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# Refinement of the critical 2p25.3 deletion region: the role of *MYT1L* in intellectual disability and obesity

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**Purpose:** Submicroscopic deletions of chromosome band 2p25.3 are associated with intellectual disability and/or central obesity. Although *MYT1L* is believed to be a critical gene responsible for intellectual disability, so far no unequivocal data have confirmed this hypothesis.

**Methods:** In this study we evaluated a cohort of 22 patients (15 sporadic patients and two families) with a 2p25.3 aberration to further refine the clinical phenotype and to delineate the role of *MYT1L* in intellectual disability and obesity. In addition, *myt1l* spatiotemporal expression in zebrafish embryos was analyzed by quantitative polymerase chain reaction and whole-mount in situ hybridization.

**Results:** Complete *MYT1L* deletion, intragenic deletion, or duplication was observed in all sporadic patients, in addition to two patients with a de novo point mutation in *MYT1L*. The familial cases comprise a 6-Mb deletion in a father and his three children and a 5′ *MYT1L* overlapping duplication in a father and his two children. Expression analysis in zebrafish embryos shows specific *myt1l* expression in the developing brain.

**Conclusion:** Our data strongly strengthen the hypothesis that *MYT1L* is the causal gene for the observed syndromal intellectual disability. Moreover, because 17 patients present with obesity/overweight, haploinsufficiency of *MYT1L* might predispose to weight problems with childhood onset.

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**Key Words:** 2p25.3 microdeletion; 2p25.3 microduplication; intellectual disability; *MYT1L*; obesity

## INTRODUCTION

Patients with deletions involving chromosome band 2p25.3 have recently been described as presenting with a nonspecific clinical phenotype that includes intellectual disability (ID), obesity/overweight, and several dysmorphic features.[1] Although the overweight has been attributed to deletion of *TMEM18* (ref. 2), it has been suggested that haploinsufficiency of *MYT1L* is responsible for the observed ID.

MYT1L belongs to the myelin transcription factor 1 (MYT1) family of neural-specific transcription factors, together with MYT1 and suppression of tumorigenicity 18 (ST18). All three members contain a MYT domain and six or seven zinc finger binding motifs in a cys-cys-his-cys pattern.[3] Although the MYT domain is characteristic for the cys-cys-his-cys transcription factor family, the zinc finger domains are the most strongly conserved throughout evolution (**Supplementary Figure S1** online). In humans, there is one N-terminal zinc finger, two tandem central zinc fingers, and three C-terminal zinc fingers. There are 13 *MYT1L* splice variants and 4 protein isoforms.

To further delineate the phenotypic spectrum associated with *MYT1L* aberrations, we describe the clinical details of 20 patients with ID who all have a submicroscopic chromosomal aberration involving chromosome band 2p25.3. This cohort

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consists of 14 patients with a microdeletion and 6 patients with a microduplication of chromosome band 2p25.3. For all patients, the breakpoints were fine-mapped using high-resolution chromosomal microarray analysis. Furthermore, in addition to our previously published case with a de novo mutation in *MYT1L*, which we identified using whole-exome sequencing,[4] we collected information on an additional patient who has a de novo *MYT1L* mutation that we detected using a high-throughput screening by molecular inversion probe (MIP) analysis.[5] Finally, to gain insight into the involvement of *MYT1L* in brain development and function, we performed expression analyses of *MYT1L* in different human tissues and of its orthologs in developing zebrafish embryos.

## MATERIALS AND METHODS

### Patients

We identified a cohort of 22 patients with aberrations involving chromosome band 2p25.3. This cohort consists of 13 sporadic patients with 2p25.3 deletions (patients 1–10) or duplications (patients 11–13); two cases with a de novo *MYT1L* mutation (patients 14 and 15), of which one was identified in a previous study[4]; and two families with a 2p25.3 chromosomal aberration. Family 1 comprises three affected siblings and their father (patients 16–19), and family 2 includes two affected children and their father (patients 20–22). Informed consent was obtained from all patients, and institutional approval was given by the local medical ethical committee. An overview of all patients and their clinical characteristics is given in **Supplementary Table S1** online. A more detailed clinical report of each patient can be found in the **Supplementary Clinical Information** online. Photographs of patients 1–3, 6–8, 13–15, and 20–22 are shown in **Figure 1**.

### Genomic microarrays

DNA was extracted from peripheral blood lymphocytes of the patients, following standard protocols. Genomic microarray analysis was performed using several well-established high-resolution microarray platforms according to the manufacturer's instructions. Data were further analyzed with arrayCGHbase to define the deleted and duplicated regions.[6]

### Molecular inversion probe analysis

MIP analysis was performed as described elsewhere.[5] Coding sequences were targeted using MIP probes, which are single-stranded DNA segments separated by a linker region, and subsequently amplified by polymerase chain reaction (PCR). MIP libraries were sequenced on an Illumina HiSeq 2000 (Illumina, San Diego, CA). *MYT1L* was 1 of 42 candidate ID genes tested in 2,491 patients with a clinical diagnosis of ID and in whom previous molecular diagnostic tests were negative. MIP libraries were sequenced with an average coverage for all 42 genes that was >220-fold. Variants were then called using the Genome Analysis Toolkit, after which they were prioritized based on diagnostic relevance using criteria described before.[4]

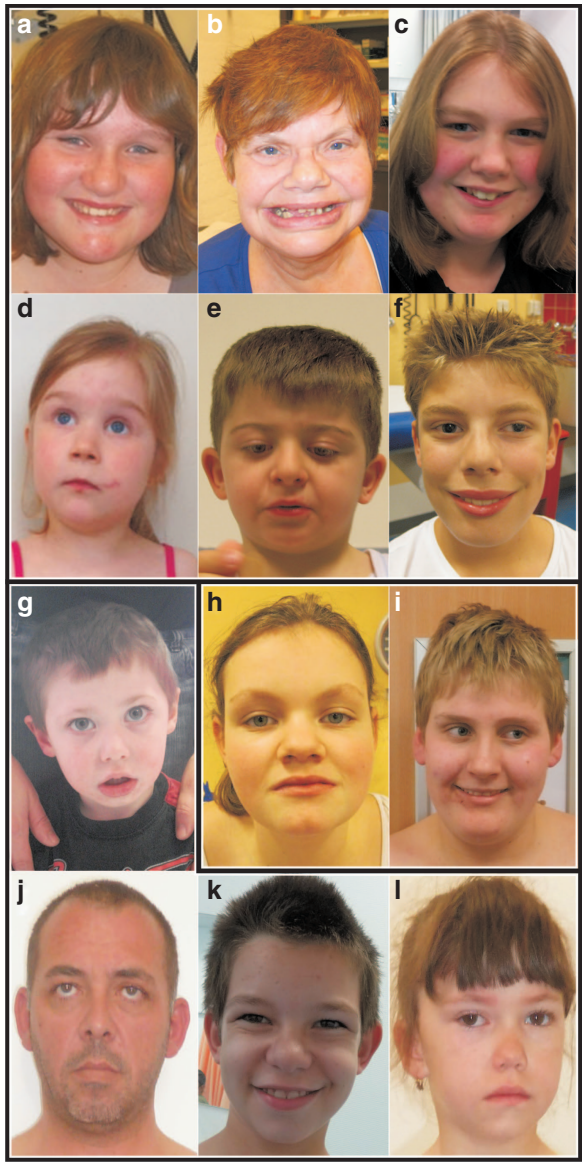


**Figure 1 Photographs of patients with a *MYT1L* aberration.** Patients with a 2p25.3 deletion include (**a**) patient 1, 13 years old; (**b**) patient 2, 50 years old; (**c**) patient 3, 13 years old; (**d**) patient 6, 4 years old; (**e**) patient 7, 4 years old, and (**f**) patient 8, 13 years old. Patients with a 2p25.3 duplication leading to loss of function include (**g**) patient 13, 3 years old; (**j**) patient 20, adult; (**k**) patient 21, 10 years old, and (**l**) patient 22, 6 years old. Patients with a point mutation in *MYT1L* include (**h**) patient 14, 12 years old and (**i**) patient 15, 13 years old. All patients present with mild to moderate intellectual disability and speech delay. Facial dysmorphisms are minor and include a long chin, broad nasal tip, and upslanting palpebral fissures.

### Expression analysis

#### *Expression of MYT1L in human tissues*

Total RNA from 15 human fetal and adult tissues was obtained commercially: whole brain, colon, heart, kidney, liver, lung, breast, and adrenal gland (all adult tissues; Stratagene Europe, Amsterdam, The Netherlands); cerebellum, brain stem, striatum, frontal cortex, occipital cortex, and parietal cortex (adult tissues; Agilent, Diegem, Belgium); and fetal whole brain (Agilent).

Expression analysis was performed, with 100 ng RNA as input, using the SurePrint G3 Human Gene Expression array version 2 (AMADID 041648; Agilent Technologies, Santa Clara, CA) according to the manufacturer's instructions. These data were normalized using the VSN package in R software (http://www.r-project.org/).

### *Expression of myt1l in zebrafish tissues*

*Zebrafish RNA isolation and complementary DNA synthesis.* Ten to 20 zebrafish embryos/larvae at different time points (between 1 and 14 days postfertilization (dpf)) were collected and, if necessary, dechorionated with pronase (2 mg/ml). After the zebrafish were euthanized with an overdose of tricaine, the samples were homogenized in 200 µl QiaZol lysis reagent (Qiagen, Hilden, Germany) and RNA was extracted according to the manufacturer's instructions. The RNA was treated with DNase using the TURBO DNA-free kit (Ambion, Foster City, CA) according to the manufacturer's protocol. Complementary DNA synthesis of these RNA samples was performed on 0.5 µg total RNA with the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions.

*Quantitative PCR.* Quantitative PCR was performed using 2× SYBR Green SsoAdvanced Supermix (BioRad Laboratories), 5 µmol/l forward and reverse primers, and 5 ng complementary DNA as input. Data were normalized by means of in-house validated internal control genes (Vanhauwaert et al., unpublished data). Expression was evaluated with the qBase PLUS software (http://www.biogazelle.com/). Primers for *myt1la* and *myt1lb* are available on request.

*Whole-mount in situ hybridization.* Whole-mount in situ hybridization was performed on 1- to 4-day-old zebrafish embryos, as previously described.[7] Digoxigenin-labeled antisense RNA

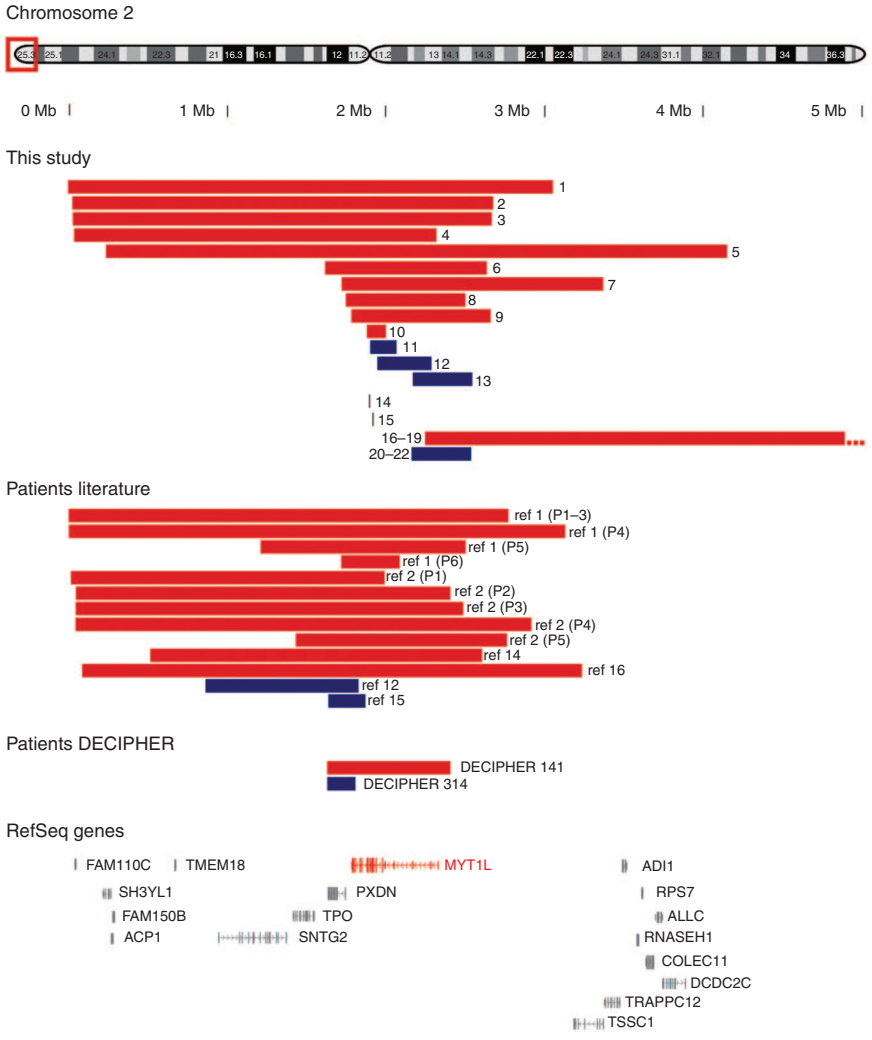


**Figure 2 Overview of the aberrations involving chromosome band 2p25.3 from this study, patients from the literature,[2,11,12,14–16] and two additional patients from the DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources, pinpointing *MYT1L* as the only commonly disrupted gene.** Red bars display deletions and blue bars represent duplications. The de novo splice-site mutations (patients 14 and 15) are depicted as short, vertical black lines. The RefSeq genes are gray. Genomic positions are according to GRCh37.

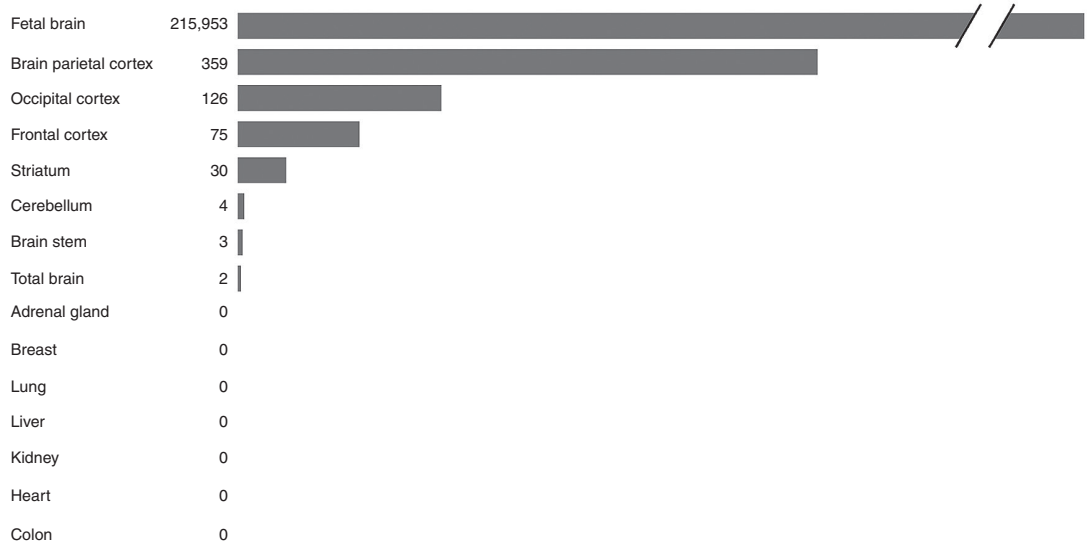


**Figure 3 *MYT1L* expression in 15 different human tissues.** *MYT1L* is predominantly expressed in the human (fetal) brain (expression array data). There is no expression of *MYT1L* in the different human organs tested other than the brain regions.

probes hybridize to complementary messenger RNA sequences in whole-mount zebrafish embryos. The tissues expressing the corresponding gene are identified by the purple stain that results from treatment with an alkaline phosphatase–conjugated antibody against digoxigenin and a chromogenic substrate.

## RESULTS

### Microdeletions and microduplications

Using genomic microarrays, a deletion involving chromosome band 2p25.3 was detected in 14 patients, that is, 10 sporadic patients (patients 1–10) and the four members of family 1 (patients 16–19). The aberrations range in size from 120 kb to 6.07 Mb and contain 1–16 genes (**Figure 2**). All aberrations lead to either partial or complete deletion of *MYT1L*.

Microduplications varying from 170 to 377 kb involving chromosome band 2p25.3 were detected in three sporadic patients (patients 11–13) and the three members of family 2 (patients 20–22). For patients 11, 12, and 13, these duplications appear to be intragenic. For family 2 (patients 20–22), the duplications comprise the 5′ region of *MYT1L*, possibly leading to a loss of function.[8]

In 7 of 10 sporadic patients with a deletion and 3 of 3 patients with a duplication, the de novo status of the aberration could be confirmed (**Supplementary Table S1** online). For the three remaining sporadic patients (patients 4, 5, and 10), no DNA from one or both parents was available to determine the inheritance pattern. The aberrations in families 1 and 2 originated de novo in the affected fathers (patients 19 and 20).

### De novo point mutations

The point mutation in patient 14 was identified in a previous study.[4] This splice-site mutation (c.2636+1G>A) occurred de novo and possibly leads to exon skipping and a truncated protein or messenger RNA decay.

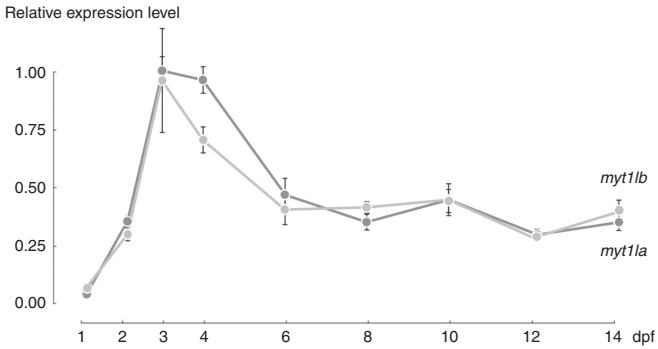


**Figure 4 Patterns of *myt1la* and *myt1lb* expression during zebrafish development (1–14 days postfertilization (dpf)).** *myt1la* and *myt1lb* show a similar temporal expression pattern in zebrafish. The expression of both genes increases during the first 3 days, with a peak in expression at 3 dpf. Error bars reflect standard deviations from the means of five biological replicates pooling 10–20 embryos per sample.

Patient 15 was identified as part of a large-scale resequencing study of candidate ID genes using MIPs. This resulted in the identification of a de novo loss-of-function mutation (c.1917T>G; p.(Tyr639*)) in exon 9, which is part of the MYT domain. This point mutation leads to interruption of the reading frame by a premature stop codon, possibly targeted for nonsense-mediated decay.

### *MYT1L* expression

The expression of *mYT1L* was evaluated in different parts of the human brain and seven other human organs (**Figure 3**). *MYT1L* expression is restricted to distinct regions of the brain and appears to be notably high in fetal brain.

Because of the high expression in early development in humans, mice, and rats (**Figure 3**; unpublished data and refs. 3,9,10), *myt1l* expression was assessed using quantitative PCR at different time points (1–14 dpf) during the embryonic

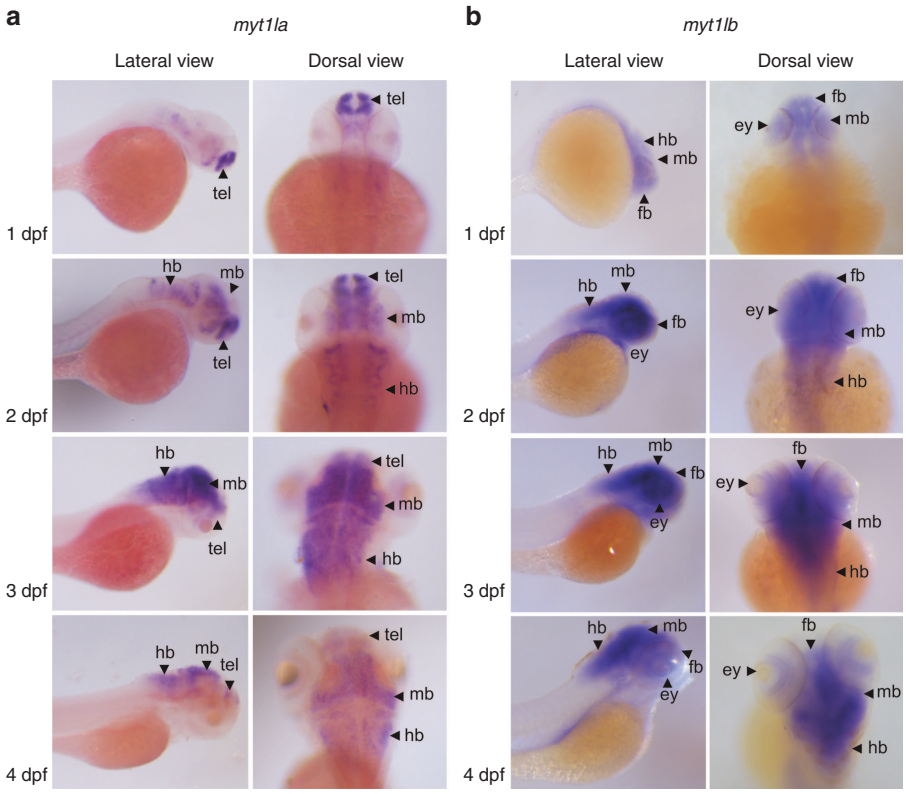


**Figure 5 Whole-mount in situ hybridization of *myt1la* and *myt1lb* in zebrafish embryos (1–4 days postfertilization (dpf)).** The spatial expression of *myt1la* and *myt1lb* in zebrafish increases over time and is restricted to the brain. Pictures of embryos during the first 4 days are shown in the lateral view (left) and the dorsal view (right). (**a**) Expression of *myt1la* starts in the telencephalon (tel; the forebrain (fb)) during the first 24 hours and extends to more posterior regions (midbrain (mb) and hindbrain (hb)). (**b**) *myt1lb* Is initially expressed in the whole central nervous system but stays limited to the different brain regions from 2 dpf on. ey, eye.

development of zebrafish (Figure 4). Both *myt1la* and *myt1lb* show a similar temporal expression pattern, with the highest peak at 3 dpf. Quantitative PCR on complementary DNA derived from eight different organs of the adult zebrafish (brain, eye, skin, liver, intestines, swim bladder, ovaries, and testes) show low but detectable levels of both *myt1l* homologs in the brain and retina and lower expression of *myt1lb* in the testes but no expression of either gene in the other organs (data not shown).

Whole-mount in situ hybridization of the embryos at four time points (1, 2, 3, and 4 dpf) correlates to the increase in *myt1l* expression until 3 dpf, after which a subsequent decline occurred (Figure 5). The expression of *myt1la* is limited to the brain, starting in the telencephalon in the first 24 hours. After 72 hours, the gene is expressed in the whole brain. *myt1lb* is modestly expressed in the brain and spinal cord at 1 dpf but increases later. Expression of *myt1la* and *myt1lb* is restricted to the brain, with an increase during the first stages of embryogenesis.

## DISCUSSION

A total of 18 cases with chromosomal aberrations restricted to chromosome band 2p25.3 have been described previously in the literature, of which 14 have a deletion and 4 have a duplication.[1,2,11–16] Although the deletions observed in these 14 patients differ in size, ranging from 0.12 to 3.15 Mb, all patients presented with ID and weight problems (obesity or overweight). The four patients with a 2p25.3 duplication and disruption of the *MYT1L* gene show significant signs of schizophrenia and paranoia,[12,13,15] whereas a girl, mosaic for 2p25.3 deletion cells, 2p25.3 duplication cells, and normal cells, only shows signs of autism.[14]

Here we report another 20 patients with either a 2p25.3 microdeletion or microduplication, all of whom share a (partial) aberration in *MYT1L*, as well as two patients with a de novo loss-of-function mutation in *MYT1L* (patients 14 and 15). Furthermore, in two patients (patients 9 and 10), *MYT1L* is the only deleted gene. Because these four patients (patients 9, 10, 14, and 15) show clinical features that are strikingly similar to those of the other patients with larger deletions, our data strongly support the hypothesis that *MYT1L* is the causal gene for the observed phenotype, with haploinsufficiency as the most likely mechanism.

For all six patients with submicroscopic 2p25.3 duplications (patients 11–13 and 20–22), one or both breakpoints are located in *MYT1L*, very likely leading to an aberrant transcript and loss of function.

All patients, including those carrying a duplication, present with mild to moderate ID and speech delay, which were

also reported in other patients with 2p25.3 aberrations.[1,2,11,14,16] Obesity or overweight is noted in 17 patients (10 of 14 patients with a deletion, 5 of 6 with a duplication, and 2 of 2 with a point mutation) and has an onset during (late) childhood (**Supplementary Table S1** online). Other prevalent symptoms are a wide range of behavioral problems (11 of 14 patients with a deletion, 6 of 6 with a duplication, and 2 of 2 with a point mutation), such as aggressive, autistiform, and hyperactive behavior; stereotypic hand movements; and sleep disturbances. Joint hyperlaxity and epilepsy were present in four cases (patients 2, 16, 17, and 19) with larger deletions (2.66–6.07 Mb), indicating that these features could be attributed to other genes in the 2p25.3 region.

The DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER) describes two additional patients (314 and 141) with an aberration of this gene.[17] Patient 314 has a 170-kb duplication affecting *PXDN* and disrupting *MYT1L*. This patient shares the clinical symptoms of ID and speech delay. Unfortunately, there is no information regarding the height or weight of the boy. Patient 141 carries a 780-kb deletion comprising *MYT1L* and *PXDN*. She is tall, but proportionate, and there are signs of general obesity. No additional information on these two cases could be obtained.

*MYT1L* is known to play a key role in the conversion of fibroblasts into functional neurons.[18,19] In addition, we and others have shown that the expression of *MYT1L* is restricted to neuronal tissues (**Figure 3**), with peaks during neurogenesis.[3,9,10] To investigate in more detail the temporal and spatial expression of *MYT1L*, we analyzed the expression of the orthologs in zebrafish. According to the Zebrafish Model Organism Database (http://zfin.org/), two zebrafish orthologs are present for *MYT1L*, that is, *myt1la* and *myt1lb*. The human MYT1L protein shows 69 and 73% homology to myt1la and myt1lb, respectively. The zinc finger domains resemble their human counterparts and are strongly conserved (~97–100%; **Supplementary Figure S1** online). The human MYT domain is more homologous to myt1la than to myt1lb (62% vs. 45% identical regions).

During the first 3 days, the process of brain development takes place. In the prehatching period of the embryo, primary neurogenesis allows the larva to perform simple behaviors. Secondary neurogenesis starts around hatching (2 dpf), when the major parts of the brain are established and begin to differentiate.[20] This may explain the evolution of *myt1la* and *myt1lb* expression in the brain. Interestingly, *myt1la* expression starts in the telencephalon, which is implicated in higher brain functions such as memory, basic emotions, and communication. This spatiotemporal expression could indicate the importance of *myt1l* function in the zebrafish brain.

In humans, MYT1L expression is merely brain specific, with a very high expression during fetal brain development and, to a lesser extent, in adult brain tissues (**Figure 3**). Several studies have already shown that *Myt1l* is predominantly expressed in the central nervous system of rats[3,9] and mice,[10] especially during development. Taking these studies together with our human and zebrafish data, we can extrapolate the hypothesis that *MYT1L* is evolutionarily conserved and pivotal for brain development.

Stevens et al.[1] hypothesized that *MYT1L* alone or a positional effect on *TMEM18* expression could account for the obesity observed in patients with 2p25.3 deletions. Since 2009, *TMEM18* has been linked to obesity through several genome-wide association studies.[21–23] It was suggested that perturbed *TMEM18* expression in the brain, and particularly in the hypothalamus, would translate into aberrant feeding behavior. Doco-Fenzy et al.[2] and d'Angelo et al.[16] reported a total of six patients carrying 2p25.3 deletions who had ID associated with obesity. It is interesting to note that in the five patients with a deletion reported by Doco-Fenzy et al., all deletions resided on the paternal allele, suggesting a paternal effect. Although obesity or overweight was noted in all patients in these studies (all had a deletion of *TMEM18*), we also observed weight problems in patients without *TMEM18* deletions (patients 7–10 and 16) or duplications (patients 11, 12, 20–22). Furthermore, both patients with a point mutation in *MYT1L* (patients 14 and 15) suffer from obesity or are overweight. Even though position effects may play a role, these findings indicate that aberrant *MYT1L* function may be a predisposing factor for overweight/obesity, the exact pathophysiological mechanism of which remains unclear.

In conclusion, we describe a cohort of 20 patients with 2p25.3 aberrations and 2 patients with de novo *MYT1L* mutations who present with mild to moderate ID, speech delay, behavioral problems, and, in the majority, overweight or obesity. Although the deletions differ in size and the duplication breakpoints do not cluster together, *MYT1L* is the one gene deleted or truncated in all cases. Furthermore, we describe two patients with a small deletion solely affecting *MYT1L* and two patients with a de novo loss-of-function mutation in *MYT1L*; our data strongly support the hypothesis that haploinsufficiency of *MYT1L* is causative for the observed phenotype in this 2p25.3 deletion syndrome.

Expression patterns of *Myt1l* are restricted to the brain region in mice, rats, and humans. The analysis of the zebrafish orthologs *myt1la* and *myt1lb* shows high expression in the brain during the early stages of brain development, correlating with the waves of primary and secondary neurogenesis. This might indicate a pivotal role for MYT1L in proper neuronal functioning and for the ID in our patients. It is yet to be determined whether and how metabolism is affected through *MYT1L* haploinsufficiency, which may then predispose to overweight.

## SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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## DISCLOSURE

The authors declare no conflict of interest.

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