Patients with KCNH1-related intellectual disability without distinctive features of Zimmermann-Laband/Temple-Baraitser syndrome

Patients with *KCNH1*-related intellectual disability without distinctive features of Zimmermann-Laband/Temple-Baraitser syndrome

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ABSTRACT

De novo missense variants in *KCNH1* encoding Kv10.1 are responsible for two clinically recognizable phenotypes: Temple-Baraitser Syndrome (TBS) and Zimmermann-Laband Syndrome (ZLS). The clinical overlap between these two syndromes suggests that they belong to a spectrum of *KCNH1*-related encephalopathies. Affected patients have severe intellectual disability (ID) with or without epilepsy, hypertrichosis and distinctive features such as gingival hyperplasia and nail hypo/aplasia (present in 20/23 reported cases).

We report a series of seven patients with ID and *de novo* pathogenic *KCNH1* variants identified by whole-exome sequencing or an epilepsy gene panel in whom the diagnosis of TBS/ZLS had not been first considered. Four of these variants, p.(Thr294Met), p.(Ala492Asp), p.(Thr493Asn) and p.(Gly496Arg), were located in the transmembrane domains S3 and S6 of Kv10.1 and one, p.(Arg693Gln), in its C-terminal cyclic nucleotide binding homology domain (CNBHD). Clinical reappraisal by the referring clinical geneticists confirmed the absence of the distinctive gingival and nail features of TBS/ZLS.

Our study expands the phenotypical spectrum of *KCNH1*-related encephalopathies to individuals with an attenuated extra-neurological phenotype preventing a clinical diagnosis of TBS or ZLS. This subtype may be related to recurrent substitutions of the Gly496, suggesting a genotype-phenotype correlation and, possibly, to variants in the CNBHD domain.

KEYWORDS

KCNH1, intellectual disability, epilepsy, Temple-Baraitser syndrome, Zimmermann-Laband syndrome

INTRODUCTION

Gain-of-function missense variants in *KCNH1* (MIM*603305) encoding the voltage gated potassium channel Kv10.1 are associated with syndromic neurodevelopmental disorders with overlapping phenotypes referred to as Temple-Baraitser syndrome (TBS;MIM#611816) and Zimmermann-Laband syndrome (ZLS;MIM#135500).[1-7] The main features of these syndromes (Table S1) are severe intellectual disability (ID) with or without early-onset epilepsy, congenital hypertrichosis, anomalies of the limbs and nails, gingival hyperplasia and mild craniofacial dysmorphic features.[1-3, 7-13] Gingival hyperplasia is a classical feature of the ZLS phenotype, present in about 50% of the published cases of *KCNH1*-related encephalopathy.[11-14] In patients with TBS, thumbs and halluces are usually long, proximally implanted and broad with hypoplastic nails and an increased radiolucency with missing ossification points in the terminal phalanx.[5, 15] The other fingers and toes may show hypoplastic distal phalanges and nails.[10] Hypo/aplasia of nails has been reported in more than 90% of published cases with *KCNH1* variants.[4, 5, 7, 15, 16] These findings in a context of severe encephalopathy are highly suggestive of TBS/ZLS.

We report on seven novel patients with *KCNH1*-related ID and lacking the distinctive features of TBS/ZLS.

METHODS

We used clinical networks (EPIGENE, EuroEPINOMICS-RES, GeneMatcher) to collect a multicentric cohort of patients with *KCNH1*-related encephalopathy, evaluated by clinical geneticists for the etiological diagnosis of ID. Referring

physicians retrospectively completed a standardized table with molecular and clinical data. We obtained written consents for genetic testing and publication of data for all patients. Retrospectively, craniofacial features, limbs and nails were assessed using patients' photographs.

Patients P1, P4 and P5 were studied on epilepsy gene panels used in different French University hospitals, including 90 to 116 genes involved in epilepsy, among 2450 individuals tested in different French university hospitals. Patients P2, P3, P6 and their parents were studied by solo or trio-WES (supplementary methods). The variant found in patient P2 was identified by performing WES in a research project on genetic ataxias. The variants in patients P3 and P6 were found by WES in the diagnosis workup of ID out of 1294 analyzed patients. Patient P7 was recruited through GeneMatcher and the *KCNH1* variant was found by WES (1/850 tested patients).

Sanger sequencing was used for confirming variants.

Sequence variations were numbered using the adenine of the ATG initiation codon as the first nucleotide (*KCNH1* GenBank accession NM_172362).

We performed an exhaustive review of the literature using the PubMed database to compile clinical data on individuals with pathogenic variants in *KCNH1* (Table S1) using the following terms: « *KCNH1*, TBS, ZLS, Temple-Baraitser, Zimmermann-Laband ». Because genetic heterogeneity has been documented,[13, 17] we excluded patients without mutation to focus on the frequency of clinical signs in proven *KCNH1*-related encephalopathy.

.INICAL DATA OF OUR 7 PATIENTS WITH *KCNH1*–RELATED ENCEPHALOPATHY

	P2	P3	P4	P5	P6	P7	TOTAl
	F	M	F	F	F	**F**	**n=7**
5 y gerian	15 y European	7.9 y Mauritian	9 y European	1.75 y European	3.5 y European	28 y European	1.75 y
210977496G>T	g.210977485C>T	g.210948724C>T	g.210977485C>T	g.210977485C>T	g.210977493G>T	g.211192276G>A	
475C>A	c.1486G>A	c.2078G>A	c.1486G>A	c.1486G>A	c.1478C>A	c.881C>T	
Ala492Asp)	p.(Gly496Arg)	p.(Arg693Gln)	p.(Gly496Arg)	p.(Gly496Arg)	p.(Thr493Asn)	p.(Thr294Met)	
PS2 PM1 PM2 '2 PP3) .2 *novo*	5 (PS2 PM1 PM2 PP2 PP3 PP5) 0 29.9 *de novo*	5 (PS2 PM1 PM2 PP2 PP3) 0 26 *de novo*	5 (PS2 PM1 PM2 PP2 PP3 PP5) 0 29.9 likely *de novo**	5 (PS2 PM1 PM2 PP2 PP3 PP5) 0 29.9 *de novo*	5 (PS2 PM1 PM2 PP2 PP3) 0 26.7 *de novo*	5 (PS2 PM1 PM2 PP2 PP3) 0 25.7 *de novo*	
vere ID	moderate ID	severe ID	mild/moderate ID	mild DD	moderate/severe ID	severe ID	**mild -**
m	10 m	10 m	12 m	9.5 m	26 m	NA	**9.5 m** ·
t achieved	21 m	35 m	20 m	18 m	not achieved	18 m	**18 m - 4.5 y**
calization	sentences	few words	sentences	canonical babbling	no words	no words	**senter words**
s	yes	no	yes	yes	no	no	**4/7**
h	12 m	NA	2 y	3 m	NA	NA	**day 1** ·
nic-clonic, myo-nic	tonic, tonic-clonic; 2 status epilepticus	NA	focal clonic, generalized tonic-clonic (total 5 seizures), myoclonic	generalized tonic-clonic, focal clonic, myoclonic	NA	NA	
veek	monthly/weekly	NA	1-3/month	8/day	NA	NA	
w background tivity, focus of left ntal spike-and-ve	normal	NA	focus of central spikes increased during sleep (close to CSWS)	few spikes in central regions, bursts of generalized polyspikes with myoclonic jerks	slow background activity	normal	
sponsive	responsive	NA	responsive	responsive with relapse	NA	NA	
etiracetam	lamotrigine, carbamazepine and clobazam	NA	carbamazepine	levetiracetam	NA	NA	
rmal	normal	normal	normal	normal	normal	normal	**norma 7/7**
derate sensori-ural deafness, opia and asti-tism	hyperopia	none	none	none	none	none	
ncal and periph-al hypotonia	truncal hypotonia, hyperreflexia, mild ataxia	hyperreflexia	NA	normal	truncal hypotonia	facial hypotonia, hyperreflexia	

PATIENTS AND RESULTS

We collected clinical and molecular data of seven unrelated patients with pathogenic *KCNH1* variants (Table 1).

Three individuals shared the same previously reported *de novo* (or assumed as *de novo*) heterozygous missense variant c.1486G>A, p.(Gly496Arg). The others carried novel *de novo* heterozygous missense variants [c.881C>T p.(Thr294Met), c.1475C>A p.(Ala492Asp), c.2078G>A p.(Arg693Gln) and c.1478C>A p.(Thr493Asn)] fulfilling the criteria of pathogenicity (Figure 1A, Table1).

All seven patients, aged 21 months to 28 years, presented with mild/moderate to severe developmental delay (DD) or ID (Table1). The age of independent walking was mildly delayed (18 to 35 months) for five patients, while the others had not achieved walking at last examination (3.5 and 4.5 years). Two out of the five patients older than 3 years were able to make sentences, one spoke a few words and the remaining three did not speak. Four patients had epilepsy starting in infancy, with generalized tonic-clonic (4/4), myoclonic (2/4), focal motor (2/4) and tonic seizures (1/4). One of them experienced status epilepticus. The epilepsy was pharmaco-responsive in all individuals: a monotherapy was effective in three patients (2/3 levetiracetam, 1/3 carbamazepine) and one individual needed a bitherapy (carbamazepine and clobazam). Cerebral MRI was normal in all cases. P1 had a moderate sensorineural deafness.

At examination, the most frequent neurological sign was truncal hypotonia, brisk tendon reflexes and ataxia. The mild dysmorphic features noted in some of the patients were regarded as nonspecific by the physicians and did not guide the

molecular diagnosis. Referring clinicians reassessed the phenotypes of their patients after the identification of the *KCNH1* variant with special attention to the morphology of limbs and gums (Figure S1). Only hypertrichosis of the back was present in P1. Almost all patients had constipation (6/7), sometimes severe, and near half of them (3/7) had gastroesophageal reflux disease (GERD).

DISCUSSION

We provide a clinical description of seven individuals with *KCNH1*-related encephalopathy without features of TBS/ZLS found by WES or with an epilepsy gene panel. Only subtle and non-distinctive dysmorphic features were noted in some of them (Figure S1). Only one patient had mild toenails hypoplasia, but this was inherited from his mother and maternal grandfather. Three patients were reported with slightly broad halluces, two with proximal placement of thumbs, including one having long fingers.

Twenty-three patients with *KCNH1*-related encephalopathy have been reported to date (Table S1).[4-9, 13-16, 18, 19] Most of them had a clinical diagnosis of TBS/ZLS based on distinctive clinical features prior to molecular analysis. Two published series reported eight patients with pathogenic *KCNH1* variants found by WES[4, 16] without prior clinical diagnosis of TBS or ZLS, but all had gingival hyperplasia and/or nail anomalies in retrospect, except one who carried the Gly496Glu variant.[16] Thus, 22/23 previously reported patients with *KCNH1*-related encephalopathy had a prospective or retrospective diagnosis of TBS/ZLS with either nail hypo/aplasia or gingival hypertrophy. In comparison, the patients of the present series stand out for their attenuated extra-

neurological phenotype and the lack of clinical clue suggesting TBS or ZLS. Do these patients have a different developmental or epileptic outcome than TBS/ZLS patients?

All 23 previously reported patients with *KCNH1*-related encephalopathy had DD/ID. When mentioned (n=16), the ID was rarely mild (n=1), mild/moderate (n=1), or moderate (n=1), and most frequently severe (n=8), severe/profound (n=2) or profound (n=3). The range of DD/ID in our patients is consistent with the literature (Table 1). Seven out of 14 previously reported patients over 18 months achieved walking at a mean age of 3.3 years, while 4/6 of our series walked at a mean age of 2 years. Ten out of 12 previous patients older than 2 years had not acquired spoken language. In our series, two individuals out of five spoke words or sentences. Thus, language abilities may be mildly better in the patients of our study.

Epilepsy was frequent in our series (n=4/7) and in the literature (n=19/22). First seizures occurred between the first day of life and the age of 2 years (mean age 1 year) in our patients, and at a median age of 0.9 year (mean age 2.5 years) in the literature (n=18). Seizures were mainly motor in nature, either focal or generalized, including clonic, tonic-clonic, tonic and myoclonic seizures, both in the literature and in our series. EEG revealed unilateral or bilateral spikes or spikes-waves in fronto-centro-temporal regions in three of our epileptic patients. In the literature, multifocal epileptiform anomalies, sometimes predominating in anterior or fronto-temporal regions[15] and foci of spikes in frontal and central areas were both reported.[16] One of our patients experienced convulsive status epi-

lepticus and one had an EEG close to continuous spikes and waves during sleep. Comparatively, status epilepticus was reported in five patients of the literature. We conclude that the severity of the neurodevelopmental involvement in patients of our series bears comparison with patients of the literature, with regard to both the level of DD/ID and the epilepsy.

Nail anomalies and gingival hypertrophy were reported in 90% and 50% of patients in the literature, respectively (Table S1). The lack of nail anomalies and gingival hyperplasia in our series is noteworthy and suggests an overestimation of their frequency in *KCNH1*-encephalopathy. We cannot exclude, however, the appearance of gingival hyperplasia with time in our patients.

Digestive disorders are a known complication of *KCNH1*-related encephalopathy, constipation and GERD affecting 60% and 25% of the patients respectively (Table S1). Though these comorbidities are not surprising in a context of severe encephalopathy, their severity in some of our patients is noteworthy.

A previous study reported four patients with *KCNH1*-related encephalopathy out of 1447 with ID (2.7‰).[4] We found three French patients out of 2450 studied on an epilepsy gene panel and three by WES out of 2144 with ID (total 6/4594). We conclude that the prevalence of the *KCNH1*-related encephalopathy in individuals with epilepsy/ID and without features of TBS/ZLS in France is about 1.3‰.

Three unrelated index cases of our series were heterozygous for the c.1486G>A p.(Gly496Arg) variant. This variant and the c.1487G>A (p.Gly496Glu) substitution affecting the same Glycine residue have already

been reported in two patients with *KCNH1*-related encephalopathy.[7, 15, 16] Interestingly, both patients also had mild extra-neurological phenotypes: the individual carrying p.(Gly496Arg) did not have nail hypo/aplasia (but gingival enlargement and moderate hypertrichosis),[7, 15] and the other with p.(Gly496Glu) only presented broad thumbs/toes but no nail aplasia, hypertrichosis, or gingival enlargement.[16] Since these patients are the only published cases of *KCNH1*-related encephalopathy lacking these key features of TBS/ZLS, this raises the question of a potential genotype-phenotype correlation associating mild or absent extra-neurological signs and variants affecting the Gly496 residue.

Functionally tested variants in *KCNH1* usually lead to a left-ward shift in voltage dependent activation, producing an increase in K^+ conductance in the negative potential-range (or a so called gain-of-function effect).[7] These variants may then contribute, as suggested by Kortum et al., to a functional blockade of voltage gated Na+ and L-type Ca^{2+} channels through stabilization of the membrane potential to a more negative voltage range, and draw parallels with the gingival hyperplasia that can be seen with prolonged use of the Na+ channel blocker phenotyoin or the Ca^{2+} channel blocker nifedipine.[7] KCNH1 is also required for resorption of the primary cilium when the cell cycle is reinitiated: hyperactivation of KCNH1 was indeed shown to increase resorption. Alterations of the sonic hedgehog (SHH) cilia-mediated pathway involved in morphogenesis were therefore proposed to lead to the skeletal and nail malformations.[17, 20]

When functionally tested in a heterologous cell system, the homotetrameric p.(Gly496Arg) mutant KCNH1 channel failed to produce depolarization-activated currents demonstrating a loss of function effect. When the p.(Gly496Arg) mutant was co-expressed with wild-type *KCNH1* to produce heterotetrameric channels, an increase in K+ conductance was seen at negative membrane potentials.[7] In addition, similarly to other studied pathogenic variants, significant reduced K+ conductance was reported at depolarizing potentials.[7]

It is tempting to hypothesize that the absence or subtlety of extra-neurological symptoms in patients with specific variants such as p.(Gly496Arg) compared to variants associated with a “classical” ZLS/TBS phenotype is reflecting tissue-specific sensitivities to a more limited gain-of-function effect of this variant. More in-depth comparative studies of the effect of specific *KCNH1* variants on SHH pathway and voltage-gated calcium channel activity is warranted, preferably in model systems recapitulating the human phenotype, such as human induced pluripotent stem cell derived neuronal and chondroprogenitor cells.

Two of our novel variants, c.1475C>A p.(Ala492Asp) and c.1478C>A p.(Thr493Asn), are located in the S6 transmembrane of Kv10.1 (Figure 1B) where other variants [c.1480A>G p.(Ile494Val) and the c.1465C>T p.(Leu489Phe)] have been previously reported in typical ZLS/TBS patients.[4, 7, 15] The c.881C>T p.(Thr294Met) variant is for its part located in the S3 transmembrane domain. The c.2078G>A p.(Arg693Gln) variant is the first reported to

affect a residue in the C-terminal cyclic nucleotide binding homology domain (CNBHD). The biochemical and electrophysiological consequences of this change have to be determined, but could impact the interaction between the *ether-a-go-go* domain and the CNBHD, leading to an impairment of the activation/deactivation channel control.[21]

To further investigate the potential genotype-phenotype correlations in *KCNH1*-related encephalopathy, the description of further thoroughly phenotyped cases will be necessary. Meanwhile, the possibility of non-syndromic *KCNH1*-related encephalopathies should be considered when designing epilepsy gene panels or interpreting whole exome data from individuals with epilepsy or non-specific ID.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

No financial assistance was received in support of the study.

ETHICS APPROVAL

This study was designed in compliance with the tenets of the Helsinki Declaration.

PATIENT CONSENT

Written consents for genetic testing and publication were obtained locally from all participants or their representatives.

AUTHOR CONTRIBUTIONS

MAM/OP: Data collection, Data analysis, Manuscript writing, Manuscript editing

SWh/SWe: Data collection, Manuscript writing, Manuscript editing

LA/DD/CK/SK/ELG/GL/CN/SV/LV/BK/BC/MN: Data collection, Manuscript editing

GB/JB/AP: Data collection

CM: Study conception, Data collection, Data analysis, Manuscript writing, Manuscript editing

All authors read and approved the final manuscript.

Figure 1. Schematic representation of all (likely) pathogenic variants identified in *KCNH1*.

A. cDNA level: Legend: in bold, the novel variants reported here.

B. protein level: Legend: Stars representing variants identified, in blue = variants identified in our series, Sn = transmembrane domains, EAG = *Ether-à-go-go* domain, CNBHD = cyclic nucleotide binding homology domain, P = Pore-lining loop.

Figure S1. Faces, hands and feet of patients P2, P4, P5, and P7.

Mild dysmorphic features could be described: long facies with high forehead, bilateral epicanthic folds, mild hypertelorism, flat nasal bridge, broad tip of nose, flat midface, full lips, small mouth, mild prognathism.

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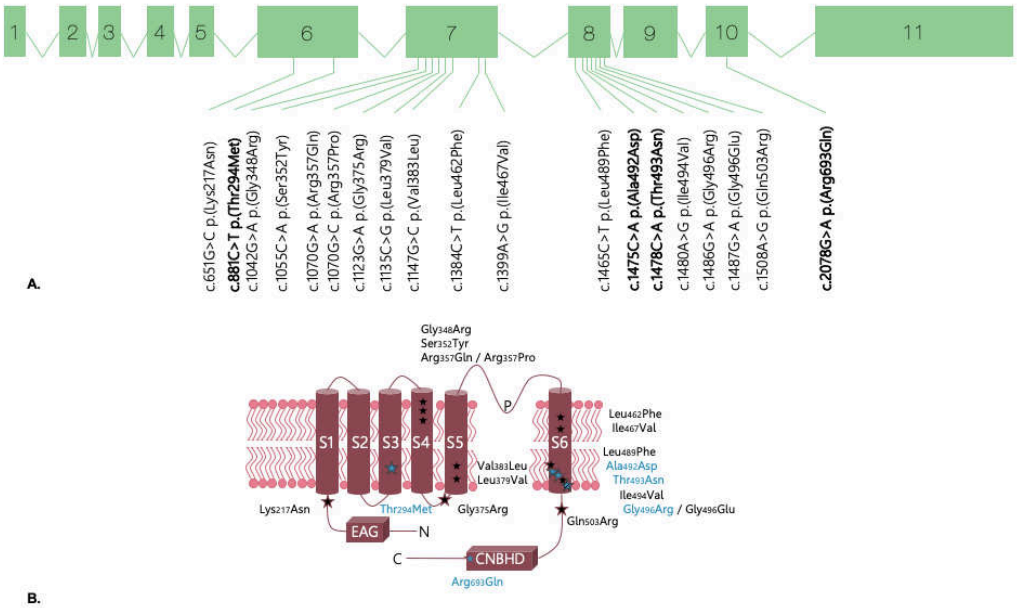

Figure 1: Schematic representation of all (likely) pathogenic variants identified in *KCNH1*.

A. cDNA level: in bold, the novel variants reported here.

B. protein level: Legend: Stars representing variants identified, In blue = variants identified in our series, Sn = transmembrane domains, EAG = *Ether-à-go-go* domain, CNBHD = cyclic nucleotide binding homology domain, P = Pore-lining loop.

Supplementary methods

The DNA of patient 1 (P1) was analyzed on a 116 epilepsy gene panel. Next-generation sequencing was performed using a SureSelectXT Custom 12-24Mb library (Agilent Technologies) on a ion proton platform (Thermo Fisher). The results were analyzed using the Torrent suite (Thermo Fisher) for alignment and variant calling. To filter and establish coverage and variant analysis, we used the Varaft annotation and filtration system (Desvignes et al., Nucleic Acids Res. 2018 Jul 2; 46: W545–W553.doi: 10.1093/nar/gky471)

The DNA of patient 4 (P4) and patient 5 (P5) was analyzed on a 90 epilepsy gene panel. A library of all coding exons and intron-exon boundaries was prepared using a SeqCap EZ Library (Roche-NimbleGen) following the manufacturer's instructions. Gene-panel sequencing was performed on a MiSeq (Illumina Inc). Sequence alignment was performed by Genodiag with BWA 0.7.12, picard-tools-1.121, GATK-3.5 (Indel realignment, base recalibration). GATK-3.5 (HaplotypeCaller) and SNPEff-4.2 were used for variant calling and annotation, adding information from gnomAD, ClinVar and HGMD.

The DNA of patients 2 (P2), 3 (P3), 6 (P6) and 7 (P7) and their parents was studied by trio-WES. A library of all coding exons and intron-exon boundaries was prepared using a SeqCap EZ MedExome (Roche-NimbleGen) following the manufacturer's instructions. Whole Exome sequencing was performed on the Illumina NextSeq 500 platform.
P2, P3 and P6: Raw reads were mapped to the human genome reference-build hg19 using the Burrows Wheeler Aligner (BWA MEM v0.717) alignment algorithm. The resulting binary alignment/map (BAM) files were further processed by Genome Analysis Tool Kit HaplotypeCaller (GATK HC v3.8). The VCF files were then annotated on Snpeff version 4.3T. Only coding non synonymous and splicing variants were consid-

ered. Variant prioritization was conducted thanks to the transmission mode (*de novo*, autosomal recessive and X-linked), and the frequency of the variants in the gnomAD database.

P7: Fastq files were aligned to human genome hg19 with bwa mem (v0.7.3). We then called SNVs and INDELs following GATKs best practices (v3.4). We achieved an average mean target coverage of 113X. Variants were annotated using ANNOVAR and filtered with in-house scripts to keep variants with at least 9 reads and with a variant read frequency over 20 percent impacting exonic sequences or splice sites (+/- 10bp from the junction) and with an allele frequency <0.5% in 1000 genomes, genome aggregation database (gnomAD, 123,136 exomes and 15,496 whole genome sequences; accessed on 11/10/2018) and in a local database. The possible functional impact of amino-acid changes was predicted by SIFT (Sorting Intolerant from Tolerant), PolyPhen-2 hvar and CADD score (Combined Annotation Dependent Depletion). The Alamut software (Interactive biosoftware) was used to study retained variant sites.

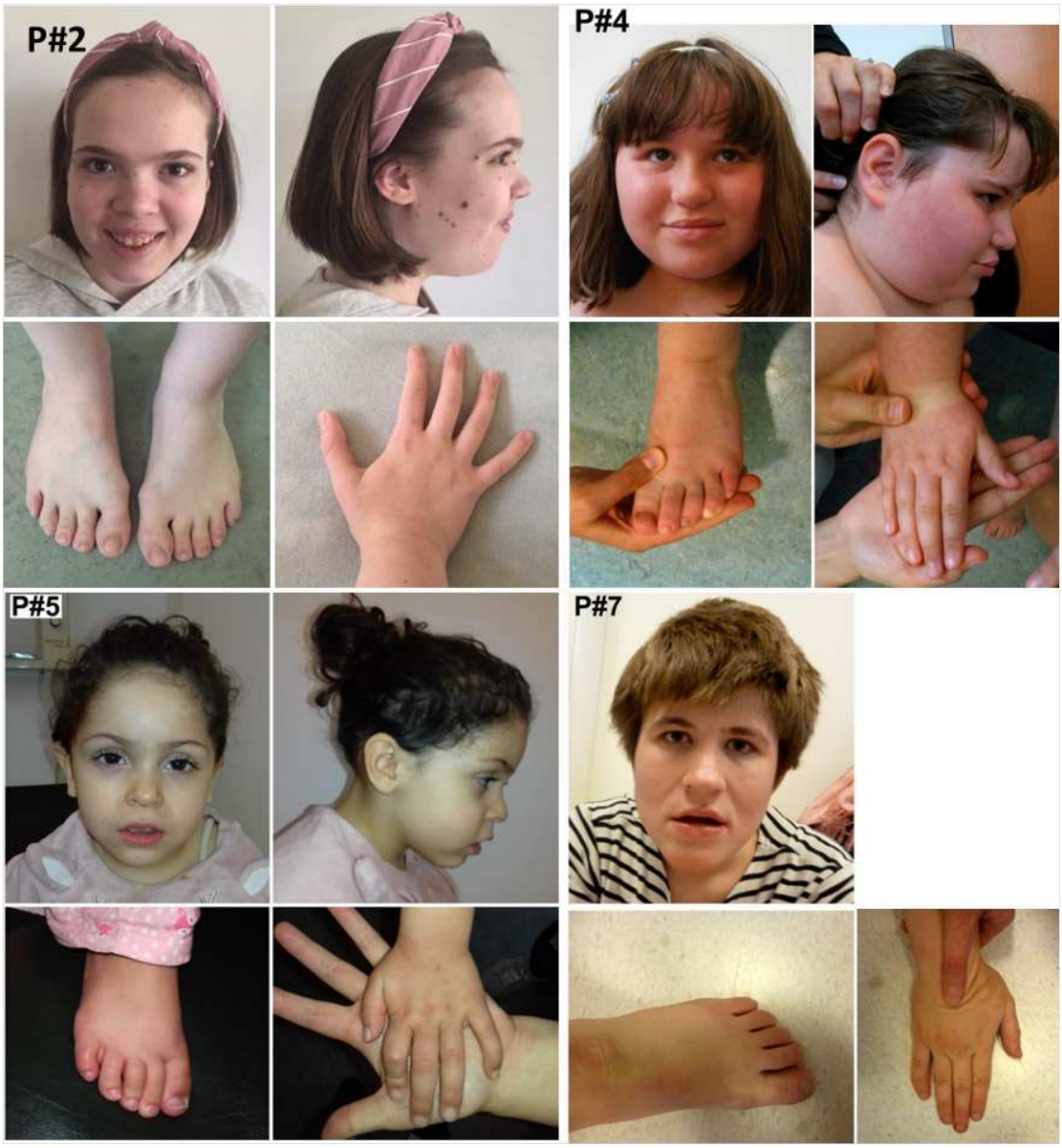

Figure S1. Faces, hands and feet of patients P2, P4, P5, and P7.
Mild dysmorphic features could be described: long facies with high forehead, bilateral epicanthic folds, mild hypertelorism, flat nasal bridge, broad tip of nose, flat midface, full lips, small mouth, mild prognathism.

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