.8 iKIR score modifies protective HLA-II drivers

We stratified the HLA-I driver negative cohort into individuals with high iKIR score and individuals with a low iKIR score and analyzed the effect of the protective HLA-II drivers within each stratum. HLA-II drivers which were carried by fewer than 10 cases were not studied as numbers were too low to permit stratification. The nine remaining protective genotypes were all modified in the same direction at all three thresholds considered (Figure 3.12 **A**, Table 2). The only genotypes for which the iKIR effect was not extremely clear (*DQA1*0201^DQB1*03:03* and *DQA1*01:02^DQB1*05:01*) were very infrequent (N=12 and N=14 cases respectively) so the number of carriers in each strata would be very low possibly explaining the lack of a clear modification. Overall, the odds of observing this iKIR modification across the 9 genotypes was very low, $P = 2.4 \times 10^{-9}$ (Figure 3.12 **B**). In addition to the stratification analysis, we also modelled the whole HLA-I negative cohort and included iKIR score as an interaction term with a given protective genotype. The iKIR interaction term was significant in 4 highly frequent genotypes and in the expected direction in all genotypes (Table 3.4). We conclude that iKIR negatively impacts the effects of all protective HLA-II genes in T1D; this effect is attributable to iKIR-HLA receptor-ligand interactions and unlikely to be observed by chance.

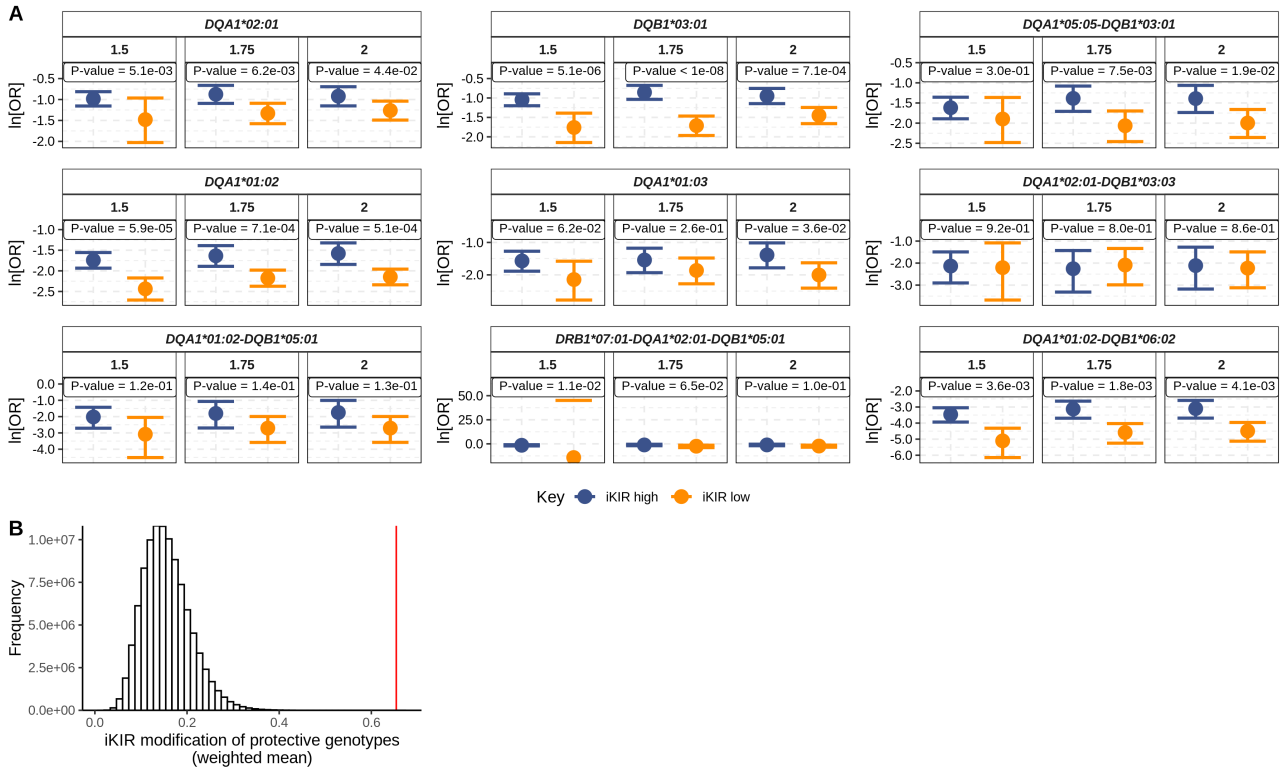


Figure 3.12: Impact of iKIR score on 9 protective HLA-II genotypes. **A** The 9 protective HLA-II genotypes investigated were all significantly modified by iKIR at the three iKIR score thresholds (definition of iKIR high and low) considered in the UK-GRID HLA-I negative subcohort (i.e. excluding individuals with HLA-I drivers). The only two genotypes for which the effect was not strong (*DQA1*0201^DQB1*03:03* and *DQA1*01:02^DQB1*05:01*) were very infrequent (N=12 and N=14 cases respectively). **B** The observed value of our test statistic (weighted mean of the difference in ln[OR] between the KIR high and the KIR low strata at threshold=1.75), indicated by the red line, lies far above the distribution (grey histogram) of the same test statistic under the null hypothesis that the iKIR score has no impact on the protective genotypes (generated by permuting the iKIR score of individuals in the cohort). Indicating that the probability of obtaining our observation by chance is extremely low ($P=2.4 \times 10^{-9}$).

Genotype	Estimate	P-value	Cases	Controls	Total
*DQA1*01:02^DQB1*06:02*	0.94	1.14E-05	26	729	755
*DQB1*03:01*	0.35	1.97E-05	361	777	1138
*DQA1*01:02*	0.33	8.35E-05	252	922	1174
*DQA1*05:05^DQB1*03:01*	0.30	3.53E-02	88	355	443
*DQA1*01:02^DQB1*05:01*	0.65	5.54E-02	14	109	123
*DQA1*02:01*	0.18	7.35E-02	256	548	804
*DQA1*01:03*	0.24	1.19E-01	67	279	346
*DRB1*07:01^DQA1*02:01^DQB1*05:01*	0.60	2.27E-01	12	52	64
*DQA1*02:01^DQB1*03:03*	0.11	7.31E-01	12	85	97

Table 3.4: iKIR score interaction terms with protective HLA-II drivers in a subcohort without HLA-I drivers. After removal of class I drivers from the cohort (N=5,420), we modelled risk of T1D for each protective genotype and included iKIR score in the model as a continuous variable interacting with the HLA-II genotype (i.e. $OUTCOME \sim HLA_class_II_genotype \times iKIR_score + GENDER$). The coefficient for the interaction term was in the expected direction for all genotypes and significant for 4 out of the 5 most frequent genotypes.

3.9 Replication: a family-based study

To validate our findings, we studied an independent dataset consisting of 700 US multiplex families from the HBDI consortium. Prior to analyzing this cohort, we conducted a power analysis using a Monte Carlo approach to assess whether we had sufficient statistical power to detect a significant iKIR score effect in this much smaller cohort (5 times smaller than the HLA-I negative UK-GRID cohort). Assuming the effect size was the same as in the UK-GRID cohort, we found that, the family cohort would not be sufficiently powered to detect a significant iKIR score modification on individual protective class II genotype (Figure 3.13 **A**). However, we estimated that there was sufficient power to detect an iKIR score modification if we considered several protective genotypes simultaneously i.e. *DQB1*03:01*, *DQA1*02:01*, *DQA1*01:02* and *DQA1*01:02^DQB1*06:02* (Figure 3.13 **B**).

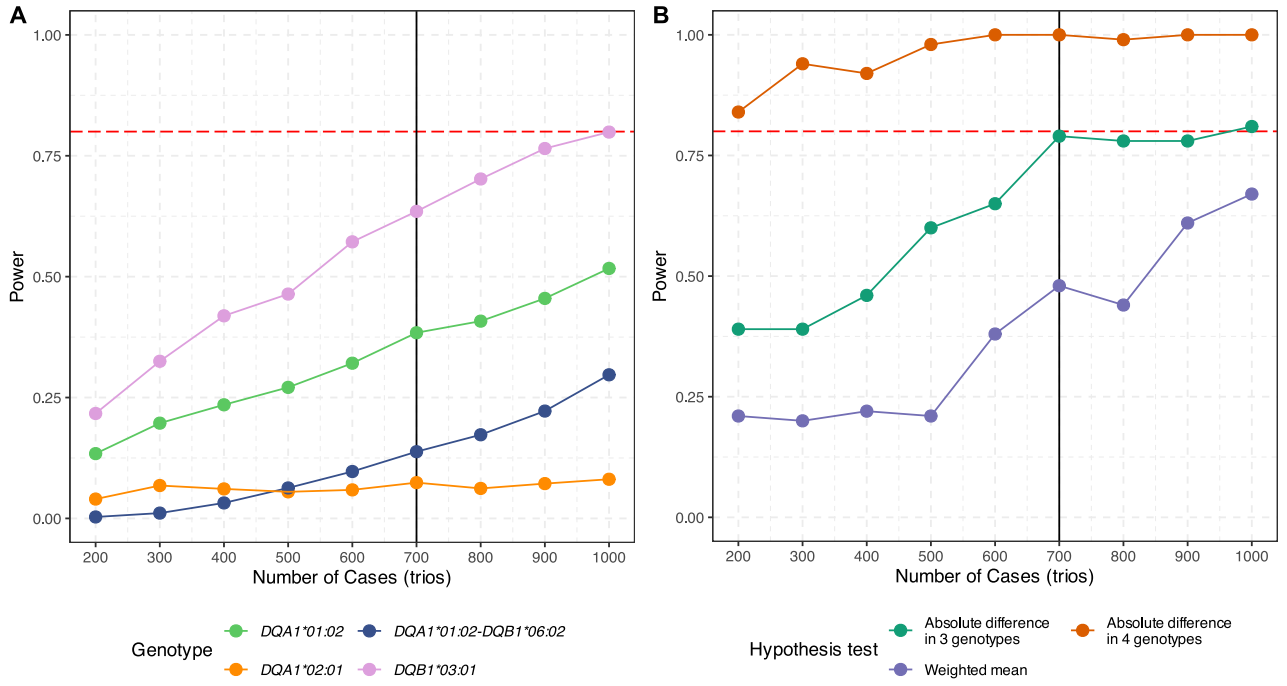


Figure 3.13: Simulation-based power analysis in the GRID cohort. **A** For each sample size s (Number of cases=Number of controls=s/2), we generated 1000 random subcohorts by resampling with replacement individuals from the GRID cohort. The sample size of the family dataset is 700 trios (indicated by a black line) which is equivalent to a case control dataset consisting of 700 cases and 700 controls. For each protective genotype and each subcohort we run a logistic regression model with stratified iKIR score (threshold=1.75) as an interaction term with the protective genotype. Power is estimated as the number of bootstraped cohorts of size s where the coefficient of interaction was significant (P<0.05). The dashed red line indicates the power standard of 0.8. Given the sample size of the family dataset, the power to detect a significant iKIR score interaction on individual protective genotypes falls considerably below the recommendation of 0.8. **B** For each sample size, we estimated the power to detect significant difference between iKIR high and iKIR low strata (assessed by permutation test) across several protective genotypes simultaneously. Power is calculated as the proportion of cohorts with a significant permutation test. Given the sample size of the family dataset, the power of detecting an iKIR difference across 4 frequent protective genotypes i.e. *DQA1*01:02*, *DQB1*03:01*, *DQA1*02:01* and *DQA1*01:02^DQB1*06:02* is higher than the recommended standard of 0.8. Sample size family dataset (vertical black line), recommended power (horizontal dashed red line).

Based on the power analysis, we tested for a significant difference in 4 frequent protective genotypes in the family dataset. Briefly, we stratified the family cohort into trios with a high iKIR score (threshold>1.75) and trios with a low iKIR score (threshold ≤1.75) based on the iKIR score of the affected child. For each genotype, we calculated the difference between the ratio of transmissions and non-transmissions in each strata (see Family-based analysis). The odds of observing an equal or greater difference between the ratios in each stratum under the null hypothesis was statistically significant P=1×10^{-5}. These conclusions remained unchanged when using different iKIR score thresholds (Table 3.5). We conclude that our finding that iKIR score impacts HLA-II mediated protection was replicated in an independent dataset.

iKIR_score **threshold**	**P-value**
1	0.00293
1.5	0.00875
1.75	1×10^{-5}
2	0.00021
2.5	0.00220

Table 3.5: Odds of observing an iKIR modification across *DQA1*01:02*, *DQB1*03:01*, *DQA1*02:01* and *DQA1*01:02^DQB1*06:02* in an independent cohort. The iKIR effect observed in the case-control cohort was validated in an independent family dataset. Trios were stratified into high (>threshold) and low (≤ threshold) iKIR score according to the iKIR score of the child in each trio. For each threshold and each genotype, we calculated the ratio of transmitted to non-transmitted genes in each stratum. The odds of observing an equal or greater difference between log ratios across 4 frequent protective genotypes (*DQA1*01:02*, *DQB1*03:01*, *DQA1*02:01* and *DQA1*01:02^DQB1*06:02*) were assessed by permutation test.

3.10 Discussion

We conducted an extensive immunogenetic analysis to study the role of iKIRs in autoimmunity. Specifically, we wanted to investigate whether functional iKIR genes have a significant impact on HLA associations in T1D. This interaction is clinically significant in other contexts; we previously reported that functional iKIR genes enhanced protective and detrimental HLA disease associations in chronic viral infections. We postulated that a similar effect might be relevant in autoimmunity. We have analyzed a large (N=11,961) case-control T1D dataset and found that a low number of functional iKIR genes (iKIR genes together with the genes encoding their corresponding HLA-I ligands), enhanced the dominant protection conferred by *DQA1*01:02^DQB1*06:02*. The effect was driven by iKIR-ligand pairs rather than HLA ligands alone, which are independently associated with T1D. The same results were observed for the other 9 protective HLA-II genotypes in our cohort. The odds of observing this effect across all protective genotypes is low (P=2.4×10^{-9}). Moving onto an independent replication cohort,

an identical result was observed with protective class II genotypes being more protective in individuals with a low number of functional iKIR genes. Again, the probability of observing this result by chance was low ($P=1 \times 10^{-5}$). In striking contrast, functional iKIR had no consistent impact on detrimental class II associations. Most detrimental genotypes showed no iKIR modification at all (see Figure A.1). In short, whilst the functional iKIR modification of protective HLA-II associations is very clear and highly statistically significant (GRID cohort: $P=2.4 \times 10^{-9}$, replication cohort: $P=1 \times 10^{-5}$), the absence of an iKIR modification of detrimental genotypes is equally clear ($P=0.19$).

Although HLA-II associations with T1D are very well studied, to the best of our knowledge there are no reports of how these associations are modified by functional iKIR genes. There are several studies investigating iKIR genes and/or functional iKIR genes in T1D (e.g. Liu et al. (2017); Middleton et al. (2006); Sanjeevi et al. (2016); Traherne et al. (2016); Zhi et al. (2011) among others) and a very large number of studies investigating HLA-II associations (Noble et al. (2010); Erlich et al. (2008); Todd et al. (1987); Hu et al. (2015); Lenz et al. (2015); Ilonen et al. (2016); Nejentsev et al. (2007) to name a few) but none reporting the interaction. Although the absence of evidence for an interaction between functional iKIR genes and HLA-II genes in candidate gene studies can easily be explained by the argument that no one was motivated to study this particular three-way genetic association, it might be wondered why it was not picked up in one of the very large "catch-all" genome wide association studies (GWAS) performed in T1D (Barrett et al., 2009; Burton et al., 2007; Hakonarson et al., 2007; Robertson et al., 2021). The reason is that three gene associations of the type we report (KIR-ligand-class II) are never studied in GWAS as the explosion in the number of multiple comparisons for comparing all triplets of variants is prohibitive and so it is to be expected that the functional iKIR modification of HLA-II associations which we report would not be found by GWAS. Whilst we are not aware of studies of functional iKIR modifications of HLA associations in T1D, we have previously reported functional iKIR modification of protective and detrimental HLA-I associations in three chronic viral infections, namely HIV-1, HCV and HTLV-1 (Boelen et al., 2018; Seich al Basatena et al., 2011).

In summary, we find evidence that iKIRs modulate HLA-II-restricted CD4+ T cell responses. In the next chapter we investigate possible mechanism(s) underlying the iKIR effect using mathematical modelling.

Chapter 4

Mathematical modelling of the iKIR effect during autoreactive T cell responses

The analysis of the UK-GRID and the HBDI cohorts reveals an important effect of iKIR genes on protective HLA-II associations. In a separate study, we have recently reported that functional iKIR genes increase T cell lifespan in humans *in vivo* (Zhang et al., 2023). In this section, we hypothesize that this iKIR-mediated increase in T cell lifespan might also explain the clinically significant difference in the risk of developing T1D between individuals with high number of functional iKIR genes and those with a low number of functional iKIR genes. If autoreactive T cells survive for longer, then protection conferred by HLA-II genotypes is expected to be weaker, in line with our immunogenetic findings. It is not obvious though why detrimental HLA associations are not in turn enhanced, and the autoreactive response also exacerbated in this risk group.

To investigate whether our hypothesis can reconcile our somewhat contradictory results, I implement a mathematical model that represents the transition from health to disease due to the autoimmune destruction of the pancreatic β cells. We find that if iKIRs increase T cell survival, then we expect a negative impact on protective HLA associations but also on detrimental HLA associations. Only when considering a density-dependent model of T cell proliferation we are able to recapitulate the findings. In indviduals carrying detrimental genotypes, i.e. with low number of T_{regs}, iKIRs have no impact on risk: the increase in survival on autoreactive effector T cells (T_{eff}) is balanced out by an increase in survival on T_{regs}. However, when T_{reg} cell numbers

reach carrying capacity, i.e. in HLA protected individuals, iKIRs increase the risk of T1D. We conclude that our hypothesis that iKIRs increase T cell survival explains our immunogenetic results.

4.1 HLA-II-mediated resistance to T1D

Understanding the mechanism by which functional iKIR modulate HLA-II associations is difficult as the mechanism underlying the HLA-II associations themselves is unclear. The mechanisms underlying HLA-II resistance against T1D have been investigated mainly for *DRB1*15:01^DQA1*01:02^DQB1*06:02*, a genotype which confers dominant protection; heterozygote carriers are protected even if they also carry detrimental HLA genotypes, e.g. *DR4*, in the other chromosome (Todd et al., 1987; Simmons et al., 2020). In this section I discuss possible hypotheses about *DQ6* mediated protection.

Epitope stealing One explanation for this dominant protection is "epitope stealing" by the DQ6 molecule, encoded by *DQA1*01:02* and *DQB1*06:02* genes(van Lummel et al., 2019). It has been suggested that DQ6 competes with the detrimental DQ8 molecule (encoded by *DR4* genotype) for identical antigenic peptides on the surface of antigen presenting cells (APCs). Though why presentation of a peptide by DQ8 should be pathogenic but stealing and presentation of the identical peptide by DQ6 be protective is unclear. Another study showing that DQ6 diverts the proinflammatory response of DQ8-restricted clones to an anti-inflammatory response when APCs present insulin peptides with DQ6 may be an explanation (Eerligh et al., 2011).

Frequency of islet-specific T_{regs} A recent study shows that healthy individuals carrying the *DRB1*15:01^DQB1*06:02* genotype have increased frequencies of islet-specific T_{regs} but also islet-specific effector T cells (T_{effs}), albeit with an altered cytokine profile, compared to healthy individuals carrying neutral or detrimental genotypes (Wen et al., 2020). Similarly, increased numbers of T_{regs} (but not T_{effs}) have been described as the main mechanism underlying the dominant protection of the *DR1* genotype Goodpasture disease (Ooi et al., 2017), a rare autoimmune disorder. In T1D, a high number of islet-specific T_{regs} may suppress antigen-specific autoreactive T cells and confer dominant protection.

Beneficial autoimmunity Finally, the increased frequency of T_{effs} in *DRB1*15:01^DQB1*06:02* protected carriers also supports the beneficial autoimmunity hypothesis (Hauben et al., 2005).

This hypothesis suggests that an effector but regulated T cell response could help clear damaged tissues and prevent autoimmunity. After birth, there is a wave of β-cell apoptosis in the neonatal pancreas (Kassem et al., 2000); an effective adaptive response might help in the clearance of damaged and dead cells to stop inflammation.

4.1.1 Islet specific Tregs deacrease as a function of HLA-II risk

HLA molecules seem to shape the islet specific TCR repertoire. As we have seen in the previous section, this might result in different numbers of protective islet-specific T_{regs} (Wen et al., 2020); individuals carrying risk HLA-II genotypes might fail to generate protective T_{regs} that control islet reactive responses whereas *DQ6+* individuals might have a high number of islet-specif T_{regs}. In Wen et al. (2020), antigen specific $CD4^+$ T cell frequency varies according to an individual's HLA-II genotype and more interestingly, although the samples come from healthy individuals, T_{reg} cell frequencies are inversely correlated with HLA-II associated T1D ln[OR] estimated in UK-GRID cohort (Figure 4.1).

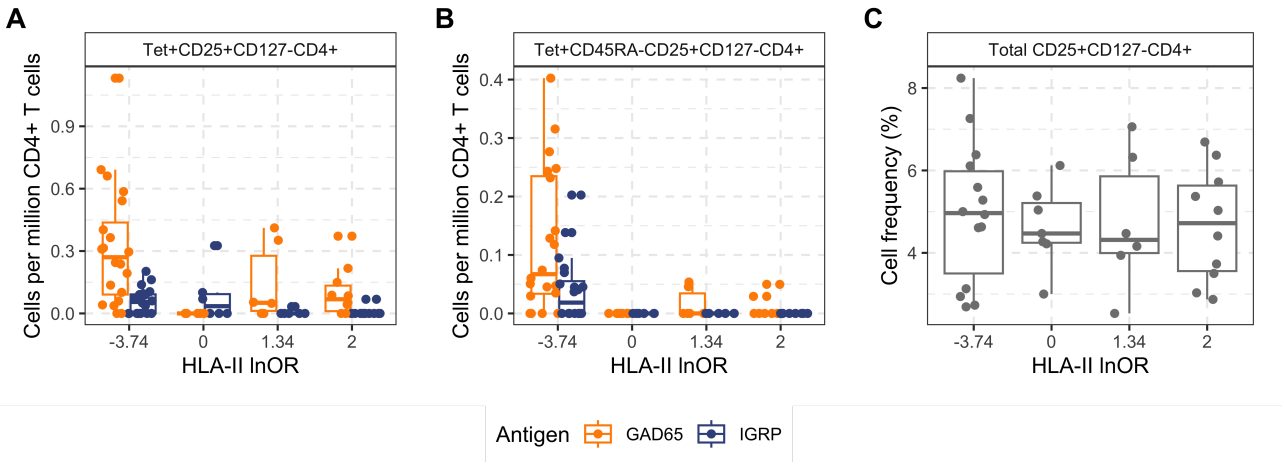


Figure 4.1: Islet-specific Treg frequency as a function of HLA-II risk in healthy individuals. A GAD65 (orange) and IGRP (blue) antigen specific T_{regs} ($Tetramer^+CD25^+CD127^-CD4^+$) among healthy individuals with different HLA-II risk genotypes. **B** Same as (**A**) but with antigen specific memory T_{regs} (memory) T_{regs} ($Tetramer^+CD45RA^-CD25^+CD127^-CD4^+$). **C** Percentage of total T_{regs} ($CD25^+CD127^-CD4^+$) among healthy individuals with different HLA-II risk genotypes. ln[OR] assigned to each individual's genotype is estimated from UK-GRID cohort. Data from (Wen et al., 2020).

4.1.2 HLA-II mediated T1D risk has no effect on overall T_{reg} frequencies in healthy individuals

If HLA-II genotype has an impact on number of islet-specific T_{regs} in healthy individuals, we speculated that this effect might also influence overall T_{reg} frequency in healthy individuals.

Such a trend has been reported in (Savastio et al., 2020). Savastio et al. (2020) analysed ICOS$^+$ T_{reg} frequency (CD4$^+$CD25$^+$FoxP3$^+$ICOS$^+$ T cells) in individuals with T1D and first-degree relatives and found a significant enrichment of ICOS$^+$ T_{regs} in first-degree relatives carrying low risk HLA-II genotypes.

To see if HLA-II T1D risk also affects total T_{reg} frequencies in healthy individuals, we requested access to genotype and phenotype data from TwinsUK registry (see Twins UK cohort). We also downloaded a public dataset containing a comprehensive list of immune traits in blood (∼80,000 traits) for a subset of TwinsUK individuals (Roederer et al., 2015). We merged the requested HLA genotypes with the 80k immunophenotypes and filtered out individuals missing HLA-II imputations and individuals with immune-related conditions or on steroid treatment (Figure 4.2 **A**). We also filtered out non-robust traits (those with correlation <0.3 in a subset of replicate samples) and focused on CD4$^+$ traits.

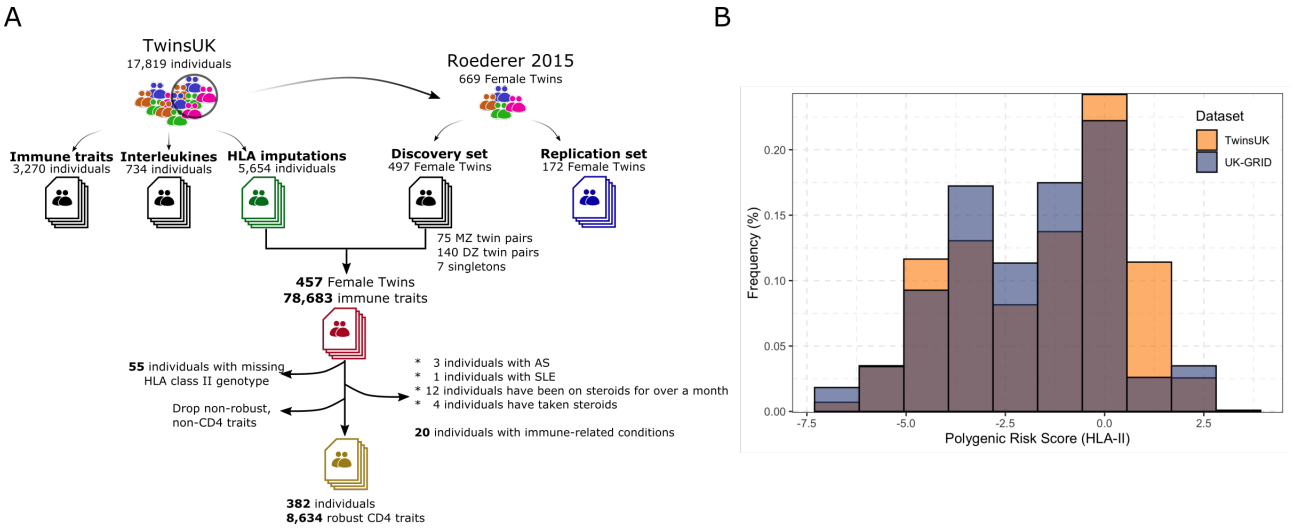


Figure 4.2: The TwinsUK dataset. Overview of the data we hold from TwinsUK dataset. **A** We got access to TwinsUK genotype (HLA imputations) and immunophenotype data (both interleukine levels and immune triats e.g. frequency of CD4$^+$ T cells). Additionally, we used a large immunophenotype dataset with more than 70k traits containing a subset of TwinsUK individuals. Both genotype and immunophenotype information was present in 457 twins. Individuals without genotype calls at any of the HLA-II loci were discarded. Individuals with autoimmune conditions (AS-Ankylosing Spondylitis, SLE-Systemic Lupus Erythematosus) or with history of steroid treatment were discarded. The filtered dataset with consisted of 382 individuals and 8,634 CD4$^+$ traits. **B** Distribution of the HLA-II polygenic risk score in the UK-GRID cohort (excluding cases) and in the TwinsUK filtered cohort.

To asses the impact of HLA-II risk on CD4$^+$ immunophenotypes, we built a polygenic risk score for each individual i as the sum of the k HLA-II genotypes identified as driver genotypes in the UK-GRID cohort (k=21, Figure 3.10):

$$HLA\text{-}PRS(i) = \sum_{j=1}^{k} \beta_j \times HLA\text{-}II_{ij} \tag{4.1}$$

where β_j is the strength of association (β coefficient) of the j HLA-II genotype as estimated in the UK-GRID cohort with a model including all drivers ($n = 31$, 21 HLA-II and 10 HLA-I):

$$OUTCOME \sim GENDER + driver_1 + driver_2 + driver_3 ... + driver_n$$

Finally, $HLA\text{-}II_{ij}$ is the presence (1) or absence (0) of the jth HLA-II driver genotype in individual i. Note that while the weights for HLA-II drivers were adjusted by HLA-I drivers, the latter are excluded from the PRS calculation as the focus here is the effect of HLA-II associated risk on immunophenotype. PRS ranged between -2.5 and 2.5 and the distribution was similar between TwinsUK individuals (N=382) and UK-GRID controls (N=5,742) (Figure 4.2 **B**).

We tested for correlation between HLA-PRS and $CD4^+$ traits using spearman correlation. Although we reduced the number of traits 10-fold, we still had 8,634 robust $CD4^+$ immunophenotypes to test. To address the multiple comparison problem, we implemented the minP multiple testing procedure (see Multiple testing procedures for dependent tests) and used the 5% quantile of the distribution of minimum p-values under the null hypothesis to keep FWER at level α, where as usual $\alpha = 0.05$.

As expected, none of the 8,634 $CD4^+$ traits remained significant after Bonferroni adjustment for multiple comparisons. Bonferroni adjusted p-values are too conservative in this case though. Cell frequencies are calculated from flow cytometry data with panel designs including multiple markers. Therefore, there is considerable co-variation between traits as cells can belong to multiple fluorescence gates. Nevertheless, when estimating the joint null distribution empirically (1e4 permutations), the minP adjusted significance level was still lower than the trait with the lowest unadjusted p-value (Figure 4.3 **A**). The most significant $CD4^+$ trait, although non-significant after multiple testing correction, was $CD25^+CD39^-CD73^+$ (Figure 4.3 **B**). CD73 is expressed in a subset of highly supressed T_{regs} ($CD4^+CD25^+$) (Jarvis et al., 2021) and is negatively correlated with $HLA\text{-}PRS$, as expected given our hypothesis; individuals with a lower PRS (carrying protective genotypes for T1D) have higher T_{reg} levels.

We also investigated the traits reported in Wen et al. (2020). The frequency of $CD4^+CD25^+CD127^-$ was not significantly correlated with $HLA\text{-}PRS$ (Figure 4.3 **C**). Wen et al. (2020) identified an enrichment of islet-specific $CD4^+CD25^+CD127^-$ T_{regs} in protected healthy individ-

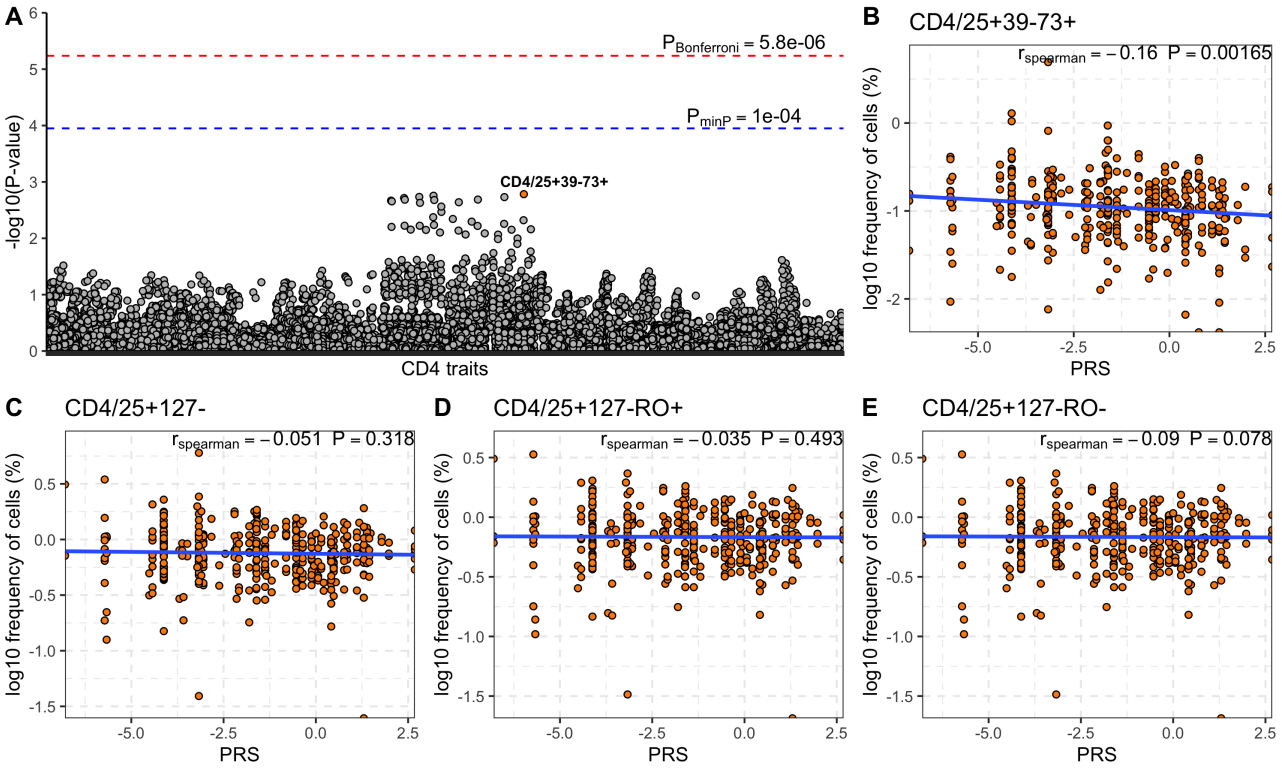


Figure 4.3: HLA-II polygenic risk score as a predictor of CD4$^+$ immunophenotypes. **A** Manhattan plot of CD4$^+$ traits p-values (spearman correlation). Dashed red line indicates the Bonferroni corrected significance level for 8,634 traits (5.8×10^{-6}) whereas the dashed blue line indicates the minP corrected significance level (5% quantile of the minP distribution under the null hypothesis). Orange dot indicates the trait with the smallest p-value. **B**, **C**, **D**, **E** Proportion of CD4$^+$ T cells as a function of PRS. Traits reported are **B** the top associated trait with PRS, **C** total T_{regs}, **D** memory T_{regs} and **E** naive T_{regs} (potentially including T_{scm}). Dots indicate cell log10 frequency levels from the parent population (CD4$^+$) in TwinsUK individuals (N=382). Blue line indicates best fit of linear regression. Spearman correlation estimates and p-values are reported in the top right of each plot.

uals, but the percentage of total CD4$^+$CD25$^+$CD127$^-$ T cells did not vary between HLA-II genotype groups, agreeing with our results in the TwinsUK. Although the percentage of CD4$^+$CD25$^+$CD127$^-$CD45RA$^-$ T cells (memory T_{regs}) is not present among the 80k immunophenotypes assessed in TwinsUK, we have frequency values for a similar trait, CD4$^+$CD25$^+$CD127$^-$CD45RO$^+$ (Gattinoni et al., 2017; Vukmanovic-Stejic et al., 2006). Again, neither the frequency of memory T_{regs} (Figure 4.3 **D**) nor the complement trait that includes naive and stem cell memory T cells (Figure 4.3 **E**), i.e. CD4$^+$CD25$^+$CD127$^-$CD45RO$^-$, were significantly correlated with PRS in this cohort.

In summary, while we failed to detect an overall impact of HLA-II autoimmune risk on overall T cell frequencies, there is evidence that HLA-II genotypes affect islet-specific T cell frequencies.

4.2 KIR effect on HLA-II associations

Broadly speaking there are two (non-exclusive) ways in which iKIR could affect HLA-II-restricted $CD4^+$ T cells (see again Figure 1.2): either by iKIR expression *directly* on $CD4^+$ T cells or *indirectly* by iKIR expression on another population (that interacts with APCs expressing HLA-II or $CD4^+$ T cells restricted by HLA-II).

Briefly, iKIRs directly expressed on T cells prolong T cell lifespan. Some studies have also reported a decrease in effector function upon iKIR ligation. Indirectly, iKIRs expressed on another population, e.g. NK cells, can modulate $CD4^+$ T cells restricted by protective HLA-II molecules and impact their survival. Given the increasing evidence for a iKIR impact on T cell survival, in our mathematical model we assume functional iKIR genes increase T cell lifespan. We do not explicitly model the direct nor the indirect pathways and so the origin of the iKIR effect is not taken into account.

4.3 Mathematical model of the transition from health to T1D

We model autoimmune response against pancreatic β-cells in the pancreatic islet with a set of four coupled differential equations describing the interactions between activated islet-specific T cells, both $CD8^+$ T_{eff} cells (C) and $CD4^+$ T_{reg} cells (R), insulin producing β-cells (B) and islet antigen levels (A) (see also Table 4.1):

$$\frac{dB}{dt} = -\delta_B C B \tag{4.2a}$$

$$\frac{dA}{dt} = \alpha_A C B - \delta_A A \tag{4.2b}$$

$$\frac{dR}{dt} = \alpha_R A - \delta_R R \tag{4.2c}$$

$$\frac{dC}{dt} = \alpha_C A - \delta_C C - \delta_i R C \tag{4.2d}$$

Variable	Definition
A	Islet antigens
B	Pancreatic β-cells
R	Islet-specific T_{regs}
C	Islet-specific T_{effs}

Table 4.1: Description of state variables included in the system of differential equations described by (4.2).

We consider that the presence of islet antigens (A) activates T_{effs} (C) but also T_{regs} (R). The activation of C and R has two opposite effects; while C mediates β-cell killing, R reduces activated C levels which in turn reduces β-cell killing[1]. The coexistence of these two antagonistic pathways triggered by the same input constitutes a type of incoherent feedforward loop (IFFL). This motif has been used to describe immune activation and tumor control (Sontag, 2017). The IFFL is coupled to a positive feedback loop: T cell mediated killing of β-cells results in more islet antigen release that in turn activates T cells (C and R) Figure 4.4. We explored additional

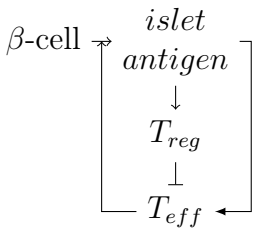


Figure 4.4: Minimal model of the T cell mediated destruction of pancreatic β cells.

structural forms of the model. We included density-dependent production of islet-specific T cells and modelled the negative feedback loop via R inhibition on C proliferation:

$$\frac{dB}{dt} = -\delta_B CB \tag{4.3a}$$

$$\frac{dA}{dt} = \alpha_A CB - \delta_A A \tag{4.3b}$$

$$\frac{dR}{dt} = \alpha_R AR(1 - \frac{R}{K_r}) - \delta_R R \tag{4.3c}$$

$$\frac{dC}{dt} = \frac{\alpha_C AC}{k + R}(1 - \frac{C}{K_c}) - \delta_C C \tag{4.3d}$$

We refer to the density-independent and the density-dependent models as **model 1** (4.2) and **model 2** (4.3) respectively.

4.3.1 Parameter estimation

Parametrization of complex biological systems is a key challenge due to the scarcity of human *in vivo* experimental estimates. Moreover, the risk associated with pancreatic biopsies implies that our molecular and cellular understanding of β-cell destruction is very limited. We provide biologically reasonable parameter ranges and, where possible, the estimates are based on quantitative experimental studies using human islets. Other parameters were taken from previous theoretical studies.

[1] T_{regs} inhibit T_{eff} function via multiple pathways. To make the model simpler we only account for the T_{regs} negative effect on T cell proliferation.

Death rates The lifespan for effector T cells is estimated to be around 5 days so death rate for both C and R is set to 0.2day^{-1}.

Proliferation rates Several studies have attempted to quantify the degree of T cell infiltration in the islets both in health and disease (Babon et al. (2016); Anderson et al. (2021); Michels et al. (2017); Denroche et al. (2021); Fasolino et al. (2022)). These estimates range between 1 and 20 T cells per islet.

β-cell killing Recent evidence shows that the rate of T cell killing is lower than expected and 2-16 cells are killed per T cell per day in a highly cooperative manner (Halle et al., 2016). However, we also need to take into account processes that we do not model explicitly, such as APC-dependent activation or T cell migration to the islet. We therefore choose killing rates that result in a biologically reasonable model behavior.

Antigen production and clearance Protein dynamics in the order of hours so we assume 1-5 islet antigens are cleared per day as in previous T1D mathematical models (Mahaffy and Edelstein-Keshet, 2007).

Carrying capacities Carrying capacities are estimated from a study on hand picked human islet (Babon et al., 2016). A maximum of 526 CD8$^+$ T cells per islet was reported. A maximum of 1,100 CD4$^+$ cells was reported in one T1D case. We expect T_{effs} to go over T_{regs}, since T_{regs} make up 5-10% of CD4$^+$ T cells.

The parameters and parameter ranges used in the simulations are listed in Table 4.2.

4.3.2 Assumptions

- The model reflects the interactions between islet cells and T cells in one islet. We assume that similar dynamics happen in all the pancreatic islets.
- We assume that seroconversion indicates the presence of an active adaptive immune response against insulin producing cells. Autoantibodies (AAb) are the first sign indicating that an individual is on the road to T1D and their appearance is strongly correlated with HLA-II genotypes (Ilonen et al., 2018). However, the role of islet autoantibodies and B cells remains unknown so we do not include them in the model (Leete and Morgan, 2021).
- We assume that all individuals in our simulated cohort are positive for at least one islet antibody due to an increase in islet antigen levels. The origin of elevated antigen levels

Equation	Parameter	Model	Definition	Units	Value	Reference
β-cell (B)	δ_{beta}	1&2	β cell killing per effector T cell	$cell^{-1}day^{-1}$	1×10^{-5} - 9×10^{-5}	Estimated
Islet antigen (A)	α_A	1&2	Rate of islet antigen release (or β-cell damage) per T_{eff} (C)	$cell^{-1}day^{-1}$	1×10^{-5}	Mahaffy and Edelstein-Keshet (2007)
	δ_A	1&2	Clearance rate of the antigen	day^{-1}	1	Mahaffy and Edelstein-Keshet (2007)
T_{regs} (R)	γ_R	1&2	Proliferation rate of T_{reg} (R) upon antigen encounter	day^{-1}	1 - 10	Babon et al. (2016)
	δ_T	1&2	Death rate of T_{regs} (R) and T_{effs} (C)	day^{-1}	0.1 - 0.2	MURPHY and CASEY. BERG (2022)
	K_R	2	Density control on T_{reg} proliferation rate	cells	1 - 10	Babon et al. (2016)
T_{effs} (C)	γ_C	1&2	Proliferation rate of T_{effs} dependent of antigen activation of T_{naive} and memory cells	day^{-1}	1 - 100	Babon et al. (2016)
	δi	1	T_{eff} suppression by T_{reg}	$cell^{-1}day^{-1}$	1	Estimated
	k	2	Threshold of T_{eff} inhibition by T_{reg}	cells	1	Estimated
	K_C	2	Density control on T_{eff} proliferation rate	cells	50 - 100	Babon et al. (2016)

Table 4.2: Parameters of both model 1 and model 2. Where possible, we provide biologically realistic ranges based on experimental data.

is unknown but viral infections and mimicry are strong candidates (García et al., 2019; Gottesman et al., 2022). We assume that all individuals are in contact with an unknown trigger that can initiate an autoreactive response.

- We assume that in the face of the same antigenic trigger, variability in the immune response parameters will determine the transition from health to disease. A balance between T_{effs} and T_{regs} is thought to determine disease outcome.

- We assume that HLA-II risk affects the proliferation rate of T_{regs}.

- There is broad agreement that 85–95% of β-cell mass is lost in more long-established T1D but many individuals retain modest numbers of residual insulin-positive cells for many

years. One example of these long standing insulin producers are Joslin medalists, individuals with T1D for more than 50 years that retain insulin production (Keenan et al., 2010). Variability in the % of β-cell mass remaining has been correlated with age at onset in several studies and has been attributed to age-related and HLA-related immunophenotypes (Leete et al., 2016). Another study though suggests that this variability might be explained by body mass index (Klinke, 2008) (a covariate that also correlates with age) but that other factors such as measure-driven technical variability due to the distinct distribution of islets in the pancreas might contribute to large variability at onset. We assume that our simulated cohort is homogeneous (similar age and body mass index) and that loss of 80% of β-cell mass indicates transition from health to T1D onset.

- Following (Mahaffy and Edelstein-Keshet, 2007), we also obviate β-cell turnover in our model. β-cells are thought to be long-lived and a stable population after birth (Keenan et al., 2010; Lam et al., 2017; Meier et al., 2008).

4.3.3 Simulations

We apply the cellular model described above to a virtual cohort of 10,000 individuals, each one defined by a different set of parameters (sampled from ranges in Table 4.2) and use numerical methods to solve the equations in Julia programming language (see Figure 4.5 **A** and **B** for realizations of each model). All simulations start with the same initial conditions, that is 100% of β-cells in the islet (1×10^3 cells) and 0 activated T cells. To initiate the autoreactive T cell response, islet antigen is also present at the start of all simulations. The presence of islet antigen at time 0 will trigger a T cell response that might or might not kill all β-cells in the islet (see Figure 4.5 **B** and **C**). Simulation time span is 2000 days. At the end of each simulation, the levels of each variable are recorded. Mean T cell levels (C and R) throughout the simulation are also recorded.

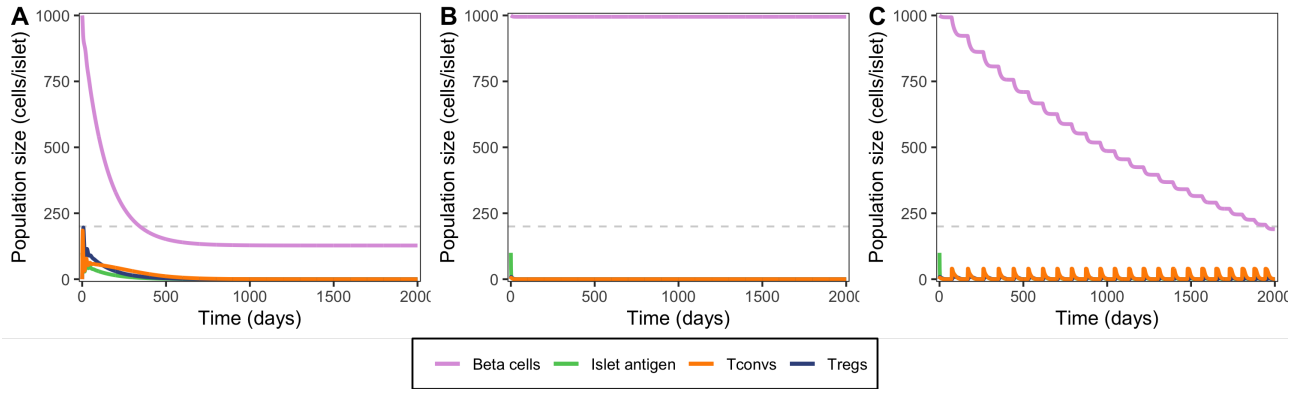


Figure 4.5: Cell population levels over time in 3 *in silico* individuals. Trajectories for one realization of model 1 (**A**) and two realizations of model 2 (**B** & **C**). **A Model 1**: Numerical solution for one *in silico* individual that develops T1D. Parameters: $\delta_{beta} = 1e-4$, $\alpha_a = 1e-5$, $\delta_a = 1$, $\gamma_R = 50$, $\delta_T = 0.2$, $\gamma_C = 150$, $\kappa_R = 1e-2$. Intial conditions: $B = 1e3$, $A = 1$, $R = C = 0$. **B Model 2**: Numerical solution for an individual that remains healthy after a rise in antigen. Parameters: $\delta_{beta} = 1e-4$, $\alpha_a = 1e-5$,$\delta_a = 1$, $\gamma_R = 7$, $\delta_T = 0.2$, $\gamma_C = 10$, $\kappa_R = 1e-2$, $K_r = 10$, $K_c = 40$. Intial conditions: $B = 1e3$, $A = 1$, $R = C = 1$. **C Model 2**: Same *in silico* individual as in B but with a 25% increase in T cell lifespan, i.e. $\delta_T = 0.16$, corresponding to a high number of functional iKIR genes. Antigen levels are shown in green. Dashed gray line indicates threshold for T1D onset.

4.4 Results T1D model

To represent the iKIR effect on T cell survival, we run the simulation twice for each parameter set. In the first simulation we set an average T cell death rate, corresponding to an average number of functional iKIR genes. In the second simulation, all the parameters of a given set are kept constant but T cell death is decreased to reflect the impact of high number of functional iKIR genes on T cell lifespan. We therefore simulated the cohort twice, resulting in 20,000 pairs. While different pairs carry different parameter sets, individuals in the same pair have the same parameters and only differ in their death rates. We refer to the individuals of the pair with the smallest death rates as the *iKIR high in silico* cohort, whereas the individuals with highest death rate as the *iKIR low* cohort.

A high number of functional iKIR genes can cause up to a two-fold increase in T cell lifespan (Zhang et al., 2023). Here we implement a moderate effect of 25% increase in lifespan. In both models, increasing T cell survival resulted in progression to T1D in a small fraction of *in silico* individuals who would otherwise stayed healthy. In the majority of *in silico* individuals, decrease in T cell death rate did not have an impact on outcome (Figure 4.6) and so the outcome of each simulated pair coincided most of the time.

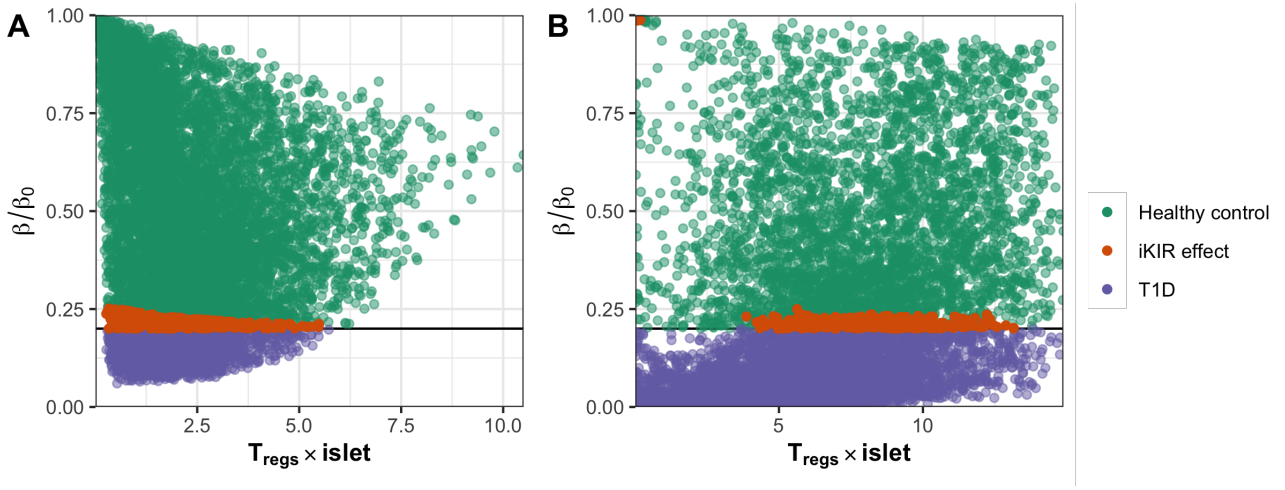


Figure 4.6: β-cell mass remaining as a function of T_{regs} levels. **A**, **B** Fraction of β-cell mass remaining as a function of the number of regulatory T_{regs} during the immune response is shown for each *in silico* individual in the simulated cohort with model 1 (**A**) and model 2 (**B**). Color code indicates outcome of the immune response: individuals that remain healthy after the immune response are shown in green, those that transition to T1D are shown in purple and individuals that have a different outcome depending on their functional iKIR gene count are shown in darkorange.

4.4.1 T_{reg} levels correlate with protection

Individuals with HLA-II protective genotypes have higher frequencies of islet-specific T_{regs}. We assumed that HLA-II-protected *in silico* individuals have high number of islet-specific T_{regs} and classified individuals into groups on the basis of mean T_{reg} numbers during the simulation. In both models, individuals with higher mean levels of activated T_{regs} tend to remain disease-free whereas individuals with low mean T_{reg} levels tend to progress to T1D (Figure 4.7). Note that in model 1, individuals with a very low number of T_{regs} typically remained healthy (T_{eff} cell frequency was also low in those cases, not shown).

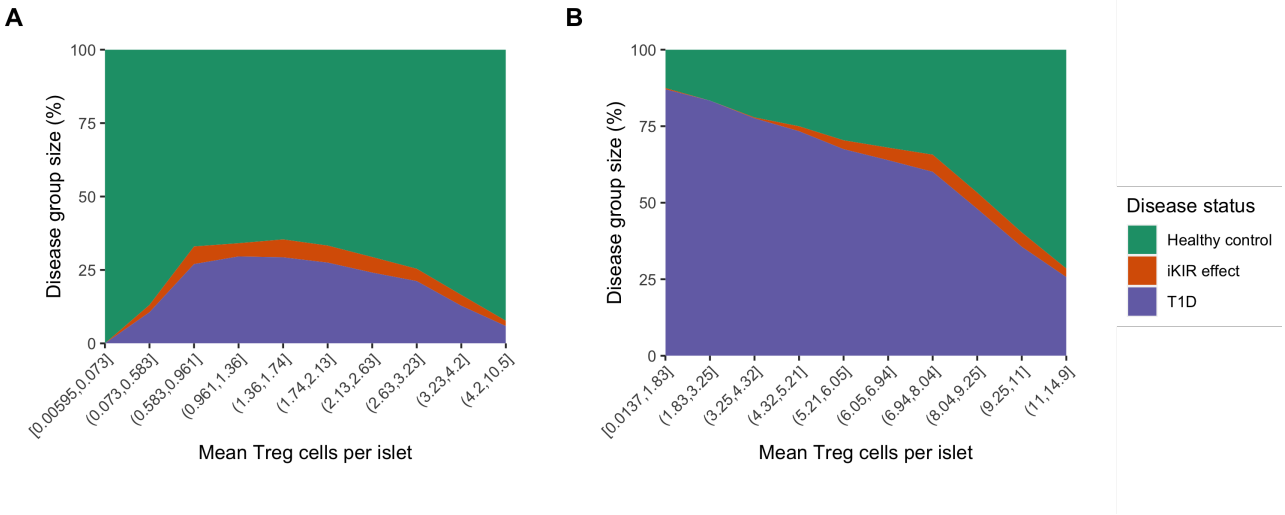


Figure 4.7: T_{reg} levels represent HLA-II risk. Mean T_{reg} levels are grouped into deciles, which results in 10 T_{reg} categories (x-axis). The proportion of each disease group is calculated within each T_{reg} category. **A** Percentages of each disease outcome in **model 1**. **B** Same plot but for **model 2**.

4.4.2 Impact of iKIRs on HLA-II associations with T1D

We then asked whether an increased T cell survival is associated with higher T1D risk in carriers of protective but not neutral nor detrimental HLA genotypes. To do that, we computed the difference in ln[OR]s between the iKIR high and iKIR low *in silico* cohorts in each T_{reg} decile group (which we assume is the equivalent of the HLA risk group in the immunogenetic data). In model 1, the difference of ln[OR]s remained constant for different groups with different numbers of T_{regs} (Figure 4.8 **A**). In model 2 though, the difference in lnORs increases as the number of T_{regs} per islet increases (Figure 4.8 **B**), which recapitulates the trend observed in the actual data (Figure 4.8 **C**).

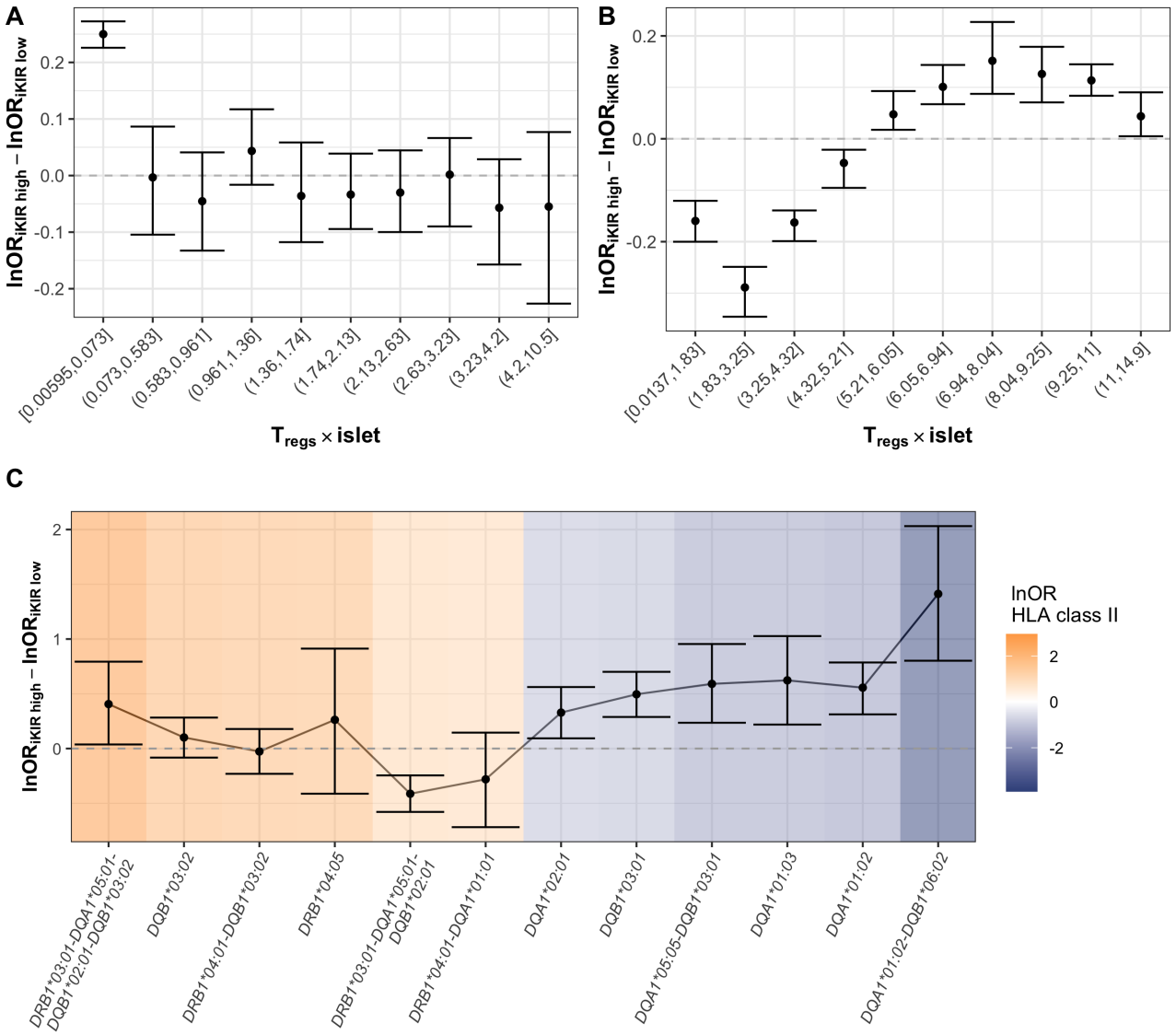


Figure 4.8: iKIR effect as a function of T_{reg} levels. **A**,**B** Difference of ln[OR]s between a cohort with low T cell death rates (iKIR high) vs and a cohorth with high T cell death rates (iKIR low) as a function of the number of T_{regs} during the immune response simulated with model 1 (**A**) and model 2 (**B**). **C** Difference in HLA-II genotype ln[OR]s between the group of UK-GRID individuals with a high iKIR score and the group with a low iKIR score in different. Color code according to the ln[OR] of the HLA-II genotype estimated from UK-GRID.

4.5 Discussion

We hypothesized that iKIR-mediated risk in HLA-protected individuals is caused by a prolonged T cell lifespan. A longer T cell lifespan is expected to aggravate autoimmune responses and so might explain our immunogenetic results.

In the context of chronic viral infections, previous studies from the lab identified a similar effect but, as expected, in the opposite direction; protective HLA associations were not weakened but enhanced by functional iKIR genes (Seich al Basatena et al., 2011; Boelen et al., 2018).

However, in both HIV-1 and HTLV-1 infection, detrimental HLA-I genotypes (*HLA-B*35* and *HLA-B*54* respectively) were also significantly enhanced. Mathematical modelling provided an explanation for these opposing and counter-intuitive effects; when T cells survive for longer, the quality of the antiviral response becomes more important and so at the population level differences between protective and detrimental HLA genotypes are magnified (Boelen et al., 2018).

In T1D though, there is no evidence for functional iKIR modification of detrimental genotypes. This difference between viral infections and T1D is striking. In the viral infections context, protective and detrimental HLA-I genotypes are thought to share a common pathway, e.g. detrimental genotypes are not actively detrimental but are absence of protection and the mechanism underlying both protection and susceptibility in chronic viral infections is the same, e.g. efficiency in targeting conserved epitopes by the different HLA molecules (Borghans et al., 2007). Given this shared biology it is unsurprising that both protective and detrimental genotypes are modified in a similar way by functional iKIR genes.

In autoimmunity the mechanism of protective and that of detrimental genotypes may be fundamentally different. For instance, the detrimental genotypes may be responsible for presenting tissue-specific autoantigens to T cells which escaped negative selection in the thymus whilst the protective genotypes may skew cells to an antiinflammatory response. That is the protective genotypes may actively prevent disease and the detrimental genotypes may actively cause disease and so a differential effect of functional iKIR can be understood. In line with this hypothesis, T cells restricted by a detrimental DQ8 HLA molecule acquire a proinflamatory phenotype when co-cultured with DQ8 homozygous APCs. When co-cultured with DQ8/DQ6 hetorezygous APCs though, T cells switch to an anti-inflamatory phenotype (Eerligh et al., 2011), providing an explanation to the dominant protection conferred by *DQ6* genotype. The finding that all protective HLA-II genotypes are similarly iKIR modified may suggest that these HLA-II genotypes share a common mechanisms of protection. This is consistent with recent work from Zhao et al. (2020), who identified shared resistant motifs among some resistant genotypes like those present in *DQ6* or *DQA1*01:03* genotypes.

In addition to epitope stealing, other mechanisms might explain *DQ6* protective effect in T1D. For example, we find evidence that HLA molecules decrease the number of islet-specific T_{regs} as a function of HLA risk associated with T1D (Wen et al., 2020). Similarly, HLA T1D risk also correlates with the loss of anergic B cells specific for insulin in healthy individuals (Smith

et al., 2015). Recent studies provide more mechanistic explanations to these HLA-dependent biases in relevant immune cell frequencies. A large portion of the HLA-II risk in T1D maps to the amino acid at position 57 in the DQB1 molecule (Todd et al., 1987), in which the presence of aspartic acid (D) correlates with protection against T1D (Morel et al., 1988). This residue position has also been recently correlated with the frequency of negatively charged sequences in the CDR3 region of the TCRs from both effector and regulatory $CD4^+$ T cells (García et al., 2021). These sequences are known to be enriched among TCRs that bind the primary epitope in T1D, insulin B:9–23 (Gioia et al., 2019). Indeed, the frequency of negatively charged sequences also correlated with the proportion of islet-specific T cells in the islets, providing a mechanistic explanation for HLA-II associations with T1D. Therefore, the nature of this residue position in DQ molecules might impose presentation constraints that bias the TCR repertoire towards higher/lower reactivity and might lower/increase the propensity for the selection of regulatory T cells. In the context of T1D, there is some evidence that overall T_{reg} frequencies correlate with T1D HLA risk genotypes (Glisic et al., 2009; Michalek et al., 2006). However, these results might be partly explained by age-dependent increase of T_{regs} (Brusko et al., 2007), which in turn reinforces the view that T_{reg}-mediated islet tolerance might be essential to prevent autoimmunity[2].

Given the relatively high prevalence of detrimental genotypes in the general population[3], one might speculate that these molecules were beneficial in past environments and so they became widespread. In this scenario, pro-T1D HLA genotypes would promote reactivity whereas anti-T1D HLA genotypes will favour immunoregulation like T_{reg}-mediated inhibition. As expected, HLA polymorphism modifies the CDR3 sequence composition in healthy individuals; interestingly, some of the most influential residues are strongly associated with autoimmune disordes (Ishigaki et al., 2022).

An alternative mechanism that could explain HLA-mediated risk is increased AICD in T_{regs} when antigen levels are high, as reported in mice experiments in autoimmune thyroiditis (Badami et al., 2019). In T1D, *in vitro* work also showed increased apoptosis levels in T_{regs} from HLA detrimental carriers (Glisic and Jailwala, 2012). Finally, the fact that CTLA-4 loci is associated with T1D and that individuals with IPEX syndrome[4] develop early-onset T1D

[2]T1D is a pediatric disease; its early onset might be explained by an environmental insult in young individuals whose tolerance mechanisms have not fully developed yet.

[3]Although only 2% of healthy controls in the UK-GRID cohort carry the *DR3/DR4* heterozygote genotype, 18% carries at least one copy of the detrimental DQ8 genotype and 26% carries DR3 genotype

[4]CTLA-4 is constitutively expressed in T_{regs}. Individuals with IPEX syndrome have mutations in FoxP3 gene, a transcription factor required for maintenance of T_{regs}.

helps secure an important role for T_{regs} in T1D ethiopathogenesis.

In an effort to elucidate the preferential iKIR modification of protective HLA associations in T1D, we implemented mathematical models that describe the interaction between islet-specific T_{reg} and T_{eff} cells during the autoimmune cell destruction of β cells. Given the central role of T_{regs} in T1D and multiple associations between T_{reg} immunophenotypes and HLA-II risk, we assumed that an individual's HLA risk to develop T1D is associated with the proliferation rate of T_{regs}.

The results presented in Figure 4.8 provide a plausible explanation for the immunogenetic findings. When T_{reg} levels are saturated, e.g. in protective HLA carriers, the increase in T cell survival results in an increase of T_{eff} but not T_{reg} population size. Consequently, in this scenario, there is an effective increase of β-cell destruction that cannot be compensated by T_{reg} suppression of T_{effs}. In unsaturated conditions though, i.e. in neutral or detrimental HLA carriers, iKIR-mediated increase of both T_{reg} and T_{eff} survival results in a zero net effect on β-cell destruction.

One limitation of this study is that we had to estimate some parameters. In addition, several known processes are considered only implicitly, e.g. antigen uptake and presentation by APCs, and so it would be difficult to experimentally estimate those. Similarly, the effect of B cells and auto-antibodies is not considered although this adaptive immune population would be equivalent to our T_{eff} population in that it is considered to be detrimental (Leete et al., 2016).

We also assumed that HLA-II risk correlates with the degree of proliferation of T_{regs}. This is based on the *DQ6* association with the frequency of islet specific T_{regs}. However, as we have seen, other studies suggest that HLA risk genotypes affect T_{reg} apoptosis levels (Glisic and Jailwala, 2012). Isotope labelling data could help to address this question by estimating the turnover rates of T_{reg} in individuals with different HLA-II genotypes. So far there are two stable-isotope labelling studies in T1D but the effect of HLA genotype on turnover was not investigated (Bollyky et al., 2013; Hao et al., 2019).

Finally, the effect of iKIRs on both T_{regs} and T_{effs} is reminiscent of recent work in mice showing that abatacept, a CTLA-4–immunoglobulin (Ig) fusion protein, blocked T follicular helper cell (T_{fh}) CD28-dependent stimulation (CTLA-4 prevents interaction between CD28 and its ligand). However, T_{reg} homeostasis also requires CD28 stimulation so abatacept not only dampened effector responses driven by T_{fh} cells but also regulatory responses (Edner et al., 2020; Wang

et al., 2022). iKIR engagement might have the same dual effect, where modulation of T lifespan affects both T_{regs} and T_{effs}.

In summary, with a minimal model of the β cell destruction by islet-specific T cells, we find a plausible mechanism that explains our immunogenetic results in the UK-GRID cohort. In the next chapter we analyse KIR expression data to investigate whether KIR^+ T cells are responsible for the iKIR effect in T1D or not.

Chapter 5

iKIR expression in T1D

We have seen that an extended T cell lifespan could explain our immunogenetic results linking functional iKIR genes with weakened HLA-II-restricted protective responses. There are two non-exclusive pathways in which KIRs affect T cell lifespan. Experiments in transgenic mice and in humans *in vitro* show that iKIR expression *directly* on T cells reduces cell death upon KIR ligation (Gati et al., 2003; Boelen et al., 2018; Roger et al., 2001; Ugolini et al., 2001). Alternatively, KIRs expressed on other immune populations like NK cells might dampen their known immunoregulatory function. Thus, other KIR expressing populations interacting with T cells might *indirectly* affect T cell lifespan. Both pathways are plausible mechanisms that could explain our immunogenetic findings.

In this Chapter we review what is known about KIR regulation of the immune response, focusing on the indirect and direct pathways that affect adaptive T cell responses. Then we move on to test the direct hypothesis. Analysis of RNA and protein KIR expression data indicates that a minority of T cells express iKIRs and that the size of this population is not altered in T1D.

5.1 KIRs during innate NK cell-mediated responses

Parts of section 5.1 have been previously published in (Mora-Bitria and Asquith, 2023).

KIRs are mainly expressed on NK cells where, along with other innate receptors, they regulate cytokine and cytotoxic responses. One of the mechanisms to modulate the NK response is the

inhibition resulting from the recognition of HLA-I ligands. NK cell inactivation by HLA-I is considered a factor that ensures the host's self-tolerance. This hypothesis is supported by the capacity of NK cells to target low self-MHC expressing cells, producing what is known as "missing self-response" (Ljunggren and Kärre, 1990). iKIRs participate in the missing self-response through interactions with their cognate HLA ligands.

However, KIR expression is independent of the host's HLA-I alleles and so NK cells may express iKIRs that lack their cognate HLA-I ligand in the host. Nonetheless, these NK cells are not alloreactive, as they are subjected to a host-tolerization process. This process is known by names such as "cell education", "licensing" (Kim et al., 2005) or "disarming" (Fernandez et al., 2005). This tolerization process ensures that developing NK cells are self-tolerant but responsive to malignant and infected cells. Different models have been proposed to explain how functional NK cell repertoires are calibrated by cognate iKIR-MHC interactions (Brodin et al., 2009; Boudreau and Hsu, 2018) but the molecular mechanism underlying the "NK cell education" process is only beginning to be understood (Wu et al., 2021).

5.2 KIRs during T cell responses

In addition to the clear role of KIRs in modulating NK cell-mediated immunity, there are multiple, non-exclusive, pathways by which these receptors potentially modulate T cell-mediated immunity (Mora-Bitria and Asquith, 2023). These pathways can be divided into two categories: "Direct pathways", in which KIR expression on a T cell directly impacts its function and survival, and "indirect pathways" in which KIR expression on another cell – e.g. an NK cell or a different T cell – indirectly affects T cell function.

5.2.1 Direct pathways

KIR engagement on T cells has been reported to have two main effects: inhibition of T cells' effector function and reduction of AICD.

Regulation of T cell effector function

Several studies have shown that iKIR engagement with their HLA ligands dampens the TCR-dependent effector response. This effect is produced both via reduction of cytokine levels

(D'Andrea et al., 1996; van der Veken et al., 2009) and impaired cytotoxicity (Bakker et al., 1998). However, the extent of this inhibition is highly heterogeneous across studies (Anfossi et al., 2004; Ferrini et al., 1994) and can be overcome by stronger stimuli (van der Veken et al., 2009). In contrast, in a previous *in vitro* study we found that the presence of functional iKIR-HLA interactions increased instead of decreased the number of $CD107a^{+}CD8^{+}$ T cells, suggesting higher degranulation potential upon iKIR ligation (Boelen et al., 2018).

While some studies argue that KIR modulation of $CD8^{+}$ T cells are ligand-independent (Alter et al., 2008; Björkström et al., 2012; Chwae et al., 2002; Young et al., 2001) others show functional impairment only in the presence of the cognate HLA (Anfossi et al., 2004; Gati et al., 2003; Guerra et al., 2002; van der Veken et al., 2009; Zajac et al., 1999). The cell type used – ex vivo KIR^{+} T cells, KIR^{+} T cell clones, transgenic KIR^{+} T cell lines –, the variability of functional KIR receptor repertoire across individuals, the effector function assessed (cytokines vs cytotoxicity) and the nature and concentration of the stimuli all might be factors underlying the discrepancies on ligand requirement. In conclusion, it seems that iKIR expression probably reduces activation-dependent T cell effector function, but it is unlikely to be a global inhibitor.

Impact on T cell survival

As opposed to the inhibitory KIR effect on T cell function, the impact on T cell survival upon KIR ligation is well established (Figure 5.1 **A**). Using mice transgenic for KIR2DL3, Ugolini *et al* showed that if the mice were also transgenic for the matching HLA ligand there was a significant accumulation of memory $KIR^{+}CD8^{+}$ T cells. However, this effect was abrogated in mice that were negative either for the ligand, the KIR or both (Ugolini et al., 2001). In addition, the size of this KIR^{+} T cell memory population increased with age, in a similar way as for the $Ly49^{+}$ T cell population in non-transgenic mice (Coles et al., 2000) and recapitulated in healthy humans (Anfossi et al., 2001).

Higher numbers of KIR^{+} T cells can be explained by either higher proliferation rates or lower death rates. Although KIR ligation has no impact on the number of cell divisions (van der Veken et al., 2009; Ugolini et al., 2001), KIR engagement resulted in reduced AICD in T cells *in vitro* (Ugolini et al., 2001), suggesting that the accumulation of memory $CD8^{+}$ T cells *in vivo* was attributable to a survival advantage of $KIR^{+}CD8^{+}$ T cells (Anfossi et al., 2001). Similarly *in vitro* experiments in mice, resulted in the inhibition of TCR-induced apoptosis in the presence of the Ly49 cognate ligand (Roger et al., 2001). An increased survival upon iKIR ligation has also been shown in $KIR^{+}CD4^{+}$ T cells in the context of ankylosing spondylitis

(Bowness et al., 2011).

Consistent with the iKIR-mediated resistance to cell death, KIR expression correlates with higher levels of the antiapoptotic molecule Bcl-2 (Young et al., 2001) and also lower caspase 8 activity (Gati et al., 2003). The requirement for iKIR ligation on this antiapoptotic effect seems to be dispensable in *in vitro* experiments using a transfected KIR3DL1 Jurkat cell line (Chwae et al., 2002). However, in human T cell clones, KIR ligation is required to reduce AICD (Arlettaz et al., 2004; Boelen et al., 2018).

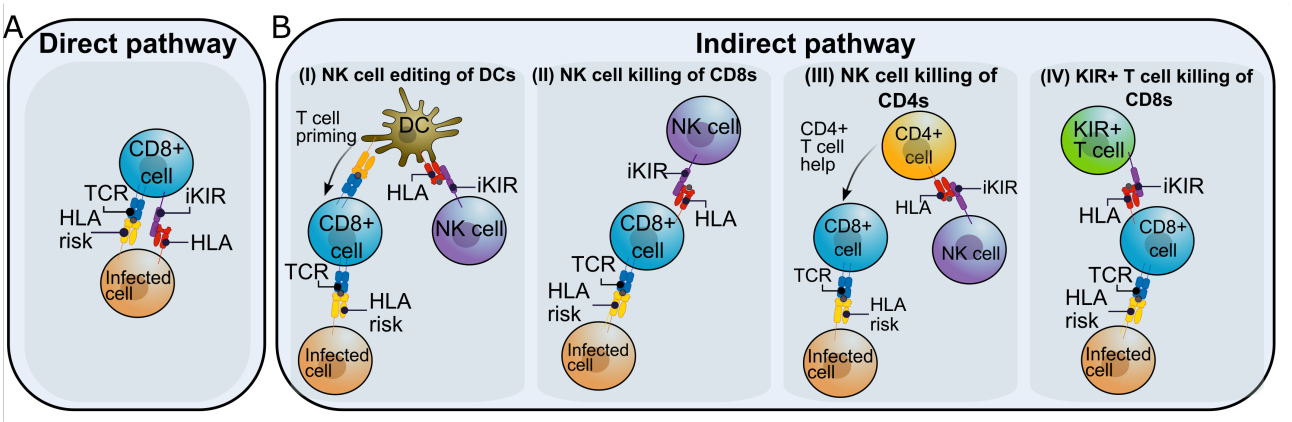


Figure 5.1: The indirect and direct pathways that affect T cell lifespan. iKIRs promote T cell survival by a number of different pathways. $CD8^+$ T cells are taken as the main responding T cell population in this figure but the same mechanisms would apply to responding $CD4^+$ T cells. The HLA-I molecule associated with disease outcome is the HLA molecule shown in yellow (interacting with the TCR in blue). **A Direct pathway**: iKIR expression on responding $CD8^+$ T cells reduces AICD and increases T cell lifespan upon ligation of the cognate KIR ligand. **B Indirect pathway**: iKIR ligation on other cells also affects $CD8^+$ T cell lifespan. (**I**) NK cells can interact with dendritic cells (DCs) and shape downstream T cell responses. (**II**) NK cells can directly kill activated $CD8^+$ T cells. (**III**) Activated $CD4^+$ T cells are also susceptible to NK cell killing. (**IV**) KIR^+CD8^+ T cells can kill activated $CD8^+$ T cells. Figure reproduced from Mora-Bitria and Asquith (2023), licensed under CC BY.

Although their discovery dates back over 3 decades ago (Moretta et al., 1990), the functional relevance of the KIR^+ T cell population remains unclear. Several reasons might explain the paucity of functional studies. First, the absence of the KIR family in murine species hinders the translation of *in vivo* studies. Second, due to the relatively wide expression of KIRs[1], it is difficult to pinpoint the immune population responsible for KIR gene associations. Finally, the antigen specificity of KIR^+ T cells is often unknown (Young et al., 2001; Pieren et al., 2021). KIR^+ T cell specificity has been identified in some settings; these specificities include tumour specific T cell clones isolated from renal cell carcinomas (Guerra et al., 2000) and melanomas (Speiser et al., 1999) as well as HLA multimer$^+$$KIR^+$ T cells specific for cy-

[1] KIR^+ T cell populations are found both within the $\gamma\delta$ (Battistini et al., 1997; Nakajima et al., 1995) and the $\alpha\beta$ T cell compartments (Ferrini et al., 1994; Mingari et al., 1996; Phillips et al., 1995). Amongst conventional $\alpha\beta$ T cells, the highest frequencies of KIR expression are found in $CD8^+$ T cells (1-25%), nevertheless low frequencies of KIR^+CD4^+ T cells (<1%) are also consistently detected (Moretta et al., 1990; Phillips et al., 1995; van Bergen et al., 2004).

tomegalovirus (CMV), HTLV-1 and HIV-1 (Alter et al., 2008; Boelen et al., 2018; van der Veken et al., 2009; Young et al., 2001; Sullivan et al., 2021).

Given the specificity of these KIR$^+$ T cell populations and their accumulation in the terminally differentiated memory compartment, it has been suggested that KIRs on T cells might be involved in the regulation of autoreactive responses (Alter et al., 2009; Cauda et al., 1994; Guerra et al., 2000), acting as an immune checkpoint that increases the activation threshold of these potentially self-reactive T cells. Finally, recent studies suggest that KIR$^+$ T cells can also play an indirect regulatory role on activated T cell populations (Pieren et al., 2021; Li et al., 2022) (see next section).

5.2.2 Indirect pathways

iKIRs can indirectly affect T cell function and survival through iKIR expression on other immune effectors (e.g. NK cells or other T cells) interacting with the responding T cell population (Figure 5.1 **B**).

NK-DC interactions

NK cells interact with DCs during the T cell priming phase, a process known as NK-DC crosstalk. There is evidence that NK-DC crosstalk can have both a positive effect – boosting the subsequent T cell response –, or a negative effect, dampening it down. Upon activation, NK cells secrete cytokines including interferon-gamma and enhance dendritic cell maturation (Piccioli et al., 2002). NK cells have also been shown to kill DCs during viral infection – known as DC editing (see Figure 5.1 **B (I)**)– which ensures robust T cell responses by selectively depleting immature DCs and sparing the most immunogenic DCs (Ferlazzo et al., 2002; Morandi et al., 2012; Piccioli et al., 2002). In contrast, some DC vaccine studies show that NK cell editing of DC during the priming phase could have an opposite effect and impair T cell responses: NK killing of immature DCs can reduce the efficacy of DC vaccinations Hayakawa et al. (2004) and affect viral control (Andrews et al., 2010; Cook and Whitmire, 2013; Mandaric et al., 2012). Whether NK-DC interactions promote or dampen effective T cell responses remains unknown but several factors such as the cytokine milieu or the density of co-stimulatory molecules might be important. For example, some studies have shown that KIRs at least partly modulate DC-NK interactions (Chiesa et al., 2003; Ferlazzo et al., 2002), so variability in the KIR-HLA interactions might contribute to the disparate results.

NK cell killing of CD4$^+$ and CD$^+$ T cells

Mice and human studies have shown that NK cells can also affect adaptive immunity by killing activated T cells (Rabinovich et al., 2003; Soderquest et al., 2011; Lang et al., 2012). Activated CD8$^+$ and CD4$^+$, including T$_{regs}$, upregulate activating ligands and/or death receptors for NK cells (Roy et al., 2008; Cerboni et al., 2007; Crouse et al., 2014; Rabinovich et al., 2003; Nielsen et al., 2012) but the exact mechanisms of NK recognition and killing of T cells are still not well defined (Waggoner et al., 2016). It has been suggested that the selectivity for activated cells indicates that NK cells might kill incorrectly activated T cells such as autoreactive T cells (Nielsen et al., 2012). Indeed, NK cells control autoreactive CD4$^+$ T cells and ameliorate inflammation in a mouse model of multiple sclerosis (Laroni et al., 2016; Lu et al., 2007). In murine LCMV infection, NK cell killing of CD4$^+$ T cells has been shown to be protective and to prevent immunopathology in the context of high viral load (Waggoner et al., 2012) (Figure 5.1 **B III**). However, in the same study, when using an intermediate viral dose, NK cell regulation results in viral persistence and immune pathology (Lang et al., 2012; Soderquest et al., 2011; Waggoner et al., 2012). Therefore, while NK cell dampening of CD4$^+$ T cell responses might be beneficial in some contexts (autoimmunity, immunopathology), it can also have long-term negative effects (including viral persistence) and result in a reduced T cell memory pool (Lu et al., 2007; Rydyznski et al., 2015; Soderquest et al., 2011). NK killing of CD8$^+$ T cells has also been described by Peppa et al (Figure 5.1 **B II**). Using PBMCs from chronic hepatitis B virus patients (HBV) they showed that, *in vitro*, NK cells selectively kill HBV-specific T cells via the engagement of death receptors (Peppa et al., 2013). In a follow up study, they show that NK regulatory function limits specific CD8$^+$ T cell responses to HBV vaccine in mouse *in vivo* and in humans *in vitro* in a PDL1-dependent manner (Diniz et al., 2022).

The role of iKIRs during this NK-T crosstalk has not been addressed. NK-T cell interactions probably take place within secondary lymphoid organs where after T cell priming, T cells undergo clonal expansion for approximately 2 days, coinciding with the peak of the NK cell response. Although perforin+ NK cells do not reside in secondary lymphoid organs (Dogra et al., 2020), upon activation during viral infections, type I interferon signalling triggers NK cell accumulation and renders them in close contact with T cells (Ali et al., 2021). Interestingly, NK cells within lymph nodes not only acquire cytolytic functions but also KIR expression (Ferlazzo et al., 2004). Therefore, activated KIR$^+$ NK cells are probably interacting with recently activated T cells in the lymph nodes. The outcome of this interaction might be modulated by the iKIR-HLA receptor-ligand system. Additionally, type I interferon also induces

KIR ligand expression, HLA-I, in activated T cells so T cells might avoid NK cell killing via iKIR engagement (Xu et al., 2014). This is consistent with recent experiments in mice showing that deficiency of a MHC-I transcriptional regulator results in NK cell elimination of T cells during inflammation (Ludigs et al., 2016).

T cells killing T cells

NK cells are not the only KIR expressing effector population that can affect T cell survival. A recent study from Li *et. al.* indicates that KIR^+ T cells can also regulate self-reactive T cell responses (Figure 5.1 **B IV**). They observed that KIR^+CD8^+ T cells are expanded in different autoimmune conditions, both in blood and within the relevant inflamed tissues (Li et al., 2022) but also in patients with severe COVID-19. A previous study also observed that activated $KIR^+CD45RA^+$ T cells expand during acute respiratory infection (including COVID-19 patients) and showed that KIR^+RA^+ T cells suppress proliferation of stimulated KIR^-CD8^+ T cells *in vitro* (Pieren et al., 2021).

To address the function of the KIR^+CD8^+ T cells in autoimmunity, Li *et. al.* isolated gliadin-reactive $CD4^+$ T cells from celiac patients and co-cultured them with KIR^+CD8^+ T cells. Upon activation, KIR^+CD8^+ T cells killed activated $CD4^+$ T cells in a perforin-dependent manner. This effect was partly abrogated by the presence of anti-HLA-I antibodies and anti-HLA-E antibodies. Whether the interaction between HLA molecules and the relevant KIR is modulating this regulatory function was not addressed and, although the KIR^+ T cells were pre-activated before blocking, classical HLA-I blockade could in principle be attributed to TCR signalling, KIR signalling, or both. Furthermore, experiments in mice lacking $Ly49^+CD8^+$T cells displayed normal antiviral responses but were more prone to autoimmunity. These observations built on previous work on mouse models of multiple sclerosis where $Ly49^+$ T cells were shown to suppress disease (Jiang et al., 1992; Saligrama et al., 2019). Based on these recent findings, Li et al. (2022) argued that KIR^+CD8^+ T cells are the counterpart of $CD4^+$ T_{regs} and might limit autoreactivity and immunopathology.

5.3 Frequency of T cells expressing iKIR transcripts in T1D patients

There is evidence that KIR$^+$ expression on T cells is higher in chronic viral infections (Alter et al., 2008; Bonorino et al., 2007; Cauda et al., 1994) although in our recent study we did not find increased frequencies of KIR$^+$ T cells in HCV viremic patients Zhang et al. (2023). Increased KIR expression has also been reported in autoimmunity. In ankylosing spondylitis, iKIR expression is dramatically increased in CD4$^+$ T cells: 1-6% of all CD4$^+$ cells are KIR3DL2$^+$ rising to 10-60% of Th17 CD4$^+$ cells in these patients (Bowness et al., 2011). In systemic lupus erythematosus (SLE), we see similar increases (Basu et al., 2009). As mentioned, the frequency of KIR$^+$CD8$^+$ T cells is also increased in multiple sclerosis, systemic lupus erythematosus, and ceoliac disease (Li et al., 2022). Finally, KIR expression is also elevated in in haploidentical bone marrow transplant recipients (Albi et al., 1996).

Whether KIR expression on CD8$^+$ and/or CD4$^+$ T cells is elevated in T1D patients has not been explored. As we have seen, KIR expression on T cells confers a survival advantage. I hypothesize that if iKIRs on T cells explain the functional iKIR gene effect on HLA associations, then KIR expression might be higher in T1D patients.

5.3.1 scRNAseq analysis

To test the direct hypothesis we analysed published scRNA-seq data of PBMC samples from 4 children postive for islet antibodies and 4 matched controls (see Kallionpaa single-cell RNA sequencing dataset). Briefly, to account for multi-mapping (multi-gene) reads, we align FASTQ files to the GRCh38 reference genome using STARsolo aligner with the option `-soloMultiMappers Uniform` (Kaminow et al., 2021). Multi-mapping reads are typically discarded during read quantification steps. Albeit at the expense of a minor decrease in precision, rescuing multimappers during the quantification step can increase sensitivity Kaminow et al. (2021), specially in cases where there is a high degree of homology between genes as is the case of KIRs (Marin et al., 2021).

After read mapping and quantification, count matrices were read into R (R Core Team, 2021) using Seurat library (Hao et al., 2021) for quality control. We filter low quality barcodes including (1) empty barcodes or barcodes with ambient RNA, (2) apoptotic/stressed cells and

(3) barcodes containing more than one cell (doublets). We computed barcode ranks for log[UMI] counts[2] (Figure 5.2 **A**) to discard empty droplets using the package `DropletUtils` (Griffiths et al., 2018). A total of 21,149 cellular barcodes passed the filtering (Figure 5.2 **B**). To remove barcodes with ambient RNA or barcodes containing more than 1 cell, barcodes with a low/high number of expressed genes or a high number of umi counts based on median absolute deviation (MAD) thresholds (Figure 5.2 **C**). Finally, barcodes with more than 5% mitochondrial reads were also filtered out. A total of 18,519 cells and 24,141 features are kept for downstream analysis.

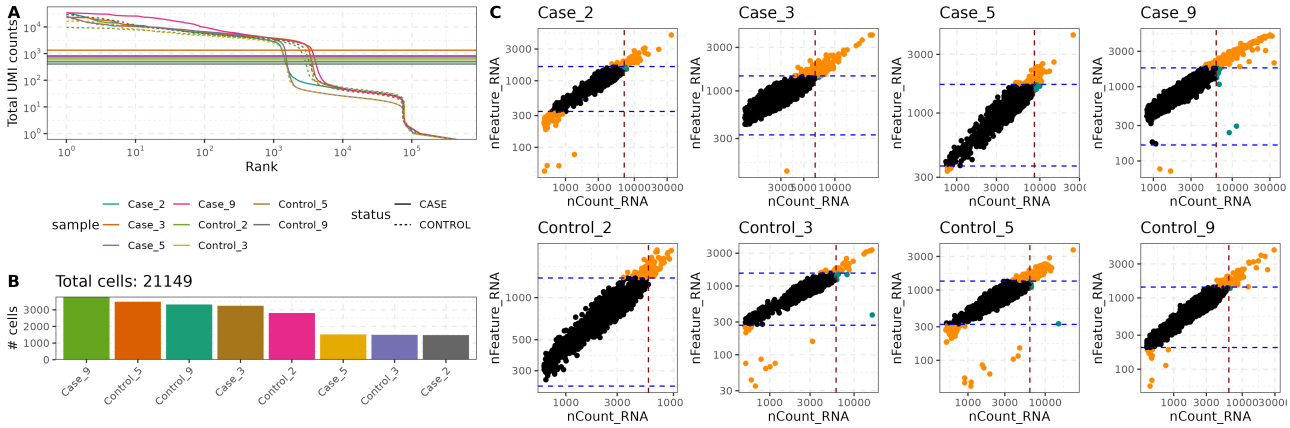


Figure 5.2: QC of scRNAseq data. Low quality barcodes are discarded using different filters. **A** Knee plot showing umi counts per barcode ranked from barcode with the highest number of umi counts to the lowest. Barcode rank curves are shown for each sample (color-coded). Horizontal line indicates the knee point for each sample. Knee point in the curve is assumed to mark the transition between two components of the total count distribution i.e. between empty droplets with little RNA and cell-containing droplets with much more RNA. Dashed curves correspond to controls and solid curves to cases. **B** Number of cellular barcodes per sample after removing empty barcodes. **C** Number of umi counts against number of detected genes (features) per barcode. Cell barcodes with high (doublets) or low (ambient RNA) number of features are shown in orange. Barcodes with a high number of umi counts are shown in green. Dashed lines indicate ± 3×MAD (median absolute deviation) thresholds in each sample.

To make counts comparable across cells and remove technical biases, feature counts of each cell are divided by total umi counts of that cell, i.e. by the library size. Normalized counts are multiplied by a scale factor equal to 10k and converted to counts per 10,000 reads to ease readability. Finally, to focus on relative differences rather than on absolute differences, scaled counts are log transformed.

[2]As explained in section subsection 2.1.3, UMIs are unique molecular identifiers that are tagged to each transcript during library preparation, before any PCR amplification. Therefore, quantification based on UMIs is more accurate than based on read counts as the former represents the number of input transcript molecules whereas the latter might contain PCR duplicates due to amplification biases.

5.3.2 Expression of KIR transcripts in memory T cells and NK cells

We used an automatic annotation method, `SingleR`, to classify qced cells (Aran et al., 2019). Briefly, given a reference dataset of single-cell or bulk samples with known labels, it classifies new cells from a test dataset based on spearman correlation between the test and the reference. We used the monaco reference dataset containing bulk sequencing data from sorted immune populations (Monaco et al., 2019). Additionally, we used the human primary cell atlas reference (hpca) dataset that contains microarray datasets from blood populations (Mabbott et al., 2013).

Few cells were annotated as terminal effector memory $CD8^+$ T cells (monaco=239, hpca=213) and just a small proportion expressed iKIR transcripts (Table 5.3.2). Nevertheless, KIR^+ terminal effector memory $CD8^+$ T cells were detected in both cases and controls with no differences in expression levels (Figure 5.3 **A**). Only 2 barcodes classified as $CD4^+$ terminal effector (hpca reference) expressed KIR transcripts. Of note, cells annotated as T_{regs} (monaco, N=1,185) did not express iKIR transcripts. Since KIR^+ T cells accumulate in the terminal memory compartment, a low number of KIR^+ T cells (both $CD8^+$ and $CD4^+$) might be expected given the age of the individuals (<2 years old). As expected, we found higher numbers of KIR^+ cells within the NK cell compartment Table 5.3.2. Again, we did not find differences in KIR expression levels between cases and controls. (Figure 5.3 **B**).

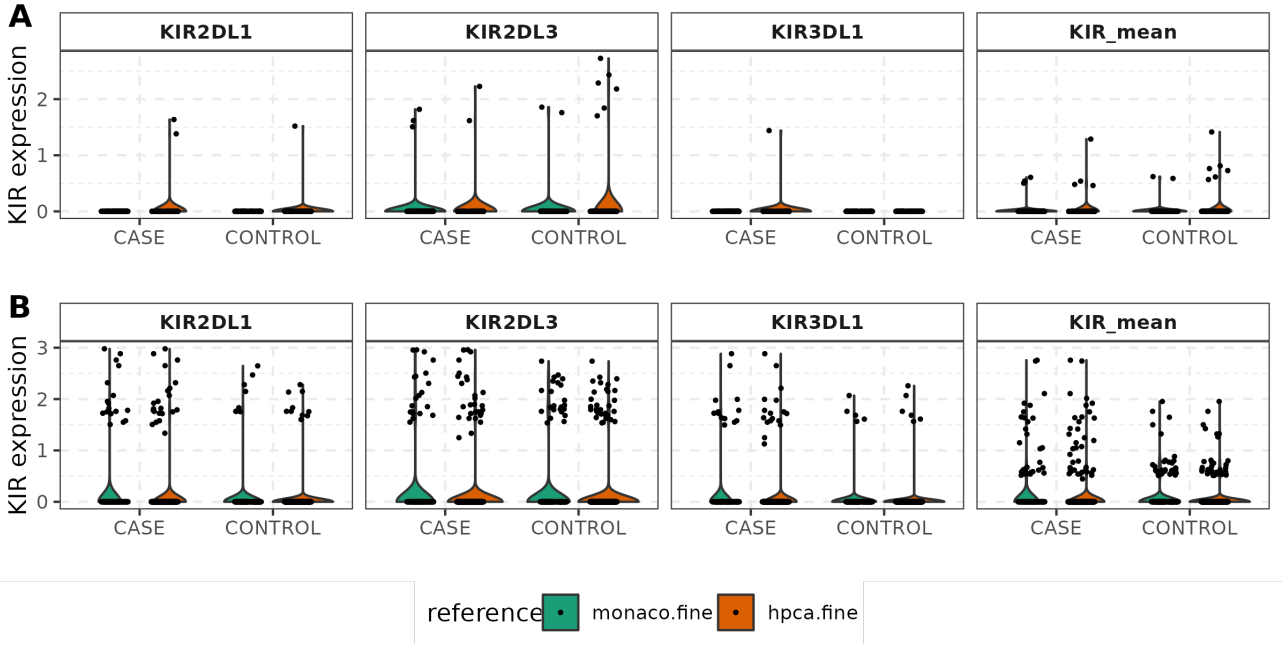


Figure 5.3: KIR gene expression within memory $CD8^+$ T cells and NK cells is similar between seropositive children and healthy controls. **A** Few $CD8^+$ terminal effector memory cells express KIR transcripts. Cells labeled as *Terminal effector CD8 T cells* (monaco, green) or as *T cell:$CD8^+$ effector memory RA* (hpca, orange) are shown split by disease status (case, control) and by KIR gene (*KIR2DL1*, *KIR2DL2/L3* and *KIR3DL1*). We also show mean expression across *KIR2DL1*, *KIR2DL2/L3* and *KIR3DL1* genes. **B** Same plot but for cells labelled as *Natural killer cells* (monaco) or *NK cells* (hpca).

	KIR2DL1		KIR2DL3		KIR3DL1		KIR		Cells	
Cell type (HPCA reference)	**T1D**	**HC**	**T1D**	**HC**	**T1D**	**HC**	**T1D**	**HC**	**T1D**	**HC**
NK_cell	3.96	2.32	5.88	5.93	3.55	1.59	8.62	7.38	986	691
NK_cell:CD56hiCD62L+	1.82	1.04	2.19	1.04	0.00	0.00	2.19	1.73	274	289
$CD4^+$_Naive	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.06	1858	1807
$CD4^+$_central_memory	0.06	0.09	0.00	0.00	0.00	0.00	0.06	0.09	1664	1133
$CD4^+$_effector_memory	0.00	0.00	0.28	0.00	0.28	0.00	0.56	0.00	360	165
$CD8^+$_Central_memory	0.00	0.49	0.00	0.49	0.00	0.00	0.00	0.98	104	205
$CD8^+$_effector_memory	0.06	0.03	0.06	0.12	0.00	0.00	0.11	0.15	1769	3235
$CD8^+$_effector_memory_RA	3.26	0.83	3.26	5.79	1.09	0.00	5.43	5.79	92	121
$CD8^+$_naive	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	373	747

Table 5.1: Percentage of cells expressing KIR transcripts within NK cell and memory T cell susbsets. Percentage of KIR2DL1, KIR2DL2/L3 and KIR3DL1 positive cells within each HPCA annotated subset is shown split by disease status (T1D= individuals at risk of type 1 diabetes, HC=matched healthy controls). KIR column represents the percentage of KIR positive cells (expressing at least one KIR). The total number of cells (regardless of KIR expression) in each NK cell and memory T cell subset is displayed in the two last columns. Percentages for other HPCA annotated immune populations can be found in Table B.2. Percentages using the Monaco reference dataset are listed in Table B.1.

5.4 KIR protein expression in T1D

In contrast to Li et al. (2022), we detected T cells expressing KIR transcripts in both cases and controls at similar frequency. In addition, KIR^+ T cells were very rare in these samples.

To confirm these results, we recruited 20 individuals including 10 T1D patients and 10 matched controls for KIR immunophenotyping flow cytometry analysis (see Cardiff cohort). Specifically, the expression of KIR2DL1, KIR2DL2/L3 and KIR3DL1 was assessed in the different $CD4^+$ and $CD8^+$ naive and memory populations as well as in NK cell subsets (see Multi-colour antibody panel). Blood samples from healthy individuals were collected and processed by Charandeep Kaur. Blood samples from T1D patients were provided by Linda Hadcocks (U of St George's) and Susan Wong (University of Cardiff). All samples were stained and analysed on the flow cytometer in the same experiment by Kelly Miners and Kristin Ladell (University of Cardiff). I performed the flow cytometry analysis in R.

5.4.1 Gating strategy

After applying compensation (see Compensation), I performed automated gating in R with the `OpenCyto` package (Finak et al., 2014). The gating hierarchy with all the gates applied is shown in (see section B.4). All gates are based on the `mindensity` function. For a given parameter, the `mindensity` function first estimates the two largest peaks in the fluorescence histogram and

then finds the cutpoint value between the two peaks where the minimum density is observed.

Mindensity gates were used to gate CD4, CD8 and NK cell populations. Within T cells, we used CD45RA and CD28 staining to define naive (Tn, CD45RA$^+$CD28$^+$) and the different memory populations, namely central memory T cells (Tcm, CD45RA$^-$CD28$^+$), T effector memory population (Tem, CD45RA$^-$CD28$^-$) and effector memory RA$^+$ T cells (Temra, CD45RA$^+$CD28$^-$). The gating strategy for the CD8$^+$ populations of a healthy donor (LD1) is shown in Figure 5.4. Events from the 20 different samples are collapsed before gating and thus a given gate, e.g. CD4vsCD8 gate, is replicated across all samples.

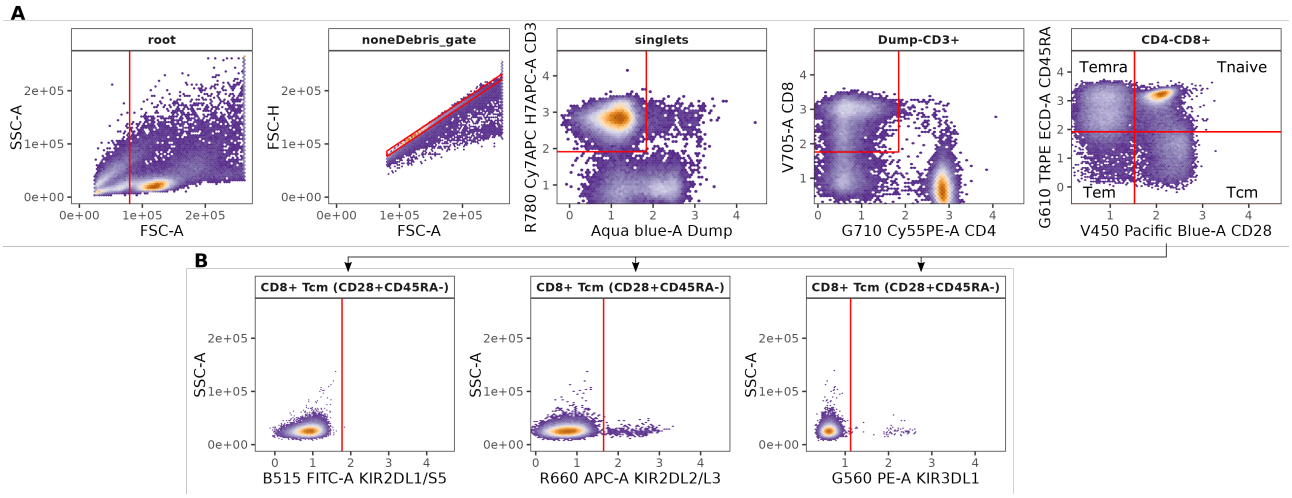


Figure 5.4: Gating strategy for enumeration of iKIR$^+$ CD8$^+$ T cell subsets. Analysis of PBMCs for a healthy donor (LD1). Each subplot shows events in the parent population, where strip names indicate parent population (root=all events). **A** From left to right, serial gating is used to identify (1) lymphocytes and discard debris (root, all events), (2) single cells in the nonDebris gate, (3) CD3$^+$ and Dump$^-$ cells in the singlet gate (excluding unwanted lineages like CD14, CD19 and also necrotic cells), (4) CD8$^+$ T cells in the Dump$^-$CD3$^+$ gate and (5) naive (Tn), central memory (Tcm), effector memory (Tem) and effector memory RA$^+$ (Temra) populations within the CD8$^+$ gate using CD45RA and CD28 staining. **B** Each of the 4 subsets defined in the CD8$^+$ gate (naive and memory subsets) was gated to determine events positive for KIR2DL1 (left), KIR2DL2/L3 (middle) and KIR3DL1 (left). In this case, only KIR gates within the Tcm population are shown but the same strategy is followed for Tem, Temra and Tn. All boundaries are determined with the 1D min density function (except for singlets) using collapsed data (see subsection 5.4.1).

An equivalent strategy was followed for CD4$^+$ T cells (see Figure B.2). For NK cells, CD56 staining was used to identify NK cells in the CD3$^-$Dump$^-$ gate. CD16 and CD56 markers were then used to gate CD56^{dim} and CD56^{bright} NK cell subsets Figure 5.5.

As a sanity check, I compared the CD4$^+$, CD8$^+$ and NK cell frequencies obtained with the automated gating strategy with frequencies obtained with manual gating in FlowJo software provided by Charandeep Kaur. There is a high degree of agreement between manual and automated CD4 and CD8 cell frequencies (Figure 5.6). However, automated gating overestimated the size of the CD56^{dim}CD16$^+$ population.

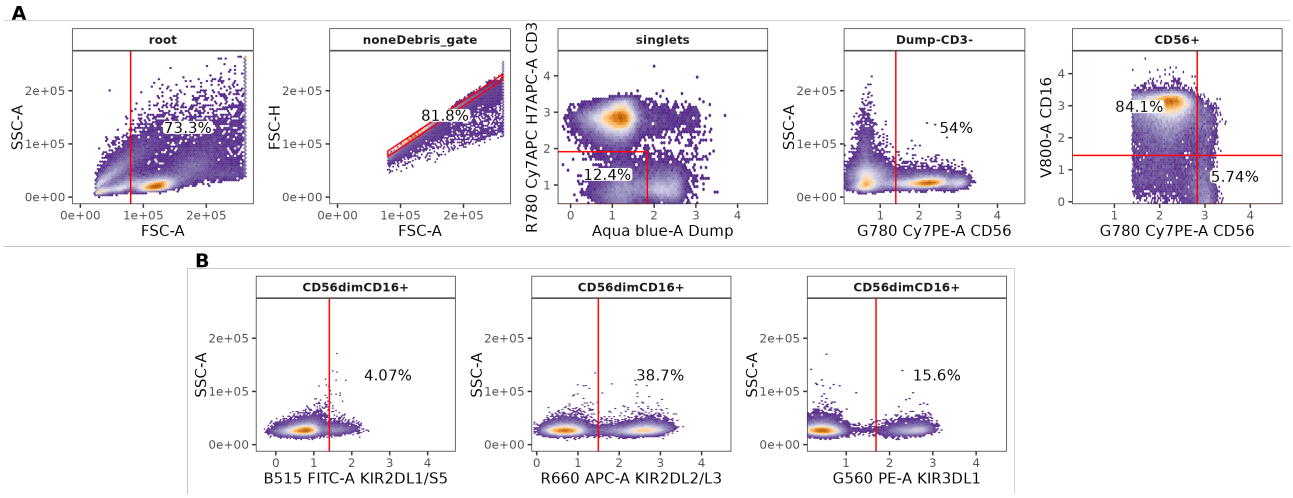


Figure 5.5: Gating strategy to enumerate iKIR$^+$ NK cells. Each subplot shows events in the parent population, where strip names indicate parent population (root=all events). **A** Similar to CD8$^+$ and CD4$^+$ gating strategy, events were gated for (1) lymphocytes, (2) single cells, (3) CD3$^-$ cells (CD3$^-$Dump$^-$), (4) NK cells using CD56 staining and finally (5) CD56^{dim}CD16$^+$ and CD56^{bright}CD16$^-$ populations using CD56 and CD16 staining. **B** Gating for iKIR$^+$ cells within the CD56^{dim}CD16$^+$ population (the same strategy is followed for CD56^{bright}CD16$^-$ population). All boundaries (red) were calculated using the min density function (except for singlets) and collapsing all the data.

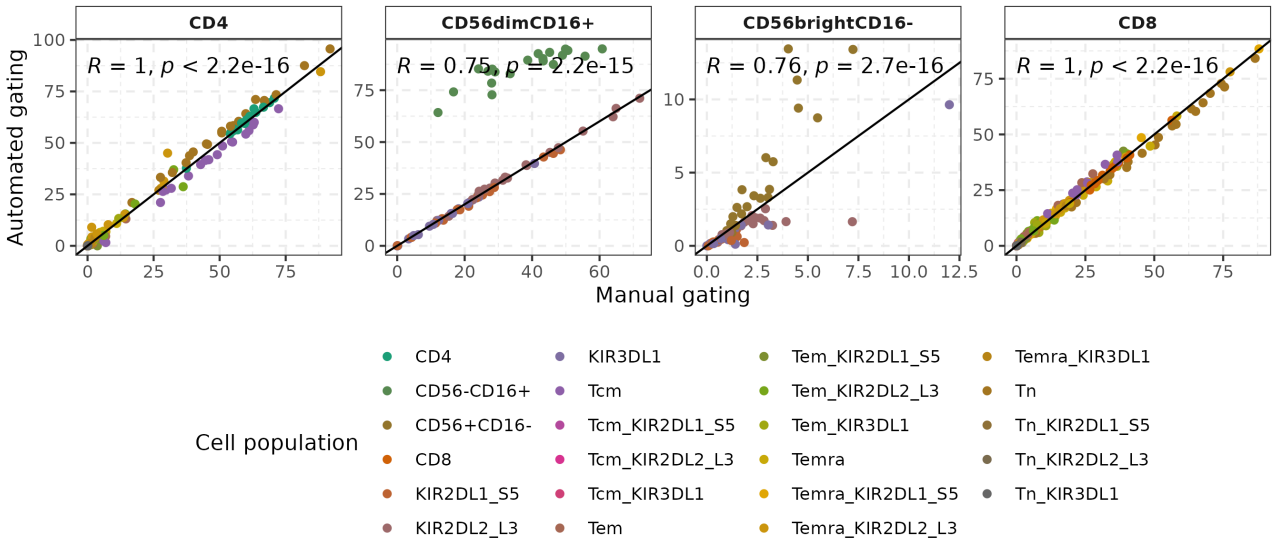


Figure 5.6: Correlation between manual and automated gating. Frequencies obtained with automated gating were compared to manual gating frequencies obtained in FlowJo for CD4$^+$, CD8$^+$, CD56^{dim}CD16$^+$ and CD56^{bright}CD16$^-$ cells and the different sub-populations downstream of these 4 parent subsets (see Figure B.1).

5.4.2 KIR expression in CD4$^+$ and CD8$^+$ T cell subsets

We compared the percentage of KIR expressing T cells between cases and controls (Figure 5.7). As expected, Temras had the highest KIR expression followed by the Tem cells. In addition, KIR2DL2/L3 showed the highest expression consistent with previous research (Zhang et al., 2023; Björkström et al., 2012). Indeed differentiation status was a significant predictor of iKIR expression, alongside the type of KIR measured (KIR2DL2/L3 showed the highest expression),

disease status (higher iKIR expression in controls) and T cell lineage (higher expression in CD8s) (Table B.4). Nevertheless, differences in KIR$^+$ cells between cases and controls were minimal and if anything controls had more iKIR expressing cells than cases (Table 5.2).

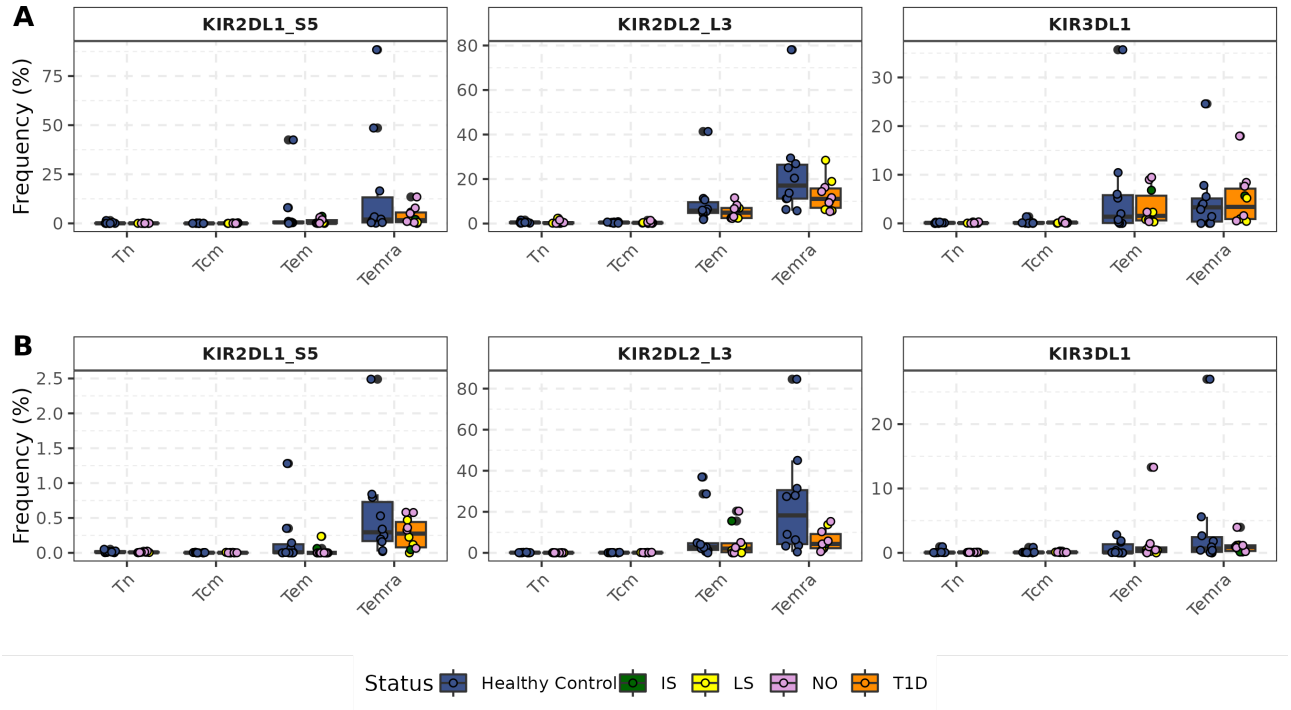


Figure 5.7: Percentage of T cells expressing different iKIR in T1D patients and healthy controls. **A** The percentage of cells in each CD8$^+$ T cell population (Tn, Tem, Tcm, Temra) expressing iKIRs quantified by flow cytometry. **B** The percentage of cells in each CD4$^+$ T cell population (Tn, Tem, Tcm, Temra) expressing iKIRs quantified by flow cytometry. **A** and **B** Dots represent frequencies for each individual in the cohort. T1D samples are color coded according to disease duration at time of collection (NO=new onset, IS=intermediate standing disease, LS=long standing disease). Boxes show medians and interquartile ranges within T1D individuals (N=10, orange, irrespective of disease duration) and healthy individuals (N=10, blue). Note that scales change between subplots.

		Healthy Control			T1D		
Lineage	**Subset**	**KIR2DL1**	**KIR2DL2L3**	**KIR3DL1**	**KIR2DL1**	**KIR2DL2L3**	**KIR3DL1**
CD4	Tn	0.01	0.09	0.04	0.01	0.04	0.07
	Tcm	0.00	0.07	0.07	0.00	0.08	0.09
	Tem	0.01	2.78	0.06	0.00	2.06	0.43
	Temra	0.29	18.23	0.67	0.28	4.33	0.89
CD8	Tn	0.04	0.50	0.11	0.04	0.20	0.07
	Tcm	0.01	0.47	0.09	0.02	0.34	0.18
	Tem	0.50	5.79	1.37	0.24	4.90	1.55
	Temra	1.96	17.07	3.30	1.63	11.08	3.39

Table 5.2: Median number of KIR$^+$ T cells between cases and controls. Median number of KIR2DL1$^+$, KIR2DL2L3$^+$ and KIR3DL1$^+$ cells between cases and controls (expressed as a percentage of the parent population). Medians are calculated within each memory subset: Tn=CD28$^+$CD45RA$^+$, Tcm=CD28$^+$CD45RA$^-$, Tem=CD28$^-$CD45RA$^-$, Temra=CD28$^-$CD45RA$^+$.

5.4.3 KIR expression in NK cell subsets

Finally, we also investigated KIR expression within the NK cell subsets (Figure 5.8). As expected the more mature $CD56^{dim}CD16^{+}$ population expressed a higher amount of KIRs (Table B.5) (Björkström et al., 2010). Again, no differences in the median frequency of KIR^{+} cells were observed between cases and controls (Table 5.4.3).

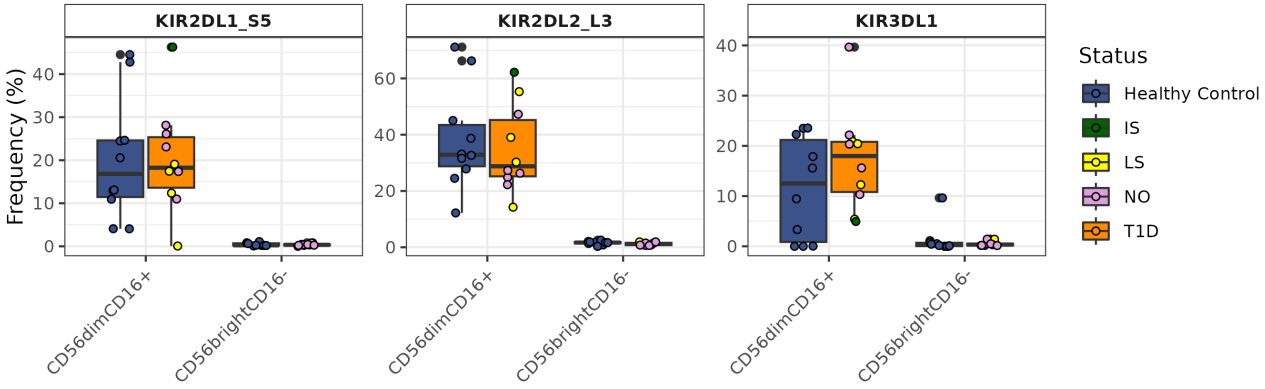


Figure 5.8: Percentage of NK cells expressing different iKIR in T1D patients and healthy controls. The percentage of cells in each NK cell population ($CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$) expressing iKIRs was quantified by flow cytometry. Dots represent frequencies for each individual in the cohort. T1D samples are color coded according to disease duration at time of collection (NO=new onset, IS=intermediate standing disease, LS=long standing disease). Boxes show medians and interquartile ranges within T1D individuals (N=10, orange, irrespective of disease duration) and healthy individuals (N=10, blue).

	Healthy Control			T1D		
Subset	**KIR2DL1**	**KIR2DL2L3**	**KIR3DL1**	**KIR2DL1**	**KIR2DL2L3**	**KIR3DL1**
$CD56^{bright}CD16^{-}$	0.24	1.67	0.29	0.34	1.16	0.34
$CD56^{dim}CD16^{+}$	16.82	32.88	12.52	18.26	28.78	17.98

Table 5.3: Median number of KIR^{+} NK cells between cases and controls. Median number of KIR2DL1+, KIR2DL2L3+ and KIR3DL1+ cells between cases and controls (expressed as a percentage of the parent population) were calculated within the $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$ subsets

5.5 Discussion

iKIRs on T cells have been shown to prolong $CD8^{+}$ and $CD4^{+}$ T cell lifetime both *in vitro* and in murine models (Ugolini and Vivier, 2000; Gati et al., 2003; Wong-Baeza et al., 2013; Ugolini et al., 2001; Boelen et al., 2018). In addition, there is evidence that KIR^{+} T cells are expanded in autoimmunity. For example, in SLE, both $KIR^{+}CD4^{+}$ and $KIR^{+}CD8^{+}$ T cell frequencies are increased (Basu et al., 2009). Furthermore, in ankylosing spondylitis, cumulative evidence indicates that ligation of KIR3DL2 on Th17 $CD4^{+}$ T cells may promote their accumulation and

survival (Bowness et al., 2011; Chan et al., 2005; Cauli et al., 2013). The aim of the present study was to test whether a similar KIR^+ T cell accumulation takes place in T1D. We found that at the RNA level, only a handful of $CD4^+$ T cells expressed KIR transcripts and none were labelled as T_{regs}. At the protein level, we did not find significant differences in iKIR expression on $CD4^+$ T cells between T1D patients and matched healthy controls. When looking at $CD8^+$ and NK subsets, where as expected KIR expression was more common, we again did not find significant differences in iKIR expression between cases and controls.

These results are in striking contrast with a recent study showing increased KIR^+CD8^+ T cell frequency in blood from patients with MS, CeD and SLE when compared to controls. Therefore, a potential caveat of our study is that we measured protein KIR expression mainly in T1D with long standing disease and the autoreactive response might have already been resolved in those individuals. Nevertheless, 3 cases were recently diagnosed – blood was collected within 1 year of diagnosis – and in the scRNAseq dataset blood samples were taken before T1D onset. Still KIR^+ T cell frequencies from these individuals were not increased when compared to healthy controls. Another difference between our study and Li et al. (2022) is that we measured KIR expression within the naive and the memory compartments rather than just in bulk T cells as Li et al. (2022). Although, we did not detect increased frequencies of T_{emras} in T1D – if anything T_{emras} were decreased in disease – it would be interesting to see whether the CeD patients have higher T_{emra} frequencies that could explain this discrepancy.

In Chapter 3, we found that functional iKIR genes were associated with T1D risk in a subset of individuals carrying protective HLA-II genotypes. We hypothesized that if the mechanism underlying this association is driven by the direct pathway – i.e. through prolonged survival of self-reactive T cells upon iKIR ligation on T cells – we would see increased frequencies of KIR^+ T cells in T1D. We did not observe differences in KIR^+ T cell frequencies between cases and controls (Figure 5.7), suggesting that the direct mechanism is unlikely to underlie our immunogenetic observations. A caveat of this study is that we did not perform KIR/HLA genotyping on the samples included in the Cardiff cohort. Similarly, coverage in scRNAseq data was too shallow to infer KIR genotypes. Consequently, in our analysis, KIR^+ cell frequencies include both functional iKIR and non-functional iKIR positive cells. We cannot rule out the possibility that functional $iKIR^+$ cells are indeed enriched in disease and this is masked by our enumeration of both functional and non-functional KIR^+ T cells. Furthermore, differences in KIR expression might only be observable at the inflammation site (Li et al., 2022). Nevertheless, Zhang et al. (2023) recently found that functional iKIR expression on $CD8^+$ T cells is not a

predictor of T cell lifespan, which is consistent with our interpretation that the direct mechanism is unlikely to underlie our immunogenetic observations. Instead, the alternative hypothesis, where iKIRs on another population"indirectly" modulate $CD4^+$ T cells restricted by protective HLA-II molecules, seems more plausible.

Probably the most likely contender for such a population is NK cells as they express high levels of iKIR: a median of 22% $CD56^{dim}CD16^+$ NK cells express iKIRs in the Cardiff cohort. NK cells are also known to interact with APCs and T cells both of which could lead to a modulation of class II restricted $CD4^+$ cell responses. NK cells kill autologous, activated but not resting $CD4^+$ and $CD8^+$ T cells (Peppa et al., 2013; Waggoner et al., 2011). *in vitro* experiments show that activated $CD4^+$ T cells upregulate HLA-E, the ligand for the inhibitory receptor CD94/NKG2A to protect themselves from NK cell killing (Takao et al., 2010; Nielsen et al., 2012). The same inhibitory receptor plays a role in the experimental autoimmune encephalomyelitis (EAE) mouse model for multiple sclerosis (Lu et al., 2007). In this model, prevention of engagement of CD94/NKG2A, either by antibody-blockade or by knockin of a mutated ligand, resulted in elimination of autoreactive T cells and improvement of EAE. Furthermore, in studies of NOD mice immunized with Complete Freund's adjuvant, NK cells decreased the numbers of autoreactive $CD8^+$ T cells preventing T1D onset (Lee et al., 2004). Similarly, NK cells can suppress $CD8^+$ T cell mediated hyperglycemia in a mouse model characterized by the transgenic expression of lymphocytic choriomeningitis virus on β-cells (Lang et al., 2020).

In summary, our results indicate that KIR expression is low in T cells, with median frequencies of 6% and 0.8% of KIR^+CD8^+ Temras and KIR^+CD4^+ T_{emras} respectively. Further, this frequency is not altered in T1D, neither before nor after disease onset. Although we cannot rule out a relevant role of the KIR^+ T cell population in T1D pathogenesis, the results are more consistent with an indirect mechanism whereby KIRs expressed on other cells modulate the T cell response against β-cells in the pancreas.

Chapter 6

Final conclusions

In this thesis, I investigated the role of iKIRs in T cell-mediated autoimmunity. Taking T1D as a case study, in Chapter 3 I tested the hypothesis that iKIRs are detrimental in the context of autoimmunity. Although no effect was detected on well-known detrimental HLA-II genotypes, the iKIR score was significantly anticorrelated with HLA-II-mediated protection in T1D.These results are in stark contrast with previous studies where iKIR score correlated with both detrimental and protective HLA-I associations in chronic viral infections (Boelen et al., 2018). During viral infection, protective and detrimental HLA-I genotypes are thought to be the result of the activation or inactivation of a single pathway (Borghans et al., 2007). For autoimmune responses, the molecular mechanism of protective and detrimental genotypes may be fundamentally different, explaining this differential effect of the iKIR score.

In Chapter 4, I provided a mathematical model providing an alternative and more parsimonious explanation of the apparently contradictory iKIR effect observed in viral and autoimmune responses. If functional iKIR genes increase both $CD8^+$ and $CD4^+$ T cell survival, then the iKIR score effect on these two populations is cancelled out; β-cell specific $CD8^+$ T cells might survive for longer, but so do $CD4^+$ T cells with suppressive function, i.e. T_{regs}. A detrimental effect only becomes apparent when $CD4^+$ T cells reach saturation, a scenario that takes place in carriers of protective HLA genotypes.

Finally, I investigated the cellular mechanism underlying the iKIR score effect in Chapter 5. The low expression of KIRs in KIR^+CD4^+ cells suggested that an indirect mechanism may explain

the immunogenetic effect of the iKIR score, with another immune population affecting HLA restricted T-cell responses. Although KIR expression studies in other autoimmune diseases detected higher numbers of KIR^+ T cells (Bowness et al., 2011; Basu et al., 2009; Li et al., 2022), the frequency of KIR^+ T cells does not seem to be altered during the natural history of T1D. Neither seropositive children for islet autoantibodies nor individuals with recent onset T1D have increased levels of KIR^+ T cell in blood.

Overall, the way I accomplished the objectives of this thesis is the following:

1. In Chapter 3, I have performed an extensive immunogenetic analysis of two diabetes cohorts and identified a modulatory effect of functional iKIR genes on HLA-II protective genotypes:

    - A weighted sum of the number of functional iKIR genes, iKIR score, modifies protective *DRB1-DQB1* haplotypes. Detrimental haplotypes were not affected by the iKIR score.
    - For the well-known dominant protective *DQ6* genotype, iKIR ligands, which are independently associated with T1D risk, detrimental genotypes in LD with *DQ6* or functional activating KIR genes could not explain the iKIR score effect.
    - All iKIR genes contributed to the iKIR score effect.
    - iKIR ligands associated with T1D confounded the iKIR score effect on HLA-II genotypes.
    - Upon discarding carriers of HLA-I drivers, the conclusions were strengthened: 8 out of 9 protective HLA-II drivers were significantly modified by the iKIR score. Again, this effect was absent in detrimental genotypes.
    - The iKIR score effect on HLA-II-mediated protection was replicated in an independent cohort.
    - I proposed that the detrimental effect of iKIR score can be explained by iKIRs, in the presence of their ligands, increasing T cell survival.

2. In Chapter 4, I have devised a mechanistic model that predicts a detrimental effect of functional iKIR genes on protective HLA genotypes if iKIR ligation increases both $CD4^+$ and $CD8^+$ T cell survival:

- A 25% increase in the lifespan of $CD4^+$ and $CD8^+$ T cells can qualitatively replicate immunogenetic findings in a simple mechanistic model describing the T-cell destruction of pancreatic β-cells.
- This prediction is contingent on density-dependent proliferation. In the absence of density-dependent proliferation, the prolonged lifespan of effectors and regulatory cells has a zero net effect on β-cell destruction.
- The model's assumption that carriers of protective genotypes have more T_{regs} is consistent with the HLA-II risk correlation with the number of islet-specific T_{regs} in healthy individuals.

3. In Chapter 5, I have quantified the expression of iKIRs in different immune cell subsets to get at the cellular mechanism underlying the iKIR score effect:
   - A minority of $CD8^+$ T cells express iKIR transcripts and only a handful of KIR expressing $CD4^+$ T cells was detected. Similarly, KIR protein expression is detected in a small $CD8^+$ and $CD4^+$ subset.
   - These results were validated by flow cytometry.
   - Similar KIR^+ T cell frequencies between T1D cases and matched controls suggest that the iKIR score effect involves an indirect mechanism, probably through NK cell-mediated killing of activated T cells.

6.1 Limitations

One limitation of the immunogenetic analysis presented in Chapter 3 is that the definition of "functional iKIR genes" is relatively simple. Although differences in iKIR binding by broad allele allotypes, e.g. KIR2DL2 vs KIR3DL2 (Moesta et al., 2008), are incorporated in the iKIR score, the different binding strengths associated with variants in the KIR3DL1 allotype group at the *KIR3DL1/S1* (Gardiner et al., 2001; Saunders et al., 2016) are not incorporated as KIR genes were not typed at allelic resolution level. In addition to binding strength, KIR polymorphism affects surface expression (Pando et al., 2003; VandenBussche et al., 2006) and there is evidence that some KIR-HLA interactions might be peptide-specific (Sim et al., 2023). However, while accounting for null alleles in the functional iKIR gene count calculation is relatively simple, it is not clear how to take into account – if available – binding avidity, peptide specificity and expression levels.

Albeit simplistic, functional iKIR gene definitions have proved very powerful in other studies (Martin et al., 2007; Ahlenstiel et al., 2008; Nakimuli et al., 2015). As mentioned earlier, we refined the definitions of these previous studies using a weighted count of functional iKIR genes, i.e. the iKIR score, in an attempt to incorporate some of the aforementioned factors in binding strength between KIR-HLA pairs. With this slightly more sophisticated definition, I saw clear and reproducible results in the T1D cohorts presented in this thesis but also in previous studies focusing on HIV-1, HCV and HTLV-1 cohorts (Boelen et al., 2018; Seich al Basatena et al., 2011). Although the iKIR score only performed slightly better than the functional iKIR count in the UK-GRID cohort, the number of iKIR genes – regardless of the ligand gene status – performed significantly worse, suggesting that the inhibitory score and inhibitory count are meaningful metrics.

Another limitation is that our mathematical model represents a greatly over-simplified view of a highly complex and still poorly understood system as the human immune system. Equally if not more intriguing is the T cell-mediated destruction of β-cells in the pancreas that takes place in around 0.5% of individuals[1]. Therefore, some of the core assumptions, like the impact of HLA-II genotype on T_{reg} frequencies, need further validation; while we observed a correlation between HLA risk and islet-specific T_{reg} levels, bulk T_{reg} frequencies in blood did not seem to correlate with HLA risk score in a much bigger cohort. An alternative explanation could be that while T_{reg} numbers might not vary significantly between HLA risk groups, their anti-inflammatory capacity might be higher when restricted by protective HLA molecules, which would be in line with the epitope stealing hypothesis (Erlich et al., 2008).

Another core assumption of the model is that KIR-HLA interactions prolong $CD8^+$ and $CD4^+$ T cell lifespan. While we recently reported that the number of functional iKIR genes correlates with $CD8^+$ T lifespan in humans *in vivo*, whether functional iKIR genes also prolong $CD4^+$ T cell survival was not assessed. Nevertheless, functional iKIR genes also correlated with a marker of cell age, CD57 (Ahmed et al., 2020), within both $CD8^+$ and $CD4^+$ subsets; this was taken as indirect evidence that iKIR genes also affect $CD4^+$ T cell lifespan *in vivo*.

Finally, I quantified KIR protein expression by flow cytometry with blood samples of individuals with clinical T1D where the immune response might have been fully resolved, potentially missing any relevant differences in iKIR expression between cases and controls. Half of the samples were collected within one year of clinical diagnosis, while the other half belonged to

[1] According to Juvenile Diabetes Research Foundation 400k individuals live with T1D in the United Kingdom.

individuals with the long-standing disease. This late sampling could explain the discrepancy between T1D findings and those in other autoimmune diseases with a systemic manifestation like SLE, or those targeting larger cell populations like MS and CeD. In these diseases, also characterised by remission-relapse cycles, $KIR^{+}CD8^{+}$ T are significantly expanded. Supporting this view, the frequency of KIR^{+} T cells is higher during active disease than during remission in CeD (Li et al., 2022).

6.2 Next steps

To address my work limitations, one first step is to incorporate allelic variation information where possible. For example, it is possible to take into account null KIR alleles to recalculate individuals' iKIR scores. KIR genotyping software such as PONG (Harrison et al., 2022) could be used to impute *KIR3DL1* alleles using the Immunochip data from the UK-GRID cohort. While other KIR genotyping tools can type variants at other KIR loci, they work with short read sequencing data and so I cannot use them to genotype the UK-GRID cohort (Marin et al., 2021; Roe et al., 2020b; Song et al., 2023). In fact, I attempted to genotype the Kallionpaa cohort using the scRNAseq data with T1K software (Song et al., 2023). I abandoned this approach as the genotype calls weren't reliable due to the low amount of reads mapping to the KIR region [data not shown].

Another route to explore is the effect of cohort age in my studies. For example, HLA risk alleles strongly influence variability at the CDR3 region of the TCR repertoire (Ishigaki et al., 2022), and in turn, TCR sequence features affect T_{reg} fate (Lagattuta et al., 2022). But, somewhat unexpectedly, our HLA risk score had no effect on immunophenotype in the TwinsUK. In this cohort, the age of individuals ranged between 41–77, so any differences driven by genetics might have disappeared due to the individuals' history of environmental pressures faced by the immune system. In line with this, unrelated older individuals display more homogenous immunophenotypes compared to younger individuals (Kaczorowski et al., 2017). Therefore it is tempting to speculate that in a younger cohort, stronger associations could be identified (Roederer et al., 2015). The findings in TwinsUK could be validated with the Milieu Intérieur cohort (The Milieu Intérieur Consortium et al., 2018), a similar cohort comprising more individuals (N=1,000) and a wider age range, from 20 to 69 years old individuals.

To further complete my research, my proposed model results can be compared to *in vivo*

measured $CD4^+$ T cell lifespan, as measured in $CD8^+$ T cells by Zhang et al. (2023). It would be interesting to check whether the number of functional iKIR genes correlates with T_{reg} lifespan in a cohort like the one resultant from an isotope labelling study (N=20) in T1D (Bollyky et al., 2013). Regarding the direct and indirect pathways, datasets that contain both immunophenotype and genotype data, like the TwinsUK and the Milieu Intérieur cohorts could be mined to pinpoint which immune traits are predicted by an individual's number of functional iKIR genes. As depicted in Figure 5.1, several mechanisms might be responsible for the iKIR-mediated effect on T cell survival. These cohorts not only harbour the potential to establish genotype-phenotype relationships – where the direction of causality is unequivocal – but also to inform functional studies *in vitro* by narrowing down the list of possible iKIR-modulated cell interactions responsible for the effect on T cell longevity.

Some of the other research questions that may lead to future work are:

- **At which disease stage do iKIRs influence HLA-II-restricted responses**? Although we did not have access to individuals' seroconversion age, we had information on age at diagnosis. In the UK-GRID cohort, iKIR had no impact on HLA associations with age at diagnosis [data not shown]. While some HLA-II genotypes are associated with age at diagnosis (Noble et al., 2010), HLA-II genotypes are first and foremost associated with seroconversion (Ilonen et al., 2016). Prospective cohorts where individuals are followed from birth and where information on seroconversion, progression and onset is available would help to answer this question.

- **Why are HLA-I associations not modulated by iKIR score**? HLA-I driver genotypes were not modified by the iKIR score in a consistent manner [Data not shown]. The complexity of HLA-I associations interpretations and the strong LD between HLA-II and HLA-I loci might have masked the iKIR effect. In contrast with HLA-II molecules, HLA-I molecules are ligands for multiple receptors including the TCR, iKIRs, aKIRs, the CD94:NKG2 family and the leukocyte immunoglobulin-like receptors (LILR), which in turn are expressed in different immune subsets, namely T cells, NK cells and DCs. In the chronic viral infection studies, only HLA-I associations attributed to T cells were studied. In T1D, the underlying mechanism of HLA-I associations is unknown. Interestingly, 5 driver alleles (*A*32:01*, *A*24:02*, *B*57:01*,*C*04:01* and *C*08:01*) carry epitopes recognized by iKIRs. Although 4 of them are significantly associated with protection, *A*24:02* is detrimental in the UK-GRID cohort and has been associated with earlier age

at diagnosis (Nejentsev et al., 2007). Immunogenetic approaches such as *fstool* (Debebe et al., 2020) could be applied in the UK-GRID cohort to identify the receptor-ligand interactions responsible for these associations. I predict that those protective associations mainly explained by TCR interactions would be affected by the iKIR score.

- **Does iKIR score affect HLA associations in other autoimmune diseases**? Other autoimmune diseases like rheumatoid arthritis are also strongly influenced by HLA genetics; the strongest associations are also found within the HLA-II loci. Therefore, it would be reassuring to observe iKIR score modulation in other autoimmune contexts.

- **Does iKIR score modulate antitumour T cell responses?** In contrast with infection and autoimmunity, effect sizes of HLA associations with risk of cancer are typically small (Li et al., 2021). This does not mean that the HLA-restricted T cell responses are trivial, but that other factors explain a higher proportion of the variation in risk across individuals. Therefore, a major drawback for immunogenetic studies in the context of malignant lesions is the requirement of large datasets for a well-powered analysis of the iKIR score interaction effect. A good starting point could be cohorts of patients undergoing checkpoint blockade; in this context, where monoclonal antibodies boost T cell activity, the quality of the HLA restriction might become more important and the power to detect the iKIR modulatory effect might be higher. Although published ICB cohorts are typically small, a recent study collated genomic and clinical information from 1,000 individuals from different ICB studies (Litchfield et al., 2021); such a cohort holds promise to answer our enquiries.

To summarize, we have identified a family of genes, the iKIR genes, that significantly impact T1D risk. Recent evidence shows that iKIRs have a large impact on $CD8^+$ T cell lifespan in humans *in vivo*. The work presented here demonstrates that this effect most likely has consequences for human health during self-reactive responses; functional iKIRs have a biologically significant impact on HLA-II-associated protection, most likely via an impact on protective HLA-II-restricted T cell responses, which manifest as a clinically significant difference in the risk of developing T1D. Given that functional iKIR genes are also relevant in other clinical settings, the iKIR-HLA receptor-ligand system appears to be an important regulator of adaptive T-cell immunity. The study of the implications of this checkpoint system not only sheds

light on the intricate interactions between innate and $CD8^+$ and $CD4^+$ adaptive responses but also has the potential to uncover new therapeutic avenues. Our work suggests that Lirilumab – a monoclonal antibody targetting KIR2DL1 and KIR2DL2/L3 receptors – could be used to modulate T cell immunity. To achieve a solid translational impact for my work, functional studies need to characterise the mechanistic link between the iKIR impact on T cell lifespan and that on disease outcome.

Bibliography

Abi-Rached, L., Moesta, A. K., Rajalingam, R., Guethlein, L. A., and Parham, P. 2010. Human-Specific Evolution and Adaptation Led to Major Qualitative Differences in the Variable Receptors of Human and Chimpanzee Natural Killer Cells. *PLoS Genet.* 6, 11 (2010), e1001192. https://doi.org/10.1371/journal.pgen.1001192

Ahlenstiel, G., Martin, M. P., Gao, X., Carrington, M., and Rehermann, B. 2008. Distinct KIR/HLA compound genotypes affect the kinetics of human antiviral natural killer cell responses. *J. Clin. Invest.* (2008), JCI32400. https://doi.org/10.1172/JCI32400

Ahmed, R., Miners, K. L., Lahoz-Beneytez, J., Jones, R. E., Roger, L., Baboonian, C., Zhang, Y., Wang, E. C., Hellerstein, M. K., McCune, J. M., Baird, D. M., Price, D. A., Macallan, D. C., Asquith, B., and Ladell, K. 2020. CD57+ Memory T Cells Proliferate In Vivo. *Cell Rep.* 33, 11 (2020), 108501. https://doi.org/10.1016/j.celrep.2020.108501

Ahn, R., Vukcevic, D., Motyer, A., Nititham, J., Squire, D. M., Hollenbach, J. A., Norman, P. J., Ellinghaus, E., Nair, R. P., Tsoi, L. C., Oksenberg, J., Foerster, J., Lieb, W., Weidinger, S., Franke, A., Elder, J. T., Jorgenson, E., Leslie, S., and Liao, W. 2021. Large-Scale Imputation of KIR Copy Number and HLA Alleles in North American and European Psoriasis Case-Control Cohorts Reveals Association of Inhibitory KIR2DL2 With Psoriasis. *Front. Immunol.* 12 (2021), 684326. https://doi.org/10.3389/fimmu.2021.684326

Albi, N., Ruggeri, L., Aversa, F., Merigiola, C., Tosti, A., Tognellini, R., Grossi, C., Martelli, M., and Velardi, A. 1996. Natural killer (NK)-cell function and antileukemic activity of a large population of CD3+/CD8+ T cells expressing NK receptors for major histocompatibility complex class I after "three-loci" HLA-incompatible bone marrow transplantation. *Blood* 87, 9 (1996), 3993–4000. https://doi.org/10.1182/blood.V87.9.3993.bloodjournal8793993

Ali, A., Canaday, L. M., Feldman, H. A., Cevik, H., Moran, M. T., Rajaram, S., Lakes,

N., Tuazon, J. A., Seelamneni, H., Krishnamurthy, D., Blass, E., Barouch, D. H., and Waggoner, S. N. 2021. Natural killer cell immunosuppressive function requires CXCR3-dependent redistribution within lymphoid tissues. *J. Clin. Invest.* 131, 18 (2021), e146686. https://doi.org/10.1172/JCI146686

Alter, G., Rihn, S., Streeck, H., Teigen, N., Piechocka-Trocha, A., Moss, K., Cohen, K., Meier, A., Pereyra, F., Walker, B., and Altfeld, M. 2008. Ligand-Independent Exhaustion of Killer Immunoglobulin-Like Receptor-Positive CD8 $^{+}$ T Cells in Human Immunodeficiency Virus Type 1 Infection. *J. Virol.* 82, 19 (2008), 9668–9677. https://doi.org/10.1128/JVI.00341-08

Alter, G., Rihn, S., Walter, K., Nolting, A., Martin, M., Rosenberg, E. S., Miller, J. S., Carrington, M., and Altfeld, M. 2009. HLA Class I Subtype-Dependent Expansion of KIR3DS1 $^{+}$ and KIR3DL1 $^{+}$ NK Cells during Acute Human Immunodeficiency Virus Type 1 Infection. *J. Virol.* 83, 13 (2009), 6798–6805. https://doi.org/10.1128/JVI.00256-09

Anderson, A. M., Landry, L. G., Alkanani, A. A., Pyle, L., Powers, A. C., Atkinson, M. A., Mathews, C. E., Roep, B. O., Michels, A. W., and Nakayama, M. 2021. Human islet T cells are highly reactive to preproinsulin in type 1 diabetes. *Proc. Natl. Acad. Sci.* 118, 41 (2021). https://doi.org/10.1073/pnas.2107208118

Anderson, S. K., Ortaldo, J. R., and McVicar, D. W. 2001. The ever-expanding Ly49 gene family: repertoire and signaling. *Immunological reviews* 181, 1 (2001), 79–89.

Andersson, S., Fauriat, C., Malmberg, J.-A., Ljunggren, H.-G., and Malmberg, K.-J. 2009. KIR acquisition probabilities are independent of self-HLA class I ligands and increase with cellular KIR expression. *Blood* 114, 1 (2009), 95–104. https://doi.org/10.1182/blood-2008-10-184549

Andrews, D. M., Estcourt, M. J., Andoniou, C. E., Wikstrom, M. E., Khong, A., Voigt, V., Fleming, P., Tabarias, H., Hill, G. R., van der Most, R. G., Scalzo, A. A., Smyth, M. J., and Degli-Esposti, M. A. 2010. Innate immunity defines the capacity of antiviral T cells to limit persistent infection. *J. Exp. Med.* 207, 6 (2010), 1333–1343. https://doi.org/10.1084/jem.20091193

Anfossi, N., Doisne, J.-M., Peyrat, M.-A., Ugolini, S., Bonnaud, O., Bossy, D., Pitard, V., Merville, P., Moreau, J.-F., Delfraissy, J.-F., Dechanet-Merville, J., Bonneville, M., Venet, A., and Vivier, E. 2004. Coordinated Expression of Ig-Like Inhibitory MHC Class I Receptors

and Acquisition of Cytotoxic Function in Human CD8 $^{+}$ T Cells. *The Journal of Immunology* 173, 12 (2004), 7223–7229. https://doi.org/10.4049/jimmunol.173.12.7223

Anfossi, N., Pascal, V., Vivier, E., and Ugolini, S. 2001. Biology of T memory type 1 cells: Biology of Tm1 cells. *Immunol Rev* 181, 1 (2001), 269–278. https://doi.org/10.1034/j.1600-065X.2001.1810123.x

Aran, D., Looney, A. P., Liu, L., Wu, E., Fong, V., Hsu, A., Chak, S., Naikawadi, R. P., Wolters, P. J., Abate, A. R., Butte, A. J., and Bhattacharya, M. 2019. Reference-based analysis of lung single-cell sequencing reveals a transitional profibrotic macrophage. *Nat. Immunol.* 20, 2 (2019), 163–172. https://doi.org/10.1038/s41590-018-0276-y

Arlettaz, L., Degermann, S., Rham, C., Roosnek, E., and Huard, B. 2004. Expression of inhibitory KIR is confined to CD8+ effector T?cells and limits their proliferative capacity. *Eur. J. Immunol.* 34, 12 (2004), 3413–3422. https://doi.org/10.1002/eji.200324756

Ashkar, A. A., Di Santo, J. P., and Croy, B. A. 2000. Interferon γ Contributes to Initiation of Uterine Vascular Modification, Decidual Integrity, and Uterine Natural Killer Cell Maturation during Normal Murine Pregnancy. *J. Exp. Med.* 192, 2 (2000), 259–270. https://doi.org/10.1084/jem.192.2.259

Babon, J. A. B., DeNicola, M. E., Blodgett, D. M., Crèvecoeur, I., Buttrick, T. S., Maehr, R., Bottino, R., Naji, A., Kaddis, J., Elyaman, W., James, E. A., Haliyur, R., Brissova, M., Overbergh, L., Mathieu, C., Delong, T., Haskins, K., Pugliese, A., Campbell-Thompson, M., Mathews, C., Atkinson, M. A., Powers, A. C., Harlan, D. M., and Kent, S. C. 2016. Analysis of self-antigen specificity of islet-infiltrating T cells from human donors with type 1 diabetes. *Nat Med* 22, 12 (2016), 1482–1487. https://doi.org/10.1038/nm.4203

Badami, E., Cexus, O. N. F., and Quaratino, S. 2019. Activation-induced cell death of self-reactive regulatory T cells drives autoimmunity. *Proc. Natl. Acad. Sci.* 116, 52 (2019), 26788–26797. https://doi.org/10.1073/pnas.1910281116

Bagwell, C. B. and Adams, E. G. 1993. Fluorescence Spectral Overlap Compensation for Any Number of Flow Cytometry Parameters. *Ann. N.Y. Acad. Sci.* 677, 1 Clinical Flow (1993), 167–184. https://doi.org/10.1111/j.1749-6632.1993.tb38775.x

Bakker, A. B., Phillips, J. H., Figdor, C. G., and Lanier, L. L. 1998. Killer cell inhibitory receptors for MHC class I molecules regulate lysis of melanoma cells mediated by NK cells,

gamma delta T cells, and antigen-specific CTL. *Journal of Immunology (Baltimore, Md.: 1950)* 160, 11 (1998), 5239–5245.

Balding, D. J., Bishop, M., and Cannings, C. (Eds.). 2007. *Handbook of Statistical Genetics* (1 ed.). Wiley. https://doi.org/10.1002/9780470061619

Barber, L. D., Percival, L., Valiante, N. M., Chen, L., Lee, C., Gumperz, J. E., Phillips, J. H., Lanier, L. L., Bigge, J. C., Parekh, R. B., et al. 1996. The inter-locus recombinant HLA-B* 4601 has high selectivity in peptide binding and functions characteristic of HLA-C. *The Journal of experimental medicine* 184, 2 (1996), 735–740.

Barbour, J. D., Sriram, U., Caillier, S. J., Levy, J. A., Hecht, F. M., and Oksenberg, J. R. 2007. Synergy or Independence? Deciphering the Interaction of HLA Class I and NK Cell KIR Alleles in Early HIV-1 Disease Progression. *PLoS Pathog.* 3, 4 (2007), e43. https://doi.org/10.1371/journal.ppat.0030043

Barrett, J. C., Clayton, D. G., Concannon, P., Akolkar, B., Cooper, J. D., Erlich, H. A., Julier, C., Morahan, G., Nerup, J., Nierras, C., Plagnol, V., Pociot, F., Schuilenburg, H., Smyth, D. J., Stevens, H., Todd, J. A., Walker, N. M., and Rich, S. S. 2009. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat Genet* 41, 6 (2009), 703–7. https://doi.org/10.1038/ng.381 Type: Journal article.

Barten, R., Torkar, M., Haude, A., Trowsdale, J., and Wilson, M. J. 2001. Divergent and convergent evolution of NK-cell receptors. *Trends Immunol.* 22, 1 (2001), 52–57. https://doi.org/10.1016/S1471-4906(00)01802-0

Basu, D., Liu, Y., Wu, A., Yarlagadda, S., Gorelik, G. J., Kaplan, M. J., Hewagama, A., Hinderer, R. C., Strickland, F. M., and Richardson, B. C. 2009. Stimulatory and Inhibitory Killer Ig-Like Receptor Molecules Are Expressed and Functional on Lupus T Cells. *The Journal of Immunology* 183, 5 (2009), 3481. https://doi.org/10.4049/jimmunol.0900034 Type: Journal article.

Battistini, L., Borsellino, G., Sawicki, G., Poccia, F., Salvetti, M., Ristori, G., and Brosnan, C. F. 1997. Phenotypic and cytokine analysis of human peripheral blood gamma delta T cells expressing NK cell receptors. *Journal of Immunology (Baltimore, Md.: 1950)* 159, 8 (1997), 3723–3730.

Beliën, J., Goris, A., and Matthys, P. 2022. Natural Killer Cells in Multiple Sclerosis: Entering the Stage. *Front. Immunol.* 13 (2022), 869447. https://doi.org/10.3389/fimmu.2022.869447

Bell, G. I., Horita, S., and Karam, J. H. 1984. A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. *Diabetes* 33, 2 (1984), 176–183. https://doi.org/10.2337/diab.33.2.176

Bender, C., Rodriguez-Calvo, T., Amirian, N., Coppieters, K. T., and von Herrath, M. G. 2020. The healthy exocrine pancreas contains preproinsulin-specific CD8 T cells that attack islets in type 1 diabetes. *Sci. Adv.* 6, 42 (2020), eabc5586. https://doi.org/10.1126/sciadv.abc5586

Benjamini, Y. and Hochberg, Y. 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological)* 57, 1 (1995), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bhatt, R. S., Berjis, A., Konge, J. C., Mahoney, K. M., Klee, A. N., Freeman, S. S., Chen, C.-H., Jegede, O. A., Catalano, P. J., Pignon, J.-C., Sticco-Ivins, M., Zhu, B., Hua, P., Soden, J., Zhu, J., McDermott, D. F., Arulanandam, A. R., Signoretti, S., and Freeman, G. J. 2021. KIR3DL3 Is an Inhibitory Receptor for HHLA2 that Mediates an Alternative Immunoinhibitory Pathway to PD1. *Cancer Immunology Research* 9, 2 (2021), 156–169. https://doi.org/10.1158/2326-6066.CIR-20-0315

Biassoni, R., Pessino, A., Malaspina, A., Cantoni, C., Bottino, C., Sivori, S., Moretta, L., and Moretta, A. 1997. Role of amino acid position 70 in the binding affinity of p50.1 and p58.1 receptors for HLA-Cw4 molecules. *Eur. J. Immunol.* 27, 12 (1997), 3095–3099. https://doi.org/10.1002/eji.1830271203

Björkström, N. K., Béziat, V., Cichocki, F., Liu, L. L., Levine, J., Larsson, S., Koup, R. A., Anderson, S. K., Ljunggren, H.-G., and Malmberg, K.-J. 2012. CD8 T cells express randomly selected KIRs with distinct specificities compared with NK cells. *Blood* 120, 17 (2012), 3455–3465. https://doi.org/10.1182/blood-2012-03-416867

Björkström, N. K., Riese, P., Heuts, F., Andersson, S., Fauriat, C., Ivarsson, M. A., Björklund, A. T., Flodström-Tullberg, M., Michaëlsson, J., Rottenberg, M. E., Guzmán, C. A., Ljunggren, H.-G., and Malmberg, K.-J. 2010. Expression patterns of NKG2A, KIR, and CD57

define a process of CD56dim NK-cell differentiation uncoupled from NK-cell education. *Blood* 116, 19 (2010), 3853–3864. https://doi.org/10.1182/blood-2010-04-281675

Boelen, L., Debebe, B., Silveira, M., Salam, A., Makinde, J., Roberts, C. h., Wang, E. C. Y., Frater, J., Gilmour, J., Twigger, K., Ladell, K., Miners, K. L., Jayaraman, J., Traherne, J. A., Price, D. A., Qi, Y., Martin, M. P., Macallan, D. C., IAVI Protocol C Investigators, Thio, C. L., Astemborski, J., Kirk, G., Donfield, S. M., Buchbinder, S., Khakoo, S. I., Goedert, J. J., Trowsdale, J., Carrington, M., Kollnberger, S., and Asquith, B. 2018. Inhibitory killer cell immunoglobulin-like receptors strengthen CD8 $^+$ T cell–mediated control of HIV-1, HCV, and HTLV-1. *Science Immunology* 3, 29 (2018), eaao2892. https://doi.org/10.1126/sciimmunol.aao2892

Bollyky, J. B., Long, S. A., Fitch, M., Bollyky, P. L., Rieck, M., Rogers, R., Samuels, P. L., Sanda, S., Buckner, J. H., Hellerstein, M. K., and Greenbaum, C. J. 2013. Evaluation of *in vivo* T cell kinetics: use of heavy isotope labelling in type 1 diabetes. *Clin. Exp. Immunol.* 172, 3 (2013), 363–374. https://doi.org/10.1111/cei.12064

Bonorino, P., Leroy, V., Dufeu-Duchesne, T., Tongiani-Dashan, S., Sturm, N., Pernollet, M., Vivier, E., Zarski, J.-P., Marche, P. N., and Jouvin-Marche, E. 2007. Features and distribution of CD8 T cells with human leukocyte antigen class I-specific receptor expression in chronic hepatitis C. *Hepatology* 46, 5 (2007), 1375–1386. https://doi.org/10.1002/hep.21850

Borghans, J. A. M., Mølgaard, A., de Boer, R. J., and Keşmir, C. 2007. HLA Alleles Associated with Slow Progression to AIDS Truly Prefer to Present HIV-1 p24. *PLoS ONE* 2, 9 (2007), e920. https://doi.org/10.1371/journal.pone.0000920

Bottini, N., Musumeci, L., Alonso, A., Rahmouni, S., Nika, K., Rostamkhani, M., MacMurray, J., Meloni, G. F., Lucarelli, P., Pellecchia, M., Eisenbarth, G. S., Comings, D., and Mustelin, T. 2004. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat Genet* 36, 4 (2004), 337–8. https://doi.org/10.1038/ng1323 Type: Journal article.

Boudreau, J. E. and Hsu, K. C. 2018. Natural Killer Cell Education and the Response to Infection and Cancer Therapy: Stay Tuned. *Trends Immunol.* 39, 3 (2018), 222–239. https://doi.org/10.1016/j.it.2017.12.001

Boudreau, J. E., Le Luduec, J.-B., and Hsu, K. C. 2014. Development of a novel multiplex PCR assay to detect functional subtypes of KIR3DL1 alleles. *PloS One* 9, 6 (2014), e99543. https://doi.org/10.1371/journal.pone.0099543

Boudreau, J. E., Mulrooney, T. J., Le Luduec, J.-B., Barker, E., and Hsu, K. C. 2016. KIR3DL1 and HLA-B Density and Binding Calibrate NK Education and Response to HIV. *Journal of Immunology (Baltimore, Md.: 1950)* 196, 8 (2016), 3398–3410. https://doi.org/10.4049/jimmunol.1502469

Boulet, S., Sharafi, S., Simic, N., Bruneau, J., Routy, J.-P., Tsoukas, C. M., and Bernard, N. F. 2008. Increased proportion of KIR3DS1 homozygotes in HIV-exposed uninfected individuals. *AIDS (London, England)* 22, 5 (2008), 595–599. https://doi.org/10.1097/QAD.0b013e3282f56b23

Bowness, P., Ridley, A., Shaw, J., Chan, A. T., Wong-Baeza, I., Fleming, M., Cummings, F., McMichael, A., and Kollnberger, S. 2011. Th17 cells expressing KIR3DL2+ and responsive to HLA-B27 homodimers are increased in ankylosing spondylitis. *J Immunol* 186, 4 (2011), 2672–80. Type: Journal article.

Brodin, P., Kärre, K., and Höglund, P. 2009. NK cell education: not an on-off switch but a tunable rheostat. *Trends Immunol.* 30, 4 (2009), 143–149. https://doi.org/10.1016/j.it.2009.01.006

Bruijnesteijn, J., Van Der Wiel, M. K. H., De Groot, N., Otting, N., De Vos-Rouweler, A. J. M., Lardy, N. M., De Groot, N. G., and Bontrop, R. E. 2018. Extensive Alternative Splicing of KIR Transcripts. *Front. Immunol.* 9 (2018), 2846. https://doi.org/10.3389/fimmu.2018.02846

Brusko, T., Wasserfall, C., McGrail, K., Schatz, R., Viener, H. L., Schatz, D., Haller, M., Rockell, J., Gottlieb, P., Clare-Salzler, M., and Atkinson, M. 2007. No Alterations in the Frequency of FOXP3+ Regulatory T-Cells in Type 1 Diabetes. *Diabetes* 56, 3 (2007), 604–612. https://doi.org/10.2337/db06-1248

Burnet, S. F. M. et al. 1959. *The clonal selection theory of acquired immunity*. Vol. 3. Vanderbilt University Press Nashville.

Burshtyn, D. N., Scharenberg, A. M., Wagtmann, N., Rajagopalan, S., Berrada, K., Yi, T., Kinet, J.-P., and Long, E. O. 1996. Recruitment of Tyrosine Phosphatase HCP by the

Killer Cell Inhibitory Receptor. *Immunity* 4, 1 (1996), 77–85. https://doi.org/10.1016/S1074-7613(00)80300-3

Burton, P. R., Clayton, D. G., Cardon, L. R., Craddock, N., Deloukas, P., Duncanson, A., Kwiatkowski, D. P., McCarthy, M. I., Ouwehand, W. H., Samani, N. J., Todd, J. A., Donnelly, P., Barrett, J. C., Burton, P. R., Davison, D., Donnelly, P., Easton, D., Evans, D., Leung, H.-T., Marchini, J. L., Morris, A. P., Spencer, C. C. A., Tobin, M. D., Cardon, L. R., Clayton, D. G., Attwood, A. P., Boorman, J. P., Cant, B., Everson, U., Hussey, J. M., Jolley, J. D., Knight, A. S., Koch, K., Meech, E., Nutland, S., Prowse, C. V., Stevens, H. E., Taylor, N. C., Walters, G. R., Walker, N. M., Watkins, N. A., Winzer, T., Todd, J. A., Ouwehand, W. H., Jones, R. W., McArdle, W. L., Ring, S. M., Strachan, D. P., Pembrey, M., Breen, G., St Clair, D., Caesar, S., Gordon-Smith, K., Jones, L., Fraser, C., Green, E. K., Grozeva, D., Hamshere, M. L., Holmans, P. A., Jones, I. R., Kirov, G., Moskvina, V., Nikolov, I., O'Donovan, M. C., Owen, M. J., Craddock, N., Collier, D. A., Elkin, A., Farmer, A., Williamson, R., McGuffin, P., Young, A. H., Ferrier, I. N., Ball, S. G., Balmforth, A. J., Barrett, J. H., Bishop, D. T., Iles, M. M., Maqbool, A., Yuldasheva, N., Hall, A. S., Braund, P. S., Burton, P. R., Dixon, R. J., Mangino, M., Stevens, S., Tobin, M. D., Thompson, J. R., Samani, N. J., Bredin, F., Tremelling, M., Parkes, M., Drummond, H., Lees, C. W., Nimmo, E. R., Satsangi, J., Fisher, S. A., Forbes, A., Lewis, C. M., Onnie, C. M., and others. 2007. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 447, 7145 (2007), 661–678. https://doi.org/10.1038/nature05911 Type: Journal article.

Béziat, V., Liu, L. L., Malmberg, J.-A., Ivarsson, M. A., Sohlberg, E., Björklund, A. T., Retière, C., Sverremark-Ekström, E., Traherne, J., Ljungman, P., Schaffer, M., Price, D. A., Trowsdale, J., Michaëlsson, J., Ljunggren, H.-G., and Malmberg, K.-J. 2013. NK cell responses to cytomegalovirus infection lead to stable imprints in the human KIR repertoire and involve activating KIRs. *Blood* 121, 14 (2013), 2678–2688. https://doi.org/10.1182/blood-2012-10-459545

Cachot, A., Bilous, M., Liu, Y.-C., Li, X., Saillard, M., Cenerenti, M., Rockinger, G. A., Wyss, T., Guillaume, P., Schmidt, J., Genolet, R., Ercolano, G., Protti, M. P., Reith, W., Ioannidou, K., De Leval, L., Trapani, J. A., Coukos, G., Harari, A., Speiser, D. E., Mathis, A., Gfeller, D., Altug, H., Romero, P., and Jandus, C. 2021. Tumor-specific cytolytic CD4 T cells mediate immunity against human cancer. *Sci. Adv.* 7, 9 (2021), eabe3348. https://doi.org/10.1126/sciadv.abe3348

Carrington, M., Nelson, G. W., Martin, M. P., Kissner, T., Vlahov, D., Goedert, J. J., Kaslow, R., Buchbinder, S., Hoots, K., and O'Brien, S. J. 1999. *HLA* and HIV-1: Heterozygote Advantage and *B*35 - Cw*04* Disadvantage. *Science* 283, 5408 (1999), 1748–1752. https://doi.org/10.1126/science.283.5408.1748

Cauda, R., Goletti, D., Lucia, M., Tumbarello, M., Rumi, C., Orengo, A., and Moretta, A. 1994. Analysis of Natural Killer (NK) Cell Subsets Defined by the Expression of Two Novel Surface Antigens (EB6 and GL183) in AIDS and AIDS-Related Conditions. *Clin. Immunol. Immunopathol.* 70, 3 (1994), 198–205. https://doi.org/10.1006/clin.1994.1029

Cauli, A., Shaw, J., Giles, J., Hatano, H., Rysnik, O., Payeli, S., McHugh, K., Dessole, G., Porru, G., Desogus, E., Fiedler, S., Hölper, S., Carette, A., Blanco-Gelaz, M. A., Vacca, A., Piga, M., Ibba, V., Garau, P., La Nasa, G., López-Larrea, C., Mathieu, A., Renner, C., Bowness, P., and Kollnberger, S. 2013. The arthritis-associated HLA-B*27:05 allele forms more cell surface B27 dimer and free heavy chain ligands for KIR3DL2 than HLA-B*27:09. *Rheumatology (Oxford, England)* 52, 11 (2013), 1952–1962. https://doi.org/10.1093/rheumatology/ket219 Type: Journal article.

Cerboni, C., Zingoni, A., Cippitelli, M., Piccoli, M., Frati, L., and Santoni, A. 2007. Antigen-activated human T lymphocytes express cell-surface NKG2D ligands via an ATM/ATR-dependent mechanism and become susceptible to autologous NK- cell lysis. *Blood* 110, 2 (2007), 606–615. https://doi.org/10.1182/blood-2006-10-052720

Chan, A. T., Kollnberger, S. D., Wedderburn, L. R., and Bowness, P. 2005. Expansion and enhanced survival of natural killer cells expressing the killer immunoglobulin-like receptor KIR3DL2 in spondylarthritis. *Arthritis Rheum* 52, 11 (2005), 3586–95. https://doi.org/10.1002/art.21395 Type: Journal article.

Chiesa, M. D., Vitale, M., Carlomagno, S., Ferlazzo, G., Moretta, L., and Moretta, A. 2003. The natural killer cell-mediated killing of autologous dendritic cells is confined to a cell subset expressing CD94/NKG2A, but lacking inhibitory killer Ig-like receptors. *Eur. J. Immunol.* 33, 6 (2003), 1657–1666. https://doi.org/10.1002/eji.200323986 _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/eji.200323986.

Chwae, Y.-J., Chang, M. J., Park, S. M., Yoon, H., Park, H.-J., Kim, S. J., and Kim, J. 2002. Molecular Mechanism of the Activation-Induced Cell Death Inhibition Mediated by a p70

Inhibitory Killer Cell Ig-Like Receptor in Jurkat T Cells. *The Journal of Immunology* 169, 7 (2002), 3726–3735. https://doi.org/10.4049/jimmunol.169.7.3726

Clayton, D. G. 2009. Prediction and interaction in complex disease genetics: experience in type 1 diabetes. *PLoS Genet* 5, 7 (2009), e1000540. https://doi.org/10.1371/journal.pgen.1000540 Type: Journal article.

Coles, M. C., McMahon, C. W., Takizawa, H., and Raulet, D. H. 2000. Memory CD8 T lymphocytes express inhibitory MHC-specific Ly49 receptors. *Eur. J. Immunol.* 30, 1 (2000), 236–244. https://doi.org/10.1002/1521-4141(200001)30:1<236::AID-IMMU236>3.0.CO;2-X

Cook, K. D. and Whitmire, J. K. 2013. The Depletion of NK Cells Prevents T Cell Exhaustion to Efficiently Control Disseminating Virus Infection. *The Journal of Immunology* 190, 2 (2013), 641–649. https://doi.org/10.4049/jimmunol.1202448

Cook, L. B., Elemans, M., Rowan, A. G., and Asquith, B. 2013. HTLV-1: Persistence and pathogenesis. *Virology* 435, 1 (2013), 131–140. https://doi.org/10.1016/j.virol.2012.09.028

Cooley, S., Xiao, F., Pitt, M., Gleason, M., McCullar, V., Bergemann, T. L., McQueen, K. L., Guethlein, L. A., Parham, P., and Miller, J. S. 2007. A subpopulation of human peripheral blood NK cells that lacks inhibitory receptors for self-MHC is developmentally immature. *Blood* 110, 2 (2007), 578–586. https://doi.org/10.1182/blood-2006-07-036228

Coppieters, K. T., Dotta, F., Amirian, N., Campbell, P. D., Kay, T. W., Atkinson, M. A., Roep, B. O., and von Herrath, M. G. 2012. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. *J. Exp. Med.* 209, 1 (2012), 51–60.

Cordell, H. J. and Clayton, D. G. 2005. Genetic association studies. *Lancet (London, England)* 366, 9491 (2005), 1121–1131. https://doi.org/10.1016/S0140-6736(05)67424-7

Cortes, A. and Brown, M. A. 2010. Promise and pitfalls of the Immunochip. *Arthritis Research & Therapy* 13, 1 (2010), 101. https://doi.org/10.1186/ar3204

Crouse, J., Bedenikovic, G., Wiesel, M., Ibberson, M., Xenarios, I., Von Laer, D., Kalinke, U., Vivier, E., Jonjic, S., and Oxenius, A. 2014. Type I Interferons Protect T Cells against NK

Cell Attack Mediated by the Activating Receptor NCR1. *Immunity* 40, 6 (2014), 961–973. https://doi.org/10.1016/j.immuni.2014.05.003

Culina, S., Lalanne, A. I., Afonso, G., Cerosaletti, K., Pinto, S., Sebastiani, G., Kuranda, K., Nigi, L., Eugster, A., Østerbye, T., Maugein, A., McLaren, J. E., Ladell, K., Larger, E., Beressi, J.-P., Lissina, A., Appay, V., Davidson, H. W., Buus, S., Price, D. A., Kuhn, M., Bonifacio, E., Battaglia, M., Caillat-Zucman, S., Dotta, F., Scharfmann, R., Kyewski, B., Mallone, R., ImMaDiab Study Group, Carel, J.-C., Tubiana-Rufi, N., Martinerie, L., Poidvin, A., JacqzAigrain, E., Corvez, L., Berruer, V., Gautier, J.-F., Baz, B., Haddadi, N., Andreelli, F., Amouyal, C., Jaqueminet, S., Bourron, O., Dasque, E., Hartemann, A., Lemoine-Yazigi, A., Dubois-Laforgue, D., Travert, F., Feron, M., Rolland, P., Vignali, V., Marre, M., Chanson, P., Briet, C., Guillausseau, P.-J., Ait-Bachir, L., Collet, C., Beziaud, F., Desforges-Bullet, V., Petit-Aubert, G., Christin-Maitre, S., Fève, B., Vatier, C., Bourcigaux, N., Lautridou, C., Lahlou, N., Bakouboula, P., Elie, C., Morel, H., Treluyer, J.-M., Gagner-ault, M.-C., Maillard, C., and Jones, A. 2018. Islet-reactive CD8 $^{+}$ T cell frequencies in the pancreas, but not in blood, distinguish type 1 diabetic patients from healthy donors. *Science Immunology* 3, 20 (2018), eaao4013. https://doi.org/10.1126/sciimmunol.aao4013

D'Andrea, A., Chang, C., Phillips, J. H., and Lanier, L. L. 1996. Regulation of T cell lymphokine production by killer cell inhibitory receptor recognition of self HLA class I alleles. *J. Exp. Med.* 184, 2 (1996), 789–794. https://doi.org/10.1084/jem.184.2.789

Daëron, M., Jaeger, S., Du Pasquier, L., and Vivier, E. 2008. Immunoreceptor tyrosine-based inhibition motifs: a quest in the past and future. *Immunol Rev* 224, 1 (2008), 11–43. https://doi.org/10.1111/j.1600-065X.2008.00666.x

Debebe, B. J., Boelen, L., Lee, J. C., IAVI Protocol C Investigators, Sanders, E. J., Anzala, O., Kamali, A., Kaleebu, P., Karita, E., Kilembe, W., Inambao, M., Lakhi, S., Allen, S., Hunter, E., Edward, V. A., Fast, P. E., Price, M. A., Gilmour, J., Tang, J., Thio, C. L., Astemborski, J., Kirk, G., Khakoo, S. I., Donfield, S. M., Goedert, J. J., and Asquith, B. 2020. Identifying the immune interactions underlying HLA class I disease associations. *eLife* 9 (2020), e54558. https://doi.org/10.7554/eLife.54558

Delaneau, O., Marchini, J., and The 1000 Genomes Project Consortium. 2014. Integrating sequence and array data to create an improved 1000 Genomes Project haplotype reference panel. *Nat Commun* 5, 1 (2014), 3934. https://doi.org/10.1038/ncomms4934

Denroche, H. C., Miard, S., Sallé-Lefort, S., Picard, F., and Verchere, C. B. 2021. T cells accumulate in non-diabetic islets during ageing. *Immunity & Ageing* 18, 1 (2021), 8. https://doi.org/10.1186/s12979-021-00221-4

Diniz, M. O., Schurich, A., Chinnakannan, S. K., Duriez, M., Stegmann, K. A., Davies, J., Kucykowicz, S., Suveizdyte, K., Amin, O. E., Alcock, F., Cargill, T., Barnes, E., and Maini, M. K. 2022. NK cells limit therapeutic vaccine–induced CD8 $^{+}$ T cell immunity in a PD-L1–dependent manner. *Sci Transl Med* 14, 640 (2022), eabi4670. https://doi.org/10.1126/scitranslmed.abi4670

Dobromylskyj, M. and Ellis, S. 2007. Complexity in cattle KIR genes: transcription and genome analysis. *Immunogenetics* 59 (2007), 463–472.

Dogra, P., Rancan, C., Ma, W., Toth, M., Senda, T., Carpenter, D. J., Kubota, M., Matsumoto, R., Thapa, P., Szabo, P. A., Li Poon, M. M., Li, J., Arakawa-Hoyt, J., Shen, Y., Fong, L., Lanier, L. L., and Farber, D. L. 2020. Tissue Determinants of Human NK Cell Development, Function, and Residence. *Cell* 180, 4 (2020), 749–763.e13. https://doi.org/10.1016/j.cell.2020.01.022

Edner, N. M., Heuts, F., Thomas, N., Wang, C. J., Petersone, L., Kenefeck, R., Kogimtzis, A., Ovcinnikovs, V., Ross, E. M., Ntavli, E., Elfaki, Y., Eichmann, M., Baptista, R., Ambery, P., Jermutus, L., Peakman, M., Rosenthal, M., and Walker, L. S. K. 2020. Follicular helper T cell profiles predict response to costimulation blockade in type 1 diabetes. *Nat. Immunol.* 21, 10 (2020), 1244–1255. https://doi.org/10.1038/s41590-020-0744-z

Eerligh, P., van Lummel, M., Zaldumbide, A., Moustakas, A. K., Duinkerken, G., Bondinas, G., Koeleman, B. P., Papadopoulos, G. K., and Roep, B. O. 2011. Functional consequences of HLA-DQ8 homozygosity versus heterozygosity for islet autoimmunity in type 1 diabetes. *Genes Immun* 12, 6 (2011), 415–27. https://doi.org/10.1038/gene.2011.24 Type: Journal article.

Erlich, H., Valdes, A. M., Noble, J., Carlson, J. A., Varney, M., Concannon, P., Mychaleckyj, J. C., Todd, J. A., Bonella, P., Fear, A. L., Lavant, E., Louey, A., Moonsamy, P., and for the Type 1 Diabetes Genetics Consortium. 2008. HLA DR-DQ Haplotypes and Genotypes and Type 1 Diabetes Risk. *Diabetes* 57, 4 (2008), 1084–1092. https://doi.org/10.2337/db07-1331

Erlich, H. A., Griffith, R. L., Bugawan, T. L., Ziegler, R., Alper, C., and Eisenbarth, G. 1991. Implication of Specific DQB1 Alleles in Genetic Susceptibility and Resistance by Identification of IDDM Siblings With Novel HLA-DQB1 Allele and Unusual DR2 and DR1 Haplotypes. *Diabetes* 40, 4 (1991), 478–481. https://doi.org/10.2337/diab.40.4.478

Falk, C. T. and Rubinstein, P. 1987. Haplotype relative risks: an easy reliable way to construct a proper control sample for risk calculations. *Ann Hum Genet* 51, 3 (1987), 227–233. https://doi.org/10.1111/j.1469-1809.1987.tb00875.x

Fasolino, M., Schwartz, G. W., Patil, A. R., Mongia, A., Golson, M. L., Wang, Y. J., Morgan, A., Liu, C., Schug, J., Liu, J., Wu, M., Traum, D., Kondo, A., May, C. L., Goldman, N., Wang, W., Feldman, M., Moore, J. H., Japp, A. S., Betts, M. R., Faryabi, R. B., Naji, A., Kaestner, K. H., and Vahedi, G. 2022. Single-cell multi-omics analysis of human pancreatic islets reveals novel cellular states in type 1 diabetes. *Nature Metabolism* 4, 2 (2022), 284–299. https://doi.org/10.1038/s42255-022-00531-x

Faure, M. and Long, E. O. 2002. KIR2DL4 (CD158d), an NK Cell-Activating Receptor with Inhibitory Potential. *The Journal of Immunology* 168, 12 (2002), 6208–6214. https://doi.org/10.4049/jimmunol.168.12.6208 arXiv:https://journals.aai.org/jimmunol/article-pdf/168/12/6208/1145562/6208.pdf

Ferlazzo, G., Thomas, D., Lin, S.-L., Goodman, K., Morandi, B., Muller, W. A., Moretta, A., and Münz, C. 2004. The Abundant NK Cells in Human Secondary Lymphoid Tissues Require Activation to Express Killer Cell Ig-Like Receptors and Become Cytolytic. *The Journal of Immunology* 172, 3 (2004), 1455–1462. https://doi.org/10.4049/jimmunol.172.3.1455

Ferlazzo, G., Tsang, M. L., Moretta, L., Melioli, G., Steinman, R. M., and Münz, C. 2002. Human Dendritic Cells Activate Resting Natural Killer (NK) Cells and Are Recognized via the NKp30 Receptor by Activated NK Cells. *The Journal of Experimental Medicine* 195, 3 (2002), 343–351. https://doi.org/10.1084/jem.20011149

Fernandez, N. C., Treiner, E., Vance, R. E., Jamieson, A. M., Lemieux, S., and Raulet, D. H. 2005. A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules. *Blood* 105, 11 (2005), 4416–4423. https://doi.org/10.1182/blood-2004-08-3156

Ferrini, S., Cambiaggi, A., Meazza, R., Sforzini, S., Marciano, S., Mingari, M. C., and Moretta, L. 1994. T cell clones expressing the natural killer cell-related p58 receptor molecule display

heterogeneity in phenotypic properties and p58 function. *Eur. J. Immunol.* 24, 10 (1994), 2294–2298. https://doi.org/10.1002/eji.1830241005

Finak, G., Frelinger, J., Jiang, W., Newell, E. W., Ramey, J., Davis, M. M., Kalams, S. A., De Rosa, S. C., and Gottardo, R. 2014. OpenCyto: an open source infrastructure for scalable, robust, reproducible, and automated, end-to-end flow cytometry data analysis. *PLoS computational biology* 10, 8 (2014), e1003806. https://doi.org/10.1371/journal.pcbi.1003806

Futas, J. and Horin, P. 2013. Natural Killer Cell Receptor Genes in the Family Equidae: Not only Ly49. *PLoS ONE* 8, 5 (2013), e64736. https://doi.org/10.1371/journal.pone.0064736

Futas, J., Oppelt, J., Janova, E., Musilova, P., and Horin, P. 2020. Complex variation in the *KLRA* (*LY49*) immunity-related genomic region in horses. *HLA* 96, 3 (2020), 257–267. https://doi.org/10.1111/tan.13939

Gao, G. F., Liu, D., Zhan, X., and Li, B. 2022. Analysis of KIR gene variants in The Cancer Genome Atlas and UK Biobank using KIRCLE. *BMC Biol.* 20, 1 (2022), 191. https://doi.org/10.1186/s12915-022-01392-2

García, A. R., Paterou, A., Lee, M., Sławiński, H., Ferreira, R., Landry, L. G., Trzupek, D., Teyton, L., Szypowska, A., Wicker, L. S., Nakayama, M., Todd, J. A., and Pekalski, M. 2021. *HLA class II mediates type 1 diabetes risk by anti-insulin repertoire selection*. Technical Report. bioRxiv. 2021.09.06.458974 pages. Type: article.

García, A. R., Paterou, A., Lee, M., Sławiński, H., Wicker, L. S., Todd, J. A., and Pękalski, M. 2019. *Peripheral tolerance to insulin is encoded by mimicry in the microbiome*. Technical Report. bioRxiv. 2019.12.18.881433 pages. Type: article.

Gardiner, C. M., Guethlein, L. A., Shilling, H. G., Pando, M., Carr, W. H., Rajalingam, R., Vilches, C., and Parham, P. 2001. Different NK cell surface phenotypes defined by the DX9 antibody are due to KIR3DL1 gene polymorphism. *Journal of Immunology (Baltimore, Md.: 1950)* 166, 5 (2001), 2992–3001. https://doi.org/10.4049/jimmunol.166.5.2992

Gati, A., Guerra, N., Gaudin, C., Da Rocha, S., Escudier, B., Lécluse, Y., Bettaieb, A., Chouaib, S., and Caignard, A. 2003. CD158 receptor controls cytotoxic T-lymphocyte susceptibility to tumor-mediated activation-induced cell death by interfering with Fas signaling. *Cancer Res.* 63, 21 (2003), 7475–7482.

Gattinoni, L., Speiser, D. E., Lichterfeld, M., and Bonini, C. 2017. T memory stem cells in health and disease. *Nat Med* 23, 1 (2017), 18–27. https://doi.org/10.1038/nm.4241

Genetics of Type 1 Diabetes in Finland, the Wellcome Trust Case Control Consortium, Todd, J. A., Walker, N. M., Cooper, J. D., Smyth, D. J., Downes, K., Plagnol, V., Bailey, R., Nejentsev, S., Field, S. F., Payne, F., Lowe, C. E., Szeszko, J. S., Hafler, J. P., Zeitels, L., Yang, J. H. M., Vella, A., Nutland, S., Stevens, H. E., Schuilenburg, H., Coleman, G., Maisuria, M., Meadows, W., Smink, L. J., Healy, B., Burren, O. S., Lam, A. A. C., Ovington, N. R., Allen, J., Adlem, E., Leung, H.-T., Wallace, C., Howson, J. M. M., Guja, C., Ionescu-Tîrgovişte, C., Simmonds, M. J., Heward, J. M., Gough, S. C. L., Dunger, D. B., Wicker, L. S., and Clayton, D. G. 2007. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat. Genet.* 39, 7 (2007), 857–864. https://doi.org/10.1038/ng2068

Gioia, L., Holt, M., Costanzo, A., Sharma, S., Abe, B., Kain, L., Nakayama, M., Wan, X., Su, A., Mathews, C., Chen, Y.-G., Unanue, E., and Teyton, L. 2019. Position β57 of I-A ^{g7} controls early anti-insulin responses in NOD mice, linking an MHC susceptibility allele to type 1 diabetes onset. *Science Immunology* 4, 38 (2019), eaaw6329. https://doi.org/10.1126/sciimmunol.aaw6329

Glisic, S. and Jailwala, P. 2012. Interaction between Treg Apoptosis Pathways, Treg Function and HLA Risk Evolves during Type 1 Diabetes Pathogenesis. *PLoS ONE* 7, 4 (2012), e36040. https://doi.org/10.1371/journal.pone.0036040

Glisic, S., Klinker, M., Waukau, J., Jailwala, P., Jana, S., Basken, J., Wang, T., Alemzadeh, R., Hagopian, W., and Ghosh, S. 2009. Genetic association of HLA DQB1 with CD4+CD25+high T-cell apoptosis in type 1 diabetes. *Genes & Immunity* 10, 4 (2009), 334–340. https://doi.org/10.1038/gene.2009.14

Gottesman, B. L., Yu, J., Tanaka, C., Longhurst, C. A., and Kim, J. J. 2022. Incidence of New-Onset Type 1 Diabetes Among US Children During the COVID-19 Global Pandemic. *JAMA Pediatrics* (2022). https://doi.org/10.1001/jamapediatrics.2021.5801

Graef, T., Moesta, A. K., Norman, P. J., Abi-Rached, L., Vago, L., Older Aguilar, A. M., Gleimer, M., Hammond, J. A., Guethlein, L. A., Bushnell, D. A., Robinson, P. J., and Parham, P. 2009. KIR2DS4 is a product of gene conversion with KIR3DL2 that introduced

specificity for HLA-A*11 while diminishing avidity for HLA-C. *J. Exp. Med.* 206, 11 (2009), 2557–2572. https://doi.org/10.1084/jem.20091010

Griffiths, J. A., Richard, A. C., Bach, K., Lun, A. T. L., and Marioni, J. C. 2018. Detection and removal of barcode swapping in single-cell RNA-seq data. *Nat. Commun.* 9, 1 (2018), 2667. https://doi.org/10.1038/s41467-018-05083-x

Guerra, N., Guillard, M., Angevin, E., Echchakir, H., Escudier, B., Moretta, A., Chouaib, S., and Caignard, A. 2000. Killer inhibitory receptor (CD158b) modulates the lytic activity of tumor-specific T lymphocytes infiltrating renal cell carcinomas. *Blood* 95, 9 (2000), 2883–2889.

Guerra, N., Michel, F., Gati, A., Gaudin, C., Mishal, Z., Escudier, B., Acuto, O., Chouaib, S., and Caignard, A. 2002. Engagement of the inhibitory receptor CD158a interrupts TCR signaling, preventing dynamic membrane reorganization in CTL/tumor cell interaction. *Blood* 100, 8 (2002), 2874–2881. https://doi.org/10.1182/blood-2002-02-0643

Guethlein, L. A., Abi-Rached, L., Hammond, J. A., and Parham, P. 2007. The expanded cattle KIR genes are orthologous to the conserved single-copy KIR3DX1 gene of primates. *Immunogenetics* 59 (2007), 517–522.

Gumperz, J. E., Barber, L. D., Valiante, N. M., Percival, L., Phillips, J. H., Lanier, L. L., and Parham, P. 1997. Conserved and variable residues within the Bw4 motif of HLA-B make separable contributions to recognition by the NKB1 killer cell-inhibitory receptor. *Journal of Immunology (Baltimore, Md.: 1950)* 158, 11 (1997), 5237–5241.

Gumperz, J. E., Litwin, V., Phillips, J. H., Lanier, L. L., and Parham, P. 1995. The Bw4 public epitope of HLA-B molecules confers reactivity with natural killer cell clones that express NKB1, a putative HLA receptor. *The Journal of Experimental Medicine* 181, 3 (1995), 1133–1144. https://doi.org/10.1084/jem.181.3.1133

Gwozdowicz, S., Nestorowicz, K., Graczyk-Pol, E., Szlendak, U., Rogatko-Koros, M., Mika-Witkowska, R., Pawliczak, D., Zubala, M., Malinowska, A., Witkowska, A., and Nowak, J. 2019. KIR specificity and avidity of standard and unusual C1, C2, Bw4, Bw6 and A3/11 amino acid motifs at entire HLA:KIR interface between NK and target cells, the functional and evolutionary classification of HLA class I molecules. *Int. J. Immunogenet.* 46, 4 (2019), 217–231. https://doi.org/10.1111/iji.12433

Hakonarson, H., Grant, S. F., Bradfield, J. P., Marchand, L., Kim, C. E., Glessner, J. T., Grabs, R., Casalunovo, T., Taback, S. P., Frackelton, E. C., Lawson, M. L., Robinson, L. J., Skraban, R., Lu, Y., Chiavacci, R. M., Stanley, C. A., Kirsch, S. E., Rappaport, E. F., Orange, J. S., Monos, D. S., Devoto, M., Qu, H. Q., and Polychronakos, C. 2007. A genome-wide association study identifies KIAA0350 as a type 1 diabetes gene. *Nature* 448, 7153 (2007), 591–4. https://doi.org/10.1038/nature06010 Type: Journal article.

Halle, S., Keyser, K. A., Stahl, F. R., Busche, A., Marquardt, A., Zheng, X., Galla, M., Heissmeyer, V., Heller, K., Boelter, J., Wagner, K., Bischoff, Y., Martens, R., Braun, A., Werth, K., Uvarovskii, A., Kempf, H., Meyer-Hermann, M., Arens, R., Kremer, M., Sutter, G., Messerle, M., and Förster, R. 2016. In Vivo Killing Capacity of Cytotoxic T Cells Is Limited and Involves Dynamic Interactions and T Cell Cooperativity. *Immunity* 44, 2 (2016), 233–245. https://doi.org/10.1016/j.immuni.2016.01.010

Hao, W., Bahnson, H. T., Speake, C., Cerosaletti, K., and Greenbaum, C. J. 2019. *In-vivo* assessment of T cell kinetics in individuals at risk for type 1 diabetes. *Clin. Exp. Immunol.* 199, 1 (2019), 50–55. https://doi.org/10.1111/cei.13375

Hao, Y., Hao, S., Andersen-Nissen, E., III, W. M. M., Zheng, S., Butler, A., Lee, M. J., Wilk, A. J., Darby, C., Zagar, M., Hoffman, P., Stoeckius, M., Papalexi, E., Mimitou, E. P., Jain, J., Srivastava, A., Stuart, T., Fleming, L. B., Yeung, B., Rogers, A. J., McElrath, J. M., Blish, C. A., Gottardo, R., Smibert, P., and Satija, R. 2021. Integrated analysis of multimodal single-cell data. *Cell* (2021). https://doi.org/10.1016/j.cell.2021.04.048

Harrison, G. F., Leaton, L. A., Harrison, E. A., Kichula, K. M., Viken, M. K., Shortt, J., Gignoux, C. R., Lie, B. A., Vukcevic, D., Leslie, S., and Norman, P. J. 2022. Allele imputation for the killer cell immunoglobulin-like receptor KIR3DL1/S1. *PLOS Comput. Biol.* 18, 2 (2022), e1009059. https://doi.org/10.1371/journal.pcbi.1009059

Hauben, E., Roncarolo, M. G., Nevo, U., and Schwartz, M. 2005. Beneficial autoimmunity in Type 1 diabetes mellitus. *Trends Immunol* 26, 5 (2005), 248–53. https://doi.org/10.1016/j.it.2005.03.004 Type: Journal article.

Hayakawa, Y., Screpanti, V., Yagita, H., Grandien, A., Ljunggren, H.-G., Smyth, M. J., and Chambers, B. J. 2004. NK cell TRAIL eliminates immature dendritic cells in vivo and limits dendritic cell vaccination efficacy. *Journal of Immunology (Baltimore, Md.: 1950)* 172, 1 (2004), 123–129. https://doi.org/10.4049/jimmunol.172.1.123

Hiby, S. E., Apps, R., Chazara, O., Farrell, L. E., Magnus, P., Trogstad, L., Gjessing, H. K., Carrington, M., and Moffett, A. 2014. Maternal *KIR* in Combination with Paternal *HLA-C2* Regulate Human Birth Weight. *The Journal of Immunology* 192, 11 (2014), 5069–5073. https://doi.org/10.4049/jimmunol.1400577

Hiby, S. E., Walker, J. J., O'Shaughnessy, K. M., Redman, C. W., Carrington, M., Trowsdale, J., and Moffett, A. 2004. Combinations of Maternal KIR and Fetal HLA-C Genes Influence the Risk of Preeclampsia and Reproductive Success. *J. Exp. Med.* 200, 8 (2004), 957–965. https://doi.org/10.1084/jem.20041214

Hoelsbrekken, S. E., Nylenna, Ø., Saether, P. C., Slettedal, I. O., Ryan, J. C., Fossum, S., and Dissen, E. 2003. Cutting edge: molecular cloning of a killer cell Ig-like receptor in the mouse and rat. *The Journal of Immunology* 170, 5 (2003), 2259–2263.

Hollenbach, J. A., Nocedal, I., Ladner, M. B., Single, R. M., and Trachtenberg, E. A. 2012. Killer cell immunoglobulin-like receptor (KIR) gene content variation in the HGDP-CEPH populations. *Immunogenetics* 64 (2012), 719–737.

Hollenbach, J. A., Pando, M. J., Caillier, S. J., Gourraud, P.-A., and Oksenberg, J. R. 2016. The killer immunoglobulin-like receptor KIR3DL1 in combination with HLA-Bw4 is protective against multiple sclerosis in African Americans. *Genes Immun* 17, 3 (2016), 199–202. https://doi.org/10.1038/gene.2016.5

Hoover, M. L. and Marta, R. T. 1997. Molecular modelling of HLA-DQ suggests a mechanism of resistance in type 1 diabetes. *Scand J Immunol* 45, 2 (1997), 193–202. https://doi.org/10.1046/j.1365-3083.1997.d01-389.x Type: Journal article.

Howson, J. M., Stevens, H., Smyth, D. J., Walker, N. M., Chandler, K. A., Bingley, P. J., and Todd, J. A. 2011. Evidence that HLA class I and II associations with type 1 diabetes, autoantibodies to GAD and autoantibodies to IA-2, are distinct. *Diabetes* 60, 10 (2011), 2635–44. https://doi.org/10.2337/db11-0131 Type: Journal article.

Hsu, K. C. 2010. Natural killer cells: alloreactive effects in hematopoietic stem cell transplantation: Natural killer cells. *ISBT Science Series* 5, n1 (2010), 282–287. https://doi.org/10.1111/j.1751-2824.2010.01413.x

Hu, X., Deutsch, A. J., Lenz, T. L., Onengut-Gumuscu, S., Han, B., Chen, W.-M., Howson, J. M. M., Todd, J. A., de Bakker, P. I. W., Rich, S. S., and Raychaudhuri, S. 2015. Additive

and interaction effects at three amino acid positions in HLA-DQ and HLA-DR molecules drive type 1 diabetes risk. *Nat. Genet.* 47, 8 (2015), 898–905. https://doi.org/10.1038/ng.3353

Huard, B. and Karlsson, L. 2000. KIR expression on self-reactive CD8+ T cells is controlled by T-cell receptor engagement. *Nature* 403, 6767 (2000), 325–328. https://doi.org/10.1038/35002105

Huhn, O., Chazara, O., Ivarsson, M. A., Retière, C., Venkatesan, T. C., Norman, P. J., Hilton, H. G., Jayaraman, J., Traherne, J. A., Trowsdale, J., Ito, M., Kling, C., Parham, P., Ghadially, H., Moffett, A., Sharkey, A. M., and Colucci, F. 2018. High-Resolution Genetic and Phenotypic Analysis of KIR2DL1 Alleles and Their Association with Pre-Eclampsia. *The Journal of Immunology* 201, 9 (2018), 2593–2601. https://doi.org/10.4049/jimmunol.1800860

Husain, B., Ramani, S. R., Chiang, E., Lehoux, I., Paduchuri, S., Arena, T. A., Patel, A., Wilson, B., Chan, P., Franke, Y., Wong, A. W., Lill, J. R., Turley, S. J., Gonzalez, L. C., Grogan, J. L., and Martinez-Martin, N. 2019. A Platform for Extracellular Interactome Discovery Identifies Novel Functional Binding Partners for the Immune Receptors B7-H3/CD276 and PVR/CD155. *Molecular & cellular proteomics: MCP* 18, 11 (2019), 2310–2323. https://doi.org/10.1074/mcp.TIR119.001433

Hyttinen, V., Kaprio, J., Kinnunen, L., Koskenvuo, M., and Tuomilehto, J. 2003. Genetic Liability of Type 1 Diabetes and the Onset Age Among 22,650 Young Finnish Twin Pairs. *Diabetes* 52, 4 (2003), 1052–1055. https://doi.org/10.2337/diabetes.52.4.1052

Ilonen, J., Kiviniemi, M., Lempainen, J., Simell, O., Toppari, J., Veijola, R., Knip, M., and Register, T. F. P. D. 2016. Genetic susceptibility to type 1 diabetes in childhood – estimation of HLA class II associated disease risk and class II effect in various phases of islet autoimmunity. *Pediatr Diabetes* 17, S22 (2016), 8–16. https://doi.org/10.1111/pedi.12327

Ilonen, J., Lempainen, J., Hammais, A., Laine, A.-P., Härkönen, T., Toppari, J., Veijola, R., Knip, M., and the Finnish Pediatric Diabetes Register. 2018. Primary islet autoantibody at initial seroconversion and autoantibodies at diagnosis of type 1 diabetes as markers of disease heterogeneity: ILONEN et al. *Pediatr Diabetes* 19, 2 (2018), 284–292. https://doi.org/10.1111/pedi.12545

Ishigaki, K., Lagattuta, K. A., Luo, Y., James, E. A., Buckner, J. H., and Raychaudhuri, S.

2022. HLA autoimmune risk alleles restrict the hypervariable region of T cell receptors. *Nat. Genet.* 54, 4 (2022), 393–402. https://doi.org/10.1038/s41588-022-01032-z

Iwasaki, A. and Medzhitov, R. 2015. Control of adaptive immunity by the innate immune system. *Nat. Immunol.* 16, 4 (2015), 343–353. https://doi.org/10.1038/ni.3123

Jarvis, L. B., Rainbow, D. B., Coppard, V., Howlett, S. K., Georgieva, Z., Davies, J. L., Mullay, H. K., Hester, J., Ashmore, T., Van Den Bosch, A., Grist, J. T., Coles, A. J., Mousa, H. S., Pluchino, S., Mahbubani, K. T., Griffin, J. L., Saeb-Parsy, K., Issa, F., Peruzzotti-Jametti, L., Wicker, L. S., and Jones, J. L. 2021. Therapeutically expanded human regulatory T-cells are super-suppressive due to HIF1A induced expression of CD73. *Communications Biology* 4, 1 (2021), 1186. https://doi.org/10.1038/s42003-021-02721-x

Jennes, W., Verheyden, S., Mertens, J. W., Camara, M., Seydi, M., Dieye, T. N., Mboup, S., Demanet, C., and Kestens, L. 2013. Inhibitory KIR/HLA incompatibility between sexual partners confers protection against HIV-1 transmission. *Blood* 121, 7 (2013), 1157–1164. https://doi.org/10.1182/blood-2012-09-455352

Jiang, H., Zhang, S. I., and Pernis, B. 1992. Role of CD8+ T cells in murine experimental allergic encephalomyelitis. *Science (New York, N.Y.)* 256, 5060 (1992), 1213–1215. https://doi.org/10.1126/science.256.5060.1213

Jiang, W., Johnson, C., Jayaraman, J., Simecek, N., Noble, J., Moffatt, M. F., Cookson, W. O., Trowsdale, J., and Traherne, J. A. 2012. Copy number variation leads to considerable diversity for B but not A haplotypes of the human KIR genes encoding NK cell receptors. *Genome Res.* 22, 10 (2012), 1845–1854. https://doi.org/10.1101/gr.137976.112

Jobim, M., Chagastelles, P., Salim, P. H., Portela, P., Wilson, T. J., Curti, A. G., Jobim, M. R., João, D. A., Nardi, N. B., Tschiedel, B., Jobim, L. F., Roesler, R., and Schwartsmann, G. 2010. Association of killer cell immunoglobulin-like receptors and human leukocyte antigen-C genotypes in South Brazilian with type 1 diabetes. *Hum Immunol* 71, 8 (2010), 799–803. https://doi.org/10.1016/j.humimm.2010.05.014

Johnsen, G. M., Størvold, G. L., Drabbels, J. J., Haasnoot, G. W., Eikmans, M., Spruyt-Gerritse, M. J., Alnæs-Katjavivi, P., Scherjon, S. A., Redman, C. W., Claas, F. H., and Staff, A. C. 2018. The combination of maternal KIR-B and fetal HLA-C2 is associated with decidua basalis acute atherosis in pregnancies with preeclampsia. *J Reprod Immunol* 129 (2018), 23–29. https://doi.org/10.1016/j.jri.2018.07.005

Kaczorowski, K. J., Shekhar, K., Nkulikiyimfura, D., Dekker, C. L., Maecker, H., Davis, M. M., Chakraborty, A. K., and Brodin, P. 2017. Continuous immunotypes describe human immune variation and predict diverse responses. *Proc. Natl. Acad. Sci.* 114, 30 (2017). https://doi.org/10.1073/pnas.1705065114

Kallionpää, H., Somani, J., Tuomela, S., Ullah, U., de Albuquerque, R., Lönnberg, T., Komsi, E., Siljander, H., Honkanen, J., Härkönen, T., Peet, A., Tillmann, V., Chandra, V., Anagandula, M. K., Frisk, G., Otonkoski, T., Rasool, O., Lund, R., Lähdesmäki, H., Knip, M., and Lahesmaa, R. 2019. Early Detection of Peripheral Blood Cell Signature in Children Developing β-Cell Autoimmunity at a Young Age. *Diabetes* 68, 10 (2019), 2024–2034. https://doi.org/10.2337/db19-0287

Kaminow, B., Yunusov, D., and Dobin, A. 2021. *STARsolo: accurate, fast and versatile mapping/quantification of single-cell and single-nucleus RNA-seq data.* preprint. Bioinformatics. https://doi.org/10.1101/2021.05.05.442755

Kaslow, R. A., Carrington, M., Apple, R., Park, L., Muñoz, A., Saah, A. J., Goedert, J. J., Winkler, C., O'Brien, S. J., Rinaldo, C., Detels, R., Blattner, W., Phair, J., Erlich, H., and Mann, D. L. 1996. Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. *Nat Med* 2, 4 (1996), 405–411. https://doi.org/10.1038/nm0496-405

Kassem, S. A., Ariel, I., Thornton, P. S., Scheimberg, I., and Glaser, B. 2000. Beta-cell proliferation and apoptosis in the developing normal human pancreas and in hyperinsulinism of infancy. *Diabetes* 49, 8 (2000), 1325–1333. https://doi.org/10.2337/diabetes.49.8.1325

Kaur, G., Gras, S., Mobbs, J. I., Vivian, J. P., Cortes, A., Barber, T., Kuttikkatte, S. B., Jensen, L. T., Attfield, K. E., Dendrou, C. A., Carrington, M., McVean, G., Purcell, A. W., Rossjohn, J., and Fugger, L. 2017. Structural and regulatory diversity shape HLA-C protein expression levels. *Nat Commun* 8, 1 (2017), 15924. https://doi.org/10.1038/ncomms15924

Keenan, H. A., Sun, J. K., Levine, J., Doria, A., Aiello, L. P., Eisenbarth, G., Bonner-Weir, S., and King, G. L. 2010. Residual Insulin Production and Pancreatic β-Cell Turnover After 50 Years of Diabetes: Joslin Medalist Study. *Diabetes* 59, 11 (2010), 2846–2853. https://doi.org/10.2337/db10-0676

Khakoo, S. I., Rajalingam, R., Shum, B. P., Weidenbach, K., Flodin, L., Muir, D. G., Canavez, F., Cooper, S. L., Valiante, N. M., Lanier, L. L., and Parham, P. 2000. Rapid Evolution of NK Cell Receptor Systems Demonstrated by Comparison of Chimpanzees and Humans. *Immunity* 12, 6 (2000), 687–698. https://doi.org/10.1016/S1074-7613(00)80219-8

Khakoo, S. I., Thio, C. L., Martin, M. P., Brooks, C. R., Gao, X., Astemborski, J., Cheng, J., Goedert, J. J., Vlahov, D., Hilgartner, M., Cox, S., Little, A.-M., Alexander, G. J., Cramp, M. E., O'Brien, S. J., Rosenberg, W. M. C., Thomas, D. L., and Carrington, M. 2004. HLA and NK Cell Inhibitory Receptor Genes in Resolving Hepatitis C Virus Infection. *Science* 305, 5685 (2004), 872–874. https://doi.org/10.1126/science.1097670 Type: Journal article.

Kim, S., Poursine-Laurent, J., Truscott, S. M., Lybarger, L., Song, Y.-J., Yang, L., French, A. R., Sunwoo, J. B., Lemieux, S., Hansen, T. H., and Yokoyama, W. M. 2005. Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature* 436, 7051 (2005), 709–713. https://doi.org/10.1038/nature03847

Klinke, D. J. 2008. Extent of Beta Cell Destruction Is Important but Insufficient to Predict the Onset of Type 1 Diabetes Mellitus. *PLoS ONE* 3, 1 (2008), e1374. https://doi.org/10.1371/journal.pone.0001374

Kucuksezer, U. C., Aktas Cetin, E., Esen, F., Tahrali, I., Akdeniz, N., Gelmez, M. Y., and Deniz, G. 2021. The Role of Natural Killer Cells in Autoimmune Diseases. *Front. Immunol.* 12 (2021), 622306. https://doi.org/10.3389/fimmu.2021.622306

Kyvik, K. O., Green, A., and Beck-Nielsen, H. 1995. Concordance rates of insulin dependent diabetes mellitus: a population based study of young Danish twins. *BMJ (Clinical research ed.)* 311, 7010 (1995), 913–917. https://doi.org/10.1136/bmj.311.7010.913

Lagattuta, K. A., Kang, J. B., Nathan, A., Pauken, K. E., Jonsson, A. H., Rao, D. A., Sharpe, A. H., Ishigaki, K., and Raychaudhuri, S. 2022. Repertoire analyses reveal T cell antigen receptor sequence features that influence T cell fate. *Nat. Immunol.* 23, 3 (2022), 446–457. https://doi.org/10.1038/s41590-022-01129-x

Lam, C. J., Jacobson, D. R., Rankin, M. M., Cox, A. R., and Kushner, J. A. 2017. β Cells Persist in T1D Pancreata Without Evidence of Ongoing β-Cell Turnover or Neogenesis. *The Journal of Clinical Endocrinology & Metabolism* 102, 8 (2017), 2647–2659. https://doi.org/10.1210/jc.2016-3806

Lang, P. A., Crome, S. Q., Xu, H. C., Lang, K. S., Chapatte, L., Deenick, E. K., Grusdat, M., Pandyra, A. A., Pozdeev, V. I., Wang, R., Holderried, T. A. W., Cantor, H., Diefenbach, A., Elford, A. R., McIlwain, D. R., Recher, M., Haussinger, D., Mak, T. W., and Ohashi, P. S. 2020. NK Cells Regulate CD8(+) T Cell Mediated Autoimmunity. *Front Cell Infect Microbiol* 10 (2020), 36. https://doi.org/10.3389/fcimb.2020.00036 Type: Journal article.

Lang, P. A., Lang, K. S., Xu, H. C., Grusdat, M., Parish, I. A., Recher, M., Elford, A. R., Dhanji, S., Shaabani, N., Tran, C. W., Dissanayake, D., Rahbar, R., Ghazarian, M., Brüstle, A., Fine, J., Chen, P., Weaver, C. T., Klose, C., Diefenbach, A., Häussinger, D., Carlyle, J. R., Kaech, S. M., Mak, T. W., and Ohashi, P. S. 2012. Natural killer cell activation enhances immune pathology and promotes chronic infection by limiting CD8 $^+$ T-cell immunity. *Proc. Natl. Acad. Sci.* 109, 4 (2012), 1210–1215. https://doi.org/10.1073/pnas.1118834109

Lanier, L. L., Corliss, B. C., Wu, J., Leong, C., and Phillips, J. H. 1998. Immunoreceptor DAP12 bearing a tyrosine-based activation motif is involved in activating NK cells. *Nature* 391, 6668 (1998), 703–707. https://doi.org/10.1038/35642

Laroni, A., Armentani, E., Kerlero de Rosbo, N., Ivaldi, F., Marcenaro, E., Sivori, S., Gandhi, R., Weiner, H. L., Moretta, A., Mancardi, G. L., and Uccelli, A. 2016. Dysregulation of regulatory CD56(bright) NK cells/T cells interactions in multiple sclerosis. *J Autoimmun* 72 (2016), 8–18. https://doi.org/10.1016/j.jaut.2016.04.003

Le Garff-Tavernier, M., Béziat, V., Decocq, J., Siguret, V., Gandjbakhch, F., Pautas, E., Debré, P., Merle-Beral, H., and Vieillard, V. 2010. Human NK cells display major phenotypic and functional changes over the life span: Human NK cell features over the life span. *Aging Cell* 9, 4 (2010), 527–535. https://doi.org/10.1111/j.1474-9726.2010.00584.x

Lee, I. F., Qin, H., Trudeau, J., Dutz, J., and Tan, R. 2004. Regulation of autoimmune diabetes by complete Freund's adjuvant is mediated by NK cells. *J Immunol* 172, 2 (2004), 937–42. https://doi.org/10.4049/jimmunol.172.2.937 Type: Journal article.

Leete, P. and Morgan, N. G. 2021. Footprints of Immune Cells in the Pancreas in Type 1 Diabetes; to "B" or Not to "B": Is That Still the Question? *Front. Endocrinol.* 12 (2021).

Leete, P., Willcox, A., Krogvold, L., Dahl-Jørgensen, K., Foulis, A. K., Richardson, S. J., and Morgan, N. G. 2016. Differential Insulitic Profiles Determine the Extent of β-Cell Destruction

and the Age at Onset of Type 1 Diabetes. *Diabetes* 65, 5 (2016), 1362–1369. https://doi.org/10.2337/db15-1615

Lenz, T. L., Deutsch, A. J., Han, B., Hu, X., Okada, Y., Eyre, S., Knapp, M., Zhernakova, A., Huizinga, T. W. J., Abecasis, G., Becker, J., Boeckxstaens, G. E., Chen, W.-M., Franke, A., Gladman, D. D., Gockel, I., Gutierrez-Achury, J., Martin, J., Nair, R. P., Nöthen, M. M., Onengut-Gumuscu, S., Rahman, P., Rantapää-Dahlqvist, S., Stuart, P. E., Tsoi, L. C., van Heel, D. A., Worthington, J., Wouters, M. M., Klareskog, L., Elder, J. T., Gregersen, P. K., Schumacher, J., Rich, S. S., Wijmenga, C., Sunyaev, S. R., de Bakker, P. I. W., and Raychaudhuri, S. 2015. Widespread non-additive and interaction effects within HLA loci modulate the risk of autoimmune diseases. *Nat. Genet.* 47, 9 (2015), 1085–1090. https://doi.org/10.1038/ng.3379

Li, J., Zaslavsky, M., Su, Y., Guo, J., Sikora, M. J., van Unen, V., Christophersen, A., Chiou, S.-H., Chen, L., Li, J., Ji, X., Wilhelmy, J., McSween, A. M., Palanski, B. A., Mallajosyula, V. V. A., Bracey, N. A., Dhondalay, G. K. R., Bhamidipati, K., Pai, J., Kipp, L. B., Dunn, J. E., Hauser, S. L., Oksenberg, J. R., Satpathy, A. T., Robinson, W. H., Dekker, C. L., Steinmetz, L. M., Khosla, C., Utz, P. J., Sollid, L. M., Chien, Y.-H., Heath, J. R., Fernandez-Becker, N. Q., Nadeau, K. C., Saligrama, N., and Davis, M. M. 2022. KIR+CD8+ T cells suppress pathogenic T cells and are active in autoimmune diseases and COVID-19. *Science (New York, N.Y.)* 376, 6590 (2022), eabi9591. https://doi.org/10.1126/science.abi9591

Li, X., Zhou, C., Chen, K., Huang, B., Liu, Q., and Ye, H. 2021. Benchmarking HLA genotyping and clarifying HLA impact on survival in tumor immunotherapy. *Mol Oncol* 15, 7 (2021), 1764–1782. https://doi.org/10.1002/1878-0261.12895

Litchfield, K., Reading, J. L., Puttick, C., Thakkar, K., Abbosh, C., Bentham, R., Watkins, T. B., Rosenthal, R., Biswas, D., Rowan, A., Lim, E., Al Bakir, M., Turati, V., Guerra-Assunção, J. A., Conde, L., Furness, A. J., Saini, S. K., Hadrup, S. R., Herrero, J., Lee, S.-H., Van Loo, P., Enver, T., Larkin, J., Hellmann, M. D., Turajlic, S., Quezada, S. A., McGranahan, N., and Swanton, C. 2021. Meta-analysis of tumor- and T cell-intrinsic mechanisms of sensitization to checkpoint inhibition. *Cell* 184, 3 (2021), 596–614.e14. https://doi.org/10.1016/j.cell.2021.01.002

Liu, S. L., Zheng, A. J., and Ding, L. 2017. Association between KIR gene polymorphisms and type 1 diabetes mellitus (T1DM) susceptibility: A PRISMA-compliant meta-

analysis. *Medicine (Baltimore)* 96, 52 (2017), e9439. https://doi.org/10.1097/MD.0000000000009439 Type: Journal article.

Ljunggren, H. G. and Kärre, K. 1990. In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol. Today* 11, 7 (1990), 237–244. https://doi.org/10.1016/0167-5699(90)90097-s

Lu, L., Ikizawa, K., Hu, D., Werneck, M. B. F., Wucherpfennig, K. W., and Cantor, H. 2007. Regulation of Activated CD4+ T Cells by NK Cells via the Qa-1–NKG2A Inhibitory Pathway. *Immunity* 26, 5 (2007), 593–604. https://doi.org/10.1016/j.immuni.2007.03.017 Type: Journal article.

Ludigs, K., Jandus, C., Utzschneider, D. T., Staehli, F., Bessoles, S., Dang, A. T., Rota, G., Castro, W., Zehn, D., Vivier, E., Held, W., Romero, P., and Guarda, G. 2016. NLRC5 shields T lymphocytes from NK-cell-mediated elimination under inflammatory conditions. *Nat Commun* 7, 1 (2016), 10554. https://doi.org/10.1038/ncomms10554

Mabbott, N. A., Baillie, J., Brown, H., Freeman, T. C., and Hume, D. A. 2013. An expression atlas of human primary cells: inference of gene function from coexpression networks. *BMC Genomics* 14, 1 (2013), 632. https://doi.org/10.1186/1471-2164-14-632

Maccari, G., Robinson, J., Hammond, J. A., and Marsh, S. G. E. 2020. The IPD Project: a centralised resource for the study of polymorphism in genes of the immune system. *Immunogenetics* 72, 1-2 (2020), 49–55. https://doi.org/10.1007/s00251-019-01133-w

Mahaffy, J. M. and Edelstein-Keshet, L. 2007. Modeling Cyclic Waves of Circulating T Cells in Autoimmune Diabetes. *SIAM J. Appl. Math.* 67, 4 (2007), 915–937. https://doi.org/10.1137/060661144

Male, V. and Moffett, A. 2023. Natural Killer Cells in the Human Uterine Mucosa. *Annu. Rev. Immunol.* 41, 1 (2023), 127–151. https://doi.org/10.1146/annurev-immunol-102119-075119

Mandaric, S., Walton, S. M., Rülicke, T., Richter, K., Girard-Madoux, M. J. H., Clausen, B. E., Zurunic, A., Kamanaka, M., Flavell, R. A., Jonjic, S., and Oxenius, A. 2012. IL-10 suppression of NK/DC crosstalk leads to poor priming of MCMV-specific CD4 T cells and prolonged MCMV persistence. *PLoS pathogens* 8, 8 (2012), e1002846. https://doi.org/10.1371/journal.ppat.1002846

Marca, V., Gianchecchi, E., and Fierabracci, A. 2018. Type 1 Diabetes and Its Multi-Factorial Pathogenesis: The Putative Role of NK Cells. *Int J Mol Sci* 19, 3 (2018), 794. https://doi.org/10.3390/ijms19030794

Marin, W. M., Dandekar, R., Augusto, D. G., Yusufali, T., Heyn, B., Hofmann, J., Lange, V., Sauter, J., Norman, P. J., and Hollenbach, J. A. 2021. High-throughput Interpretation of Killer-cell Immunoglobulin-like Receptor Short-read Sequencing Data with PING. *PLOS Comput. Biol.* 17, 8 (2021), e1008904. https://doi.org/10.1371/journal.pcbi.1008904

Marin-Acevedo, J. A., Kimbrough, E. O., and Lou, Y. 2021. Next generation of immune checkpoint inhibitors and beyond. *Journal of Hematology & Oncology* 14, 1 (2021), 45. https://doi.org/10.1186/s13045-021-01056-8

Marsh, S. G. E., Parham, P., Dupont, B., Geraghty, D. E., Trowsdale, J., Middleton, D., Vilches, C., Carrington, M., Witt, C., Guethlein, L. A., Shilling, H., Garcia, C. A., Hsu, K. C., and Wain, H. 2003. Killer-cell immunoglobulin-like receptor (KIR) nomenclature report, 2002. *Immunogenetics* 55, 4 (2003), 220–226. https://doi.org/10.1007/s00251-003-0571-z

Martin, A. M., Freitas, E. M., Witt, C. S., and Christiansen, F. T. 2000. The genomic organization and evolution of the natural killer immunoglobulin-like receptor (KIR) gene cluster. *Immunogenetics* 51, 4-5 (2000), 268–280. https://doi.org/10.1007/s002510050620

Martin, M. P., Gao, X., Lee, J.-H., Nelson, G. W., Detels, R., Goedert, J. J., Buchbinder, S., Hoots, K., Vlahov, D., Trowsdale, J., Wilson, M., O'Brien, S. J., and Carrington, M. 2002. Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. *Nat. Genet.* 31, 4 (2002), 429–434. https://doi.org/10.1038/ng934

Martin, M. P., Qi, Y., Gao, X., Yamada, E., Martin, J. N., Pereyra, F., Colombo, S., Brown, E. E., Shupert, W. L., Phair, J., Goedert, J. J., Buchbinder, S., Kirk, G. D., Telenti, A., Connors, M., O'Brien, S. J., Walker, B. D., Parham, P., Deeks, S. G., McVicar, D. W., and Carrington, M. 2007. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat. Genet.* 39, 6 (2007), 733–740. https://doi.org/10.1038/ng2035

Maxwell, L., Wallace, A., Middleton, D., and Curran, M. 2002. A common KIR2DS4 deletion variant in the human that predicts a soluble KIR molecule analogous to the KIR1D molecule observed in the rhesus monkey: Maxwell et al : A predicted soluble KIR2DS4 deletion variant.

Tissue Antigens 60, 3 (2002), 254–258. https://doi.org/10.1034/j.1399-0039.2002.600307.x

Mehers, K. L., Long, A. E., van der Slik, A. R., Aitken, R. J., Nathwani, V., Wong, F. S., Bain, S., Gill, G., Roep, B. O., Bingley, P. J., and Gillespie, K. M. 2011. An increased frequency of NK cell receptor and HLA-C group 1 combinations in early-onset type 1 diabetes. *Diabetologia* 54, 12 (2011), 3062–3070. https://doi.org/10.1007/s00125-011-2299-x

Meier, J. J., Butler, A. E., Saisho, Y., Monchamp, T., Galasso, R., Bhushan, A., Rizza, R. A., and Butler, P. C. 2008. β-Cell Replication Is the Primary Mechanism Subserving the Postnatal Expansion of β-Cell Mass in Humans. *Diabetes* 57, 6 (2008), 1584–1594. https://doi.org/10.2337/db07-1369

Michalek, J., Vrabelova, Z., Hrotekova, Z., Kyr, M., Pejchlova, M., Kolouskova, S., Faresjö, M., and Stechova, K. 2006. Immune Regulatory T Cells in Siblings of Children Suffering from Type 1 Diabetes Mellitus. *Scand. J. Immunol.* 64, 5 (2006), 531–535. https://doi.org/10.1111/j.1365-3083.2006.01837.x

Michels, A. W., Landry, L. G., McDaniel, K. A., Yu, L., Campbell-Thompson, M., Kwok, W. W., Jones, K. L., Gottlieb, P. A., Kappler, J. W., Tang, Q., Roep, B. O., Atkinson, M. A., Mathews, C. E., and Nakayama, M. 2017. Islet-Derived CD4 T Cells Targeting Proinsulin in Human Autoimmune Diabetes. *Diabetes* 66, 3 (2017), 722–734. https://doi.org/10.2337/db16-1025

Middleton, D., Halfpenny, I., Meenagh, A., Williams, F., Sivula, J., and Tuomilehto-Wolf, E. 2006. Investigation of KIR gene frequencies in type 1 diabetes mellitus. *Hum Immunol* 67, 12 (2006), 986–90. https://doi.org/10.1016/j.humimm.2006.08.295 Type: Journal article.

Mingari, M. C., Schiavetti, F., Ponte, M., Vitale, C., Maggi, E., Romagnani, S., Demarest, J., Pantaleo, G., Fauci, A. S., and Moretta, L. 1996. Human CD8+ T lymphocyte subsets that express HLA class I-specific inhibitory receptors represent oligoclonally or monoclonally expanded cell populations. *Proc. Natl. Acad. Sci.* 93, 22 (1996), 12433–12438. https://doi.org/10.1073/pnas.93.22.12433

Moayyeri, A., Hammond, C. J., Valdes, A. M., and Spector, T. D. 2013. Cohort Profile: TwinsUK and healthy ageing twin study. *Int J Epidemiol* 42, 1 (2013), 76–85. https://doi.org/10.1093/ije/dyr207

Moesta, A. K., Norman, P. J., Yawata, M., Yawata, N., Gleimer, M., and Parham, P. 2008. Synergistic polymorphism at two positions distal to the ligand-binding site makes KIR2DL2 a stronger receptor for HLA-C than KIR2DL3. *Journal of Immunology (Baltimore, Md.: 1950)* 180, 6 (2008), 3969–3979. https://doi.org/10.4049/jimmunol.180.6.3969

Mogami, S., Hasegawa, G., Nakayama, I., Asano, M., Hosoda, H., Kadono, M., Fukui, M., Kitagawa, Y., Nakano, K., Ohta, M., Obayashi, H., Yoshikawa, T., and Nakamura, N. 2007. Killer cell immunoglobulin-like receptor genotypes in Japanese patients with type 1 diabetes. *Tissue Antigens* 70, 6 (2007), 506–510. https://doi.org/10.1111/j.1399-0039.2007.00956.x

Monaco, G., Lee, B., Xu, W., Mustafah, S., Hwang, Y. Y., Carré, C., Burdin, N., Visan, L., Ceccarelli, M., Poidinger, M., Zippelius, A., Pedro De Magalhães, J., and Larbi, A. 2019. RNA-Seq Signatures Normalized by mRNA Abundance Allow Absolute Deconvolution of Human Immune Cell Types. *Cell Rep.* 26, 6 (2019), 1627–1640.e7. https://doi.org/10.1016/j.celrep.2019.01.041

Mora, T. and Walczak, A. M. 2016. *Quantifying lymphocyte receptor diversity.* preprint. Immunology. https://doi.org/10.1101/046870

Mora-Bitria, L. and Asquith, B. 2023. Innate receptors modulating adaptive T cell responses: KIR-HLA interactions and T cell-mediated control of chronic viral infections. *Immunogenetics* (2023). https://doi.org/10.1007/s00251-023-01293-w

Morandi, B., Mortara, L., Chiossone, L., Accolla, R. S., Mingari, M. C., Moretta, L., Moretta, A., and Ferlazzo, G. 2012. Dendritic cell editing by activated natural killer cells results in a more protective cancer-specific immune response. *PloS One* 7, 6 (2012), e39170. https://doi.org/10.1371/journal.pone.0039170

Morel, P. A., Dorman, J. S., Todd, J. A., McDevitt, H. O., and Trucco, M. 1988. Aspartic acid at position 57 of the HLA-DQ beta chain protects against type I diabetes: a family study. *Proc. Natl. Acad. Sci.* 85, 21 (1988), 8111–8115. https://doi.org/10.1073/pnas.85.21.8111

Moretta, A., Tambussi, G., Bottino, C., Tripodi, G., Merli, A., Ciccone, E., Pantaleo, G., and Moretta, L. 1990. A novel surface antigen expressed by a subset of human CD3- CD16+ natural killer cells. Role in cell activation and regulation of cytolytic function. *J. Exp. Med.* 171, 3 (1990), 695–714. https://doi.org/10.1084/jem.171.3.695

Moretta, A., Vitale, M., Bottino, C., Orengo, A. M., Morelli, L., Augugliaro, R., Barbaresi, M., Ciccone, E., and Moretta, L. 1993. P58 molecules as putative receptors for major histocompatibility complex (MHC) class I molecules in human natural killer (NK) cells. Anti-p58 antibodies reconstitute lysis of MHC class I-protected cells in NK clones displaying different specificities. *J. Exp. Med.* 178, 2 (1993), 597–604. https://doi.org/10.1084/jem.178.2.597

MURPHY, K. M. W. and CASEY. BERG, L. J. 2022. *JANEWAY'S IMMUNOBIOLOGY.* W W NORTON, S.l. OCLC: 1264714311.

Mölder, F., Jablonski, K. P., Letcher, B., Hall, M. B., Tomkins-Tinch, C. H., Sochat, V., Forster, J., Lee, S., Twardziok, S. O., Kanitz, A., Wilm, A., Holtgrewe, M., Rahmann, S., Nahnsen, S., and Köster, J. 2021. Sustainable data analysis with Snakemake. *F1000Research* 10 (2021), 33. https://doi.org/10.12688/f1000research.29032.1

Müller, V., de Boer, R. J., Bonhoeffer, S., and Szathmáry, E. 2018. An evolutionary perspective on the systems of adaptive immunity: Evolution of adaptive immunity. *Biol. Rev.* 93, 1 (2018), 505–528. https://doi.org/10.1111/brv.12355

Nakajima, H., Tomiyama, H., and Takiguchi, M. 1995. Inhibition of gamma delta T cell recognition by receptors for MHC class I molecules. *Journal of Immunology (Baltimore, Md.: 1950)* 155, 9 (1995), 4139–4142.

Nakimuli, A., Chazara, O., Hiby, S. E., Farrell, L., Tukwasibwe, S., Jayaraman, J., Traherne, J. A., Trowsdale, J., Colucci, F., Lougee, E., Vaughan, R. W., Elliott, A. M., Byamugisha, J., Kaleebu, P., Mirembe, F., Nemat-Gorgani, N., Parham, P., Norman, P. J., and Moffett, A. 2015. A KIR B centromeric region present in Africans but not Europeans protects pregnant women from pre-eclampsia. *Proc Natl Acad Sci U S A* 112, 3 (2015), 845–50. https://doi.org/10.1073/pnas.1413453112 Type: Journal article.

Nejentsev, S., Howson, J. M., Walker, N. M., Szeszko, J., Field, S. F., Stevens, H. E., Reynolds, P., Hardy, M., King, E., Masters, J., Hulme, J., Maier, L. M., Smyth, D., Bailey, R., Cooper, J. D., Ribas, G., Campbell, R. D., Clayton, D. G., Todd, J. A., and Wellcome Trust Case Control, C. 2007. Localization of type 1 diabetes susceptibility to the MHC class I genes HLA-B and HLA-A. *Nature* 450, 7171 (2007), 887–92. https://doi.org/10.1038/nature06406 Type: Journal article.

Nerup, J., Platz, P., Andersen, O., Christy, M., Lyngsøe, J., Poulsen, J., Ryder, L., Thomsen, M., Nielsen, L., and Svejgaard, A. 1974. HL-A ANTIGENS AND DIABETES MELLI-

TUS. *The Lancet* 304, 7885 (1974), 864–866. https://doi.org/10.1016/S0140-6736(74)91201-X

Nielsen, N., Ødum, N., Ursø, B., Lanier, L. L., and Spee, P. 2012. Cytotoxicity of CD56bright NK Cells towards Autologous Activated CD4+ T Cells Is Mediated through NKG2D, LFA-1 and TRAIL and Dampened via CD94/NKG2A. *PLoS ONE* 7, 2 (2012), e31959. https://doi.org/10.1371/journal.pone.0031959 Type: Journal article.

Nikitina-Zake, L., Rajalingham, R., Rumba, I., and Sanjeevi, C. B. 2004. Killer cell immunoglobulin-like receptor genes in Latvian patients with type 1 diabetes mellitus and healthy controls. *Ann. N.Y. Acad. Sci.* 1037 (2004), 161–169. https://doi.org/10.1196/annals.1337.027

Nisticò, L., Buzzetti, R., Pritchard, L. E., Van der Auwera, B., Giovannini, C., Bosi, E., Larrad, M. T., Rios, M. S., Chow, C. C., Cockram, C. S., Jacobs, K., Mijovic, C., Bain, S. C., Barnett, A. H., Vandewalle, C. L., Schuit, F., Gorus, F. K., Tosi, R., Pozzilli, P., and Todd, J. A. 1996. The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Belgian Diabetes Registry. *Hum. Mol. Genet.* 5, 7 (1996), 1075–1080. https://doi.org/10.1093/hmg/5.7.1075

Noble, J. A. and Valdes, A. M. 2011. Genetics of the HLA region in the prediction of type 1 diabetes. *Curr Diab Rep* 11, 6 (2011), 533–42. https://doi.org/10.1007/s11892-011-0223-x Type: Journal article.

Noble, J. A., Valdes, A. M., Cook, M., Klitz, W., Thomson, G., and Erlich, H. A. 1996. The role of HLA class II genes in insulin-dependent diabetes mellitus: molecular analysis of 180 Caucasian, multiplex families. *Am. J. Hum. Genet.* 59, 5 (1996), 1134–1148.

Noble, J. A., Valdes, A. M., Varney, M. D., Carlson, J. A., Moonsamy, P., Fear, A. L., Lane, J. A., Lavant, E., Rappner, R., Louey, A., Concannon, P., Mychaleckyj, J. C., Erlich, H. A., and Type 1 Diabetes Genetics, C. 2010. HLA class I and genetic susceptibility to type 1 diabetes: results from the Type 1 Diabetes Genetics Consortium. *Diabetes* 59, 11 (2010), 2972–9. https://doi.org/10.2337/db10-0699 Type: Journal article.

Ooi, J. D., Petersen, J., Tan, Y. H., Huynh, M., Willett, Z. J., Ramarathinam, S. H., Eggenhuizen, P. J., Loh, K. L., Watson, K. A., Gan, P. Y., Alikhan, M. A., Dudek, N. L., Handel, A., Hudson, B. G., Fugger, L., Power, D. A., Holt, S. G., Coates, P. T., Gregersen, J. W., Purcell, A. W., Holdsworth, S. R., La Gruta, N. L., Reid, H. H., Rossjohn, J., and Kitching,

A. R. 2017. Dominant protection from HLA-linked autoimmunity by antigen-specific regulatory T cells. *Nature* 545, 7653 (2017), 243–247. https://doi.org/10.1038/nature22329 Type: Journal article.

Ordonez, D., Gómez-Lozano, N., Rosales, L., and Vilches, C. 2011. Molecular characterisation of KIR2DS2* 005, a fusion gene associated with a shortened KIR haplotype. *Genes & Immunity* 12, 7 (2011), 544–551.

Osman, A. E., Eltayeb-ELSheikh, N., Mubasher, M., Al Harthi, H., Alharbi, S., Hamza, M. A., and ElGhazali, G. 2016. Investigation of activating and inhibitory killer cell immunoglobulin-like receptors and their putative ligands in type 1 diabetes (T1D). *Hum Immunol* 77, 1 (2016), 110–114. https://doi.org/10.1016/j.humimm.2015.10.019

Ott, J. 1989. Statistical properties of the haplotype relative risk. *Genet Epidemiol* 6, 1 (1989), 127–130. https://doi.org/10.1002/gepi.1370060124

Pallmer, K. and Oxenius, A. 2016. Recognition and Regulation of T Cells by NK Cells. *Front. Immunol.* 7 (2016). https://doi.org/10.3389/fimmu.2016.00251

Pando, M. J., Gardiner, C. M., Gleimer, M., McQueen, K. L., and Parham, P. 2003. The Protein Made from a Common Allele of *KIR3DL1* (*3DL1*004*) Is Poorly Expressed at Cell Surfaces due to Substitution at Positions 86 in Ig Domain 0 and 182 in Ig Domain 1. *The Journal of Immunology* 171, 12 (2003), 6640–6649. https://doi.org/10.4049/jimmunol.171.12.6640

Park, Y., Choi, H., Park, H., Park, S., Yoo, E.-K., Kim, D., and Sanjeevi, C. B. 2006. Predominance of the group A killer Ig-like receptor haplotypes in Korean patients with T1D. *Ann. N.Y. Acad. Sci.* 1079 (2006), 240–250. https://doi.org/10.1196/annals.1375.037

Pelak, K., Need, A. C., Fellay, J., Shianna, K. V., Feng, S., Urban, T. J., Ge, D., De Luca, A., Martinez-Picado, J., Wolinsky, S. M., Martinson, J. J., Jamieson, B. D., Bream, J. H., Martin, M. P., Borrow, P., Letvin, N. L., McMichael, A. J., Haynes, B. F., Telenti, A., Carrington, M., Goldstein, D. B., Alter, G., and on behalf of NIAID Center for HIV/AIDS Vaccine Immunology (CHAVI). 2011. Copy Number Variation of KIR Genes Influences HIV-1 Control. *PLoS Biol.* 9, 11 (2011), e1001208. https://doi.org/10.1371/journal.pbio.1001208

Peppa, D., Gill, U. S., Reynolds, G., Easom, N. J., Pallett, L. J., Schurich, A., Micco, L., Nebbia, G., Singh, H. D., Adams, D. H., Kennedy, P. T., and Maini, M. K. 2013. Up-regulation of a death receptor renders antiviral T cells susceptible to NK cell–mediated deletion. *J. Exp. Med.* 210, 1 (2013), 99–114. https://doi.org/10.1084/jem.20121172

Phillips, J. H., Gumperz, J. E., Parham, P., and Lanier, L. L. 1995. Superantigen-Dependent, Cell-Mediated Cytotoxicity Inhibited by MHC Class I Receptors on T Lymphocytes. *Science* 268, 5209 (1995), 403–405. https://doi.org/10.1126/science.7716542

Piccioli, D., Sbrana, S., Melandri, E., and Valiante, N. M. 2002. Contact-dependent Stimulation and Inhibition of Dendritic Cells by Natural Killer Cells. *J. Exp. Med.* 195, 3 (2002), 335–341. https://doi.org/10.1084/jem.20010934

Pieren, D. K. J., Smits, N. A. M., Hoeboer, J., Kandiah, V., Postel, R. J., Mariman, R., van Beek, J., van Baarle, D., de Wit, J., and Guichelaar, T. 2021. Regulatory KIR+ RA+ T cells accumulate with age and are highly activated during viral respiratory disease. *Aging Cell* 20, 6 (2021), e13372. https://doi.org/10.1111/acel.13372

Pollock, N. R., Harrison, G. F., and Norman, P. J. 2022. Immunogenomics of Killer Cell Immunoglobulin-Like Receptor (KIR) and HLA Class I: Coevolution and Consequences for Human Health. *The Journal of Allergy and Clinical Immunology: In Practice* (2022), S2213219822004810. https://doi.org/10.1016/j.jaip.2022.04.036

Pontikos, N., Smyth, D. J., Schuilenburg, H., Howson, J. M., Walker, N. M., Burren, O. S., Guo, H., Onengut-Gumuscu, S., Chen, W. M., Concannon, P., Rich, S. S., Jayaraman, J., Jiang, W., Traherne, J. A., Trowsdale, J., Todd, J. A., and Wallace, C. 2014. A hybrid qPCR/SNP array approach allows cost efficient assessment of KIR gene copy numbers in large samples. *BMC Genomics* 15 (2014), 274. https://doi.org/10.1186/1471-2164-15-274 Type: Journal article.

Power, C. and Elliott, J. 2006. Cohort profile: 1958 British birth cohort (National Child Development Study). *Int J Epidemiol* 35, 1 (2006), 34–41. https://doi.org/10.1093/ije/dyi183

Pugliese, A., Boulware, D., Yu, L., Babu, S., Steck, A. K., Becker, D., Rodriguez, H., DiMeglio, L., Evans-Molina, C., Harrison, L. C., Schatz, D., Palmer, J. P., Greenbaum, C., Eisenbarth, G. S., Sosenko, J. M., and the Type 1 Diabetes TrialNet Study Group. 2016. HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02 Haplotype Protects Autoantibody-Positive Relatives

From Type 1 Diabetes Throughout the Stages of Disease Progression. *Diabetes* 65, 4 (2016), 1109–1119. https://doi.org/10.2337/db15-1105

Pyo, C.-W., Guethlein, L. A., Vu, Q., Wang, R., Abi-Rached, L., Norman, P. J., Marsh, S. G. E., Miller, J. S., Parham, P., and Geraghty, D. E. 2010. Different Patterns of Evolution in the Centromeric and Telomeric Regions of Group A and B Haplotypes of the Human Killer Cell Ig-Like Receptor Locus. *PLoS ONE* 5, 12 (Dec. 2010), e15115. https://doi.org/10.1371/journal.pone.0015115

Qi, Q., Liu, Y., Cheng, Y., Glanville, J., Zhang, D., Lee, J.-Y., Olshen, R. A., Weyand, C. M., Boyd, S. D., and Goronzy, J. J. 2014. Diversity and clonal selection in the human T-cell repertoire. *Proc. Natl. Acad. Sci.* 111, 36 (2014), 13139–13144. https://doi.org/10.1073/pnas.1409155111

Qi, Y., Martin, M. P., Gao, X., Jacobson, L., Goedert, J. J., Buchbinder, S., Kirk, G. D., O'Brien, S. J., Trowsdale, J., and Carrington, M. 2006. KIR/HLA pleiotropism: protection against both HIV and opportunistic infections. *PLoS pathogens* 2, 8 (2006), e79. https://doi.org/10.1371/journal.ppat.0020079

Qin, H., Wang, Z., Du, W., Lee, W. H., Wu, X., Riggs, A. D., and Liu, C. P. 2011. Killer cell Ig-like receptor (KIR) 3DL1 down-regulation enhances inhibition of type 1 diabetes by autoantigen-specific regulatory T cells. *Proc Natl Acad Sci U S A* 108, 5 (2011), 2016–21. https://doi.org/10.1073/pnas.1019082108 Type: Journal article.

R Core Team. 2021. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rabinovich, B. A., Li, J., Shannon, J., Hurren, R., Chalupny, J., Cosman, D., and Miller, R. G. 2003. Activated, But Not Resting, T Cells Can Be Recognized and Killed by Syngeneic NK Cells. *The Journal of Immunology* 170, 7 (2003), 3572–3576. https://doi.org/10.4049/jimmunol.170.7.3572

Rajagopalan, S. and Long, E. O. 2012. KIR2DL4 (CD158d): an activation receptor for HLA-G. *Frontiers in immunology* 3 (2012), 258.

Rajalingam, R., Parham, P., and Abi-Rached, L. 2004. Domain shuffling has been the main mechanism forming new hominoid killer cell Ig-like receptors. *The Journal of Immunology* 172, 1 (2004), 356–369.

Redondo, M. J., Jeffrey, J., Fain, P. R., Eisenbarth, G. S., and Orban, T. 2008. Concordance for islet autoimmunity among monozygotic twins. *N Engl J Med* 359, 26 (2008), 2849–50. https://doi.org/10.1056/NEJMc0805398 Type: Journal article.

Rich, S. S. 2017. Genetics and its potential to improve type 1 diabetes care. *Curr Opin Endocrinol Diabetes Obes* 24, 4 (2017), 279–284. https://doi.org/10.1097/MED.0000000000000347 Type: Journal article.

Risch, N. 1987. Assessing the role of HLA-linked and unlinked determinants of disease. *Am J Hum Genet* 40, 1 (1987), 1–14. Type: Journal article.

Robertson, C. C., Inshaw, J. R. J., Onengut-Gumuscu, S., Chen, W.-M., Santa Cruz, D. F., Yang, H., Cutler, A. J., Crouch, D. J. M., Farber, E., Bridges, S. L., Edberg, J. C., Kimberly, R. P., Buckner, J. H., Deloukas, P., Divers, J., Dabelea, D., Lawrence, J. M., Marcovina, S., Shah, A. S., Greenbaum, C. J., Atkinson, M. A., Gregersen, P. K., Oksenberg, J. R., Pociot, F., Rewers, M. J., Steck, A. K., Dunger, D. B., Type 1 Diabetes Genetics Consortium, Wicker, L. S., Concannon, P., Todd, J. A., and Rich, S. S. 2021. Fine-mapping, trans-ancestral and genomic analyses identify causal variants, cells, genes and drug targets for type 1 diabetes. *Nat. Genet.* 53, 7 (2021), 962–971. https://doi.org/10.1038/s41588-021-00880-5

Robinson, J., Barker, D. J., Georgiou, X., Cooper, M. A., Flicek, P., and Marsh, S. G. E. 2019. IPD-IMGT/HLA Database. *Nucleic Acids Res.* 48, D1 (2019), D948–D955. https://doi.org/10.1093/nar/gkz950 arXiv:https://academic.oup.com/nar/article-pdf/48/D1/D948/31697215/gkz950.pdf

Robinson, J., Halliwell, J. A., McWilliam, H., Lopez, R., and Marsh, S. G. E. 2012. IPD—the Immuno Polymorphism Database. *Nucleic Acids Res.* 41, D1 (2012), D1234–D1240. https://doi.org/10.1093/nar/gks1140

Roe, D., Rosoff, H., Ruan, D. F., Khan, Z., Dogra, P., Carroll, J., Hunkapiller, J., Mohindra, R., Srivastava, M. K., Nabet, B. Y., Chandler, G. S., Albert, M. L., McCarthy, M. I., Mellman, I., Horowitz, A., and Hammer, C. 2022. Natural killer cell educating KIR/HLA combinations impact survival in anti-PD-L1 treated cancer patients. *medRxiv* (2022). https://doi.org/10.1101/2022.12.06.22282592 arXiv:https://www.medrxiv.org/content/early/2022/12/07/2022.12.06.22282592.full.pdf

Roe, D., Vierra-Green, C., Pyo, C.-W., Eng, K., Hall, R., Kuang, R., Spellman, S., Ranade, S., Geraghty, D. E., and Maiers, M. 2017. Revealing complete complex KIR haplotypes phased

by long-read sequencing technology. *Genes & Immunity* 18, 3 (2017), 127–134. https://doi.org/10.1038/gene.2017.10

Roe, D., Vierra-Green, C., Pyo, C.-W., Geraghty, D. E., Spellman, S. R., Maiers, M., and Kuang, R. 2020a. A Detailed View of KIR Haplotype Structures and Gene Families as Provided by a New Motif-Based Multiple Sequence Alignment. *Front. Immunol.* 11 (2020), 585731. https://doi.org/10.3389/fimmu.2020.585731

Roe, D., Williams, J., Ivery, K., Brouckaert, J., Downey, N., Locklear, C., Kuang, R., and Maiers, M. 2020b. Efficient Sequencing, Assembly, and Annotation of Human KIR Haplotypes. *Front. Immunol.* 11 (2020), 582927. https://doi.org/10.3389/fimmu.2020.582927

Roederer, M., Quaye, L., Mangino, M., Beddall, M. H., Mahnke, Y., Chattopadhyay, P., Tosi, I., Napolitano, L., Terranova Barberio, M., Menni, C., Villanova, F., Di Meglio, P., Spector, T. D., and Nestle, F. O. 2015. The genetic architecture of the human immune system: a bioresource for autoimmunity and disease pathogenesis. *Cell* 161, 2 (2015), 387–403. https://doi.org/10.1016/j.cell.2015.02.046

Roger, J., Chalifour, A., Lemieux, S., and Duplay, P. 2001. Cutting Edge: Ly49A Inhibits TCR/CD3-Induced Apoptosis and IL-2 Secretion. *The Journal of Immunology* 167, 1 (2001), 6–10. https://doi.org/10.4049/jimmunol.167.1.6

Roy, S., Barnes, P. F., Garg, A., Wu, S., Cosman, D., and Vankayalapati, R. 2008. NK Cells Lyse T Regulatory Cells That Expand in Response to an Intracellular Pathogen. *The Journal of Immunology* 180, 3 (2008), 1729–1736. https://doi.org/10.4049/jimmunol.180.3.1729

Ruggeri, L., Capanni, M., Casucci, M., Volpi, I., Tosti, A., Perruccio, K., Urbani, E., Negrin, R. S., Martelli, M. F., and Velardi, A. 1999. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. *Blood* 94, 1 (1999), 333–339.

Ruggeri, L., Capanni, M., Urbani, E., Perruccio, K., Shlomchik, W. D., Tosti, A., Posati, S., Rogaia, D., Frassoni, F., Aversa, F., Martelli, M. F., and Velardi, A. 2002. Effectiveness of Donor Natural Killer Cell Alloreactivity in Mismatched Hematopoietic Transplants. *Science* 295, 5562 (2002), 2097–2100. https://doi.org/10.1126/science.1068440

Rumpret, M., Drylewicz, J., Ackermans, L. J. E., Borghans, J. A. M., Medzhitov, R., and Meyaard, L. 2020. Functional categories of immune inhibitory receptors. *Nature*

reviews. Immunology 20, 12 (December 2020), 771—780. https://doi.org/10.1038/s41577-020-0352-z

Rumpret, M., von Richthofen, H. J., Peperzak, V., and Meyaard, L. 2021. Inhibitory pattern recognition receptors. *J. Exp. Med.* 219, 1 (2021), e20211463.

Rydyznski, C., Daniels, K. A., Karmele, E. P., Brooks, T. R., Mahl, S. E., Moran, M. T., Li, C., Sutiwisesak, R., Welsh, R. M., and Waggoner, S. N. 2015. Generation of cellular immune memory and B-cell immunity is impaired by natural killer cells. *Nat Commun* 6, 1 (2015), 6375. https://doi.org/10.1038/ncomms7375 Number: 1 Publisher: Nature Publishing Group.

Rückert, T., Lareau, C. A., Mashreghi, M.-F., Ludwig, L. S., and Romagnani, C. 2022. Clonal expansion and epigenetic inheritance of long-lasting NK cell memory. *Nat. Immunol.* 23, 11 (2022), 1551–1563. https://doi.org/10.1038/s41590-022-01327-7

Saligrama, N., Zhao, F., Sikora, M. J., Serratelli, W. S., Fernandes, R. A., Louis, D. M., Yao, W., Ji, X., Idoyaga, J., Mahajan, V. B., Steinmetz, L. M., Chien, Y.-H., Hauser, S. L., Oksenberg, J. R., Garcia, K. C., and Davis, M. M. 2019. Opposing T cell responses in experimental autoimmune encephalomyelitis. *Nature* 572, 7770 (2019), 481–487. https://doi.org/10.1038/s41586-019-1467-x

Sambrook, J. G., Sehra, H., Coggill, P., Humphray, S., Palmer, S., Sims, S., Takamatsu, H.-H., Wileman, T., Archibald, A. L., and Beck, S. 2006. Identification of a single killer immunoglobulin-like receptor (KIR) gene in the porcine leukocyte receptor complex on chromosome 6q. *Immunogenetics* 58, 5-6 (2006), 481–486. https://doi.org/10.1007/s00251-006-0110-9

Sanjeevi, S., Sun, C., Kanungo, A., and Sanjeevi, C. B. 2016. Killer immunoglobulin receptor genes and their HLA-C ligand are associated with Type 1 diabetes in an Eastern Indian population. *Diabet Med* 33, 1 (2016), 91–6. https://doi.org/10.1111/dme.12815 Type: Journal article.

Santin, I., de Nanclares, G. P., Calvo, B., Gaafar, A., Castaño, L., GEPV-N Group, and Bilbao, J. R. 2006. Killer cell immunoglobulin-like receptor (KIR) genes in the Basque population: association study of KIR gene contents with type 1 diabetes mellitus. *Hum Immunol* 67, 1-2 (2006), 118–124. https://doi.org/10.1016/j.humimm.2006.02.036

Saunders, P. M., Pymm, P., Pietra, G., Hughes, V. A., Hitchen, C., O'Connor, G. M., Loiacono, F., Widjaja, J., Price, D. A., Falco, M., Mingari, M. C., Moretta, L., McVicar, D. W., Rossjohn, J., Brooks, A. G., and Vivian, J. P. 2016. Killer cell immunoglobulin-like receptor 3DL1 polymorphism defines distinct hierarchies of HLA class I recognition. *The Journal of Experimental Medicine* 213, 5 (2016), 791–807. https://doi.org/10.1084/jem.20152023

Savastio, S., Cadario, F., D'Alfonso, S., Stracuzzi, M., Pozzi, E., Raviolo, S., Rizzollo, S., Gigliotti, L., Boggio, E., Bellomo, G., Basagni, C., Bona, G., Rabbone, I., Dianzani, U., and Prodam, F. 2020. Vitamin D Supplementation Modulates ICOS+ and ICOS- Regulatory T Cell in Siblings of Children With Type 1 Diabetes. *The Journal of Clinical Endocrinology and Metabolism* 105, 12 (2020), dgaa588. https://doi.org/10.1210/clinem/dgaa588

Schwartz, J. C. and Hammond, J. A. 2018. The unique evolution of the pig LRC, a single KIR but expansion of LILR and a novel Ig receptor family. *Immunogenetics* 70, 10 (2018), 661–669. https://doi.org/10.1007/s00251-018-1067-1

Seich al Basatena, N.-K., MacNamara, A., Vine, A. M., Thio, C. L., Astemborski, J., Usuku, K., Osame, M., Kirk, G. D., Donfield, S. M., Goedert, J. J., Bangham, C. R., Carrington, M., Khakoo, S. I., and Asquith, B. 2011. KIR2DL2 Enhances Protective and Detrimental HLA Class I-Mediated Immunity in Chronic Viral Infection. *PLoS Pathog.* 7, 10 (2011), e1002270. https://doi.org/10.1371/journal.ppat.1002270

Shastry, A., Sedimbi, S. K., Rajalingam, R., Nikitina-Zake, L., Rumba, I., Wigzell, H., and Sanjeevi, C. B. 2008. Combination of KIR 2DL2 and HLA-C1 (Asn 80) confers susceptibility to type 1 diabetes in Latvians. *Int J Immunogenet* 35, 6 (2008), 439–46. https://doi.org/10.1111/j.1744-313X.2008.00804.x Type: Journal article.

Sim, M. J. W., Brennan, P., Wahl, K. L., Lu, J., Rajagopalan, S., Sun, P. D., and Long, E. O. 2023. *Innate receptors with high specificity for HLA class I–peptide complexes*. preprint. Immunology. https://doi.org/10.1101/2023.02.06.527249

Simmons, K. M., Mitchell, A. M., Alkanani, A. A., McDaniel, K. A., Baschal, E. E., Armstrong, T., Pyle, L., Yu, L., and Michels, A. W. 2020. Failed Genetic Protection: Type 1 Diabetes in the Presence of HLA-DQB1*06:02. *Diabetes* 69, 8 (2020), 1763–1769. https://doi.org/10.2337/db20-0038

Singal, D. P. and Blajchman, M. A. 1973. Histocompatibility (HL-A) Antigens, Lymphocyto-

toxic Antibodies and Tissue Antibodies in Patients with Diabetes Mellitus. *Diabetes* 22, 6 (1973), 429–432. https://doi.org/10.2337/diab.22.6.429

Smigoc Schweiger, D., Mendez, A., Kunilo Jamnik, S., Bratanic, N., Bratina, N., Battelino, T., Brecelj, J., and Vidan-Jeras, B. 2014. Genetic risk for co-occurrence of type 1 diabetes and celiac disease is modified by HLA-C and killer immunoglobulin-like receptors. *Tissue Antigens* 84, 5 (2014), 471–478. https://doi.org/10.1111/tan.12450

Smith, M. J., Packard, T. A., O'Neill, S. K., Henry Dunand, C. J., Huang, M., Fitzgerald-Miller, L., Stowell, D., Hinman, R. M., Wilson, P. C., Gottlieb, P. A., and Cambier, J. C. 2015. Loss of Anergic B Cells in Prediabetic and New-Onset Type 1 Diabetic Patients. *Diabetes* 64, 5 (2015), 1703–1712. https://doi.org/10.2337/db13-1798

Smith, S. D., Dunk, C. E., Aplin, J. D., Harris, L. K., and Jones, R. L. 2009. Evidence for Immune Cell Involvement in Decidual Spiral Arteriole Remodeling in Early Human Pregnancy. *The American Journal of Pathology* 174, 5 (2009), 1959–1971. https://doi.org/10.2353/ajpath.2009.080995

Soderquest, K., Walzer, T., Zafirova, B., Klavinskis, L. S., Polić, B., Vivier, E., Lord, G. M., and Martín-Fontecha, A. 2011. Cutting Edge: CD8 $^{+}$ T Cell Priming in the Absence of NK Cells Leads to Enhanced Memory Responses. *The Journal of Immunology* 186, 6 (2011), 3304–3308. https://doi.org/10.4049/jimmunol.1004122

Sojka, D. K. 2020. Uterine Natural Killer Cell Heterogeneity: Lessons From Mouse Models. *Front. Immunol.* 11 (2020), 290. https://doi.org/10.3389/fimmu.2020.00290

Sollis, E., Mosaku, A., Abid, A., Buniello, A., Cerezo, M., Gil, L., Groza, T., Güneş, O., Hall, P., Hayhurst, J., Ibrahim, A., Ji, Y., John, S., Lewis, E., MacArthur, J. A. L., McMahon, A., Osumi-Sutherland, D., Panoutsopoulou, K., Pendlington, Z., Ramachandran, S., Stefancsik, R., Stewart, J., Whetzel, P., Wilson, R., Hindorff, L., Cunningham, F., Lambert, S. A., Inouye, M., Parkinson, H., and Harris, L. W. 2023. The NHGRI-EBI GWAS Catalog: knowledgebase and deposition resource. *Nucleic Acids Res.* 51, D1 (2023), D977–D985. https://doi.org/10.1093/nar/gkac1010

Song, L., Bai, G., Liu, X. S., Li, B., and Li, H. 2023. Efficient and accurate KIR and HLA genotyping with massively parallel sequencing data. *Genome Res.* (2023), gr.277585.122. https://doi.org/10.1101/gr.277585.122

Sontag, E. D. 2017. A Dynamic Model of Immune Responses to Antigen Presentation Predicts Different Regions of Tumor or Pathogen Elimination. *Cell Systems* 4, 2 (2017), 231–241.e11. https://doi.org/10.1016/j.cels.2016.12.003

Speiser, D. E., Pittet, M. J., Valmori, D., Dunbar, R., Rimoldi, D., Liénard, D., MacDonald, H. R., Cerottini, J. C., Cerundolo, V., and Romero, P. 1999. In vivo expression of natural killer cell inhibitory receptors by human melanoma-specific cytolytic T lymphocytes. *The Journal of Experimental Medicine* 190, 6 (1999), 775–782. https://doi.org/10.1084/jem.190.6.775

Spielman, R. S., McGinnis, R. E., and Ewens, W. J. 1993. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am. J. Hum. Genet.* 52, 3 (1993), 506–516.

Stern, M., Ruggeri, L., Capanni, M., Mancusi, A., and Velardi, A. 2008. Human leukocyte antigens A23, A24, and A32 but not A25 are ligands for KIR3DL1. *Blood* 112, 3 (2008), 708–710. https://doi.org/10.1182/blood-2008-02-137521

Sullivan, L. C., Nguyen, T. H., Harpur, C. M., Stankovic, S., Kanagarajah, A. R., Koutsakos, M., Saunders, P. M., Cai, Z., Gray, J. A., Widjaja, J. M., Lin, J., Pietra, G., Mingari, M. C., Moretta, L., Samir, J., Luciani, F., Westall, G. P., Malmberg, K. J., Kedzierska, K., and Brooks, A. G. 2021. Natural killer cell receptors regulate responses of HLA-E–restricted T cells. *Science Immunology* 6, 58 (2021), eabe9057. https://doi.org/10.1126/sciimmunol.abe9057

Takahashi, T., Yawata, M., Raudsepp, T., Lear, T. L., Chowdhary, B. P., Antczak, D. F., and Kasahara, M. 2004. Natural killer cell receptors in the horse: evidence for the existence of multiple transcribed LY49 genes. *Eur. J. Immunol.* 34, 3 (2004), 773–784. https://doi.org/10.1002/eji.200324695

Takao, S., Ishikawa, T., Yamashita, K., and Uchiyama, T. 2010. The rapid induction of HLA-E is essential for the survival of antigen-activated naive CD4 T cells from attack by NK cells. *J Immunol* 185, 10 (2010), 6031–40. https://doi.org/10.4049/jimmunol.1000176 Type: Journal article.

The Milieu Intérieur Consortium, Patin, E., Hasan, M., Bergstedt, J., Rouilly, V., Libri, V., Urrutia, A., Alanio, C., Scepanovic, P., Hammer, C., Jönsson, F., Beitz, B., Quach, H., Lim, Y. W., Hunkapiller, J., Zepeda, M., Green, C., Piasecka, B., Leloup, C., Rogge, L., Huetz,

F., Peguillet, I., Lantz, O., Fontes, M., Di Santo, J. P., Thomas, S., Fellay, J., Duffy, D., Quintana-Murci, L., and Albert, M. L. 2018. Natural variation in the parameters of innate immune cells is preferentially driven by genetic factors. *Nat. Immunol.* 19, 3 (2018), 302–314. https://doi.org/10.1038/s41590-018-0049-7

Thöns, C., Senff, T., Hydes, T. J., Manser, A. R., Heinemann, F. M., Heinold, A., Heilmann, M., Kim, A. Y., Uhrberg, M., Scherbaum, N., Lauer, G. M., Khakoo, S. I., and Timm, J. 2017. HLA-Bw4 80(T) and multiple HLA-Bw4 copies combined with KIR3DL1 associate with spontaneous clearance of HCV infection in people who inject drugs. *J Hepatol* 67, 3 (2017), 462–470. https://doi.org/10.1016/j.jhep.2017.03.040

Todd, J. A., Bell, J. I., and McDevitt, H. O. 1987. HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* 329, 6140 (1987), 599–604. https://doi.org/10.1038/329599a0

Traherne, J. A., Jiang, W., Valdes, A. M., Hollenbach, J. A., Jayaraman, J., Lane, J. A., Johnson, C., Trowsdale, J., and Noble, J. A. 2016. KIR haplotypes are associated with late-onset type 1 diabetes in European-American families. *Genes Immun* 17, 1 (2016), 8–12. https://doi.org/10.1038/gene.2015.44

Traherne, J. A., Martin, M., Ward, R., Ohashi, M., Pellett, F., Gladman, D., Middleton, D., Carrington, M., and Trowsdale, J. 2010. Mechanisms of copy number variation and hybrid gene formation in the KIR immune gene complex. *Hum. Mol. Genet.* 19, 5 (2010), 737–751. https://doi.org/10.1093/hmg/ddp538

Trefny, M. P., Rothschild, S. I., Uhlenbrock, F., Rieder, D., Kasenda, B., Stanczak, M. A., Berner, F., Kashyap, A. S., Kaiser, M., Herzig, P., Poechtrager, S., Thommen, D. S., Geier, F., Savic, S., Jermann, P., Alborelli, I., Schaub, S., Stenner, F., Früh, M., Trajanoski, Z., Flatz, L., Mertz, K. D., Zippelius, A., and Läubli, H. 2019. A Variant of a Killer Cell Immunoglobulin-like Receptor Is Associated with Resistance to PD-1 Blockade in Lung Cancer. *Clinical Cancer Research* 25, 10 (05 2019), 3026–3034. https://doi.org/10.1158/1078-0432.CCR-18-3041 arXiv:https://aacrjournals.org/clincancerres/article-pdf/25/10/3026/2050664/3026.pdf

Ugolini, S., Arpin, C., Anfossi, N., Walzer, T., Cambiaggi, A., Förster, R., Lipp, M., Toes, R. E., Melief, C. J., Marvel, J., and Vivier, E. 2001. Involvement of inhibitory NKRs in

the survival of a subset of memory-phenotype CD8+ T cells. *Nat. Immunol.* 2, 5 (2001), 430–435. https://doi.org/10.1038/87740

Ugolini, S. and Vivier, E. 2000. Regulation of T cell function by NK cell receptors for classical MHC class I molecules. *Curr. Opin. Immunol.* 12, 3 (2000), 295–300. https://doi.org/10.1016/S0952-7915(00)00090-X

Uhrberg, M. 2005. Shaping the human NK cell repertoire: an epigenetic glance at KIR gene regulation. *Mol. Immunol.* 42, 4 (2005), 471–475. https://doi.org/10.1016/j.molimm.2004.07.029

Uhrberg, M., Valiante, N. M., Shum, B. P., Shilling, H. G., Lienert-Weidenbach, K., Corliss, B., Tyan, D., Lanier, L. L., and Parham, P. 1997. Human Diversity in Killer Cell Inhibitory Receptor Genes. *Immunity* 7, 6 (1997), 753–763. https://doi.org/10.1016/S1074-7613(00)80394-5

Valiante, N. M. and Parham, P. 1997. Natural killer cells, HLA class I molecules, and marrow transplantation. *Biology of Blood and Marrow Transplantation: Journal of the American Society for Blood and Marrow Transplantation* 3, 5 (1997), 229–235.

Valés-Gómez, M., Reyburn, H. T., Erskine, R. A., and Strominger, J. 1998. Differential binding to HLA-C of p50-activating and p58-inhibitory natural killer cell receptors. *Proc. Natl. Acad. Sci.* 95, 24 (1998), 14326–14331. https://doi.org/10.1073/pnas.95.24.14326

van Bergen, J., Thompson, A., van der Slik, A., Ottenhoff, T. H. M., Gussekloo, J., and Koning, F. 2004. Phenotypic and Functional Characterization of CD4 T Cells Expressing Killer Ig-Like Receptors. *The Journal of Immunology* 173, 11 (2004), 6719–6726. https://doi.org/10.4049/jimmunol.173.11.6719

van der Slik, A. R., Alizadeh, B. Z., Koeleman, B. P. C., Roep, B. O., and Giphart, M. J. 2007. Modelling KIR-HLA genotype disparities in type 1 diabetes. *Tissue Antigens* 69 Suppl 1 (2007), 101–105. https://doi.org/10.1111/j.1399-0039.2006.762_5.x

van der Slik, A. R., Koeleman, B. P., Verduijn, W., Bruining, G. J., Roep, B. O., and Giphart, M. J. 2003. KIR in type 1 diabetes: disparate distribution of activating and inhibitory natural killer cell receptors in patients versus HLA-matched control subjects. *Diabetes* 52, 10 (2003), 2639–42. https://doi.org/10.2337/diabetes.52.10.2639 Type: Journal article.

van der Veken, L. T., Diez Campelo, M., van der Hoorn, M. A. W. G., Hagedoorn, R. S., van Egmond, H. M. E., van Bergen, J., Willemze, R., Falkenburg, J. H. F., and Heemskerk, M. H. M. 2009. Functional Analysis of Killer Ig-Like Receptor-Expressing Cytomegalovirus-Specific CD8 $^{+}$ T Cells. *The Journal of Immunology* 182, 1 (2009), 92–101. https://doi.org/10.4049/jimmunol.182.1.92

van Lummel, M., Buis, D. T. P., Ringeling, C., de Ru, A. H., Pool, J., Papadopoulos, G. K., van Veelen, P. A., Reijonen, H., Drijfhout, J. W., and Roep, B. O. 2019. Epitope Stealing as a Mechanism of Dominant Protection by HLA-DQ6 in Type 1 Diabetes. *Diabetes* 68, 4 (2019), 787–795. https://doi.org/10.2337/db18-0501 Type: Journal article.

VandenBussche, C. J., Dakshanamurthy, S., Posch, P. E., and Hurley, C. K. 2006. A Single Polymorphism Disrupts the Killer Ig-Like Receptor 2DL2/2DL3 D1 Domain. *The Journal of Immunology* 177, 8 (2006), 5347–5357. https://doi.org/10.4049/jimmunol.177.8.5347

Vely, F., Peyrat, M.-A., Couedel, C., Morcet, J.-F., Halary, F., Davodeau, F., Romagne, F., Scotet, E., Saulquin, X., Houssaint, E., Schleinitz, N., Moretta, A., Vivier, E., and Bonneville, M. 2001. Regulation of Inhibitory and Activating Killer-Cell Ig-Like Receptor Expression Occurs in T Cells After Termination of TCR Rearrangements. *The Journal of Immunology* 166, 4 (2001), 2487–2494. https://doi.org/10.4049/jimmunol.166.4.2487

Vivier, E. and Daëron, M. 1997. Immunoreceptor tyrosine-based inhibition motifs. *Immunol. Today* 18, 6 (1997), 286–291. https://doi.org/10.1016/S0167-5699(97)80025-4

Vukcevic, D., Traherne, J. A., Naess, S., Ellinghaus, E., Kamatani, Y., Dilthey, A., Lathrop, M., Karlsen, T. H., Franke, A., Moffatt, M., Cookson, W., Trowsdale, J., McVean, G., Sawcer, S., and Leslie, S. 2015. Imputation of KIR Types from SNP Variation Data. *Am J Hum Genet* 97, 4 (2015), 593–607. https://doi.org/10.1016/j.ajhg.2015.09.005 Type: Journal article.

Vukmanovic-Stejic, M., Zhang, Y., Cook, J. E., Fletcher, J. M., McQuaid, A., Masters, J. E., Rustin, M. H. A., Taams, L. S., Beverley, P. C. L., Macallan, D. C., and Akbar, A. N. 2006. Human CD4+ CD25hi Foxp3+ regulatory T cells are derived by rapid turnover of memory populations in vivo. *The Journal of Clinical Investigation* 116, 9 (2006), 2423–2433. https://doi.org/10.1172/JCI28941

Waggoner, S. N., Cornberg, M., Selin, L. K., and Welsh, R. M. 2011. Natural killer cells act

as rheostats modulating antiviral T cells. *Nature* 481, 7381 (2011), 394–8. Type: Journal article.

Waggoner, S. N., Cornberg, M., Selin, L. K., and Welsh, R. M. 2012. Natural killer cells act as rheostats modulating antiviral T cells. *Nature* 481, 7381 (2012), 394–398. https://doi.org/10.1038/nature10624

Waggoner, S. N., Reighard, S. D., Gyurova, I. E., Cranert, S. A., Mahl, S. E., Karmele, E. P., McNally, J. P., Moran, M. T., Brooks, T. R., Yaqoob, F., and Rydyznski, C. E. 2016. Roles of natural killer cells in antiviral immunity. *Current Opinion in Virology* 16 (2016), 15–23. https://doi.org/10.1016/j.coviro.2015.10.008

Wallace, A. E., Fraser, R., and Cartwright, J. E. 2012. Extravillous trophoblast and decidual natural killer cells: a remodelling partnership. *Hum Reprod Update* 18, 4 (2012), 458–471. https://doi.org/10.1093/humupd/dms015 arXiv:https://academic.oup.com/humupd/article-pdf/18/4/458/1928515/dms015.pdf

Wang, C. J., Petersone, L., Edner, N. M., Heuts, F., Ovcinnikovs, V., Ntavli, E., Kogimtzis, A., Fabri, A., Elfaki, Y., Houghton, L. P., Hosse, R. J., Schubert, D. A., Frei, A. P., Ross, E. M., and Walker, L. S. K. 2022. Costimulation blockade in combination with IL-2 permits regulatory T cell sparing immunomodulation that inhibits autoimmunity. *Nat Commun* 13, 1 (2022), 6757. https://doi.org/10.1038/s41467-022-34477-1

Welsh, R. M. and Waggoner, S. N. 2013. NK cells controlling virus-specific T cells: Rheostats for acute vs. persistent infections. *Virology* 435, 1 (2013), 37–45. https://doi.org/10.1016/j.virol.2012.10.005

Wen, X., Yang, J., James, E., Chow, I. T., Reijonen, H., and Kwok, W. W. 2020. Increased islet antigen-specific regulatory and effector CD4(+) T cells in healthy individuals with the type 1 diabetes-protective haplotype. *Sci Immunol* 5, 44 (2020). https://doi.org/10.1126/sciimmunol.aax8767 Type: Journal article.

Westfall, P. H. and Young, S. S. 1993. *Resampling-based multiple testing: Examples and methods for p-value adjustment*. Vol. 279. John Wiley & Sons.

Wherry, E. J. and Kurachi, M. 2015. Molecular and cellular insights into T cell exhaustion. *Nat. Rev. Immunol.* 15, 8 (2015), 486–499.

Winter, C. C., Gumperz, J. E., Parham, P., Long, E. O., and Wagtmann, N. 1998. Direct Binding and Functional Transfer of NK Cell Inhibitory Receptors Reveal Novel Patterns of HLA-C Allotype Recognition1. *The Journal of Immunology* 161, 2 (07 1998), 571–577. https://doi.org/10.4049/jimmunol.161.2.571 arXiv:https://journals.aai.org/jimmunol/article-pdf/161/2/571/1082357/im149800571o.pdf

Wong-Baeza, I., Ridley, A., Shaw, J., Hatano, H., Rysnik, O., McHugh, K., Piper, C., Brackenbridge, S., Fernandes, R., Chan, A., Bowness, P., and Kollnberger, S. 2013. KIR3DL2 Binds to HLA-B27 Dimers and Free H Chains More Strongly than Other HLA Class I and Promotes the Expansion of T Cells in Ankylosing Spondylitis. *The Journal of Immunology* 190, 7 (2013), 3216–3224. https://doi.org/10.4049/jimmunol.1202926 Type: Journal article.

Wu, Z., Park, S., Lau, C. M., Zhong, Y., Sheppard, S., Sun, J. C., Das, J., Altan-Bonnet, G., and Hsu, K. C. 2021. Dynamic variability in SHP-1 abundance determines natural killer cell responsiveness. *Sci. Signal.* 14, 708 (2021), eabe5380. https://doi.org/10.1126/scisignal.abe5380

Xu, H. C., Grusdat, M., Pandyra, A. A., Polz, R., Huang, J., Sharma, P., Deenen, R., Köhrer, K., Rahbar, R., Diefenbach, A., Gibbert, K., Löhning, M., Höcker, L., Waibler, Z., Häussinger, D., Mak, T. W., Ohashi, P. S., Lang, K. S., and Lang, P. A. 2014. Type I Interferon Protects Antiviral CD8+ T Cells from NK Cell Cytotoxicity. *Immunity* 40, 6 (2014), 949–960. https://doi.org/10.1016/j.immuni.2014.05.004

Yawata, M., Yawata, N., Draghi, M., Little, A.-M., Partheniou, F., and Parham, P. 2006. Roles for HLA and KIR polymorphisms in natural killer cell repertoire selection and modulation of effector function. *The Journal of Experimental Medicine* 203, 3 (2006), 633–645. https://doi.org/10.1084/jem.20051884

Young, N. T., Uhrberg, M., Phillips, J. H., Lanier, L. L., and Parham, P. 2001. Differential Expression of Leukocyte Receptor Complex-Encoded Ig-Like Receptors Correlates with the Transition from Effector to Memory CTL. *The Journal of Immunology* 166, 6 (2001), 3933–3941. https://doi.org/10.4049/jimmunol.166.6.3933

Yu, W., Jiang, N., Ebert, P. J., Kidd, B. A., Müller, S., Lund, P. J., Juang, J., Adachi, K., Tse, T., Birnbaum, M. E., Newell, E. W., Wilson, D. M., Grotenbreg, G. M., Valitutti, S., Quake, S. R., and Davis, M. M. 2015. Clonal Deletion Prunes but Does Not Eliminate Self-Specific

$\alpha\beta$ CD8+ T Lymphocytes. *Immunity* 42, 5 (2015), 929–941. https://doi.org/10.1016/j.immuni.2015.05.001

Zajac, A. J., Vance, R. E., Held, W., Sourdive, D. J., Altman, J. D., Raulet, D. H., and Ahmed, R. 1999. Impaired anti-viral T cell responses due to expression of the Ly49A inhibitory receptor. *Journal of Immunology (Baltimore, Md.: 1950)* 163, 10 (1999), 5526–5534.

Zhang, Y., Yan, A. W., Boelen, L., Hadcocks, L., Salam, A., Padrosa Gispert, D., Spanos, L., Mora Bitria, L., Nemat-Gorgani, N., Traherne, J. A., Roberts, C. H., Koftori, D. A., Taylor, G. P., Forton, D., Norman, P. J., Marsh, S. G., Busch, R., Macallan, D., and Asquith, B. 2023. KIR-HLA interactions extend human CD8+ T cell lifespan in vivo. *The Journal of Clinical Investigation* (2023), e169496. https://doi.org/10.1172/JCI169496

Zhao, L. P., Papadopoulos, G. K., Kwok, W. W., Moustakas, A. K., Bondinas, G. P., Carlsson, A., Elding Larsson, H., Ludvigsson, J., Marcus, C., Samuelsson, U., Wang, R., Pyo, C. W., Nelson, W. C., Geraghty, D. E., and Lernmark, 2020. Next-Generation HLA Sequence Analysis Uncovers Seven HLA-DQ Amino Acid Residues and Six Motifs Resistant to Childhood Type 1 Diabetes. *Diabetes* 69, 11 (2020), 2523–2535. https://doi.org/10.2337/db20-0374 Type: Journal article.

Zheng, X., Shen, J., Cox, C., Wakefield, J. C., Ehm, M. G., Nelson, M. R., and Weir, B. S. 2014. HIBAG–HLA genotype imputation with attribute bagging. *Pharmacogenomics J* 14, 2 (2014), 192–200. https://doi.org/10.1038/tpj.2013.18 Type: Journal article.

Zhi, D., Sun, C., Sedimbi, S. K., Luo, F., Shen, S., and Sanjeevi, C. B. 2011. Killer cell immunoglobulin-like receptor along with HLA-C ligand genes are associated with type 1 diabetes in Chinese Han population. *Diabetes Metab Res Rev* 27, 8 (2011), 872–7. https://doi.org/10.1002/dmrr.1264 Type: Journal article.

Zohar, O., Reiter, Y., Bennink, J. R., Lev, A., Cavallaro, S., Paratore, S., Pick, C. G., Brooker, G., and Yewdell, J. W. 2008. Cutting Edge: MHC Class I–Ly49 Interaction Regulates Neuronal Function. *The Journal of Immunology* 180, 10 (2008), 6447–6451. https://doi.org/10.4049/jimmunol.180.10.6447

Appendix A

Immunogenetic analysis of a T1D case control cohort

A.1 iKIR score modifies the *DQ6* protective association

Threshold	Group	ln[OR]	2.50%	97.50%	P-value	N genotype +		N genotype -	
						Cases	Controls	Cases	Controls
Unstratified	**Whole cohort**	-3.741	-4.026	-3.477	1.05E-157	54	1545	6165	4197
1.5	**iKIR high**	-3.486	-3.792	-3.207	1.52E-03	48	1190	4730	3598
	iKIR low	-4.955	-5.887	-4.233		6	355	1435	599
1.75	**iKIR high**	-3.250	-3.628	-2.909	4.61E-04	32	734	2795	2487
	iKIR low	-4.292	-4.749	-3.890		22	811	3370	1710
2	**iKIR high**	-3.278	-3.684	-2.915	4.09E-03	28	640	2424	2091
	iKIR low	-4.037	-4.439	-3.677		28	923	3874	2263
2.5	**iKIR high**	-3.345	-3.767	-2.970	1.89E-02	26	622	2291	1934
	iKIR low	-4.037	-4.439	-3.677		28	923	3874	2263

Table A.1: *DQ6* protection in T1D is enhanced amongst individuals with a low iKIR score. The cohort was stratified into individuals with high or low iKIR score using different iKIR score thresholds (1.5, 1.75, 2.0 and 2.5). The protective effect of *DQ6* was evaluated independently in each stratum using multivariate logistic regression with gender included as a covariate. *DQ6* is significantly protective (ln[OR]= -3.74, P=1.05e-157) in the cohort (Group=Whole cohort, unstratified analysis). Regression coefficients, p-values and cohort sizes are reported for the different strata. P-value for the unstratified analysis calculated using Wald-test, all other p-values calculated using a permutation test.

A.2 HLA drivers in T1D

	Genotype	ln[OR]	P-value	N cases	N controls
Protective	*DQA1*01:02^DQA1*01:03*	-4.24	2.63E-09	2	125
	*DQA1*01:02^DQB1*06:03*	-3.85	4.30E-11	3	128
	*DQA1*01:02-DQB1*06:02*	-3.74	1.10E-157	54	1545
	*DQA1*01:01^DQA1*05:05-DQB1*03:01-DQB1*05:01*	-3.08	2.00E-13	6	118
	*DRB1*14:01^DQA1*01:04^DQB1*05:03*	-2.94	5.10E-30	16	265
	*DQA1*02:01^DQB1*03:03*	-2.60	3.10E-63	46	524
	*DQA1*01:02^DQB1*05:01*	-2.08	5.20E-37	43	304
	*DRB1*07:01^DQA1*02:01^DQB1*05:01*	-2.06	3.90E-28	33	232
	*DRB1*04:07^DQA1*03:03^DQB1*03:01*	-1.95	3.20E-15	19	121
	*DQA1*01:02*	-1.80	5.60E-249	526	2051
	*DQA1*05:05^DQB1*03:01*	-1.70	9.20E-104	213	933
	*DRB1*10:01^DQA1*01:05^DQB1*05:01*	-1.67	1.20E-08	14	69
	*B*57:01*	-1.67	5.90E-52	103	474
	*DQA1*01:03*	-1.51	8.80E-63	161	618
	*DQA1*02:01*	-1.37	8.50E-149	573	1639
	*DQB1*03:01*	-1.16	4.10E-129	759	1773
	*C*08:02*	-0.94	4.00E-29	215	484
	*A*26:01*	-0.63	9.50E-09	137	232
	*C*04:01*	-0.54	9.20E-22	593	882
	*A*32:01*	-0.53	1.30E-11	283	432
	*A*11:01*	-0.50	9.60E-16	472	687
	Genotype	**ln[OR]**	**P-value**	**N cases**	**N controls**
Detrimental	*A*24:02*	0.45	1.10E-19	1284	820
	*B*18:01*	0.73	9.20E-30	827	397
	*A*30:02*	1.08	3.20E-20	305	98
	*DRB1*04:01-DQA1*01:01*	1.25	2.10E-39	522	146
	*DRB1*03:01^DQA1*05:01^DQB1*02:01*	1.34	7.10E-257	3724	1612
	*B*39:06*	1.48	1.10E-31	356	78
	*DRB1*04:05*	1.86	7.90E-28	270	40
	*DRB1*04:01^DQB1*03:02*	2.01	0.00E+00	2849	583
	*DQB1*03:02*	2.04	0.00E+00	3996	1085
	*DRB1*03:01^DQA1*05:01^DQB1*02:01^DQB1*03:02*	2.94	7.20E-264	2177	159

Table A.2: HLA-I and HLA-II genotypes most closely identified with outcome in the GRID cohort. This table shows the "driver" genotypes which were independently associated with outcome. We report the natural log of the odds ratio (ln[OR]) and associated p-value for each genotype in multiple logistic regression in the whole cohort with gender as an additional covariate (i.e. the coefficients and p-values are derived in a model containing one genotype at a time). We also report the number of cases and the number of controls carrying this genotype.

A.3 Detrimental genotypes are not modified by iKIR score

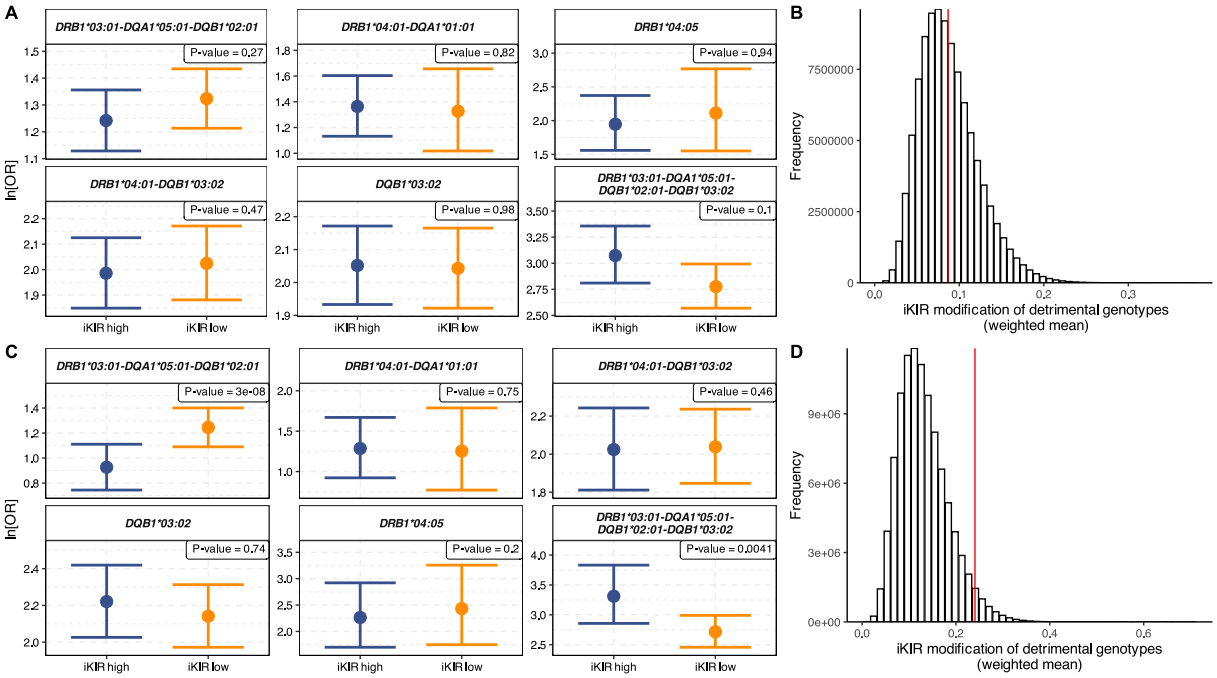


Figure A.1: Functional iKIR do not consistently modify detrimental genotypes. **A** In the whole cohort, for most detrimental genotypes, iKIR have no impact on the detrimental effect (the ln[OR] of the detrimental genotype is very similar in the strata with a high iKIR score (blue) and the strata with a low iKIR score (yellow) and the 95% confidence intervals are overlapping). Two genotypes (*DRB1*03:01^DQA1*05:01^DQB1*02:01* and *DRB1*03:01^DQA1*05:01^DQB1*02:01^DQB1*03:02*) show some evidence of iKIR modification but the modifications are in opposite directions. **B** The observed value of our test statistic (weighted mean of the difference in ln[OR] between the KIR high and the KIR low strata at threshold=1.75), indicated by the red line, is entirely consistent with the distribution (grey histogram) of the same test statistic under the null hypothesis that the iKIR score has no impact on the detrimental genotypes (generated by permuting the iKIR score of individuals in the cohort). Indicating that the probability of obtaining our observation by chance is high (P=0.36) and that there is no evidence to reject the null hypothesis of no iKIR modification. **C** Similarly in the cohort with HLA class I drivers removed. Most genotypes are not modified and where there is modification then results are in opposite directions. **D** In the cohort without HLA class I drivers the observed value of our test statistic (indicated by the red line), whilst still overlapping, is more of an outlier from the distribution (grey histogram) of the same test statistic under the null hypothesis that the iKIR score has no impact on the detrimental genotypes. This decrease in the test statistic is driven entirely by one genotype which is strongly iKIR modified (*DRB1*03:01^DQA1*05:01^DQB1*02:01*). Overall, there is some evidence for iKIR modification in this subcohort (P=0.02) but it is far from convincing. Examining the apparent iKIR modification of *DRB1*03:01^DQA1*05:01^DQB1*02:01* we found that it was explained by the negative correlation of *DRB1*03:01^DQA1*05:01^DQB1*02:01* with the protective genotype *DQA1*01:02^DQB1*06:02*, which is modified by functional iKIR score since, upon exclusion of *DQB1*0602 carriers*, *DRB1*03:01^DQA1*05:01^DQB1*02:01* was no longer iKIR modified (the converse was not the case i.e. *DQA1*01:02^DQB1*06:02* was still significantly modified by functional iKIR when *DRB1*03:01^DQA1*05:01^DQB1*02:01* carriers were excluded from the cohort). In the corresponding permutation test the p value becomes even less significant (P=0.19). We conclude that there is no evidence that detrimental class II drivers are modified by functional iKIR.

Appendix B

iKIR expression in T1D

B.1 Expression of KIR transcripts in T and NK cells

	KIR2DL1		KIR2DL3		KIR3DL1		KIR		Cells	
Cell type (Monaco reference)	**T1D**	**HC**	**T1D**	**HC**	**T1D**	**HC**	**T1D**	**HC**	**T1D**	**HC**
Unknown	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	10
Central memory CD8 T cells	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.14	528	708
Classical monocytes	1.47	0.00	1.47	0.00	1.47	0.00	1.47	0.00	68	54
Effector memory CD8 T cells	0.56	0.00	0.00	0.00	0.00	0.00	0.56	0.00	180	45
Exhausted B cells	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	45	87
Follicular helper T cells	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	87	151
Intermediate monocytes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	34	18
Low-density basophils	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	1
MAIT cells	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	64	80
Myeloid dendritic cells	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11	9
Naive B cells	0.27	0.00	0.27	0.00	0.00	0.00	0.27	0.00	377	573
Naive CD4 T cells	0.07	0.03	0.00	0.10	0.00	0.00	0.07	0.13	2914	3031
Naive CD8 T cells	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.11	883	884
Natural killer cells	5.19	3.17	7.99	7.41	3.79	2.38	10.94	9.79	713	378
Non classical monocytes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17	5
Non-Vd2 gd T cells	1.89	2.62	2.36	6.10	1.65	0.87	4.01	6.98	424	344
Non-switched memory B cells	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	126	223
Plasmacytoid dendritic cells	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16	23
Progenitor cells	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5	9
Switched memory B cells	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	48	109
T regulatory cells	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	479	706
Terminal effector CD4 T cells	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	53	99
Terminal effector CD8 T cells	0.00	0.00	2.14	2.02	0.00	0.00	2.14	2.02	140	99
Th1 cells	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	477	692
Th1/Th17 cells	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	129	219
Th17 cells	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	61	102
Th2 cells	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	274	507
Vd2 gd T cells	0.43	0.39	0.87	0.98	0.58	0.20	1.30	1.38	692	508

Table B.1: Percentage of cells expressing KIR transcripts within immune subsets annotated with Monaco reference. Percentage of KIR2DL1, KIR2DL3 and KIR3DL1 positive cells within each Monaco annotated subset is shown splitted by disease status (T1D= individuals at risk of type 1 diabetes, HC=matched healthy controls). KIR column represents the percentage of KIR positive cells (expressing at least one KIR). The total number of cells (regardless of KIR expression) in each subset is displayed in the two last columns.

	KIR2DL1		KIR2DL3		KIR3DL1		KIR		Cells	
Cell type (HPCA reference)	**T1D**	**HC**	**T1D**	**HC**	**T1D**	**HC**	**T1D**	**HC**	**T1D**	**HC**
Unknown	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16	117
B_cell	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18	43
B_cell:CXCR4-_centrocyte	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	3
B_cell:Germinal_center	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1	3
B_cell:Memory	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	26	29
B_cell:Naive	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	379	581
B_cell:Plasma_cell	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	3
B_cell:immature	0.61	0.00	0.61	0.00	0.00	0.00	0.61	0.00	165	280
CMP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1	0
DC:monocyte-derived:Schuler_treatment	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	1
GMP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1	1
Macrophage:monocyte-derived:M-CSF	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	1
Monocyte	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	2
Monocyte:CD14+	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1	3
Monocyte:CD16^{+}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	45	15
Monocyte:CD16^{-}	1.33	0.00	1.33	0.00	1.33	0.00	1.33	0.00	75	33
Monocyte:S._typhimurium_flagellin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3	14
Monocyte:anti-FcgRIIB	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1	4
Monocyte:leukotriene_D4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	1
NK_cell	3.96	2.32	5.88	5.93	3.55	1.59	8.62	7.38	986	691
NK_cell:CD56hiCD62L+	1.82	1.04	2.19	1.04	0.00	0.00	2.19	1.73	274	289
NK_cell:IL2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10	4
Neutrophil:commensal_E._coli_MG1655	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5	7
Platelets	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2	7
Pre-B_cell_CD34-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6	4
Pro-B_cell_CD34+	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3	17
T_cell:CD4^{+}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2	20
T_cell:CD4^{+}_Naive	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.06	1858	1807
T_cell:CD4^{+}_central_memory	0.06	0.09	0.00	0.00	0.00	0.00	0.06	0.09	1664	1133
T_cell:CD4^{+}_effector_memory	0.00	0.00	0.28	0.00	0.28	0.00	0.56	0.00	360	165
T_cell:CD8^{+}	0.33	2.30	1.17	3.45	0.17	2.30	1.51	4.60	598	87
T_cell:CD8^{+}_Central_memory	0.00	0.49	0.00	0.49	0.00	0.00	0.00	0.98	104	205
T_cell:CD8^{+}_effector_memory	0.06	0.03	0.06	0.12	0.00	0.00	0.11	0.15	1769	3235
T_cell:CD8^{+}_effector_memory_RA	3.26	0.83	3.26	5.79	1.09	0.00	5.43	5.79	92	121
T_cell:CD8^{+}_naive	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	373	747
T_cell:gamma-delta	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7	1

Table B.2: Percentage of cells expressing KIR transcripts within immune subsets annotated with HPCA reference. Percentage of KIR2DL1, KIR2DL3 and KIR3DL1 positive cells within each Monaco annotated subset is shown splitted by disease status (T1D= individuals at risk of type 1 diabetes, HC=matched healthy controls). KIR column represents the percentage of KIR positive cells (expressing at least one KIR). The total number of cells (regardless of KIR expression) in each subset is displayed in the two last columns.

B.2 Multi-colour antibody panel

Antibody	Fluorochrome
KIR2DL2/L3	R660 APC-A
CD3	R780 Cy7APC H7APC-A
KIR2DL1/S5	B515 FITC-A
CD28	V450 Pacific Blue-A
Dump	Aqua blue-A
CD8	V705-A
KIR3DL1	G560 PE-A
CD45RA	G610 TRPE ECD-A
CD56	G780 Cy7PE-A
CD16	V800-A
CD4	G710 Cy55PE-A

Table B.3: Flow cytometry panel to assess iKIR expression in $CD4^+$, $CD8^+$ and NK cell populations

B.3 Compensation

Fluorescence signal of each fluorochrome will be detected in a dedicated fluorescence channel (or *primary* detector). However, the emission spectrum of a given fluorochrome might overlap another one. Consequently, if wide enough, the signal emitted by one fluorochrome might be captured by multiple *secondary* channels.

To clean-up interfering signals from other fluorochromes to detectors different from the one assigned to the dye, we need to apply compensation. For that, we calculate the spillover of fluorescence to secondary detectors using single stained controls assuming that the median fluorescent intensity (MFI) of a population that is said to be negative for a particular florescent probe will be equal to the MFI of cells that have bound the probe that the sample is a positive control for, for all other colors. We can then create a spillover matrix by solving a system of linear equations so that this is true for all parameters. Spillover matrix is calculated from single stained controls following the algorithm for spillover calculation as in Bagwell and Adams (Bagwell and Adams, 1993).

B.4 Gating strategy

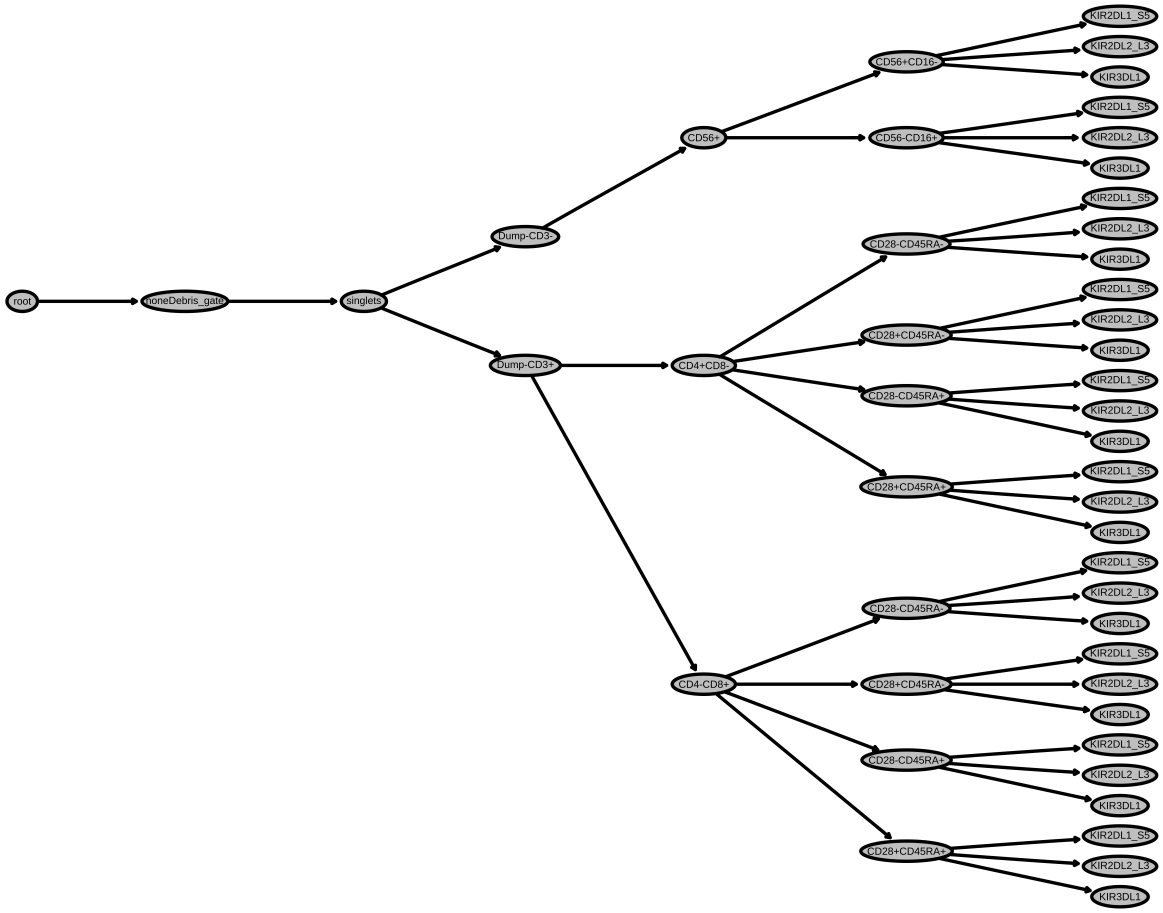


Figure B.1: Automated gating hierarchy. Gating scheme for the different $CD4^+$, $CD8^+$ and NK cell populations is shown. Different nodes indicate different cell populations. Arrows indicate parent-child relationship between populations and sub-populations.

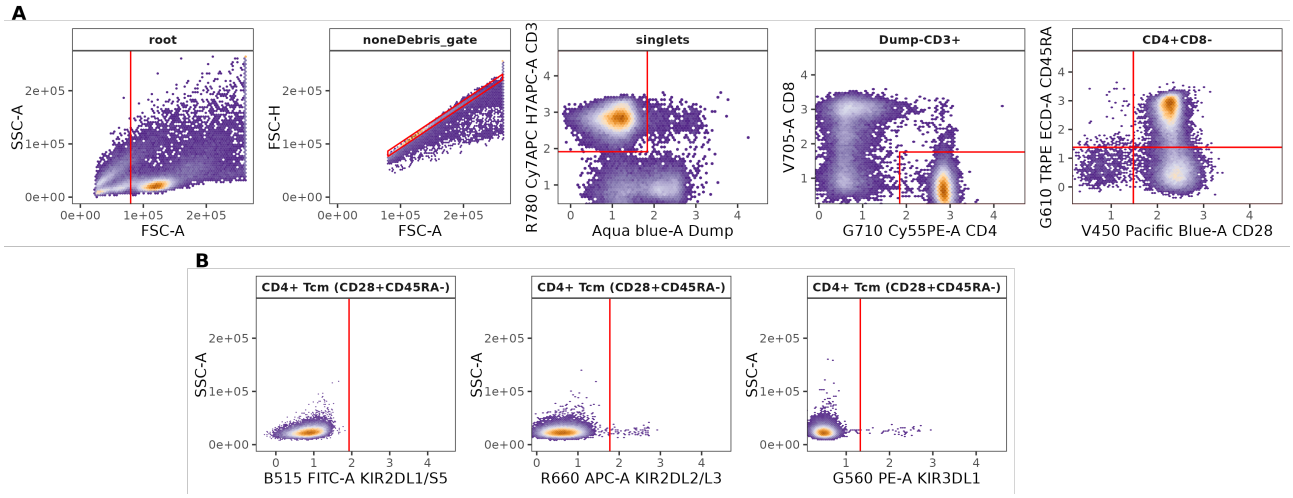


Figure B.2: Gating strategy for enumeration of iKIR+ CD4$^+$ memory subsets. Analysis of PBMCs for a healthy donor (LD1). Each subplot shows events in the parent population, where strip names indicate parent population (root=all events). **A** From left to right, serial gating is used to identify (1) lymphocytes and discard debris (root, all events), (2) single cells in the nonDebris gate, (3) CD3$^+$ and Dump$^-$ cells in the singlet gate (excluding unwanted lineages CD14, CD19 and necrotic cells), (4) CD4$^+$ T cells in the Dump$^-$CD3$^+$ gate and (5) naive, Tcm, Tem and Temra populations whithin the CD4$^+$ gate using CD45RA and CD28 staining. **B** Each of the 4 subsets defined in the CD4$^+$ gate (naive and memory subsets) was gated to determine events positive for KIR2DL1 (left), KIR2DL2/L3 (middle) and KIR3DL1 (left). In this case, only KIR gates within the Tcm population are shown but the same strategy is followed for Tem, Temra and Tn. All boundaries are determined with the 1D min density function using collapsed data (see text).

B.5 KIR expression in CD4$^+$ and CD8$^+$ T cell subsets

	Estimate	P-value
Differentiation stage	0.06	3.92E-16
KIR KIR2DL2_L3 (vs KIR2DL1)	0.04	5.12E-06
KIR KIR3DL1 (vs KIR2DL1)	-8.55E-05	9.93E-01
Status T1D (vs HC)	-0.03	8.49E-04
Lineage CD8 (vs CD4)	0.02	1.08E-02
Sex Male (vs Female)	-0.01	8.76E-02

Table B.4: Multiple linear regression model to identify predictors of iKIR expression on T cells.

B.6 KIR expression in NK cell subsets

	Estimate	P-value
CD56$^{\text{bright}}$CD16$^-$ (vs CD56$^{\text{dim}}$CD16$^+$)	-0.23	1.44E-21
KIR KIR2DL2_L3 (vs KIR2DL1)	0.09	3.58E-04
KIR KIR3DL1 (vs KIR2DL1)	-0.03	0.27

Table B.5: Multiple linear regression model to identify predictors of iKIR expression on NK cells.