Expanding the clinical phenotype of the ultra-rare Skraban-Deardorff syndrome: Two novel individuals with WDR26 loss-of-function variants and a literature review

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Expanding the clinical phenotype of the ultra-rare Skraban-Deardorff syndrome: two novel individuals with *WDR26* loss-of-function variants and a literature review

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ABSTRACT

De novo variants in the *WDR26* gene leading to haploinsufficiency have recently been associated with Skraban-Deardorff syndrome. This condition is an ultrarare autosomal dominant neurodevelopmental disorder characterized by a broad range of clinical signs, including intellectual disability (ID), developmental delay (DD), seizures, abnormal facial features, feeding difficulties and minor skeletal anomalies.

Currently, 18 cases have been reported in the literature and for only 15 of them a clinical description is available. Here, we describe a child with Skraban-Deardorff syndrome associated with the *WDR26* pathogenic *de novo* variant NM_025160.6:c.69dupC, p.(Gly24ArgfsTer48), and an adult associated with the pathogenic *de novo* variant c.1076G>A, p.(Trp359Ter). The adult patient was a 29-year-old female with detailed information on clinical history and pharmacological treatments since birth, providing an opportunity to map disease progression and patient management.

By comparing our cases with published reports of Skraban-Deardorff syndrome, we provide a genetic and clinical summary of this ultrarare condition, describe the clinical management from childhood to adult age, and further expand on the clinical phenotype.

INTRODUCTION

Haploinsufficiency of the WD Repeat Domain 26 (*WDR26*) gene has been associated with Skraban-Deardorff syndrome (MIM #617616), an ultrarare autosomal dominant disorder characterized by intellectual disability (ID), developmental delay (DD), seizures and common facial features, including depressed nasal root and broad nasal tip, anteversion of nares, prominent smile, widely spaced teeth, and gingival abnormalities [Nguengang Wakap et al., 2020; Hennekam, 2011]. Happy and friendly demeanour and wide-based and/or stiff-legged gait are often observed [Skraban et al., 2017].

WDR26 is an evolutionary conserved gene and is highly intolerant to loss–of-function variation (pLI = 1.0; Z-score = 3.58, gnomAD ver.2.1.1). This suggests haploinsufficiency as underlying mechanism. The exact pathogenic mechanisms are still unclear, although a role in MAPK, Wnt, and PI3K signalling pathways has been suggested [Clevers and Nusse, 2012; Ye et al., 2016].

To date, only 15 affected patients (ten females and five males) have been identified and clinically described [Skraban et al., 2017]. Another case with a nonsense variant [Jiao et al., 2019], and two cases with missense variants have been identified by whole-exome sequencing (WES), but without detailed descriptions of the clinical phenotypes [Iossifov et al., 2014; Takata et al., 2018]. Notably, 12 of the 18 *WDR26* causative variants previously reported were truncating, further supporting haploinsufficiency as the molecular mechanism of the disease (Figure 1A).

Here, we report two novel loss-of-function *de novo* variants in *WDR26* in one paediatric and one adult patient with Skraban-Deardorff syndrome. Detailed clinical characterization of the two cases allowed us to identify new features of Skraban-Deardorff syndrome. Furthermore, we profile the history of this disease and provide insights useful for the management of affected individuals.

METHODS

Whole exome sequencing, prioritization, and variant calling

DNA was extracted from total blood using the ReliaPrep Blood gDNA Miniprep kit (Promega, Madison, WY, USA) following manufacturer's protocol and quantified with a NanoDrop spectrophotometer (Thermo Fisher Scientifics, Waltham, MA, USA).

Array-CGH was performed using a 60K whole-genome oligonucleotide microarray (Agilent Technologies, Santa Clara, California, USA).

Patients were enrolled in the Autism Sequencing Consortium (ASC) project and their gDNA samples were sequenced at the Broad Institute on Illumina HiSeq sequencers as previously described [De Rubeis et al., 2014; Satterstrom et al., 2020].

WES raw data of the trio were processed and analyzed using an in-house implemented pipeline previously described [Flex et al., 2019; Bauer et al., 2018] which is based on the GATK Best Practices [Van der Auwera et al., 2013]. The UCSC GRCh37/hg19 version of genome assembly was used as a reference for reads alignment by means of BWA-MEM [Li and Durbin, 2009] tool and the subsequent variant calling with HaplotypeCaller (GATK v3.7)[Van der Auwera et al., 2013]. We used SnpEff v.4.3 [Cingolani et al., 2012] and dbNSFP v.3.5 [Liu et al., 2016] tools for variants functional annotation, including Combined Annotation Dependent Depletion (CADD) v.1.3 [Kircher et al., 2014], Mendelian Clinically Applicable Pathogenicity (M-CAP) v.1.0 [Jagadeesh et al., 2016] and Intervar v.0.1.6 for functional impact prediction [Li and Wang, 2017]. Thereby, the analysis was narrowed to variants which affect coding sequences or splice site regions. Moreover, high-quality variants were filtered against public databases (dbSNP150 and gnomAD ver.2.0.1) so that only variants with unknown frequency or having MAF <0.1%, as well as variants occurring with frequency <1% in our population-matched database (~2000 exomes) were considered.

A further variant stratification in conformity with the American College of Medical Genetics and Genomics (ACMG) guideline [Li and Wang, 2017], considering also mode of inheritance and functional *in-silico* prediction of impact, allowed us considering the final set of variants for possible associations with the phenotype. All variants are referred to GRCh37 annotation and to NM_025160.6.

Identified variants were confirmed by Sanger sequencing using standard conditions and the following primers: 5'- ctcctccgtggtggtagtgg; 5'- gacgactccccgttctgg (for c.69dupC, p.(Gly24ArgfsTer48)) and 5'-gagtctcttgaccccaggcg; 5'-cagtttcctcatctgactgcagg (for c.1076G>A, p.(Trp359Ter)).

In silico prediction of variants impact

Variants were analysed with the VarSome tool [Kopanos et al., 2019] as a starting point for further analysis. This allowed evaluation of at least nine *in silico* predictors simultaneously. Variants frequencies were evaluated using Genome Aggregation Database (GnomAD) Browser version 2.1.1.

Ethical committee

Informed consent was obtained from participating families and the study protocol was approved by the internal Ethics Committee of University of Turin (n. 0060884) and University of Skopje (n. 03-6116/7), according to the Declaration of Helsinki.

RESULTS

Clinical description of patient 1

Patient 1 was an 8 year-old Caucasian female, born at 40th weeks of gestation via spontaneous vaginal delivery as a second child from a non-consanguineous parents from Macedonia (Apgar 9/10; birth weight 3,000 g, 50th centile; birth length 51 cm, 50th centile) (Supplementary Figure 1A). The pregnancy was uncomplicated and prenatal ultrasounds were normal; the family history was unremarkable.

The patient could hold her head at 5 months, sat at 7 months and walked at 17 months; the control of the sphincters was reached at 4 years of age. Language delay was also reported. Previous evaluation included normal basic metabolic screenings and normal karyotype.

Her medical history was significant for bilateral foot deformity with *metatarsus varus* (Figure 1B), recurrent serous otitis media requiring tympanostomy tubes, hypertrophic adenoids, and food allergies at age of 7 years old. Her hearing Brain Stem Evoked Response Audiometry (BERA) examination showed tympanometry curve type “B” on the right, and type “C” on the left ear. Beside the report of neonatal hypotonia, the child had normal muscle strength accompanied by dyspraxia and wide-based gait.

At 4 years old, she was evaluated for possible absence seizures (described by the mother as staring episodes with frequent blinking and mouth chewing) however the sleep-deprived EEG was inconclusive. A second EEG assessment (with hyperventilation/photic stimulation) could not be performed to obtain a definitive diagnosis [Sadleir et al., 2009].

The patient had a playful and affectionate demeanour yet became shy with strangers. Aggressiveness was occasionally observed. At the last evaluation, she had not acquired significant verbal language, her communication was limited to a few words and she often communicated with gestures. The presence of an adult, especially her mother, was required for every activity. Memory could not be evaluated because of lack of verbalization and expressive aphasia.

The proband had prominent brows with long lashes, large irises and upslanted palpebral fissures, depressed nasal root and broad nasal tip, mild anteverted nares, full cheeks, slightly long philtrum, wide mouth, widely spaced teeth, abnormal gums, and gingival hyperplasia (Figure 1B). Pictures from childhood along with computational comparison with previously reported cases are provided in Figures 1B and D.

Clinical description of patient 2

Patient 2 was enrolled in the ASC project at 25 years old, after a long diagnostic odyssey, and received her diagnosis 4 years later. A detailed description of the medical reports, performed tests and administered drugs is provided in Figure 2 and Table S1.

She was the second child (Supplementary Figure 1A) of non-consanguineous Caucasian parents from Italy, born at 38^{th} gestational weeks after a complicated pregnancy due to placental insufficiency and sclerosis of the chorionic villi. Parents were healthy without history of neuropsychiatric disorders, beside report of ID of the maternal first cousin.

At postnatal day 13, a slight increase in the volume of the heart left cavity, the right ventricle and of the pulmonary artery were reported together with a perimembranous ventricular septal defect. Since then, the patient was clinically followed due to severe developmental and motor delays.

At two years old, computed tomography (CT) scan (Figure 2B) and magnetic resonance imaging (MRI, not available) exam showed reduced dimension of the splenium of the corpus callosum and periventricular leukomalacia, with a final diagnosis of suspected connatal malformative encephalopathy. Routine metabolic screening was normal.

The first neuropsychiatric evaluation was performed at 3 years of age, when several manual and oral motor disabilities (semi-open mouth, frequent movement of protrusion of the tongue without drooling,

absent chewing, oral-motor apraxia) were reported. She also presented two types of generalized epilepsy: motor tonic-clonic, and non-motor absence crisis (when seizures were more frequent, the patient fell asleep or remained confused at awake) with a duration of about 10 seconds each.

Walking began at 3 years of age, with slow and unstable wide-based stiff-legged gait, often requiring support from a wheelchair. Fine motor skills were particularly compromised, as well as her muscle coordination presented with dysmetria and dyspraxia. Dysphagia was identified during the infancy and continued lifelong, with the preferential consumption of liquid foods.

At 22 years old, her brain MRI showed a modest enlargement of cerebral ventricles with alterations of the periventricular white matter suggestive of neonatal hypoxia. The corpus callosum was modestly thinner, while pineal gland was normal (Figure 2B).

As an adult, she showed good spatial orientation only in familiar places and became agitated in new ones. She was unable to discriminate right from left. Interestingly she showed hypersensitivity to smell and touch, and she was not been able to filter different sensorial stimuli.

Throughout her life the patient had a poorly organized recreational activity, extremely poor social interaction, and deep anxiety. The Griffith scale [Griffith et al., 1999] reported medium severity global delay, reduced ability of visual attention, impaired visual monitoring, and ability of considering many things at the same time. The attention span was limited, due to extremely low motivation. Memory could not be evaluated because of lack of verbalization. Taking in consideration all these features a diagnosis of autism spectrum disorder (ASD) associated with language disorder and severe intellectual disability was reached.

Concerning patient's perspective with health outcome, a complete habilitative daily program, based on behavioral therapy in a small ASD group, has been provided from Adult autism center and Social Agency to improve social skill but very little improvement has been obtained, so the treatment was further based on care and support [Keller et al., 2020].

She had a happy and friendly demeanour for the most part, but could become agitated and scared, especially in case of new situations.

She presented good facial mimic with lively and expressive look, wide mouth with thin and elongated lips, decreased Cupid's bow, abnormal gingiva, widely spaced teeth, as well as large and protruding tongue.

Skeletal findings included bilateral clinodactyly of fifth finger and partial agenesis of distal part of the second finger of left hand. Pictures from childhood to adulthood and a computational comparison with already reported cases are provided in Figure 1B and C.

A detailed clinical characterization of the two patients together with the clinical phenotype of the previously reported Skraban-Deardorff patients is presented in Figure 3 and divided by different systems.

Molecular findings

Our probands were part of a large cohort of 686 predominantly Caucasian patients affected by ASD with or without ID collected for genetic screening and analysed by whole exome sequencing as previously reported [De Rubeis et al., 2014; Satterstrom et al., 2020].

In patient 1, we identified the *de novo* frameshift variant c.69dupC, p.(Gly24ArgfsTer48) in *WDR26*. The single nucleotide insertion occurred in the first exon of 14 coding exons, causing a frameshift variant that is predicted to trigger non-sense mediated decay. In patient 2, we found the *de novo* nonsense variant c.1076G>A, p.(Trp359Ter) in *WDR26* introducing a premature stop codon, that predicted to activate non-sense mediated decay. Both variants were classified as "pathogenic" according to ACMG criteria (PVS1, PS2, PM2) [Li and Wang, 2017]. Moreover, concerning the *de novo* nonsense variant identified in patient 2, eight out of nine *in silico* tools predicted this change as damaging, adding the PP3 supporting criteria to ACMG classification. Both variants were absent in the Genome Aggregation Database (gnomAD ver 2.1.1) or in the 1000 Genomes browser. No further clinically relevant variants were identified.

DISCUSSION

We report one paediatric and one adult case with *de novo* loss-of-function variants in *WDR26* associated with the ultra-rare Skraban-Deardorff syndrome. Our patients showed overlapping phenotype with reported cases. However, patient 2 had several additional features, further expanding the Skraban-Deardorff syndrome-related phenotype (Table S2). It is interesting to note that many of these newly observed characteristics appeared after puberty, suggesting a possible evolution of the disease that has not been reported

yet. Indeed, almost all of the described cases are paediatric, with the exception of two cases of 21 and 34-years old [Skraban et al., 2017].

The consensus phenotype for Skraban-Deardorff syndrome includes intellectual disability, delayed or absent speech, autistic features, dysphagia, wide-base and/or stiff-legged gait, skeletal anomalies, recurrent otitis media and brain MRI anomalies (prevalently affecting corpus callosum). The similarity of facial phenotype is striking, underlying the consistent facial gestalt in this disorder and the relevance of dysmorphological evaluation in the diagnostic path of ASD patients.

Notably, seizures have been observed in all cases, mainly generalised motor, and non-motor absences seizures. The latter (also called *petit mal seizures*) are hard to diagnose, especially in non-verbal children [Crunelli and Leresche, 2002]. Other condition that might further bias the diagnosis are the frequent otitis media that could give the impression that the child is not responsive due to hearing problems. For this reason, we suggest that a more accurate phenotyping of the type of epilepsy would provide relevant insights for a more effective management of these patients.

Beside the absence epilepsy in patient 2, a frequent sleep disturbance and tactile and olfactory hypersensitivity were reported. It is intriguing to observe that these clinical features can be tracked to the thalamus [Cope et al., 2009; Courtiol and Wilson, 2015]. Indeed, thalamus transmits the sensory information with a rhythmic pattern in the thalamocortical tract, typical tuned down during sleep. It appears that in absence epilepsy and also in people having sleeping difficulties, this thalamocortical pattern is changed and amplified into a distortion range [Sorokin et al., 2017]. This information could help in further understanding the mechanisms underlying the disorder and open new avenues for treatment [Fogerson and Huguenard, 2016; Paz et al., 2013].

Hypotonia has been reported in 9 out of 12 cases [Skraban et al., 2017]. Surprisingly, our patients showed remarkable muscle strength and often hypertonia; hypertonic limbs with brisk reflexes were also previously reported for one case [Skraban et al., 2017]. We can speculate that this ambiguous muscle tone phenotype, as well as the diverse type of walking, could be related to the brain MRI findings, predominantly affecting the white matter such as corpus callosum anomalies [Hofman et al., 2020; Edwards et al., 2014], enlarged ventricles, white matter volume loss, and mild cerebellar hypoplasia [Skraban et al., 2017].

Furthermore, the combination of wide-based gait and spastic and/or stiff-leg gait are often reported. They indicate the involvement of the corticospinal tract and the upper motor neurons. The inclusion of a spinal MRI in Skraban-Deardorff patients may elucidate the origin of these symptoms.

Skeletal findings included metatarsus varus in patient 1, and bilateral clinodactyly of the fifth finger, and partial agenesis of distal part of the second finger of left hand in patient 2. Moreover, as described for one patient [Skraban et al., 2017], a foot malformation was present (right flat and supine foot and left cavus and metatarsus varus) (Figure 1B). Steopathia striata of the distal femurs, *pes cavus* or hip dysplasia that were described in some cases, where not identified in our patients.

The immune system seems also often affected in Skraban-Deardorff with a variety of presentations starting from hypersensitivity reactions to food and airborne allergies, to weak immune response presented by frequent *otitis media*.

Patient 2 showed also additional features such as hypothyroidism, renal acidosis, and various gynaecological abnormalities. Given the facial and behavioural similarity between individuals with Skraban-Deardorff and individuals diagnosed with Angelman syndrome (MIM #105830), Kabuki syndrome (MIM #147920; reports of hypothyroidism; premature thelarche) or Cornelia de Lange syndrome (MIM #122470; renal anomalies), it is reasonable that Skraban-Deardorff phenotype might result from altered chromatin regulation, as suggested by [Skraban et al., 2017].

WDR26 is located on chromosomal region 1q42, which can be disrupted in 1q41q42 microdeletion syndrome, a chromosomal anomaly resulting in severe developmental delay and/or intellectual disability, typical facial dysmorphic features, brain anomalies, seizures, cleft palate, clubfeet, nail hypoplasia and congenital heart disease [Rosenfeld et al., 2011]. Many features of this syndrome overlapped with the one observed for *WDR26*-related disease and a comparison has already been provided previously [Skraban et al., 2017].

WDR26 gene encodes a WD40 repeat protein 26 (Wdr26), a member of the WD repeat (WDR) protein family. The WDR is one of the most abundant protein-protein interactions domain, with its central peptide-binding pocket [Stirnimann et al., 2010].

The mechanism by which *WDR26* haploinsufficiency leads to human developmental disorders is unknown. However, multiple roles have been proposed for *WDR26* and some studies suggest that it could play several roles in the regulation of MAPK, Wnt, and PI3K pathway signalling.

WDR26 is important for the degradation of β-catenin in the canonical Wnt pathway and it has been suggested that the gene is important for the Wnt signalling during embryogenesis [Goto et al., 2016]. Alterations in Wnt have been implicated in both neurodevelopmental and neurodegenerative disorders. Interestingly, the impairment of this pathway has also been involved in cardiovascular, kidney, lung, allergy, skeletal and oral diseases [Clevers and Nusse, 2012]. WDR26 is also involved in the regulation of Gβγ-mediated PI3K/AKT signalling, acting as a scaffolding protein to promote the interactions between Gβγ, PI3Kβ and AKT2[Ye et al., 2016]. The PI3K-Akt-mTOR cascade has been broadly implicated in both neurodevelopmental and neuropsychiatric diseases[Wang et al., 2017]. Therefore, since the *WDR26* haploinsufficiency affects many systems, an intriguing possibility is that the impaired activation of the Wnt and/or PI3K/AKT cascade could be involved in the onset of the disease.

In summary, we report an extended clinical description of two novel Skraban-Deardorff syndrome cases, adding new information about the clinical spectra and the evolvement of the disease from childhood to adulthood and providing a review of the available literature related to the disease. This deep clinical characterization and description of pharmacological treatments might inform management of Skraban-Deardorff patients and give suggestion for further functional characterization of the gene and related disorder.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA SHARING AND DATA ACCESSIBILITY

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

AUTHORS CONTRIBUTION

Lisa Pavinato initiated and designed the study, wrote, and edited the manuscript. Lisa Pavinato, Slavica Trajkova and Elisa Giorgio interpreted exome data collected the cases and performed variant confirmation. Giovanni Battista Ferrero, Enrico Grosso, Roberto Keller, Francesca Clementina Radio, Aleksandar Petlichkovski and Slavica Trajkova performed the clinical evaluation. Alessandro Bruselles, Silvia De Rubeis, Paola Dimartino, Joseph Buxbaum, Tommaso Pippucci, and Marco Tartaglia perfomed the exome data analysis. Alfredo Brusco coordinated the study. All authors critically reviewed the manuscript and provided expert feedback.

WEB RESOURCES

Autism Sequencing Consortium exome analysis browser, https://asc.broadinstitute.org/

Face2Gene, https://www.face2gene.com/

gnomAD Browser, v2.1.1, https://gnomad.broadinstitute.org/

National Center for Biotechnology Information (NCBI), https://www.ncbi.nlm.nih.gov/

OMIM, https://omim.org/

SFARI gene, https://gene.sfari.org/

Varsome, https://varsome.com/

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Legends to figures

Figure 1. WDR26 pathogenic variants and facial features in Skraban-Deardorff syndrome patients.

(A) WDR26 protein structure reported by the National Center for Biotechnology Information (NCBI) (NP_079436.4) contains two domain types: a C-terminal LIS homology domain (CTLH, amino acid residues 156-231; light blue), and several WD repeat domains (yellow); here, six WD domains are shown, but from five to 14 have been estimated. Currently, twenty pathogenic (red) / likely pathogenic (orange) variants (ACMG criteria), including the two here described (p.(Gly24ArgfsTer48) and p.(Trp359Ter) in bold) are reported (five frameshifts, five premature stop codons, seven missense and one splice site variants).

(B) Our probands' pictures at different ages from childhood to adulthood show the evolution of facial appearance and dysmorphisms. Below feet malformations include flatfoot, forefoot varus and metatarsus varus.

(C) Facial features analysis by Face2Gene software (FDNA Inc., Boston, USA) [Gurovich et al., 2019]. Heat map (on the left) were automatically generated by Face2Gene and shows overlapping features with 15 published Skraban-Deardorff syndrome cases [Skraban et al., 2017] and compared with our Patient 1 at 7 yrs. and Patient 2 at 25 yrs.; overlapping facial regions are indicated by the coloured halo from red to blue. A computational face comparison with already reported cases carrying *WDR26* loss-of-function variants is shown on the right.

Figure 2. Timeline of the Patient 2 diagnostic odyssey, treatments and neuroradiology.

(A) Summary of the key medical reports, performed tests and administered drugs is reported. The first medical record dates to 13 post-natal days, when cardiovascular defects were noted. The patient was subjected to a long diagnostic odyssey before finding a molecular diagnosis thanks to trio whole exome sequencing, which happened at 29 years old. (B) On the left, brain computer tomography (CT) scan (T1 sagittal section) at 2 years of age showed reduced dimension of the splenium of the corpus callosum and periventricular leukomalacia. On the right: At 22 years old, MRI axial T2 flair showed an enlargement of cerebral ventriculi with features resembling neonatal hypoxia (left, white arrows); a modest reduction in corpus callosum size was also apparent (right, white arrow).

Figure 3. Phenotypic comparison between previously reported Skraban-Deardorff syndrome cases and our patients.

Graphical representation of the clinical features previously reported in other Skraban-Deardorff syndrome cases (light blue) and in our patients (pink and magenta). Numbers indicate the patients showing the phenotype, considering a total number of 17 cases (15 reported in Skraban *et al. 2017* work and our cases). Patients reported by [Iossifov et al., 2014; Takata et al., 2018; Jiao et al., 2019] were excluded from this count, as detailed clinical information were not available.

American Journal of Medical Genetics part A – Original research

Expanding the clinical phenotype of the ultra-rare Skraban-Deardorff syndrome: two novel individuals with *WDR26* loss-of-function variants and a literature review

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Supplementary data

Table S1. Pharmacological treatments (Patient 2)

Table S2. Additional features observed in our patients

Figure S1. Pedigree and IGV or Sanger sequencing confirmation of variants

Table S1. Pharmacological treatments

Drug	Dosage	Method of administration	Starting age	Clinical indication	Response to treatment
Sodium bicarbonate	10mEq three times/day	oral intake	3	renal tubular acidosis	symptoms improvement
Valproate	1000 mg	oral intake	3	seizures	no seizures
Phenobarbital	100 mg	oral intake	3	seizures	no seizures
Leuprolide acetate	3.75 mg every 28 days	intramuscular injection	8,8	central precocious puberty and advanced bone age	stabilization at 12 years of age
Melatonin	3mg/day	oral intake	10	insomnia	responder
Omeprazole	20 mg /day	oral intake	15	gastroesophageal reflux	symptoms improvement
Mesalazine	n.a.	oral intake	18	colitis	responder
Dydrogesterone	10 mg once a day	oral intake	23	amenorrhea and low progesterone levels	n.a.
Calcium folinate	5 mg/day	oral intake	25	folate deficit	n.a.

Lormetazepam	2 mg/day	oral intake	26	insomnia	responder
Mirtazapine	15 mg/day	oral intake	27	insomnia	responder
Folic acid supplement	n.a.	n.a.	22	high homocysteine levels	responder
Vitamin B supplement	n.a.	n.a.	n.a.	n.a.	n.a.
n.a. = not available					

489

490

Table S2. Additional features observed in our cases

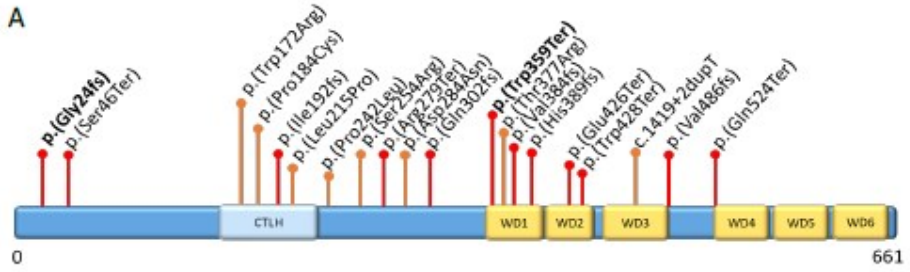
	Features not previously reported in other cases
Pubertal development and endocrinology	Early pubertal development
	Advanced bone age at 8 years
	Idiopathic hyperprolactinemia
	Small uterus
	Nabothian cyst
	Multifollicolar ovaries
	Low progesterone levels
	Hypothyroidism
	Amenorrhea/Spaniomenorrhea
Senses	Hypersensitivity of smell
	Hypersensitivity of touch
	Inability to filter different sensorial stimula
Biochemical	Iron deficiency anemia
	MTHFR defect, with low folate and high homocysteine levels
	Hyperuricemia
Neurological and behavioural	Absence of mental flexibility
	Limited verbal comprehension
	Limited attention span
Facial features	Thin and elongated lips
	Good facial mimic
	Flat and protuding tongue
Gastointestinal and genitourinary systems	Antral erosive gastropathy
	Hemorrhoidal congestion
	Intestinal bleeding
	Intestinal villi atrophy
	Eosinofilic colitis
	Dysphagia
	Disorders of alvo
	Meteorism

	Renal distal tubular acidosis
	Kidney stones
Immunological features	Chronic bronchitis with broncospasm
	Frequent infections of lower respiratory tract
Skeletal features	Flatfoot
Motor skills	Impaired fine motor skills
	Low visual-motor coordination
	Dysmetria
	Dyspraxia
	Oral-motor apraxia
Other	Hair fragility
	Poor spatial orientation
	Hirsutism

Legend to Supplementary Figure.

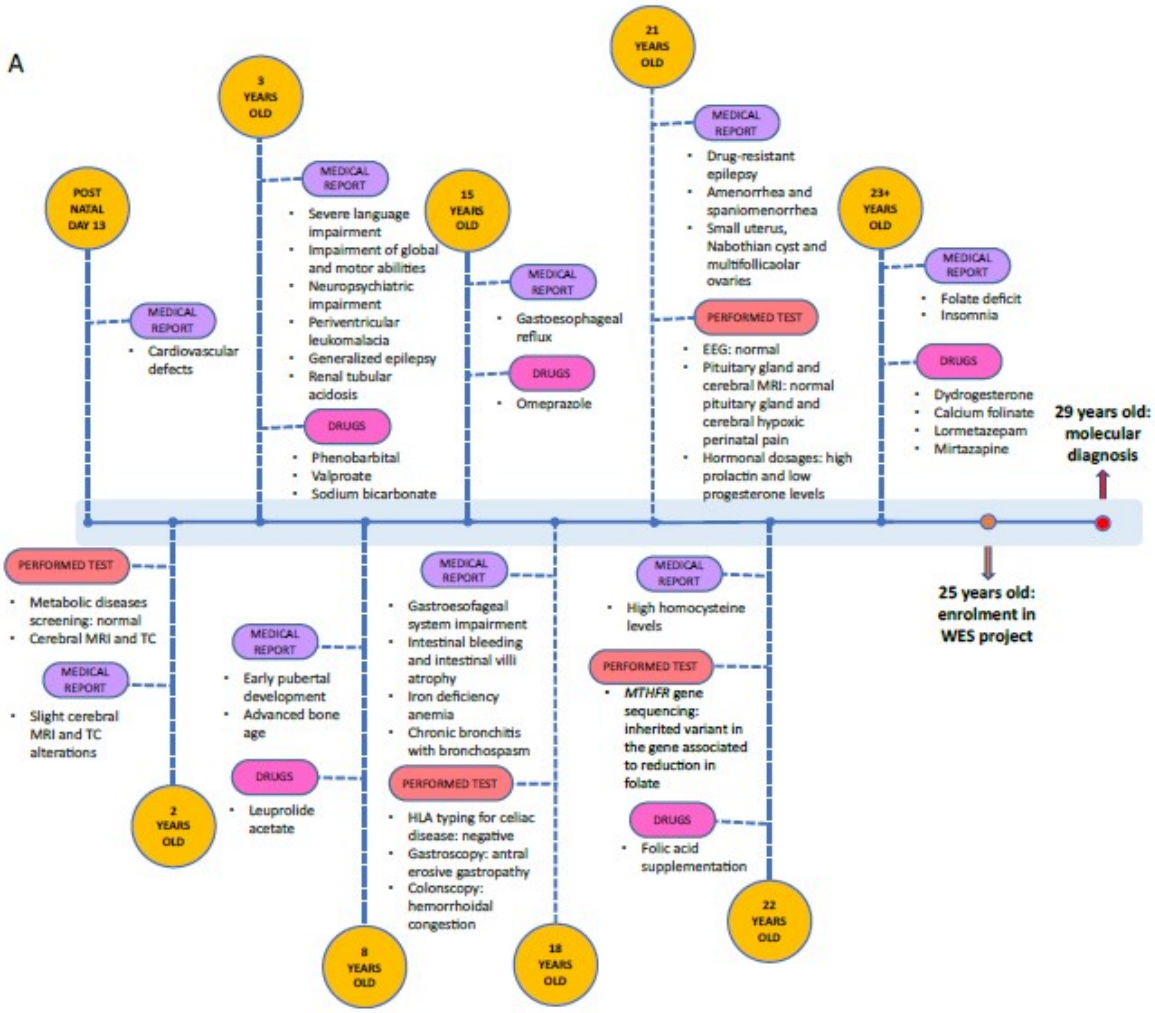
Figure S1. Pedigree and Sanger sequencing confirmation of variants

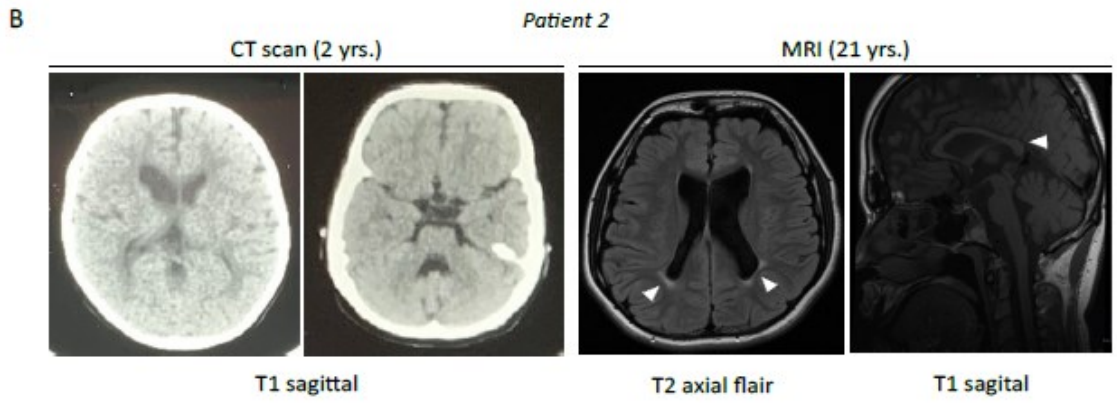
(A) Pedigree of Patient 1 family and confirmation of the variant by Sanger sequencing. (B) Pedigree of Patient 2 family and confirmation of the variant by Sanger sequencing.

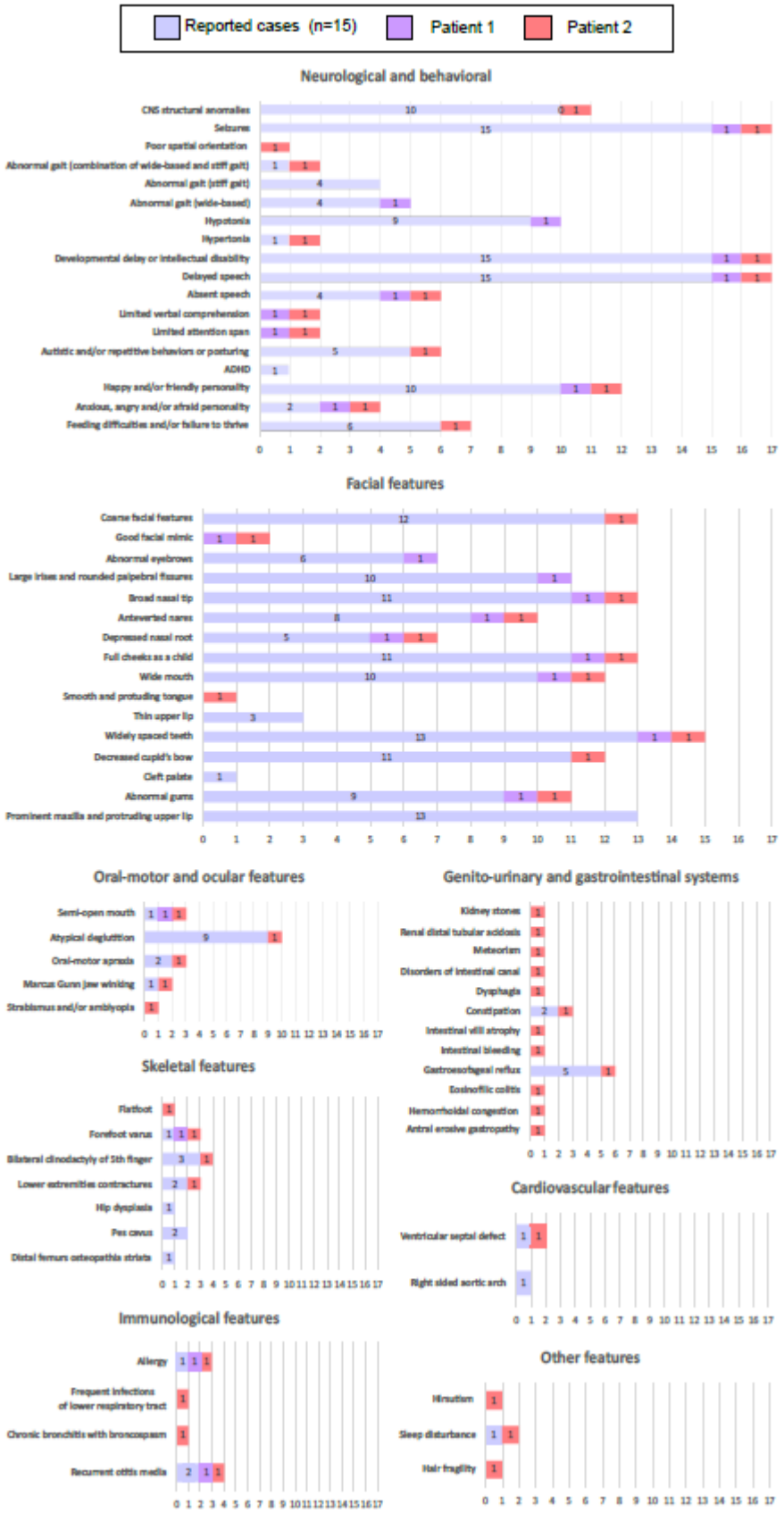

A
p.(Gly24fs)
p.(Ser46Ter)
p.(Trp172Arg)
p.(Pro184Cys)
p.(Ile192fs)
p.(Leu215Pro)
p.(Pro242Leu)
p.(Ser254Arg)
p.(Arg279Ter)
p.(Asp284Asn)
p.(Gln302fs)
p.(Trp359Ter)
p.(Thr377Arg)
p.(Val384fs)
p.(His389fs)
p.(Glu426Ter)
p.(Trp428Ter)
c.1419+2dupT
p.(Val486fs)
p.(Gln524Ter)
CTLH
WD1
WD2
WD3
WD4
WD5
WD6
0
661


B
Patient 1
Patient 2
Skeletal features

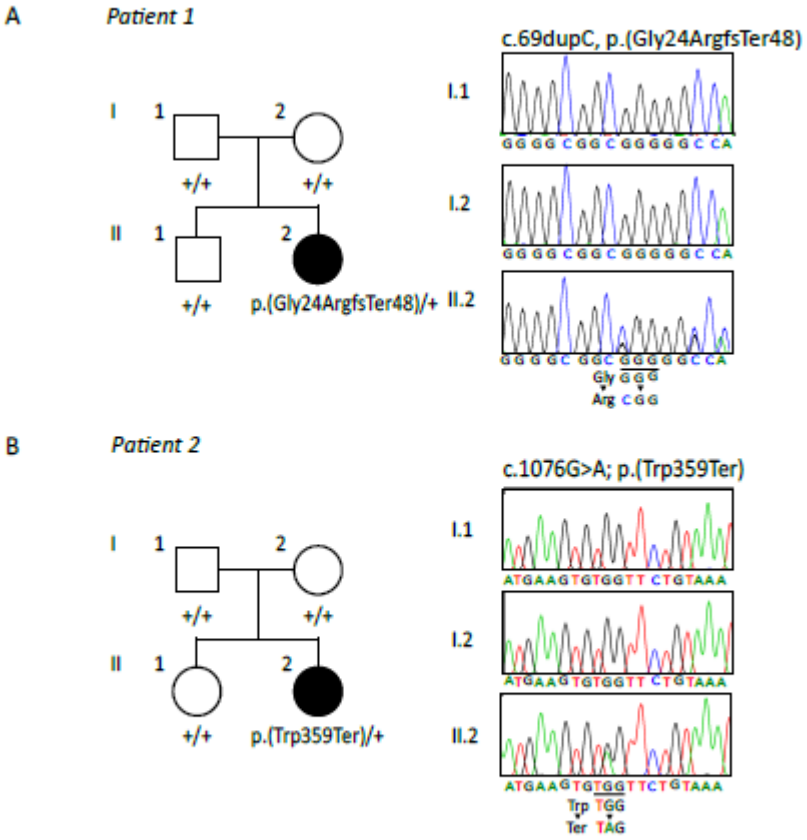

C
Our case
Reported cases
Facial feature of Skraban-Deardorff patients
Patient 1
Patient 2


A
POST NATAL DAY 13
MEDICAL REPORT
Cardiovascular defects
3 YEARS OLD
MEDICAL REPORT
Severe language impairment
Impairment of global and motor abilities
Neuropsychiatric impairment
Periventricular leukomalacia
Generalized epilepsy
Renal tubular acidosis
DRUGS
Phenobarbital
Valproate
Sodium bicarbonate
15 YEARS OLD
MEDICAL REPORT
Gastroesophageal reflux
DRUGS
Omeprazole
21 YEARS OLD
MEDICAL REPORT
Drug-resistant epilepsy
Amenorrhea and spaniomenorrhea
Small uterus, Nabothian cyst and multifollicaolar ovaries
PERFORMED TEST
EEG: normal
Pituitary gland and cerebral MRI: normal pituitary gland and cerebral hypoxic perinatal pain
Hormonal dosages: high prolactin and low progesterone levels
23+ YEARS OLD
MEDICAL REPORT
Folate deficit
Insomnia
DRUGS
Dydrogesterone
Calcium folinate
Lormetazepam
Mirtazapine
29 years old: molecular diagnosis
25 years old: enrolment in WES project
PERFORMED TEST
Metabolic diseases screening: normal
Cerebral MRI and TC
MEDICAL REPORT
Slight cerebral MRI and TC alterations
2 YEARS OLD
MEDICAL REPORT
Early pubertal development
Advanced bone age
DRUGS
Leuprolide acetate
8 YEARS OLD
MEDICAL REPORT
Gastroesofageal system impairment
Intestinal bleeding and intestinal villi atrophy
Iron deficiency anemia
Chronic bronchitis with bronchospasm
PERFORMED TEST
HLA typing for celiac disease: negative
Gastroscopy: antral erosive gastropathy
Colonscopy: hemorrhoidal congestion
18 YEARS OLD
MEDICAL REPORT
High homocysteine levels
PERFORMED TEST
MTHFR gene sequencing: inherited variant in the gene associated to reduction in folate
DRUGS
Folic acid supplementation
22 YEARS OLD


B
Patient 2
CT scan (2 yrs.)
MRI (21 yrs.)
T1 sagittal
T2 axial flair
T1 sagital


Reported cases (n=15)
Patient 1
Patient 2
Neurological and behavioral
CNS structural anomalies
Seizures
Poor spatial orientation
Abnormal gait (combination of wide-based and stiff gait)
Abnormal gait (stiff gait)
Abnormal gait (wide-based)
Hypotonia
Hypertonia
Developmental delay or intellectual disability
Delayed speech
Absent speech
Limited verbal comprehension
Limited attention span
Autistic and/or repetitive behaviors or posturing
ADHD
Happy and/or friendly personality
Anxious, angry and/or afraid personality
Feeding difficulties and/or failure to thrive
Facial features
Coarse facial features
Good facial mimic
Abnormal eyebrows
Large irises and rounded palpebral fissures
Broad nasal tip
Anteverted nares
Depressed nasal root
Full cheeks as a child
Wide mouth
Smooth and protuding tongue
Thin upper lip
Widely spaced teeth
Decreased cupid's bow
Cleft palate
Abnormal gums
Prominent maxilla and protruding upper lip
Oral-motor and ocular features
Semi-open mouth
Atypical deglutition
Oral-motor apraxia
Marcus Gunn jaw winking
Strabismus and/or amblyopia
Genito-urinary and gastrointestinal systems
Kidney stones
Renal distal tubular acidosis
Meteorism
Disorders of intestinal canal
Dysphagia
Constipation
Intestinal villi atrophy
Intestinal bleeding
Gastroesofageal reflux
Eosinofilic colitis
Hemorrhoidal congestion
Antral erosive gastropathy
Skeletal features
Flatfoot
Forefoot varus
Bilateral clinodactyly of 5th finger
Lower extremities contractures
Hip dysplasia
Pes cavus
Distal femurs osteopathia striata
Cardiovascular features
Ventricular septal defect
Right sided aortic arch
Immunological features
Allergy
Frequent infections of lower respiratory tract
Chronic bronchitis with bronchospasm
Recurrent otitis media
Other features
Hirsutism
Sleep disturbance
Hair fragility

501


A
Patient 1
c.69dupC, p.(Gly24ArgfsTer48)
I
1
2
+/+
+/+
II
1
2
+/+
p.(Gly24ArgfsTer48)/+
I.1
I.2
II.2
B
Patient 2
c.1076G>A; p.(Trp359Ter)
I
1
2
+/+
+/+
II
1
2
+/+
p.(Trp359Ter)/+
I.1
I.2
II.2
Trp TGG
Ter TAG

502

503