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Phenotypic spectrum of the recurrent TRPM3 p.(Val837Met) substitution in seven individuals with global developmental delay and hypotonia

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Abstract

*TRPM3* encodes a transient receptor potential cation channel of the melastatin family, expressed in the central nervous system and in peripheral sensory neurons of the dorsal root ganglia. The recurrent substitution in *TRPM3*: c.2509G>A, p.(Val837Met) has been associated with syndromic intellectual disability and seizures. In this report, we present the clinical and molecular features of seven previously-unreported individuals, identified by exome sequencing, with the recurrent p.(Val837Met) variant and global developmental delay. Other shared clinical features included congenital hypotonia, dysmorphic facial features (broad forehead, deep-set eyes and down turned mouth), exotropia and musculoskeletal issues (hip dysplasia, hip dislocation, scoliosis). Seizures were observed in 2/7 individuals (febrile seizure in one and generalized tonic-clonic seizures with atonic drops in another), and epileptiform activity was observed electroencephalographically in an additional two individuals. This report extends the affected individuals to 16 who are heterozygous for the *de novo* recurrent substitution p.(Val837Met). In contrast with the initial report, epilepsy was not a mandatory feature in this series. *TRPM3* pathogenic variation should be considered in individuals with global developmental delays, moderate-severe intellectual disability with, or without, childhood-onset epilepsy.

Word count excluding references and abstract: 1,573

Key words (3-5): global developmental delay, intellectual disability, seizures, *TRPM3*, Genematcher
1. INTRODUCTION

The transient receptor potential, melastatin-related (TRPM) family of gated channels (TRPM1-8) are nonselective Ca\(^{2+}\)-permeable cation channels with varied functions including temperature sensation, regulation of cell adhesion and modulation of cellular magnesium and calcium levels(Clapham, 2003; Farooqi et al., 2011; Thiel et al., 2017). Pathogenic variation in TRPM channels are responsible for several human diseases, including intestinal hypomagnesemia (TRPM6; OMIM 602014), progressive familial heart block (TRPM4; OMIM 604559), congenital stationary night blindness (TRPM1; OMIM 613216), erythroderma variabilis et progressiva (TRPM4; OMIM 618531) and susceptibility to amyotrophic lateral sclerosis (TRPM7, TRPM2) (Audo et al., 2009; Hermosura et al., 2008; Hermosura et al., 2005; Kruse et al., 2009; Li et al., 2009; Schlingmann et al., 2002; van Genderen et al., 2009; Walder et al., 2002; Wang et al., 2019). Both gain- and loss-of-function variants have been implicated as mechanisms of clinical disease(Zhao & Rohacs, 2021).

Recently, missense variation in TRPM3 has been implicated in a syndrome characterized by moderate to severe intellectual disability (ID), congenital hypotonia, distinctive craniofacial features, and epilepsy (Dyment et al., 2019). Seven of eight individuals reported in the initial series were heterozygous for a recurrent substitution, described here as TRPM3: (NM_020952.4), c.2509G>A, NP_066003.3:p.(Val837Met) (Dyment et al., 2019). Subsequently, other individuals with a similar phenotype, though lacking epilepsy, were reported with the same
recurrent variant (de Sainte Agathe et al., 2020; Gauthier et al., 2021). Although initial reports did not include any functional studies, subsequent in vitro experiments in heterologous expression systems demonstrated pathogenic gain-of-function through altered gating and conductance (Held et al., 2018; Van Hoeymissen et al., 2020; Zhao et al., 2020). The channels expressing the recurrent substitution exhibit increased basal activity, enhanced sensitivity to the TRPM3 agonist pregnenolone sulfate, increased thermal sensitivity, and increased permeability via a non-canonical or “alternative” conductance pathway previously identified in TRPM3 (Van Hoeymissen et al., 2020; Vriens et al., 2014; Zhao et al., 2020).

In this report, we describe the clinical features of an additional seven individuals with the recurrent TRPM3 p.(Val837Met) substitution, increasing the total number to 16 affected individuals described to date. In contrast to the initial report, epilepsy appears to be a non-mandatory feature; we further note a craniofacial appearance that highlights shared features among the affected individuals based on an expanded number of clinical photographs available.

2. MATERIAL AND METHODS

We compiled a cohort of individuals with de novo pathogenic TRPM3 variants using a distributed case-matching approach (GeneMatcher)(Sobreira et al., 2015). The study was approved by CHEO REB#21/57X. Clinical and genetic data was provided by co-authors in accordance with local research and ethics requirements. Written consent was provided for individuals providing photographic images.
Comment on TRPM3 Nomenclature: There are over 25 isoforms of TRPM3 (Oberwinkler & Philipp, 2014). We present the recurrent variant as NM_020952.4:c.2509G>A, NP_066003.3:p.(Val837Met) and NC_000009.11:g.73213379C>T (Dyment et al., 2019). This nomenclature has been used by others (de Sainte Agathe et al., 2020; Gauthier et al., 2021) including Human Gene Mutation Database (HGMD) (Stenson et al., 2017) and OMIM (https://www.omim.org/). Others have reported this same variant as NM_001007471.2:c.2986G>A, NP_001007472.2:p.(Val990Met) (Van Hoeymissen et al., 2020; Zhao et al., 2020). In ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) the nomenclature used for this variant is NM_001366145.2:c.3004G>A; NP_001353074.1:p.(Val1002Met).

3. RESULTS

3.1 The recurrent p.(Val837Met) variant

The clinical details of the 7 individuals ascertained by GeneMatcher are presented in Table 1, and the Supplementary text. The individuals ranged from five months of age to fifteen years old (average age was 3.6 years with four individuals aged three years or less). Six individuals were female and one was male. Growth was within normal limits for six of the seven individuals. One individual in the series (Figure 1F) had below-average stature (SD -3.1SD), head circumference of -2.86SD, and weight of -2.7SD. All seven individuals reported global developmental delay, and five individuals had a history of hypotonia. The three individuals of sufficient age to undergo formal cognitive assessment had a diagnosis of severe ID. Of the five individuals’ age two years or older, one was non-ambulatory, and four had achieved independent ambulation at an average age of 3.6 years (range: 1.5 - 5 years). Of the same five individuals, two were non-verbal, and the remaining three individuals spoke their first words at the respective ages of 18 months, 3 years,
and 4 years. Two individuals were able to communicate in short sentences. Aggression and temper tantrums were ongoing concerns for two individuals.

In this series, only two of seven affected individuals experienced clinical seizures. One individual had a febrile seizure at 14 months of age; another had generalized tonic-clonic seizures with atonic drops from the age of 19 months. Only the latter individual was receiving anti-epileptic medication, valproic acid, at the time of their most recent assessment, and seizures were described as well-controlled. EEGs were abnormal in four of six individuals (Table 1).

Craniofacial features were variable, and affected individuals were considered distinctive to mildly dysmorphic (Figure 1). Common features included a broad forehead, deep-set eyes, and downturned mouth. The overall facial gestalt was not felt to be specifically “recognizable” although shared features were appreciated (Figure 1). Other reported clinical features among the seven patients reported in this study included strabismus (n=4), nystagmus (n=1), scoliosis (n=2), hip dysplasia (n=1), hip dislocations (n=2) and talipes (n=3). No individual had a history of glaucoma or cataracts.

3.2 Previously published cases of the recurrent variant (n=9)

We obtained clinical updates regarding two previously-published individuals: Proband 1 of Dyment et al., 2019 (Dyment et al., 2019) (Figure 1, Panel A, B), and the proband reported by de Sainte Agathe et al, 2020 (de Sainte Agathe et al., 2020) (Supplementary Text). The individuals are now 19 years and 7 years, 8 months old, respectively. Neither have experienced seizures since their initial reports and neither has required antiepileptic medication. Individual 1 takes
risperidone for management of food-seeking behaviour. ID was characterized as severe in Individual 1, and moderate-to-severe in the proband reported by de Sainte-Agathe (de Sainte Agathe et al., 2020).

When we consider the presentations in this series combined with the published cases (Table 1), the sex ratio was 1:1, and in all 16 individuals the recurrent variant had occurred de novo. All experienced some degree of global developmental delay, and hypotonia was noted in 12/16 (75%) cases. ID could be assessed in twelve individuals, and was present in each, ranging in severity from moderate to severe ID. Seizures were reported in only about half of individuals (10/16; 62.5%), and those that did have seizures were described as stable or well-controlled with, or without, medication. Age at seizure onset, when reported, averaged two years and nine months (n=7). Seizures were varied in type and included generalized tonic-clonic, febrile, absence, infantile spasms, and electrical status epilepticus of sleep. Medications used included levetiracetam, diazepam, clobazam and valproic acid. Distinctive or dysmorphic facial features were observed in 9/16 (56%) individuals, although features were not specifically recognizable. Decreased pain and/or decreased heat sensation were reported in 4/16 (previously published(de Sainte Agathe et al., 2020; Dyment et al., 2019; Gauthier et al., 2021)) and not observed in this series.

4. DISCUSSION

Including this report, the recurrent de novo TRPM3: (NM_020952.4), c.2509G>A, p.(Val837Met) variant has been observed in sixteen individuals with global developmental delay and/or intellectual disability(de Sainte Agathe et al., 2020; Dyment et al., 2019; Gauthier et al., 2021). TRPM3 is known to be expressed in the central nervous system (Oberwinkler and Philipp,
2014) and variant-level functional data has shown the mutated channel to have altered gating and
cconductance characteristics, consistent with a gain-of-function model of disease (Van
Hoeymissen et al., 2020; Zhao et al., 2020). This body of evidence would appear to meet the bar
for TRPM3 to be considered a bone-fide disease-associated gene.

The core phenotypes common to the 16 individuals reported to date include global
developmental delay, moderate-to-severe ID, medically-manageable epilepsy in about half of
cases, and a scattering of inconsistent minor anomalies including strabismus, scoliosis, hip
dislocation, and talipes. As such the phenotype is consistent with that of an intellectual disability
syndrome though variable in the extent of the cognitive, language and motor deficits. Autistic-
like stereotypies were also reported in 7/14 (50%) individuals, and brain imaging showed non-
specific anomalies including white matter hyperintensities and mild volume loss. Cerebellar
hypoplasia was notable in a previously published case and heterotopias in another (Individual
#7); however these have so far been in the only reported individuals.

In the initial series, a second, putatively-pathogenic TRPM3 variant, c.3305C>A, p.(Pro937Gln),
was also identified in a single individual with the core clinical features compatible with TRPM3-
associated syndrome (Dyment et al., 2019). Subsequent functional studies of that substitution,
p.(Pro937Gln), also produced results consistent with gain-of-function of the mutant channel,
although with comparatively less agonist sensitivity, lower basal activity, and increased heat
activation in comparison with p.(Val837Met)(Zhao et al., 2020). Another individual with a
different, novel variant at NM_020952:c.3650G>C, p.(Ser1202Thr) has also been reported(Kang
et al., 2021). Functional evidence in support of its pathogenicity has not yet been performed. It
appears likely that other non-random *de novo* TRPM3 variants may similarly be associated with gain of function and an accompanying clinical phenotype; further studies in additional patients will be required to establish whether this is in fact the case.

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CONFLICT OF INTEREST: The authors declare no conflict of interest

AUTHOR CONTRIBUTIONS:
MAL, PG, AW, SS, AB, HH, HGK, KA-Y, JL, NJ, AM, EG, MM, SD;A, CC, CP, RC, JO, HZ-L, FT-M-T, AG, DH, BK, HM, dSA, LB, AMI recruited the individuals for the TRPM3 study, provided clinical description, contributed to the first draft of the manuscript and critically revised the manuscript. DD, TV and JV contributed to the study design, first draft of the manuscript and critically revised the manuscript.

DATA AVAILABILITY STATEMENT
Data that support the findings of this study are available on request from the corresponding authors.

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FIGURE LEGEND

Figure 1. Images of individuals with the TRPM3, p.(Val837Met). Figure 1 highlights the distinctive facial features including broad forehead, deep set eyes, downturned mouth, and small chin observed in many individuals carrying the recurrent variant. Panel A-B, Individual 1. Panel C-D, Individual 2. Panel E, Individual 4. Panel F. Individual 6. Panel G, Individual 7 at 1 year of age. Panel H, Individual 7 at 19 years of age. Panels I-J, updated images of the proband from the
<table>
<thead>
<tr>
<th>Recurrent variant only</th>
<th>Total of 9 individuals from the literature*</th>
<th>Individual 1</th>
<th>Individual 2</th>
<th>Individual 3</th>
<th>Individual 4</th>
<th>Individual 5</th>
<th>Individual 6</th>
<th>Individual 7</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy complications (eg IUGR?)</td>
<td>NR</td>
<td>No polyhydramnios, gestational diabetes</td>
<td>left asymmetric ventriculomegaly, concern for club foot</td>
<td>none</td>
<td>left club foot</td>
<td>premature rupture of membranes</td>
<td>none</td>
<td>4 of 7 reported (57%)</td>
<td></td>
</tr>
<tr>
<td>Term delivery?</td>
<td>term (n=9)</td>
<td>full term</td>
<td>37 weeks and 3 days</td>
<td>full term</td>
<td>full term</td>
<td>39 weeks and 2 days</td>
<td>34 weeks</td>
<td>40 weeks and 4 days</td>
<td>Term delivery 16/16 (100%)</td>
</tr>
<tr>
<td>Delivery complications</td>
<td>CS (n=2)</td>
<td>none</td>
<td>Elective cesarean section due to polyhydramnios</td>
<td>cesarean section</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>prolonged</td>
<td>CS in 4/16 (25%), prolonged labor in 1/16 (6%)</td>
</tr>
<tr>
<td>Birth weight</td>
<td>NL (n=8)</td>
<td>3203g</td>
<td>3295g</td>
<td>3750g</td>
<td>3742g</td>
<td>2955g</td>
<td>NR</td>
<td>3600g</td>
<td>NL in 14/14 reported (100%)</td>
</tr>
<tr>
<td>Sex</td>
<td>2 females: 7 males</td>
<td>female</td>
<td>male</td>
<td>female</td>
<td>female</td>
<td>female</td>
<td>female</td>
<td>female</td>
<td>8 females : 8 males</td>
</tr>
<tr>
<td>Measurements</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at last assessment</td>
<td>13 years (4.75-38 years)</td>
<td>2 years old</td>
<td>5 months</td>
<td>16 months</td>
<td>36 months</td>
<td>6 years</td>
<td>15 years</td>
<td>15 years</td>
<td>10.1 years (5months-38 years)</td>
</tr>
<tr>
<td>height</td>
<td>NL</td>
<td>84.3cm (-0.31SD)</td>
<td>67.5cm (+0.7SD)</td>
<td>0.82cm (+1.25SD)</td>
<td>93cm (-0.15SD)</td>
<td>108cm (-1.57SD)</td>
<td>142.2cm (-3.15SD)</td>
<td>143.2cm (-2.85SD)</td>
<td>Range -3.15SD to 1.2SD</td>
</tr>
<tr>
<td>weight (kg)</td>
<td>NL</td>
<td>12.2kg (+0.05SD)</td>
<td>6.85kg (-0.56SD)</td>
<td>11.3kg (+0.62SD)</td>
<td>16kg (+1.08SD)</td>
<td>16kg (-1.85SD)</td>
<td>36kg (-2.71SD)</td>
<td>65.1kg (+0.84SD)</td>
<td>Range -2.7SD to 1.85SD</td>
</tr>
<tr>
<td>OFC (cm)</td>
<td>NL</td>
<td>48cm (+0.39SD)</td>
<td>44.6cm (+1.22SD)</td>
<td>45cm (+.01SD; 12 months)</td>
<td>52cm (+1.59SD)</td>
<td>50.5cm(-0.24SD)</td>
<td>51cm (-2.86SD)</td>
<td>53.2cm (+1.55SD at 6years)</td>
<td>Range -2.86SD to 2.1SD</td>
</tr>
<tr>
<td>Psychomotor development</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of first independent steps</td>
<td>4.6 years (n=4, range 4-5 years)</td>
<td>not walking yet</td>
<td>NA</td>
<td>rolling after 8 months, does not sit, does not stand or walk at 16 months</td>
<td>16-18 months</td>
<td>5 years</td>
<td>5 years</td>
<td>3 years</td>
<td>8 of 15 over 1 year of age (53%) can ambulate independently; Range 18 months to 5 years; Average 4.1 years</td>
</tr>
<tr>
<td>Age of first words</td>
<td>5 years (n=2; the other 7 individuals had no speech)</td>
<td>no single words yet</td>
<td>NA</td>
<td>vocalizations at 15 months but does not speak</td>
<td>17-19 months</td>
<td>averbal</td>
<td>4 years</td>
<td>mama/dada at 3yrs; minimal progress</td>
<td>5 of 15 over 1 year of age (33%); Range 19 months to 5 years; Average 3.7 years</td>
</tr>
<tr>
<td>Ability to combine words (#)</td>
<td>1 in 9</td>
<td>N/A</td>
<td>NA</td>
<td>no</td>
<td>24 months</td>
<td>No</td>
<td>5 years</td>
<td>Not achieved</td>
<td>3 of 15 (20%) over 1 year of age</td>
</tr>
<tr>
<td>Recurrent variant only</td>
<td>Total of 9 individuals from the literature*</td>
<td>Individual</td>
<td>Individual</td>
<td>Individual</td>
<td>Individual</td>
<td>Individual</td>
<td>Individual</td>
<td>Individual</td>
<td>Totals</td>
</tr>
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<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Any diagnosis of global developmental delay</td>
<td>9 of 9</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>16 of 16 (100%)</td>
</tr>
<tr>
<td>Intellectual disability</td>
<td>9 of 9 (moderate to severe)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Severe ID</td>
<td>Severe ID</td>
<td>Severe ID</td>
<td>12 of 12 (100%) for those able to be assessed</td>
</tr>
<tr>
<td>Behavior</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autism spectrum disorder (Y/N)</td>
<td>6 of 9</td>
<td>no</td>
<td>NA</td>
<td>NA</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>7 of 14 (50%)</td>
<td></td>
</tr>
<tr>
<td>Epilepsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any Seizure and type</td>
<td>7-8 of 9; variable; absence, infantile spasms, GTC, ESES, focal with loss of awareness</td>
<td>none</td>
<td>none</td>
<td>febrile seizure</td>
<td>none</td>
<td>none</td>
<td>no</td>
<td>GTCS -&gt; drop attacks</td>
<td>10 of 16 (62.5%); each with varied semiology</td>
</tr>
<tr>
<td>Age of first seizure</td>
<td>Range 9 months to 7 years (average 3.7 years)</td>
<td>possible seizure-like activity at 20 months</td>
<td>NA</td>
<td>14 months</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>19 months</td>
<td>Range 9 months to 7 years; Average 3.1 years</td>
</tr>
<tr>
<td>Current antiepileptic drug</td>
<td>levetiracetam, clobazam and diazepam used</td>
<td>None</td>
<td>NA</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>valproic acid</td>
<td>Varied anti-epileptic medications used</td>
</tr>
<tr>
<td>Seizures controlled (Y/N)</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
<td>yes</td>
<td>NA</td>
<td>NA</td>
<td>no</td>
<td>yes</td>
<td>Controlled in 9 of 10 who have experienced seizures</td>
</tr>
<tr>
<td>EEG findings</td>
<td>Variable; confirmed seizure activity in 7 of 9</td>
<td>48th EEG at 20 months of age: occasional focal spikes in the central midline and right central regions that are sleep potentiated; background poorly sustained</td>
<td>normal at 6 months</td>
<td>bilateral temporal sharp waves and slowing; background slowing</td>
<td>normal x 2</td>
<td>A focus of right temporop-occipital spikes increased by sleep associated with slow waves. A focus of asynchronous spikes in the left temporop-occipital region</td>
<td>normal at 13 years</td>
<td>Early hypsarrhythmia; progressed to slow background with some bilateral sharp waves</td>
<td>Variable</td>
</tr>
<tr>
<td>Recurrent variant only</td>
<td>Total of 9 individuals from the literature*</td>
<td>Individual</td>
<td>Individual</td>
<td>Individual</td>
<td>Individual</td>
<td>Individual</td>
<td>Individual</td>
<td>Individual</td>
<td>Totals</td>
</tr>
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<td>------------------------</td>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>Total of 16 (%)</td>
</tr>
<tr>
<td>Other clinical features</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Hypotonia (congenital)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>yes-axial</td>
<td>yes - mild</td>
<td>yes</td>
<td>no</td>
<td>no - lower limb stiffness</td>
<td>yes - truncal</td>
<td></td>
<td>12 of 16 (75%)</td>
</tr>
<tr>
<td>Movement disorders</td>
<td></td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>Stereotypies: hyperventilation and hand rubbing</td>
<td>no</td>
<td>no</td>
<td>Jerking or athetoid movements in 2 of 16 (12.5%)</td>
<td></td>
</tr>
<tr>
<td>Pain insensitivity (Y/N)?</td>
<td></td>
<td>no</td>
<td>NA</td>
<td>NR</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>3 of 14 (21%) reported</td>
</tr>
<tr>
<td>Heat insensitivity</td>
<td></td>
<td>NR</td>
<td>NA</td>
<td>NR</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>2 of 13 (15%) reported</td>
</tr>
<tr>
<td>Ophthalmological issues</td>
<td></td>
<td>intermittent exotropia</td>
<td>nystagmus</td>
<td>NR</td>
<td>No</td>
<td>Divergent strabismus</td>
<td>right strabismus, bilateral exotropia</td>
<td>left strabismus</td>
<td>Strabismus 9/15 (60%)</td>
</tr>
<tr>
<td>Dysmorphisms</td>
<td></td>
<td>yes</td>
<td>yes (slightly)</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>7 of 15 (46%) reported</td>
</tr>
<tr>
<td>If yes, craniofacial features</td>
<td></td>
<td>broad forehead and low set ears commented in 2 individuals</td>
<td>midface hypoplasia, mild frontal bossing, high anterior hairline, widow’s peak, short nose with low bridge, possible thin upper lip, prominent nasolabial folds</td>
<td>deformational plagiocephaly, low set ears, and bilateral 4-finger crease</td>
<td>plagiocephaly due to torticollis and mild frontal bossing. Face asymmetric. Mild hypertelorism</td>
<td>broad forehead with a wide inner canthus</td>
<td>frontal bossing, ears in posterior rotation, synophris, small mouth</td>
<td>telecanthus, upslanting palpebral fissures, narrow nasal bridge, prominent and pointed chin</td>
<td>hypertelorism, broad nasal bridge, prominent eyes, open-mouthed appearance with everted lower lip, prominent maxilla &amp; upper teeth, mild brachycephaly, small ears</td>
</tr>
<tr>
<td>Other congenital anomalies</td>
<td></td>
<td></td>
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<td>Recurrent variant only</td>
<td>Total of 9 individuals from the literature*</td>
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</tr>
<tr>
<td></td>
<td>Individual 1</td>
<td>Individual 2</td>
<td>Individual 3</td>
<td>Individual 4</td>
<td>Individual 5</td>
<td>Individual 6</td>
<td>Individual 7</td>
<td>Totals</td>
<td></td>
</tr>
<tr>
<td>hip dysplasia</td>
<td>yes (1 in 8)</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>4 of 15 (26%)</td>
<td></td>
</tr>
<tr>
<td>Other?</td>
<td>varied (1)</td>
<td>scoliosis (2), talipes (2), vertebral anomalies (2), cryptorchidism (1), unilateral hearing loss (1), GERD (1), neonatal hypoglycemia (1), trigonocephaly (1) and plagiocephaly (1)</td>
<td>pes equinovarus</td>
<td>congenital right hip dislocation, torticollis</td>
<td>None</td>
<td>Bilateral recurvatum of the knees, Equine varus feet</td>
<td>Severe scoliosis, tapering fingers. Bilateral congenital clubfoot, tracheal stenosis; 2 years: surgical intestinal resection for NEC</td>
<td>long tapered digits, scoliosis</td>
<td>varied (4), talipes (5), vertebral anomalies (2), cryptorchidism (1), unilateral hearing loss (1), GERD (2), neonatal hypoglycemia (1), trigonocephaly (1) and plagiocephaly (1)</td>
</tr>
</tbody>
</table>

**Investigations**

<p>| MRI or other imaging | Normal in 5 of 9 (55%); 3 with mild volume loss, 3 with periventricular white matter intensities and 1 with cerebellar atrophy | Brain MRI: prominent cavum septum pellucidum et vergae, otherwise normal | Brain MRI: small brain for age, delayed myelination | asymmetric enlargement of left lateral ventricle and mild diffuse gyral prominence | Normal | Brain MRI: occipital arachnoid cyst, normal renal and cardiac ultrasound, normal skeletal X-rays | Brain MRI: Grey matter heterotopia near the left lateral ventricle, thinning of the posterior part of corpus callosum | Normal in 8 of 16 (50%); 5 with mild volume loss, 3 with periventricular white matter intensities, 1 with heterotopia and 1 with cerebellar atrophy |
| Microarray          | normal in 8 of 8 tested | normal | normal | normal | normal | normal | normal | Normal in 16 of 16 (100%) |
| Fragile X testing   | normal in 7 of 7 tested | N/A | no | NR | no | normal | no | Normal in 12 of 12 reported (100%) |</p>
<table>
<thead>
<tr>
<th>Recurrent variant only</th>
<th>Total of 9 individuals from the literature*</th>
<th>Individual</th>
<th>Individual</th>
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<th>Individual</th>
<th>Totals</th>
</tr>
</thead>
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<tr>
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<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Metabolic studies</td>
<td>performed in 2 of 9 and reported normal</td>
<td>N/A</td>
<td>normal</td>
<td>normal</td>
<td>plasma amino acids, urine organic acids, CMP, CBC, TSH, CK normal</td>
<td>NR</td>
<td>not performed</td>
<td>not performed</td>
</tr>
<tr>
<td>Glucose level</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
<td>congenital hypoglycemia</td>
<td>NR</td>
<td>normal</td>
<td>NR</td>
<td>no</td>
</tr>
<tr>
<td>Insulin</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
<td>NR</td>
<td>normal</td>
</tr>
<tr>
<td>Any other testing</td>
<td>ID panel (n=3), mecp2, craniosynostosis panel</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Karyotype, Angelman syndrome, Rett syndrome, intellectual disability panel (56 genes)</td>
<td>Karyotype, FLNA sequencing; normal</td>
<td>Multiple single genes and panels</td>
<td>ID panel (n=5), MECP2, craniosynostosis panel, PWS (1), AS (2), FLNA (1)</td>
</tr>
</tbody>
</table>

* are summed from Dyment et al (2019), de Sainte Agathe (2020) and Gauthier et al (2021); CS, cesarian section, NR, not reported; NL, Normal Limits; PWS, Prader Willi syndrome testing; AS, Angelman syndrome testing