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SHORT REPORT

Further confirmation of the *MED13L* haploinsufficiency syndrome

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***MED13L* haploinsufficiency syndrome has been described in two patients and is characterized by moderate intellectual disability (ID), conotruncal heart defects, facial abnormalities and hypotonia. Missense mutations in *MED13L* are linked to transposition of the great arteries and non-syndromal intellectual disability. Here we describe two novel patients with *de novo* *MED13L* aberrations. The first patient has a *de novo* mutation in the splice acceptor site of exon 5 of *MED13L*. cDNA analysis showed this mutation results in an in-frame deletion, removing 15 amino acids in middle of the conserved MED13L N-terminal domain. The second patient carries a *de novo* deletion of exons 6–20 of *MED13L*. Both patients show features of the *MED13L* haploinsufficiency syndrome, except for the heart defects, thus further confirming the existence of the *MED13L* haploinsufficiency syndrome.**

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INTRODUCTION

MED13L (Mediator Complex Subunit 13 like) is one of the many subunits of the Mediator transcription co-activator complex. The complex has been shown to associate with general transcription factors and RNA polymerase II to regulate transcription. The mediator complex is the essential co-activator acting as a bridge between transcription factors bound at upstream DNA regulatory elements and the transcription machinery.[1]

MED13L was found to map to the breakpoints of a patient with intellectual disability (ID) and transposition of the great arteries. Subsequent sequencing of the gene in a larger cohort of patients with transposition of the great arteries identified three additional missense mutations in *MED13L*.[2] A large resequencing study of homozygous regions in consanguineous patients identified a homozygous missense variant in *MED13L* as a cause of nonsyndromic ID.[3]

Asadollahi *et al*[4] presented two patients with *MED13L* haploinsufficiency. These patients suffered from a similar phenotype of hypotonia, moderate ID, conotruncal heart defects and specific dysmorphic facial features, including macroglossia. Their findings showed that *MED13L* haploinsufficiency in contrast to the previously observed missense mutations cause a distinct syndromic phenotype.

Here, we describe two new patients with *de novo MED13L* aberrations who are phenotypically similar to the patients described by Asadollahi *et al*[4] but lack the cardiac phenotype.

PATIENTS AND METHODS

Ethical approval

Informed consent for whole-exome sequencing and subsequent Sanger sequencing as a part of the diagnostic process (approved by the Medical Ethical Committee of the University Medical Center Utrecht) was obtained for case 1 and her parents. Informed consent for array CGH was obtained for case 2 and her parents. Consent to publish clinical photographs was obtained for both cases included in this study.

Patient 1

The first patient is the second child of healthy non-consanguineous Caucasian parents. She was born after a twin pregnancy of 37 weeks with a weight of 3020 g. After birth, a right-sided clubfoot was noted for which she was treated at the age of 11 weeks. Her developmental milestones were delayed: she walked independently at the age of 2½ years and spoke her first sentences at age 3½ years. Because of her developmental delay, she was seen at the age of 14 months by the neurologist who observed hypertonia of all extremities. Her older sister and her twin brother were healthy. Apart from a paternal great uncle who has an intellectual deficit of unknown cause, the family history is unremarkable. On clinical examination at the age of 16 months, height was 75 cm (−1 SD), weight: 8.5 kg (−1 SD) and headsize: 43.5 cm (−1.5 SD). She had a slightly asymmetric face with short, upslanted palpebral fissures, a bulbous nasal tip and protrusion of her tongue (Figure 1; Table 1). She had bilateral accessory nipples, abnormal palmar creases with an extra phalangeal crease of the index fingers. Auscultation of heart and lungs was normal. The right foot showed results of the correction of the clubfoot, and both feet showed a sandal gap. On neurological investigation, she had normal reflexes and no hypertonia of the extremities was observed. No murmur could be heard at cardiac auscultation, an ECG was unremarkable and echocardiography showed a structurally and functionally normal heart. Additional investigations (including MRI of the brain, karyotype, array CGH analysis, metabolic and ophthalmological investigations) were normal.

Patient 2

The second patient was born after an uneventful pregnancy of 42 weeks with a weight of 3300 g. Prenatal karyotyping indicated by increased nuchal translucency showed a normal male karyotype. The neonatal period was complicated by feeding problems, eczema and gastroesofageal reflux, which all improved after induction of bottle feeding. Grommets were inserted three times because of

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recurrent ear infections. Hearing and vision were normal. He had a motor- and speech developmental delay (walking independently at the age of 24 months, and spoke only 10 words at the age of 4 years). There were no behavioral problems.

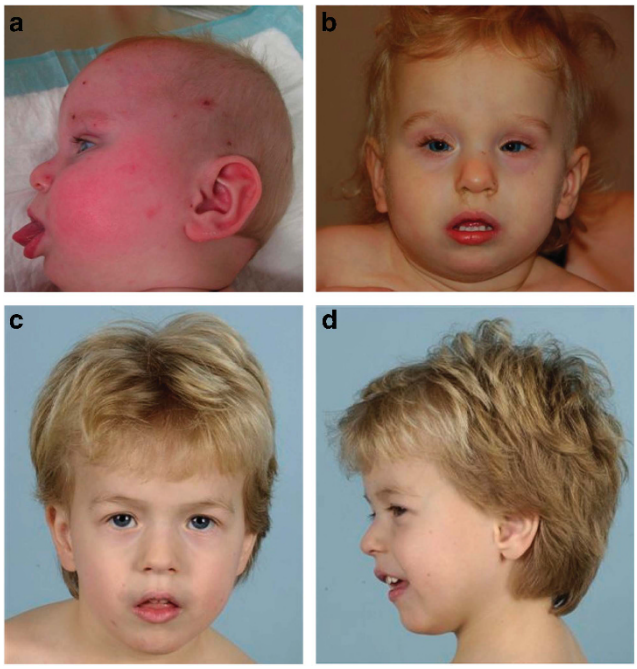

Figure 1 Facial features of patient 1 and 2 with *MED13L* aberrations: (**a**) patient 1 at 6 months and (**b**) 17 months of age. Note macroglossia, facial asymmetry, upslanting palpebral fissures, flat nasal bridge and dark circles under eyes. (**c** and **d**) Patient 2 at age 4½ years: slightly different phenotype: note broad nasal bridge, open mouth appearance.

Parents were non-consanguineous and family history is non-contributory. On clinical examination (at the age of 4 years and 2 months) height was: 97 cm (−2.25 SD), weight: 14.55 kg (0 SD) and OFC: 50 cm (−0.5 SD). Facial dysmorphisms include small eyelids and mild retrognathia (Figure 1; Table 1). Cardiac screening was unremarkable; no murmur was present at auscultation, ECG was normal and echocardiography showed a structurally and functionally normal heart with a small persistent foramen ovale (PFO). Metabolic screening and DNA analysis for Fragile X syndrome showed no abnormalities.

Whole-exome sequencing and array CGH analysis

Trio-based whole-exome sequencing was performed on patient 1 and parents as described previously[5] with the alterations that sequencing was performed on the Solid 5500 platform and enrichment was performed using the Agilent Sureselect kit (v4; Agilent, Santa Clara, CA, USA). The obtained average coverage was 69, 77 and 66 × for the patient, father and mother, respectively. Filtering variants with a predicted effect at the protein level using a *de novo* hypothesis yielded 11 candidate mutations, of which one could be validated by Sanger sequencing (*MED13L*: NC_000012.11:g.116460407C>A). Filtering with a recessive hypothesis yielded no sound candidate variants. No further rare variants in the *MED13L* coding regions were identified.

Array CGH was performed on an Agilent 180 K oligo-array (amadid 023363) on patient 2 plus the parents.

Splicing analysis

cDNA was generated using High Capacity cDNA Reverse Transcription Kit (Life Technologies, Carlsbad, CA, USA) on RNA isolated from lymphocytes using Trizol reagent (Life Technologies). Subsequently, PCRs were performed using cDNA specific primers located in the different exons of *MED13L* (sequence available upon request), followed by excision of the bands from an agarose gel and purification of DNA (QIAquick, Qiagen, Hilden, Germany) followed by Sanger sequencing.

Table 1 Summary of clinical features of the patients with *MEDL13* haploinsufficiency syndrome

	Asadollahi et al[4]		*Present cases*	
	Patient 1	*Patient 2*	*Patient 1*	*Patient 2*
Sex	F	F	F	M
Cardiac manifestations	+	+	−	−
Supra cardial total anomalous pulmonary venous connection	+	−	−	−
Pulmonary atresia	+	−	−	−
Ventricular septal defect	+	−	−	−
Persistent foramen ovale	−	−	−	+
Tetralogy of Fallot	−	+	−	−
Neurological manifestations	+	+	+	+
Developmental delay	+	+	+	+
Motor delay	+	+	+	+
Speech delay	+	+	+	+
Truncal muscular hypotonia	+	+	−	−
Hypertonia of extremities	+	−	+	−
Dysmorphic features	+	+	+	+
Hypotonic open-mouth appearance	+	+	+	+
Macroglossia	+	−	+	−
Mild facial asymmetry	+	−	+	−
Upslanted palpebral fissures	+	+	+	−
Bulbous nasal tip	+	+	+	−
Extra phalangeal crease of the index fingers	−	−	+	−
Accesory nipples	Not reported	Not reported	+	−
MED13L aberration	*De novo* deletion exon 2	deletion exon 3 a-d 4	*De novo* mutation c.480-1G>T	*De novo* deletion exons 6–20

Abbreviations: F, female; M, male; *MED13L*, mediator complex subunit 13 like.

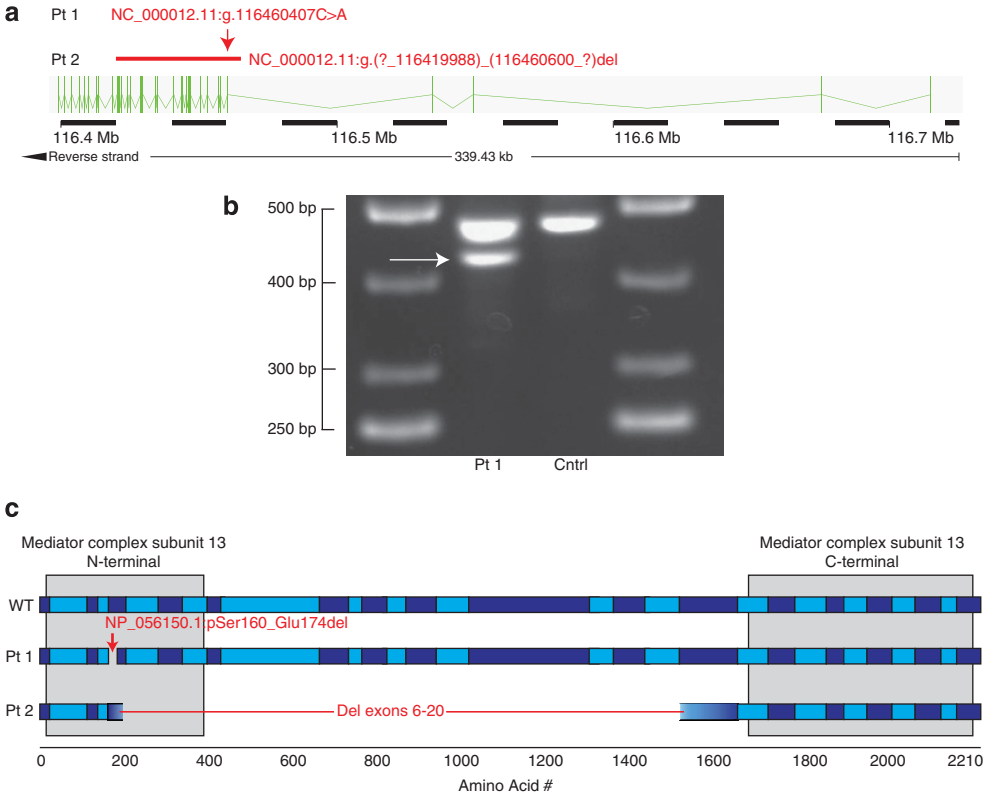

Figure 2 MED13L abberations. (**a**) Genomic structure of human *MED13L* with indicated *de novo* splice site mutation in patient 1 and the minimal *de novo* deletion of patient 2. (**b**) Agarose gel results of a PCR on cDNA with primers located on exons 4 and 7. Pt1 denotes cDNA from patient 1 and Ctrl cDNA from a control cDNA sample. The cDNA PCR of patient 1 shows an additional lower band (indicated by arrow). The predicted size of the wildtype band is 467 bp. Sanger sequencing of the band proved an in-frame partial deletion of exon 5. (**c**) Protein structure of human MED13L, with the conserved protein domains indicated. The splice site mutation in patient 1 results in a deletion of 15 amino acids (160–174) in the conserved MED13L N-terminal domain. The minimal deleted region of patient 2 encompasses exons 6–20 in the middle of the MED13L protein.

Databases

Mutations and phenotypic details from both patients were submitted to Decipher.[6]

RESULTS

We performed trio-based whole-exome sequencing on patient 1 and her parents. We identified a single *de novo* mutation in a consensus splice acceptor site of exon 5 of *MED13L* (NM_015335.4:c.480-1G>T; exons numbered as in NC_000012.11; Figure 2a). Analysis of cDNA derived from lymphocyte RNA from this patient identified a single alternatively spliced *MED13L* transcript not present in controls (Figure 2b). Subsequent sequencing of the transcript identified a cryptic splice acceptor site, 45 nucleotides into exon five, which was used in the patient but not in controls. This alternative splicing results in an in-frame deletion of 45 base pairs in the cDNA and deletion of 15 amino acids in the middle of the MED13L N-terminal domain (NM_015335.4:r.480_524del and NP_056150.1:pSer160_Glu174del; Figure 2c), which is a highly conserved domain of unknown function.[3] The deletion of multiple amino acids in a highly conserved domain is likely to affect MED13L protein function.

Array CGH analysis revealed a deletion of 41 kb containing and disrupting the distal part of the *MED13L* gene (NC_000012.11:g.(?_116419988)_(116460600_?)del). Additional array studies in both parents revealed that the deletion had arisen *de novo*. This deletion removes exons 6–20, of the 31 exons of the *MED13L* gene, likely resulting in loss of protein function (Figure 2c).

DISCUSSION

Recently, the *MED13L* haploinsufficiency syndrome has been characterized by moderate ID, conotruncal heart defects, facial abnormalities and hypotonia.[4] Missense mutations in *MED13L* were already reported in a cohort of patients with congenital heart defects.[2] As parents of those cases were not studied and no phenotype details were available, the relevance of these three missense variants remains unclear. The cases reported here show a similar phenotype as the previously reported syndromal cases[4] (Table 1). They both have a delay in motor and speech development and both show a hypotonic open-mouth appearance on clinical examination. Patient 1 also has an asymmetric face with macroglossia and bulbous nasal tip as was decribed in the previous report. Both our cases, however, lack the previously reported cardiac phenotype. This shows reduced penetrance of the cardiac anomalies as part of this syndrome.

In summary, we report two new cases with ID and facial dysmorphic features caused by *MED13L* haploinsufficiency. Both cases do (apart from the frequently identified small PFO in patient two) not show cardiac anomalies, thereby underlining the reduced penetrance of the cardiac phenotype in of *MED13L* haploinsufficiency syndrome. Our study thus confirms the existence of a *MED13L* haploinsufficiency syndrome characterized by ID and specific dysmorphic facial features with macroglossa and shows a reduced penetrance of the cardiac anomalies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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