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A new microdeletion syndrome involving TBC1D24, ATP6V0C and PDPK1 causes epilepsy, microcephaly and developmental delay.

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A new microdeletion syndrome involving *TBC1D24*, *ATP6V0C* and *PDPK1* causes epilepsy, microcephaly and developmental delay.

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Running title: *TBC1D24* microdeletions and seizures

Abstract

Purpose: Contiguous gene deletions are known to cause several neurodevelopmental syndromes, many of which are caused by recurrent events on chromosome 16. However, chromosomal microarray studies (CMA) still yield copy number variants (CNV) of unknown clinical significance. We sought to characterize eight individuals with overlapping 205 kb to 504 kb 16p13.3 microdeletions that are distinct from previously published deletion syndromes.

Methods: Clinical information on the patients and bioinformatic scores for the deleted genes were analyzed.

Results: All individuals in our cohort displayed developmental delay, intellectual disability and various forms of seizures. Six individuals were microcephalic and two had strabismus. The deletion was absent in all 13 parents who were available for testing. The area of overlap encompasses seven genes including *TBC1D24*, *ATP6V0C* and *PDPK1* (also known as *PDK1*). Bi-allelic *TBC1D24* mutations are known to cause nonsyndromic deafness, epileptic disorders, or DOORS syndrome (deafness, onychodystrophy, osteodystrophy, mental retardation, seizures). Sanger sequencing of the non-deleted *TBC1D24* allele did not yield any additional mutations.

Conclusion: We propose that 16p13.3 microdeletions resulting in simultaneous haploinsufficiencies of *TBC1D24*, *ATP6V0C* and *PDPK1* cause a novel rare contiguous gene deletion syndrome of microcephaly, developmental delay, intellectual disability and epilepsy.

Introduction

Chromosomal microarray (CMA) technology has facilitated the discovery of multiple new microdeletion syndromes previously invisible on conventional karyotypes. However, classification of small deletions as pathogenic can be challenging. Many genes are still poorly characterized and functional data are often unavailable. Therefore, collecting a group of individuals with phenotypic and cytogenetic data can aid in the interpretation of a copy number variant (CNV), especially for very rare variants.

Autosomal recessive mutations in *TBC1D24* (MIM613577) lead to epilepsy (familial infantile myoclonic epilepsy (FIME), MIM 605021; early-infantile epileptic encephalopathy 16 (EIEE16), MIM 615338), non-syndromic hearing loss (either recessive, DFNB86, MIM 614617, or dominant, DFNA65, MIM 616044) or DOORS syndrome (deafness, onychodystrophy, osteodystrophy, mental retardation, and seizures, MIM 220500). We noted that carriers of *TBC1D24* mutations may have a susceptibility to epilepsy notably in the mother of a patient with DOORS syndrome who carries a loss-of-function mutation [1], and this was eventually noted in other families (detailed in Banuelos et al. [2]). We thus sought to identify the phenotype associated with microdeletions of *TBC1D24* and surrounding genes. We here report on eight individuals with epilepsy and developmental delay who share overlapping microdeletions at 16p13.3 including *TBC1D24*, *ATP6V0C* and *PDPK1*.

Materials and Methods

Cytogenetic laboratories were contacted to identify individuals with microdeletions encompassing *TBC1D24*. Patients were identified in the cytogenetics laboratories of the institutions where Dr. Campeau was a faculty member (Baylor College of Medicine) and currently is (CHU Sainte-Justine), but also in other centers across the world. Ten individuals had eligible microdeletions and treating clinicians were then approached to recruit patients, provide clinical details and DNA samples. Eight individuals were enrolled in the study after informed consent was obtained (on consent forms approved by the Baylor College of Medicine and the CHU Sainte-Justine Internal Review Boards). *TBC1D24* Sanger sequencing was performed in all individuals except individual 6 (no DNA available) according to published protocols [1]. Heterozygous *TBC1D24* deletion was confirmed in individual 2 by real-time PCR on genomic DNA (data not shown).

Clinical information was collected with a standardized questionnaire. Given the clinical manifestations of DOORS syndrome, specific questions were included on dental anomalies, hearing deficits, dysmorphic facial features, and abnormalities of the hands, nails and feet. Physicians were asked to provide details on seizure disorders and brain imaging.

CNVs and deleted genes were visualized using the UCSC genome browser human assembly hg19 [3]. Haploinsufficiency scores (%HI) for the deleted genes were obtained from the DECIPHER database [4] (Supplementary Material). pLI scores were drawn from the ExAC database [5] (Supplementary Material). Modeling the probability of autosomal dominant inheritance P(AD) was done with the DOMINO tool [6] (Supplementary Material). PubMed, Google Scholar, and OMIM were used for the literature review until February 2018.

Clinical data on individuals (see also Supplementary Material)

Individual 1 was referred at 8 years for seizures, microcephaly and developmental delay. She is the only child from a non-consanguineous union. She was born at term after an uneventful pregnancy. She attends a mainstream school with one-to-one support. Her major difficulties are comprehension and mathematics. At 23 months, she presented with a cluster of generalized tonic clonic seizures that were treated with levetiracetam and sodium valproate. She has been seizure free on levetiracetam monotherapy for 5 years. At 5 years, an MRI was reported as normal. At 8 years, her height and weight were at the 9th percentile, while her head circumference (HC) measured 1.5 cm below the 0.4th percentile for age. She was not dysmorphic (Fig. 1, A, B).

Individual 2 came to the attention of a neurometabolic clinic at the age of 6 years. He was born at term to non-consanguineous parents. Early on, he was noted to have feeding difficulties, failure to thrive and microcephaly with increased tone. At 13 months, he presented with seizures including generalized tonic-clonic and atonic seizures and head drops. His early developmental milestones were met normally, but at 6 years, he was not yet toilet trained, his speech was limited to single words and he was able to follow simple verbal commands. He attended kindergarten in an inclusion classroom and received speech, physical, occupational, and applied behavior analysis therapy. His physical exam was remarkable for short stature ($<5^{th}$ percentile) and a HC at the 2^{nd} to 5^{th} percentile. There was no dysmorphism.

Individual 3 is the first of three brothers of non-consanguineous parents. He was born at term after a normal pregnancy. At birth, length, weight and HC were at the 25^{th} percentile. During

early childhood, he was found to have hyperacusis, hypotonia and developmental delays (sitting at 9 months, walking after 21 months of age). He attends a specialized classroom. At 30 months, he developed myoclonic astatic epilepsy and was subsequently hospitalized for epileptic encephalopathy. He was treated with valproic acid and lamotrigine. He has been seizure-free since the age of 5 years with normalization of EEG patterns resulting in the discontinuation of valproic acid. On physical exam at 8.5 years, he had microcephaly, prognathism, small teeth with only two permanent teeth, and tapering fingers.

Individual 4 is a 15-year-old male who was born at term to non-consanguineous parents. He had speech delay and significant learning difficulties. At 15 years, IQ testing (score 51-62) confirmed mild intellectual disability. He exhibits sexualized behavior and has a diagnosis of autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD). From 15 months, he had convulsions consisting of generalized tonic-clonic seizures that were initially associated with febrile illnesses. From 2 years, he was treated with valproic acid; later clobazam and sulthiame were added. At 5.3 years, his height and weight were above the 90^{th} percentile. He was microcephalic with a HC at the 2^{nd} percentile, but otherwise without dysmorphic features. A neurological exam was normal, including an EEG and an MRI of the brain.

Individual 5 is a 21-year-old male with intellectual disability. At 13 years, he scored below the 1^{st} percentile on the Wechsler Intelligence Scale for Children (WISC-IV). At 10 months, he was diagnosed with generalized tonic-clonic seizures, later he also had episodes of absence and myoclonic or atonic seizures. He has been seizure free for more than one year on a combination treatment of levetiracetam, rufinamide, and clonazapam. An MRI at 13 years revealed a small

tubular structure in the right frontal lobe that was interpreted as a normal venous variant. His HC measured at the 2nd percentile at 14 years, with height and weight at the 3rd percentile at 17 years. Mildly dysmorphic features included posteriorly rotated ears and a pointed chin (Fig. 1, C, D).

Individual 6 is a 39-year-old man with intellectual disability and significant emotional behavioral concerns with mania and bipolar episodes necessitating multiple psychiatric hospitalizations. From age 3 years, he had generalized tonic-clonic seizures that have been well controlled with the exception of break-through seizures at 14 and 25 years. He is treated with phenytoin, buspirone, lorazepam, clonazepam, lamotrigine, olanzapine, and zonisamide. He had corrective surgery for strabismus and multiple dental operations. A brain MRI at 31 years was significant for microcephaly with a thickening of the calvarium and minimal vermian atrophy, which may be secondary to chronic phenytoin use. On physical exam, he had normal height and weight, a tubular nose, and slightly enlarged testicles (Fig. 1, E, F).

Individual 7 was born after a normal pregnancy to healthy non-consanguineous parents. He was diagnosed with hearing loss, strabismus (Fig. 1, G, H), and nystagmus with normal vision at 2 years. At 5.5 years, his developmental status was estimated at about 2 years; formal testing was unsuccessful. He is treated for ADHD. Since the age of 13 months, he suffered from generalized tonic-clonic seizures that are moderately controlled with oxcarbazepine, levetiracetam and valproic acid. MRIs at 2.5 and 4.5 years demonstrated stable cerebral and cerebellar atrophy. At 5.5 years, he was of normal height and weight with a HC at the 2nd to 5th percentile.

Individual 8 was born at 32 weeks estimated gestational age via Caesarean section for non-reassuring fetal heart tracing. At birth, her height and weight measured at the 10th percentile, whereas head growth was preserved at the 50th percentile. At 6.5 years, she measured at the 10th percentile for height and weight with a HC below the 3rd percentile. Gross motor and language development is delayed and her IQ was measured at 58 with the Culture Fair Intelligence Test (CFT-R). She experienced her first febrile seizure at 18 months, followed by a cluster of febrile and afebrile tonic seizures at 20 months and 2.4 years. She experienced two more seizure clusters of myoclonic seizures lasting up to seven days requiring polytherapy of valproic acid, clobazam, and levetiracetam and has been seizure-free on this combination for 2 years. On physical exam, she has a high forehead, a long tubular nose with a broad nasal ridge and epicanthal folds.

Results

Clinical and cytogenetic data were available on eight individuals (Table 1, Supplementary Material). All eight suffered from childhood onset epilepsy, mostly generalized tonic-clonic seizures (six individuals). All eight individuals also have variable developmental delays ranging from mild to moderate and affecting speech, and fine and gross motor skills, with three being diagnosed with ADHD and one with ASD. Cranial MRI findings were normal for five individuals and non-specific in three. Interestingly, some features observed in this cohort, such as microcephaly (six individuals), hypotonia (two individuals), hearing loss (one individual) and visual impairment (two individuals), have been previously associated with biallelic *TBC1D24* mutations. Four individuals had mild dysmorphic features (Table 1, Fig. 1). The three Caucasian individuals for whom images are available (Fig. 1) share facial similarities such as a sloping forehead, a long tubular nose with a prominent columella and a prominent chin.

CMA identified overlapping microdeletions on the short arm of chromosome 16 (16p13.3; Fig. 2). There is no overlap with the 16p13.3[7 8] and 16p11.2 [9 10] deletion syndromes. The smallest deletion (individual 8) contains 13 genes and the largest (individual 3) 25 genes (Supplementary Table 1). Parental testing in six families determined the deletion to be a *de novo* event. For individual 1, her tested mother is not a carrier. In individual 3, the deletion was present in 83% of cells, suggesting a post-zygotic event. The deletions do not share a common break point and range in size from 205 kb to 504 kb with a minimally overlapping region (MOR) of 112 kb that includes seven genes (UCSC genome browser hg19) *TBC1D24* (MIM 613577), *ATP6V0C* (MIM 108745), *AMDHD2* (amidohydrolase domain containing 2), *CEMP1* (Cementum protein 1, MIM 611113), *MIR3168* (microRNA 3168), *PDPK1* (or *PDK1*, 3-phosphoinositide dependent protein kinase-1, MIM 605213), and *DQ577714* (piRNA38825).

We next looked at bioinformatic prediction scores. A %HI score of less than 10% is predictive of haploinsufficiency of a heterozygously deleted gene. A pLI score of ≥0.9 is indicative of intolerance to loss-of-function mutations and haploinsufficiency. A P(AD) of ≥0.95 is highly associated with autosomal dominant inheritance through haploinsufficiency, gain-of-function or dominant-negative effects. Of the genes within the MOR, *PDPK1* reaches the lowest %HI at 27% and the highest pLI score at 0.95. DOMINO predicts *PDPK1* to "very likely" cause autosomal dominant conditions with a P(DA) of 0.986. However, none of the genes in the MOR reach significant %HI scores of less than 10% (Table 2). Complete Sanger sequencing of the non-deleted *TBC1D24* allele did not detect any pathogenic mutations and therefore excludes an AR epilepsy phenotype in this cohort (data not shown).

Discussion

Several factors favor a causative link between microdeletions at 16p13.3 and the clinical manifestations in this group. The phenotype is very homogeneous with all individuals suffering from epilepsy and variable degrees of developmental delay. In addition, the majority is microcephalic and none have additional malformations or major medical problems. In all six for whom this data were available, the deletion occurred *de novo*. Furthermore, CNVs containing the MOR have not been identified in normal controls in several large-scale studies [11-13]. Only one additional case with a comparable deletion was found in a cohort of 29,085 cases with intellectual disability, developmental delay and/or ASD, but clinical information is not available (see supplemental table 7 in [12]). The microdeletion was absent in two additional cohorts, one of 5,531 cases that were sent to a diagnostic laboratory for clinical testing [14] and one including 1,133 children with severe developmental disorders [15].

Our results suggest that 16p13.3 microdeletions encompassing *TBC1D24*, *ATP6V0C* and *PRPK1* genes represent a novel contiguous gene deletion epileptic syndrome. *TBC1D24*, a known epilepsy gene, encodes a member of the Tre2-Bub2-Cdc16 (TBC) domain-containing RAB-specific GTPase-activating proteins. Analysis of the crystal structure of the drosophila orthologue Skywalker (Sky) identified a cationic pocket that is preserved in human TBC1D24. This pocket is necessary for binding to the lipid membrane via phosphoinositides phosphorylated at the 4 and 5 positions. Abrogation of the cationic pocket by introduction of two human *TBC1D24* pathogenic variants found in DOORS syndrome led to impaired synaptic vesicle trafficking and seizures in drosophila [16]. *TBC1D24* is the only gene in the MOR that is associated with autosomal dominant and recessive human disease phenotypes.

ATP6V0C (ATPase, H+ transporting, lysosomal 16kDa, V0 subunit C) is a component of vacuolar ATPase (V-ATPase), a multi-subunit enzyme that mediates acidification of eukaryotic intracellular organelles. It is present in endosomes, lysosomes, clathrin-coated vesicles and the Golgi complex, where it is essential to acidification and maintenance of endocytic and exocytic pathways [17]. Experiments in zebrafish embryos suggest a neuron-specific expression of the zebrafish ortholog atp6v0c2 where it is associated with presynaptic vesicles and involved in neurotransmitter storage [18].

PDPK1 (also known as PDK1) is a highly conserved protein kinase that is involved in many different signalling pathways (reviewed in [19]). Similar to TBC1D24, it is able to bind to phosphatidylinositol 3,4,5-trisphosphate or phosphatidylinositol 3,4-bisphosphate produced at the plasma membrane where it fulfills an important function in cell migration [20]. While homozygous *Pdpk1* knockout mice die on embryonic day E9.5 [21], mice with residual PDK1 activity (10-30%) are viable and fertile, albeit of a smaller size [22]. The reduced interaction of PDPK1 with phosphoisonitides leads to a decrease in PKB/mTORC1/BRSK signaling, decreased neuronal cell size *in vivo* and shorter cortical neuron length *in vitro* [23]. To date, evidence on direct interactions between the three main genes of interest has not been published.

Other genes in the MOR are less likely to play a causative role in the pathogenesis of this recurrent deletion. The enzyme AMDHD2 is involved in a degradation pathway that tightly regulates N-glycolylneuraminic acid (Neu5Gc) [24], a protein that is incorporated at low levels into the surface glycoproteins of several human tissues [25]. However, loss-of-function mutations of metabolic disorders are usually well tolerated in the carrier state. Cementum protein 1 (CEMP1) is a marker of cementoblast-related cells and plays a role in cementoblast differentiation in periodontal ligament. It is not expressed in brain [26]. Expression studies in

hepatocellular carcinoma (HCC) suggest a role of MIR-3178 as a tumor suppressor by inhibiting cell proliferation, angiogenesis, invasion, and migration of HCC tumor endothelial cells [27]. The potential role of MIR-3178 in other organ systems and during development has not yet been studied. For DQ577714, to date, no investigations detailing the function of its gene product have been published.

While individuals with recessive *TBC1D24* mutations have more severe phenotypes than our cohort, in some families with recessive epilepsy or DOORS syndrome, carriers or obligate carriers also suffered from a milder form of childhood epilepsy [1 2 28 29]. In the ExAC database, the number of expected loss-of-function (LoF) variants (n=10.7) corresponds to the number of observed LoF variants (n=10) for *TBC1D24*, which seems to contradict our suggestion that haploinsufficiency for *TBC1D24* may predispose to epilepsy. However, it is important to note that the incidence of epilepsy is relatively high in the general population (7 per 1000 [30]) and the ExAC dataset only excludes severe childhood-onset disorders. It is therefore possible that some *TBC1D24* heterozygous LoF or deleterious missense variants may lower the threshold for the development of mild forms of epilepsy in some families. In animal studies, *Tbc1d24* has been shown to be important for neuronal migration and cortical maturation by facilitating the transition of migrating neurons into a bipolar shape [31]. *PDPK1* is also involved in neuronal differentiation in mice [23]. The third candidate gene within the MOR, *ATP6V0C*, like *TBC1D24*, can regulate vesicular trafficking. While heterozygous *Atp6v0c* knockout mice are phenotypically normal [32], homozygous embryos develop only to the blastocyst stage and die shortly after implantation [33].

In recent years, several exome sequencing studies have been conducted in patient cohorts with severe epilepsy, developmental delays or both who often remained undiagnosed after a standard

genetic evaluation with CMA and targeted gene sequencing [15 34-39]. Different *de novo* frameshift variants in *ATP6V0C* were found in one individual in a study performing exome sequencing of 80 patients with Dravet syndrome [34] and in one individual from a cohort of 4,293 families undergoing exome sequencing for severe developmental delay [39]. Details on their phenotypes were not provided and the variants were not validated by functional assays. In the Dravet syndrome study, the authors conducted targeted sequencing of *ATP6V0C* in 67 additional families and did not identify other mutations. One proband in a cohort of 1,133 children with severe developmental delay was found to have a *de novo* missense variant in *PDPK1* by exome sequencing, but no phenotype information was provided (Table S2 in [15]). *De novo* variants in either gene were absent from other studies with cohort sizes ranging from 50 to 293 trios [35 37 38] and none of the above cited studies listed *de novo* variants in *TBC1D24*. Neither of the three genes emerged as a strong individual candidate gene for either severe epilepsy or developmental delay in these studies, however further large-scale cohort studies or functional assays are needed to explore the possible contribution of *PDPK1* and *ATP6V0C* LoF variants to developmental delay and epilepsy phenotypes.

In conclusion, while haploinsufficiency of *TBC1D24*, *ATP6V0C* or *PDPK1* may be tolerated individually (larger cohorts will be useful to provide a definitive answer), our results suggest that haploinsufficiency for a combination of these genes leads to developmental delay and epilepsy as observed in this cohort. Future studies are needed to further refine the MOR and elucidate the individual and cumulative effect of the genes implicated in this phenotype.

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Figure legends

Table 1

Clinical information and deletion size on eight individuals with overlapping microdeletions of chromosome 16p13.3. ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; DD, developmental delay; FFT, failure to thrive; ID, intellectual disability; Ind., individual; kb, kilobases.

Table 2

Bioinformatic prediction scores for seven genes. See text for explanation; NA, not available.

Fig. 1

Four individuals with microdeletion 16p13.3 and mild dysmorphic features: Individuals 1 (A, B), 5 (C, D), 6 (E, F), and 7 (G, H) from left to right; note the shared features in individuals 1, 5 and 6 as described in the text.

Fig. 2

Schematic of microdeletions observed in the cohort. The borders of the minimal overlapping region (MOR) are demarcated by dotted lines encompassing seven genes.

Montreal, June 5th 2018

CONFLICT OF INTEREST STATEMENT

The authors have nothing to disclose.

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Figure 1_color

Figure 2_color

Click here to access/download;Figure;201805 TBC1D24 microdeletions_figure 2.tiff

Figure 1_black and white

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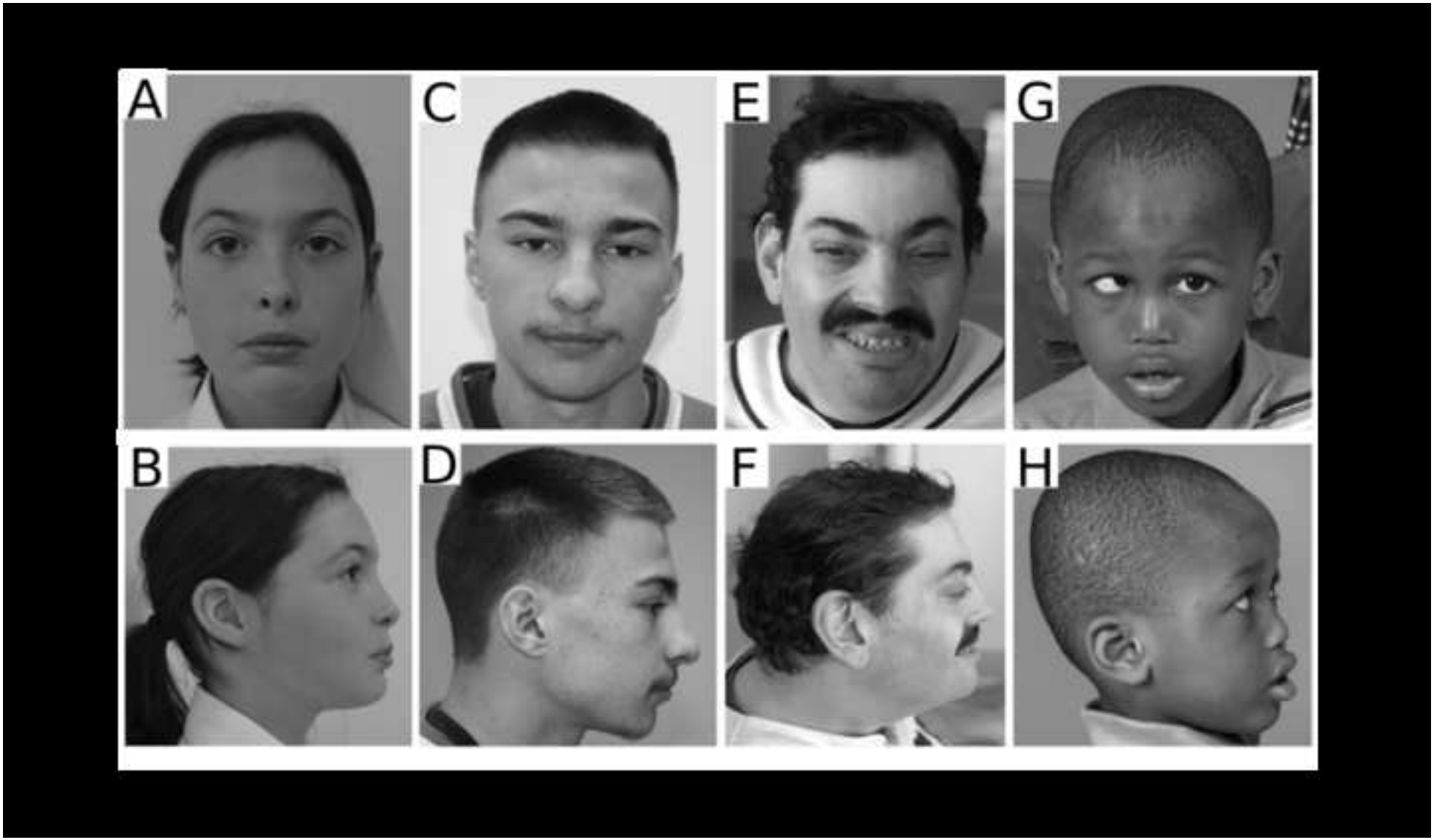

Figure 2_black and white

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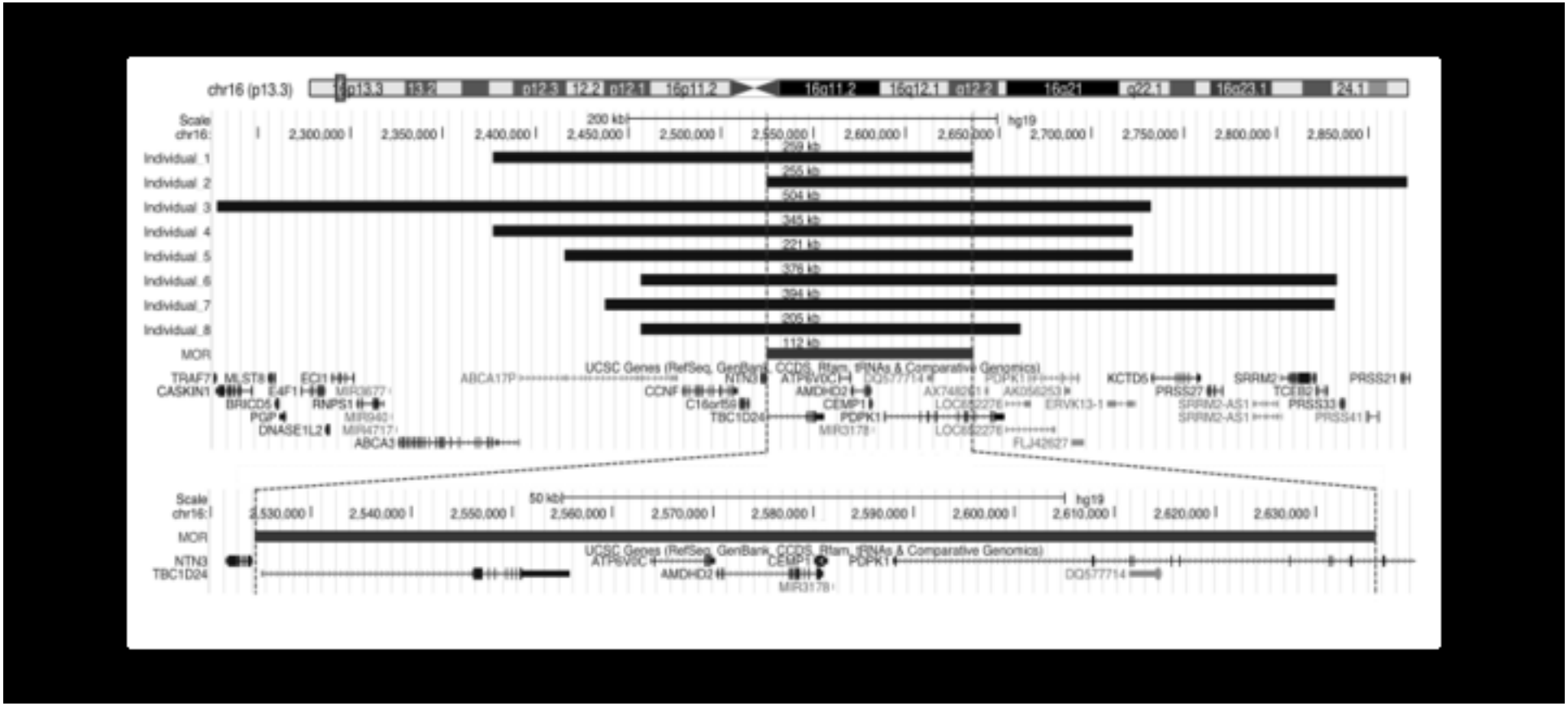

Table 1

Clinical information and deletion size on eight individuals with overlapping microdeletions of chromosome 16p13.3. ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; DD, developmental delay; FTT, failure to thrive; ID, intellectual disability; Ind., individual; kb, kilobases.

Table 2

Bioinformatic prediction scores for seven genes. See text for explanation; NA, not available.

Table 1: Clinical information and deletion size on eight individuals with overlapping microdeletions of chromosome 16p13.3

Ind.	Gender	Age	Development	Seizure disorder	Micro-cephaly	Additional features	Brain imaging	Deletion size [kb]
1	Female	13	DD	Generalized tonic-clonic	Yes, < 0.4th percentile	None	Normal	259
2	Male	6	DD, ADHD, insomnia	Generalized tonic-clonic, atonic	No	FTT, hypotonia, short stature	Normal	255
3	Male	8	DD	Myoclonic astatic	Yes, <3rd percentile	Tapering fingers, prognathism, hypotonia	Normal	504
4	Male	15.5	Mild ID (IQ 51-62), ADHD, ASD	Generalized tonic-clonic	Yes, 2nd percentile	None	Normal	345
5	Male	17	ID (<1st %ile on WISC-IV)	Generalized tonic-clonic, myoclonic, atonic, absence	No	Pointed chin, posteriorly rotated ears, short stature	Small stable venous anomaly	221
6	Male	39	ID, bipolar disorder	Generalized tonic clonic	Yes	Strabismus, vision loss, tubular nose	Thickening of calvarium	376
7	Male	5.5	DD, ADHD	Generalized tonic-clonic	Yes, 2nd - 5th percentile	Hearing loss, strabismus, nystagmus	Cerebral & cerebellar atrophy	394
8	Female	6.5	DD (IQ 58)	Tonic, myoclonic	Yes, <3rd percentile	Beaked nose	Normal	205

Table 2: Bioinformatic prediction scores for seven genes.

Gene	%HI (DECIPHER)	pLI score (EXAC)	P(AD) (DOMINO)
TBC1D24	55.45	0	0.337 (likely recessive)
ATP6V0C	51.76	0.73	0.29 (likely recessive)
AMDHD2	64.16	0	0.063 (likely recessive)
CEMP1	99.15	0	0.109 (likely recessive)
MIR3178	NA	NA	NA
PDPK1	27.04	**0.95**	**0.986** (very likely dominant)
DQ577714	NA	NA	NA

Supplementary material

A new microdeletion syndrome involving *TBC1D24, ATP6V0C* and *PDPK1* causes epilepsy and developmental delay

Contains:

1. Methods: Scientific basis for the used bioinformatics tools
2. Detailed clinical information on individuals
3. Supplementary table 1: Bioinformatic prediction scores for all deleted genes
4. Supplementary information on gene function for *TBC1D24*, *ATP6V0C* and *PDPK1*
5. Bibliography for supplementary material

Methods: Scientific basis for the used bioinformatics tools

The haploinsufficiency score %HI is calculated based on a computational algorithm developed by Huang *et al*. (2010). The group compiled a list of human genes that cause disease by haploinsufficiency (HI) and compared them to a group of genes with tolerated loss-of-function copy number variants (CNVs) in two or more individuals from a cohort of healthy controls (haplosufficient = HS genes). The HI genes were found to differ from the HS genes in the degree of conservation between the coding sequence of human and macaque genes, the number of promoter variants, the presence of paralogs with lower sequence similarity, the length of the spliced transcript and 3' UTR, the expression pattern during early development and in specific tissues, the number of interaction partners in both protein-protein interaction networks and gene interaction networks, and their interaction with other known HI genes and cancer genes. From these variables, the group developed a model using the degree of human-macaque conservation, promoter

conservation, embryonic expression and interaction with known HI genes to calculate the %HI where a low percentage number (e.g. 0-10%) indicates that a gene is more likely to exhibit haploinsufficiency, whereas a high value indicates that a gene is more likely to tolerate a loss-of-function variant or deletion. In validation sets composed from known human and mouse HI genes for which the information used for the algorithm was available, calculation of %HI correctly predicted 22.2% (87 of 392) and 24.5% of HI genes. In the group of human recessive genes, 39 of 606 genes (~6.4%) were predicted as being haploinsufficient.

The pLI score is based on exome sequencing data of more than 60k individuals generated by the Exome Aggregation Consortium (ExAC) (Lek *et al*., 2016). By comparing the expected number of missense and nonsense variants based on a selection neutral, sequence-context based mutational model to the observed variants in any given gene, the group calculated a Z score named probability of being loss-of-function (LoF) intolerant (pLI) score. The pLI score allows classifying genes in one of three groups: if the observed number of variants equals the number of expected variants, the pLI score equals zero and the gene is likely tolerant to LoF variants. High pLI scores of 0.9 or greater indicate intolerance to LoF variants, whereas recessive genes score at 0.5 or lower. When analyzing pLI scores of known disease genes, the correlation is highest with HI genes causing severe disease phenotypes.

The DOMINO tool was developed in 2017 to calculate the probability P(AD) that any given gene is associated with an autosomal dominant (AD) phenotype irrespective of the type of variant found (Quinodoz *et al.*, 2017). A machine learning approach was used to develop the algorithm that considers eight weighted measures including the number of

interactions with known AD genes from different training sets compiled by the group, from ExAC, the probability to be intolerant to homozygous loss-of-function variants, the missense Z score and the ratio between the number of donor site variants and synonymous variants present, the average PhyloP score for mammals across the transcriptional start site, and a high mRNA half-life (> 10 hr) in mouse embryonic stem cells. The algorithm was then validated on 26 AD genes not included in the training set and was found to correctly identify genes with an AD phenotype with 88.5% specificity and 78.1% sensitivity. No information was given on the rate of false positive attribution of autosomal recessive genes as being associated with an AD phenotype.

Detailed clinical information on individuals

Individual 1 was referred at the age of 8 years for seizures, microcephaly and developmental delay. She is the only child from a non-consanguineous union. She was born at term after an uneventful pregnancy. She started walking at 13 months and her development was normal until 2 years of age. Her development has not been formally evaluated, but she attends a mainstream school with one-to-one support. Her major difficulties are comprehension and mathematics and she needs some support with activities of daily life. At the age of 23 months, she presented with a cluster of generalized tonic clonic seizures that were treated with levetiracetam and sodium valproate. She has been seizure free on levetiracetam monotherapy for 5 years. Suspicion of mild hypoplasia of the corpus callosum were raised on an initial MRI scan at age 2 years. However a repeat scan at age 5 years was reported to be normal. At 8 years of age, her height (119.6 cm) and weight (19.9 kg) were at the 9th to 25th percentile, and 2nd to

9th percentile, respectively, while her head circumference measured 1.5cm <0.4th percentile for age (48 cm). She was not dysmorphic (Fig. 1, A, B).

Individual 2 came to the attention of a neurometabolic clinic at the age of 6 years. He was born at term without complications to non-consanguineous parents as the second of three children. Early on, he was noted to have feeding difficulties, failure to thrive and microcephaly with increased tone. At 13 months of age, he presented with seizures including generalized tonic-clonic and atonic seizures and head drops. His early developmental milestones were met normally, but at 6 years, he was not yet toilet trained, his speech was limited to single words and he was able to follow simple verbal commands. He attended kindergarten in an inclusion classroom and received speech, physical, occupational and applied behavior analysis therapy. The formal developmental assessment is not available. Clinical evaluation included a muscle biopsy at 3 yrs of age that demonstrated no abnormalities. Electron transport chain analysis showed decreased function of Complex I to < 5% of the control sample. mtDNA quantification and sequencing was normal. Sequencing of UBE3A, CDKL5 and Complex I nDNA including NDUF V1, A7, S3, A1, AF4, AF2, S5, S4, S7, S6, and S8 did not yield any pathogenic variants. Urinary amino acids and organic acids, guanidinoacetate, acylcarnitine profile and coenzyme Q 10 levels were normal. At 6 yrs, his physical exam was remarkable for dysarthria, muscle hypotonia, stereotypic movements (rocking and hand flapping), short stature (106 cm, $<5^{th}$ percentile) and a head circumference at the 2^{nd} to 5^{th} percentile (47.6 cm). He was exclusively toe walking and had a lordotic stance. There were no dysmorphic features.

Individual 3 is the first of three brothers of non-consanguineous parents. He was born at term after a normal pregnancy. At birth, length, weight and head circumference were at the 25th percentile. During the first few months of life, he cried frequently, particularly in response to loud noises. He was found to have hyperacusis, hypotonia and developmental delays (sitting at 9 months, walking after 21 months of age). He attends a specialized classroom and has poor handwriting. At 30 months of age, he developed myoclonic astatic epilepsy and was subsequently hospitalized for epileptic encephalopathy and microcephaly. An extensive work-up including an MRI of the brain, *ARX* and *PQBP1* sequencing, determination of thyroid hormones and a basic metabolic panel yielded normal results. He was treated with valproic acid and lamotrigine. He has been seizure-free since the age of 5 years with normalization of EEG patterns resulting in the discontinuation of the valproic acid treatment. On physical exam at the age of 8½ years, his height was 126 cm (25th percentile) and his head circumference 48 cm (<3rd percentile). He had prognathism, small teeth with only two permanent teeth and tapering fingers.

Individual 4 is a 15-year-old individual who was born at term to non-consanguineous parents. He walked at 14 months, but had speech delay using complete sentences only at the age of 3 years. At school, he experienced significant learning difficulties associated with poor concentration qualifying for a diagnosis of ADHD. At 15 years of age, he was tested with the Wechsler Intelligence Scale for Children (WISC-V) and found to have a mild intellectual disability with an IQ of 51-62 (verbal comprehension index 57-73,

visual spatial index 59-75, fluid reasoning index 56-71, working memory index 64-78, processing speed index 59-78). He attends a special needs class. He exhibits sexualized behavior and has a diagnosis of autism spectrum disorder (ASD) based on increased sensitivity to sensory stimulation, behavioral rigidity, encyclopedic knowledge of football and an inability to read other person's emotions. From the age of 15 months, he had convulsions consisting of generalized tonic-clonic seizures that were initially associated with febrile illnesses. From age 2, he was treated with valproic acid; later clobazam and sulthiame were added. At 5 years and 4 months his height (125 cm) and weight (24.5 kg) were above the 98th percentile and at the 90th percentile respectively. He was microcephalic with a head circumference at the 2nd percentile (49 cm), but otherwise without dysmorphic features. By age 10 years, growth velocity and weight development had diminished and he measured between the 75th and 90th percentile for height (181 cm) the 25th to 50th percentile for weight (62.4 kg), and the 2nd percentile for head circumference (53 cm) at last follow-up at 16 years of age. A neurological exam was normal, including an EEG and an MRI of the brain. A multigene panel for 343 genes associated with epilepsy confirmed the heterozygous deletion of *TBC1D24* and also resulted in two variants of unknown significance, one each in *CLCN2* and *GRIN1*, that were both inherited from his unaffected mother.

Individual 5 is a 17-year-old male with intellectual disability. At 13 years of age, he scored below the 1st percentile on the WISC-IV. At age 10 months, he was diagnosed with generalized tonic-clonic seizures, later he also had episodes of absence and myoclonic or atonic seizures. He has been seizure free for more than one year on a

combination treatment of levetiracetam, rufinamide, and clonazapam. An MRI at 13 years confirmed a previously identified, stable hypointense tubular structure extending from the right frontal cortex to the anterior portion of the body of the right lateral ventricle consistent with a developmental venous anomaly. A small area with a cystic appearance involving the pineal gland consistent with a small pineal cyst was unchanged in size compared to the prior MRI study at 6 years of age. His head circumference (51.5 cm) measured at the 2nd percentile at 14 years, and his weight (51 kg) at the 3rd percentile with a height (159 cm) below the 3rd percentile at 17 years. Mildly dysmorphic features included posteriorly rotated ears and a pointed chin (Fig. 1, C, D).

Individual 6 is a 39-year-old man with intellectual disability, history of seizures, and significant emotional behavioral concerns with intermittent aggressive behavior, and manic and bipolar episodes necessitating multiple psychiatric hospitalizations. He was born at term after an uncomplicated pregnancy. His early development was delayed as he began crawling at 11 months and walking at 17 months. He started talking late, although no details are available. Since the age of 3 years, he had generalized tonic-clonic seizures that have been overall well controlled with the exception of break-through seizures at age 14 and 25. He is treated with phenytoin, buspirone, lorazepam, clonazepam, lamotrigine, olanzapine, and zonisamide. He had corrective surgery for strabismus and multiple dental operations. He has a non-specified vision loss requiring corrective lenses. A CT of the abdomen and pelvis with contrast at the age of 38 years was normal. A brain MRI without contrast at the age of 31 years was significant for microcephaly with a thickening of the calvarium that was disproportionately greater in the frontal bone near the base of

the skull. The metopic suture bony margins were still visualized. Minimal vermian atrophy was noted. These changes were attributed to chronic phenytoin use and remained stable compared to CTs at the ages of 34 and 38 years. Fragile X testing was done for slightly enlarged testicles and was normal. On physical exam by B.S., he had normal height (171 cm) and weight (66.7 kg), proptotic eyes, a tubular nose, and slightly enlarged testicles (Fig. 1, E, F).

Individual 7 was born after a normal pregnancy as the third of four sons to healthy non-consanguineous parents from Ivory Coast. At 5 ½ years of age, his developmental status was estimated at about 2 years; formal testing was attempted, but unsuccessful due to lack of cooperation. He is treated with amphetamine/dexamphetamine for ADHD. Since the age of 13 months, he suffered from generalized tonic-clonic seizures that are moderately controlled with oxcarbazepine, levetiracetam and valproic acid. Two MRIs at 2½ and 4½ years demonstrated stable cerebral and cerebellar atrophy. At age 2 years, he was found to have hearing loss and nystagmus with normal vision. On physical exam, he was non-dysmorphic (Fig. 1, G, H) with a head circumference (48.9 cm) at the 2^{nd} to 5^{th} percentile, height (106 cm) at the 10^{th} percentile and weight (18 kg) at the 25^{th} percentile. Plasma amino acids and urine organic acids were normal.

Individual 8 was born at 32 weeks estimated gestational age (EGA) via Caesarean section for non-reassuring fetal heart tracing. At birth, her height and weight measured at the 10^{th} percentile, whereas head growth was preserved at the 50^{th} percentile. Her height (114 cm) and weight (17.5 kg) remained around the 10^{th} percentile until her last follow-up at 6.5

years. Head growth decelerated with the head circumference below the 3rd percentile (48 cm) at 6.5 years. Gross motor and language development was delayed. Her IQ was measured at 58 with the Culture Fair Intelligence Test (CFT-R), but at 7.5 years, she is attending first grade in a regular classroom with one-on-one support. She experienced her first febrile seizure at 18 months, followed by a cluster of febrile and afebrile tonic seizures at 20 months of age. After a second cluster of mostly myoclonic seizures at age 2.4 years, valproic acid treatment was initiated and continued for two seizure-free years. She experienced two more seizure clusters of myoclonic seizures lasting up to seven days requiring polytherapy of valproic acid, clobazam, and levetiracetam and has been seizure-free on this combination for 2 years. On physical exam, she has a high forehead, a long tubular nose with a broad nasal ridge and epicanthal folds.

Supplementary table 1

Bioinformatic prediction scores for all deleted genes. Bars under individual's column signifies deletion, significant values (haploinsufficiency score %HI <10%, pLI >0.9, P(DA) > 0.95) in bold; genes in the MOR are underlined; NA, not available.

UCSC gene	USCS hg19 position chr16:2227976-2832412	%HI DECIPHER	pLI score ExAC	P(DA) DOMINO
CASKIN1	chr16:2,227,184-2,246,465	69.64	1	0.544
MLST8	chr16:2,255,178-2,258,736	28.26	0.63	0.724
BRICD5	chr16:2,259,254-2,261,069	86.33	0	0.094
PGP	chr16:2,261,603-2,264,822	49.01	0.76	0.307
E4F1	chr16:2,273,567-2,285,743	42.22	0.43	0.266
DNASE1L2	chr16:2,286,424-2,288,712	74.92	0	0.066
ECI1	chr16:2,289,873-2,301,602	59.31	0	0.114
RNPS1	chr16:2,303,100-2,318,413	16.82	**0.95**	0.789
MIR3677	chr16:2,320,714-2,320,773	NA	NA	NA
MIR940	chr16:2,321,748-2,321,841	NA	NA	NA
MIR4717	chr16:2,324,621-2,324,692	NA	NA	NA
ABCA3	chr16:2,325,879-2,390,747	64.63	0	0.073
ABCA17P	chr16:2,390,923-2,476,700	NA	NA	NA
CCNF	chr16:2,479,395-2,508,859	48.91	0.02	0.313
C16orf59	chr16:2,511,122-2,514,293	87.27	0	0.149
NTN3	chr16:2,521,703-2,524,106	75.14	0	0.218
TBC1D24	chr16:2,525,147-2,555,734	55.45	0	0.337
ATP6V0C	chr16:2,563,871-2,570,224	51.76	0.73	0.29
AMDHD2	chr16:2,570,363-2,580,955	64.16	0	0.063
CEMP1	chr16:2,580,036-2,581,409	99.15	0	0.109
MIR3178	chr16:2,581,923-2,582,006	NA	NA	NA
PDPK1	chr16:2,587,965-2,653,191	27.04	**0.95**	**0.986**
DQ577714	chr16:2,611,468-2,614,643	NA	NA	NA
AX748261	chr16:2,642,515-2,644,557	NA	NA	NA
LOC652276	chr16:2,653,385-2,680,495	NA	NA	NA
AK056253	chr16:2,685,551-2,688,726	NA	NA	NA
FLJ42627	chr16:2,688,983-2,696,130	NA	NA	NA
ERVK13-1	chr16:2,708,390-2,723,440	NA	NA	NA
KCTD5	chr16:2,732,495-2,759,031	57.45	0.54	0.372
PRSS27	chr16:2,763,073-2,770,552	72.79	0	0.073
SRRM2-AS1	chr16:2,787,077-2,802,601	NA	NA	NA
SRRM2	chr16:2,802,627-2,818,262	19.08	NA	0.948
TCEB2	chr16:2,821,415-2,827,297	47.67	0.02	0.209
PRSS33	chr16:2,833,954-2,836,708	79.37	0	0.109
PRSS41	chr16:2,848,486-2,855,133	NA	NA	NA

Supplementary information on gene function

Gene orthologues and phenotype (in brackets) in select species

Sources:

FlyBase (FlyBase.org)
WormBase Version WS262 (http://www.wormbase.org/#012-34-5)
Mouse Genome Informatics (http://www.informatics.jax.org/)
The Zebrafish Information Network (ZFIN.org)

Gene	Drosophila melanogaster	Danio rerio	Caenorhabditis elegans	Mus musculus
TBC1D24	skywalker (see summary)	*tbc1d24* (no information)	*tbc-7*/*C31H2.1* (no information)	*Tbc1d24* (no information)
ATP6V0C	*Vha16-1* (**knockout lethal)**	*atp6v0ca* *atp6c0cb* (see summary)	*vha-1* *vha-2/3* (no information)	*Atp6v0c* (see summary)
AMDHD2	*Dmel\CG17065* (knockout viable)	*amdhd2* (no information)	*F59B2.3* (no information)	*Amdhd2* (no information)
CEMP1	no orthologue identified with protein-protein BLAST			
PDPK1	*pdk1* (see summary)	*pdpk1a* *pdpk1b* (no information)	*pdk-1* (see summary)	*Pdpk1* (see summary)

Additional information on the gene function for *TBC1D24*, *ATP6V0C* and *PDPK1*

TBC1D24 encodes a member of the Tre2-Bub2-Cdc16 (TBC) domain-containing RAB-specific GTPase-activating proteins. It has been shown to negatively regulate small GTPases such as ARF6 and RAB35, which orchestrate vesicular trafficking (Falace *et al.*, 2010). In rat brain, TBC1D24 was shown to be important for neuronal migration and maturation (Falace *et al.*, 2014). Analysis of the crystal structure of the drosophila orthologue Skywalker (Sky) identified a cationic pocket that is preserved in human TBC1D24. This pocket is necessary for binding to the lipid membrane via phosphoinositides phosphorylated at the 4 and 5 positions. Abrogation of the cationic pocket by introduction of two human *TBC1D24* pathogenic variants, at positions Arg40

and Arg242, found in DOORS syndrome led to impaired synaptic vesicle trafficking and seizures in drosophila (Fischer *et al.*, 2016) whereas homozygous loss-of-function variants are embryonic lethal (Uytterhoeven *et al.*, 2011).

ATP6V0C (ATPase, H+ transporting, lysosomal 16kDa, V0 subunit C) is a component of vacuolar ATPase (V-ATPase), a multi-subunit enzyme that mediates acidification of eukaryotic intracellular organelles. It is present in endosomes, lysosomes, clathrin-coated vesicles and the Golgi complex, where it is essential to acidification and maintenance of endocytic and exocytic pathways (Mangieri *et al.*, 2014). While heterozygous knockout mice are phenotypically normal (Inoue *et al.*, 1999), homozygous embryos develop only to the blastocyst stage and die shortly after implantation (Sun-Wada *et al.*, 1999). In drosophila larvae, the only *ATP6V0C* orthologue *Vha-1* is upregulated in the sensory organ precursor (SOP), which later develops into the mechano-sensory organ, indicating that *Vha-1* may play a role in proneural patterning (Tognon *et al.*, 2016). Two zebrafish orthologues, atp6v0ca and atp6v0cb, share important protein homology to human ATP6V0C protein of 90% and 93%, respectively (NCBI, https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins). Zebrafish atp6v0ca plays an important role during the development of the eye and melanophores (Nuckels *et al.*, 2009), as well as for the maintenance of the notochord (Ellis *et al.*, 2013). Loss of atp6v0ca function leads to embryonal lethality (Nuckels *et al.*, 2009). In contrast, atp6v0cb (also known as atp6v0c2) is specifically expressed in mature, post-mitotic neurons and associated with presynaptic vesicles. Morpholino knockdown experiments of atp6v0cb did not affect neurogenesis, but instead suggested a role in neuronal excitability

and neurotransmitter storage (Chung *et al.*, 2010). In humans, recessive mutations in V-ATPase subunits *ATP6V1E1, ATP6V1A, ATP6V0A2* cause cutis laxa, recessive mutations in *ATP6V1B1* and *ATP6V0A4* cause renal tubular acidosis, and recessive mutations in *ATP6V0A3* cause osteopetrosis (see OMIM for details). X-linked recessive mutations in *ATP6AP2* cause intellectual disability or parkinsonism, and X-linked recessive mutations in the assembly chaperone *VMA21* cause a myopathy. Finally, interestingly, dominant mutations in *ATP6V1B2* or *ATP6V1A* cause epileptic syndromes.

PDPK1 (also known as PDK1) is a highly conserved protein kinase that serves as a key regulator in many signaling pathways that control cell responses to chemotaxis, cell migration and invasion (reviewed in Gagliardi *et al.*, 2015). As TBC1D24, PDPK1 is able to bind to phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P3) or phosphatidylinositol 3,4-bisphosphate (PtdIns(3,4)P2) produced at the plasma membrane where it binds and phosphorylates other protein kinases (Gagliardi *et al.*, 2015). The down-stream effectors vary depending on the cell type. In endothelial cells for example, PDK1 promotes the disassembly of focal adhesions by modulating integrin endocytosis, an important function in cell migration (di Blasio *et al.*, 2015). In *C. elegans*, *pdk1* is part of the insulin/insulin-like growth factor signaling (IIS) cascade, which is essential for *C. elegans* development, learning and reproduction (reviewed in Murphy and Hu, 2013). Pdk1 is widely expressed in head and tail neurons, pharynx and intestinal cells (Paradis et al., 1999). Loss-of-function mutant nematodes are viable, and exhibit a dauer constitutive phenotype and increased life span (Paradis et al., 1999). Homozygous loss-of-function variants of drosophila *dPDK-1* lead to larval lethality and an increase in cellular

apoptosis (Cho *et al.*, 2001) whereas flies with hypomorphic variants are viable, but exhibit developmental delay, reduction in body size through a decrease in cell size and male infertility (Rintelen *et al.*, 2001). While homozygous *Pdpk1* knockout mice die on embryonic day E9.5 (Lawlor *et al.*, 2002), mice with residual PDK1 activity (10-30%) are viable and fertile, albeit of a smaller size than their unaffected litter mates (Bayascas *et al.*, 2008) similar to the findings in *drosophila*. Their brain is also proportionally smaller in size, with decreased neuronal cell size and deficient neuronal differentiation *in vitro* (Zurashvili *et al.*, 2013).

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