Received: 7 November 2019 | Revised: 16 January 2020 | Accepted: 28 January 2020

DOI: 10.1002/ajmg.a.61515

ORIGINAL ARTICLE

AMERICAN JOURNAL OF medical genetics PART A WILEY

Nine newly identified individuals refine the phenotype associated with *MYT1L* mutations

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Abstract

Both point mutations and deletions of the *MYT1L* gene as well as microdeletions of chromosome band 2p25.3 including *MYT1L* are associated with intellectual disability, obesity, and behavioral problems. Thus, *MYT1L* is assumed to be the—at least mainly—causative gene in the 2p25.3 deletion syndrome. Here, we present comprehensive descriptions of nine novel individuals bearing *MYT1L* mutations; most of them single nucleotide variants (SNVs). This increases the number of known individuals with causative deletions or SNVs of *MYT1L* to 51. Since eight of the nine novel patients bear mutations affecting *MYT1L* only, the total number of such individuals now nearly equals the number of individuals with larger microdeletions affecting additional genes, allowing for a comprehensive phenotypic comparison of these two patient groups. For example, 55% of the individuals with mutations affecting *MYT1L* only were overweight or obese as compared to 86% of the individuals with larger microdeletions. A similar trend was

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observed regarding short stature with 5 versus 35%, respectively. However, these differences were nominally significant only after correction for multiple testing, further supporting the hypothesis that *MYT1L* haploinsufficiency is central to the 2p25.3 deletion phenotype. Most importantly, the large number of individuals with *MYT1L* mutations presented and reviewed here allowed for the delineation of a more comprehensive clinical picture. Seizures, postnatal short stature, macrocephaly, and microcephaly could be shown to be over-represented among individuals with *MYT1L* mutations.

KEYWORDS

chromosomal microarray, intellectual disability, microdeletion 2p25.3, *MYT1L*, obesity, whole exome sequencing

1 | INTRODUCTION

Heterozygous mutations in the myelin transcription factor-1 like (*MYT1L*) gene are responsible for autosomal dominant mental retardation-39 (MRD39, OMIM # 616521) which is characterized by intellectual disability/developmental delay (ID/DD), diverse behavioral manifestations including aggressive behavior and autistic features as well as obesity or overweight. The facial phenotype does not seem to be recognizable. Up to now, the most frequent type of variation described for *MYT1L* are microdeletions which often include additional genes while considerably less individuals with single nucleotide variants (SNVs) of *MYT1L* have been reported (Al Tuwaijri & Alfadhel, 2019; Becker, Jaggard, & Horrocks, 2010; Blanchet et al., 2017; Bonaglia, Giorda, & Zanini, 2014; de Ligt et al., 2012; De Rocker et al., 2015; Doco-Fenzy et al., 2014; Loid et al., 2018; Mayo et al., 2015; Rio et al., 2013; Stevens et al., 2011; Wang et al., 2016). *MYT1L* has been shown to be the main causative gene in such submicroscopic deletions of chromosome band 2p25.3 (Blanchet et al., 2017; De Rocker et al., 2015). A previous comparison of individuals with *MYT1L* deletion versus *MYT1L* SNVs identified no significant difference regarding the main clinical features (Blanchet et al., 2017).

Here, we present a comprehensive clinical characterization of nine novel individuals from eight families bearing *MYT1L* mutations. Six of the novel variants are SNVs or small indels and two are microdeletions. To our knowledge, this increases the number of known individuals with *MYT1L* mutations to 51. Based on this large number, certain features such as postnatal short stature could be identified as significantly over-represented among individuals with *MYT1L* mutations. Additionally, an even distribution between individuals carrying mutations affecting *MYT1L* only (SNVs, indels, and small microdeletions) and individuals with larger microdeletions affecting additional genes is achieved. A comparison of the proportions of individuals with certain clinical features between these two groups was performed.

2 | MATERIALS AND METHODS

All individuals presented here were originally ascertained during genetic counseling or genetic diagnostics with ID/DD as the most frequent and prominent reason. Additional reasons for ascertainment were behavioral issues (two individuals) and, for example, microcephaly, seizures or obesity (one individual each).

Individuals 1 and 2 were identified in a study to elucidate new candidate ID genes by trio whole exome sequencing (WES) in a cohort of 311 individuals with unexplained ID and their unaffected parents. The inclusion criteria were ID/DD with or without additional features (e.g., craniofacial dysmorphism, organ malformation, etc.) which could not be attributed to a clinically recognizable syndrome by an experienced clinical geneticist. Clinically relevant chromosomal aberrations were excluded by chromosome microarray analysis (CMA). Written informed consent to the study and to the publication of the clinical photographs was obtained from the legal representatives. All investigations were performed in accordance with the protocols of the Declaration of Helsinki and were approved by the local institutional review board (Ethics Committee of the Medical Faculty of the University of Bonn, approvals 131/08 and 024/12). WES was performed as published previously (Schafgen et al., 2016).

Trio WES with DNA samples of individuals 3, 4, 5, and 8 as well as of their healthy parents was performed after written informed consent as described previously (Hempel et al., 2015).

WES of individuals 6a (son) and 6b (father) as well as their unaffected mother/wife and sister/daughter was performed after written consent as published previously (Youssefian et al., 2019). Genomic variants were filtered and prioritized according to the following criteria: heterozygous presence in the affected father and son and absence in the unaffected mother and daughter; gene reported to be involved in neurologic or mental disorders; frequency in publicly available variant databases <0.01%; and in silico predicted pathogenic effect of the variant.

Sanger sequencing was performed by standard methods to confirm all *MYT1L* sequence variants and their de novo status or in the case of individuals 6a and b its parental origin. Primer sequences are available upon request. The Combined Annotation Dependent Depletion tool (CADD, version GRCh37-v1.4, [Kircher et al., 2014]) was used to determine the potential pathogenicity of the identified variants.

After informed consent, CMA of individual 7 (8×60K ISCA v2.0 oligonucleotide array [BlueGnome], backbone resolution ~0.25 Mb) identified a 0.43 Mb deletion at chromosome band 2p25.3 which was

TABLE 1 Summary of findings of Individuals 1–9 and published individuals with genetic variation affecting MYT1L

	Mutations affecting *MYT1L* only										Microdeletions of *MYT1L* and additional genes		All
Individual #	**Individual 1**	**Individual 2**	**Individual 3**	**Individual 4**	**Individual 5**	**Individual 6a**	**Individual 6b (Father of 6a)**	**Individual 7**	**Published SNV/indel carriers (*n* = 14)[1] and published carriers of *MYT1L* only microdeletions (*n* = 2)[2]**	**Total mutations affecting *MYT1L* only (*n* = 24)**	**Individual 8**	**Total large microdeletions (*n* = 27) including 26 published carriers[3]**	***n* = 51**
Mutation (genomic, cDNA NM_015025.2, protein alteration *MYT1L* or microdeletion size [Mb]	chr2: g.1921058C>A c.1531G>T p.(Gly511*)	chr2: g.1844617T>C c.2769-2A>G splice site	chr2: g.1914112C>T c.1711G>A p.(Gly571Arg)	chr2: g.1947036G>A c.223C>T p.(Arg75*)	ch2: g.1915795C>G c.1700G>C p.(Arg567Pro)	chr2: g.1812896T>A c.3118A>T p.(Lys1040*)	chr2: g.1812896T>A c.3118A>T p.(Lys1040*)	arr 2p25.3 (2,231,592-2,659,881)x1 0.43Mb (del *MYT1L* exons 1, 2)	13 SNVs, 1 indel, (6 missense, 3 frameshift, 3 nonsense, 2 splice site); 2 microdeletions	20 SNVs, 1 indel (8 missense, 3 frameshift, 7 nonsense, 3 splice site), 3 microdeletions;	chr2:41,608-1,907,065 1.87Mb		18 SNVs, 3 indels, 30 microdeletions
Age at last examination (years)	12	8	3	6	1.6	27	56	9	3–28	1–28	4	3–57	1–57
Gender	m	f	f	f	m	m	m	f	f:10, m:6	f:14, m:10	m	f:13, m:14	f:27, m:24
Weight (kg) at last examination	40 (−0.5 *SD*)	78.4 (+3.8 *SD*)	20.8 (+2.2 *SD*)	25.6 (+0.9 *SD*)	14 (+1.3 *SD*)	72 (+0.1 *SD*)	87 (+1.4 *SD*)	33.1 kg (+0.2 *SD*)	>2*SD*: 5/13 (38.5%)	>2*SD*: 7/21 (33.3%)	22 (+1.7 *SD*)	>2*SD*: 10/22 (45.5%)	>2*SD*: 17/43 (39.5%)
Height (cm) at last examination	Normal 154.5 (0 *SD*)	Tall stature 154 (+3.6 *SD*)	Normal 100.6 (+0.2 *SD*)	Normal 120.7 (+0.1 *SD*)	Normal 87 (+0.7 *SD*)	Normal 172 (−1.3 *SD*)	Normal 182 (+0.2 *SD*)	Normal 134 (−0.7 *SD*)	Tall stature: 0/13 (0%) Short stature: 1/13 (7.7%)	Tall stature: 1/21 (4.8%) Short stature: 1/21 (4.8%)	Normal 111 (+1.3 *SD*)	Tall stature: 1/23 (4.3%) Short stature: 8/23 (34.8%)	Tall stature: 2/44 (4.5%) Short stature: 9/44 (20.5%)
OFC (cm) at last examination	Normal 52.5 (−1.7 *SD*) (microcephaly at age 6 yrs)	Normal 54 (+1.4 *SD*)	Macrocephaly 52.6 (+2.1 *SD*)	Normal 50 (−1.0 *SD*)	Normal 50 (+0.9 *SD*)	n.r.	n.r.	Normal 51 (−1.2 *SD*)	Macrocephaly: 2/13 (15.4%) microcephaly: 2/13 (15.4%)	Macrocephaly: 3/19 (15.8%) microcephaly: 2/19 (10.5%)	Normal 52.5 (+1.0 *SD*)	Macrocephaly: 2/18 (11.1%) microcephaly: 1/18 (5.6%)	Macrocephaly: 5/37 (13.5%) microcephaly: 3/37 (8.1%)
BMI/weight for height at last examination	Normal 16.8 kg/m^2 (−0.7 *SD*)	Obesity 33.1 kg/m^2 (+3.2 *SD*)	Overweight 20.8 kg/m (+2.5 *SD*)	Normal 17.6 kg/m^2 (+1.1 *SD*)	Normal 14 kg/m (+1.6 *SD*)	Normal 24.3 kg/m^2 (+0.9 *SD*)	Overweight 26.3 kg/m^2 (+1.4 *SD*)	Normal 18.4 kg/m^2 (+0.7 *SD*)	Overweight: 3/14 (21.4%) obesity: 6/14 (42.9%)	Overweight: 5/22 (22.7%) obesity: 7/22 (31.8%)	Normal 20.8 kg/m (+1.5 *SD*)	Overweight: 8/22 (36.4%) obesity: 11/22 (50%)	Overweight: 13/44 (29.5%) obesity: 18/44 (40.9%)
Intellectual disability	+	+	+	+	+	+	+	Borderline	16/16 (100%)	24/24 (100%)	+	25/26 (96.2%)	49/50 (98%)
Developmental delay	+	+	+	+	+	+	n.r.	+	16/16 (100%)	23/23 (100%)	+	21/21 (100%)	44/44 (100%)
Muscular hypotonia	+	+	–	+	+	+	+	–	2/4 (50%)	8/12 (66.7%)	+	4/6 (66.7%)	12/18 (66.7%)
Seizures	–	–	+	–	+ (febrile)	+ (febrile)	+	–	4/5 (80%)	8/13 (61.5%)	–	8/9 (88.9%)	16/22 (72.2%)
ASD	–	–	–	+	–	–	–	+	10/10 (100%)	12/18 (66.7%)	–	4/19 (21.1%)	16/37 (43.2%)
Behavioral anomalies	+	+	+	+	–	+	+	+	16/16 (100%)	23/24 (95.8%)	+	19/19 (100%)	42/43 (97.7%)
Mild craniofacial dysmorphism	–	–	+	+	+	+	+	+	4/12 (33.3%)	10/20 (50%)	+	13/25 (52%)	23/45 (51.1%)
Mild somatic dysmorphism	–	+	–	–	–	–	–	+	3/8 (37.5%)	5/16 (31.3%)	–	10/19 (50%)	15/35 (42.9%)

Note: Differences in total numbers are due to patients for whom the corresponding phenotypic feature was not reported.

Abbreviations: ASD, autism spectrum disorder; n.r.: not reported; OFC, occipitofrontal circumference; yrs: years.

[1]de Rocker et al. patients 14, 15; Blanchet et al. patients 1–9, Al Tuwaijri & Alfadhel, Loid et al., Wang et al.

[2]de Rocker et al. patient 10, Mayo et al. patient 1.

[3]Rocker et al. patient 1, 3–9, 16–19, Stevens et al. patient 1–6, Blanchet et al. patient 10, Bonaglia et al. patient 1, Doco-Fenzy et al. patient 1,3,5, Rio et al. twin 1, Becker et al. patient 1, DECIPHER patient 141.

confirmed by FISH using a BAC probe (RP11-90H11). The deletion involves part of the *MYT1L* gene. Parental testing for the 2p25.3 deletion was negative.

The reference sequence for *MYT1L* was NM_015025.2. The nomenclature of sequence variants follows the recommendations of HGVS (den Dunnen & Antonarakis, 2000). All data interpretation was based on the GRCh37/hg19 human genome assembly.

Statistical significance of the differences in frequencies between two patient groups with different mutation types was calculated for five features (presence or absence of short stature, microcephaly and overweight/obesity at last examination, presence or absence of seizures, and autism spectrum disorder; ASD) using Fisher's exact test. Bonferroni correction for multiple testing (n = 5) yielded a corrected p value of $p \leq .01$ as significance threshold. Age distribution at last examination was compared between the "*MYT1L* only" patient group (median/*M* = 13.07, *SD* = 11.43) and the group of patients with "larger microdeletions" (*M* = 18.35, *SD* = 17.4). This comparison did not reveal significant difference between the two groups (t test, p = .22). For one patient, no information on age was provided in the original study (Wang et al., 2016).

Overweight and obesity are defined according to the definitions of the World Health Organization (de Onis & Lobstein, 2010) as follows: Below 5 years: weight for height (kg/m) > +2*SD*/≤ +3*SD*: overweight, > +3*SD*: obesity; Ages 5–19: BMI (kg/m^2) > +1*SD*/≤ +2*SD*: overweight, > +2*SD*: obesity; adults: BMI ≥ 25: overweight, BMI ≥ 30: obesity.

3 | CASE REPORTS AND RESULTS

3.1 | Case reports and genetic testing

Main findings and measurements at last examination are given in Table 1.

Individual 1 is the third child of healthy, non-consanguineous German parents. He was born by spontaneous vaginal delivery at 40 weeks of gestation (gw 40) after an uncomplicated pregnancy and was small for gestational age (weight: 2,380 g [−2.8 *SD*], length: 49 cm [−1.5 *SD*], occipitofrontal circumference [OFC]: 33 cm [−2.0 *SD*]).

At the age of 1 year, muscular hypotonia and a delayed motor development became obvious. He walked without support at the age of 23 months. His expressive and receptive speech development was delayed with first words at the age of 2.5 years. MFE (Munich functional development diagnostics) at 4 years showed a developmental level corresponding to 16–33 months, the Denver developmental scale at an age of 6 years showed a level of 2–3 years. At the age of 12 years, he still used 2-to-3-word sentences with slurred and partly incomprehensible pronunciation but his vocabulary comprised more than 100 words. He still had significant fine and gross motor skill deficits. No formal intelligence test was performed but his ID was estimated to be in the moderate range.

He had mild behavioral abnormalities with aggressive outbursts and fixation on certain persons and things. He needed stable rules in his daily routines.

At the age of 2 years, a temporary mild hypothyroidism was diagnosed and he was treated with L-thyroxine for 2 years. Examinations of his eyes, teeth, metabolic system, sleep-EEG, brain MRI, and audiology gave normal results.

He attended a school for children with special needs. At the first clinical genetic assessment at the age of 6 $^{6}/_{12}$ years, he presented with microcephaly (OFC 49.5 cm, −2.2 *SD*) and clinodactyly (Figure 1). Other measurements were normal (weight 20 kg, −1.0 *SD*; height 116 cm, −1.1 *SD*; BMI 14.9 kg/m^2, −0.4 *SD*). His parents had normal head circumferences (mother 55 cm, father 57.5 cm). In the last clinical assessment at 12 $^{5}/_{12}$ years, all measurements were in the normal range. He has red hair like his healthy sister, a thin upper lip and no noticeable dysmorphic features (Figure 1).

Conventional karyotyping, FISH (whole chromosome paint 21), microdeletion and microduplication screening (MLPA Kit P245, MRC Holland) and chromosomal microarray (CMA) gave normal results. Trio WES revealed a heterozygous de novo nonsense mutation in *MYT1L* [NM_015025.2:c.1531G>T, p.(Gly511*); CADD 42].

Individual 2 is the second child of healthy, non-consanguineous German parents. The pregnancy was uneventful except for gestational diabetes and minor nicotine consumption. Individual 2 was born by spontaneous vaginal delivery at gw 41 + 3 with a weight of 3,100 g (−1.2 *SD*), a length of 52 cm (−0.2 *SD*) and an OFC of 34 cm (−1.0 *SD*). A nuchal loop was reported. The Apgar scores were 8/10/10.

Her motor development was delayed with crawling at 13 months, sitting without support at 14 months and walking without support at 20 months. Truncal hypotonia was apparent since the age of 4 months and poor fine and gross motor abilities were reported at the age of 8 years.

She spoke her first words at 8 months with a subsequent a delay of expressive speech development (first two-word sentences at the age of 2.5 years, 3-to-5-word sentences at her first clinical genetic assessment at the age of 6 $^{2}/_{12}$ years). At this time, she presented with tall stature and obesity (height: 132 cm,+2.5 *SD*; weight: 44.8 kg,+3.4 *SD*; BMI: 25.7 kg/m^2,+3.2 *SD*) and a normal OFC (50 cm, −1.0 *SD*). She was diagnosed with mild ID (SON-R at 4 years: IQ 59) and showed behavioral problems with aggressive outbursts. The parents reported frequent nose bleeding. Obesity started in her second year of life, the parents reported a missing sense of satiation. At the last clinical genetic assessment at the age of 8 years, she still presented with tall stature and obesity. She had minor, nonspecific dysmorphic features including narrow palpebral fissures (possibly due to her obesity), a short neck, tapering fingers, sandal gap, and short distal phalanges of the toes with small nails (Figure 1). Her skin shows striae distensae and regions of yellow-brown discoloration at her neck along skin folds and in the armpits. She presented with a rough voice, increased noise and pain sensitivity, insomnia with nocturnal awakenings, and persisting muscular hypotonia. According to the mother, she measured 170 cm (+3.7 *SD*) and 106 kg (+4.0 *SD*, BMI: 36.7 kg/m^2, +3.3 *SD*) at the age of 10 $^{9}/_{12}$ years and menarche was at age 10 years. She attends a special school for mentally handicapped children and cannot read nor write, except her name.

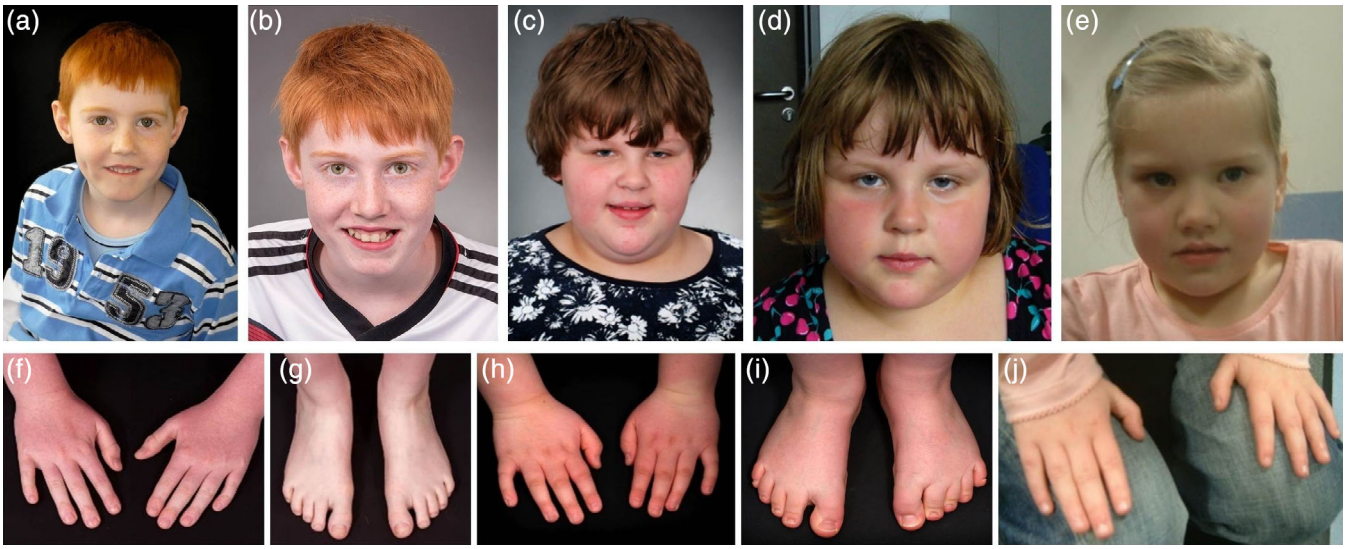

FIGURE 1 Portrait and photographs of hands/feet of Individuals 1, 2, and 7. Facial appearance at the age of 6 (a) and 12 years (b), hands (f) and feet (g) of Individual 1. Besides thin upper lip and fifth finger clinodactyly, no dysmorphisms were present. Facial appearance at the age of 6 (c) and 8 years (d), hands (h) and feet (i) of Individual 2. Note obesity and minor dysmorphic features including narrow palpebral fissures, a short neck, tapering fingers, sandal gap, and short distal phalanges of the toes with small nails. Facial appearance (e) and hands (j) of Individual 7 at the age of 9 years. Note mild facial dysmorphism with a long face, cupid bows, and upslanting palpebral fissures [Color figure can be viewed at wileyonlinelibrary.com]

EEGs, brain MRI, audiometry, basic metabolic investigations, conventional karyotyping, fragile X-syndrome and Prader Willi/Angelman-syndrome diagnostics, and CMA gave normal results. WES revealed two heterozygous de novo splice site mutations in *MYT1L* [NM_015025.2:c.2769-2A>G; CADD 33] and *SETD1B* [NM_015048.1:c.3126+2C>T; CADD 10.9].

Individual 3, a 2 $^{4}/_{12}$ years old girl, is the second child of healthy non-consanguineous parents. She was born after an uncomplicated pregnancy by vaginal delivery at gw 40 + 1 with normal measurements (weight: 3,530 g [+0.1 *SD*], length: 53 cm [+0.6 *SD*], OFC: 36 cm [+0.8 *SD*]). After a reportedly normal development in the first months of life, a first generalized febrile seizure occurred at the age of 10 months, followed by recurrent febrile and nonfebrile seizures which responded well to treatment with Valproate. Motor development was delayed with rolling over at 9–10 months, unaided sitting at 15 months, and unsupported walking at 23 months with a gait that continued to be unstable. She had a speech delay with ~20 words but was raised bilingually. She seemed to tire very easily, while her muscle tone was normal. She got agitated easily and had difficulties to concentrate. There were no organic malformations.

At the last examination at age 3 years and 5 months we saw an agitated and restless girl with a clumsy gait who could speak only single words and follow simple instructions. She was overweight, macrocephalic, and slightly dysmorphic with low-set ears, synophrys (familial), large palpebral fissures, and long eyelashes.

Extensive metabolic screening in blood, cerebrospinal fluid and urine and cranial MRIs at the ages of 10 months, 20 months, and 2 $^{4}/_{12}$ years gave normal results. Panel analysis of genes associated with epileptic encephalopathy could not identify disease causing gene mutations.

Trio WES resulted in the discovery of a de novo missense variant in *MYT1L* [NM_015025.2:c.1711G>A, p.(Gly571Arg), CADD 29.4].

Individual 4 is the first and only child of healthy unrelated parents of German origin. She was born at term after uneventfully pregnancy by vacuum extraction due to abnormal CTG. Birth measurements were normal (weight 3,560 g [+0.2 *SD*], length 53 cm [+0.6 *SD*], OFC 35.5 cm [+0.5 *SD*]). Surgeries for bilateral inguinal hernia were performed at ages of 7 and 8 weeks. A delay in motor development was noted at the age of 6 months. Supported by physiotherapy, she started to roll over, to sit unaided at and to walk without support at the age of 9, 14, and 22 months, respectively. Her gait was unstable and clumsy with frequent falling and poor balance; joint instability was treated with leg braces. Gross and fine motor skills and slightly delayed speech development improved over time. Her intelligence was described as mildly impaired; formal testing has not been performed. Abnormal behavior included auto-stimulation and hand stereotypies with fluttering and washing movements. Over time, aggression and auto-aggression became more prominent. She was shy and reclusive with particularly reduced her interactions in noisy situations. The parents reported that she has difficulties falling asleep.

At the last examination at the age of 6 $^{4}/_{12}$ years, we saw a friendly, open-minded girl with normal measurements. She spoke in correct sentences and could follow simple tasks. The gait was clumsy with inwardly rotated feet. Arms were always held in slight flexion at the elbows, and hands in palmar flexion. Further examination revealed joint hypermobility, axial muscular hypotonia and limb hypertonia, increased tendon reflexes and poor balance. Minor dysmorphic features included a long face, thick and horizontal eyebrows, small vermillion, pointed chin, and simple ears.

Extended metabolic work up in blood, urine and CSF as well as MRI of brain and spinal cord gave normal results. SSEP showed slightly elevated latency in response to right n. tibialis stimulation. CMA gave normal results.

Trio WES identified a de novo nonsense mutation in *MYT1L* [NM_015025.2:c.223C>T, p.(Arg75*), CADD 35.0].

Individual 5 is the second child of healthy unrelated parents of German origin. He was born at gw 38 by cesarean section due to macrosomia. Birth measurements were: weight 4,230 g, +2.1 *SD*; length 53 cm, +0.8 *SD*; OFC 37 cm, +1.5 *SD*. Perinatally, there was a short episode of shallow breathing which was most likely due to an aspiration. Delay in motor development, muscular hypotonia and poor eye fixation were noted at the age of 7 months. At the age of 9 months, he experienced a fever-associated seizure treated successfully with levetiracetam.

At the last examination at the age of 19 months, we saw a friendly boy with normal measurements. He showed a predominantly truncal muscular hypotonia, strabismus, and mildly dysmorphic features: a high and broad forehead, flat midface, deep-set eyes, a broad and flat nasal bridge, small mouth with downturned corners, puffy hands and feet, tapering fingers, rather short fifth fingers as well as two small cafe-au-lait spots on the upper leg. Severe developmental delay was apparent. The boy was able to stand on all fours and to sit, but could not crawl, stand, nor walk. His fine motor skills were not age-appropriate, but he had started sorting things or clapping them together. His receptive and expressive speech development was severely delayed: He made sounds but did not form words.

Extended metabolic work up in blood, urine and CSF as well as cranial MRI and EEG (under treatment with levetiracetam) gave normal results.

Trio-WES resulted in the discovery of a de novo missense mutation in *MYT1L* [NM_015025.2:c.1700G>C, p.(Arg567Pro), CADD 29.4].

Individual 6a was delivered at gw 37 + 2 by cesarean section after a normal pregnancy. Birth length (47 cm, −1.5 *SD*) and head circumference (32 cm, −1.7 *SD*) were within the lower range, and his weight was 3,700 g (+1.2 *SD*). His development was delayed, especially concerning speech and language. At 2 $^{3}/_{12}$ years he spoke <25 words clearly and did not form sentences. Receptive language was impaired with difficulties in playing with other children. Hearing impairment was excluded. His motor development was mildly delayed (independent sitting and walking at the age of 11 and 19 months, respectively). He tended to display destructive behavior. Two febrile seizures occurred at Ages 3 and 5 years. EEG showed abnormal peak waves but was inconclusive. He had a history of hypotonia and feeding problems, systolic heart murmurs and mild gastroesophageal reflux. He was diagnosed with attention deficit hyperactivity disorder and attended a special school for children with language and communication disabilities. ASD was excluded by neuropsychological tests (autism diagnostic interview—revised, autism-spectrum quotient, child autism rating scale).

He was referred for clinical genetic assessment at the age of 27 years with normal measurements. Dysmorphic facial features included a broad nasal bridge, wide nasal tip, short philtrum, large ears with hyperplastic ear lobes, wide mouth, irregularly spaced teeth, relatively small hands, and micro-orchidism. Hypotonia and hyperactivity were observed. He had myopia and frequent otitis.

He had been institutionalized from 17 to 22 years of age, during which occasional aggressive and self-injurious behaviors were reported. After institutionalization, he lived at home with psychological and social assistance and is unemployed. Neuropsychological tests including Wechsler Adult Intelligence Scale/WAIS and Folstein test revealed mild ID.

Conventional karyotyping of blood lymphocytes and fragile-X-syndrome diagnostics gave normal results.

Individual 6b is the father of Individual 6a. According to his older sister, he was delivered at term after an uncomplicated pregnancy with a weight of ~3.2 kg (−1 *SD*) as only known birth measure. He suffered from epilepsy until puberty.

At clinical genetic assessment at the age of 56 years, he was overweight and presented with mild dysmorphic craniofacial features: mild strabismus, bulbous, rounded nasal tip, short and broad philtrum, wide mouth, widely spaced teeth, thick lower lip, and an elongated neck. Hypotonia and hyperactivity were observed. Aggressive outbursts in stressful situations were reported. He is employed under supervision as a warehouseman. Neuropsychological tests (see above) revealed mild ID.

A heterozygous nonsense variant [NM_015025.2:c.3118A>T, p.(Lys1040*), CADD 46.0] in *MYT1L* was identified both in the affected father and son, but not in the unaffected mother and daughter.

Individual 7 is the first of three children of healthy unrelated parents. She was born after uneventful pregnancy at gw 38 by normal delivery. Her birth weight was 3,130 g (−0.1 *SD*) and her OFC at birth was 33.5 cm (−0.6 *SD*). Her birth length is unknown. Her OFC at 6 weeks was 37 cm (−0.3 *SD*) and at 33 weeks it was 41.5 cm (−2.4 *SD*).

She had normal gross motor developmental milestones, but her fine motor skills were delayed. Her early speech development was normal but her speech remained unclear and needed speech and language therapy because of pronunciation difficulties. She was referred to the pediatrician at the age of 4 ½ years because of concerns about sensory behaviors, poor fine motor skills and issues regarding social interactions. A cognitive assessment showed a verbal IQ of 75 and a performance IQ of 67. She had autistic features with ritualistic behavior and a very good memory for images. She was diagnosed with atypical autism. At the last examination at the age of 9 years, measurements were normal. She had mild facial dysmorphism with a long face, cupid bows, and upslanting palpebral fissures (Figure 1). First signs of puberty were early, at the age of 9 years. She had increased TSH with normal T4 and no indication for thyroid treatment at that stage.

CMA identified a 0.43 Mb deletion on the short arm of chromosome 2 at p25.3 which was confirmed by FISH studies. It affects only Exons 1 and 2 of the *MYT1L* gene. The deletion was shown to be de novo.

Individual 8 is the fourth child of healthy non-consanguineous parents and was born after an uneventful pregnancy at gw 37 + 6 GW by

TABLE 2 Frequency comparison of findings between the two patient groups with different mutation types of MYT1L ("*MYT1L* only" microdeletions and SNVs vs. "larger microdeletions") for five features (presence or absence at last examination of short stature, microcephaly and overweight/obesity, presence or absence of seizures, and ASD) using Fisher's exact test

Feature	All individuals	*MYT1L* only	Larger microdeletions	*p* value
Short stature at last examination	20.5% (9/44)	4.8% (1/21)	34.8% (8/23)	.023
Microcephaly at last examination	11.4% (4/35)	17.6% (3/17)	5.6% (1/18)	.338
Overweight/obesity at last examination	70.5% (31/44)	54.5% (12/22)	86.4% (19/22)	.045
Seizures	76.2% (16/21)	66.7% (8/12)	88.9% (8/9)	.338
ASD	43.2% (16/37)	66.7% (12/18)	21.1% (4/19)	.008

Note: None of the five comparisons were below significance threshold/corrected *p* value of $p \leq .01$ after Bonferroni correction for multiple testing. Nominally significant *p* values are underlined.
Abbreviations: ASD, autism spectrum disorder; SNVs, single nucleotide variants.

re-re-cesarean section with normal measurements (weight 3,120 g, −0.4 *SD*; length 51 cm, 0 *SD*; OFC 34.5 cm, −0.2 *SD*). After an unremarkable development in the first months of life, muscular hypotonia, and delays in motor and speech development became obvious. He started sitting with 1 year and walking at the age of 2.5 years. At the age of 3 $^{10}/_{12}$ years he spoke only two words with an impaired receptive language development. He hardly understood and followed instructions and did not interact with other children. He had myopia and strabismus, but did not accept wearing glasses.

The last examination at age 4 $^{2}/_{12}$ years showed a friendly but reclusive boy with normal measurements and minor dysmorphic features including thick eyebrows, low set ears, retrognathia, and very soft skin. Extended blood analysis including metabolic analysis, cranial MRT, EEG as well as chromosome analysis, and fragile X testing gave normal results.

Trio WES identified a de novo heterozygous deletion of ~1.87 Mb (chr2:41,608-1,907,065) in 2p25.3 involving i.a. the genes *TPO* and *PXDN* and the exons 14–25 of the *MYT1L* gene.

3.2 | Frequency comparison of findings in two patient groups with different mutation types

To our knowledge, our study increases the number of published individuals with *MYT1L* deletions or SNVs to 51 (Al Tuwaijri & Alfadhel, 2019; Becker et al., 2010; Blanchet et al., 2017; Bonaglia et al., 2014; De Rocker et al., 2015; Doco-Fenzy et al., 2014; Loid et al., 2018; Mayo et al., 2015; Rio et al., 2013; Stevens et al., 2011; Wang et al., 2016, DECIPHER patient 141). Thirty of these carry microdeletions, 3 of which affect only *MYT1L* and 27 of which affect additional genes. Twenty-one individuals carry 20 different *MYT1L* SNVs (8 missense and 12 potential loss of function mutations, i.e., nonsense [$n = 6$], frameshift [$n = 3$], or splice mutations/exon skipping [$n = 3$]). Thus, 24 known individuals carry mutations affecting *MYT1L* only (i.e., 21 SNVs and three microdeletions) and 27 individuals carry larger microdeletions affecting additional genes. These numbers may allow for a more comprehensive analysis of possible differences between the clinical appearance of individuals with variants affecting only *MYT1L* and that of individuals with microdeletions affecting additional genes. These two groups of individuals will be referred to as "*MYT1L* only" and "larger microdeletions" from now on. Significance of the frequency differences between the two groups of individuals was calculated for five features (presence or absence at last examination of short stature, microcephaly and overweight/obesity, presence or absence of seizures, and ASD) using Fisher's exact test with a Bonferroni-corrected *p* value of $p \leq .01$ for multiple testing as significance threshold. The findings are summarized in Table 2.

4 | DISCUSSION

In 2011, *MYT1L* was first proposed as the causative gene in 2p25.3 microdeletions and *MYT1L* haploinsufficiency as a cause for ID (Stevens et al., 2011). Based on six individuals with differently sized heterozygous de novo deletions and ID, obesity or overweight and/or a square-shaped stature and five additional patients with overlapping deletions from the DECIPHER database (https://decipher.sanger.ac.uk), a smallest region of deletion overlap containing only *MYT1L* was defined. In 2012, a de novo *MYT1L* splice site mutation was identified as part of a larger study in a girl with ID, autistic features, normal growth, and mild dysmorphism (de Ligt et al., 2012). After three publications on seven more individuals with microdeletions encompassing *MYT1L* and global DD/ID, behavioral difficulties/hyperactivity and overweight/obesity (Bonaglia et al., 2014; Doco-Fenzy et al., 2014; Rio et al., 2013), de Rocker and colleagues published an extensive study on 22 individuals bearing either chromosomal aberrations or SNVs affecting *MYT1L* (De Rocker et al., 2015). Seventeen of these 22 individuals presented with obesity/overweight. Since four individuals with *MYT1L* SNVs or microdeletions exclusively affecting *MYT1L* were strikingly similar to the individuals with larger deletions, the authors hypothesized that *MYT1L* is the causal gene for both observed phenotypes. A further case report described an individual with a de novo intragenic deletion of *MYT1L* and DD, microcephaly, behavioral problems, and stereotypies, but without obesity at an age of 4.5 years (Mayo et al., 2015). In 2017, Blanchet and colleagues published the next larger series of novel patients with ID/DD, reviewed the clinical phenotype and compared the clinical pictures of SNV mutation carriers with that of microdeletion carriers (Blanchet

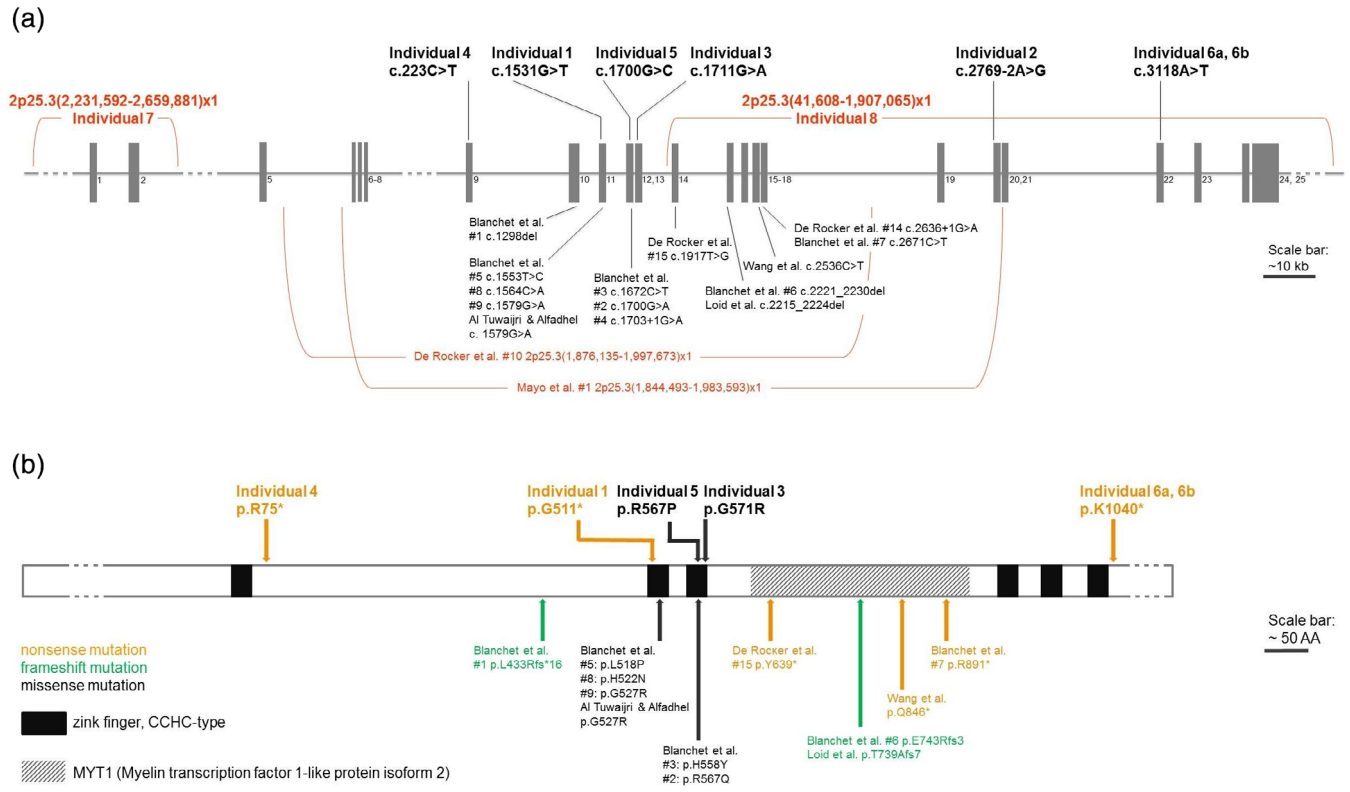

FIGURE 2 Genomic (a) and domain structure (b) of *MYT1L* with localizations of the mutations identified within this study (n = 9; upper panel) and reported in previous studies (n = 15, lower panel). Those mutations that are localized in the coding region are also shown within the domain structure (b). The respective color in the online version corresponds to the type of mutation: orange (nonsense), green (frameshift), and black (missense) [Color figure can be viewed at wileyonlinelibrary.com]

et al., 2017). In addition to one individual with a microdeletion, nine individuals with de novo *MYT1L* SNVs were reported. The authors then compared the proportions of individuals with the phenotypes ID, gross motor delay, speech delay, autism, overweight/obesity, or hyperphagia between 28 published and own individuals with deletions affecting *MYT1L* and 11 published and own individuals with *MYT1L* SNVs (Bonaglia et al., 2014; De Rocker et al., 2015; Doco-Fenzy et al., 2014; Stevens et al., 2011). No significant differences between the groups were found for any of the clinical features, supporting the hypothesis that *MYT1L* haploinsufficiency is central to the 2p25.3 deletion phenotype. The overall proportion of individuals with overweight or obesity was 85%.

Here, we present nine novel individuals with *MYT1L* mutations. The clinical findings of these novel individuals largely confirmed the above-named clinical picture. All nine individuals had developmental delay and (borderline) ID, six had muscular hypotonia, and four had (febrile) seizures. Behavioral anomalies were seen in seven of them and ASD in two. Only three of the nine individuals developed overweight/obesity postnatally. Thus, the frequency for overweight/obesity among these novel individuals is lower than reported before with 33% as compared to, for example, the 85% reported earlier (Blanchet et al., 2017). Severe congenital malformations were absent and craniofacial dysmorphism was generally mild.

Seven individuals carry *MYT1L* SNVs, two of which are familial and identical (Individuals 6a and b), increasing the number of known *MYT1L* SNV carriers by more than half. Individuals 1, 4, 6a, and 6b carry nonsense variants in *MYT1L* and Individuals 3 and 5 missense variants. A splice site mutation was detected in Individual 2 in addition to a putative de novo splice site mutation of *SETD1B*. We are aware of three published individuals with de novo missense or frameshift variants of *SETD1B* and epilepsy, developmental delay, ID, autistic behavior and craniofacial dysmorphic features, and in one case obesity (Den et al., 2019; Hiraide et al., 2018). Thus, an additional effect of the *SETD1B* variant in Individual 2 cannot completely be excluded in spite of its low CADD score. However, several lines of evidence point to an—if any—minor effect: First, all published individuals with de novo *SETD1B* variants had severe myoclonic seizures which are not present in Individual 2. Second, none of them had muscular hypotonia which is present in Individual 2. Additionally, the three published *SETD1B* variants affect the terminal SET domain of the protein while the putative splice site mutation (rs749218728) in Individual 2 is located much further upstream. According to earlier studies, this variant most likely causes nonsense mediated mRNA decay (NMD) (Coban-Akdemir et al., 2018; Den et al., 2019) which in turn has been postulated not to be damaging (Den et al., 2019). In two of the nine individuals, de novo microdeletions affecting *MYT1L* were detected: one of them affecting only two exons of *MYT1L* (Individual 7) and one

of them 12 exons of *MYT1L* and 12 additional RefSeq genes (Individual 8).

To our knowledge, our study increases the number of published individuals with *MYT1L* deletion or SNVs to 51. While a causal role of *MYT1L* for the well-known clinical features ID/DD, ASD, behavioral problems, and overweight/obesity is clearly confirmed, the large number of individuals presented and reviewed here allows for a more comprehensive picture and the highlighting of additional, less frequent clinical features associated with *MYT1L* mutations. Several clinical findings which have only been mentioned in passing if at all in earlier reviews are shown to be considerably over-represented among individuals with *MYT1L* mutations. Of the 21 individuals with information on the presence or absence of seizures, 16 were affected (76.2%, Table 2). Even if none of the individuals without explicit information had had seizures, the resulting minimal frequency of 31.4% would still be high. Other features concern the growth pattern of individuals with *MYT1L* mutations besides their weight/BMI. For these features, explicit information is available for most individuals. Thus, the observation seems quite robust that postnatal short stature, macrocephaly and microcephaly with frequencies of 20.5, 13.5, and 8.1%, respectively, are considerably over-represented among individuals with *MYT1L* mutations.

Additionally, the sample size may allow for a more comprehensive analysis of possible clinical differences between individuals with deletion and SNV variation affecting only *MYT1L* and individuals with larger microdeletions affecting additional genes. Twenty-one individuals carry *MYT1L* SNVs and three carry microdeletions which affect only *MYT1L*. In contrast to those 24 individuals with genetic variation of *MYT1L* only (the "*MYT1L* only" group), 27 carry microdeletions which affect additional genes (the "larger microdeletions" group). It should be kept in mind that these larger microdeletions are quite heterogeneous in their size, localization and gene content. Consequently, the putative effects of larger deletions may vary.

As a matter of course, we agree with the conclusion of earlier publications that *MYT1L* haploinsufficiency is central to the etiology of the clinical features of individuals with microdeletions of 2p25.3 affecting *MYT1L*. One important example is overweight or obesity which affected all reported individuals with microdeletions affecting *MYT1L* in the first review, (Stevens et al., 2011). De Rocker et al. found overweight/obesity of childhood onset in 71% of their deletion patients and both of their SNV patients (De Rocker et al., 2015) and Blanchet and colleagues in 85% of their novel and reviewed patients (Blanchet et al., 2017). However, only three out of the nine novel individuals presented here were affected. Taken together with all previously published individuals, this results in an overall proportion of individuals with overweight/obesity of 70.5% (31/44) with overweight in 29.5%, and obesity in 40.9%. As stated above, we wanted to explore if the numbers of both "*MYT1L* only" individuals and "larger microdeletion" individuals are now large enough to reach statistical significance for potential differences between these two patient groups. While 54.5% (12/22) of the "*MYT1L* only" individuals are overweight/obese, the proportion among individuals with "larger microdeletions" is 86.4% (19/22). This difference is nominally significant only (p = .045) and does not reach significance after correction for multiple testing.

Regarding the less frequently discussed features, postnatal microcephaly yielded no frequency difference between the two groups (*MYT1L* only: 3/17, larger microdeletions 1/18, p = .338). However, especially postnatal short stature may merit closer attention. It was reported in three out of six individuals by Stevens and colleagues but was not addressed in subsequent studies. Of all 44 individuals with postnatal height measurements reported now, nine (20.5%) had postnatal short stature. Interestingly, only one out of 21 (4.8%) "*MYT1L*

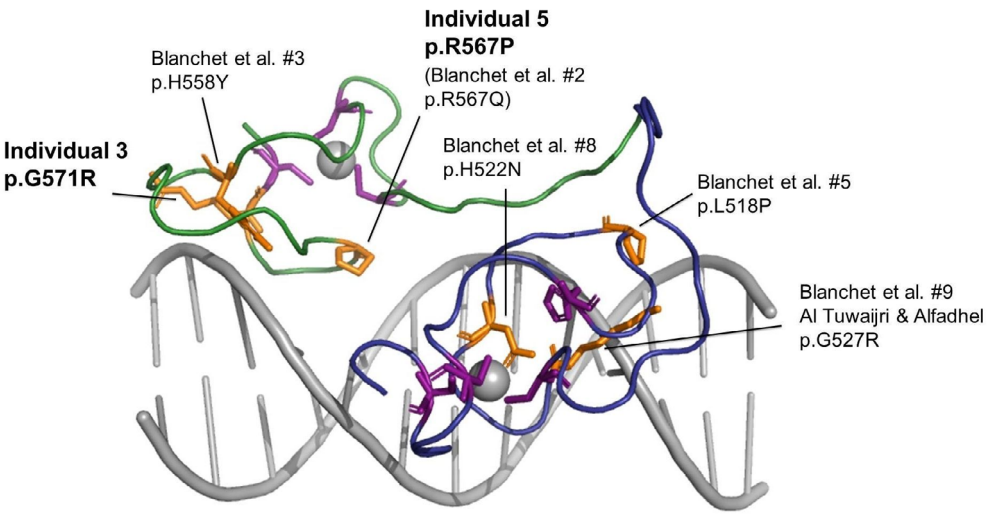

FIGURE 3 Model of the second (blue) and third (green) zinc fingers of MYT1L bound to DNA. The gray spheres represent the zinc ions, with the CCHC zinc ligands shown in purple. All reported missense variants (depicted in orange) identified in eight individuals are localized in the zinc finger domains. The missense variants of Individual 5 and Individual #2 from Blanchet et al. affect the same amino acid (position 567). The figure shows the missense variant of Individual 5 (p.R567P). The image was created using Pymol Version 2.3 (https://www.pymol.org/) [Color figure can be viewed at wileyonlinelibrary.com]

only" individuals, but eight out of 23 (34.8%) for the "larger microdeletions" group had short stature (p = .023). Again, this difference is only nominally significant. This clearly shows that the differences between the two groups are very small if they exist at all, underscoring the importance of *MYT1L* haploinsufficiency for the etiology of the clinical features of individuals with microdeletions of 2p25.3 affecting *MYT1L*. However, the Bonferroni correction used here is quite conservative as it is generally used for independent tests while two of the tests included in the correction here (height and BMI/weight) are not entirely independent. Additionally, insufficient power of the sample analyzed here cannot be excluded. Thus, our data may constitute a—however to date definitely insignificant—hint at a higher overall incidence of postnatal short stature and overweight/obesity in the patient group with additional deleted genes. Additional studies with even larger samples of individuals with different *MYT1L* variation are necessary.

Seizures were described in 76.2% of all individuals with no difference between groups (*MYT1L* only: 8/12, larger microdeletions 8/9, p = .338). While the overall proportion of different behavioral anomalies is very similar for both groups (95.8 vs. 100%), there seems to be a difference for the presence of ASD. This proportion seems to be significantly higher in the "*MYT1L* only" group with 66.7% (12/18) than in the "larger microdeletions" group with 21.1% (4/19, p = .008). However, this should be interpreted with utmost caution since ASD is much more prone to ascertainment differences than features which are determined more easily and more comprehensively such as body measurements. The overall proportion of individuals with reported ASD is 43.2% and thus slightly higher than the one reported by Blanchet and colleagues.

We were also interested in the localization of the many different mutations within *MYT1L* (Figure 2). The nonsense and frameshift variants do not seem to show any clustering or preferential localizations. Even the rather N-terminal nonsense mutations of Individuals 6a and 6b do not seem to be associated with a distinguishable clinical picture. A possible explanation for this may be mRNA NMD as opposed to truncation which would more likely give rise to distinguishable clinical pictures caused by proteins of different lengths and domain contents. However, this hypothesis could not be analyzed for lack of suitable patient material. Equally, we could not find evidence for preferential deletions of certain regions of the gene neither for deletions within the gene nor for deletions affecting the N- or the C-terminus of the gene (Figure 2) or for different deletion localizations being associated with different clinical pictures. Interestingly, all seven known missense variants (identified in eight individuals) are localized in the second and third zinc finger domains and therefore are likely to interfere with DNA binding (Figures 2 and 3).

In summary, we present nine novel individuals from eight families with different types of mutations affecting *MYT1L*, seven of them with SNVs affecting *MYT1L* and two of them with microdeletions affecting either *MYT1L* only or *MYT1L* and additional genes. Seven of the individuals carry de novo mutations and one SNV is shared by an affected son and his affected father in whose case the origin of the mutation could not be analyzed.

Our study gives the most comprehensive picture of the clinical appearance of individuals with *MYT1L* mutations so far. The typical clinical picture of ID/DD together with frequent overweight or obesity is confirmed. Overweight/obesity is present in 70.5% of individuals and thus slightly less frequent than reported before. Altogether, the number of affected individuals now is 51, allowing for the most comprehensive clinical picture yet. Thus, several clinical findings such as seizures, short stature, macrocephaly, and microcephaly which have not been in the focus so far could be shown to be clearly overrepresented among individuals with *MYT1L* mutations.

ACKNOWLEDGMENTS

We thank the patients and their families for their support.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

I.C.W., J.B., K.C., and H.E. designed the study and wrote the manuscript. K.C., L.M.Y., E.M., F.D., E.W., M.B., R.C., T.H., J.J., J.D., and M.H. gathered and provided patient data and wrote case reports. I.C.W. and K.U.L. performed statistical analyses. I.C.W., J.B., H.H., S.P., A.M.Z., T.M.Z., D.W., D.L., T.B., and H.E. analyzed exome data. I.C.W., J.B., S.P., and H.E. analyzed the mutational spectrum of *MYT1L*. I.C.W. made Table 1 and analyzed previously published data for review. I.C.W., J.B., K.C., and H.E. made tables and figures.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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How to cite this article: Windheuser IC, Becker J, Cremer K, et al. Nine newly identified individuals refine the phenotype associated with *MYT1L* mutations. *Am J Med Genet Part A*. 2020;1–11. https://doi.org/10.1002/ajmg.a.61515