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

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37 **ABSTRACT**

38 *De novo* variants in the *WDR26* gene leading to haploinsufficiency have recently been associated with
39 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
40 (DD), seizures, abnormal facial features, feeding difficulties and minor skeletal anomalies.

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

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

51

52 INTRODUCTION

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

59 *WDR26* is an evolutionary conserved gene and is highly intolerant to loss-of-function variation (pLI =
60 1.0; Z-score = 3.58, gnomAD ver.2.1.1). This suggests haploinsufficiency as underlying mechanism. The exact
61 pathogenic mechanisms are still unclear, although a role in MAPK, Wnt, and PI3K signalling pathways has
62 been suggested [Clevers and Nusse, 2012; Ye et al., 2016].

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

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

73

74 METHODS

75 Whole exome sequencing, prioritization, and variant calling

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

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

81 Patients were enrolled in the Autism Sequencing Consortium (ASC) project and their gDNA samples
82 were sequenced at the Broad Institute on Illumina HiSeq sequencers as previously described [De Rubeis et al.,
83 2014; Satterstrom et al., 2020].

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

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

100 Identified variants were confirmed by Sanger sequencing using standard conditions and the following
101 primers: 5'-ctcctcgtggtgtagtgg; 5'-gacgactccccgttctgg (for c.69dupC, p.(Gly24ArgfsTer48)) and 5'-gagtctctt-
102 gaccccaggcg; 5'-cagtttctcatctgactgcagg (for c.1076G>A, p.(Trp359Ter)).

103

104 ***In silico* prediction of variants impact**

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

108

109 **Ethical committee**

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

113

114 **RESULTS**

115 **Clinical description of patient 1**

116 Patient 1 was an 8 year-old Caucasian female, born at 40th weeks of gestation via spontaneous vaginal
117 delivery as a second child from a non-consanguineous parents from Macedonia (Apgar 9/10; birth weight
118 3,000 g, 50th centile; birth length 51 cm, 50th centile) (Supplementary Figure 1A). The pregnancy was uncom-
119 plicated and prenatal ultrasounds were normal; the family history was unremarkable.

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

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

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

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

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

141

142 **Clinical description of patient 2**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

191

192 **Molecular findings**

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

196 In patient 1, we identified the *de novo* frameshift variant c.69dupC, p.(Gly24ArgfsTer48) in *WDR26*.
197 The single nucleotide insertion occurred in the first exon of 14 coding exons, causing a frameshift variant that
198 is predicted to trigger non-sense mediated decay. In patient 2, we found the *de novo* nonsense variant
199 c.1076G>A, p.(Trp359Ter) in *WDR26* introducing a premature stop codon, that predicted to activate non-
200 sense mediated decay. Both variants were classified as “pathogenic” according to ACMG criteria (PVS1, PS2,
201 PM2) [Li and Wang, 2017]. Moreover, concerning the *de novo* nonsense variant identified in patient 2, eight
202 out of nine *in silico* tools predicted this change as damaging, adding the PP3 supporting criteria to ACMG
203 classification. Both variants were absent in the Genome Aggregation Database (gnomAD ver 2.1.1) or in the
204 1000 Genomes browser. No further clinically relevant variants were identified.

205

206 **DISCUSSION**

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

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

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

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

225 Beside the absence epilepsy in patient 2, a frequent sleep disturbance and tactile and olfactory hy-
226 persensitivity were reported. It is intriguing to observe that these clinical features can be tracked to the thal-
227 amus [Cope et al., 2009; Courtiol and Wilson, 2015]. Indeed, thalamus transmits the sensory information
228 with a rhythmic pattern in the thalamocortical tract, typical tuned down during sleep. It appears that in ab-
229 sence epilepsy and also in people having sleeping difficulties, this thalamocortical pattern is changed and
230 amplified into a distortion range [Sorokin et al., 2017]. This information could help in further understanding
231 the mechanisms underlying the disorder and open new avenues for treatment [Fogerson and Huguenard,
232 2016; Paz et al., 2013].

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

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

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

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

250 Patient 2 showed also additional features such as hypothyroidism, renal acidosis, and various gynae-
251 cological abnormalities. Given the facial and behavioural similarity between individuals with Skraban-
252 Deardorff and individuals diagnosed with Angelman syndrome (MIM #105830), Kabuki syndrome (MIM
253 #147920; reports of hypothyroidism; premature thelarche) or Cornelia de Lange syndrome (MIM #122470;
254 renal anomalies), it is reasonable that Skraban-Deardorff phenotype might result from altered chromatin
255 regulation, as suggested by [Skraban et al., 2017].

256 *WDR26* is located on chromosomal region 1q42, which can be disrupted in 1q41q42 microdeletion
257 syndrome, a chromosomal anomaly resulting in severe developmental delay and/or intellectual disability,
258 typical facial dysmorphic features, brain anomalies, seizures, cleft palate, clubfeet, nail hypoplasia and con-
259 genital heart disease [Rosenfeld et al., 2011]. Many features of this syndrome overlapped with the one ob-
260 served for *WDR26*-related disease and a comparison has already been provided previously [Skraban et al.,
261 2017].

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

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

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

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

283

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290

291 **CONFLICT OF INTEREST**

292 The authors declare no potential conflict of interest.

293

294 **DATA SHARING AND DATA ACCESSIBILITY**

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

297

298 **AUTHORS CONTRIBUTION**

299 Lisa Pavinato initiated and designed the study, wrote, and edited the manuscript. Lisa Pavinato, Slavica
300 Trajkova and Elisa Giorgio interpreted exome data collected the cases and performed variant confirmation.
301 Giovanni Battista Ferrero, Enrico Grosso, Roberto Keller, Francesca Clementina Radio, Aleksandar Petlichko-
302 vski and Slavica Trajkova performed the clinical evaluation. Alessandro Bruselles, Silvia De Rubeis, Paola Di-
303 martino, Joseph Buxbaum, Tommaso Pippucci, and Marco Tartaglia performed the exome data analysis. Al-
304 fredo Brusco coordinated the study. All authors critically reviewed the manuscript and provided expert feed-
305 back.

306

307 **WEB RESOURCES**

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

309 Face2Gene, <https://www.face2gene.com/>

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

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

312 OMIM, <https://omim.org/>

313 SFARI gene, <https://gene.sfari.org/>

314 Varsome, <https://varsome.com/>

315

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439

440 **Legends to figures**

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

442 (A) WDR26 protein structure reported by the National Center for Biotechnology Information (NCBI)
443 (NP_079436.4) contains two domain types: a C-terminal LIS homology domain (CTLH, amino acid residues
444 156-231; light blue), and several WD repeat domains (yellow); here, six WD domains are shown, but from
445 five to 14 have been estimated. Currently, twenty pathogenic (red) / likely pathogenic (orange) variants
446 (ACMG criteria), including the two here described (p.(Gly24ArgfsTer48) and p.(Trp359Ter) in bold) are re-
447 ported (five frameshifts, five premature stop codons, seven missense and one splice site variants).

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

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

457

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

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

467

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

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

475

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

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480 tina Radio⁵, Tommaso Pippucci⁶, Paola Dimartino⁷, Marco Tartaglia⁵, Aleksandar Petlichkovski⁸, Silvia De Ru-
481 beis^{9,10,11,12}, Joseph Buxbaum^{9,10,11,12,13,14,15}, Giovanni Battista Ferrero¹⁶, Roberto Keller¹⁷, Alfredo Brusco¹

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483

484 **Supplementary data**

485 **Table S1.** Pharmacological treatments (Patient 2)

486 **Table S2.** Additional features observed in our patients

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

488

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 | in-somnia | responder |
| Mirtazapine | 15 mg/day | oral intake | 27 | in-somnia | 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. |
| <i>n.a. = not available</i> | | | | | |

489

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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 Multifollicular ovaries Low progesterone levels Hypothyroidism Amenorrhea/Spaniomenorrhoea |
| 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 |
| Gastrointestinal and genitourinary systems | Antral erosive gastropathy Hemorrhoidal congestion Intestinal bleeding Intestinal villi atrophy Eosinophilic colitis Dysphagia Disorders of alvo Meteorism |

| | |
|-------------------------------|--|
| | Renal distal tubular acidosis |
| | Kidney stones |
| Immunological features | Chronic bronchitis with bronchospasm |
| | 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 |

491

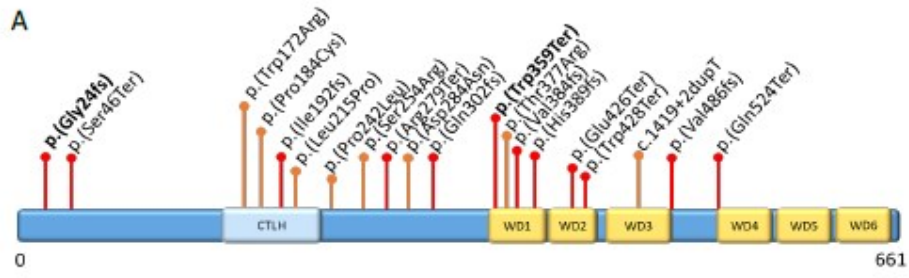
492 **Legend to Supplementary Figure.**

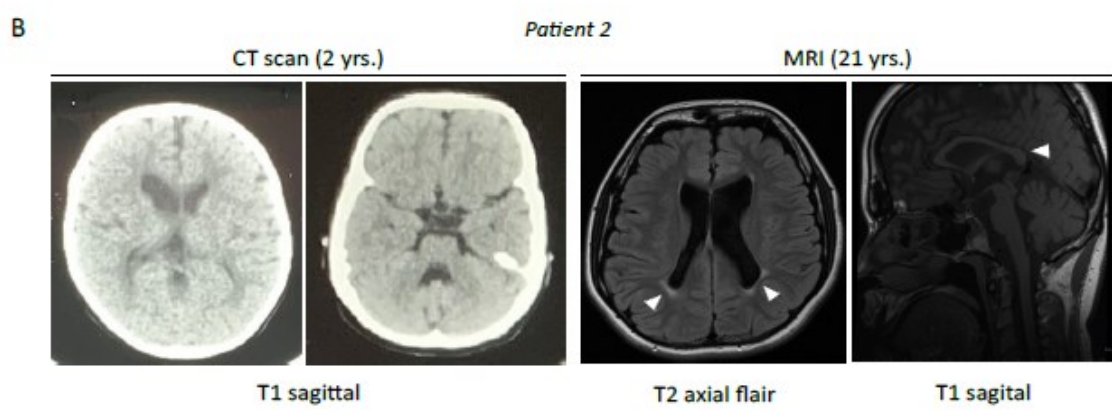
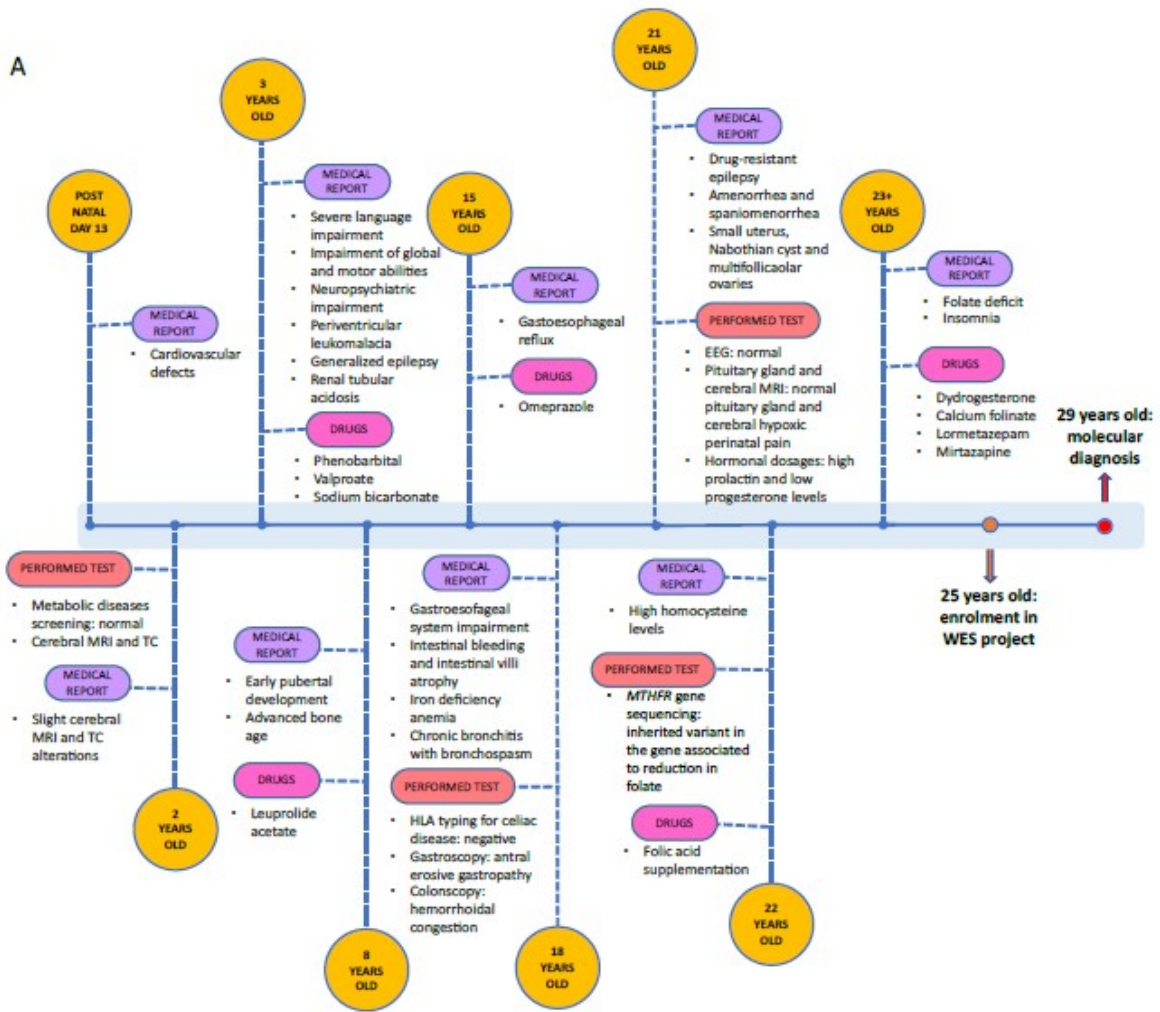
493 **Figure S1.** Pedigree and Sanger sequencing confirmation of variants

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

495 2 family and confirmation of the variant by Sanger sequencing.

496





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