RESEARCH NOTE

Whole exome sequencing reveals a *MLL de novo* mutation associated with mild developmental delay and without 'hairy elbows': expanding the phenotype of Wiedemann–Steiner syndrome

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[Steel D., Salpietro V., Phadke R., Pitt M., Gentile G., Massoud A., Batten L., Bashamboo A., McElreavey K., Saggar A. and Kinali M. 2015 Whole exome sequencing reveals a *MLL de novo* mutation associated with mild developmental delay and without 'hairy elbows': expanding the phenotype of Wiedemann–Steiner syndrome. *J. Genet.* **94**, 755–758]

Introduction

Wiedemann–Steiner syndrome (WSS; MIM: 605130) is a rare autosomal dominant condition characterized by developmental delay, short stature and dysmorphic features (Wiedemann *et al.* 1989; Koenig *et al.* 2010). Hypertrichosis cubiti (i.e., excessive hair on the elbows) has been regarded as one of the most prominent feature of WSS and this condition has sometimes been also called as 'hairy elbows syndrome' (Polizzi *et al.* 2005). Here, we report the case of a young girl, admitted to our institution due to hypotonia and developmental delay, and underwent whole exome sequencing (WES). A heterozygous *de novo* nonsense mutation (c.4897C>T/p.R1633*) in the *MLL* gene (MIM: 159555) was identified, previously not reported among *MLL* pathogenics for WSS, suggesting the diagnosis of WSS by *MLL* haploinsufficiency as causative mechanism. Although, most of the classical features of the condition were present, the diagnosis was not suspected until WES was performed, as she did not have hairy elbows. We suggest that the phenotypic spectrum of WSS may be wider than previously assumed. Clinicians should consider testing children with short stature, developmental delay and hypotonia even in the absence of hypertrichosis cubiti.

Materials and method

Clinical summary

The girl was initially admitted to our institution when she was 21 month old because of hypotonia, developmental delay and dysmorphic features. She was the fifth child of first cousin consanguineous parents. She was born at term weighing 3.3 kg (50th centile) following an uneventful pregnancy. There was no family history of significance and all four siblings were healthy. At birth, a small hairy patch was noticed on her lower back. Ultrasound of the spine was performed to exclude spina bifida and was reported as normal and this

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Keywords. Wiedemann–Steiner syndrome; whole exome sequencing; hairy elbows; hypertrichosis cubiti; *MLL* gene; KMT2A; developmental delay; children.

hairy patch later disappeared. At one month old she presented to her local hospital with poor growth, despite apparently satisfactory breastfeeding. At this point in time she was also noted to have pectus excavatum and bilateral eyelid ptosis. Her parents described her as a placid baby who did not move much. In view of her poor growth and delayed development, she was referred from the United Arab Emirates, her home country, to the United Kingdom for specialist genetic and neurological review. She attended our clinic at the age of eight months. Moderate developmental delay was noticed: she was not yet able to sit unsupported and had significant head lag, although she could roll on either direction. There was a social smile but no babble. She was small, weighing 5.2 kg (far below the 0.4th centile) with a head circumference of 46 cm (2nd centile). There was no specific history of feeding problems but barium swallow confirmed a degree of gastro-oesophageal reflux. Examination revealed several dysmorphic features including hypertelorism, bilateral ptosis with a compensatory retrocollis, epicanthic folds, low-set ears and an anteverted nose (figure 1). She was hypotonic overall with an extensor pattern of tone, and there were no significant contractures. Cranial nerve examination identified no facial weakness. Except for the hairy patch on her lower back there was no other hirsutism noticed elsewhere. Hands and feet were normal. The anterior fontanel was patent. Ophthalmological work-up did not reveal any eye and/or visual problems except a mild intermittent squint. Cardiovascular examination and echocardiography were normal. She had noisy breathing, with a history of cyanotic episodes during sleep, and a sleep study confirmed significant obstructive sleep apnoea. Nasal continuous positive airways pressure (nCPAP) was used for several months at night but was successfully discontinued by the time she was 15 months old. She was initially managed with high-calorie milk feeds under dietetic supervision and regular physiotherapy. Weight gain improved significantly, and by 16 months she weighed 9.15 kg (25–50th centile) with a head circumference of 47 cm (75–90th centile). Her hypotonia also became less marked. She made steady developmental progress, sitting independently at one year old and crawling at 15 months, and started to babble, although she still showed moderate global delay.

Investigations

All initial metabolic investigations were normal. These included full blood count, electrolytes, renal function, liver function, thyroid function, creatine kinase, lactate, carnitine and acyl carnitine profile, lipid profile, folate, biotinidase, urine and serum amino acids, urine mucopolysaccharides, urine oligosaccharides, transferrin glycoforms and anticholinesterase antibodies. Electromyography (EMG) showed a nonspecific myopathic pattern, so quadriceps muscle biopsy was arranged. This showed mild nonpecific myopathic changes with some of persistence foetal myosin. Initial genetic investigations were also normal, including karyotype (46 XX), genomic comparative hybridization (CGH) testing and mitochondrial DNA sequencing. Several genes were tested to rule out specific suspected diagnoses: congenital myasthenic syndrome (*CHAT*, *DOK7* and *RAPSN*), genitopatellar syndrome (*KAT6B*) and blepharophimosis and epicanthus inversus syndrome (*FOXL2*). They were all normal. Muscle respiratory chain enzyme analysis was normal for complexes I–IV.

Whole exome sequencing

At this point when no diagnosis had been reached and as the parents were planning for the next pregnancy identification of a heritable defect was considered a priority. Exon enrichment was performed using Agilent SureSelect Human All Exon V4. Paired-end sequencing was performed on the Illumina HiSeq2000 platform using TruSeq v3 chemistry. Read files (Fastq) were generated from the sequencing platform via the manufacturer's proprietary software. Reads were mapped using the Burrows-Wheeler Aligner and local realignment of the mapped reads around potential insertion/deletion (indel)

Figure 1. The patient at 21 months of age. Note hypertelorism, epicanthic folds, low-set ears and anteverted nose.

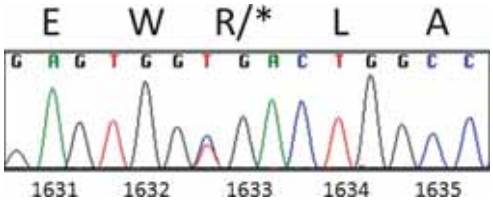

Figure 2. The *de novo* heterozygous nonsense mutation in the *MLL* gene that is predicted to result in a truncated protein. The electropherogram shows the c.4897C>T mutant peak, whose correspondent amino acid sequence and position are indicated above and below it (p.R1633*).

sites was carried out with the GATK ver. 1.6. Duplicate reads were marked using Picard ver. 1.62. Additional BAM file manipulations were performed with Samtools 0.1.18. Single-nucleotide polymorphism (SNP) and indel variants were called using the GATK Unified Genotyper for each sample. SNP novelty was determined against dbSNP138. Novel variants were analysed by a range of web-based bioinformatics tools using the EnsEMBL SNP Effect Predictor (http://www.ensembl.org/homosapiens/userdata/uploadvariations). Analyses of the variant datasets from these patients using a panel of gene prioritization software (http://homes.esat.kuleuven.be/~bioiuser/gpp/tools.php). All variants were screened manually against the Human Gene Mutation Database Professional [Biobase] (http://www.biobase-international.com/product/hgmd). *In silico* analysis was performed to determine the potential pathogenicity of the variants. Potentially pathogenic mutations were verified using classic Sanger sequencing.

Results

The exome sequencing revealed a heterozygous *de novo* nonsense c.4897C>T mutation on exon 15 (GRCh38/hg38) of the *MLL* (KMT2A; ENST00000389506) gene, which is predicted to result in a p.Arg1633Ter truncation of the protein resulting from a premature termination codon (figure 2). Nonsense mutations have a high likelihood of impairment of protein functionality (Yamaguchi-Kabata *et al.* 2008). The identified mutation was not present in dbSNP and has been not detected in 200 unrelated control exome studies, nor observed by the 1000 Genomes Project. No other gene mutations/variants that could be linked to the phenotype were identified in the proband. The mutation was confirmed by traditional sanger sequencing and was not found in DNA extracted from leukocytes of the unaffected parents. *De novo* mutations in this gene have been previously reported in association with WSS and classified as pathogenic or likely pathogenic (Jones *et al.* 2012; Mendelsohn *et al.* 2014; Strom *et al.* 2014), suggesting for most of them haploinsufficiency as the disease mechanism.

Discussion

WSS was first described in 1989 by Wiedemann *et al.* (1989) with the case of a child with short stature, hypertelorism and other dysmorphic features and moderate developmental delay. A second case was identified by Steiner and Marques (2000), and several other cases of WSS have since been reported (Visser *et al.* 2002; Koç *et al.* 2007; Koenig *et al.* 2010; Mendelsohn *et al.* 2014).

Jones *et al.* (2012) identified heterozygous mutations of the *MLL* gene in five out of six children with clinical features of WSS. Each individual had a different mutation but all five had haploinsufficiency of the protein product, a histone-modification enzyme.

In WSS patients studied by Jones *et al.* (2012) as well in our patient, the MLL mutations identified are predicted to lead to the premature termination of the protein product with a high likelihood of functional impact. The proband was found to have a heterozygous *de novo* nonsense c.4897C>T mutation on exon 15 of the *MLL* gene, which is predicted to result in a p.Arg1633Ter truncation of the protein due to a premature termination codon at amino acid position 1633 and her phenotype is therefore likely to be due to *MLL* haploinsufficiency, as has been reported by Jones *et al.* (2012). Additionally, the mutations identified in WSS patients both by us and Jones *et al.* (2012), have not been found in their unaffected parents, supporting the notion that the disease-causing alleles have arisen *de novo*. Notably, also intragenic deletion and missense variations of the *MLL* gene have been recently reported as the molecular cause of phenotypes within the WSS-related clinical spectrum (Mendelsohn *et al.* 2014; Strom *et al.* 2014).

Although Wiedemann's original description did not include hypertrichosis cubiti, this has been a consistent feature in most of the cases subsequently reported, including all the three discussed by Koenig *et al.* (2010), and all five of those found to have mutations affecting MLL in Jones *et al.* (2012). Notably, the sixth case of the study by Jones *et al.* (2012) who had no MLL mutation was also the only one who did not display hairy elbows. However, missense mutations in the *MLL* gene have been recently found in patients with developmental delay, microphthalmia, distinctive skeletal and facial features who did not display hairy elbow (Strom *et al.* 2014), further supporting the heterogeneity of the WSS-related clinical spectrum.

While hypertrichosis cubiti has been associated with a range of other clinical findings (Flannery *et al.* 1989; Polizzi *et al.* 2005), they have been regarded as a key diagnostic feature of WSS. In fact, the condition is listed in the Online Mendelian Inheritance in Man (OMIM) database as 'hairy elbows, short stature, facial dysmorphism and developmental delay' (OMIM Entry #605130: Hairy elbows, short stature, facial dysmorphism, and developmental delay. O'Neill MJF, http://www.omim.org/entry/605130, accessed 12/11/14). The proband presented distinctive facial features,

short stature, hypotonia and developmental delay; mutations and/or copy number variations in a broad variety of genes have been identified in both human and experimental studies to be variably responsible for the above combined features (Cao *et al.* 2014; Pavone *et al.* 2014; Portin 2014; Singh *et al.* 2014). We had therefore not suspected WSS in the proband, and the diagnosis was made only on whole exome sequencing after many other avenues had been explored, including metabolic tests, CGH and mitochondrial DNA sequencing. One reason for this was the absence of hairy elbows. Other classic features, such as hypertelorism, ptosis, narrow palpebral fissures, hypotonia and developmental delay were present. On the other hand, proband presented a small hairy patch on her lower back at birth which subsequently disappeared. However, the proband was younger at diagnosis when compared to most of other described cases, it is possible that hypertrichosis cubiti might develop with advancing age. Interestingly, the proband's hypotonia was most marked during her first year of life, subsequently improving; a similar improvement with age has already been described in two girls with a WSS-related phenotype (Visser *et al.* 2002), before genetic testing was available. WSS is a rare, sporadic syndrome and to date it has only been considered as a differential in children displaying hairy elbows. The case reported here suggests that this syndrome remains a possible diagnosis in the absence of hypertrichosis cubiti in children with short stature, dysmorphism and developmental delay, making this genetic condition likely underestimated. Additionally, our report together with other reported patients (Visser *et al.* 2002), suggest that the developmental delay can improve during the natural history of this syndrome and that milder and incomplete (i.e. without hairy elbows) presentations of WSS have to be taken into consideration. In conclusion, on the basis of present and previous findings (Mendelsohn *et al.* 2014; Strom *et al.* 2014), we suggest that the prevalence and phenotypic spectrum of WSS may be broader than had been believed; clinicians should therefore consider testing the *MLL* gene in children with short stature, developmental delay, peculiar facial features and hypotonia, even in the absence of hypertrichosis cubiti.

Acknowledgements

We gratefully acknowledge all the clinicians and the laboratory researchers who made this study possible.

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Received 22 March 2015, in revised form 26 April 2015; accepted 5 May 2015
Unedited version published online: 6 May 2015
Final version published online: 13 November 2015