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Supplemental Data

WDR26 Haploinsufficiency Causes a Recognizable Syndrome of Intellectual Disability, Seizures, Abnormal Gait, and Distinctive Facial Features

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Supplemental Note: Case Reports

Individual 1 (PPMD01P, GEA055P, USA)
Individual 1 was initially evaluated at 20 months of age due to concern for short stature and developmental delay. She was the product of a fraternal twin gestation conceived via in-vitro fertilization to a then 32-year-old G4P030 -> P2 mother. Mom had three prior first trimester miscarriages of unknown etiology. Family history is otherwise unremarkable. The pregnancy was uncomplicated and prenatal ultrasounds were normal. Prenatal screening was declined. She was born at 35 weeks gestation via spontaneous vaginal delivery. Birth weight was 4 lbs. 10 oz. (<10% for gestational age); twin brother birth weight 6 lbs. 2 oz. Her neonatal history was unremarkable. Her development has been delayed: she sat at 10 months, crawled at 15 months, and walked at 2.5 years. Language began to develop at 16-17 months, however, at 5 years she has only six words and some inconsistent signs. She has made slow developmental progress without regression. She exhibits some stereotypical behaviors including hand flapping and teeth grinding. She is playful and affectionate. Her medical history includes hypotonia, torticollis with positional plagiocephaly, strabismus, Marcus Gun jaw winking, recurrent otitis media s/p tympanostomy tubes, gastroesophageal reflux, and constipation. She developed febrile seizures at 17 months and subsequent EEG showed multifocal sharps and slow wave discharges. Brain MRI at 13 months showed a prominent sulcal pattern with dilation of the lateral ventricles and mild diminished white matter volume.

Growth parameters at 20 months showed head sparing failure to thrive: height <5% (50% for 10.5 months), weight 2% (50% for 9 months), and head circumference at 21%. At follow-up evaluation at 27 months of age, her growth parameters were similar: height <5% (50% for 16 months), weight 2% (50% for 12 months), and head circumference 12%. Growth parameters at 4 years were significant for height <5% (50% for 28 months) and weight <5% (50% for 23.5 months)(head circumference not obtained). Physical exam at that time was significant for a full dark hair, mildly coarse features, full cheeks, full brows with long lashes, large appearing irises, mildly depressed and slightly short nasal bridge, mildly anteverted nares, bulbous nasal tip, slightly long philtrum, widely spaced teeth, thin upper lip with decreased cupid's bow, prominent maxilla, mild micrognathia, mildly low tone, and mildly spastic gait.

Her genetic evaluation included normal female karyotype and normal chromosomal microarray (Quest-Affymetrix CytoScan HD 2.6M probes). She had negative sequencing and deletion/duplication analysis for the following genes: NIPBL, SMC3, RAD21, HDAC8, and SMC1A. Russell-Silver testing (11p15 methylation and UPD7 analysis) was normal and X-inactivation analysis was uninformative. Clinical whole exome sequencing identified a de novo heterozygous variant of unknown significance p.Glu426* (c.1276 G>T) in *WDR26*. No additional suspicious variants were reported.

Individual 2 (UNEZ01P, Netherlands)
Individual 2 is a 34-year old female at the time of evaluation. Her medical history is significant for severe mental retardation, spastic diplegia (left side most pronounced), febrile seizures at age 17 months, suspicion of seizures from age 7-8 years, dysarthric speech, constipation, sleep disturbances, double arch of teeth (primary and permanent dentition), exotropia/amblyopia, and vascular disturbances in the left foot (purple discoloration). Brain MRI showed enlarged lateral ventricles and thin corpus callosum. Her height is 10%, weight <3%, and head circumference 16%. Physical exam is

significant for coarse facial features, proptosis, full nasal tip, small nares, short philtrum, full lips, wide mouth with decreased cupid's bow, prominent maxilla, widely spaced teeth, and mild micrognathia. She had full cheeks, anteverted nares, and a short depressed nasal root in childhood. Clinical whole exome sequencing revealed a *de novo* variant p.His389Profs*6 (c.1161_1162del) in *WDR26*. In addition, *de novo* variants in *CDC7* c.1070A>G, p.Asp357Gly) and *NTNG2* (c.1426G>A, p.Ala476Thr) were noted, but were less suspicious, based on predictions and were subsequently felt less likely with the identification of additional individuals with *WDR26* pathogenic variants.

Individual 3 (OFPW01P, USA)
Individual 3 was 2.5 years at initial evaluation. She was the product of a full term, uncomplicated pregnancy. She was born via repeat C-section. Birth weight was 6 pounds and 4 ounces and birth length 18 inches. She was observed in the NICU for 3 days due to initial breathing issues, briefly requiring oxygen. Hypotonia was noted at a few weeks of age, eventually prompting evaluation by Neurology at 10 months of age. Previous evaluation included normal basic metabolic studies, normal karyotype, normal microarray analysis, and normal Prader-Willi/Angelman methylation studies. She had initial difficulty with solid foods, but now does well with a variety of foods. She developed prolonged febrile seizures at 2 yo and still has episodes of intermittent body stiffening. EEG immediately after the start of the seizures was abnormal, but more recent EEG was much improved. Brain MRI was normal. At 3 years 2 months she has no words used appropriately but can follow several step commands. She is always very happy and smiling. She started walking at 3 years of age and has a stiff gait. She has mild hypotonia and head nods when concentrating or tired. Growth parameters at 3 years 2 months of age were as follows: head circumference is 15%, her length is 29%, and her weight is 5%. Physical exam is significant for mildly coarse facial features with full cheeks, mild proptosis, large appearing irises, depressed nasal root, mild anteversion of nares, small nares, mild micrognathia, prominent maxilla, low set attached gingiva, and hypotonia. Clinical whole exome sequencing revealed a de novo variant p.Val486Glufs*9 (c.1457delT) in *WDR26.* No additional suspicious variants were reported.

Individual 4 (UNNV01P, Netherlands)
Individual 4 was a 2 year 1 month old female at the time of evaluation and is currently 6 yo. She developed febrile seizures at 11 months that continued until 5.5 years (3-4 episodes). She was later evaluated at 6 years for possible seizures, but does not carry a definitive diagnosis. Brain MRI and EEG were normal. Her medical history is also significant for congenital abducens paralysis requiring surgical intervention and mixed myopia and hyperopia. She has mild hypotonia and a mildly wide ataxic gait. She has significant developmental delay with first words at 24 months, sitting at 12 months, and walking at 2 years. She is described as happy and smiling. Growth parameters at 2 years 1 month were significant for height +0.5 SD, weight -1.5 SD, and head circumference -1 SD. Physical exam is significant for mildly coarse facies, full cheeks, proptosis, sparse lateral brows, full nasal tip, wide mouth with decreased cupid's bow, full lips, maxillary prominence, short philtrum, widely spaced teeth, gingival hyperplasia, mild prognathism, pes planus, sandal gap, and hypotonia. Whole exome sequencing revealed a de novo variant p.Leu215Pro (c.644T>C) in *WDR26.* No other suspicious variants were identified.

Individual 5 (IILW01P, USA)
Individual 5 was a 3 year 4 month old female at the time of evaluation. She was the product of a 37.5 week twin gestation. She had two episodes concerning for seizures at

2.5 years. EEG was normal. Brain MRI showed an arachnoid cyst, white matter volume loss, periventricular leukomalacia, and pineal cyst. She has hypotonia and a mildly wide gait. Her development has been delayed. She crawled at 18 months and walked between 2 and 2.5 years. Her first words were at 18 months and she has 4-5 words at 3 years 4 months. She has abnormal social interactions with some stereotypies on exam (clapping and hand flapping). Growth parameters at 3 years and 2 months were weight 3%, height just below 3%, and head circumference 10% for age. Physical exam is significant for large appearing irises, depressed nasal bridge, mild anteversion of the nares, full nasal tip, prominent smile, full cheeks, prominent maxilla, decreased cupid's bow, low set attached gingiva, pectus excavatum, and wide based gait. Williams syndrome and, to some extent, Angelman syndrome were considered at initial evaluation. Following normal chromosome microarray and Rett/Angelman syndrome disorders panel, clinical exome sequencing was pursued, which revealed a de novo variant p.Gln302Aspfs*22 (c.904_905delCA) in *WDR26.* A paternally heterozygous paternally inherited variant in *RNASEH2D* (c. 529G>A, p.Ala177Thr) was also noted but felt to be less consistent with clinical features and inheritance.

Individual 6 (PPMD02P, GEA131P, USA)
Individual 6 was a 24 month old female at the time of her last evaluation. She was the product of a full term, spontaneous gestation. Family history is significant for her biological mother having learning differences resulting in adoption by foster parents. Pregnancy was complicated by lack of prenatal care and multiple maternal drug exposures, requiring a 90 day NICU stay for withdrawal. There were concerns for seizures in the newborn period, requiring Phenobarbital. EEG was normal. Brain MRI showed a pineal cyst and FLAIR abnormality in the bilateral medial temporal lobes (artifact vs. delayed myelination). She has a history of elevated transaminases (ALT >1000) of unknown etiology that resolved spontaneously. Liver biopsy showed non-specific inflammation. Her medical history is also significant for a wandering left eye, hypotonia, sacral dimple, g-tube dependence, failure to thrive, and gastroesophageal reflux. She has had mild developmental delay. Early milestones are unclear, but she walked at 17 months. At 24 months she had about 15-20 words. She has some stereotypical behaviors including rocking and abnormal hand movements. She has made slow developmental gains without regression.

Growth parameters at her first genetics evaluation at our institution were significant for height 6%, weight 21%, and head circumference 4% for age. Growth parameters at her last visit (24 months of age) were significant for height 13%, weight 7%, and head circumference 8%. Physical exam from that time was significant for fuller cheeks, mildly upslanting palpebral fissures, large appearing irises, prominent nasal tip with mild anteversion of the nares, thin upper lip, shorter philtrum, maxillary prominence, mildly wide spaced teeth, wider mouth, decreased cupid's bow, sacral dimple, bilateral fifth finger clinodactyly, and a wide based and intermittently crouched gait.

Genetic evaluation included the following normal studies: genome wide SNP array, karyotype, chromosomal breakage studies, and multiple single gene analysis (*NIPBL, SMC1A, SMC3, RAD21, HDAC8, SRCAP*, and *EP300*). Exome sequencing revealed three variants of uncertain significance in the following genes: *ARID1B* (c.1016_1021dupTGGCGG; p.Val339_Ala340dup; maternally inherited), *ZMYND11* (c.470A>G; p.Glu157Gly; maternally inherited), and *WDR26* (c.850G>A; p.Asp284Asn; de novo). The variants in *ARID1B* and *ZMYND11* were felt to be less likely as causative

based on inheritance, pathogenic mechanism, poor conservation of residue and/or poor clinical correlation with previously reported phenotypes.

Individual 7 (SCLB01P, USA)
Individual 7 was a 4 year old male at the time of his last evaluation. He was the product of a 34 week gestation that was complicated by placenta previa, treated with bedrest starting at 22 weeks gestation, and diet-controlled gestational diabetes. He was delivered at 34 weeks due to maternal hemorrhage and spent 10 days in the NICU. Parental concern was first raised at 8 months of age due to poor weight gain. At a year of age he started developmental services due to delay. He sat at 11 months, army crawled at 14 months, 4-pt crawled at 16-17 months, and started walking at 22 months. His initial gait was wide-based and stiff-legged. At 4 years he has no words. Brain MRI showed a pineal cyst and area of signal intensity in cerebellum, ventricular size asymmetry with contour abnormality along the left lateral ventricle and associated mild white matter signal abnormality suggesting a prior insult, well-circumscribed focus non-enhancing T2 signal abnormality in the right cerebellar hemisphere, mild thinning of corpus callosum, mild vermian and left cerebellar hemisphere hypoplasia. He had a history of febrile seizures (one episode at 2 years and one at 3 years) and an episode of body stiffening concerning for a seizure at 3.5 years. EEG showed spike and wave discharges over the vertex and he was started on Depakote. Medical history is also significant for a right-sided aortic arch, eustachian tube dysfunction, and hypotonia. He has some abnormal behaviors (biting, hair pulling) and mimicking behaviors, but is typically friendly with new people.

Growth parameters at 4 years of age were significant for height 9%, weight 9%, and head circumference 1% for age. Physical exam is significant for slender build with little subcutaneous fat, mildly coarse features, midface retrusion, large appearing irises with inferior scleral reveal, abnormal lateral brows, depressed nasal root, prominent maxilla, widely spaced teeth, open mouth posture, full lips with decreased cupid's bow, fused and broad secondary alveolar ridges, low set attached gingiva, and bilateral fifth finger clinodactyly.

Genetic evaluation included a normal microarray, fragile X testing, *ATRX* analysis, and Angelman syndrome methylation testing. Whole exome sequencing (GeneDx) revealed three maternally inherited variant of uncertain significance in *CHD7* (c.5995 G>A, p.Ala1999Thr), *EFHC1* (c.520 A>G, p.Ile174Val), and *MBD5* (c.139T>G, p.Leu47Val) as well as a *de novo* variant in *WDR26* (c.137 C>A, p.Ser46*).

Individual 8 (DNCW01P, France)
Individual 8 is a 21 year old male. Pregnancy was complicated by IUGR (birth weight 2800g, length 47 cm, head circumference 33 cm). His medical history is significant for seizures, ventricular septal defect, failure to thrive, short stature, scoliosis, knee contractures, gastroesophageal reflux, left hemiparesis, inguinal hernia, cryptorchidism, gingival hypertrophy, conjunctival problems, chalazions, and delayed primary dental eruption. X-rays showed osteopathia striata. Brain MRI showed opercular dysplasia and pachygyria. His developmental milestones were delayed with sitting at 12 months and walking at 26 months. He had less than 10 words at 9 years of age. He has severe intellectual disability. Growth parameters at 19 years of age were globally small (weight -5SD, height -4SD, and head circumference <-3SD). Physical exam was significant for mild temporal narrowing, mildly coarse facies, abnormal lateral brows, short nasal bridge, mild anteversion of the nares, full nasal tip, prominent maxilla, full cheeks in

childhood, widely spaced teeth, gingival hyperplasia, open mouth posture, scoliosis, and knee contractures. Whole exome trio sequencing revealed de novo variants in two genes with a SnpEff impact of moderate or greater confirmed by Sanger sequencing. A missense variant *INTS12* c.887T>C (p.Phe296Ser), which had a nonsignificant ExAC missense constraint probability (p=0.312; z-score=0.49), and was considered an unlikely causal candidate. In contrast, a de novo nonsense variant was noted in *WDR26* (c.1570C>T, p.Gln524*), which had a pLI=0 and was considered a strong causal candidate.

Individual 9 (DDD273958, GSEK01P, GEA212P, UK)
Individual 9 is a 14 year old boy, the second child to healthy non-consanguineous Scottish parents. He was born at term by emergency section due to fetal distress with a birth weight of 4.33kg and there were no neonatal problems. He was described as difficult to feed with a poor suck. He had febrile convulsions until the age of 2 years. He has not had brain imaging. He was noted at the age of 3 years to have global developmental delay and marked speech delay. He has asthma and eczema but is in good health. He attends a special school and at the age of 14 years, speaks in short sentences but is difficult to understand. He is a happy, friendly boy who is sociable and well liked. He has some anxiety and hand clasping. He walks with a stiff gait and has tight achilles tendons and high arches of the feet and brisk knee and ankle reflexes. Growth parameters at 14 years were appropriate for age. Physical exam was significant for mildly coarse facial features, bright blue large appearing irises, sparse lateral brows, short nasal bridge with full tip, full cheeks, prominent maxilla with short philtrum, full lips, wide spaced teeth with low set attached gingiva, wide mouth with decreased cupid's bow, fifth finger clinodactyly, and pes cavus. Whole exome sequencing revealed a de novo variant in *WDR26* (c.762T>G; p.Ser254Arg).

Individual 10 (EUCT01P, DDD273377, UK)
Individual 10 is a 7 year old female, the second child of non-consanguineous parents. She was the product of a full term uncomplicated pregnancy; birth weight 2700g and head circumference 34 cm. She was noted to have a cleft soft palate on newborn exam. She had some early issues with poor feeding (recurrent aspiration pneumonia) and poor growth (requiring nasogastric and then long term PEG feeds). In early life she had puffy feet, constipation and recurrent otitis media. She developed tonic-clonic and absence seizures at the age of 3 years and was treated with valproate. EEG showed intermittent discrete spike/sharp wave discharges over the occipital area. Brain MRI showed left sided middle cranial fossa arachnoid cyst, relatively prominent cerebral ventricles and subarachnoid spaces, generalized thinning of the corpus callosum, and generalized lack of brain bulk. She has intellectual disability and developmental delay. Her first words were at 2 years and at age 7 she has around four words and also communicates using Makaton signs She sat at 11 months, bottom shuffled at 17 months, and walked at 24 months. She is described as being happy and affectionate. Growth parameters at 7 years were appropriate for age. Physical exam was significant for mildly coarse facies, brachycephaly, hypertelorism, large appearing irises with inferior reveal, upslanting palpebral fissures, sparse lateral brows, posteriorly rotated ears, mild anteversion of nares, full nasal tip, full lips with decreased cupid's bow, wide mouth, low set attached gingiva, decreased range of motion in hips and knees, inverted nipples, and hypertonic limbs with brisk reflexes. She walks with a wide-based gait. Stickler syndrome and Angelman syndromes were considered. Whole exome sequencing revealed a de novo variant in *WDR26* (c.1284G>A; p.Trp428*).

Individual 11 (OKAS01P; USA)
Individual 11 is an 8-year old female, the second child of non-consanguineous parents. The pregnancy was complicated by oligohydramnios and IUGR, prompting induction of labor at 35.5 weeks gestation. She had hypotonia, feeding difficulties, and failure to thrive in infancy and has since been found to have an excessive appetite and high metabolic demand. She was diagnosed with absence seizures at about 8 years. EEG showed generalized as well as multifocal epileptiform activities and is currently on Zonisamide and Keppra. Brain MRI showed a diminutive vs. hypoplastic corpus callosum. She has an abnormal gait, ADHD, and a tic disorder that is exacerbated by stimulants. Her medical history is also significant for chronic otitis media s/p tympanostomy tubes, gastroesophageal reflux, food allergies, and eczema. She has intellectual disability and developmental delay. She sat at 11 months, crawled at 14 months, and walked at 20 months. Her first words were at 13 months. She was putting two words together by 5 years and talking in short sentences at 8 years. She is in regular classes with a full time aid and has had great success with ABA therapy. She is described as happy and friendly, but anxious at times. Growth parameters at 8 years were appropriate for age. Physical exam revealed mildly coarse facial features, mild bitemporal narrowing, large appearing irises, mildly upslanting palpebral fissures, penciled brow, mild posteriorly rotated ears, full nasal tip, prominent maxilla, short philtrum, widely spaced teeth, full lips, wide mouth with decreased cupid's bow, low set attached gingiva, and pes cavus. Clinical whole exome sequencing (GeneDx) revealed a de novo variant in *WDR26* (c.1419+2dupT; splice site). No additional suspicious variants were reported.

Individual 12 (F47009, 51810, DGDW01P, Germany)
Individual 12 is the first child of healthy, non-consanguineous parents with an unremarkable family history. The pregnancy was complicated by pre-eclampsia. This girl was born at 38 weeks gestation with normal birth measurements [weight 2390 g (-1.9 SD), length: 47 cm (-1.5 SD) and OFC: 32 cm (-1.8 SD)]. At 10 months of age, the parents noted she was unable to crawl, and at 12 months of age, she had her first epileptic seizure. At that time, Down syndrome was excluded (karyotype: 46,XX). Her epilepsy has successfully been treated with valproate. Brain MRI did not reveal any anomalies. Her last examination was at age of 5.5 years demonstrated body measurements to be normal except for OCF, which showed microcephaly [height: 110 cm (-0.9 SD), weight: 15.8 kg (-1.8 SD), OFC: 47.5 cm (-2.9 SD)]. She is a very friendly and happy girl. She presented with upward slanting palpebral fissures, short nose with anteverted nostrils and a wide mouth with diastema. She was unable to speak a single word at 5.5 years and used some signs to express her wishes.
At the age of 22 months, the clinical diagnosis of Kleefstra syndrome was considered. A chromosome microarray and sequencing of the *EHMT1* gene revealed normal results. Subsequent exome sequencing identified two potential pathogenic variants: *WDR26* (novel *de novo* c.835C>T; p.(Arg279*)) and *TACC3* (novel *de novo* c.197C>T; p.(Thr66Met). The probability of LoF intolerance (pLi) for *TACC3* of 0.09 and the Z score for missense variants of -1.04. suggested that the *TACC3* variant was a less likely pathogenic candidate than the nonsense change in *WDR26*.

Individual 13 (IIJB01P, USA)
Individual 13 first presented for genetics evaluation at 4 years 7 months of age. He had previous diagnoses of gastroesophageal reflux, successfully treated as an infant, reactive airway disease, which has improved over time, and febrile seizures. He was

seen by orthopedics at 16 months for mild left hip dysplasia and motor delay. Soon after he began crawling and then pulling to a stand and cruising. Evaluation by a developmental pediatrician at 18 months was notable for no speech and subsequent audiology testing was normal. Neurology evaluation at 20 months noted limited walking (a few steps at a time), absent speech, and an otherwise normal neurologic exam. CPK and Fragile X testing were normal. Brain MRI at age 2-3 years was unremarkable. His dymorphology exam was notable for plagiocephaly with frontal bossing, hypertelorism with wavy palpebral fissures, low-set cupped and thickened ears, prominent nasal bridge, thin upper lip, and irregular right palmar creases. Diagnoses entertained included 22q11.2 deletion syndrome, Kabuki syndrome, and FG syndrome. He returned to genetics clinic for follow-up at 7 years 10 months of age. He continued to be nonverbal and was using an adaptive communication device. He has a history of about 20 febrile seizures, with many occurring in the previous year. Because of abnormal running, he was seen by orthopedics and diagnosed with moderate forefoot varus and accommodative shoes were prescribed. Exam was notable for hypertelorism, prominent ears, inverted nipples, and short fingers. No specific diagnoses were suspected. Chromosomal microarray was normal. Trio clinical exome sequencing identified a de novo frameshift variant in *WDR26* (c.574dupA); no other variants were reported.

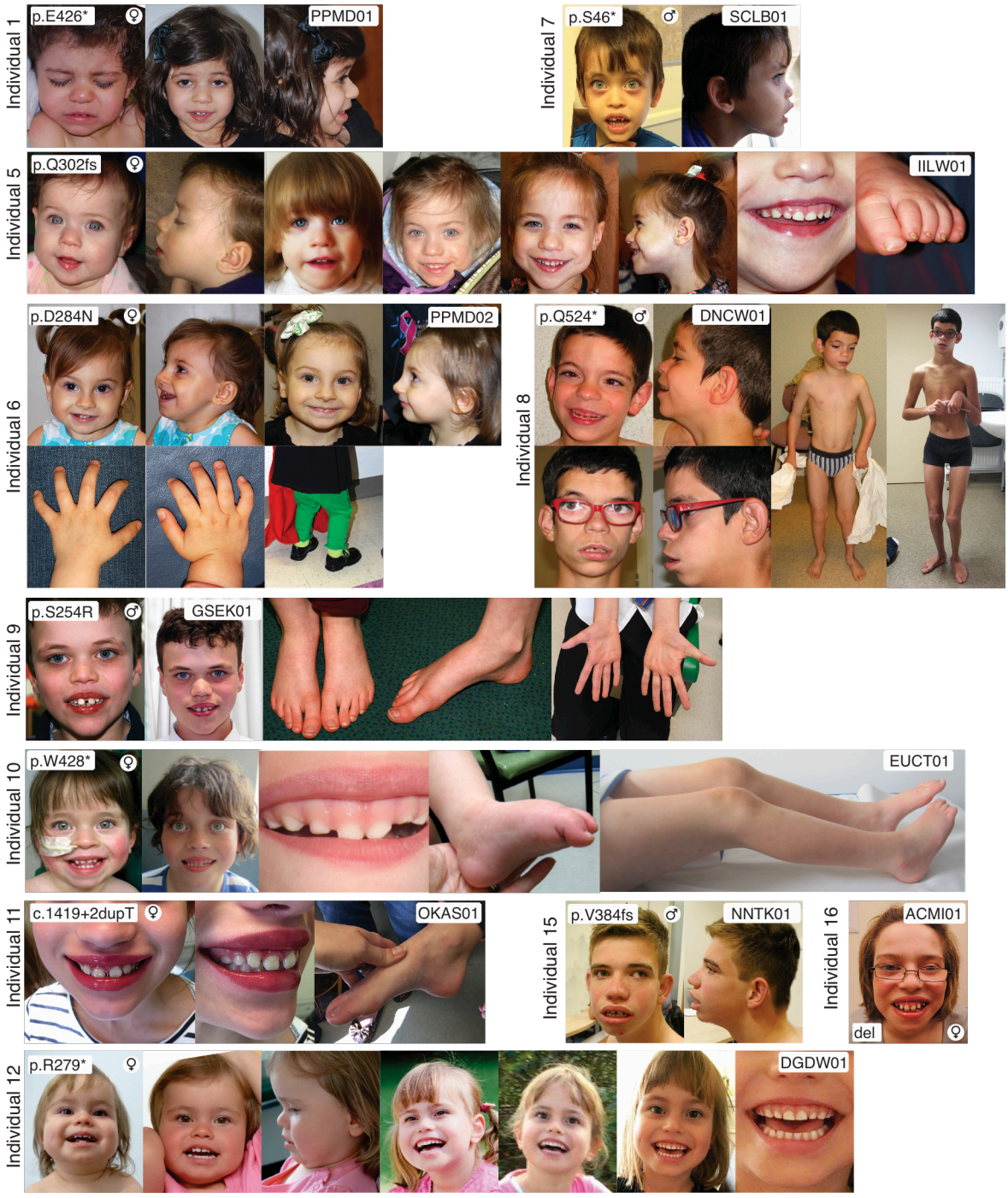

Figure S1. Detailed facial and clinical features of individuals with pathogenic *WDR26* variants. Each individual is noted with a number that corresponds to that used throughout the manuscript. Images are clustered for each individual. Included on the top left of each cluster is the variant identified and sex and an additional study identifier, also noted in Table S1 is noted in the top right. When available, frontal and facial profiles, varied ages, mouth, feet and stance are shown to display common findings.

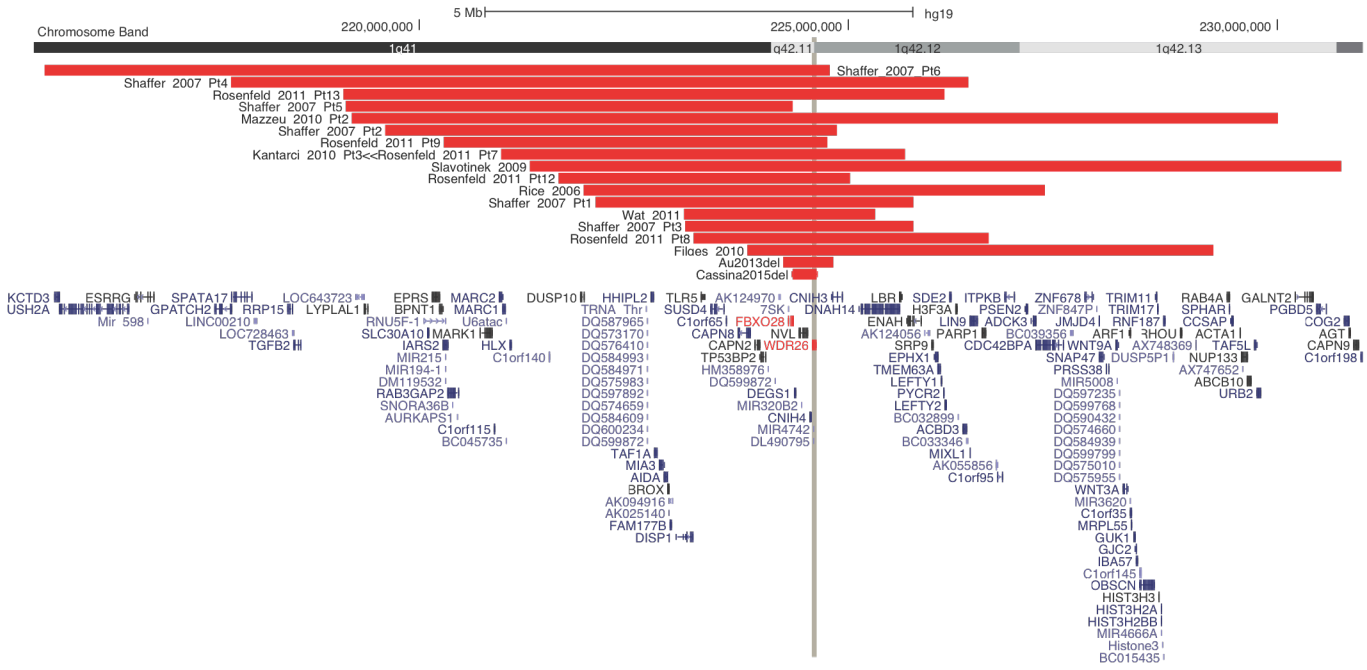

Figure S2. Chromosomal microdeletions involving the region 1q41q42.13. Demonstrated are microdeletions for which clinical and/or facial photographic information is available. Positions are based on hg19 coordinates (for detailed coordinates, see Table S1), with publication and subject identifiers noted. Genes in the region are noted beneath, with *FBX028* and *WDR26* in red to facilitate visualization. A grey bar spans the coordinates for *WDR26* across all deletions and genes to clarify locations of *WDR26* with respect to breakpoint ends. Of note, microdeletions for all reported subjects, with the exception of Patient 5 from Shaffer et al, 2007, include the *WDR26* gene, while this patient's breakpoint lies centromeric to *WDR26*.