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MEIS2 Involvement in Cardiac Development, Cleft Palate, and Intellectual Disability

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MEIS2 has been associated with cleft palate and cardiac septal defects as well as varying degrees of intellectual disability. We present a female patient with a more severe phenotype compared to previous reported patients. She has multiple congenital malformations; cleft palate and congenital heart defect characterized by septal defects and aortic coarctation. She has severe feeding problems, facial dysmorphism, severely delayed gross motor and verbal development, and autism spectrum disorder. Facial dysmorphism consisting of bitemporal narrowing, arched and laterally extended eyebrows, mild upslanting palpebral fissures, deep-set eyes, a tented upper lip, thin upper vermilion, full lower vermilion, broad first ray of hands and feet, a gap between the first and second toes, and syndactyly of toe II–III. Exome sequencing revealed a non-frameshift deletion (c.998_1000del: p.Arg333del) of three base pairs in the *MEIS2* homeodomain. The more severe phenotype is most probably due to dominant-negative mechanisms. This is the first report showing a de novo small intragenic mutation in *MEIS2* and further confirms the important role of this gene in normal development.

Key words: cleft palate; cleft lip; cardiopathy; heart; intellectual disability; next generation sequencing (NGS)

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INTRODUCTION

MEIS2 is a homeodomain-containing transcription factor of the TALE superfamily. Cytogenetic studies have identified *MEIS2* as a candidate gene for congenital malformations of the heart and palate. In 2007, a first patient with atrial septal defect type secundum (ASD II), cleft palate, moderate developmental delay, severe speech delay, and mild motor delay was described with a 5.3 Mb deletion in 15q14 [Erdogan et al., 2007]. This was followed by two more patients with cleft palate and ventricle septal defect (VSD), respectively showing a deletion of 5.6 Mb and 123 kb in 15q14 [Chen et al., 2008; Crowley et al., 2010]. *MEIS2* was the only gene overlapping in all of these patients, making it the primary candidate gene for cleft palate, cardiac septal defects, and varying degrees of developmental delay. More recently, nine patients, including a family with four patients, demonstrate that *MEIS2* disruption alone can cause cleft palate and cardiac defects (VSD) [Chen et al., 2008; Crowley et al., 2010; Johansson et al., 2014]. One of these also suffered from autism spectrum disorder, albeit with mild intellectual disability (ID) and without cleft palate or cardiopathy [Johansson et al., 2014]. Interestingly, most of these patients had delayed motor development, but varying degrees of cognitive disability, ranging from normal to moderate ID. It remains uncertain whether the ID observed in the other individuals is related to a deletion of nearby genes, or related to haploinsufficiency of the *MEIS2* gene. We here report on the first patient with an intragenic *MEIS2* mutation detected by exome sequencing.

CLINICAL REPORT

A female patient is the second child of healthy, unrelated parents, and family history is negative regarding congenital malformations. She was born at term after an uneventful pregnancy, with weight 3650 g (25th–50th centile), length 51 cm (50th centile), and head

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circumference 36 cm (50th–75th centile). Multiple congenital malformations were present, a cleft of the soft and posterior part of the hard palate, a congenital heart defect (a large perimembranous, inlet-to-outlet VSD, an ASD II, a small left ventricular outflow tract without obstruction, and aortic coarctation). Surgical correction with coarctectomy and VSD closure was done. She also had congenital lobar emphysema of the left upper lobe for which a lobectomy was performed at the age 1 month. There were severe feeding problems with gastro-esophageal reflux, oral aversion, aerophagia, and achalasia necessitating gastrostomy and Botox infiltrations. On examination, she had arched and laterally extended eyebrows, mild upslanting palpebral fissures, deep-set eyes, a tented upper lip, thin upper vermilion, full lower vermilion. There was bitemporal narrowing. She had a broad first ray of hands and feet, a gap between the first and second toes and syndactyly of toe II–III (Fig. 1). Growth parameters at 5 years 8 months were height 108.5 cm (10th centile) and weight 15.8 kg (3rd centile).

Her gross motor and verbal development were severely delayed. She could sit at 17 months and walk at age 30 months. At the age of 4 years she pronounces single words. Mentally she scored equivalent to age 25 months at the age of 50 months (4 years 2 months), with an IQ in the range of 35–49, placing her in the moderate group of intelligence disability. The diagnosis of autism spectrum disorder was made at the age of 3 years. Cerebral echography and MRI could not detect any abnormalities. Previously performed array-CGH (1 Mb resolution) was normal.

MATERIALS AND METHODS

Exome Analysis

Informed consent for trio analysis by exome analysis was obtained from the parents. Library construction for all samples was prepared using TruSeq DNA Library Preparation Kit (Illumina, Inc., San Diego, CA) in which platform-specific adaptors and unique DNA indexes were ligated. For each sample, 1 μg genomic DNA was sheared by sonication to approximately 300 bp fragments, followed by end-repair, adenylation, and adapter ligation steps. DNA sequencing libraries were subsequently enriched with the SeqCap EZ Human Exome Library v3.0 (Roche, NimbleGen), and 2 × 100 bp paired-end reads were generated on the Illumina HiSeq2000 platform with 3–4 exome-seq samples pooled per lane of a sequencing flow-cell. Sheared DNA, whole genome libraries, and enriched exome-seq libraries were validated using DNA-1000 chips on the BioAnalyser (Agilent), and library concentrations were determined using the dsDNA Broad Range Assay using the Qubit (Invitrogen).

Data analysis was done using commercial and in-house developed software (Genomics Core/UZ Leuven). Exome sequences were obtained from both parents and the patient. As our hypothesis was a de novo mutation, the patient was filtered against all in-house (n = 72) exomes, allowing the exclusion of local rare variants. All non-reference calls were excluded in the parents, reference calls were excluded in the patient. According to Ensembl (www.ensembl.org) only exonic, exonic/splicing, and splicing variants were

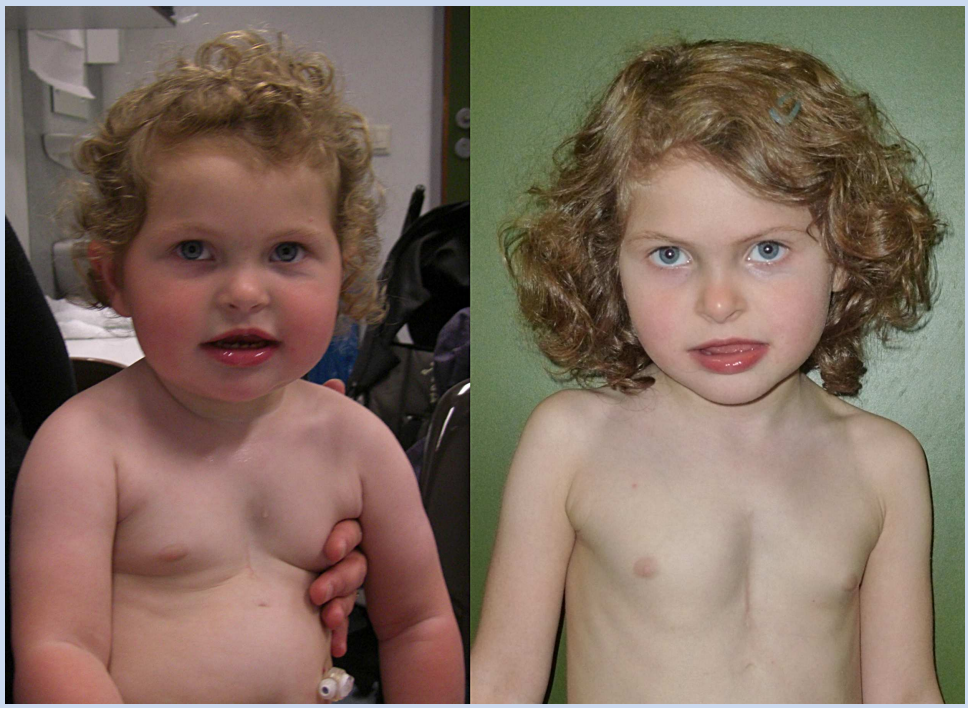

FIG. 1. Clinical pictures at the age of 2 and 5 years showing mild dysmorphic facial features: bitemporal narrowing, arched and laterally extended eyebrows, mild upslanting palpebral fissures, deep-set eyes, a tented upper lip, thin upper vermilion, and full lower vermilion. Pectus excavatum is most likely due to cardiac surgery performed at a young age. [Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/journal/10. 1002/(ISSN) 1552–4833].

included. Synonymous variants were excluded. Variants occurring with a frequency of <1% in the 1000 genomes project or with an unknown frequency were included. Variants occurring in *HLA* and *MUC* genes were excluded. Splicing site changes occurring at less than 5 positions were considered as possible candidates.

RESULTS

Exome Sequencing

After variant filtering as outlined in the methods section, we identified variants in 48 candidate genes (Table SI). This gene list was manually curated using functional data and genotype–phenotype correlations for the implicated genes. We thus identified a de novo non-frameshift deletion of three base pairs in the *MEIS2* gene NM_170674.2 c.998_1000del:p.Arg333del as the most likely cause. This causes a deletion of three nucleotides GAA and thus deletion of the amino acid Arginine (Arg). Results were confirmed by Sanger sequencing (Fig. 2).

DISCUSSION

We report on the first patient who carries a small intragenic mutation in the *MEIS2* gene, confirming the previous association

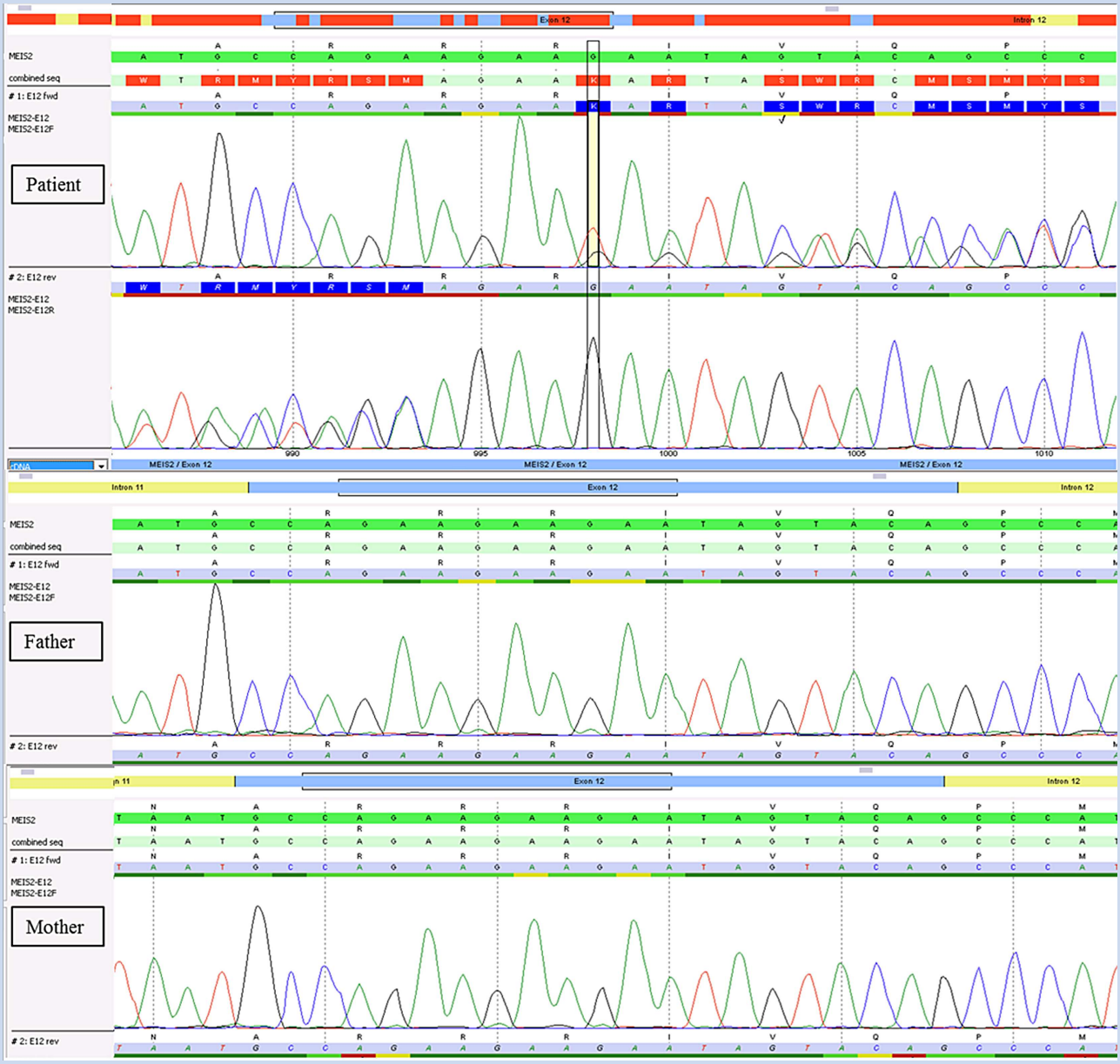

FIG. 2. Sanger sequencing of the patient, father, and mother showing a a de novo non-frameshift deletion in the patient of three base pairs in the *MEIS2* gene.

TABLE I. Main Phenotypic Features and Involved Genes of Patients with *MEIS2* Haploinsufficiency

Publication	Case number	Sex	Chromosomal aberration	Genes involved	Cardiac malformation	Clefting	Psychomotor development
Erdogan et al. [2007]	1	F	5.3 Mb del	*ACTC, GREM1, CX36,**MEIS2**, ARHGAP11A, CHRNA7, CHRM5*	ASD II	palate	severe speech delay, mild motor delay
Chen et al. [2008]	2	M	5.6 Mb del	***MEIS2***, many other genes	VSD	palate	delayed, epilepsy
Crowley et al. [2010]	3	M	123 kb del	***MEIS2***	VSD	cleft soft palate	bilateral moderate hearing loss
Johansson et al. [2014]	4	F	58 kb dup	***MEIS2***	NONE	submucous cleft palate	mild ID
	5	F	58 kb dup	***MEIS2***	NONE	submucous cleft palate	delayed
	6	M	58 kb dup	***MEIS2***	NONE	open cleft palate	delayed
	7	F	58 kb dup	***MEIS2***	NONE	open cleft palate	mild ID
	8	F	0.6 Mb del	*C15orf41, CSNK1A1P1,**MEIS2***	VSD	open cleft palate	normal
	9	M	0.6 Mb del	*C15orf41, CSNK1A1P1, LOC145845,**MEIS2***	NONE	bilateral cleft lip and palate	delayed
	10	M	1.0 Mb del	*C15orf41, CSNK1A1P1, LOC145845,**MEIS2***	NONE	NONE	mild ID, ASD
	11	M	1.9 Mb del	*C15orf41, CSNK1A1P1, LOC145845,**MEIS2**, TMCO5A, SPRED1, FAM98B, RASGRP1, C15orf53*	VSD	NONE	delayed
	12	F	4.8 Mb del	*C15orf41, CSNK1A1P1, LOC145845,**MEIS2**, TMCO5A, SPRED1, FAM98B, RASGRP1, C15orf53, C15orf54*	VSD	submucous cleft palate, bifid uvula	delayed
Current case	13	F	c.998_1000del:p.Arg333del	***MEIS2***	ASD II, VSD, LVOTO, CoA	soft and hard cleft palate	severe gross motor and verbal delay, ASD

ASD II, Atrial Septal Defect II; VSD, Ventricular Septal Defect; LVOTO, Left Ventricular Outflow Tract Obstruction, CoA, Coarctation of the Aorta, ID, intellectual disability; ASD, Autism Spectrum Disorder.

of *MEIS2* with cleft palate and cardiac septal defects. No other in-frame deletions or point mutations have been described until now. Of interest, the present patient had moderate ID, severe gross motor delay, and autism spectrum disorder. Previously reported patients with an intragenic *MEIS2* deletion also had delayed motor development, but varying degrees of delay in mental development, ranging from normal to moderate. One of these patients also suffered from autism spectrum disorder as diagnosed in our patient, albeit with a mild ID and without cleft palate or cardiopathy [Johansson et al., 2014] Table I.

MEIS2 is a transcription factor and most likely has a role in the stabilization of the homeoprotein-DNA complex. It binds to HOX or PBX proteins to form dimers and multimers. The arginine residue deleted is located in the homeodomain. This single amino acid deletion could therefore interfere with DNA binding. Arg333 is highly conserved across all species and isoforms. In addition, according to the Protein Databank in Europe (PDBePISA) database, the Arg residue mutated in the present patient is involved in a multimer contact [Krissinel and Henrick, 2007 (http://www.ebi.ac.uk/pdbe/prot_int/pistart .html)]. Any hydrogen bonds that could be made by the wild type residue to other monomers would therefore be lost and affect the multimeric compound. Therefore, the more pronounced ID observed in the present patient may possibly be due to another mechanism than merely haploinsufficiency. Thus, the deletion of the Arg residue may have an additional dominant negative effect.

Functional studies showed that *MEIS2B,* one of two orthologs of *MEIS2* in zebrafish, is important in heart formation, regulation, and function. Knockdown of *MEIS2B* in zebrafish embryos lead to defective cardiac morphogenesis with no midline formation of the linear heart tube, severe defects in heart looping, pericardial edema, and a significantly reduced heart rate [Glickman and Yelon, 2002; Paige et al., 2012]. Interestingly, expression of *MEIS2B* in the heart field of developing mutant zebrafish embryos closely resembles that of *GATA4*, a known cardiac transcription factor [Paige et al., 2012]. Mutations in *GATA4* is well-known in causing primarily cardiac septal defects, ranging from ASD and VSD to atrio-ventricular septal defects (AVSD) [Garg et al., 2003]. Regarding ID and developmental delay; *MEIS2B* expression is observed in the developing hindbrain of somites [Zerucha and Prince, 2001], and *MEIS2* is a principal key factor in patterning of the hindbrain [Waskiewicz et al., 2001] as well as normal mesencephalic development in mice and chick embryos [Shim et al., 2007; Vennemann et al., 2008; Agoston and Schulte, 2009].

This report builds on the previous publications that *MEIS2* should be considered in patients with cleft palate, septal cardiac defects, and ID. Further functional studies regarding the role of *MEIS2* in neuronal pathways and mental development will be necessary to explore the exact mechanisms involved in this intricate mechanism.

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