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SHORT COMMUNICATION

De novo MEIS2 mutation causes syndromic developmental delay with persistent gastro-esophageal reflux

Atsushi Fujita[1], Bertrand Isidor[2,3], Hugues Piloquet[4], Pierre Corre[5], Nobuhiko Okamoto[6], Mitsuko Nakashima[1], Yoshinori Tsurusaki[1], Hirotomo Saitsu[1], Noriko Miyake[1] and Naomichi Matsumoto[1]

MEIS2 **aberrations are considered to be the cause of intellectual disability, cleft palate and cardiac septal defect, as *MEIS2* copy number variation is often observed with these phenotypes. To our knowledge, only one nucleotide-level change—specifically, an in-frame *MEIS2* deletion—has so far been reported. Here, we report a female patient with a *de novo* nonsense mutation (c.611C>G, p.Ser204*) in *MEIS2*. She showed severe intellectual disability, moderate motor/verbal developmental delay, cleft palate, cardiac septal defect, hypermetropia, severe feeding difficulties with gastro-esophageal reflux and constipation. By reviewing this patient and previous patients with *MEIS2* point mutations, we found that feeding difficulty with gastro-esophageal reflux appears to be one of the core clinical features of *MEIS2* haploinsufficiency, in addition to intellectual disability, cleft palate and cardiac septal defect.**
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INTRODUCTION

Meis homeobox 2 (*MEIS2*; also known as *MRG1*, NM_170677.4) encodes a homeobox (HOX) protein belonging to the three amino acid loop extension (TALE) superfamily. TALE homeobox proteins are highly conserved transcription factors.[1] MEIS functions as a HOX co-factor that cooperatively binds deoxyribonucleic acid (DNA) to pre-B cell leukemia transcription factor (PBX) and/or HOX proteins to increase the affinity and specificity of their DNA binding activity.[2,3] In mice, *Meis2* is expressed in embryonic neural crest cells (multipotent and migratory cells), particularly in the heart, central nervous system, upper and lower jaw, and intestinal tract.[4] *Meis2* null mice are embryonic lethal at E13.5–14.5, with defects of craniofacial cartilage and heart derived from neural crest cells, which is also observed in knockdown zebrafish.[4–6] The detailed phenotype of heterozygous knockout mice is unknown. Human *MEIS2* is highly expressed in fetal and adult brain and lymphoid organs including spleen, lymph node, thymus and appendix.[7,8] Further, *MEIS2* mutations in humans may lead to cardiac septal defects, cleft palate and intellectual disability (ID), as observed with *MEIS2* haploinsufficiency in six sporadic and one familial deletion cases (123 kb–5.6 Mb), and in another family with a 58-kb duplication that results in a frame shift mutation.[9–12] Only one *MEIS2* mutation at the nucleotide level has been found in a female patient: c.998_1000del (p.Arg333del).[13] This 3-bp deletion at the homeodomain may interfere with DNA binding. Moreover, this female patient also presented with various anomalous features and developmental abnormalities. Here, we report on another *MEIS2* mutation case of specific clinical features and discuss the clinical consequence of *MEIS2* mutations.

CASE REPORT

The patient is the first child of non-consanguineous healthy French parents. She was born after a full-term uneventful pregnancy. Her birth length was 46 cm (−2.5SD), weight 3270 g (mean) and head circumference 33.5 cm (−1SD). Cleft palate, atrial septal defect (ASD) and ventricular septal defect (VSD) were noted at birth. ASD and VSD were surgically corrected 2 weeks after birth, and her cleft palate was operated on twice (at 7 and 23 months). She presented with mild morphological features including a large forehead, mild trigonocephaly, sparse eyebrow, deeply set eyes, large and low-set ears, full cheeks and thin upper lip vermilion (Figures 1a–c). She had a serious feeding difficulty with severe gastro-esophageal reflux requiring gastrostomy at 3 months. Her motor development was delayed: sitting at 11 months and independent gait at 2 years 9 months. Severe hypermetropia was also noticed. At 2 years 9 months, her height was 95 cm (+1SD), weight 12.5 kg (mean) and head circumference 46.8 cm (−1.5SD). Her speech was delayed with only a few words at 2 years 9 months and severe constipation was noted. At 18 months,

[1]Department of Human Genetics, Yokohama City University Graduate School of Medicine, Kanagawa, Japan; [2]Service de Génétique Médicale, CHU de Nantes, Nantes, France; [3]INSERM, UMR-S 957, Nantes, France; [4]Service de Pédiatrie, CHU de Nantes, Nantes, France; [5]Service de Stomatologie, CHU de Nantes, Nantes, France and [6]Department of Medical Genetics, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan
Correspondence: Dr N Miyake or Professor N Matsumoto, Department of Human Genetics, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama, Kanagawa 236-0004, Japan.
E-mail: nmiyake@yokohama-cu.ac.jp or naomat@yokohama-cu.ac.jp
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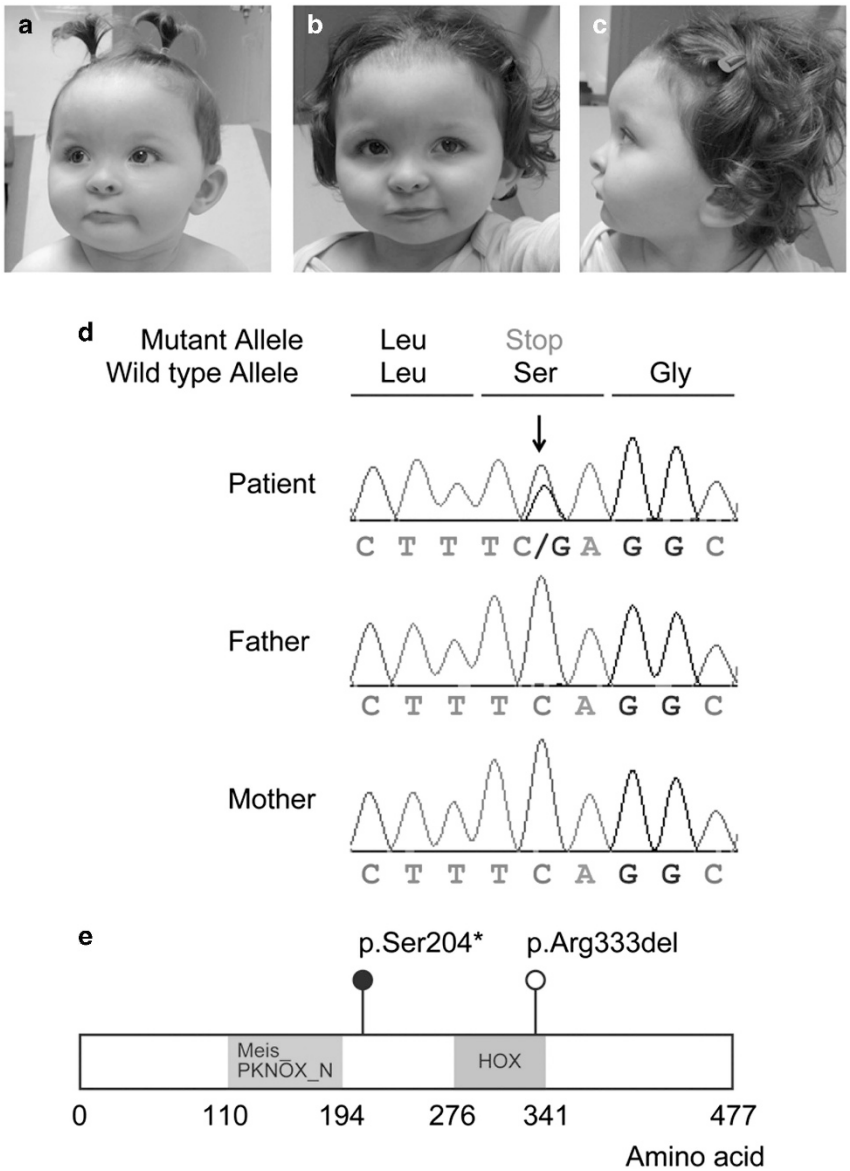

Figure 1 Patient's facial features and *MEIS2* mutation. Facial photographs at 13 months (**a**) and 20 months (**b** and **c**). (**d**) Electoropherograms for the c.611C>G (p.Ser204*) mutation in *MEIS2*. Arrow indicates the mutation. (**e**) Schematic representation of MEIS2 protein with its domain and the mutation. Open and filled circles indicate a previously reported mutation and the current mutation, respectively. The protein contains a N-terminal of Homeobox Meis and PKNOX1 (Meis_PKNOX_N) domain and a homeobox (HOX) domain predicted by SMART (http://smart.embl.de/). A full color version of this figure is available at the *Journal of Human Genetics* journal online.

brain magnetic resonance imaging and comparative genomic hybridization array (Agilent 60k, Agilent Technologies, Santa Clara, CA, USA) were all normal.

MATERIALS AND METHODS

Whole-exome sequencing was performed for the patient only. Blood leukocytes were obtained from the patient and her parents with their informed consent. Genomic DNA was extracted by standard methods, partitioned using the SureSelect Human All Exon v5 Kit (Agilent Technologies), and sequenced by Illumina HiSeq2000 (Illumina, San Diego, CA, USA) with 101-bp paired-end reads. Variants were selected by excluding synonymous variants and variants registered in dbSNP137, our in-house exome database of 575 Japanese individuals, and the NHLBI Exome Sequencing Project (ESP6500, http://evs.gs.washington.edu/EVS/) (MAF$\geqslant$0.01). The remaining variants were then evaluated depending on whether they fitted an X-linked or autosomal dominant model (*de novo* occurrence), and were found in the known mutant genes registered in the Human Gene Mutation database Professional (http://www.biobase-international.com/product/hgmd). Candidate variants were confirmed by Sanger sequencing. Parentage was tested using nine microsatellite markers with Gene Mapper software v4.1.1 (Life Technologies Inc., Carlsbad, CA, USA). This study was approved by the institutional review board of Yokohama City University School of Medicine.

RESULTS AND DISCUSSION

Mean whole-exome sequencing read depth in protein coding regions of RefSeq was 102×, with 93.9% of target regions covered by 20 reads

Table 1 Clinical features of patients with *MEIS2* alterations

Reference	*Erdogan* et al.[9]	*Chen* et al.[10]	*Crowley* et al.[11]	*Johansson* et al.[12]									*Louw* et al.[13]	*Present case*
Case number	1	2	3	4-1	4-2	4-3	4-4	5-1	5-2	6	7	8	9	10
Sex	Female	Male	Male	Female	Female	Male	Female	Female	Male	Male	Male	Female	Female	Female
MEIS2 alteration	5.3 Mb del	5.6 Mb del	123 kb del	58 kb dup[a]				0.6 Mb del		1.0 Mb del	1.9 Mb del	4.8 Mb del	p.Arg333del[b]	p.Ser204*
Inheritance	*De novo*	*De novo*	Somatic mosaic (60% of cells)	*De novo*	Maternal	Maternal	Maternal	*De novo*	Maternal	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*
Cleft lip and cleft palate	Cleft palate	Cleft palate	Cleft soft palate	Submucous cleft palate	Submucous cleft palate	Open cleft palate	Open cleft palate	Open cleft palate	Bilateral cleft lip and palate	None	None	Submucous cleft palate, bifid uvula	Soft and hard cleft palate	Cleft palate
Cardiac malformation	ASD	VSD	VSD	None	None	None	None	VSD	None	None	VSD	VSD	ASD, VSD, LVOTO, CoA	ASD, VSD
Psychomotor development	Moderate ID	Delayed, epilepsy, ID	N.A.	Mild ID	Delayed	Delayed	Mild ID	Normal	Delayed	Mild ID, autism spectrum disorder	Delayed, LD	Delayed, LD	Moderate ID, autism spectrum disorder	Severe ID
Verbal developmental delay	+	+	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	+	+
Motor developmental delay	Mild	N.A.	N.A.	+	+	+	+	+	+	+	+	+	+	+
Walked at age	18 months	N.A.	N.A.	22 months	24 months	14 months	15 months	3 years	21 months	20 months	27 months	23 months	30 months	33 months
Gastro-esophageal reflux	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	+	+
Other features	Muscular hypotonia, upper respiratory infection	Prolapse of epiglottis	Bilateral moderate hearing loss					Agenesis of the right typanic membrane	A gracile corpus callosum				Congenital lobar emphysema, syndactyly	Severe hypermetropia, severe constipation

Abbreviatons: ASD, atrial septal defect; CoA, coarctation of the aorta; ID, intellectual disability; LD, learning difficulty; LVOTO, left ventricular outflow tract obstruction; N.A., information not available; VSD, ventricular septal defect.
[a]58-kb duplication including exon 9 of *MEIS2* resulted in a frame shift mutation.
[b]Louw *et al.* suggest that this mutation may interfere with DNA binding.

or more. Through our variant selection, we successfully narrowed down the variants to a heterozygous nonsense *MEIS2* mutation (c.611C>G, p.Ser204*). In addition, using Sanger sequencing we confirmed that the mutation occurred *de novo* with no discrepant parentage (Figure 1d).

Similar to patients with *MEIS2* haploinsufficiency, two patients possessing *MEIS2* point mutations commonly show cleft palate, cardiac septal defect and ID (Table 1).[9–13] Therefore, we reconfirm these clinical features as the *MEIS2* alteration phenotype. In addition, severe gastro-esophageal reflux requiring gastrostomy was observed commonly in both patients with a *MEIS2* mutation (Table 1).[13] MEIS proteins play an important role in neural crest cell development.[2,4] Neural crest cell-related diseases are collected under the term "neurocristopathy", and include the 22q11.2 deletion syndrome, CHARGE syndrome and Hirschsprung disease, which is a congenital intestinal motility disorder.[14,15] Like *MEIS2* alterations, digestive symptoms (gastro-esophageal reflux, esophagitis or chronic constipation) are observed in 24% (50/211) of patients with 22q11.2 deletions,[16] and gastro-esophageal reflux is observed in 89% (32/36) of patients with CHARGE syndrome.[17] Therefore, digestive symptoms may be a part of the phenotype caused by a loss-of-function mutation in *MEIS2*, and may result from neuronal dysfunction and/or abnormal number of neurons as seen in the neurocristopathy.[15,17] Nevertheless, to fully understand the disease accumulation of patients with MEIS2 aberrations is highly encouraged.

In summary, we have identified a novel nonsense *MEIS2* mutation in a female patient with cleft palate, cardiac septal defect, severe ID, developmental delay and feeding difficulty with gastro-esophageal reflux. Our findings strongly suggest neurocristopathy be included in the clinical features associated with *MEIS2* alterations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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