

## ARTICLE

# Dosage changes of *MED13L* further delineate its role in congenital heart defects and intellectual disability

Reza Asadollahi<sup>1</sup>, Beatrice Oneda<sup>1</sup>, Frenny Sheth<sup>2</sup>, Silvia Azzarello-Burri<sup>1</sup>, Rosa Baldinger<sup>1</sup>, Pascal Joset<sup>1</sup>, Beatrice Latal<sup>3</sup>, Walter Knirsch<sup>4</sup>, Soaham Desai<sup>5</sup>, Alessandra Baumer<sup>1</sup>, Gunnar Houge<sup>6</sup>, Joris Andrieux<sup>7</sup> and Anita Rauch<sup>\*,1</sup>

A chromosomal balanced translocation disrupting the *MED13L* (Mediator complex subunit13-like) gene, encoding a subunit of the Mediator complex, was previously associated with transposition of the great arteries (TGA) and intellectual disability (ID), and led to the identification of missense mutations in three patients with isolated TGA. Recently, a homozygous missense mutation in *MED13L* was found in two siblings with non-syndromic ID from a consanguineous family. Here, we describe for the first time, three patients with copy number changes affecting *MED13L* and delineate a recognizable *MED13L* haploinsufficiency syndrome. Using high resolution molecular karyotyping, we identified two intragenic *de novo* frameshift deletions, likely resulting in haploinsufficiency, in two patients with a similar phenotype of hypotonia, moderate ID, conotruncal heart defect and facial anomalies. In both, Sanger sequencing of *MED13L* did not reveal any pathogenic mutation and exome sequencing in one patient showed no evidence for a non-allelic second hit. A further patient with hypotonia, learning difficulties and perimembranous VSD showed a 1 Mb *de novo* triplication in 12q24.2, including *MED13L* and *MAP1LC3B2*. Our findings show that *MED13L* haploinsufficiency in contrast to the previously observed missense mutations cause a distinct syndromic phenotype. Additionally, a *MED13L* copy number gain results in a milder phenotype. The clinical features suggesting a neurocristopathy may be explained by animal model studies indicating involvement of the Mediator complex subunit 13 in neural crest induction.

*European Journal of Human Genetics* (2013) 21, 1100–1104; doi:10.1038/ejhg.2013.17; published online 13 February 2013

**Keywords:** congenital heart defect; intellectual disability; *MED13L*; copy number changes; neurocristopathy

## INTRODUCTION

*MED13L*, Mediator complex subunit13-like, is a subunit of the so-called Mediator complex that functions with DNA-binding transcription factors and RNA polymerase II for gene transcription.<sup>1</sup> Muncke *et al.*<sup>2</sup> cloned *MED13L* using a positional cloning approach to identify the gene on chromosome 12 interrupted by a translocation breakpoint in a patient with dextro-looped transposition of the great arteries (dTGA) and intellectual disability (ID). They designated the gene as *PROSIT240* due to the protein similarity to the human thyroid hormone receptor-associated protein 240. Independently, Musante *et al.*<sup>3</sup> cloned the gene by RT-PCR, 5-prime RACE of human fetal brain and lymphoblastoid cell line cDNA libraries, which they called THRAP2. Eventually, it was shown that *MED13L* is a component of the Mediator complex in HeLa cells.<sup>1</sup>

From the family of Mediator complex, *MED12* is known to be involved in the etiology of Opitz–Kaveggia syndrome (FG syndrome, MIM no. 305450)<sup>4</sup> and Lujan–Fryns syndrome (MIM no. 309520),<sup>5</sup> X-linked disorders characterized by ID, hypotonia and minor anomalies such as macrocephaly or high forehead and rarely congenital heart defects. For the other members of the Mediator

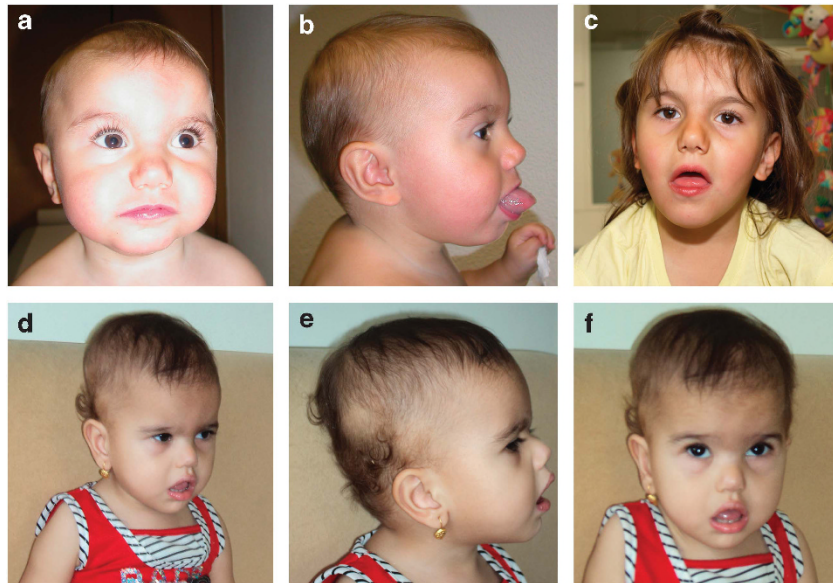
complex, the following disease associations have been reported so far: an 800 kb heterozygous deletion including *MED13* in a patient with ID, cataract and hearing loss,<sup>6</sup> association of infantile cerebral and cerebellar atrophy with a homozygous missense mutation in *MED17*,<sup>7</sup> co-segregation of a missense mutation in *MED23* with non-syndromic autosomal recessive ID<sup>8</sup> and a homozygous missense mutation of *MED25* in a family with Charcot–Marie–Tooth neuropathy.<sup>9</sup> These findings highlight the importance of the Mediator complex in embryonic development and in particular of the nervous system. Concerning *MED13L*, to date, three heterozygous missense mutations in patients with dTGA, one translocation disrupting *MED13L* between exons 1 and 2 in a patient with ID and dTGA<sup>2</sup> and recently, a homozygous missense mutation in two patients of a consanguineous family with non-syndromic ID were found (Table 1).<sup>10</sup> Furthermore, a breakpoint disrupting chromosome 12 near *MED13L* was reported in a patient with Noonan-like phenotype with unknown contribution to the patient's phenotype.<sup>3</sup> Here, we describe for the first time, three patients with copy number changes affecting *MED13L* and delineate a recognizable *MED13L* haploinsufficiency syndrome.

<sup>1</sup>Institute of Medical Genetics, University of Zurich, Zurich, Switzerland; <sup>2</sup>FRIGE's Institute of Human Genetics, FRIGE House, Satellite, Ahmedabad, India; <sup>3</sup>Child Development Center, University Children's Hospital Zurich, Zurich, Switzerland; <sup>4</sup>Division of Cardiology, University Children's Hospital Zurich, Zurich, Switzerland; <sup>5</sup>Shree Krishna Hospital, Karamsad, India; <sup>6</sup>Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, University of Bergen, Bergen, Norway; <sup>7</sup>Institut de Génétique Médicale, Hôpital Jeanne de Flandre, CHRU de Lille, Lille, France

\*Correspondence: Professor A Rauch, Institute of Medical Genetics, University of Zurich, Schorenstrasse 16, Schwerzenbach, Zurich CH-8603, Switzerland. Tel: +41 44 6557051; Fax: +41 44 6557220; E-mail: anita.rauch@medgen.uzh.ch

Received 17 October 2012; revised 9 January 2013; accepted 16 January 2013; published online 13 February 2013





**Figure 1** Facial features of patients 1 and 2 with *MED13L* haploinsufficiency. (a, b) patient 1 at 13 months and, (c) at 4.5 years of age. Note broad forehead with bitemporal narrowing, mild facial asymmetry, long eyelashes and upslanting palpebral fissures, flat nasal root, bulbous nose, deep philtrum and macroglossia. (d, e and f) patient 2 at 2 years and 9 months of age with notable similarity in facial gestalt.

Despite neonatal muscular hypotonia and frequent vomiting, she did not have neonatal feeding problems and was breastfed for more than 1 year. She could sit at the age of 8–9 months and walked without support when she was 30 months old. However, her gait was unsteady for a long time. Her language development was normal and she had good social skills. She gained bladder and bowel control at the age of 5 years.

At 6 and half years of age, her length and head circumference were 117 cm (25th centile) and 50.5 cm (10th–25th centile), respectively. Physical examination revealed a broad nasal bridge and mild pectus excavatum, only. She had satisfactory performance on most levels in her first year of school. The only cardiac malformation was a perimembranous VSD that closed spontaneously.

#### Microarray and confirmatory studies

DNA, extracted from peripheral blood, was analyzed with a Cytogenetics 2.7 array (Affymetrix Inc, Santa Clara, CA, USA) in patient 1, a Human Genome CGH Microarray 60 K (Agilent Technologies, Santa Clara, CA, USA) in patient 2 and an Affymetrix Genome-Wide Human SNP Array 6.0 in patient 3 according to the manufacturers' protocols. Customized MLPA was performed using synthetic probes for exon 2 of *MED13L* and the SALSA MLPA kit P300 Human DNA reference<sup>2</sup> (MRC-Holland, Amsterdam, The Netherlands) in patient 1. Customized qPCR for exons 3 and 4 was performed using SYBR green in patient 2.

#### Exome sequencing

Whole-exome sequencing on genomic DNA of patient 1 was performed using the SureSelect XT HumanAllExon 50 Mb Kit (Agilent Technologies) with 75-bp forward reads on a SOLiD 5500xl System (ABI/Life Technologies). The average depth of coverage was  $40\times$  and about 80% of the targeted bases were assessed by  $\geq 5$  independent sequence reads; only non-silent exonic and splice site variants in genes known to cause developmental disorders (according to HGMD and OMIM) were considered.

#### Mutation analysis

All exons of *MED13L* and candidate nucleotide variants from exome sequencing were analyzed after PCR amplification from the patient's DNA by

Sanger sequencing using an ABI Genetic Analyzer 3730 (Applied Biosystems, Foster City, CA, USA).

#### RESULTS

In patient 1, chromosome studies showed a normal 46,XX karyotype and FISH-testing of the 22q11.2 DiGeorge region revealed no microdeletion. Copy number profiling with the 2.7 array revealed only 10 rare CNVs, including a 17 kb deletion encompassing exon 2 of *MED13L* (Supplementary Figure 1A) ([g.(115,155,757\_115,158,648)\_115,175,505\_115,176,337]del NCBI Build 36.1). No CNV including *MED13L* exons was observed among our 820 control individuals. Deletion of exon 2 was confirmed as *de novo* by customized MLPA and *in silico* analysis determined that the deletion is out of frame.

Considering the possibility of a second hit in this patient, whole-exome sequencing was performed with the evaluation of mutations located in the known disease-causing genes related to congenital anomalies and/or ID. A missense heterozygous mutation (c.1468 G>A, p.G490S) within the *ATRX* gene (ENSG00000085224) was detected, but turned out to be maternally inherited and was predicted as benign by PolyPhen-2 classification. In spite of the limitations in the exome sequencing data, deletion of *MED13L* is the most likely cause of this patient's phenotype.

In patient 2, by means of Agilent 60 K Microarray, a 115 kb deletion encompassing exons 3 and 4 of *MED13L* was detected, which is out of frame too (g.(114,906,957\_114,960,998)\_115,075,825\_115,116,716) del NCBI Build 36.1). The deletion was confirmed as *de novo* by customized qPCR. Sanger sequencing of all exons and flanking introns of *MED13L* in patients 1 and 2 revealed no pathogenic mutation.

In patient 3, the 6.0 array in the patient and her parents showed a 1 Mb *de novo* triplication in 12q24.2 spanning from 114 508 443 to 115 527 724 (*Homosapiens*, build 36/hg18), which includes the whole *MED13L* gene, several non-protein-coding RNA genes and

microtubule-associated protein 1 light chain 3 beta-2 gene (*MAP1LC3B2*) (Supplementary Figure 1B) ([g.(114507371\_114,508,443)\_(115,527,724\_115528576)tri NCBI Build 36.1]).

## DISCUSSION

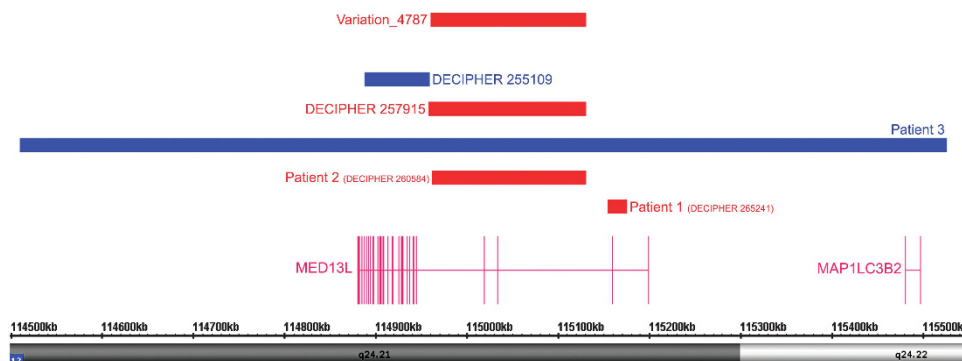
We report the first three patients with copy number variants of *MED13L*, two intragenic out-of-frame *de novo* deletions, and a 1 Mb triplication of the whole gene and the flanking regions (Figure 2). The two patients with out-of-frame deletions showed a distinct phenotype with similar facial dysmorphism, conotruncal congenital heart defect, hypotonia and moderate developmental delay (Figure 1). Facial anomalies included upslanting palpebral fissures, flat nasal root with bulbous tip, deep philtrum, micrognathia, large, low set ears and broad forehead. As both deletions are out-of-frame, it is likely that they disrupt the affected allele completely, resulting in nonsense-mediated mRNA decay of the transcript. Following the recent report of a homozygous missense mutation of *MED13L* in a family with non syndromic ID,<sup>10</sup> which suggests a recessive inheritance pattern, the gene was sequenced in patients 1 and 2 but revealed no pathogenic mutation. Furthermore, considering the two hit hypothesis, exome sequencing was performed in patient 1. However, it did not reveal any strong finding possibly contributing to the patient's phenotype. Absence of an obvious second hit in patient 1 in addition to the phenotypic similarity to the second patient with intragenic deletion strongly suggests that the phenotype in these patients is indeed caused by *MED13L* haploinsufficiency. The third patient with the triplication had a milder phenotype with mild hypotonia, a clinically insignificant cardiac defect and mild developmental delay only. Thus, like in the cases with 22q11.2 duplication or triplication, overexpression of the gene seems to lead to a much milder phenotype than deletions, yet with an overlapping clinical spectrum.<sup>11</sup> On the other hand, the triplication of our patient in addition to *MED13L* encompasses several non-protein-coding RNA genes and the gene *MAP1LC3B2* with unknown function. A larger *de novo* duplication of 2.3 Mb spanning 12q24.21q24.23 containing 16 genes together with *MED13L* has been reported in a girl with syndromic ID without cardiac abnormality.<sup>12</sup> However, several of these affected genes are expressed in the brain and/or are involved in embryogenesis and thus, it is likely that a combination effect of them is contributing to their patient's phenotype. Of note, there are two further *de novo* CNVs affecting

*MED13L* in the DECIPHER database, including a gain of 60 kbp within exons 5–28 in a female patient with ID, developmental delay, auricular tags and macrostomia (DECIPHER no. 255109), and the other one is a 120 kbp *de novo* loss encompassing exons 3 and 4 in a male patient with ID and developmental delay (DECIPHER no. 257915). If the intragenic duplication would be in tandem, though, it would be unlikely to cause gain of function but may rather disrupt the gene.

Notably, a variant deletion of exons 3 and 4 was reported in 6 out of 95 controls using BAC Array CGH by Wong *et al.* 2007,<sup>13</sup> which is likely a false-positive finding, as we did not observe it in our 820 control individuals (Variation\_4787; Figure 2).

Our findings suggest that abnormal *MED13L* dosage can affect both cardiac and neurological development, although both DECIPHER entries not available for this study may indicate reduced penetrance for heart defects. As some heterozygous missense mutations apparently result in isolated heart defects<sup>2</sup> while one homozygous missense mutation was reported to cause non-syndromic autosomal recessive ID,<sup>10</sup> these mutations may affect tissue-specific distinct functions of the protein. In fact, according to Scansite (<http://scansite.mit.edu>) and pfam (<http://pfam.sanger.ac.uk/>) predictions, the missense mutations observed in isolated dTGA patients are located in the N- or C-terminal Mediator complex subunit 13 domains, whereas the homozygous ID mutation does not overlap with any special domain or motif.

The complex phenotype observed in *MED13L* haploinsufficiency is in line with the contribution of different Mediator complex subunits to transcriptional activation of developmentally regulated genes in model organisms,<sup>14–16</sup> which can have remarkably gene-specific, and even tissue-specific functions.<sup>17</sup> mRNA expression of *MED13L* in human has been detected in fetal brain and heart, and adult tissues including skeletal muscle, brain (especially, cerebellum), heart and aorta, kidney and peripheral blood leukocytes.<sup>2,3</sup> Animal studies imply a specific role for MED13, similar in size and ~50% identical to MED13L, in regulating transcription of Wnt and Notch target genes, and Hh signal transduction,<sup>18–20</sup> which are involved in neural crest induction.<sup>21</sup> Neural crest cell migration has an important role in the development of the heart, the nervous system and the facial mesenchyme, which could explain the syndromic signs of *MED13L* haploinsufficiency and the clinical overlap with DiGeorge



**Figure 2** Copy number variants of *MED13L* in our patients. Patient 1 (DECIPHER no. 265241) with a *de novo* deletion of exon 2 (red bar), patient 2 (DECIPHER no. 260584) with a *de novo* deletion encompassing exons 3 and 4 (red bar) and patient 3 with a *de novo* triplication of the region 114 508 443–115 527 724 (Homo sapiens, build 36/hg18) (blue bar), which includes the whole *MED13L* gene, several non-protein-coding RNA genes and *MAP1LC3B2*. Patients 255 109 and 257 915 are from the DECIPHER database with 60 kbp *de novo* gain within exons 5–28 of *MED13L* and 120 kbp *de novo* loss within its exons 3 and 4, respectively (<https://decipher.sanger.ac.uk>). Variation\_4787 from the Database of Genomic Variants (build 36) (<http://projects.tcag.ca/variation>, build 36) is shown in the upper part. Variation\_4787, a 173 kbp deletion observed in 6 out of 95 controls using BAC Array CGH, is likely a false-positive finding due to a misbehaving BAC. This variation was not observed in our 820 control individuals.

syndrome.<sup>22</sup> Of note, a pathogenic role for neural crest cells was also suspected for Lujan-Fryns Syndrome with the involvement of *MED12*, another member of the Mediator complex.<sup>23</sup>

In addition, *MED13L* and other subunits of the Mediator complex were recently shown to have a role in Rb/E2F-induced growth inhibition.<sup>24</sup> So far, no increased tumor susceptibility was observed in patients with *MED13L* defects, however, that may be due to the limited number of patients known and their young age.

In conclusion, our findings show that *MED13L* haploinsufficiency in contrast to the previously observed missense mutations cause a distinct syndromic phenotype. In addition, like in other haploinsufficiency syndromes, a *MED13L* copy number gain results in a milder phenotype.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### ACKNOWLEDGEMENTS

We sincerely thank the affected individuals and their families for participation and their permission to publish the results. We are grateful to Dr Cédric Le Caignec (Service de Génétique Médicale, Nantes, France) and Dr Björn Menten (Centrum Medische Genetica, Ghent, Belgium) for accepting the citation of their DECIPHER Consortium cases (numbers 255109 and 257915, respectively) in our discussion. We also thank the DECIPHER Consortium. This research was supported by grants from the Swiss National Science Foundation (SNF 320030\_135669) and the Forschungskredit of the University of Zurich, grant number 54220201.

- 1 Sato S, Tomomori-Sato C, Parmely TJ *et al*: A set of consensus mammalian mediator subunits identified by multidimensional protein identification technology. *Mol Cell* 2004; **14**: 685–691.
- 2 Muncke N, Jung C, Rudiger H *et al*: Missense mutations and gene interruption in *PROSIT240*, a novel TRAP240-like gene, in patients with congenital heart defect (transposition of the great arteries). *Circulation* 2003; **108**: 2843–2850.
- 3 Musante L, Bartsch O, Ropers HH, Kalscheuer VM: cDNA cloning and characterization of the human *THRAP2* gene which maps to chromosome 12q24, and its mouse ortholog *Thrap2*. *Gene* 2004; **332**: 119–127.
- 4 Risheg H, Graham Jr. JM, Clark RD *et al*: A recurrent mutation in *MED12* leading to R961W causes Opitz-Kaveggia syndrome. *Nat Genet* 2007; **39**: 451–453.
- 5 Schwartz CE, Tarpey PS, Lubs HA *et al*: The original Lujan syndrome family has a novel missense mutation (p.N1007S) in the *MED12* gene. *J Med Genet* 2007; **44**: 472–477.
- 6 Boutry-Kryza N, Labalme A, Till M *et al*: An 800 kb deletion at 17q23.2 including the *MED13* (*THRAP1*) gene, revealed by aCGH in a patient with a SMC 17p. *Am J Med Genet A* 2012; **158A**: 400–405.
- 7 Kaufmann R, Straussberg R, Mandel H *et al*: Infantile cerebral and cerebellar atrophy is associated with a mutation in the *MED17* subunit of the transcription preinitiation Mediator complex. *Am J Hum Genet* 2010; **87**: 667–670.
- 8 Hashimoto S, Boissel S, Zarhrate M *et al*: *MED23* mutation links intellectual disability to dysregulation of immediate early gene expression. *Science* 2011; **333**: 1161–1163.
- 9 Leal A, Huehne K, Bauer F *et al*: Identification of the variant Ala335Val of *MED25* as responsible for *CMT2B2*: molecular data, functional studies of the SH3 recognition motif and correlation between wild-type *MED25* and *PMP22* RNA levels in *CMT1A* animal models. *Neurogenetics* 2009; **10**: 275–287.
- 10 Najmabadi H, Hu H, Garshasbi M *et al*: Deep sequencing reveals 50 novel genes for recessive cognitive disorders. *Nature* 2011; **478**: 57–63.
- 11 Yobb TM, Somerville MJ, Willatt L *et al*: Microduplication and triplication of 22q11.2: a highly variable syndrome. *Am J Hum Genet* 2005; **76**: 865–876.
- 12 Ruiter M, Koolen DA, Pfundt R *et al*: A novel 2.3 Mb microduplication of 12q24.21q24.23 detected by genome-wide tiling-path resolution array comparative genomic hybridization in a girl with syndromic mental retardation. *Clin Dysmorphol* 2006; **15**: 133–137.
- 13 Wong KK, deLeeuw RJ, Dosaanj NS *et al*: A comprehensive analysis of common copy-number variations in the human genome. *Am J Hum Genet* 2007; **80**: 91–104.
- 14 Wang JC, Walker A, Blackwell TK, Yamamoto KR: The *Caenorhabditis elegans* ortholog of *TRAP240*, *CeTRAP240/let-19*, selectively modulates gene expression and is essential for embryogenesis. *J Biol Chem* 2004; **279**: 29270–29277.
- 15 Lim J, Lee OK, Hsu YC, Singh A, Choi KW: *Drosophila* *TRAP230/240* are essential coactivators for *Atonal* in retinal neurogenesis. *Dev Biol* 2007; **308**: 322–330.
- 16 Ito M, Okano HJ, Darnell RB, Roeder RG: The *TRAP100* component of the *TRAP*/Mediator complex is essential in broad transcriptional events and development. *EMBO J* 2002; **21**: 3464–3475.
- 17 Conaway RC, Conaway JW: Function and regulation of the Mediator complex. *Curr Opin Genet Dev* 2011; **21**: 225–230.
- 18 Yoda A, Kouike H, Okano H, Sawa H: Components of the transcriptional Mediator complex are required for asymmetric cell division in *C. elegans*. *Development* 2005; **132**: 1885–1893.
- 19 Janody F, Treisman JE: Requirements for Mediator complex subunits distinguish three classes of notch target genes at the *Drosophila* wing margin. *Dev Dyn* 2011; **240**: 2051–2059.
- 20 Janody F, Martirosyan Z, Benlali A, Treisman JE: Two subunits of the *Drosophila* mediator complex act together to control cell affinity. *Development* 2003; **130**: 3691–3701.
- 21 Stuhlmiller TJ, Garcia-Castro MI: Current perspectives of the signaling pathways directing neural crest induction. *Cel Mol Life Sci* 2012; **69**: 3715–3737.
- 22 Calmont A, Ivins S, Van Bueren KL *et al*: *Tbx1* controls cardiac neural crest cell migration during arch artery development by regulating *Gbx2* expression in the pharyngeal ectoderm. *Development* 2009; **136**: 3173–3183.
- 23 Lin AE, Pober BR, Mullen MP, Slavotinek AM: Cardiovascular malformations in Fryns syndrome: is there a pathogenic role for neural crest cells? *Am J Med Genet A* 2005; **139**: 186–193.
- 24 Angus SP, Nevins JR: A role for Mediator complex subunit *MED13L* in Rb/E2F-induced growth arrest. *Oncogene* 2012; **31**: 4709–4717.

Supplementary Information accompanies the paper on European Journal of Human Genetics website (<http://www.nature.com/ejhg>)