Université de Montréal

Clarification of the role of the *TBC1D24* gene in human genetic conditions

Par

Bettina E. Mucha-Le Ny

Programme des sciences biomédicales

Faculté de Médecine

Mémoire présenté à la Faculté des études supérieures en vue de l'obtention du grade de maîtrise en Sciences biomédicale, option médecine expérimental

Mai 4 2020

Université de Montréal

Programme des sciences biomédicales, Faculté de Médecine

Ce mémoire intitulé

Clarification of the role of the *TBC1D24* gene in human genetic conditions

Présenté par

Bettina E. Mucha-L eNy

A été évaluée par un jury composé des personnes suivantes

Dr Sébastien Jacquemont

Président-rapporteur

Philippe. Campeau

Directeur de recherche

Myriam Srour

Membre du jury

Résumé

Des variants pathogéniques du gène *TBC1D24* sont associés à des maladies génétiques dont la majorité sont transmises d'une façon autosomique récessive. Les phénotypes sont variables en termes de présentation clinique et de sévérité. Les formes les plus sévères causent une encéphalopathie épileptique (EIEE16) ou le syndrome DOORS qui est marqué par une surdité, des anomalies des ongles et des doigts, un déficit intellectuel et des convulsions qui sont souvent difficiles à contrôler. D'autres formes d'épilepsie incluent EPRPDC (Rolandic epilepsy with paroxysmal exercise-induce dystonia and writer's cramp), FIME (familial infantile myoclonic epilepsy), et PME (progressive myoclonus epilepsy). Une variant faux-sens spécifique est associée à une surdité autosomique dominante (DFNA65) qui se développe à l'âge adulte. Nous avons écrit un guide de pratique clinique qui inclut une revue de la littérature sur les phénotypes publiés chez les individus avec des variantes pathogénique du gène *TBC1D24* avec de recommandations pour le suivi clinique de ces patients.

De plus, une cohorte de huit patients avec déficience intellectuelle et épilepsie qui partagent une microdélétion sur le chromosome 16p13.3 contenant le gène *TBC1D24* a été assemblée et caractérisée afin de définir un nouveau syndrome génétique. La région critique contient *TBC1D24*, *ATP6V0C* et *PDPK1*. Le phénotype similaire entre les huit individus suggère que l'haploinsuffisance pour *TBC1D24, ATP6V0C* et *PDPK1* cause un nouveau syndrome génétique. L'etude des gènes essentiels pour le phénotype dans cette cohorte aide dans l'identification des nouveaux gènes candidates pour la déficience intellectuelle et épilepsie.

Mots-clés: DOORS, FIME, PME, EPRPDC, *TBC1D24*, microdélétion, épilepsie, surdité, déficit intellectuel

Abstract

Pathogenic variants in the *TBC1D24* gene are associated with genetic disorders, the majority of which are transmitted in an autosomal recessive manner. The phenotypes are variable in terms of clinical presentation and severity. The most severe forms cause epileptic encephalopathy (EIEE16) or DOORS syndrome which is marked by deafness, abnormalities of the nails and fingers, intellectual deficit and convulsions which are often difficult to control. Other forms of epilepsy include EPRPDC (Rolandic epilepsy with paroxysmal exercise-induce dystonia and writer's cramp), FIME (familial infantile myoclonic epilepsy), and PME (progressive myoclonus epilepsy). A specific missense variant is associated with autosomal dominant deafness (DFNA65) which develops in adulthood. A review of the literature of the published phenotypes observed in individuals with pathogenic variants in the *TBC1D24* gene is presented here with recommendations for the clinical management of these patients.

In addition, a group of eight patients with intellectual disability and epilepsy who share a microdeletion on chromosome 1613.3 containing the *TBC1D24* gene were characterized in order to define a new genetic syndrome. The critical region contains *TBC1D24*, *ATP6V0C* and *PDPK1*. The significantly similar phenotype shared by the eight individuals suggests that haploinsufficiency for *TBC1D24*, *ATP6V0C* and *PDPK1* causes a new genetic syndrome. Knowledge of the genes essential for the phenotype in this cohort helps in the identification of new candidate genes for intellectual disability and epilepsy.

Keywords: DOORS, FIME, PME, EPRPDC, *TBC1D24*, microdeletion, epilepsy, deafness, intellectual disability

Table of Contents

List of tables

List of figures

List of abbreviations

AD	autosomal dominant
ADHD	attention deficit hyperactivity disorder
AH	alternating hemiplegia
AR	autosomal recessive
ASD	autism spectrum disorder
CFT-R	Culture Fair Intelligence Test
CIL	contact inhibition of locomotion
CMA	chromosomal microarray
CNC	cranial neural crest
CNV	copy number variant
CT	computerized tomography
DD	developmental delay
DEE	developmental and epileptic encephalopathies
DIV	day in vitro
DOORS	deafness, onychodystrophy, osteodystrophy, mental retardation, and seizures
EEG	electroencephalogram
EIEE16	early infantile epileptic encephalopathy 16
EIP	exercise-induced dystonia
EPC	epilepsia partialis continua
EPRPDC	Rolandic epilepsy with paroxysmal exercise-induce dystonia and writer's cramp
ExAC	Exome Aggregation Consortium
FIME	familial infantile myoclonic epilepsy
FLAIR	fluid-attenuated inversion recovery
fs	frameshift
FTT	failure to thrive
HC	head circumference

HI	haploinsufficiency
ILAE	International League Against Epilepsy
ID	intellectual disability
IQ	intelligence quotient
kb	kilobase
LoF	loss of function
MOR	minimal overlapping region
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
mtDNA	mitochondrial deoxyribonucleic acid
NCBI	National Center for Biotechnology Information
NCSE	nonconvulsive status epilepticus
nDNA	nuclear deoxyribonucleic acid
NGS	next generation sequencing
PME	Progressive Myoclonus Epilepsy
SUDEP	sudden unexplained death in epilepsy
UTR	untranslated region
VUS	variant of unknown significance
WISC	Wechsler Intelligence Scale for Children

This thesis is dedicated my children Quentin and Juliette and my husband Jérôme.

Remerciements

Thank you, Philippe, for your persistence and patience.

Thanks also to the past and present members of the Campeau lab: You always made me feel welcome and included. I wish you all the best for your future endeavors!

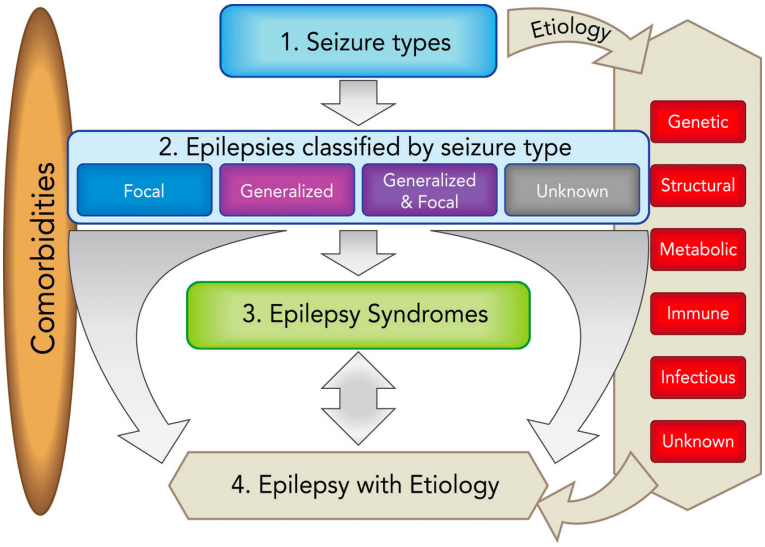
Chapter 1 – Introduction

The genetics of epilepsy

When a patient presents with seizures, neurologists strive to make a precise diagnosis for their patient in order to optimize treatment and offer clinical guidance to the family. In 2010, the ILAE issued a revised terminology for organization of epilepsies (Berg et al., 2010) and suggested to distinguish epilepsy forms by their etiology into genetic, structural/metabolic and unknown cause. “The concept of genetic epilepsy is that the epilepsy is, as best as understood, the direct result of a known or presumed genetic defect(s) in which seizures are the core symptom of the disorder” (Berg et al., 2010). This is contrasted with structural or metabolic epilepsies where different underlying conditions which include structural brain anomalies such as tumors or congenital brain malformations, primary genetic conditions, metabolic conditions, infectious processes and autoimmune reactions increase the risk of developing epilepsy. A general framework for epilepsy classification was later proposed by the ILAE Classification Task Force in 2016 (figure 1) which illustrates that the seizure type does not allow for a determination of the underlying cause without additional investigations (Scheffer et al., 2016). The same seizure type observed in two different patients may well be caused by two different etiologies. Vice versa, pathogenic variants in the same gene may cause different epilepsies, a principle known as phenotypic pleomorphy (Nolan & Fink, 2018) or phenotypic heterogeneity.

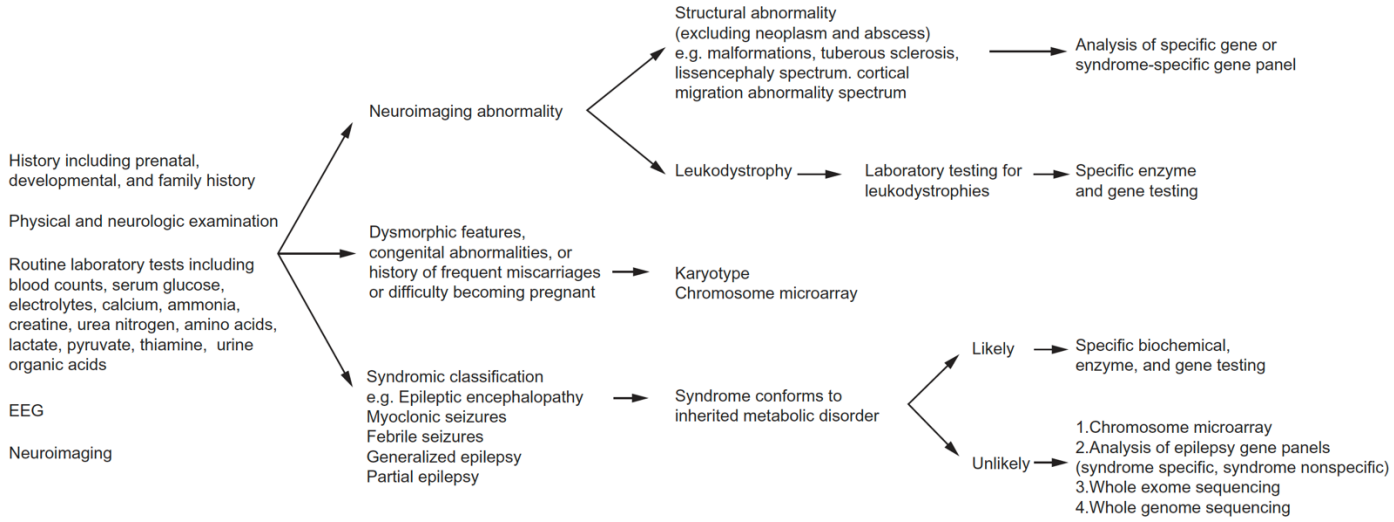
In general, the evaluation of an individual with epilepsy by the neurologist is comprehensive and investigates the different possible underlying etiologies as mentioned above. A proposed algorithm aims to identify the patients best suited for genetic analysis (see figure 2) (Nolan & Fink, 2018), although other authors suggest to consider molecular genetic testing for the known epilepsy genes as a first-line diagnostic tool (Symonds & McTague, 2020).

Figure 1. – Framework for epilepsy classification (Scheffer et al., 2016)



"The etiological framework can also be used for acute seizures. The term "genetic" refers to the etiology in an individual if there is an epilepsy syndrome that is known to be primarily genetic based on evidence from family and twin studies. Although the underlying gene may be identified for some individuals, in most cases, the underlying genetic mutation will not be known." (Scheffer et al., 2016)

Figure 2. – Approach to genetic evaluation of subjects with epilepsy (Nolan & Fink, 2018)



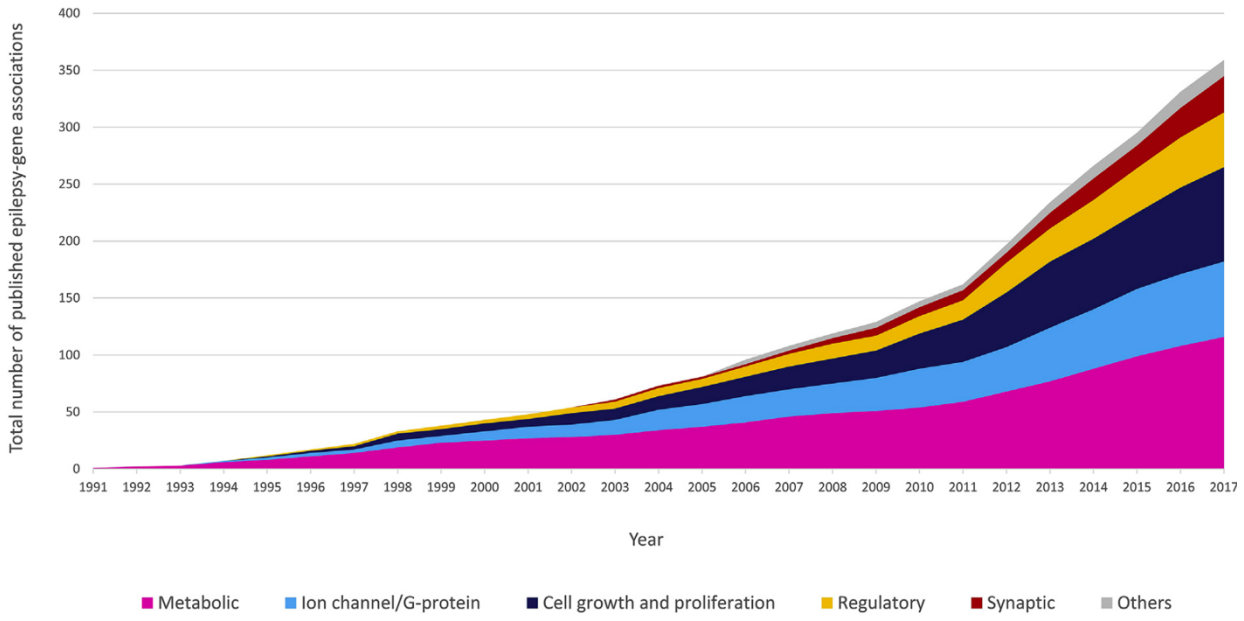
For severe forms of epilepsy, the distinction between developmental and epileptic encephalopathies (DEE) and a secondary epilepsy associated with a severe neurodevelopmental disorder (NDD) can be challenging. Epilepsy can occur with many complex developmental disorders and convulsions can frequently be seen as a presenting symptom during episodes of decompensation in the context of severe inborn errors of metabolism. Genetic syndromes caused by larger structural chromosomic rearrangements also increase the risk of a child to develop a seizure disorder, for example in Pallister-Killian syndrome (MIM **#** 601803), but dysmorphic features and congenital structural malformations are the primary features. Smaller copy number variations (CNV) have also been identified as genetic risk factors. Deletions and duplications can be found in 5-10 % of patients with childhood epilepsy (Hebbar & Mefford, 2020). In a study including 1255 patients with "epilepsy plus" defined as epilepsy with comorbid features, including intellectual disability, psychiatric symptoms, and other neurological and non-neurological features, 10.9% had a pathogenic CNV, most commonly deletion 1p36, deletion 15q11.2, deletion 15q11.3, deletion 16p11.2, deletion 16p13.1, and deletion or duplication 22q11.21 (Coppola et al., 2019).

For epilepsy disorders that are primarily characterized by seizures, *CHRNA4* was the first gene linked to a "pure" form of epilepsy, autosomal dominant nocturnal frontal lobe epilepsy (ENFL1, MIM**#** 600513) (Steinlein et al., 1995). The gene codes for a ligand-gated ion channel and opened the gateway for the subsequent discovery of many additional ion channels important in the pathogenesis of epilepsies, a group of conditions that was collectively termed channelopathies.

With the advent of next generation gene sequencing (NGS), the analysis of large numbers of known epilepsy and new candidate genes became cost-effective. NGS can be applied for use of preselected gene panels, exome sequencing or genome sequencing – the two former on a clinical basis, the latter at this time most commonly in a research setting. As a consequence, the discovery of epilepsy genes has increased at a rapid pace as illustrated in figure 3 (Symonds &

McTague, 2020) and has expanded the knowledge on possible underlying disease mechanisms in epilepsy, particularly for the most severe forms, commonly referred to as epileptic encephalopathies (McTague, Howell, Cross, Kurian, & Scheffer, 2016).

Figure 3. – Epilepsy gene discovery 1991-2017 (Symonds & McTague, 2020)



Pathogenic variants have been identified in many genes involved in various neuronal cell functions. These include genes linked to chromatin remodeling and DNA repair, transcriptional regulation and intracellular signaling (C. A. Ellis, Petrovski, & Berkovic, 2020). The genes involved in intracellular signaling can be further sub-divided, for example into ion channel components, synaptic support proteins and receptor molecules. Figure 4 from McTague et al. (2016) summarizes graphically the many different levels of neuronal development and function that have been shown to be implicated in the development of seizures in the presence of pathogenic variants in one of the coding genes.

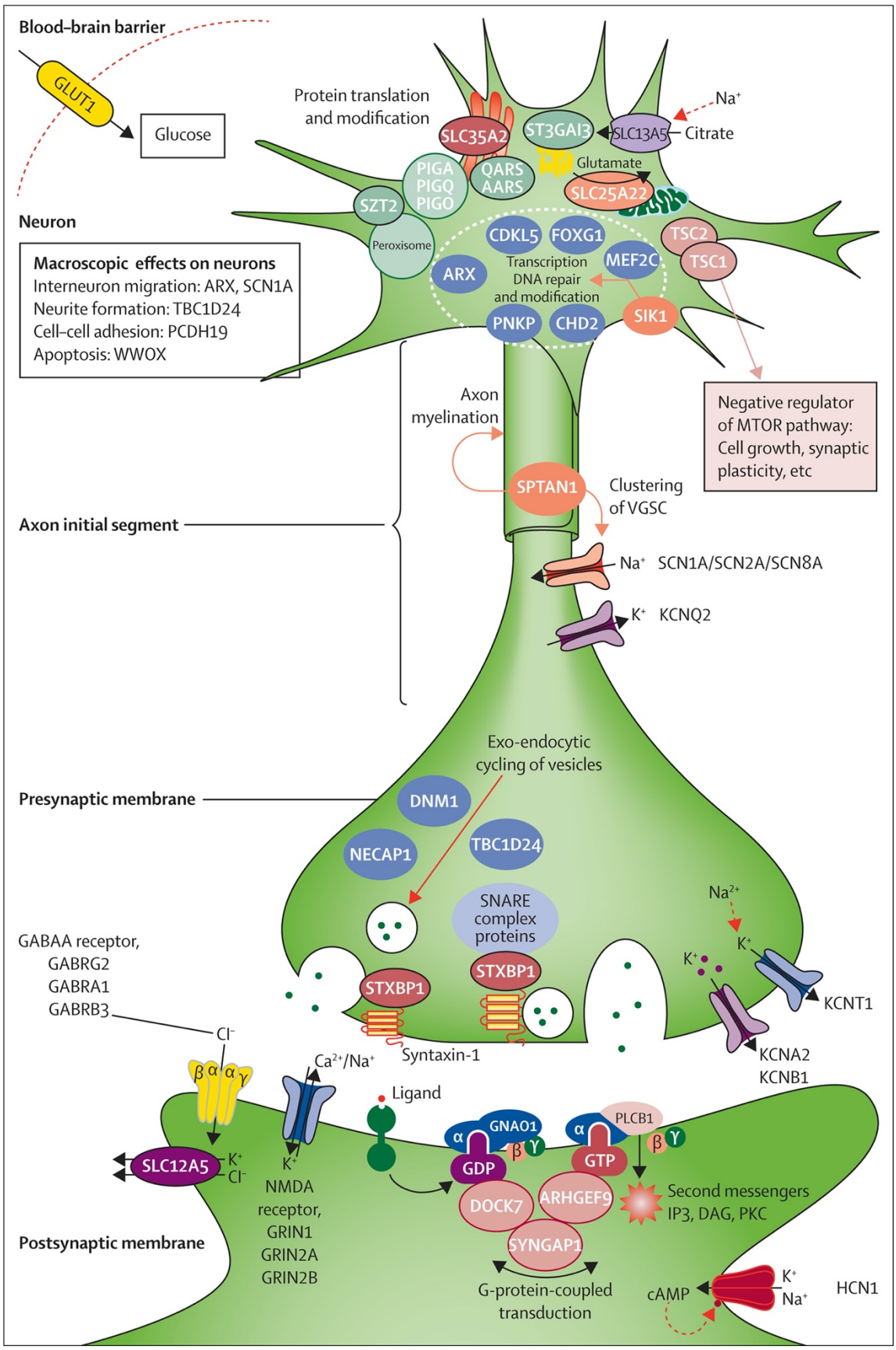

Blood–brain barrier
GLUT1
Glucose
Neuron
Protein translation and modification
SLC35A2
ST3GAL3
SLC13A5
Na+
Citrate
Glutamate
SLC25A22
PIGA
PIGQ
PIGO
QARS
AARS
SZT2
Peroxisome
CDKL5
FOXG1
TSC2
TSC1
ARX
Transcription
DNA repair
and modification
MEF2C
SIK1
PNKP
CHD2
Macroscopic effects on neurons
Interneuron migration: ARX, SCN1A
Neurite formation: TBC1D24
Cell–cell adhesion: PCDH19
Apoptosis: WWOX
Negative regulator of MTOR pathway: Cell growth, synaptic plasticity, etc
Axon myelination
SPTAN1
Clustering of VGSC
Axon initial segment
Na+ SCN1A/SCN2A/SCN8A
K+ KCNQ2
Exo-endocytic cycling of vesicles
Presynaptic membrane
DNM1
NECAP1
TBC1D24
SNARE complex proteins
STXBP1
STXBP1
Syntaxin-1
Na2+
K+
K+
KCNT1
KCNA2
KCNB1
GABAA receptor,
GABRG2
GABRA1
GABRB3
Cl−
β α α γ
Ca2+/Na+
Ligand
SLC12A5
K+
Cl−
K+
NMDA receptor,
GRIN1
GRIN2A
GRIN2B
Postsynaptic membrane
α
GNAO1
β γ
GDP
α
PLCB1
β γ
GTP
DOCK7
ARHGEF9
Second messengers
IP3, DAG, PKC
SYNGAP1
G-protein-coupled transduction
cAMP
K+
Na+
HCN1

Figure 4. – Disease mechanisms in childhood epileptic encephalopathies (McTague et al., 2016)

(Figure on previous page) “Neuron, axon, presynaptic, and post-synaptic compartments. Many areas of abnormal neuronal function, including DNA repair, transcriptional regulation, axon myelination, metabolite and ion transport, and peroxisomal function, in addition to channelopathies and synaptic dysfunction, are implicated in childhood epileptic encephalopathies.“ (McTague et al., 2016)

TBC1D24 is implicated in presynaptic vesicular trafficking (see section “The TBC1D24 protein and its function – current knowledge”). Other genes coding for proteins thought to be important for synaptic vesicular trafficking are *RUSC2* (MIM 617773), *PLAA* (MIM 603873), and *VAMP2* (MIM 185881). The associated human diseases, autosomal recessive mental retardation 61 (MRT61, MIM# 617773), neurodevelopmental disorder with progressive microcephaly, spasticity, and brain anomalies (NDMSBA, MIM# 617527), and neurodevelopmental disorder with hypotonia and autistic features with or without hyperkinetic movements (NEDHAHM, MIM# 618760), respectively, all present with variable degrees of developmental delay and other neurological symptoms such as autism spectrum disorder and seizures. In addition, dysmorphic features are part of the presentation of MRT61 and NDMSBA. However, the exact functions of these genes are still not completely understood, and more research is needed.

The yield of genetic testing varies from study to study and seems to largely depend on the studied cohort (Symonds & McTague, 2020). In a recent review, Symonds and McTague (2020) summarized the results of 24 studies including ~13.000 patients with NGS: In 17% of patients, a molecular cause was established due to pathogenic variants in one of 210 genes. The yield was increased with the use of larger panels and in patients with earlier onset (under age 6 months) or more severe seizures (up to 50%).

Identifying an underlying genetic cause of a clinically characterized seizure disorder can have multiple benefits. Especially for young children, the clinical prognosis and treatment options

may be shaped by the identified gene defect. For example, in Dravet syndrome, an early onset epileptic encephalopathy most commonly caused by heterozygous *de novo* loss-of-function variants in *SCN1A* coding for the alpha subunit of the neuronal type 1 sodium channel, an important trigger for epileptic activity is a raised body temperature which can occur due to fevers, but also warm baths or exercise (Verbeek et al., 2015). Another example constitutes the focal onset reflex seizures triggered by contact with water in boys with truncating variants in the *SYN1* gene (Peron, Baratang, Canevini, Campeau, & Vignoli, 2018). Furthermore, antiepileptic drugs that further block sodium channel activity such as carbamazepine are contraindicated. In the future, gene specific treatment may be become more widely available and some children are already benefitting by inclusion in clinical trials based on the underlying gene defect.

Many gene defects causing epileptic encephalopathies with significant developmental delays, morbidity and mortality are *de novo* events, but some conditions are transmitted in an autosomal recessive manner. The risk of recurrence differs significantly in those situations and is estimated at about 1% due to germline mosaicism for *de novo* events compared to 25% for each subsequent pregnancy in autosomal recessive conditions. Appropriate genetic counseling is therefore essential to families touched by these devastating neurological diseases. Furthermore, a definitive genetic diagnosis can put an end to a diagnostic odyssey for the affected children and their families that often includes invasive testing and repeat sedation for imaging studies.

Ultimately, clinical studies with large gene panels in patients with a variety of neurological presentations will expand the spectrum of phenotypes associated with any given gene. This will further increase our knowledge of phenotypic heterogeneity as illustrated in chapter 3, a review of *TBC1D2*4-related disorders, thus expanding our understanding about the complex connections between different organ systems and yielding important avenues in the study of gene functions and possibly genotype-phenotype correlations.

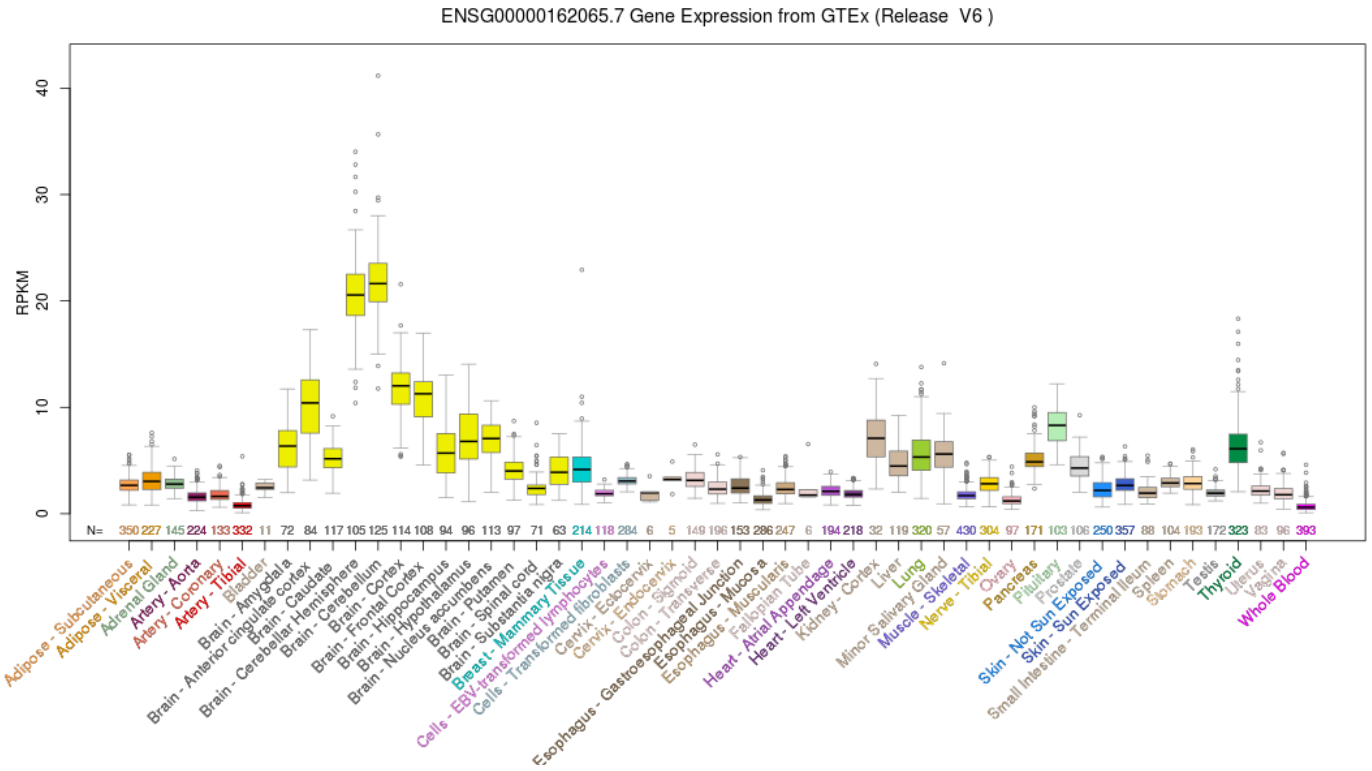
The TBC1D24 protein and its function – current knowledge

TBC1D24 encodes a member of the Tre2-Bub2-Cdc16 (TBC) domain-containing RAB-specific GTPase-activating proteins. The protein exists in two isoforms of 553 and 559 amino acids that differ in the inclusion of 6 amino acids coded by micro-exon 3. It features two functional domains, an N-terminal TBC domain followed by 82 residues that link to a C-terminus TLDc domain. Pathogenic variants causing different human conditions are distributed throughout the gene (Tona et al., 2019).

TBC domains can be found in other human proteins and are necessary for a protein's interaction with small GTPases. However, the TBC domain of TBC1D24 is lacking the "arginine finger" necessary to promote GTP hydrolysis in other Rab proteins casting doubt on its functionality (Falace et al., 2010). While the combination of the two functional domains is unique in the human genome, the protein sequence itself is highly conserved in evolution with paralogues, among others, in drosophila and Caenorhabditis elegans. In humans, the protein is highly expressed in brain tissue, particularly the cerebellum (figure 5).

Since the first link between pathogenic variants in *TBC1D24* and a human disease phenotype was established in 2010 (Falace et al., 2010), different groups have contributed different pieces of the puzzle using animal models and *in vitro* approaches to elucidate the function of TBC1D24 although many open questions remain.

Figure 5. – RNA-Seq Expression Data from GTEx for TBC1D24

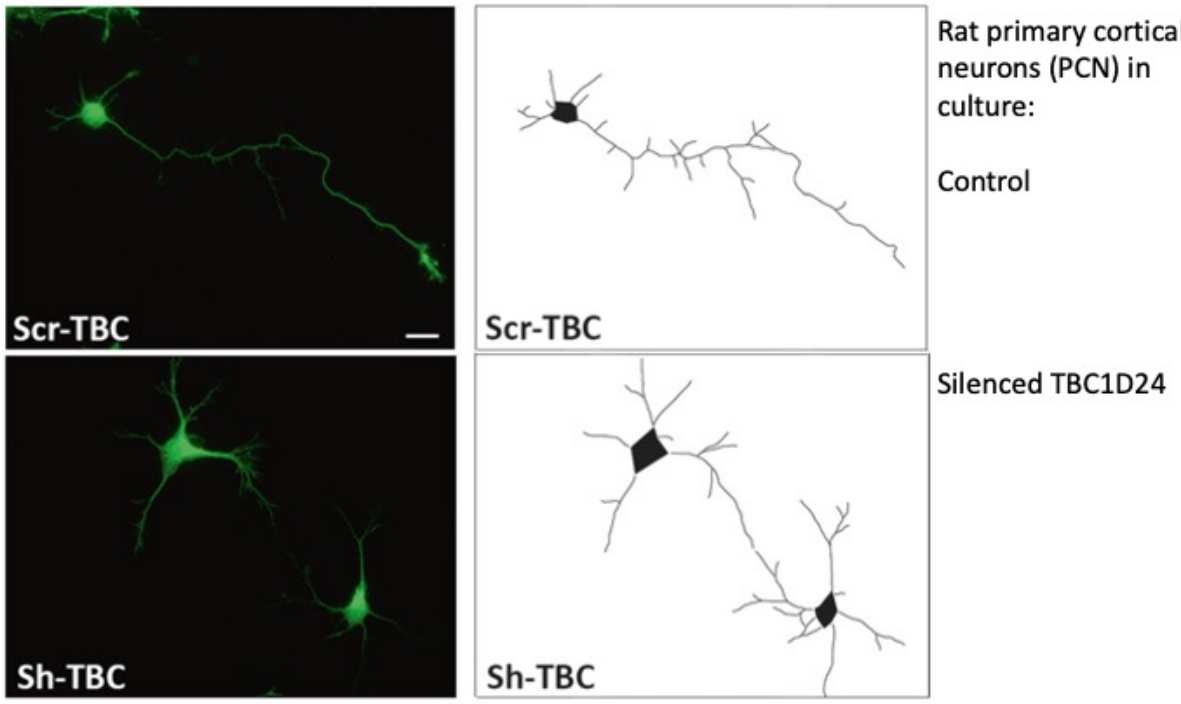


A potential role for TBC1D24 for neuronal function and development

In initial overexpression studies of TBC1D24 in COS-7 cells derived from monkey kidney cells, TBC1D24 was shown to co-localize with ARF6 (ADP ribosylation factor 6), a small GTPase with a role in vesicular trafficking at the plasma membrane (Falace et al., 2010). Further experiments in primary cortical neurons from mouse embryos suggested that TBC1D24 negatively regulates the activity of small GTPases such as ARF6 and RAB35 (Falace et al., 2010). Almost concomitantly, results from experiments with the *TBC1D24* orthologue Skywalker (*sky*) in drosophila lead to further insights into the protein's important role in presynaptic function and suggested a role in vesicle sorting for the Sky protein (Uytterhoeven, Kuenen, Kasprowicz, Miskiewicz, & Verstreken, 2011).

Transient *in utero* knock-down of TBC1D24 in developing rat brain by using inhibitory RNA leads to delayed migration of neural progenitor cells from the ventricular zone to the cortical plate (Falace et al., 2014). The formation of apical and basal dendrites was diminished both after *in utero* silencing as well as in an *in vitro* model. The group suggested that these effects were caused by a loss of ARF6 regulation (Falace et al., 2014).

Figure 6. – TBC1D24 expression increases in PCN at early stages of development and its silencing leads to a reduced neurite arborization (Aprile et al., 2019)



"Representative images of control and TBC1D24-silenced GFP-positive PCNs transfected at 0 day in vitro (DIV) and analyzed at 5 DIV. The respective manual tracings are shown on the right of each image" and show a "strong reduction of total neurite length, with a selective impairment of the putative axon" in cells after silencing of TBC1D24. Scale bar, 20 µm. (Aprile et al., 2019)

Aprile et al. contributed additional information on the role of TBC1D24 in axonal development and showed that loss of TBD1D24 in rat primary cortical neurons (PCN) *in vitro* and *in vivo* lead

to a defect in axonal specifications and projections (figure 6) with decreased excitability which were likely mediated by a dysregulation of ARF6 function (Aprile et al., 2019). These findings were confirmed in neurons differentiated from human induced pluripotent stem cells (hiPSCs) of a previously described patient with severe epileptic encephalopathy caused by homozygosity for the missense variant p.Asp11Gly, but were not observed in cells derived from a patient with a milder phenotype (FIME) with compound heterozygosity for p.Asp147His and p.Asp509Val.

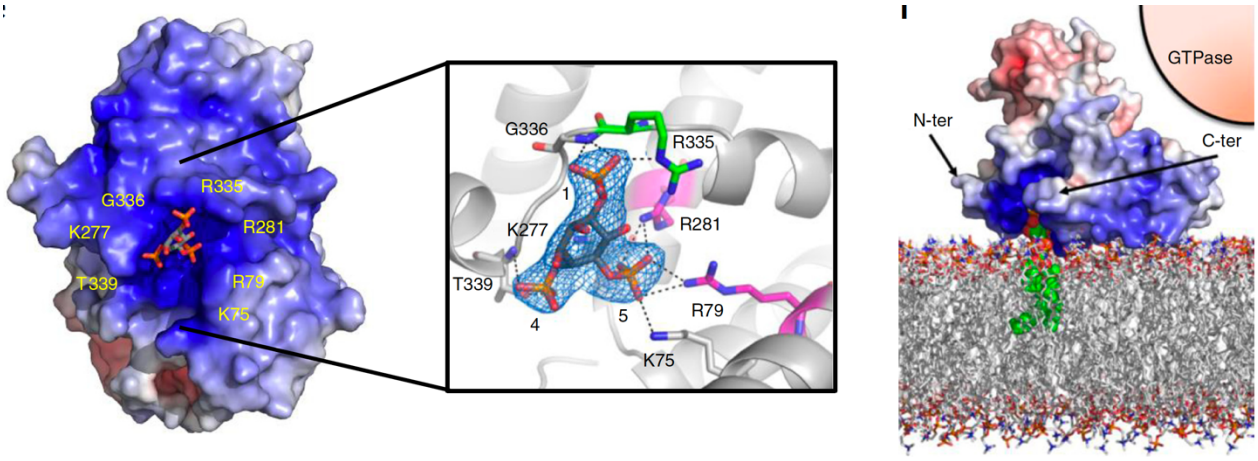
Lin et al. aimed to explore a potential post-synaptic function of TBC1D24 and the effect a loss of TBC1D24 may have on adult rodent behavior in order to uncover evidence of the TBC1D24 protein's importance for learning and memory (Lin et al., 2020). The group found TBC1D24 expressed in postsynaptic sites of murine cultured excitatory hippocampal neurons and confirmed interaction with ARF6, but not RAB35. Knock-down of TBC1D24 by inhibitory RNA in adult hippocampal neurons lead to a decrease of excitatory synapses and dendritic spines *in vitro* and *in vivo*. Adult mice showed behavioral changes after virus-induced depletion of TB1D24 in the hippocampus with increased anxiety and impaired fear learning. The group then generated a knock-in mouse model with the human pathogenic variant Phe251Leu, localized within the TBC domain and previously found in affected members of a consanguineous Arab family with infantile myoclonic epilepsy (FIME) and intellectual disability in some (Corbett et al., 2010). Homozygosity in mice led to increased neuronal excitability, seizures and early death, while heterozygous carriers were seizure-free, but had similar histological and behavioral changes as previously found in the viral animal model. This study was the first to investigate the role of TBC1D24 in post-synaptic neurons and the effect of TBC1D24 depletion on animal behavior (Lin et al., 2020).

The putative function of the TBC domain

The analysis of the crystal structure of the TBC domain of the drosophila orthologue Skywalker (Sky) identified a cationic pocket that is preserved in human TBC1D24, but that is not present in other TBC domain containing proteins (Fischer et al., 2016). In a liposome flotation assay with a

truncated Sky protein containing only the TBC domain (Sky_{1-353}) this pocket was found necessary for binding to the lipid membrane via phosphoinositides phosphorylated at the 4 and 5 positions (figure 7). Abrogation of the cationic pocket by introduction of two human *TBC1D24* pathogenic variants found in DOORS syndrome into the Sky_{1-353} construct at positions Arg40 and Arg242 led to impaired synaptic vesicle trafficking and seizures in drosophila (Fischer et al., 2016). Previous studies with homozygous loss-of-function variants proved embryonic lethal in fly larvae (Uytterhoeven et al., 2011).

Figure 7. – The cationic pocket of the TBC domain of Sky binds phosphoinositides (Fischer et al., 2016).

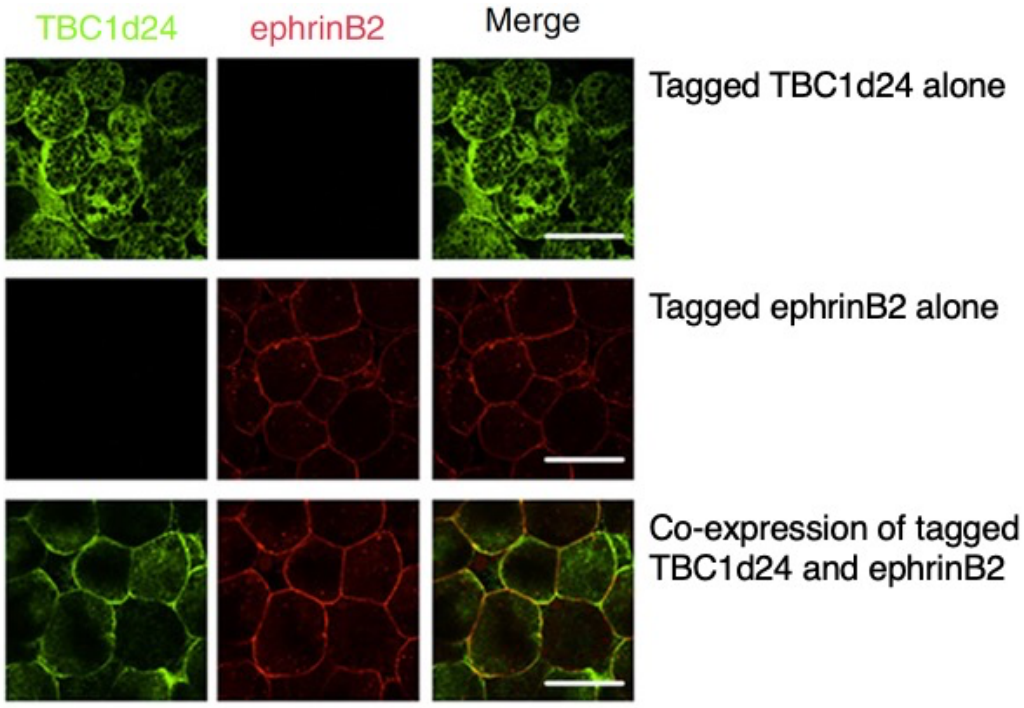


Left: "Electrostatic surface representation of the structure of $Sky_{1–353}$ in complex with inositoltriphosphate (IP_3). The box represents a close-up view of the IP_3-binding site. IP_3 is represented in dark-gray sticks, and the $2F_o - F_c$ electron density map (at 1.3σ) is shown as a blue mesh." (Fischer et al., 2016)

Right: "Docking model for binding of $Sky_{1–353}$ to a $PI(4,5)P_2$-containing biological membrane. A 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) bilayer containing a molecule of $PI(4,5)P_2$ was generated, and the $Sky_{1–353}$–IP_3 crystal structure was docked on this membrane by superimposing the protein-bound IP_3 group on the membrane-embedded $PI(4,5)P_2$. Ter, terminus." (Fischer et al., 2016)

A group interested in the process of cranial neural crest (CNC) cell migration during early development identified TBC1d24 as a new binding partner for ephrinB2 through ephrinB2 overexpression studies in *Xenopus* embryos (Yoon et al., 2018). Eph/ephrin signaling is involved in the regulating of cell–cell interaction events including the directional migration of CNC cells. 35 amino acids (amino acids 220 to 254) in the TBC domain were identified as crucial for ephrinB2/TBC1d24 interaction mediated by Dishevelled (Dsh) with a shift of TBC1d24 from the cytoplasm to a membrane-bound localisation (figure 8).

Figure 8. – TBC1d24 interacts with ephrinB2 (Yoon et al., 2018)



Immunofluorescence microscopic analysis (IF) shows that membrane localization of tagged TBC1d24 was induced by wild-type ephrinB2. Animal caps were dissected at stage 10 and then immunostained for ephrinB2 (red) and TBC1d24 (green). Bar, 50 µm (modified from (Yoon et al., 2018)

TBC1d24 negatively regulates Rab35, a member of the Ras superfamily of small GTP-binding proteins, through interaction at amino acid residues 151 to 184 of the TBC domain, but was

shown to only have weak affinity to ARF6 contrary to previous findings (Falace et al., 2010). Knock-down of TBC1d24 with morpholinos led to CNC cell migration defects in *Xenopus* embryos by disrupting contact inhibition of locomotion (CIL) likely through the regulation of E-cadherin recycling. This study provided new additional details on the function of TBC1D24 during neurodevelopment (Yoon et al., 2018).

The putative function of the TLDc domain

TBC1D24 shares the highly conserved TLDc domain with other proteins, one of which is OXR1 (oxidation resistance 1) (Finelli, Sanchez-Pulido, Liu, Davies, & Oliver, 2016). As summarized in Finelli et al. (2016), OXR1 confers resistance to oxidative stress in Escherichia coli, a function that appears to be dependent on the presence of the TLDc domain, while loss of OXR1 results in early lethality and severe neurodegeneration. Finelli et al. therefore postulated that the TLDc domain plays a crucial role in cellular oxidative stress response and assessed a potential neuroprotective effect by expressing different TLDc containing proteins including TBC1D24 in neuronal cultures subjugated to oxidative stress. While a construct with a missense variant in TBC1D24 described in a family with familial infantile myoclonic epilepsy (p.Ala515Val) was able to rescue cultured neuronal cells treated with arsenite, expression of mutant TBC1D24 carrying a nonsense variant (p.Cys156ter) lead to significant cell death suggesting a crucial role of the TLDc domain in neuroprotection (Finelli et al., 2016). The exact function and role of the TLDc domain in oxidative stress response was not investigated.

Lüthy et al. modeled the TLDc structure from crystalized drosophila TLDc protein ($Sky_{401–587}$) and predicted destabilizing effects on the domain for some human *TBC1D24* variants associated with exercise-induced dystonia (Luthy et al., 2019). Expression of the pathogenic human $TBC1D24^{Gly501Arg}$ variant in mutant Drosophila larvae lacking the *TBC1D24* paralogue *sky* resulted in sustained and coordinated movement defects, but not seizures. Exposure to oxidative stress lead to vesicle trafficking defects. Treatment with the radical scavengers N-acetylcysteine amide (AD4) or α-tocopherol (vitamin E) rescued both the movement disorder as well as the vesicle

defects in drosophila larvae suggesting that the TLDc domain acts as a sensor to oxidative stress and pathogenic variants that destabilize or affect the conformation of the TLDc domain cause hypersensitivity to reactive oxygen species (Luthy et al., 2019).

In summary, TBC1D24 appears to play an important role during development in neuronal cell migration and later for neuronal function through regulation of vesicular trafficking. While multiple interaction partners at the TBC domain have been identified, the precise role of the TLDc remains elusive but appears to be important in oxidative stress response. The function of TBC1D24 in the ear and its importance for intact hearing has not yet been investigated. Furthermore, the results to date have not yet shed any conclusive evidence on the cause of the remarkable phenotypic variability of pathogenic *TBC1D24* variants in human disease ranging from isolated hearing loss to severe epileptic encephalopathy and dysmorphic features in DOORS syndrome.

Introduction to *TBC1D24*-related disorders

Pathogenic variants in the *TBC1D24* gene were first associated with a human disease phenotype in 2010, when missense variants were identified in a large family with autosomal recessive Familial Infantile Myoclonic Epilepsy (FIME) (Falace et al., 2010). Subsequently, pathogenic variants in *TBC1D24* were found in a variety of different phenotypes with a mild presentation of isolated autosomal dominant hearing loss (Azaiez et al., 2014; L. Zhang et al., 2014) to autosomal recessive DOORS (*d*eafness, *o*nychodystrophy, *o*steodystrophy, mental *r*etardation, and *s*eizures) syndrome, a severe multi-system disorder (Campeau et al., 2014). With the exception of one specific missense variant causing autosomal dominant hearing loss, the other five annotated disease phenotypes are all autosomal recessive conditions. Given this important phenotypic pleomorphy, an extensive literature review was undertaken to better understand the clinical presentation of *TBC1D24*-related disorders. The results were made available to the public in 2015 with an updated version published in 2017 (Mucha, Hennekam, Sisodiya, & Campeau, 2017). The review is summarized in chapter 3 and includes manuscripts published prior to December 31, 2019.

Chromosomal microarray (CMA) has emerged as a standard clinical test in the last 10 years. Test indications include developmental delay or intellectual disability, seizure disorders, and congenital malformations. Recurrent microdeletion and -duplication syndromes are a frequent cause of developmental delay and can now be routinely diagnosed. Classification of new and rare copy number variations (CNVs) into benign or pathogenic can be challenging. When small microdeletions encompassing *TBC1D24* were first identified on CMA of individuals with mild developmental delay and seizures, pathogenicity could not be determined, and they were classified as variants of unknown significance (VUS). Genetic counseling on risk recurrence and reproductive choices is not possible without a confirmed diagnosis. In an effort to provide a definitive diagnosis to affected families and to possibly further expand the clinical phenotypes associated with *TBC1D24*-related disorders, we collected and analysed clinical data of eight individuals with overlapping microdeletions on chromosome 16q13.3 containing the *TBC1D24* gene. The results were published in 2019 (Mucha et al., 2019) and are reproduced in chapter 4.

Chapter 2 – MATERIALS, METHODS AND PATIENT INFORMATION

Literature review on *TBC1D24*-related disorders (chapter 3)

To review the spectrum of clinical phenotypes associated with pathogenic variants in the *TBC1D24* gene, a detailed review of the literature was undertaken using the online search tools PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Google Scholar (https://scholar.google.ca/). Additional information was obtained from the entries related to *TBC1D24* (*613577, #614617, #616044, #220500, #68105, #615338, and #605021) in the Online Mendelian Inheritance in Man® database (https://omim.org/). The search for publications was limited to a 10-year time period from January 1, 2010 (year when the first *TBC1D24*-related disorder was described) to December 31, 2019. Older publications were included in the review when they were relevant, e.g. to obtain more information on a previously published patient or family for which later publications had determined a *TBC1D24*-related disorder.

Search terms used were “TBC1D24”, “TBC1D24 AND epilepsy", “TBC1D24 AND FIME", “TBC1D24 AND PME”, “TBC1D24 AND encephalopathy”, “TBC1D24 AND genotype”, “TBC1D24 AND phenotype” and “TBC1D24 AND hearing". A total of 67 articles were identified. Only publications in English were retained for analysis, none were published in the French language. Manuscripts were evaluated based on clinical description of patients confirmed to have pathogenic or likely pathogenic variants in *TBC1D24*. Publications with data exclusively on experimental animal models were not included in the analysis. 40 original publications and case studies fulfilled the inclusion criteria and were retained for analysis.

Supplementary material for manuscript "A new microdeletion syndrome involving *TBC1D24, ATP6V0C* and *PDPK1* causes epilepsy and developmental delay" (Mucha et al., 2019) (Chapter 4)

Scientific basis for the used bioinformatics tools

The haploinsufficiency score %HI is calculated based on a computational algorithm developed by Huang *et al*. (Huang, Lee, Marcotte, & Hurles, 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 1.5 cm <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, <5th percentile) and a head circumference at the 2nd to 5th 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 2nd to 5th percentile, height (106 cm) at the 10th percentile and weight (18 kg) at the 25th 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 10th percentile, whereas head growth was preserved at the 50th percentile. Her height (114 cm) and weight (17.5 kg) remained around the 10th 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 information on gene function

Tableau 1. – Gene orthologues and phenotype (in brackets) in select species

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)

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)

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, Noumi, Nagata, Murakami, & Kanazawa, 1999), homozygous embryos develop only to the blastocyst stage and die shortly after implantation (Sun-Wada et al., 2000). 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, Ng, Darland, & Gross, 2009), as well as for the maintenance of the notochord (K. Ellis, Bagwell, & Bagnat, 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, di Blasio, & Primo, 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 & Hu, 2013). Pdk1 is widely expressed in head and tail neurons, pharynx and intestinal cells (Paradis, Ailion, Toker, Thomas, & Ruvkun, 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, Stocker, Thomas, & Hafen, 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).

Chapter 3 – *TBC1D24*-related disorders

Clinical characteristics

TBC1D24 (MIM *613577, last updated in August 2019) is located on chromosome 16p13.3. As of December 2019, six different human genetic conditions were associated with pathogenic variants in *TBC1D24* in the Online Mendelian Inheritance in Man® database:

- DOORS (*d*eafness, *o*nychodystrophy, *o*steodystrophy, mental *r*etardation, and *s*eizures) syndrome (MIM #220500): an autosomal recessive disorder characterized by profound sensorineural hearing loss, onychodystrophy, osteodystrophy, intellectual disability/developmental delay, and seizures.

- Familial infantile myoclonic epilepsy (FIME, MIM # 605021): an autosomal recessive form of epilepsy causing early-onset myoclonic seizures, focal epilepsy, dysarthria, and mild-to-moderate intellectual disability.

- Early infantile epileptic encephalopathy 16 (EIEE16, MIM #615338): a severe autosomal recessive neurologic disorder characterized by onset of seizures in the first weeks or months of life; seizures are often refractory to treatment and many children die in infancy.

- Rolandic epilepsy with paroxysmal exercise-induce dystonia and writer's cramp (EPRPDC, MIM #608105): an autosomal recessive form of epilepsy that is relatively benign and limited to childhood, while the exercise-induced dystonia may persist into adulthood.

- Autosomal recessive non-syndromic hearing loss, DFNB86 (MIM #614617): an autosomal recessive form of non-syndromic deafness with profound prelingual hearing loss.

- Autosomal dominant non-syndromic hearing loss, DFNA65 (MIM #616044): an autosomal dominant form of slowly progressive deafness with onset in the third decade, initially affecting the high frequencies.

Pathogenic bi-allelic *TBC1D24* variants have also been identified in a seventh condition, Progressive Myoclonus Epilepsy (PME) (Muona et al., 2015). PME is characterized by action myoclonus, tonic-clonic seizures, progressive neurologic decline, and ataxia. Collectively, these conditions are referred to as *TBC1D24*-related disorders and comprise a continuum on a phenotypic spectrum of disease expression.

While initial reports suggested specific phenotypes associated with pathogenic *TBC1D24* variants, the publication of more and more case reports and case series suggest rather a phenotypic spectrum ranging from a mild form of FIME to DOORS syndrome (Balestrini et al., 2016; Luthy et al., 2019; J. Zhang et al., 2019). Movement disorders both in children and in adults are increasingly being recognized as complications of biallelic *TBC1D24* pathogenic variants and include Parkinsonism (Banuelos et al., 2017), ataxia and dysarthria (Balestrini et al., 2016), and exercise-induced dystonia (Luthy et al., 2019). Features associated with DOORS syndrome can also be seen in patients that do not clinically fulfill the strict diagnostic criteria of DOORS syndrome. These include axial hypotonia, hearing loss, visual impairment, mild dysmorphic facial features, developmental delay or intellectual disability, and microcephaly (Balestrini et al., 2016).

TBC1D24-related disorders are overall rare. Fewer than 50 families with DOORS syndrome are known. In a study that was limited to children with epileptic encephalopathy and which included 360 patients only one patient had pathogenic biallelic *TBC1D24* variants (de Kovel et al., 2016). In a Chinese study, 19 patients with various forms of *TBC1D24*-related epilepsy were identified out of 2174 patients tested over a 40 month period (J. Zhang et al., 2019), a proportion of 0.87% of all patients tested. In two cohorts with hearing loss (see also below), *TBC1D24* variants have not emerged as a major underlying cause, although these studies were limited to individuals of Moroccan (Bakhchane et al., 2015) and Arab-Israeli (Danial-Farran et al., 2018) descent.

A growing body of literature suggests increased susceptibility to seizure disorders in heterozygous carriers of *TBC1D24* pathogenic variants as compared to the population frequency (estimated at 7 per 1000 individuals). Seizure disorders have been seen in heterozygous family members in families with different *TBC1D24*-related disorders. A confirmed carrier (p.Asp70Tyr) in a family investigated for autosomal recessive hearing loss had seizures starting at 3 years of age (Rehman et al., 2014). A family history of seizures was also reported in two families with DOORS syndrome, a mother (p.His336Glnfs*12) with absence seizures in childhood (Campeau et al., 2014) and a father with non-specified seizures (see supplemental information) (Balestrini et al., 2016). In a further five families with *TBC1D24* pathogenic variants, the family history was positive for seizure disorders; unfortunately, family members were unavailable for sequencing (Balestrini et al., 2016; Strazisar, Neubauer, Paro Panjan, & Writzl, 2015). In a family with an atypical neurologic phenotype in the proband, both the patient's mother as well as her brother had seizures in childhood and adolescence respectively. Both were confirmed carriers for a novel pathogenic variant (p.Pro135Leu) (Banuelos et al., 2017). Finally, Zhang et al. published a case study of epilepsy in patients with *TBC1D24* variants (J. Zhang et al., 2019): the father of a child with Dravet syndrome (patient 12) and the brother of another proband with unclassified epilepsy (patient 15) had febrile seizures. Unfortunately, neither was available for sequencing, but the father may be counted as a presumptive carrier of a heterozygous *TBC1D24* variant. As outlined in chapter 4, heterozygous *de novo* microdeletions on chromosome 16 encompassing among other genes *TBC1D24* are also associated with seizure disorders (Mucha et al., 2019) suggesting that haploinsufficiency for *TBC1D24* is likely to contribute to the described phenotype. Recent evidence from a heterozygous mouse model with *TBC1D24* haploinsufficiency further corroborates this hypothesis (Finelli et al., 2019): *in vitro* studies of cultured hippocampal and cortical neurons from mice haploinsufficient for *TBC1D24* showed significantly reduced dendritic and axonal growth compared to WT controls, increased susceptibility to oxidative stress and impaired endocytosis (see also chapter 5 Discussion).

DOORS Syndrome

The cardinal features of DOORS syndrome include profound sensorineural hearing loss, onychodystrophy, osteodystrophy, global developmental delay or intellectual disability, and seizures (Campeau et al., 2014; James, Miranda, Culver, Hall, & Golabi, 2007), although expressivity is variable leading to differences in clinical presentation.

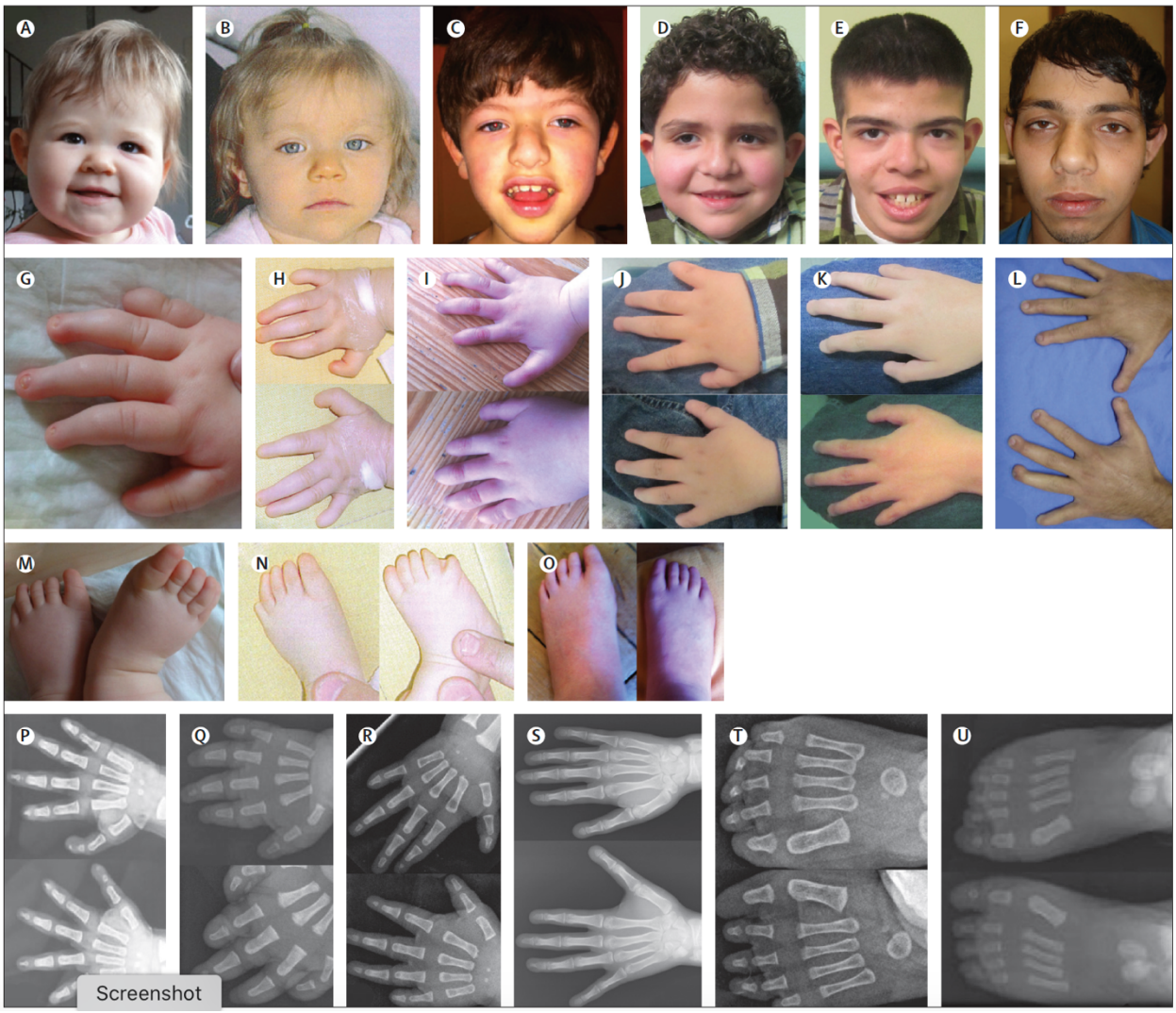
The neurological presentation is characterized by global developmental delay, seizures and sensorineural hearing loss that is often profound and prelingual. The use of hearing aids is suggested, and one patient has benefited from cochlear implants (Campeau et al., 2014). Most children experience global developmental delay that may be mild (Santos, Reis-Rego, Coutinho, & Almeida, 2019), but is often severe and leads to significant intellectual disability (previously referred to as mental retardation) (Balestrini et al., 2016). The motor and language skills were severely delayed in a child with DOORS syndrome who was also diagnosed with autism spectrum disorder (Nomura, Koyama, Yokoyama, Awaya, & Yokochi, 2009). Multiple probands with DOORS syndrome had hypotonia (Balestrini et al., 2016).

Most individuals with DOORS syndrome present with seizures that usually start in the first year of life. The seizures are more often generalized tonic-clonic, but myoclonic, partial, and absence seizures also occur. The frequency or severity of seizure episodes may increase with time and are sometimes difficult to control even with multiple antiepileptic medications. Treatment refractory seizures increase the risk for status epilepticus and death (Balestrini et al., 2016). On MRI, hyperintense T_2 signal anomalies may be observed in the cerebellar hemispheres and the frontal region (Balestrini et al., 2016; Campeau & Hennekam, 2014; Nomura et al., 2009). Visual impairment has also been noted in six out of 14 patients in a case series and peripheral neuropathy in one (Balestrini et al., 2016).

In addition to neurological abnormalities, DOORS syndrome is characterized by dysmorphic features and physical findings on exam (figure 9). One of the hallmark signs, the onycho-osteodystrophy, affects the hands and feet equally. The nails for the fingers and toes are small or absent (onychodystrophy) and the distal phalanges are hypoplastic (osteodystrophy) in most

individuals causing brachydactyly. A triphalangeal thumb is present in one third of affected individuals.

Figure 9. – Physical features of participants with TBC1D24 mutations (Campeau et al., 2014)



"(A–F) Some individuals have a wide base of the nose and bulbous end of the nose (individual numbers from tables corresponding to each panel: A=3, B=6, C=5a, D=2b, E=2a, F=7). (G–L) Hands in individuals with *TBC1D24* mutations have triphalangeal thumbs, brachydactyly, short terminal phalanges, and hypoplasia or aplasia of the nails (G=3, H=6, I=5a, J=2b, K=2a, I=7). (M–O) Feet with short terminal phalanges and hypoplasia or aplasia of the nails (M=3, N=6, O=5a). (P–S) Radiographs of the hands in individuals with *TBC1D24* mutations. Note the triphalangeal thumbs and the short terminal phalanges (P=4, Q=5a,

R=6, S=1). (T,U) Radiographs of the feet in individuals with *TBC1D24* mutations, showing short terminal phalanges (T=6, U=5a)." (Campeau et al., 2014)

In addition, non-specific dysmorphic facial features may be noted and include most commonly a wide nasal base and a bulbous nose. More rarely, a narrow forehead, narrow or high arched palate, broad alveolar ridge, nevus simplex on glabella and nose, and short frenulum can also be found. Cranial anomalies include microcephaly in one third of individuals. One patient had sagittal craniosynostosis and several patients had other cranial abnormalities such as frontal bossing, trigonocephaly, or brachycephaly (Balestrini et al., 2016; Campeau et al., 2014). Additional anomalies have been seen in children with DOORS syndrome but are rare and not observed in a larger proportion of patients with confirmed pathogenic *TBC1D24* variants. Congenital heart defects are mostly limited to atrial or ventricular septal defects (James et al., 2007), but one patient with a more severe congenital heart defect, a double outlet right ventricle, has been described (Campeau et al., 2014). Additional findings include dental anomalies (delayed eruption, wide spacing, and abnormal shape, size, and number of teeth), non-specific skeletal anomalies (e.g., calcaneal deformities) and renal and urinary tract anomalies (e.g., hydronephrosis and nephrocalcinosis) (Campeau et al., 2014).

The standard investigations for an underlying cause of seizures and developmental delay often include metabolic studies. Interestingly, some patients with DOORS syndrome have been found to have elevated levels of urinary 2-oxoglutaric acid. These may be consistently elevated or can fluctuate between normal and elevated values over multiple measurements (Campeau et al., 2014). A causal correlation between pathogenic *TBC1D24* variants and urinary 2-oxoglutaric acid has yet to be established.

Familial Infantile Myoclonic Epilepsy (FIME)

A causative link between familial infantile myoclonic epilepsy (FIME) and the TBC1D24 gene was first established in 2010 (Falace et al., 2010). FIME is characterized by early-onset myoclonic seizures, but other findings may include focal epilepsy and febrile convulsions (de Falco et al.,

2001). In this previously studied family, seven members had well-controlled seizures and normal intelligence. Furthermore, the MRI of the brain was normal in six, while one had periventricular nodular heterotopia.

The presentation can be variable with four siblings of a consanguineous Arab-Israeli family showing dysarthria, mild-to-moderate intellectual disability, and cortical thickening and cerebellar atrophy with high T_2 and FLAIR on MRI (Afawi et al., 2013; Corbett et al., 2010). In two brothers with FIME, both had moderate intellectual disability with speech delay (Poulat et al., 2015). None of the published patients had hearing loss or physical features of DOORS syndrome.

Progressive Myoclonus Epilepsy (PME)

Progressive myoclonic epilepsy (PME) is characterized by action myoclonus, tonic-clonic seizures, progressive neurologic decline, and ataxia. The first case of a child with PME and biallelic pathogenic variants in *TBC1D24* was described in 2015 (Muona et al., 2015). The infant's seizure characteristics changed with age: Tonic seizures started at 36 hours of life, myoclonus was noted at eight months of age and tonic-clonic seizures at 3.5 years. Developmental delay with later regression was then noted. Additional neurologic symptoms included ataxia, spasticity, supranuclear gaze palsy, and visual decline. Although the initial clinical diagnosis was of an epileptic encephalopathy, a florid PME pattern was apparent by age nine years. There were no digital anomalies or deafness (Sam Berkovic, MD, personal communication). Two additional patients were identified in a group of 19 children with epilepsy and biallelic variants in *TBC1D24* (J. Zhang et al., 2019). Both patients had prominent myoclonus and MRI findings of cerebral and cerebellar atrophy with hyperintense T2 signals in the cerebellum. Patient 10 had a normal neonatal hearing screen but was diagnosed with sensorineural deafness at 9 years of age. She has developmental delay and developed ataxia after a severe episode of epilepsia partialis continua (EPC). Patient 11 had a normal hearing test and mild developmental delay with preserved language. Overall, only few patients with biallelic *TBC1D24* variants and PME have been clinically characterized in the literature.

Early-Infantile Epileptic Encephalopathy 16 (EIEE16)

The terminology “epileptic encephalopathy” pertains to the observation that “epileptic activity itself may contribute to severe cognitive and behavioral impairments above and beyond what might be expected from the underlying pathology alone (e.g., cortical malformation), and that these can worsen over time” (Berg et al., 2010).

Early-infantile epileptic encephalopathy 16 (EIEE16) falls on the severe spectrum of *TBC1D24*-related disorders. The genetic cause was first identified in 2013 in a Turkish family that had been previously linked to chromosome 16pter-p13.3 (Duru, Iseri, Selcuk, & Tolun, 2010; Guven & Tolun, 2013). The children in that family suffered from severe and progressive myoclonic epilepsy, dystonia that was initially episodic but progressed to persistent, and pyramidal signs (Duru et al., 2010). All died prior to the age of 8 years and had significant developmental delays and developmental regression including feeding difficulties, generalized hypotonia with inability to walk and absent language (Guven & Tolun, 2013).

Subsequently, more patients with EIEE16 and pathogenic *TBC1D24* variants have been identified (Balestrini et al., 2016; Lozano et al., 2016; Strazisar et al., 2015; Trivisano et al., 2017; J. Zhang et al., 2019) allowing for the identification of common features in this condition. EIEE16 is characterized by early onset (within first days or months of life) myoclonic epilepsy that may be treatment resistant. Patients experience severe global developmental delays and regression. Other neurological symptoms may include episodic dystonia, hemiparesis, autonomic signs, and lethargy evolving to chronic dystonia and generalized hypotonia. Brain imaging by MRI may initially be normal, but during disease progression cerebral and cerebellar atrophy develop with acquired microcephaly on physical exam. With time, children become unresponsive to visual and auditory stimuli, but not all patients have documented hearing tests. In one study, three of five patients with EIEE16 had profound sensorineural hearing loss at birth while two had normal documented hearing (J. Zhang et al., 2019). One additional patient (sibling I-2) had bilateral sensorineural hearing loss at the 50 dB threshold on brainstem auditory evoked potentials test in infancy (Strazisar et al., 2015) suggesting that hearing loss may be a common feature in *TBC1D24*-related epileptic encephalopathy.

Rolandic epilepsy with paroxysmal exercise-induce dystonia and writer's cramp (EPRPDC)

Most recently, compound heterozygous pathogenic *TBC1D24* variants were identified in six patients from four families with a particular epilepsy phenotype, Rolandic epilepsy with paroxysmal exercise-induce dystonia and writer's cramp (EPRPDC) (Luthy et al., 2019). Linkage to the *TBC1D24* locus had been established previously in three members of an Italian family (Guerrini et al., 1999). Although only few patients are known, 20-year follow-up in the three Italian individuals suggests that focal motor seizures can be well controlled on anticonvulsive therapy and are limited to infancy and early childhood. Prolonged physical activity triggers exercise-induced dystonia (EIP), which persisted into adulthood in two patients, but with decreased frequency due to lifestyle changes. Brain MRI was normal in all six patients (Luthy et al., 2019). The three adult patients had normal neurodevelopmental assessments during childhood (Guerrini et al., 1999). It is unclear whether any of the published patients had hearing tests or physical exams to assess for features of DOORS syndrome.

Autosomal Recessive Non-Syndromic Hearing Loss, DFNB86

Homozygous missense variants in *TBC1D24* were first found in four consanguineous Pakistani families with isolated autosomal recessive non-syndromic hearing loss DFNB86 (Rehman et al., 2014). The hearing loss was profound and of prelingual onset with hearing thresholds above 90 dB for all test frequencies. Importantly, in one family, one homozygous patient and one individual with a heterozygous *TBC1D24* pathogenic variant also had febrile seizures, but re-evaluation was not possible in two of the families. Subsequently, pathogenic compound heterozygous variants were identified in three Moroccan families with DFNB86 out of 136 families with hearing impairment. It is unknown whether the affected individuals had a targeted physical exam for other *TBC1D24*-related symptoms or a personal or family history of seizures (Bakhchane et al., 2015). In a large study of 168 Arab-Israeli families with isolated hearing loss, a homozygous missense variant was found in one consanguineous family. The authors reported

the absence of other TBC1D24-associated symptoms on physical exam (Danial-Farran et al., 2018).

Autosomal Dominant Non-Syndromic Hearing Loss, DFNA65

Concomitantly with the publication of autosomal recessive hearing loss, two studies linked the same autosomal dominant missense variant in *TBC1D24* to DFNA65 (Azaiez et al., 2014; L. Zhang et al., 2014). The affected patients from a large family of European descent (Azaiez et al., 2014) and an extended pedigree of Chinese origin (L. Zhang et al., 2014) suffered from slowly progressive sensorineural deafness with onset in the third decade, initially affecting the high frequencies. No additional patients have been published since 2016, but two relatives of two patients with generalized tonic-clonic seizures and pathogenic *TBC1D24* variants had a history of hearing loss (Balestrini et al., 2016). The brother of patient 16 and the maternal grandmother of patient 31 had hearing impairment, but unfortunately neither was available for *TBC1D24* sequencing to determine carrier status (supplemental material, Balestrini et al., 2016).

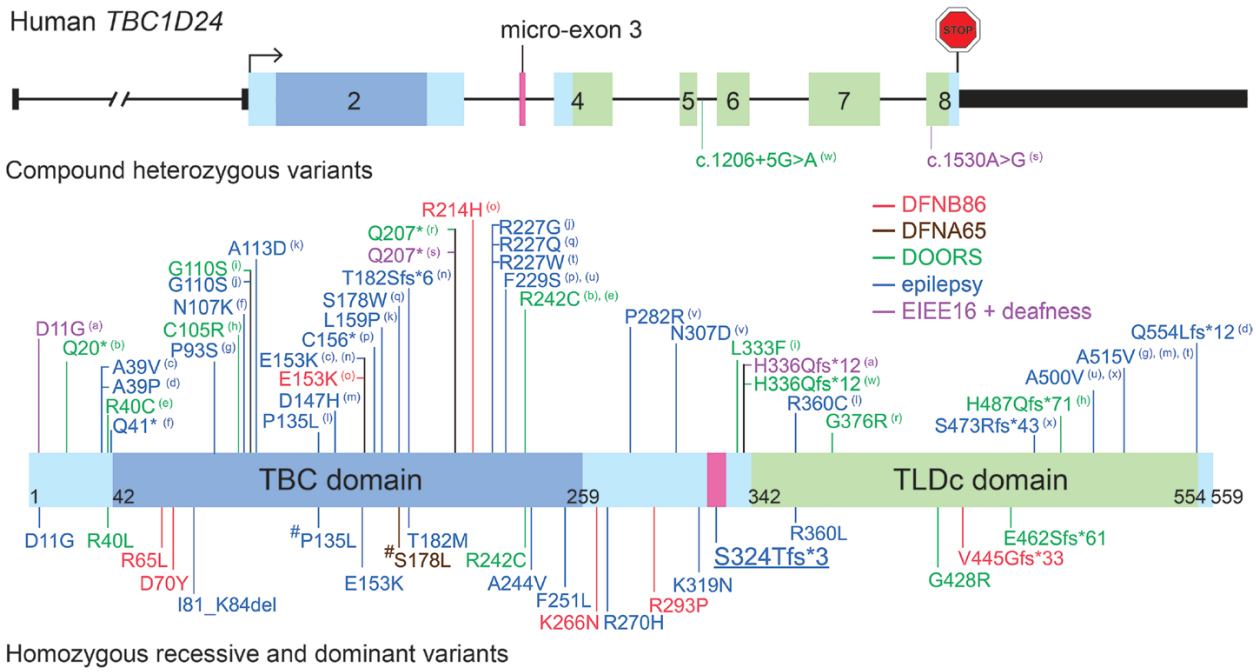
Genotype-Phenotype Correlations

The pathogenic *TBC1D24* variants that cause DOORS syndrome, epilepsy, DFNB86, and DFNA65 are located throughout the gene (Figure 10, from Tona et al., 2019) and clear genotype-phenotype correlations have not yet been established given the rarity of *TBC1D24*-related disorders and the lack of larger patient cohorts.

A first study investigating a group of 48 patients with epilepsy and pathogenic *TBC1D24* variants suggested that loss-of-function variants caused by frameshift, nonsense or splice-site variants are associated with a more severe epilepsy phenotype with drug-resistance and early death unless the variant is localized in the last exon (Balestrini et al., 2016). This association is illustrated in four patients with the same frameshift variant (p.His336Glnfs*12) that was identified in conjunction with different additional variants in two simplex cases with DOORS syndrome and difficult to control seizures and one sibling pair with EIEE16 and early death. However, the expression of additional *TBC1D24*-related symptoms diverged between patients. In contrast, in families with more than one affected child, the disease phenotype was similar between siblings and did not show any significant differences. In this same study, the presence of features of DOORS syndrome did not predict the epilepsy phenotype. No clear pattern emerged for missense variants with the exception of missense variants located prior to or within the TBC domain: these conferred a higher risk for early lethality. Balestrini et al. concluded that the analysis of genotype-phenotype correlations for *TBC1D24*-related disorders is hampered by the heterogeneity of pathogenic variants for simplex cases which are often compound heterozygous with individuals from different families rarely sharing exactly the same pathogenic variants (Balestrini et al., 2016).

Figure 10 (from Tona et al., 2019) illustrates the location of previously identified pathogenic *TBC1D24* variants throughout the gene and their location within confirmed or putative functional domains. At least two pathogenic variants (p.Gly110Ser and p.His336Glnfs*12) have been associated with both DOORS syndrome and epilepsy phenotypes.

Figure 10. – Structure of the human *TBC1D24* gene and the location of pathogenic variants in the encoded protein (Tona et al., 2019).



"The eight annotated exons of human *TBC1D24* are depicted with the seven protein-coding exons (colored rectangles). Exon 2 (blue) encodes the TBC domain (deep blue). Exons 4 to 8 encode the TLDc domain (light green). Alternatively spliced micro-exon 3 is shown in pink. The 5' untranslated sequence of exon 1 and part of exon 2 and 3' untranslated region of exon 8 are in black. A right-pointing arrow above exon 2 indicate the location of the translation start codon. A red stop sign marks the location of the translation stop codon. Two reported pathogenic splice-site variants (mutations) of human *TBC1D24* are drawn below the gene structure. To date, an additional 55 variants that alter the TBC1D24 protein are reported and have been depicted based upon the amino acid sequence of the longest isoform of *TBC1D24* (NM 001199107). Variants are grouped by color depending on the clinical phenotype. They have been associated with DFNB86 non-syndromic recessive deafness (red font), dominantly inherited non-syndromic deafness DFNA65 (brown), DOORS syndrome (green), epilepsy (blue) and EIEE16 epilepsy with deafness (purple). The same lowercase and superscripted letter written in parentheses identifies the two variants in compound heterozygosity. Homozygous recessive and dominant variants are drawn under the protein structure. The one-letter code for amino acids is used in this figure. For example, P135L indicates a leucine residue substituted for the wild-type proline. Two dominant variants are marked with a #. An asterisk indicates a stop codon, fs indicates a translation frameshift and the

number after an asterisk indicates the number of mutant amino acids residues encoded in a different translation reading frame before the premature translation stop codon." (Tona et al., 2019)

Additionally, one variant (p.Glu153Lys) has been seen in diverging phenotypes: a girl with recurrent attacks of alternating hemiplegia (AH) and recurrent episodes of epilepsia partialis continua (EPC) (Ragona et al., 2017), siblings with multifocal polymyoclonus and neurodevelopmental delay (Ngoh et al., 2017), brothers with infantile-onset myoclonic epilepsy (FIME) (Poulat et al., 2015), and two siblings with DFNB86 (Bakhchane et al., 2015). While the phenotypic expression held true between siblings, intrafamilial variance is significant. This may be due to the nature of the second pathogenic variant (Table 2) or genetic modifier genes elsewhere in the genome.

Tableau 2. – Genotype-phenotype comparison for p.Glu153Lys

Phenotype	**Variant 1**	**Variant 2**	**Reference**
AH, EPC	c.457G > A; p.Glu153Lys	c.116C > T; p.Ala39Val	Ragona et al., 2017
PME, DD	c.457G > A; p.Glu153Lys	c.545del; p.Thr182Serfs*6	Ngoh et al., 2017
FIMA, DD	c.457G > A; p.Glu153Lys	c.457G > A; p.Glu153Lys	Poulat et al., 2015
DFNB86	c.457G > A; p.Glu153Lys	c.641G>A; p.Arg214His	Bakhchane et al., 2015

AH, alternating hemiplegia; DD, developmental delay; EPC, epilepsia partialis continua; FIMA, familial infantile-onset myoclonic epilepsy; PME, progressive myoclonic epilepsy

The frameshift variant on the second allele may explain the more severe phenotype of progressive myoclonic epilepsy in the sibling pair described by Ngoh et al. as outlined above (Balestrini et al., 2016). Furthermore, the p.Arg214His variant identified in the siblings with non-syndromic hearing loss is a known single nucleotide polymorphism (SNP, rs200324356). It has an overall allele frequency of 0.01% with the highest allele frequency in the Ashkenazi Jewish population (0.04%) and an allele frequency of 0.03% in the African population (gnomAD

browser, https://gnomad.broadinstitute.org/). In their healthy Moroccan control samples, Bakhchane et al. calculated an even higher allele frequency of 2% and postulated that the p.Arg214His variant acts as a hypomorphic allele (Bakhchane et al., 2015). Functional studies on these missense variants are needed to conclusively explain phenotypic differences between individuals with the same missense variants.

Luthy et al. (2019) noted that all six patients with EPRPDC had pathogenic variants in the TLD_c domain between amino acids 500 and 511 and suggested an important role of these amino acid residues for the specific clinical phenotype. Two of their patients, both of Han Chinese ancestry, had the same compound heterozygous pathogenic variants, p.Ile81_Lys84del and p.Ala500Val. Two additional patients with the p.Ala500Val on one allele have been described in the literature. A 2-year old boy presented at three months of age with bilateral rhythmic, myoclonic seizures involving the peri-oral region and/or limbs that lasted up to 30 minutes and did not cause loss of consciousness (Balestrini et al., 2016). He was compound heterozygous with another missense variant on the second allele, p.Phe229Ser. He had normal neuroimaging and additional clinical features including hypotonia, nystagmus, and tongue fasciculations. He is not known for dystonia, but may have been too young to exhibit this phenotype at the time of follow-up since most patients started to experience EID after age 2 years (Guerrini et al., 1999; Luthy et al., 2019). A four year old girl was compound heterozygous for p.Ala500Val in combination with a frameshift variant, p.Ser473Argfs*43 (J. Li et al., 2018). Her presentation was more severe than the patients described by Luthy et al. (2019) with nonconvulsive status epilepticus (NCSE), cerebellar ataxia, ophthalmoplegia and significant global developmental delay. Her brain MRI was also abnormal and showed mild atrophic changes in both hemispheres, as well as the cerebellum. This is in contrast with the normal brain imaging in all six patients with EPRPDC (see above). It is possible that her more severe phenotype compared to the relatively benign seizures in EPRPDC is determined by the frameshift variant leading to a premature stop codon as outlined previously in this chapter.

Finally, in the Chinese patient cohort, Zhang et al. identified a common variant c.241_252del shared by nine of 19 patients suggesting a common ancestral variant in the East Asian population. Furthermore, six patients had the same c.116C > T (p.Ala39Val) variant. However, the authors noted differences in the patients' phenotypes regarding the severity of developmental delay, MRI findings and seizure type and were unable to determine any genotype-phenotype correlation (J. Zhang et al., 2019).

In summary, while phenotypic expressivity is consistent among affected family members, intrafamilial variance is significant. Although more and more patients with *TBC1D24*-related disorders are being identified, the location of a pathogenic variant cannot yet be used to precisely predict the expected phenotype, especially for missense variants.

Chapter 4 – A new microdeletion syndrome involving *TBC1D24, ATP6V0C* and *PDPK1* causes epilepsy, microcephaly and developmental delay

Explanation of author contributions to the manuscript (Mucha et al., 2019)

Bettina E. Mucha analyzed the clinical information of the individuals included in the study, evaluated the submitted images for dysmorphic features (independent of Philippe M. Campeau) and conducted the literature review of the individually discussed genes. She also wrote the complete first draft of the manuscript as well as subsequent versions including the final published manuscript after co-author feedback.

Norbert Fonya Ajeawung and Sirinart Molidperee performed *TBC1D24* Sanger sequencing for all individuals except individual 6 and *TBC1D24* genomic DNA real-time PCR for individual 2. Siddharth Banka, Mary Kay Koenig, Rhamat B Adejumo, Marianne Till, Michael Harbord, Renee Perrier, Emmanuelle Lemyre, Renee-Myriam Boucher, Brian G Skotko, Jessica L Waxler, Mary Ann Thomas, Jennelle C Hodge, Jozef Gecz, Jillian Nicholl, Lesley McGregor, Tobias Linden, Sanjay M Sisodiya, Damien Sanlaville, and Sau W Cheung provided clinical information and microarray data on the eight individuals included in the study. Gary G Chen provided analytic support on the microdeletion analysis. Philippe M. Campeau provided supervision and guidance throughout the project and served as the 2nd rater for dysmorphic features of the submitted patient images.

All co-authors reviewed the manuscript prior to publication.

Authors and affiliations

Bettina E Mucha[1], Siddharth Banka[2], Norbert Fonya Ajeawung[3], Sirinart Molidperee[3], Gary G Chen[4], Mary Kay Koenig[5], Rhamat B Adejumo[5], Marianne Till[6], Michael Harbord[7], Renee Perrier[8], Emmanuelle Lemyre[9], Renee-Myriam Boucher[10], Brian G Skotko[11], Jessica L Waxler[11], Mary Ann Thomas[8], Jennelle C Hodge[12], Jozef Gecz[13], Jillian Nicholl[14], Lesley McGregor[14], Tobias Linden[15], Sanjay M Sisodiya[16], Damien Sanlaville[6], Sau W Cheung[17], Carl Ernst[4], Philippe M Campeau[9]

1. Division of Medical Genetics, Department of Specialized Medicine, McGill University Health Centre, Montreal, QC, Canada.
2. Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, M13 9PL, Manchester, UK.
3. Centre de Recherche du CHU Sainte-Justine, Montreal, QC, Canada
4. Department of Psychiatry, McGill University, Montreal, Canada.
5. Department of Pediatrics, Division of Child & Adolescent Neurology, The University of Texas McGovern Medical School, Houston, Texas, USA.
6. Service de Génétique CHU de Lyon-GH Est, Lyon, France.
7. Department of Pediatrics, Flinders Medical Centre, Bedford Park, South Australia, Australia.
8. Department of Medical Genetics, University of Calgary, Calgary, AB, Canada.
9. Department of Pediatrics, Université de Montréal, Montreal, QC, Canada.
10. Department of Pediatrics, CHU Laval, Quebec City, QC, Canada.
11. Division of Medical Genetics, Department of Pediatrics, Massachusetts General Hospital, Boston, MA, USA.
12. Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA.
13. Adelaide Medical School and Robinson Research Institute, The University of Adelaide, Adelaide, Australia.
14. South Australian Clinical Genetics Service, SA Pathology, Women's and Children's

Hospital, Adelaide, Australia.
15. Klinik für Neuropädiatrie und Stoffwechselerkrankungen, Zentrum für Kinder- und Jugendmedizin, Oldenburg, Germany.
16. Department of Clinical and Experimental Epilepsy, Institute of Neurology, University College London, London, and Chalfont Centre for Epilepsy, Bucks, United Kingdom.
17. Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA.

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 (Campeau et al., 2014), and this was eventually noted in other families (detailed in Banuelos et al., 2017). 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 (Campeau et al., 2014). 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 (Kent et al., 2002). Haploinsufficiency scores (%HI) for the deleted genes were obtained from the DECIPHER database (Huang et al., 2010) (Supplementary Material). pLI scores were drawn from the ExAC database (Lek et al., 2016) (Supplementary Material). Modeling the probability of autosomal dominant inheritance P(AD) was done with the DOMINO tool (Quinodoz et al., 2017) (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 (<5th percentile) and a HC at the 2nd to 5th 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 25th 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 90th percentile. He was microcephalic with a HC at the 2nd 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 1st 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.

Figure 11. – Fig. 1: Four individuals with microdeletion 16p13.3 and mild dysmorphic features

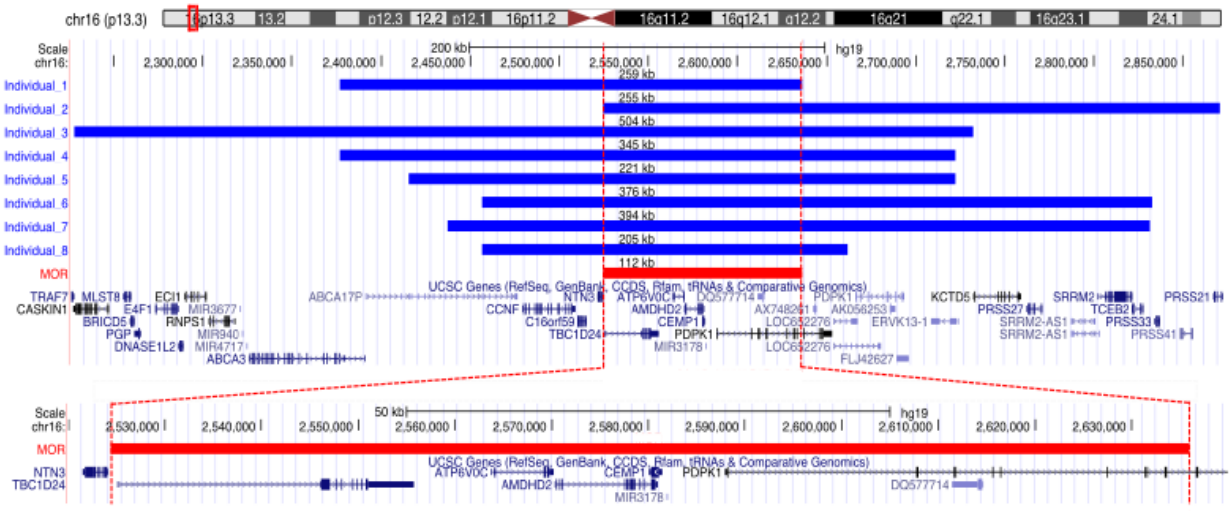
Individuals 1 (A, B), 5 (C, D), 6 (E, F), and 7 (G, H) from left to right; note the shared features in individuals 1, 5 and 6 as described in the text.

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.

Figure 12. – 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.



CMA identified overlapping microdeletions on the short arm of chromosome 16 (16p13.3; Fig. 2). There is no overlap with the 16p13.3 (Bartsch et al., 2006; Nelson, Quinonez, Ackley, Iyer, & Innis, 2011) and 16p11.2 (Bijlsma et al., 2009; Ghebranious, Giampietro, Wesbrook, & Rezkalla, 2007) 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).

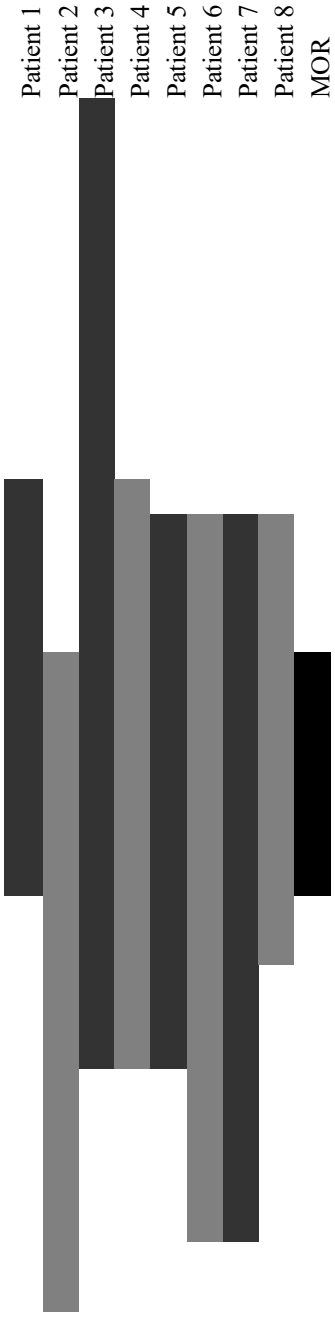
Tableau 3. – Table 1: Clinical information and deletion size on eight individuals with overlapping microdeletions of chromosome 16p13.3.

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

ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; DD, developmental delay; FFT, failure to thrive; ID, intellectual disability; Ind., individual; kb, kilobases.

Tableau 4. – 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

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).

Tableau 5. – 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

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

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 (Coe et al., 2014; Cooper et al., 2011; Männik et al., 2015). 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 (Coe et al., 2014)). The microdeletion was absent in two additional cohorts, one of 5,531 cases that were sent to a diagnostic laboratory for clinical testing (Vulto-van Silfhout et al., 2013) and one including 1,133 children with severe developmental disorders (Fitzgerald et al., 2015).

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 (Fischer et al., 2016). *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 (Mangieri et al., 2014). 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 (Chung et al., 2010).

PDPK1 (also known as PDK1) is a highly conserved protein kinase that is involved in many different signalling pathways (reviewed in (Gagliardi et al., 2015)). 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 (di Blasio et al., 2015). 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 (Bayascas et al., 2008). 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* (Zurashvili et al., 2013). 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) (Bergfeld, Pearce, Diaz, Pham, & Varki, 2012), a protein that is incorporated at low levels into the surface glycoproteins of several human tissues (Diaz et al., 2009). 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 (Alvarez-Pérez, Narayanan, Zeichner-David, Carmona, & Arzate, 2006). 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 (W. Li et al., 2015). 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 (Balestrini et al., 2016; Banuelos

et al., 2017; Campeau et al., 2014; Zara et al., 2000). 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 (Hirtz et al., 2007)) 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 (Falace et al., 2014). *PDPK1* is also involved in neuronal differentiation in mice (Zurashvili et al., 2013). The third candidate gene within the MOR, *ATP6V0C*, like *TBC1D24*, can regulate vesicular trafficking. While heterozygous *Atp6v0c* 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., 2000).

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 (Bowling et al., 2017; Carvill et al., 2014; Fitzgerald et al., 2015; Gilissen et al., 2014; Hamdan et al., 2017; Helbig et al., 2016; Study, 2017). Different *de novo* frameshift variants in *ATP6V0C* were found in one individual in a study performing exome sequencing of 80 patients with Dravet syndrome (Carvill et al., 2014) and in one individual from a cohort of 4,293 families undergoing exome sequencing for severe developmental delay (Study, 2017). 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 (Fitzgerald et al., 2015)). *De novo* variants in either gene were absent from other studies with cohort sizes ranging from 50 to 293 trios (Bowling et al., 2017; Gilissen et al., 2014; Hamdan et al., 2017) 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.

Chapter 5 – Discussion

Suggested initial management and follow-up of patients with *TBC1D24*-related disorders

While more and more clinical information becomes available on all aspects of *TBC1D24*-related phenotypes, patient cohorts are too small to determine formal management guidelines and best practices. However, the knowledge gained from the published literature as summarized in chapter 2 can be used to guide clinicians in charge of a newly diagnosed patient. This chapter will discuss possible aspects that clinicians should take into consideration when managing a patient with a *TBC1D24*-related disorder.

TBC1D24 sequencing and deletion/duplication testing has been included in many commercial genetic testing panels that target epilepsy, syndromic and non-syndromic hearing loss, or developmental delay and autism. These tests can be ordered by medical specialists other than clinical geneticists such as neurologists or developmental pediatricians. Once an individual is diagnosed with a *TBC1D24*-related disorder, e.g. in the context of seemingly isolated hearing loss or epilepsy, it is important to establish the extent of the disease. Given the significant overlap between *TBC1D24*-related epilepsy, hearing loss and developmental delay, the core evaluation of a newly diagnosed individual should at a minimum include an audiology evaluation and a thorough developmental assessment.

Although supportive literature is currently lacking, evaluation for other known complications of DOORS syndrome should be considered. Given the recurrence risk of 25% for this severe autosomal recessive disorder, a consultation with a clinical geneticist and/or genetic counselor early after diagnosis is essential, if not already done. A detailed physical exam by a clinical geneticist is also necessary to determine whether the individual has features of DOORS syndrome. This may help in determining the need and extent of subsequent investigations such as a detailed eye exam by an ophthalmologist, a dental examination, renal ultrasound and an echocardiogram. Since some patients with DOORS syndrome have been found to have a

predisposition to otitis media secondary to narrow ear canals (Campeau et al., 2014), the threshold for a referral to an otolaryngologist should be low. If not already done, the newly diagnosed patient should be referred for a neurology consultation, especially in the context of parental concerns for a possible seizure disorder.

Following the initial work-up, the identified medical concerns need to be addressed. Hearing aids should be considered as needed for hearing loss to promote speech development and improve communication. Cochlear implants at age one year have been beneficial in one individual with DOORS syndrome (Campeau et al., 2014). Longitudinal data on the risk of later development or progression of hearing loss is lacking and it appears prudent to conduct yearly audiology evaluations even if the initial hearing screen was normal. All individuals identified with hearing impairment and especially individuals heterozygous for the *TBC1D24* pathogenic variant known to cause autosomal dominant deafness should avoid excessive ambient noise as it may exacerbate hearing loss.

Visual impairment from high myopia or cataracts requires management by an ophthalmologist and congenital heart defects, although a rare feature in DOORS syndrome, should be managed by a pediatric cardiologist according to the underlying diagnosis. Possible renal anomalies include cystic dysplasia or aplasia. Referral to a nephrologist to monitor and protect renal function is essential.

Seizures require symptomatic pharmacologic management but may be difficult to control or treatment refractory. No controlled studies have compared the efficacy of different antiepileptic drugs in *TBC1D24*-related disorders and a variety of different anticonvulsive agents have been tried individually and in combination. In the cohort of 48 patients studied by Balestrini et al., the most successful option to reduce seizure activity was valproic acid combined with phenobarbital, but seizure control could only be achieved in 19 patients (Balestrini et al., 2016). In 14 of 19 patients with epilepsy, seizure activity was reduced by valproic acid and clobazam, but none of the patients in this group were completely seizure free (J. Zhang et al., 2019). All patients in the cohort studied by Zhang et al. had epilepsia partialis continua (EPC) which could be terminated by sleep or chloral hydrate. Overall, mortality in

patients with *TBC1D24*-related disorders, especially DOORS syndrome, is high (Balestrini et al., 2016; James et al., 2007) and reached 19% (nine of 48 patients) at an average age of 37 months in the cohort described by Balestrini et al. Two siblings out of 12 patients with DOORS syndrome died before age 1 years (Campeau et al., 2014). Complications leading to death are mostly linked to status epilepticus (two of 19 patients, Zhang et al. 2019), infections (four patients in Balestrini et al., 2016) and respiratory distress (James et al., 2007). Sudden unexpected death (SUDEP) has been suspected in at least three patients (Balestrini et al., 2016; Trivisano et al., 2017). The pathomechanism leading to SUDEP is unclear and no specific prevention measures exist.

Although brain MRI may be normal at the time of initial presentation, many individuals with severe forms of epilepsy develop cerebral and cerebellar atrophy with advancing age. However, structural brain abnormalities are rare and imaging studies are unlikely to contribute to the diagnosis and management of *TBC1D24*-related disorders. The risks and benefits of sedation to obtain a high-quality brain MRI in young children and individuals with intellectual disabilities or developmental delay have to be taken into account and the decision of pursuing brain imaging should be taken on a case by case basis by medical experts.

Developmental delay is highly prevalent in individuals with *TBC1D24*-related disorders and ranges from mild to severe, although some individuals with FIME are intellectually normal (Falace et al., 2010). Early intervention and rehabilitation by physiotherapy, speech and language pathologists and occupational therapists has been shown to improve outcomes in children with developmental delays of various etiologies and should be offered to individuals with *TBC1D24*-related disorders at risk for delays.

In summary, a cure or causal treatment for *TBC1D24*-related disorders is not available and all intervention has to remain symptomatic at this time.

16p13.3 microdeletion syndrome

Following the publication of eight patients with a microdeletion syndrome (see chapter 4) (Mucha et al., 2019), the effect of haploinsufficiency for *TBC1D24* was studied *in vivo* in a mouse model with a heterozygous copy of disrupted Tbc1d24 termed *Tbc1d24*^{tm1b} in collaboration with the Campeau and Rossignol labs at the CHUSJ (Finelli et al., 2019). *Tbc1d24*^{tm1b} mice developed normally in the early post-natal period and had a grossly normal brain anatomy compared to their wildtype litter mates. Hearing was not impaired. However, dendritic and axonal growth of neurons from the hippocampus and cortex was reduced after 5 days of *in vitro* culture. Furthermore, these cells also responded with an increased rate of cell death after oxidative stress compared to wildtype cells. When investigating synaptic function in hippocampal primary neurons from *Tbc1d24*^{tm1b} mice compared to wildtype controls, a significant difference in the frequency of miniature excitatory post-synaptic currents (mEPCSs) was detected with a 3-fold decrease, while synaptic charge and the density of synaptic contacts was unaltered indicating a selective defect in mEPSC frequency in *Tbc1d24*^{tm1b} neurons. The authors suggested that TBC1D24 has an important function in maintaining normal activity of excitatory nerve terminals. Additional experiments in the presynaptic neurons showed a significantly slower endocytic process after stimulation of *Tbc1d24*^{tm1b} neurons compared to wildtype and a defect in recycling during stimulation. The authors concluded that their results "from the *Tbc1d24tm1b* model of TBC1D24 haploinsufficiency indicate an important function for the gene in neuronal development and survival alongside a critical role in presynaptic SV recycling in mammalian neurons" (Finelli et al., 2019). The results from this study also further support our own conclusion that the 16p13.3 microdeletions resulting in simultaneous haploinsufficiencies of *TBC1D24*, *ATP6V0C* and *PDPK1* are the underlying etiology for the phenotypes observed in the patient cohort described in chapter 4.

Chapter 6 – Conclusion

TBC1D24-related disorders may be rare, but emerging knowledge about the great diversity of phenotypic presentations allows a glimpse at the complex function of the TBC1D24 protein. Multiple studies in diverse animal models have started to contribute pieces of the puzzle and hint at the different roles that TBC1D24 plays in different tissues at different times of development. However, additional studies are needed and may be guided by the features observed in the human disease phenotypes. In turn, the results from *in vitro* and *in vivo* studies may help guide treatment decisions for children and adults with severe neurological presentations and difficult to treat epilepsy in the future.

Bibliography

Afawi, Z., Mandelstam, S., Korczyn, A. D., Kivity, S., Walid, S., Shalata, A., . . . Jackson, G. D. (2013). TBC1D24 mutation associated with focal epilepsy, cognitive impairment and a distinctive cerebro-cerebellar malformation. *Epilepsy Res, 105*(1-2), 240-244. doi:10.1016/j.eplepsyres.2013.02.005

Alvarez-Pérez, M. A., Narayanan, S., Zeichner-David, M., Carmona, B. R., & Arzate, H. (2006). Molecular cloning, expression and immunolocalization of a novel human cementum-derived protein (CP-23). *Bone, 38*(3), 409-419.

Aprile, D., Fruscione, F., Baldassari, S., Fadda, M., Ferrante, D., Falace, A., . . . Fassio, A. (2019). TBC1D24 regulates axonal outgrowth and membrane trafficking at the growth cone in rodent and human neurons. *Cell Death Differ, 26*(11), 2464-2478. doi:10.1038/s41418-019-0313-x

Azaiez, H., Booth, K. T., Bu, F., Huygen, P., Shibata, S. B., Shearer, A. E., . . . Smith, R. J. (2014). TBC1D24 mutation causes autosomal-dominant nonsyndromic hearing loss. *Hum Mutat, 35*(7), 819-823. doi:10.1002/humu.22557

Bakhchane, A., Charif, M., Salime, S., Boulouiz, R., Nahili, H., Roky, R., . . . Barakat, A. (2015). Recessive TBC1D24 Mutations Are Frequent in Moroccan Non-Syndromic Hearing Loss Pedigrees. *PloS one, 10*(9), e0138072. doi:10.1371/journal.pone.0138072

Balestrini, S., Milh, M., Castiglioni, C., Luthy, K., Finelli, M. J., Verstreken, P., . . . Sisodiya, S. M. (2016). TBC1D24 genotype-phenotype correlation: Epilepsies and other neurologic features. *Neurology, 87*(1), 77-85. doi:10.1212/wnl.0000000000002807

Banuelos, E., Ramsey, K., Belnap, N., Krishnan, M., Balak, C., Szelinger, S., . . . Schrauwen, I. (2017). Case Report: Novel mutations in TBC1D24 are associated with autosomal dominant tonic-clonic and myoclonic epilepsy and recessive Parkinsonism, psychosis, and intellectual disability. *F1000Res, 6*, 553. doi:10.12688/f1000research.10588.1

Bartsch, O., Rasi, S., Delicado, A., Dyack, S., Neumann, L. M., Seemanova, E., . . . Kalscheuer, V. M. (2006). Evidence for a new contiguous gene syndrome, the chromosome 16p13.3 deletion syndrome alias severe Rubinstein-Taybi syndrome. *Hum Genet, 120*(2), 179-186. doi:10.1007/s00439-006-0215-0

Bayascas, J. R., Wullschleger, S., Sakamoto, K., García-Martínez, J. M., Clacher, C., Komander, D., . . . Lipina, C. (2008). Mutation of the PDK1 PH domain inhibits protein kinase B/Akt, leading to small size and insulin resistance. *Mol Cell Biol, 28*(10), 3258-3272.

Berg, A. T., Berkovic, S. F., Brodie, M. J., Buchhalter, J., Cross, J. H., van Emde Boas, W., . . . Scheffer, I. E. (2010). Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia, 51*(4), 676-685. doi:10.1111/j.1528-1167.2010.02522.x

Bergfeld, A. K., Pearce, O. M., Diaz, S. L., Pham, T., & Varki, A. (2012). Metabolism of Vertebrate Amino Sugars with N-Glycolyl Groups ELUCIDATING THE INTRACELLULAR FATE OF THE NON-HUMAN SIALIC ACID N-GLYCOLYLNEURAMINIC ACID. *Journal of Biological Chemistry, 287*(34), 28865-28881.

Bijlsma, E., Gijsbers, A., Schuurs-Hoeijmakers, J., Van Haeringen, A., van de Putte, D. F., Anderlid, B.-M., . . . Delle Chiaie, B. (2009). Extending the phenotype of recurrent rearrangements of 16p11. 2: deletions in mentally retarded patients without autism and in normal individuals. *European journal of medical genetics, 52*(2), 77-87.

Bowling, K. M., Thompson, M. L., Amaral, M. D., Finnila, C. R., Hiatt, S. M., Engel, K. L., . . . Gray, D. E. (2017). Genomic diagnosis for children with intellectual disability and/or developmental delay. *Genome medicine, 9*(1), 43.

Campeau, P. M., & Hennekam, R. C. (2014). DOORS syndrome: phenotype, genotype and comparison with Coffin-Siris syndrome. *Am J Med Genet C Semin Med Genet, 166c*(3), 327-332. doi:10.1002/ajmg.c.31412

Campeau, P. M., Kasperaviciute, D., Lu, J. T., Burrage, L. C., Kim, C., Hori, M., . . . Sisodiya, S. M. (2014). The genetic basis of DOORS syndrome: an exome-sequencing study. *Lancet Neurol, 13*(1), 44-58. doi:10.1016/s1474-4422(13)70265-5

Carvill, G. L., Weckhuysen, S., McMahon, J. M., Hartmann, C., Møller, R. S., Hjalgrim, H., . . . Petrou, S. (2014). GABRA1 and STXBP1: novel genetic causes of Dravet syndrome. *Neurology, 82*(14), 1245-1253.

Cho, K. S., Lee, J. H., Kim, S., Kim, D., Koh, H., Lee, J., . . . Chung, J. (2001). Drosophila phosphoinositide-dependent kinase-1 regulates apoptosis and growth via the phosphoinositide 3-kinase-dependent signaling pathway. *Proc Natl Acad Sci U S A, 98*(11), 6144-6149. doi:10.1073/pnas.101596998

Chung, A. Y., Kim, M. J., Kim, D., Bang, S., Hwang, S. W., Lim, C. S., . . . Huh, T. L. (2010). Neuron-specific expression of atp6v0c2 in zebrafish CNS. *Developmental Dynamics, 239*(9), 2501-2508.

Coe, B. P., Witherspoon, K., Rosenfeld, J. A., Van Bon, B. W., Vulto-van Silfhout, A. T., Bosco, P., . . . Vissers, L. E. (2014). Refining analyses of copy number variation identifies specific genes associated with developmental delay. *Nature genetics, 46*(10), 1063.

Cooper, G. M., Coe, B. P., Girirajan, S., Rosenfeld, J. A., Vu, T. H., Baker, C., . . . Hannig, V. (2011). A copy number variation morbidity map of developmental delay. *Nature genetics, 43*(9), 838.

Coppola, A., Cellini, E., Stamberger, H., Saarentaus, E., Cetica, V., Lal, D., . . . Marini, C. (2019). Diagnostic implications of genetic copy number variation in epilepsy plus. *Epilepsia, 60*(4), 689-706. doi:10.1111/epi.14683

Corbett, M. A., Bahlo, M., Jolly, L., Afawi, Z., Gardner, A. E., Oliver, K. L., . . . Gecz, J. (2010). A focal epilepsy and intellectual disability syndrome is due to a mutation in TBC1D24. *Am J Hum Genet, 87*(3), 371-375. doi:10.1016/j.ajhg.2010.08.001

Danial-Farran, N., Brownstein, Z., Gulsuner, S., Tammer, L., Khayat, M., Aleme, O., . . . Shalev, S. A. (2018). Genetics of hearing loss in the Arab population of Northern Israel. *Eur J Hum Genet, 26*(12), 1840-1847. doi:10.1038/s41431-018-0218-z

de Falco, F. A., Majello, L., Santangelo, R., Stabile, M., Bricarelli, F. D., & Zara, F. (2001). Familial infantile myoclonic epilepsy: clinical features in a large kindred with autosomal recessive inheritance. *Epilepsia, 42*(12), 1541-1548. doi:10.1046/j.1528-1157.2001.26701.x

de Kovel, C. G., Brilstra, E. H., van Kempen, M. J., Van't Slot, R., Nijman, I. J., Afawi, Z., . . . Koeleman, B. P. (2016). Targeted sequencing of 351 candidate genes for epileptic

encephalopathy in a large cohort of patients. *Mol Genet Genomic Med, 4*(5), 568-580. doi:10.1002/mgg3.235

di Blasio, L., Gagliardi, P. A., Puliafito, A., Sessa, R., Seano, G., Bussolino, F., & Primo, L. (2015). PDK1 regulates focal adhesion disassembly by modulating endocytosis of alphavbeta3 integrin. *J Cell Sci, 128*(5), 863-877. doi:10.1242/jcs.149294

Diaz, S. L., Padler-Karavani, V., Ghaderi, D., Hurtado-Ziola, N., Yu, H., Chen, X., . . . Varki, N. M. (2009). Sensitive and specific detection of the non-human sialic Acid N-glycolylneuraminic acid in human tissues and biotherapeutic products. *PloS one, 4*(1), e4241.

Duru, N., Iseri, S. A., Selcuk, N., & Tolun, A. (2010). Early-onset progressive myoclonic epilepsy with dystonia mapping to 16pter-p13.3. *J Neurogenet, 24*(4), 207-215. doi:10.3109/01677063.2010.514368

Ellis, C. A., Petrovski, S., & Berkovic, S. F. (2020). Epilepsy genetics: clinical impacts and biological insights. *Lancet Neurol, 19*(1), 93-100. doi:10.1016/s1474-4422(19)30269-8

Ellis, K., Bagwell, J., & Bagnat, M. (2013). Notochord vacuoles are lysosome-related organelles that function in axis and spine morphogenesis. *J Cell Biol, 200*(5), 667-679. doi:10.1083/jcb.201212095

Falace, A., Buhler, E., Fadda, M., Watrin, F., Lippiello, P., Pallesi-Pocachard, E., . . . Cardoso, C. (2014). TBC1D24 regulates neuronal migration and maturation through modulation of the ARF6-dependent pathway. *Proc Natl Acad Sci U S A, 111*(6), 2337-2342. doi:10.1073/pnas.1316294111

Falace, A., Filipello, F., La Padula, V., Vanni, N., Madia, F., De Pietri Tonelli, D., . . . Zara, F. (2010). TBC1D24, an ARF6-interacting protein, is mutated in familial infantile myoclonic epilepsy. *Am J Hum Genet, 87*(3), 365-370. doi:10.1016/j.ajhg.2010.07.020

Finelli, M. J., Aprile, D., Castroflorio, E., Jeans, A., Moschetta, M., Chessum, L., . . . Oliver, P. L. (2019). The epilepsy-associated protein TBC1D24 is required for normal development, survival and vesicle trafficking in mammalian neurons. *Hum Mol Genet, 28*(4), 584-597. doi:10.1093/hmg/ddy370

Finelli, M. J., Sanchez-Pulido, L., Liu, K. X., Davies, K. E., & Oliver, P. L. (2016). The Evolutionarily Conserved Tre2/Bub2/Cdc16 (TBC), Lysin Motif (LysM), Domain Catalytic (TLDc) Domain Is Neuroprotective against Oxidative Stress. *J Biol Chem, 291*(6), 2751-2763. doi:10.1074/jbc.M115.685222

Fischer, B., Luthy, K., Paesmans, J., De Koninck, C., Maes, I., Swerts, J., . . . Versees, W. (2016). Skywalker-TBC1D24 has a lipid-binding pocket mutated in epilepsy and required for synaptic function. *Nat Struct Mol Biol, 23*(11), 965-973. doi:10.1038/nsmb.3297

Fitzgerald, T., Gerety, S., Jones, W., Van Kogelenberg, M., King, D., McRae, J., . . . Ambridge, K. (2015). Large-scale discovery of novel genetic causes of developmental disorders. *Nature, 519*(7542), 223.

Gagliardi, P. A., di Blasio, L., & Primo, L. (2015). PDK1: A signaling hub for cell migration and tumor invasion. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 1856*(2), 178-188.

Ghebranious, N., Giampietro, P. F., Wesbrook, F. P., & Rezkalla, S. H. (2007). A novel microdeletion at 16p11. 2 harbors candidate genes for aortic valve development, seizure

disorder, and mild mental retardation. *American Journal of Medical Genetics Part A, 143*(13), 1462-1471.

Gilissen, C., Hehir-Kwa, J. Y., Thung, D. T., van de Vorst, M., van Bon, B. W., Willemsen, M. H., . . . Schenck, A. (2014). Genome sequencing identifies major causes of severe intellectual disability. *Nature, 511*(7509), 344.

Guerrini, R., Bonanni, P., Nardocci, N., Parmeggiani, L., Piccirilli, M., De Fusco, M., . . . Casari, G. (1999). Autosomal recessive rolandic epilepsy with paroxysmal exercise-induced dystonia and writer's cramp: delineation of the syndrome and gene mapping to chromosome 16p12-11.2. *Ann Neurol, 45*(3), 344-352. doi:10.1002/1531-8249(199903)45:3<344::aid-ana10>3.0.co;2-9

Guven, A., & Tolun, A. (2013). TBC1D24 truncating mutation resulting in severe neurodegeneration. *J Med Genet, 50*(3), 199-202. doi:10.1136/jmedgenet-2012-101313

Hamdan, F. F., Myers, C. T., Cossette, P., Lemay, P., Spiegelman, D., Laporte, A. D., . . . Cadieux-Dion, M. (2017). High rate of recurrent de novo mutations in developmental and epileptic encephalopathies. *The American Journal of Human Genetics, 101*(5), 664-685.

Hebbar, M., & Mefford, H. C. (2020). Recent advances in epilepsy genomics and genetic testing. *F1000Res, 9*. doi:10.12688/f1000research.21366.1

Helbig, K. L., Hagman, K. D. F., Shinde, D. N., Mroske, C., Powis, Z., Li, S., . . . Helbig, I. (2016). Diagnostic exome sequencing provides a molecular diagnosis for a significant proportion of patients with epilepsy. *Genetics in Medicine, 18*(9), 898.

Hirtz, D., Thurman, D., Gwinn-Hardy, K., Mohamed, M., Chaudhuri, A., & Zalutsky, R. (2007). How common are the “common” neurologic disorders? *Neurology, 68*(5), 326-337.

Huang, N., Lee, I., Marcotte, E. M., & Hurles, M. E. (2010). Characterising and predicting haploinsufficiency in the human genome. *PLoS genetics, 6*(10), e1001154.

Inoue, H., Noumi, T., Nagata, M., Murakami, H., & Kanazawa, H. (1999). Targeted disruption of the gene encoding the proteolipid subunit of mouse vacuolar H+-ATPase leads to early embryonic lethality. *Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1413*(3), 130-138.

James, A. W., Miranda, S. G., Culver, K., Hall, B. D., & Golabi, M. (2007). DOOR syndrome: clinical report, literature review and discussion of natural history. *Am J Med Genet A, 143a*(23), 2821-2831. doi:10.1002/ajmg.a.32054

Kent, W. J., Sugnet, C. W., Furey, T. S., Roskin, K. M., Pringle, T. H., Zahler, A. M., & Haussler, D. (2002). The human genome browser at UCSC. *Genome research, 12*(6), 996-1006.

Lawlor, M. A., Mora, A., Ashby, P. R., Williams, M. R., Murray-Tait, V., Malone, L., . . . Alessi, D. R. (2002). Essential role of PDK1 in regulating cell size and development in mice. *Embo j, 21*(14), 3728-3738. doi:10.1093/emboj/cdf387

Lek, M., Karczewski, K. J., Minikel, E. V., Samocha, K. E., Banks, E., Fennell, T., . . . MacArthur, D. G. (2016). Analysis of protein-coding genetic variation in 60,706 humans. *Nature, 536*(7616), 285-291. doi:10.1038/nature19057

Li, J., Liu, R., Feng, H., Zhang, J., Wang, D., Wang, Y., . . . Fan, Y. (2018). Novel TBC1D24 Mutations in a Case of Nonconvulsive Status Epilepticus. *Front Neurol, 9*, 623. doi:10.3389/fneur.2018.00623

Li, W., Shen, S., Wu, S., Chen, Z., Hu, C., & Yan, R. (2015). Regulation of tumorigenesis and metastasis of hepatocellular carcinoma tumor endothelial cells by microRNA-3178 and

underlying mechanism. *Biochemical and biophysical research communications, 464*(3), 881-887.

Lin, L., Lyu, Q., Kwan, P. Y., Zhao, J., Fan, R., Chai, A., . . . Lai, K. O. (2020). The epilepsy and intellectual disability-associated protein TBC1D24 regulates the maintenance of excitatory synapses and animal behaviors. *PLoS Genet, 16*(1), e1008587. doi:10.1371/journal.pgen.1008587

Lozano, R., Herman, K., Rothfuss, M., Rieger, H., Bayrak-Toydemir, P., Aprile, D., . . . Fassio, A. (2016). Clinical intrafamilial variability in lethal familial neonatal seizure disorder caused by TBC1D24 mutations. *Am J Med Genet A, 170*(12), 3207-3214. doi:10.1002/ajmg.a.37933

Luthy, K., Mei, D., Fischer, B., De Fusco, M., Swerts, J., Paesmans, J., . . . Guerrini, R. (2019). TBC1D24-TLDc-related epilepsy exercise-induced dystonia: rescue by antioxidants in a disease model. *Brain, 142*(8), 2319-2335. doi:10.1093/brain/awz175

Mangieri, L. R., Mader, B. J., Thomas, C. E., Taylor, C. A., Luker, A. M., Tonia, E. T., . . . Shacka, J. J. (2014). ATP6V0C knockdown in neuroblastoma cells alters autophagy-lysosome pathway function and metabolism of proteins that accumulate in neurodegenerative disease. *PLoS One, 9*(4), e93257.

Männik, K., Mägi, R., Macé, A., Cole, B., Guyatt, A. L., Shihab, H. A., . . . Reigo, A. (2015). Copy number variations and cognitive phenotypes in unselected populations. *Jama, 313*(20), 2044-2054.

McTague, A., Howell, K. B., Cross, J. H., Kurian, M. A., & Scheffer, I. E. (2016). The genetic landscape of the epileptic encephalopathies of infancy and childhood. *Lancet Neurol, 15*(3), 304-316. doi:10.1016/s1474-4422(15)00250-1

Mucha, B. E., Banka, S., Ajeawung, N. F., Molidperee, S., Chen, G. G., Koenig, M. K., . . . Campeau, P. M. (2019). A new microdeletion syndrome involving TBC1D24, ATP6V0C, and PDPK1 causes epilepsy, microcephaly, and developmental delay. *Genet Med, 21*(5), 1058-1064. doi:10.1038/s41436-018-0290-3

Mucha, B. E., Hennekam, R. C. M., Sisodiya, S., & Campeau, P. M. (2017). TBC1D24-Related Disorders. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews((R))*. Seattle (WA): University of Washington, Seattle

Muona, M., Berkovic, S. F., Dibbens, L. M., Oliver, K. L., Maljevic, S., Bayly, M. A., . . . Lehesjoki, A. E. (2015). A recurrent de novo mutation in KCNC1 causes progressive myoclonus epilepsy. *Nat Genet, 47*(1), 39-46. doi:10.1038/ng.3144

Murphy, C. T., & Hu, P. J. (2013). Insulin/insulin-like growth factor signaling in C. elegans. *WormBook*, 1-43. doi:10.1895/wormbook.1.164.1

Nelson, M., Quinonez, S., Ackley, T., Iyer, R. K., & Innis, J. W. (2011). Multiple congenital anomalies and developmental delay in a boy associated with a de novo 16p13.3 deletion. *Am J Med Genet A, 155A*(3), 612-617. doi:10.1002/ajmg.a.33808

Ngoh, A., Bras, J., Guerreiro, R., McTague, A., Ng, J., Meyer, E., . . . Kurian, M. A. (2017). TBC1D24 Mutations in a Sibship with Multifocal Polymyoclonus. *Tremor Other Hyperkinet Mov (N Y), 7*, 452. doi:10.7916/d8q52vbv

Nolan, D., & Fink, J. (2018). Genetics of epilepsy. *Handb Clin Neurol, 148*, 467-491. doi:10.1016/b978-0-444-64076-5.00030-2

Nomura, T., Koyama, N., Yokoyama, M., Awaya, A., & Yokochi, K. (2009). DOOR syndrome concomitant with non-convulsive status epilepticus and hyperintense cerebellar cortex on T2-weighted imaging. *Brain Dev, 31*(1), 75-78. doi:10.1016/j.braindev.2008.03.006

Nuckels, R. J., Ng, A., Darland, T., & Gross, J. M. (2009). The vacuolar-ATPase complex regulates retinoblast proliferation and survival, photoreceptor morphogenesis, and pigmentation in the zebrafish eye. *Invest Ophthalmol Vis Sci, 50*(2), 893-905. doi:10.1167/iovs.08-2743

Paradis, S., Ailion, M., Toker, A., Thomas, J. H., & Ruvkun, G. (1999). A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in Caenorhabditis elegans. *Genes Dev, 13*(11), 1438-1452. doi:10.1101/gad.13.11.1438

Peron, A., Baratang, N. V., Canevini, M. P., Campeau, P. M., & Vignoli, A. (2018). Hot water epilepsy and SYN1 variants. *Epilepsia, 59*, 2162-2165.

Poulat, A. L., Ville, D., de Bellescize, J., Andre-Obadia, N., Cacciagli, P., Milh, M., . . . Lesca, G. (2015). Homozygous TBC1D24 mutation in two siblings with familial infantile myoclonic epilepsy (FIME) and moderate intellectual disability. *Epilepsy Res, 111*, 72-77. doi:10.1016/j.eplepsyres.2015.01.008

Quinodoz, M., Royer-Bertrand, B., Cisarova, K., Di Gioia, S. A., Superti-Furga, A., & Rivolta, C. (2017). DOMINO: Using Machine Learning to Predict Genes Associated with Dominant Disorders. *The American Journal of Human Genetics, 101*(4), 623-629.

Ragona, F., Castellotti, B., Salis, B., Magri, S., DiFrancesco, J. C., Nardocci, N., . . . Granata, T. (2017). Alternating Hemiplegia and Epilepsia Partialis Continua: A new phenotype for a novel compound TBC1D24 mutation. *Seizure, 47*, 71-73. doi:10.1016/j.seizure.2017.03.003

Rehman, A. U., Santos-Cortez, R. L., Morell, R. J., Drummond, M. C., Ito, T., Lee, K., . . . Friedman, T. B. (2014). Mutations in TBC1D24, a gene associated with epilepsy, also cause nonsyndromic deafness DFNB86. *Am J Hum Genet, 94*(1), 144-152. doi:10.1016/j.ajhg.2013.12.004

Rintelen, F., Stocker, H., Thomas, G., & Hafen, E. (2001). PDK1 regulates growth through Akt and S6K in Drosophila. *Proc Natl Acad Sci U S A, 98*(26), 15020-15025. doi:10.1073/pnas.011318098

Santos, M., Reis-Rego, A., Coutinho, M., & Almeida, E. S. C. (2019). DOOR syndrome: A case report and its embryological basis. *Int J Pediatr Otorhinolaryngol, 117*, 57-60. doi:10.1016/j.ijporl.2018.11.015

Scheffer, I. E., French, J., Hirsch, E., Jain, S., Mathern, G. W., Moshé, S. L., . . . Zuberi, S. M. (2016). Classification of the epilepsies: New concepts for discussion and debate-Special report of the ILAE Classification Task Force of the Commission for Classification and Terminology. *Epilepsia Open, 1*(1-2), 37-44. doi:10.1002/epi4.5

Steinlein, O. K., Mulley, J. C., Propping, P., Wallace, R. H., Phillips, H. A., Sutherland, G. R., . . . Berkovic, S. F. (1995). A missense mutation in the neuronal nicotinic acetylcholine

receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nat Genet, 11*(2), 201-203. doi:10.1038/ng1095-201

Strazisar, B. G., Neubauer, D., Paro Panjan, D., & Writzl, K. (2015). Early-onset epileptic encephalopathy with hearing loss in two siblings with TBC1D24 recessive mutations. *Eur J Paediatr Neurol, 19*(2), 251-256. doi:10.1016/j.ejpn.2014.12.011

Study, T. D. D. D. (2017). Prevalence and architecture of de novo mutations in developmental disorders. *Nature, 542*(7642), 433.

Sun-Wada, G.-H., Murata, Y., Yamamoto, A., Kanazawa, H., Wada, Y., & Futai, M. (2000). Acidic endomembrane organelles are required for mouse postimplantation development. *Developmental biology, 228*(2), 315-325.

Symonds, J. D., & McTague, A. (2020). Epilepsy and developmental disorders: Next generation sequencing in the clinic. *Eur J Paediatr Neurol, 24*, 15-23. doi:10.1016/j.ejpn.2019.12.008

Tognon, E., Kobia, F., Busi, I., Fumagalli, A., De Masi, F., & Vaccari, T. (2016). Control of lysosomal biogenesis and Notch-dependent tissue patterning by components of the TFEB-V-ATPase axis in Drosophila melanogaster. *Autophagy, 12*(3), 499-514. doi:10.1080/15548627.2015.1134080

Tona, R., Chen, W., Nakano, Y., Reyes, L. D., Petralia, R. S., Wang, Y. X., . . . Friedman, T. B. (2019). The phenotypic landscape of a Tbc1d24 mutant mouse includes convulsive seizures resembling human early infantile epileptic encephalopathy. *Hum Mol Genet, 28*(9), 1530-1547. doi:10.1093/hmg/ddy445

Trivisano, M., Bellusci, M., Terracciano, A., De Palma, L., Pietrafusa, N., Valeriani, M., . . . Specchio. (2017). TBC1D24 gene mutations are associated with high risk of sudden unexpected death. *Epilepsy Behav, 72*, 208-209. doi:10.1016/j.yebeh.2017.04.010

Uytterhoeven, V., Kuenen, S., Kasprowicz, J., Miskiewicz, K., & Verstreken, P. (2011). Loss of skywalker reveals synaptic endosomes as sorting stations for synaptic vesicle proteins. *Cell, 145*(1), 117-132. doi:10.1016/j.cell.2011.02.039

Verbeek, N. E., Wassenaar, M., van Campen, J. S., Sonsma, A., Gunning, B., Knoers, N., . . . Kasteleijn-Nolst Trenité, D. (2015). Seizure precipitants in Dravet syndrome: What events and activities are specifically provocative compared with other epilepsies? *Epilepsy Behav, 47*, 39-44. doi:10.1016/j.yebeh.2015.05.008

Vulto-van Silfhout, A. T., Hehir-Kwa, J. Y., Bon, B. W., Schuurs-Hoeijmakers, J. H., Meader, S., Hellebrekers, C. J., . . . Webber, C. (2013). Clinical significance of de novo and inherited copy-number variation. *Human mutation, 34*(12), 1679-1687.

Yoon, J., Hwang, Y. S., Lee, M., Sun, J., Cho, H. J., Knapik, L., & Daar, I. O. (2018). TBC1d24-ephrinB2 interaction regulates contact inhibition of locomotion in neural crest cell migration. *Nat Commun, 9*(1), 3491. doi:10.1038/s41467-018-05924-9

Zara, F., Gennaro, E., Stabile, M., Carbone, I., Malacarne, M., Majello, L., . . . Bricarelli, F. D. (2000). Mapping of a locus for a familial autosomal recessive idiopathic myoclonic epilepsy of infancy to chromosome 16p13. *Am J Hum Genet, 66*(5), 1552-1557. doi:10.1086/302876

Zhang, J., Chen, J., Zeng, Q., Zhang, L., Tian, X., Yang, X., . . . Zhang, Y. (2019). Infantile epilepsy with multifocal myoclonus caused by TBC1D24 mutations. *Seizure, 69*, 228-234. doi:10.1016/j.seizure.2019.05.010

Zhang, L., Hu, L., Chai, Y., Pang, X., Yang, T., & Wu, H. (2014). A dominant mutation in the stereocilia-expressing gene TBC1D24 is a probable cause for nonsyndromic hearing impairment. *Hum Mutat, 35*(7), 814-818. doi:10.1002/humu.22558

Zurashvili, T., Cordón-Barris, L., Ruiz-Babot, G., Zhou, X., Lizcano, J. M., Gómez, N., . . . Bayascas, J. R. (2013). Interaction of PDK1 with phosphoinositides is essential for neuronal differentiation but dispensable for neuronal survival. *Molecular and cellular biology, 33*(5), 1027-1040.

Annexes

Annex I: *TBC1D24*-Related Disorders

Annex II: A New Microdeletion Syndrome Involving *TBC1D24*, *ATP6V0C*, and *PDPK1* Causes Epilepsy, Microcephaly, and Developmental Delay

Annex III: Supplementary material to annex II

NCBI Bookshelf. A service of the National Library of Medicine, National Institutes of Health.

Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2020.

TBC1D24-Related Disorders

Bettina E Mucha, MD
Medical Genetics Service
Sainte-Justine Hospital
Montreal, Canada
Email: bettina.elizabe.mucha-le.ny@umontreal.ca

Raoul CM Hennekam, MD, PhD
University of Amsterdam
Amsterdam, Netherlands
Email: r.c.hennekam@amc.uva.nl

Sanjay Sisodiya, MD, PhD
University College London
London, United Kingdom
Email: s.sisodiya@ucl.ac.uk

Philippe M Campeau, MD
Medical Genetics Service
Sainte-Justine Hospital
Montreal, Canada
Email: p.campeau@umontreal.ca

Initial Posting: February 26, 2015; Last Update: December 7, 2017.

Estimated reading time: 23 minutes

Summary

Clinical characteristics. ***TBC1D24***-related disorders comprise a continuum of features that were originally described as distinct, recognized phenotypes:

- DOORS syndrome (*d*eafness, *o*nychodystrophy, *o*steodystrophy, mental *r*etardation, and *s*eizures). Profound sensorineural hearing loss, onychodystrophy, osteodystrophy, intellectual disability / developmental delay, and seizures
- Familial infantile myoclonic epilepsy (FIME). Early-onset myoclonic seizures, focal epilepsy, dysarthria, and mild-to-moderate intellectual disability
- Progressive myoclonus epilepsy (PME). Action myoclonus, tonic-clonic seizures, progressive neurologic decline, and ataxia
- Early-infantile epileptic encephalopathy 16 (EIEE16). Epileptiform EEG abnormalities which themselves are believed to contribute to progressive disturbance in cerebral function
- Autosomal recessive nonsyndromic hearing loss, DFNB86. Profound prelingual deafness
- Autosomal dominant nonsyndromic hearing loss, DFNA65. Slowly progressive deafness with onset in the third decade, initially affecting the high frequencies

Diagnosis/testing. The diagnosis of a ***TBC1D24***-related disorder is established in an individual with biallelic ***TBC1D24*** pathogenic variants when the mode of inheritance is autosomal recessive (i.e., DOORS syndrome, FIME, PME, EIEE16, and DFNB86), and in an individual with a heterozygous ***TBC1D24*** pathogenic variant when the mode of inheritance is autosomal dominant (DFNA65).

Management. *Treatment of manifestations:* Hearing aids or cochlear implants as needed for hearing loss; early educational intervention and physical, occupational, and speech therapy for developmental delay; symptomatic

pharmacologic management for seizures; routine management of visual impairment and renal and cardiac anomalies.

Surveillance: Neurology evaluations with EEGs depending on seizure frequency and/or progression of clinical manifestations; yearly audiologic evaluation to assess for possible progression of hearing loss and/or the efficacy of hearing aids; yearly dental evaluation.

Agents/circumstances to avoid: Excessive ambient noise, which may exacerbate hearing loss in heterozygotes for a ***TBC1D24*** pathogenic variant that causes DFNA65.

Evaluation of relatives at risk: Molecular genetic testing for the familial ***TBC1D24*** pathogenic variant(s) in older and younger sibs of a proband in order to identify as early as possible those who would benefit from early treatment of seizures and/or hearing loss.

Genetic counseling. Most ***TBC1D24***-related disorders are inherited in an autosomal recessive manner (DOORS syndrome, FIME, PME, EIEE16, and DFNB86). For autosomal recessive inheritance: At conception, each sib of an affected individual has a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier. Carrier testing for at-risk relatives requires prior identification of the ***TBC1D24*** pathogenic variants in the family. Prenatal testing is possible for pregnancies at increased risk if the ***TBC1D24*** pathogenic variants have been identified in an affected family member.

GeneReview Scope

***TBC1D24*-Related Disorders: Included Phenotypes** [1]
• DOORS syndrome • Familial infantile myoclonic epilepsy (FIME) • Progressive myoclonus epilepsy (PME) • Early-infantile epileptic encephalopathy 16 (EIEE16) • Autosomal recessive nonsyndromic hearing loss, DFNB86 • Autosomal dominant nonsyndromic hearing loss, DFNA65

For synonyms and outdated names see Nomenclature.

1. For other genetic causes of these phenotypes see Differential Diagnosis.

Diagnosis

TBC1D24-related disorders comprise a continuum of recognized phenotypes:

- DOORS syndrome (*d*eafness, *o*nychodystrophy, *o*steodystrophy, mental *r*etardation, and *s*eizures)
- Familial infantile myoclonic epilepsy (FIME)
- Progressive myoclonus epilepsy (PME)
- Early-infantile epileptic encephalopathy 16 (EIEE16)
- Autosomal recessive nonsyndromic hearing loss (DFNB86)
- Autosomal dominant nonsyndromic hearing loss (DFNA65)

No formal diagnostic criteria have been published for any of the***TBC1D24***-related disorders.

Suggestive Findings

A ***TBC1D24***-related disorder **should be suspected** in individuals with the following features of the recognized phenotypes that comprise a phenotypic continuum. (Information on additional features appears in Clinical Characteristics).

DOORS syndrome

- *D*eafness (profound sensorineural hearing loss)
- *O*nychodystrophy (short/absent nails)
- *O*steodystrophy (short phalanges)
- Intellectual disability / developmental delay (formerly known as mental *r*etardation)
- *S*eizures
- 2-oxoglutaric aciduria

Familial infantile myoclonic epilepsy (FIME). Early-onset myoclonic seizures

Progressive myoclonus epilepsy (PME). Action myoclonus, tonic-clonic seizures, progressive neurologic decline, and ataxia

Early-infantile epileptic encephalopathy 16 (EIEE16)

- Early-onset seizures (unresponsive to medication) that can include myoclonic seizures or malignant migrating partial seizures of infancy
- Extrapyramidal signs (e.g., dystonia), hemiparesis, and/or autonomic signs
- Neurologic deterioration and early death
- Progressive diffuse cerebral atrophy

Autosomal recessive nonsyndromic hearing loss, DFNB86. Prelingual nonsyndromic sensorineural deafness (See Deafness and Hereditary Hearing Loss Overview.)

Autosomal dominant nonsyndromic hearing loss, DFNA65. Adult-onset nonsyndromic sensorineural deafness (See Deafness and Hereditary Hearing Loss Overview.)

Establishing the Diagnosis

The diagnosis of a ***TBC1D24***-related disorder **is established** in a proband by identification of (Table 1):

- Biallelic pathogenic variants in ***TBC1D24*** on molecular genetic testing when the mode of inheritance is autosomal recessive (i.e., DOORS syndrome, FIME, PME, EIEE16, and DFNB86);
- A heterozygous ***TBC1D24*** pathogenic variant when the mode of inheritance is autosomal dominant (DFNA65).

Molecular genetic testing approaches can include **single-gene testing**, use of a **multigene panel**, and **more comprehensive genomic testing**:

- **Single-gene testing.** Sequence analysis of ***TBC1D24*** is performed first and followed by gene-targeted deletion/duplication analysis if only one or no pathogenic variant is found.

  Note: (1) The diagnostic yield appears to be highest in individuals with all five typical features of DOORS syndrome [Campeau et al 2014]. (2) The proportion of epilepsy caused by pathogenic variants in ***TBC1D24***

appears to be small, and clinically distinctive signs and symptoms have not yet been identified.

- **A multigene panel** that includes ***TBC1D24*** and other genes of interest (see Differential Diagnosis) may be considered. Note: (1) The genes included in the panel and the diagnostic sensitivity of the testing used for each gene vary by laboratory and are likely to change over time. (2) Some multigene panels may include genes not associated with the condition discussed in this *GeneReview*; thus, clinicians need to determine which multigene panel is most likely to identify the genetic cause of the condition at the most reasonable cost while limiting identification of variants of uncertain significance and pathogenic variants in genes that do not explain the underlying phenotype. (3) In some laboratories, panel options may include a custom laboratory-designed panel and/or custom phenotype-focused exome analysis that includes genes specified by the clinician. (4) Methods used in a panel may include sequence analysis, deletion/duplication analysis, and/or other non-sequencing-based tests.

  For an introduction to multigene panels click here. More detailed information for clinicians ordering genetic tests can be found here.

- **More comprehensive genomic testing** (when available) including exome sequencing, mitochondrial sequencing, and genome sequencing may be considered. Such testing may provide or suggest a diagnosis not previously considered (e.g., mutation of a different gene or genes that results in a similar clinical presentation).

  For an introduction to comprehensive genomic testing click here. More detailed information for clinicians ordering genomic testing can be found here.

Table 1.

Molecular Genetic Testing Used in ***TBC1D24***-Related Disorders

Gene [1]	Method	Proportion of Probands with Pathogenic Variants [2] Detectable by Method
TBC1D24	Sequence analysis [3]	• DOORS syndrome: 9/18 families w/all 5 major features [4] • FIME: rare [5, 6] • PME: rare [5, 7] • EIEE16: rare [5, 8] • DFNB86: rare [5, 9] • DFNA65: rare [5, 10]
	Gene-targeted deletion/duplication analysis [11]	Unknown [12]
Unknown [13]		

EIEE = early-infantile epileptic encephalopathy; FIME = familial infantile myoclonic epilepsy; PME = progressive myoclonus epilepsy

1. See Table A. Genes and Databases for chromosome locus and protein.
2. See Molecular Genetics for information on allelic variants detected in this gene.
3. Sequence analysis detects variants that are benign, likely benign, of uncertain significance, likely pathogenic, or pathogenic. Pathogenic variants may include small intragenic deletions/insertions and missense, nonsense, and splice site variants; typically, exon or whole-gene deletions/duplications are not detected. For issues to consider in interpretation of sequence analysis results, click here.

4. Campeau et al [2014]
5. Although a significant proportion of individuals with this phenotype have a genetic etiology, ***TBC1D24*** is a rare cause.
6. Three families reported [Corbett et al 2010, Falace et al 2010, Afawi et al 2013]
7. One family reported [Muona et al 2015]
8. Three families reported [Guven & Tolun 2013, Milh et al 2013, Lozano et al 2016]; one individual in a cohort of 359 individuals with epileptic encephalopathy [de Kovel et al 2016]
9. Recessive deafness; five families reported [Rehman et al 2014, Bakhchane et al 2015]
10. Dominant deafness; two families reported [Azaiez et al 2014, Zhang et al 2014]
11. Gene-targeted deletion/duplication analysis detects intragenic deletions or duplications. Methods used may include quantitative PCR, long-range PCR, multiplex ligation-dependent probe amplification (MLPA), and a gene-targeted microarray designed to detect single-exon deletions or duplications.
12. No data on detection rate of gene-targeted deletion/duplication analysis are available.
13. Genetic heterogeneity for DOORS syndrome is likely. Exome analysis in some of these families did not reveal a commonly mutated gene [Campeau et al 2014], but an extension of this study is ongoing.

Clinical Characteristics

Clinical Description

While initial reports suggested specific phenotypes associated with pathogenic variants in ***TBC1D24***, several recent publications suggest that the features represent a phenotypic spectrum ranging from a mild form of familial infantile myoclonic epilepsy (FIME) to a combination of epilepsy with variable other features, to DOORS syndrome [Balestrini et al 2016]. Features seen in individuals with biallelic ***TBC1D24*** pathogenic variants who do not have DOORS syndrome include parkinsonism [Banuelos et al 2017], ataxia, dysarthria, axial hypotonia, hearing loss, visual impairment, mild dysmorphic facial features, developmental delay or intellectual disability, and microcephaly [Balestrini et al 2016].

DOORS Syndrome

The five major features of DOORS syndrome are profound sensorineural hearing loss, onychodystrophy, osteodystrophy, intellectual disability / developmental delay, and seizures [James et al 2007, Campeau et al 2014].

The sensorineural hearing loss is often profound and prelingual. Some have benefited from cochlear implants.

The onychoosteodystrophy affects the hands and feet equally. Small or absent nails (onychodystrophy) and hypoplastic terminal phalanges (osteodystrophy) are noted in most individuals. A triphalangeal thumb is present in one third of affected individuals.

The intellectual disability (previously referred to as mental retardation) can vary significantly in degree but is often severe [Balestrini et al 2016]. The motor and language skills were most delayed in two children where such details are available [Nomura et al 2009, Girish et al 2011]. One child had autism spectrum disorder [Nomura et al 2009].

Seizures, present in most individuals with DOORS syndrome, usually start in the first year of life. The seizures are more often generalized tonic-clonic, but myoclonic, partial, and absence seizures also occur. Occasionally their frequency or severity increases. In several instances, the seizures have been difficult to control even with multiple antiepileptic medications, and have led to status epilepticus and death.

On MRI hyperintense T_2-weighted signal anomalies may be observed in the cerebellar hemispheres and the frontal region [Campeau et al 2014].

Nonspecific dysmorphic features. A wide nasal base and a bulbous nose are the most common facial dysmorphisms. Other findings in a minority of individuals include narrow forehead, narrow or high arched palate, broad alveolar ridge, short frenulum, and nevus simplex on the glabella and nose.

Other. In individuals with DOORS syndrome the additional anomalies noted are the following:

- Microcephaly in one third of individuals
- Other cranial anomalies (sagittal craniosynostosis in 1 individual; frontal bossing, trigonocephaly, or brachycephaly in several other affected individuals)
- Dental anomalies (delayed eruption, wide spacing, and abnormal shape, size, and number)
- Congenital heart defects (double outlet right ventricle)
- Skeletal anomalies (e.g., calcaneal deformities)
- Hypothyroidism
- Renal and urinary tract anomalies (e.g., hydronephrosis, nephrocalcinosis) [Campeau et al 2014]
- Elevated levels of urinary 2-oxoglutaric acid, which can fluctuate between normal and elevated over time [Patton et al 1987, van Bever et al 2007, Campeau et al 2014]
- Visual impairment in six of 14 individuals [Balestrini et al 2016]
- Peripheral neuropathy in one individual with confirmed ***TBC1D24*** pathogenic variants [Balestrini et la 2016] and three individuals who either did not undergo genetic testing or in whom no ***TBC1D24*** pathogenic variant was identified

Familial Infantile Myoclonic Epilepsy (FIME)

FIME is characterized by early-onset myoclonic seizures.

Findings include focal epilepsy, dysarthria, mild-to-moderate intellectual disability, and cortical thickening and cerebellar atrophy with high T_2-weighted and FLAIR on MRI (in 4 sibs of an Israeli Arab family [Corbett et al 2010, Afawi et al 2013]).

Intellect may be normal: all seven members of an Italian family who had FIME and biallelic ***TBC1D24*** pathogenic variants also had normal intelligence. Six had normal brain MRI and one had periventricular nodular heterotopia [Zara et al 2000, de Falco et al 2001, Falace et al 2010].

Progressive Myoclonus Epilepsy (PME)

PME is characterized by action myoclonus, tonic-clonic seizures, progressive neurologic decline, and ataxia.

For the child described with PME caused by biallelic pathogenic variants in ***TBC1D24***, tonic seizures started at age 36 hours. Developmental delay with later regression was then noted. Myoclonus started at age eight months and tonic-clonic seizures at age 3.5 years. Ataxia, spasticity, supranuclear gaze palsy, and visual decline were also noted. Although the initial clinical diagnosis was of an epileptic encephalopathy, a florid PME pattern was apparent by age nine years [Muona et al 2015]. There were no digital anomalies or deafness [Sam Berkovic, MD, personal communication].

Early-Infantile Epileptic Encephalopathy 16 (EIEE16)

Epileptic encephalopathies are defined by the International League Against Epilepsy (ILAE) as conditions in which epileptiform EEG abnormalities themselves are believed to contribute to progressive disturbance in cerebral function [Engel 2001, Berg et al 2010].

Findings may include:

- Myoclonic epilepsy with episodic dystonia, hemiparesis, autonomic signs, and lethargy evolving to chronic

dystonia, progressive diffuse cerebral atrophy, and early death (in 5 Turkish families [Duru et al 2010, Guven & Tolun 2013]);

- Malignant migrating partial seizures in infancy with progressive diffuse cerebral atrophy of the gray matter (sparing the posterior fossa) and early death (in 2 French sibs [Milh et al 2013]).

Autosomal Recessive Nonsyndromic Hearing Loss, DFNB86

Findings observed include profound prelingual deafness with hearing thresholds above 90 dB for all test frequencies (in 2 consanguineous Pakistani families; 1 affected family member and 1 individual with a heterozygous ***TBC1D24*** pathogenic variant also had seizures [Rehman et al 2014]) (see Deafness and Hereditary Hearing Loss Overview).

Autosomal Dominant Nonsyndromic Hearing Loss, DFNA65

Findings include slowly progressive deafness with onset in the third decade, initially affecting the high frequencies (in 1 Chinese family [Zhang et al 2014] and in a family of European descent [Azaiez et al 2014]) (see Deafness and Hereditary Hearing Loss Overview).

Heterozygotes, in the context of autosomal recessive disease. Two unrelated individuals with generalized tonic-clonic seizures and biallelic pathogenic ***TBC1D24*** variants both had a family history of hearing loss, but the relatives with hearing loss were not tested for a heterozygous ***TBC1D24*** pathogenic variant. In one family, the affected individual's brother had hearing loss and in the other family the affected individual's maternal grandmother had hearing loss [Balestrini et al 2016].

Increasing evidence points to an elevated susceptibility to seizure disorders in apparently unaffected individuals who have a heterozygous ***TBC1D24*** pathogenic variant (i.e., a "carrier") as compared to the population frequency estimated at seven per 1,000 individuals.

- In a family with autosomal recessive hearing loss, an individual with a heterozygous pathogenic p.Asp70Tyr variant developed seizures starting at age three years [Rehman et al 2014].
- A family history of seizures was also reported in two families with DOORS syndrome, including a mother who was heterozygous for a c.1008delT variant with absence seizures in childhood [Campeau et al 2014] and a heterozygous father [Balestrini et al 2016, supplemental material].
- Family history was positive in an additional five families with ***TBC1D24*** pathogenic variants; family members were unavailable for sequencing [Stražišar et al 2015, Balestrini et al 2016, supplemental material].
- Finally, in a family with an atypical neurologic phenotype in the proband, both the affected individual's mother and her brother had seizures in childhood and adolescence, respectively. Both were confirmed to have a heterozygous novel pathogenic variant (p.Pro135Leu) [Banuelos et al 2017].

Genotype-Phenotype Correlations

The ***TBC1D24*** pathogenic variants that cause DOORS syndrome, FIME, EIEE16, DFNB86, and DFNA65 are located throughout the gene; no pattern has emerged to date. Most pathogenic variants causing one phenotype have not been demonstrated to cause the others, either within the same family or in different families. However, a heterozygous frameshift variant (c.1008delT) coupled with another pathogenic variant affecting the other ***TBC1D24*** allele was identified in four affected individuals, two with DOORS and one sib pair with EIEE16 and early death.

In general, loss-of-function variants (frameshift, nonsense, or splice site) are associated with a more severe epilepsy phenotype with drug resistance and early death, except when the loss-of-function variant is located in the last exon. Pathogenic missense variants in or before the TBC domain are also associated with a higher risk of lethality.

The diagnosis of DOORS does not allow for a prediction of the epilepsy type [Balestrini et al 2016]. The location of

new pathogenic variants cannot yet be used to predict a phenotype. This may change as more pathogenic variants are identified.

Nomenclature

The acronym "DOOR syndrome" was coined in 1975 [Cantwell 1975]. Subsequently, Qazi & Nangia [1984] suggested adding an S (DOORS syndrome) because of the seizures present in most individuals. Other terms used for this condition include digito-reno-cerebral syndrome [Eronen et al 1985] and Eronen syndrome [Le Merrer et al 1992].

EIEE16 has also been referred to as malignant migrating partial seizures of infancy (MMPSI) [Milh et al 2013], which is also known as epilepsy of infancy with migrating focal seizures.

Prevalence

The prevalence of ***TBC1D24***-related disorders is very low. Fewer than 50 families with DOORS syndrome are known, and fewer than five each for the other ***TBC1D24***-related disorders. A targeted sequencing study of a cohort of 359 individuals with epileptic encephalopathy identified one person with biallelic ***TBC1D24*** variants [de Kovel et al 2016].

Genetically Related (Allelic) Disorders

All the phenotypes known to be associated with pathogenic variants in ***TBC1D24*** are included in this *GeneReview*.

Differential Diagnosis

DOORS Syndrome

Table 2.

Disorders to Consider in the Differential Diagnosis of DOORS Syndrome

Differential Diagnosis Disorder	Gene(s)	MOI	Features of Differential Diagnosis Disorder	
			Overlapping w/DOORS syndrome	Distinguishing from DOORS syndrome
Coffin-Siris syndrome (see also *ARID1B*-Related Disorder)	*ARID1A* *ARID1B* *SMARCA4* *SMARCB1* *SMARCE1* *SOX11*	AD [1]	ID/DD, aplastic or hypoplastic nails & terminal phalanges, seizures	Variably seen: coarse face, generalized hypertrichosis, scoliosis (some), gingival overgrowth (some), & 5th finger hypoplasia
Dominant deafness-onychodystrophy syndrome (OMIM 124480)	*ATP6V1B2*	AD	Congenital sensorineural deafness, onychodystrophy	Dental anomalies (conical, hypoplastic teeth) (some); absence of ID/DD & seizures
Nicolaides-Baraitser syndrome	*SMARCA2*	AD [1]	Severe ID/DD, seizures	Coarse face, prominent finger joints & broad distal phalanges, scoliosis (some)
Temple-Baraitser syndrome (OMIM 611816)	*KCNH1*	AD [1]	Severe ID/DD, seizures, nail hypoplasia/aplasia limited to 1st rays (thumb, great toe)	Broad and proximally implanted thumbs, long great toes

Differential Diagnosis Disorder	Gene(s)	MOI	Features of Differential Diagnosis Disorder	
			Overlapping w/DOORS syndrome	Distinguishing from DOORS syndrome
Zimmermann-Laband syndrome (OMIM 135500 and 616455)	*KCNH1* *ATP6B1B2*	AD	Variable ID/DD, seizures in ZLS due to pathogenic variants in *KCNH1*, hypoplasia or aplasia of nails & terminal phalanges, hearing loss (some)	Coarse face, hypertrichosis, gingival overgrowth, scoliosis; no seizures in individuals w/ZLS caused by pathogenic variants in *ATP6B1B2*
Mabry syndrome (OMIM 239300)	*PIGV*	AR	Severe ID/DD, seizures, short terminal phalanges, and nail hypoplasia	Hyperphosphatasia; absence of 2-oxoglutaric aciduria & deafness
Kaufman oculocerebrofacial syndrome (OMIM 244450)	*UBE3B*	AR	ID/DD, hearing loss (some), microcephaly, nail dysplasia	Blepharophimosis; hypoplastic/absent terminal phalanges rarely seen
Fetal anticonvulsant syndrome	n/a	n/a	ID/DD, nail hypoplasia	Dental abnormalities w/delayed eruption, talipes equinovarus, otitis media w/effusion; absence of hearing loss & seizures

AD = autosomal dominant; AR = autosomal recessive; ID/DD = intellectual disability / developmental delay; MOI = mode of inheritance; XL = X-linked

1. Pathogenic variants are typically (or always) *de novo*.

Table 3.

Other Conditions with 2-Oxoglutaric Aciduria

Differential Diagnosis Disorder	Gene	MOI	Features of Differential Diagnosis Disorder	
			Overlapping w/*TBC1D24*-related disorders	Distinguishing from *TBC1D24*-related disorders
Combined D-2- and L-2-hydroxyglutaric aciduria (OMIM 615182)	*SLC25A1*	AR	Severe DD, seizures	Severe neonatal encephalopathy w/early death, no skeletal manifestations, ↑ D-2- & L-2-hydroxyglutaric acid
3-methylcrotonyl-CoA carboxylase 1 deficiency (OMIM 210200)	*MCCC1*	AR	2-oxoglutaric aciduria in 1 individual; ID, DD, seizures	Urinary excretion of 3-hydroxyisovalerate & 3-methylcrotonylglycine, metabolic decompensation
2-ketoglutarate dehydrogenase deficiency (OMIM 203740)	Unknown	AR	Elevated 2-oxoglutaric acid	Progressive neurodegenerative disorder; development initially normal

AR = autosomal recessive; DD = developmental delay; ID = intellectual disability; MOI = mode of inheritance

Familial Infantile Myoclonic Epilepsy (FIME) and Progressive Myoclonus Epilepsy (PME)

Table 4.

Disorders to Consider in the Differential Diagnosis of ***TBC1D24***-Related FIME and ***TBC1D24***-Related PME

Differential Diagnosis Disorder	**Gene(s)**	**MOI**	**Features of Differential Diagnosis Disorder**	
			Overlapping w/*TBC1D24*-related FIME/PME	**Distinguishing from *TBC1D24*-related FIME/PME**
Juvenile myoclonic epilepsy (OMIM 254770)	*EFHC1*	AD	Myoclonic seizures, generalized tonic-clonic seizures	Typical 3-Hz polyspike EEG, normal intelligence, later mean age of onset (~10 yrs), myoclonic seizures (jerks) typically in the morning
Early-infantile myoclonic encephalopathy 3 (OMIM 609304)	*SLC25A22*	AR	Myoclonic refractory seizures, early onset age	Burst suppression on EEG, abnormal visual evoked potentials, spasticity
Unverricht-Lundborg disease	*CSTB*	AR	Generalized tonic-clonic seizures, myoclonic seizures	No or mild decline in intellectual performance, EEG always abnormal, later onset age
Progressive myoclonus epilepsy, Lafora type	*EPM2A* *NHLRC1*	AR	Generalized myoclonus and/or generalized tonic-clonic seizures	Progressive neurologic degeneration in previously healthy adolescents; Lafora bodies
Neuronal ceroid-lipofuscinoses	*ATP13A2* *CLN3* *CLN5* *CLN6* *CLN8* *CTSD* *CTSF* *DNAJC5* *GRN* *KCTD7* *MFSD8* *PPT1* *TPP1*	AR [1]	Myoclonus, seizures	Progressive intellectual & motor deterioration w/vision loss
MERRF	*MT-TF* *MT-TI* *MT-TK* *MT-TL1* *MT-TP*	Mat	Myoclonus, generalized epilepsy, hearing loss, ataxia	Normal early development, ragged-red fibers on muscle biopsy, lactic acidosis; cardiomyopathy in some
POLG-related disorders	*POLG*	AR AD	Myoclonus, seizures, ataxia	Variable phenotype that may incl ophthalmoplegia, neuropathy, liver dysfunction
SCN1A-related seizure disorders	*SCN1A*	AD	Myoclonic epilepsy, generalized tonic-clonic/	Not assoc w/hearing loss

Differential Diagnosis Disorder	Gene(s)	MOI	Features of Differential Diagnosis Disorder	
			Overlapping w/*TBC1D24*-related FIME/PME	**Distinguishing from *TBC1D24*-related FIME/PME**
			hemiclonic & focal seizures	
Action myoclonus-renal failure syndrome	*SCARB2*	AR	Progressive myoclonic epilepsy	Onset in late teens or early 20s w/tremors, proteinuria, & development of renal failure possible
PRICKLE1-related progressive myoclonus epilepsy with ataxia	*PRICKLE1*	AR	Myoclonic seizures, generalized convulsive seizures, ataxia	Normal intellect

AD = autosomal dominant; AR = autosomal recessive; Mat = maternal; MOI = mode of inheritance

1. Neuronal ceroid-lipofuscinosis (NCL) is inherited in an autosomal recessive manner with the exception of adult NCL, which can be inherited in either an autosomal recessive or an autosomal dominant manner.

Early-Infantile Epileptic Encephalopathy (EIEE)

EIEE is a rare form of epilepsy in which affected children develop intractable seizures in the first weeks or months of life resulting in severe developmental disabilities or death in infancy.

EIEE is genetically heterogeneous. Causes can include the following:

- Rare copy number variations [Mefford et al 2011]
- Mutation of an individual gene that may be rare, with fewer than five affected families identified (e.g., *ST3GAL3* (EIEE15), *GNAO1* (EIEE17), or *SZT2* (EIEE18)
- Mutation of an individual gene that may affect hundreds of individuals (e.g., *SCN1A* (Dravet syndrome) [Brunklaus et al 2012]
- Metabolic causes of epileptic encephalopathies such as glycine encephalopathy, biotinidase deficiency, organic acidemias, cerebral folate deficiency, and pyridoxine-dependent epilepsy [Yu & Pearl 2013]

See Epileptic encephalopathy, early-infantile – OMIM Phenotypic Series to view genes associated with this phenotype in OMIM.

Hereditary Hearing Loss and Deafness

For the differential diagnosis of hereditary hearing loss and deafness, see Hereditary Deafness and Hearing Loss Overview.

Management

Evaluations Following Initial Diagnosis

To establish the extent of disease and needs in an individual diagnosed with a ***TBC1D24***-related disorder, the following evaluations are recommended if they have not already been completed:

DOORS syndrome

- Audiology evaluation

- Ophthalmology evaluation
- Dental examination
- Otolaryngology consultation
- Echocardiogram
- Renal ultrasound examination
- Neurology consultation
- Consultation with a clinical geneticist and/or genetic counselor

FIME, PME, EIEE16, and ***TBC1D24*-related nonsyndromic deafness syndromes (DFNA65** and **DFNB86).** One should consider some of the above evaluations depending on the signs and symptoms, since clinical overlap between all ***TBC1D24***-related conditions exists.

Treatment of Manifestations

Deafness. Consider hearing aids or cochlear implants as needed for hearing loss (see Hereditary Hearing Loss and Deafness Overview). Cochlear implants at age one have been beneficial in one individual with DOORS syndrome [Campeau et al 2014].

Visual impairment should be managed in the standard fashion.

Heart defects should be managed according to the anomalies detected.

Renal anomalies. Refer to an urologist or nephrologist, as indicated.

Seizures. Symptomatic pharmacologic management is warranted, as no controlled studies have compared the efficacy of different antiepileptic drugs in ***TBC1D24***-related disorders. A variety of different antiepileptic agents have been used to achieve seizure control [Balestrini et al 2016].

The management of epilepsy in many genetic epilepsies is complex; general recommendations from the UK National Institute for Health and Care Excellence are available online.

Developmental Delay / Intellectual Disability Management Issues

The following information represents typical management recommendations for individuals with developmental delay / intellectual disability in the United States; standard recommendations may vary from country to country.

Ages 0-3 years. Referral to an early intervention program is recommended for access to occupational, physical, and speech therapy. In the US, early intervention is a federally funded program available in all states.

Ages 3-5 years. In the US, developmental preschool through the local public school district is recommended. Before placement, an evaluation is made to determine needed services and therapies and an individualized education plan (IEP) is developed.

Ages 5-21 years

- In the US, an individualized education program (IEP) based on the individual's level of function should be developed by the local public school district. Affected children are permitted to remain in the public school district until age 21.
- Discussion about transition plans including financial, vocation/employment, and medical arrangements should begin at age 12 years. Developmental pediatricians can provide assistance with transition to adulthood.

All ages. Consultation with a developmental pediatrician is recommended to ensure the involvement of appropriate community, state, and educational agencies and to support parents in maximizing quality of life.

Consideration of private supportive therapies based on the affected individual's needs is recommended. Specific recommendations regarding type of therapy can be made by a developmental pediatrician.

In the US:

- Developmental Disabilities Administration (DDA) enrollment is recommended. DDA is a public agency that provides services and support to qualified individuals. Eligibility differs by state but is typically determined by diagnosis and/or associated cognitive/adaptive disabilities.
- Families with limited income and resources may also qualify for supplemental security income (SSI) for their child with a disability.

Motor Dysfunction

Gross motor dysfunction

- Physical therapy is recommended to maximize mobility and to reduce the risk for later-onset orthopedic complications (e.g., contractures, scoliosis, hip dislocation).
- Consider use of durable medical equipment as needed (e.g., wheelchairs, walkers, bath chairs, orthotics, adaptive strollers).

Fine motor dysfunction. Occupational therapy is recommended for difficulty with fine motor skills that affect adaptive function such as feeding, grooming, dressing, and writing.

Communication issues. Consider evaluation for alternative means of communication (e.g., Augmentative and Alternative Communication [AAC]) for individuals who have expressive language difficulties.

Social/Behavioral Concerns

Children may qualify for and benefit from interventions used in treatment of autism spectrum disorder, including applied behavior analysis (ABA). ABA therapy is targeted to the individual child's behavioral, social, and adaptive strengths and weaknesses and is typically performed one on one with a board-certified behavior analyst.

Consultation with a developmental pediatrician may be helpful in guiding parents through appropriate behavior management strategies or providing prescription medications when necessary.

Surveillance

Neurology evaluations with EEGs are appropriate, depending on seizure frequency and/or progression of clinical manifestations. Individuals with epilepsy, irrespective of cause, should have periodic ECGs as interictal and ictal abnormalities may predispose to sudden unexplained death in epilepsy (SUDEP).

Perform yearly audiologic evaluation to assess for possible progression of hearing loss and/or the efficacy of hearing aids.

Yearly dental evaluation is appropriate.

Agents/Circumstances to Avoid

Heterozygotes for a ***TBC1D24*** pathogenic variant causing autosomal dominant deafness, DFNA65 should avoid excessive ambient noise as it may exacerbate hearing loss.

Evaluation of Relatives at Risk

Molecular genetic testing for the familial ***TBC1D24*** pathogenic variant(s) in older and younger sibs of a proband is appropriate in order to identify as early as possible those who would benefit from early treatment of seizures and/or deafness.

See Genetic Counseling for issues related to testing of at-risk relatives for genetic counseling purposes.

Pregnancy Management

In general, no information on specific prenatal presentations is available.

Polyhydramnios is often noted when a fetus has DOORS syndrome [James et al 2007]. A subsequent affected pregnancy in one family with DOORS syndrome was terminated due to an elevated nuchal translucency of 5.1 mm at 12 weeks' estimated gestational age [Balestrini et al 2016].

Therapies Under Investigation

Search ClinicalTrials.gov in the US and EU Clinical Trials Register in Europe for access to information on clinical studies for a wide range of diseases and conditions. Note: There may not be clinical trials for this disorder.

Genetic Counseling

Genetic counseling is the process of providing individuals and families with information on the nature, inheritance, and implications of genetic disorders to help them make informed medical and personal decisions. The following section deals with genetic risk assessment and the use of family history and genetic testing to clarify genetic status for family members. This section is not meant to address all personal, cultural, or ethical issues that individuals may face or to substitute for consultation with a genetics professional. —ED.

Mode of Inheritance

Most ***TBC1D24***-related disorders (DOORS syndrome, FIME, PME, EIEE16, and DFNB86) are inherited in an autosomal recessive manner.

Risk to Family Members – Autosomal Recessive Inheritance

Parents of a proband

- The parents of an affected child are obligate heterozygotes (i.e., carriers of one ***TBC1D24*** pathogenic variant).
- Heterozygotes (carriers) are typically asymptomatic and are not at risk of developing an autosomal recessive ***TBC1D24***-related disorder. It is possible that some heterozygotes (carriers) could have an elevated susceptibility to seizure disorders related to certain ***TBC1D24*** pathogenic variants, but genotype-phenotype correlation is lacking and no risk estimates are available.

Sibs of a proband

- At conception, each sib of an affected individual has a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier.
- Heterozygotes (carriers) are asymptomatic and are not at risk of developing the disorder.

Offspring of a proband

- Individuals with ***TBC1D24***-related DOORS syndrome, ***TBC1D24***-related PME, and EIEE16 have not reproduced.

- The offspring of an individual with a FIME or DFNB86 are obligate heterozygotes (carriers) for a pathogenic variant in ***TBC1D24***.

Other family members. Each sib of the proband's parents is at a 50% risk of being a carrier of a ***TBC1D24*** pathogenic variant.

Carrier (Heterozygote) Detection

Carrier testing for at-risk relatives requires prior identification of the ***TBC1D24*** pathogenic variants in the family.

Related Genetic Counseling Issues

See Management, Evaluation of Relatives at Risk for information on evaluating at-risk relatives for the purpose of early diagnosis and treatment.

The following points are noteworthy:

- Communication with individuals who are members of the Deaf community and who sign requires the services of a skilled interpreter.
- Members of the Deaf community may view deafness as a distinguishing characteristic and not as a handicap, impairment, or medical condition requiring a "treatment" or "cure," or to be "prevented."
- Many deaf people are interested in obtaining information about the cause of their own deafness, including information on medical, educational, and social services, rather than information about prevention, reproduction, or family planning. It is, therefore, important to ascertain and address the questions and concerns of the family/individual.
- The use of certain terms is preferred: probability or chance vs risk; deaf and hard-of-hearing vs hearing impaired. Terms such as "abnormal" should be avoided.

Family planning

- The optimal time for determination of genetic risk, clarification of carrier status, and discussion of the availability of prenatal testing is before pregnancy.
- It is appropriate to offer genetic counseling (including discussion of potential risks to offspring and reproductive options) to young adults who are affected, are carriers, or are at risk of being carriers.

DNA banking is the storage of DNA (typically extracted from white blood cells) for possible future use. Because it is likely that testing methodology and our understanding of genes, allelic variants, and diseases will improve in the future, consideration should be given to banking DNA of affected individuals.

Prenatal Testing and Preimplantation Genetic Diagnosis

Once the ***TBC1D24*** pathogenic variants have been identified in an affected family member, prenatal testing for a pregnancy at increased risk and preimplantation genetic diagnosis are possible.

Differences in perspective may exist among medical professionals and within families regarding the use of prenatal testing, particularly if the testing is being considered for the purpose of pregnancy termination rather than early diagnosis. While most centers would consider decisions regarding prenatal testing to be the choice of the parents, discussion of these issues is appropriate.

Resources

GeneReviews staff has selected the following disease-specific and/or umbrella support organizations and/or registries

for the benefit of individuals with this disorder and their families. GeneReviews is not responsible for the information provided by other organizations. For information on selection criteria, click here.

- **American Epilepsy Society (AES)**
  www.aesnet.org
- **American Society for Deaf Children (ASDC)**
  800 Florida Avenue Northeast
  Suite 2047
  Washington DC 20002-3695
  Phone: 800-942-2732 (Toll-free Parent Hotline); 866-895-4206 (toll free voice/TTY)
  Fax: 410-795-0965
  Email: info@deafchildren.org; asdc@deafchildren.org
  www.deafchildren.org
- **Canadian Epilepsy Alliance**
  Canada
  Phone: 1-866-EPILEPSY (1-866-374-5377)
  www.epilepsymatters.com
- **Epilepsy Foundation**
  8301 Professional Place East
  Suite 200
  Landover MD 20785-7223
  Phone: 800-332-1000 (toll-free)
  Email: ContactUs@efa.org
  www.epilepsy.com
- **National Association of the Deaf (NAD)**
  8630 Fenton Street
  Suite 820
  Silver Spring MD 20910
  Phone: 301-587-1788; 301-587-1789 (TTY)
  Fax: 301-587-1791
  Email: nad.info@nad.org
  www.nad.org
- **National Institute of Neurological Disorders and Stroke (NINDS)**
  PO Box 5801
  Bethesda MD 20824
  Phone: 800-352-9424 (toll-free); 301-496-5751; 301-468-5981 (TTY)
  Epilepsy Information Page

Molecular Genetics

Information in the Molecular Genetics and OMIM tables may differ from that elsewhere in the GeneReview: tables may contain more recent information. —ED.

Table A.

TBC1D24-Related Disorders: Genes and Databases

Gene	Chromosome Locus	Protein	Locus-Specific Databases	HGMD	ClinVar
TBC1D24	16p13.3	TBC1 domain family member 24	**TBC1D24** @ LOVD	**TBC1D24**	**TBC1D24**

Data are compiled from the following standard references: gene from HGNC; chromosome locus from OMIM; protein from UniProt. For a description of databases (Locus Specific, HGMD, ClinVar) to which links are provided, click here.

Table B.

OMIM Entries for **TBC1D24**-Related Disorders (View All in OMIM)

220500	DEAFNESS, ONYCHODYSTROPHY, OSTEODYSTROPHY, MENTAL RETARDATION, AND SEIZURES SYNDROME; DOORS
605021	MYOCLONIC EPILEPSY, FAMILIAL INFANTILE; FIME
613577	TBC1 DOMAIN FAMILY, MEMBER 24; **TBC1D24**
614617	DEAFNESS, AUTOSOMAL RECESSIVE 86; DFNB86
615338	EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 16; EIEE16
616044	DEAFNESS, AUTOSOMAL DOMINANT 65; DFNA65

Gene structure. Transcript variant 1 (NM_001199107.1) encodes the longest isoform, and is composed of eight exons, with exon 1 being noncoding. Exon 3 is spliced out in isoform 2 (encoded by transcript NM_020705.2), which is expressed predominantly in non-neural tissues [Guven & Tolun 2013]. For a detailed summary of gene and protein information, see Table A, **Gene**.

Pathogenic variants

Table 5.

TBC1D24 Pathogenic Variants Discussed in This *GeneReview*

DNA Nucleotide Change	Predicted Protein Change	Reference Sequences
c.119G>T	p.Arg40Leu	NM_001199107.1 NP_001186036.1
c.208G>T	p.Asp70Tyr	
c.404C>T	p.Pro135Leu	
c.533C>T	p.Ser178Leu	
c.724C>T	p.Arg242Cys	
c.1008delT	p.His336GlnfsTer12	

Variants listed in the table have been provided by the authors. *GeneReviews* staff have not independently verified the classification of variants.
GeneReviews follows the standard naming conventions of the Human Genome Variation Society (varnomen.hgvs.org). See Quick Reference for an explanation of nomenclature.

Normal gene product. TBC1D24 is a Tre2–Bub2–Cdc16 (TBC) domain-containing RAB GTPase-activating protein, which catalyzes the hydrolysis of GTP by small GTPases, thus regulating the proper transport of intracellular vesicles. **TBC1D24** is the only TBC/RabGAP protein with a TLDc domain (TBC, LysM, Domain catalytic) of unknown

function but thought to be involved in oxidative stress resistance and perhaps to have some enzymatic activity.

TBC1D24 has been demonstrated to interact with ARF6 when both proteins are overexpressed in cell culture [Falace et al 2010, Falace et al 2014]. In *C. elegans*, C31H2.1 (a **TBC1D24** ortholog) was implicated in synaptic function by an RNAi screen [Sieburth et al 2005]. In *Drosophila*, the ortholog Skywalker (Sky) facilitates endosomal trafficking in synaptic vesicles by facilitating GTP hydrolysis by Rab35, thus controlling synaptic vesicle rejuvenation and neurotransmitter release [Uytterhoeven et al 2011]. Analysis of the crystal structure of 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 [Fischer et al 2016]. Whether human **TBC1D24** is able to also facilitate Rab protein-mediated GTP hydrolysis remains to be determined.

Abnormal gene product. ***TBC1D24***-related disorders that are inherited in an autosomal recessive manner are thought to be the result of reduced function or loss of function of **TBC1D24**. Abrogation of the cationic pocket by introducing two human pathogenic variants p.Arg40 and p.Arg242 led to impaired synaptic vesicle trafficking and seizures in *Drosophila* [Fischer et al 2016]. Functional studies of other causative variants are limited and to date, no studies have been conducted examining the variant causing autosomal dominant hearing loss, p.Ser178Leu.

References

Literature Cited

Afawi Z, Mandelstam S, Korczyn AD, Kivity S, Walid S, Shalata A, Oliver KL, Corbett M, Gecz J, Berkovic SF, Jackson GD. **TBC1D24** mutation associated with focal epilepsy, cognitive impairment and a distinctive cerebro-cerebellar malformation. Epilepsy Res. 2013;105:240–4. [PubMed: 23517570]

Azaiez H, Booth KT, Bu F, Huygen P, Shibata SB, Shearer AE, Kolbe D, Meyer N, Black-Ziegelbein EA, Smith RJ. **TBC1D24** mutation causes autosomal-dominant nonsyndromic hearing loss. Hum Mutat. 2014;35:819–23. [PMC free article: PMC4267685] [PubMed: 24729539]

Bakhchane A, Charif M, Salime S, Boulouiz R, Nahili H, Roky R, Lenaers G, Barakat A. Recessive **TBC1D24** mutations are frequent in Moroccan non-syndromic hearing loss pedigrees. PLoS One. 2015;10:e0138072. [PMC free article: PMC4570774] [PubMed: 26371875]

Balestrini S, Milh M, Castiglioni C, Lüthy K, Finelli MJ, Verstreken P, Cardon A, Stražišar BG, Holder JL, Lesca G, Mancardi MM. **TBC1D24** genotype–phenotype correlation Epilepsies and other neurologic features. Neurology. 2016;87:77–85. [PMC free article: PMC4932231] [PubMed: 27281533]

Banuelos E, Ramsey K, Belnap N, Krishnan M, Balak C, Szelinger S, Siniard AL, Russell M, Richholt R, De Both M, Piras I. Case report: novel mutations in **TBC1D24** are associated with autosomal dominant tonic-clonic and myoclonic epilepsy and recessive Parkinsonism, psychosis, and intellectual disability. F1000Res. 2017;6:553. [PMC free article: PMC5473401] [PubMed: 28663785]

Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, Engel J, French J, Glauser TA, Mathern GW, Moshé SL, Nordli D, Plouin P, Scheffer IE. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. Epilepsia. 2010;51:676–85. [PubMed: 20196795]

Brunklaus A, Ellis R, Reavey E, Forbes GH, Zuberi SM. Prognostic, clinical and demographic features in SCN1A mutation-positive Dravet syndrome. Brain. 2012;135:2329–36. [PubMed: 22719002]

Campeau PM, Kasperaviciute D, Lu JT, Burrage LC, Kim C, Hori M, Powell BR, Stewart F, Félix TM, van den Ende J, Wisniewska M, Kayserili H, Rump P, Nampoothiri S, Aftimos S, Mey A, Nair LD, Begleiter ML, De Bie I, Meenakshi G, Murray ML, Repetto GM, Golabi M, Blair E, Male A, Giuliano F, Kariminejad A, Newman WG, Bhaskar SS, Dickerson JE, Kerr B, Banka S, Giltay JC, Wieczorek D, Tostevin A, Wiszniewska J, Cheung

SW, Hennekam RC, Gibbs RA, Lee BH, Sisodiya SM. The genetic basis of DOORS syndrome: an exome-sequencing study. Lancet Neurol. 2014;13:44–58. [PMC free article: PMC3895324] [PubMed: 24291220]

Cantwell RJ. Congenital sensori-neural deafness associated with onycho-osteo dystrophy and mental retardation (D.O.O.R. syndrome). Humangenetik. 1975;26:261–5. [PubMed: 1132883]

Corbett MA, Bahlo M, Jolly L, Afawi Z, Gardner AE, Oliver KL, Tan S, Coffey A, Mulley JC, Dibbens LM, Simri W, Shalata A, Kivity S, Jackson GD, Berkovic SF, Gecz J. A focal epilepsy and intellectual disability syndrome is due to a mutation in **TBC1D24**. Am J Hum Genet. 2010;87:371–5. [PMC free article: PMC2933342] [PubMed: 20797691]

de Falco FA, Majello L, Santangelo R, Stabile M, Bricarelli FD, Zara F. Familial infantile myoclonic epilepsy: clinical features in a large kindred with autosomal recessive inheritance. Epilepsia. 2001;42:1541–8. [PubMed: 11879364]

de Kovel CG, Brilstra EH, Kempen MJ, Slot R, Nijman IJ, Afawi Z, De Jonghe P, Djémié T, Guerrini R, Hardies K, Helbig I. Targeted sequencing of 351 candidate genes for epileptic encephalopathy in a large cohort of patients. Mol Genet Genomic Med. 2016;4:568–80. [PMC free article: PMC5023942] [PubMed: 27652284]

Duru N, Iseri SA, Selcuk N, Tolun A. Early-onset progressive myoclonic epilepsy with dystonia mapping to 16pter-p13.3. J Neurogenet. 2010;24:207–15. [PubMed: 21087195]

Engel J Jr. International League Against E. A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. Epilepsia. 2001;42:796–803. [PubMed: 11422340]

Eronen M, Somer M, Gustafsson B, Holmberg C, Fraser FC, Preus M. New syndrome: A digito-reno-cerebral syndrome. Am J Med Genet. 1985;22:281–5. [PubMed: 4050858]

Falace A, Buhler E, Fadda M, Watrin F, Lippiello P, Pallesi-Pocachard E, Baldelli P, Benfenati F, Zara F, Represa A, Fassio A, Cardoso C. **TBC1D24** regulates neuronal migration and maturation through modulation of the ARF6-dependent pathway. Proc Natl Acad Sci U S A. 2014;111:2337–42. [PMC free article: PMC3926028] [PubMed: 24469796]

Falace A, Filipello F, La Padula V, Vanni N, Madia F, De Pietri Tonelli D, de Falco FA, Striano P, Dagna Bricarelli F, Minetti C, Benfenati F, Fassio A, Zara F. **TBC1D24**, an ARF6-interacting protein, is mutated in familial infantile myoclonic epilepsy. Am J Hum Genet. 2010;87:365–70. [PMC free article: PMC2933335] [PubMed: 20727515]

Fischer B, Lüthy K, Paesmans J, De Koninck C, Maes I, Swerts J, Kuenen S, Uytterhoeven V, Verstreken P, Versées W. Skywalker-**TBC1D24** has a lipid-binding pocket mutated in epilepsy and required for synaptic function. Nat Struct Mol Biol. 2016;23:965–73. [PubMed: 27669036]

Girish M, Mujawar N, Salodkar A. DOOR syndrome. Indian Pediatr. 2011;48:479–81. [PubMed: 21743113]

Guven A, Tolun A. **TBC1D24** truncating mutation resulting in severe neurodegeneration. J Med Genet. 2013;50:199–202. [PubMed: 23343562]

James AW, Miranda SG, Culver K, Hall BD, Golabi M. DOOR syndrome: clinical report, literature review and discussion of natural history. Am J Med Genet A. 2007;143A:2821–31. [PubMed: 17994565]

Le Merrer M, David A, Goutieres F, Briard ML. Digito-reno-cerebral syndrome: confirmation of Eronen syndrome. Clin Genet. 1992;42:196–8. [PubMed: 1424243]

Lozano R, Herman K, Rothfuss M, Rieger H, Bayrak-Toydemir P, Aprile D, Fruscione F, Zara F, Fassio A. Clinical intrafamilial variability in lethal familial neonatal seizure disorder caused by **TBC1D24** mutations. Am

J Med Genet A. 2016;170:3207–14. [PubMed: 27541164]

Mefford HC, Yendle SC, Hsu C, Cook J, Geraghty E, McMahon JM, Eeg-Olofsson O, Sadleir LG, Gill D, Ben-Zeev B, Lerman-Sagie T, Mackay M, Freeman JL, Andermann E, Pelakanos JT, Andrews I, Wallace G, Eichler EE, Berkovic SF, Scheffer IE. Rare copy number variants are an important cause of epileptic encephalopathies. Ann Neurol. 2011;70:974–85. [PMC free article: PMC3245646] [PubMed: 22190369]

Milh M, Falace A, Villeneuve N, Vanni N, Cacciagli P, Assereto S, Nabbout R, Benfenati F, Zara F, Chabrol B, Villard L, Fassio A. Novel compound heterozygous mutations in **TBC1D24** cause familial malignant migrating partial seizures of infancy. Hum Mutat. 2013;34:869–72. [PubMed: 23526554]

Muona M, Berkovic SF, Dibbens LM, Oliver KL, Maljevic S, Bayly MA, Joensuu T, Canafoglia L, Franceschetti S, Michelucci R, Markkinen S, Heron SE, Hildebrand MS, Andermann E, Andermann F, Gambardella A, Tinuper P, Licchetta L, Scheffer IE, Criscuolo C, Filla A, Ferlazzo E, Ahmad J, Ahmad A, Baykan B, Said E, Topcu M, Riguzzi P, King MD, Ozkara C, Andrade DM, Engelsen BA, Crespel A, Lindenau M, Lohmann E, Saletti V, Massano J, Privitera M, Espay AJ, Kauffmann B, Duchowny M, Møller RS, Straussberg R, Afawi Z, Ben-Zeev B, Samocha KE, Daly MJ, Petrou S, Lerche H, Palotie A, Lehesjoki AE. A recurrent de novo mutation in KCNC1 causes progressive myoclonus epilepsy. Nat Genet. 2015;47:39–46. [PMC free article: PMC4281260] [PubMed: 25401298]

Nomura T, Koyama N, Yokoyama M, Awaya A, Yokochi K. DOOR syndrome concomitant with non-convulsive status epilepticus and hyperintense cerebellar cortex on T2-weighted imaging. Brain Dev. 2009;31:75–8. [PubMed: 18440741]

Patton MA, Krywawych S, Winter RM, Brenton DP, Baraitser M. DOOR syndrome (deafness, onycho-osteodystrophy, and mental retardation): elevated plasma and urinary 2-oxoglutarate in three unrelated patients. Am J Med Genet. 1987;26:207–15. [PubMed: 3812564]

Qazi QH, Nangia BS. Abnormal distal phalanges and nails, deafness, mental retardation, and seizure disorder: A new familial syndrome. J Pediatr. 1984;104:391–4. [PubMed: 6707793]

Rehman AU, Santos-Cortez RL, Morell RJ, Drummond MC, Ito T, Lee K, Khan AA, Basra MA, Wasif N, Ayub M, Ali RA, Raza S. University of Washington Center for Mendelian Genomics, Nickerson DA, Shendure J, Bamshad M, Riazuddin S, Billington N, Khan SN, Friedman PL, Griffith AJ, Ahmad W, Riazuddin S, Leal SM, Friedman TB. Mutations in **TBC1D24**, a gene associated with epilepsy, also cause nonsyndromic deafness DFNB86. Am J Hum Genet. 2014;94:144–52. [PMC free article: PMC3882911] [PubMed: 24387994]

Sieburth D, Ch'ng Q, Dybbs M, Tavazoie M, Kennedy S, Wang D, Dupuy D, Rual JF, Hill DE, Vidal M, Ruvkun G, Kaplan JM. Systematic analysis of genes required for synapse structure and function. Nature. 2005;436:510–7. [PubMed: 16049479]

Stražišar B.G., Neubauer D., Panjan D.P., Writzl K. Early-onset epileptic encephalopathy with hearing loss in two siblings with **TBC1D24** recessive mutations. Eur J Paediatr Neurol. 2015;19(2):251–256. [PubMed: 25557349]

Uytterhoeven V, Kuenen S, Kasprowicz J, Miskiewicz K, Verstreken P. Loss of skywalker reveals synaptic endosomes as sorting stations for synaptic vesicle proteins. Cell. 2011;145:117–32. [PubMed: 21458671]

van Bever Y, Balemans W, Duval EL, Jespers A, Eyskens F, van Hul W, Courtens W. Exclusion of OGDH and BMP4 as candidate genes in two siblings with autosomal recessive DOOR syndrome. Am J Med Genet A. 2007;143A:763–7. [PubMed: 17343268]

Yu JY, Pearl PL. Metabolic causes of epileptic encephalopathy. Epilepsy Res Treat. 2013;2013:124934. [PMC free article: PMC3674738] [PubMed: 23762547]

Zara F, Gennaro E, Stabile M, Carbone I, Malacarne M, Majello L, Santangelo R, de Falco FA, Bricarelli FD. Mapping of a locus for a familial autosomal recessive idiopathic myoclonic epilepsy of infancy to chromosome 16p13. Am J Hum Genet. 2000;66:1552–7. [PMC free article: PMC1378007] [PubMed: 10741954]

Zhang L, Hu L, Chai Y, Pang X, Yang T, Wu H. A dominant mutation in the stereocilia-expressing gene **TBC1D24** is a probable cause for nonsyndromic hearing impairment. Hum Mutat. 2014;35:814–8. [PubMed: 24729547]

Chapter Notes

Author Notes

Philippe M Campeau is Clinical Assistant Professor in Pediatrics at the Sainte-Justine Hospital of the University of Montreal. He is a clinical geneticist with interest in skeletal dysplasias and inborn errors of metabolism, and a principal investigator whose laboratory focuses on understanding the pathophysiology of newly identified skeletal dysplasia genes.

Acknowledgments

The authors would like to thank the families who participated in our study on DOORS syndrome and made this work possible.

Revision History

- 7 December 2017 (ma) Comprehensive update posted live
- 26 February 2015 (me) Review posted live
- 31 July 2014 (pmc) Original submission

Bookshelf ID: NBK274566 PMID: 25719194

Genetics inMedicine | ARTICLE

Corrected: Correction

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

Bettina E. Mucha, MD[1], Siddharth Banka, MD[2], Norbert Fonya Ajeawung, PhD[3], Sirinart Molidperee, BA[3], Gary G. Chen, PhD[4], Mary Kay Koenig, MD[5], Rhamat B. Adejumo, MD[5], Marianne Till, MD[6], Michael Harbord, MD[7], Renee Perrier, MD[8], Emmanuelle Lemyre, MD[9], Renee-Myriam Boucher, MD[10], Brian G. Skotko, MD[11], Jessica L. Waxler, MSc, CGC[11], Mary Ann Thomas, MD[8], Jennelle C. Hodge, PhD[12], Jozef Gecz, MD[13], Jillian Nicholl, MD[14], Lesley McGregor, MD[14], Tobias Linden, MD[15], Sanjay M. Sisodiya, MD[16,17], Damien Sanlaville, MD[6], Sau W. Cheung, PhD[18], Carl Ernst, PhD[4] and Philippe M. Campeau, MD[9]

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 (CNVs) 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* pathogenic variants are known to cause nonsyndromic deafness, epileptic disorders, or DOORS syndrome (deafness, onychodystrophy, osteodystrophy, mental retardation, seizures). Sanger sequencing of the nondeleted *TBC1D24* allele did not yield any additional pathogenic variants.

Conclusions: 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.

Genetics in Medicine (2019) 21:1058–1064; https://doi.org/10.1038/s41436-018-0290-3

Keywords: Microdeletion; TBC1D24; Microcephaly; Epilepsy; 16p13.3

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 pathogenic variants in *TBC1D24* (MIM 613577) lead to epilepsy (familial infantile myoclonic epilepsy (FIME, MIM 605021) early-infantile epileptic encephalopathy 16 (EIEE16, MIM 615338), nonsyndromic 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* pathogenic variants may have a susceptibility to epilepsy notably in the mother of a patient with DOORS syndrome who carries a loss-of-function pathogenic variant,[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

Correspondence: Philippe M. Campeau (p.campeau@umontreal.ca). #Affiliations are listed at the end of the paper.

Submitted 5 June 2018; accepted: 17 August 2018
Published online: 24 September 2018

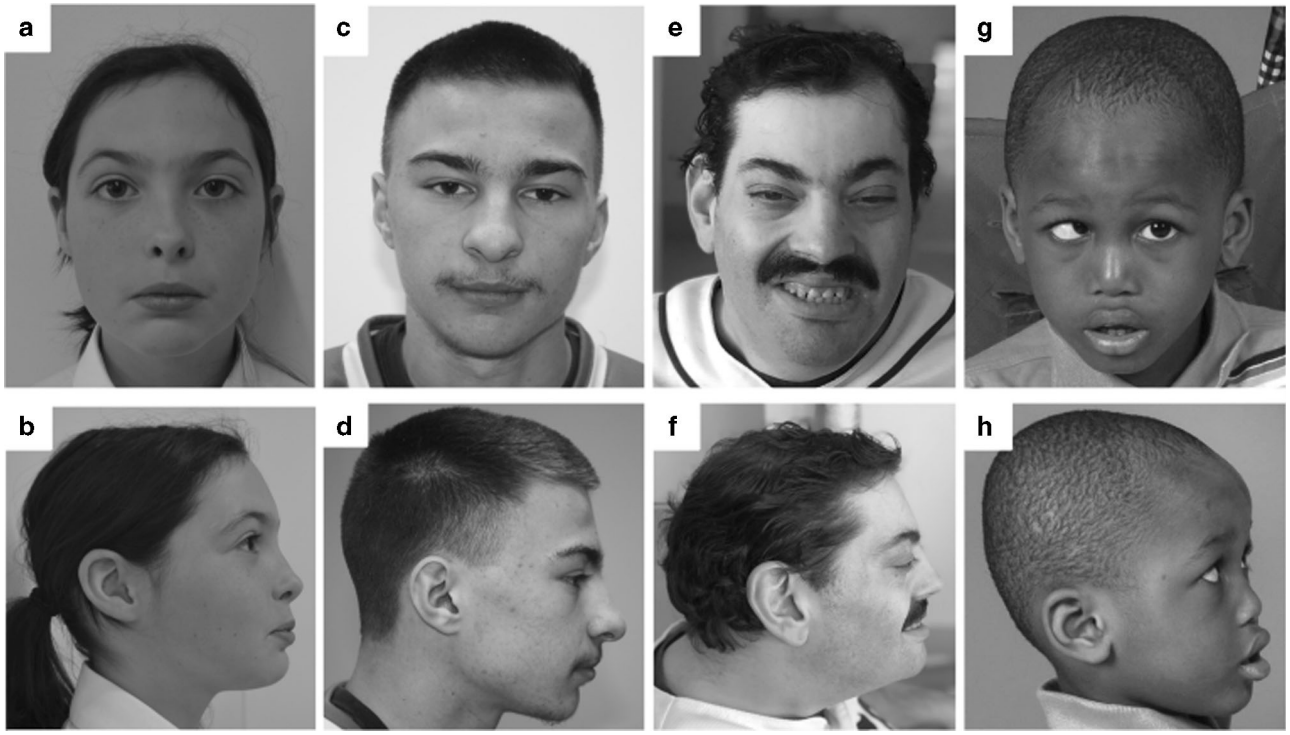


Fig. 1 Four individuals with microdeletion 16p13.3 and mild dysmorphic features. Individuals (**a**, **b**) 1, (**c**, **d**) 5, (**e**, **f**) 6, and (**g**, **h**) 7 from left to right; note the shared features in individuals 1, 5, and 6 as described in the text.

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 P.M.C. was a faculty member (Baylor College of Medicine) and currently is (Centre hospitalier universitaire [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, and 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). Consent to publish photographs was obtained from each family where a photo is shown. 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 polymerase chain reaction (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 University of California–Santa Cruz (UCSC) genome browser human assembly hg19 (ref. [3]). Haploinsufficiency scores (% HI) for the deleted genes were obtained from the DECIPHER database[4] (Supplementary Material). Probability of loss-of-function intolerance (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

Individual 1 was referred at 8 years for seizures, microcephaly, and developmental delay. She is the only child from a nonconsanguineous 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, a magnetic resonance image (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 nonconsanguineous 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 (<5th percentile) and a HC at the 2nd to 5th percentile. There was no dysmorphism.

Individual 3 is the first of three brothers of nonconsanguineous parents. He was born at term after a normal pregnancy. At birth, length, weight, and HC were at the 25th 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 electroencephalogram (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 nonconsanguineous 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 90th percentile. He was microcephalic with a HC at the 2nd 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 1st 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 1 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. **1c, 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 breakthrough 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. **1e, f**).

Individual 7 was born after a normal pregnancy to healthy nonconsanguineous parents. He was diagnosed with hearing loss, strabismus (Fig. **1g, 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 nonreassuring 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 7 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.

For more information, see Supplementary Material.

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 nonspecific in three. Interestingly, some features observed in this cohort, such as microcephaly (six individuals), hypotonia (two individuals), hearing loss (one individual),

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

Ind.	Gender	Age	Development	Seizure disorder	Microcephaly	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

ADHD attention deficit hyperactivity disorder, *ASD* autism spectrum disorder, *DD* developmental delay, *FTT* failure to thrive, *ID* intellectual disability, *Ind.* individual, *kb* kilobases.

and visual impairment (two individuals), have been previously associated with biallelic *TBC1D24* pathogenic variants. 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 (refs. [7,8]) and 16p11.2 (refs. [9,10]) deletion syndromes. The smallest deletion (individual 8) contains 13 genes and the largest (individual 3) contains 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 postzygotic 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 pathogenic variants 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(AD) 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 nondeleted *TBC1D24* allele did not detect any pathogenic variants 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 ref. [12]). The

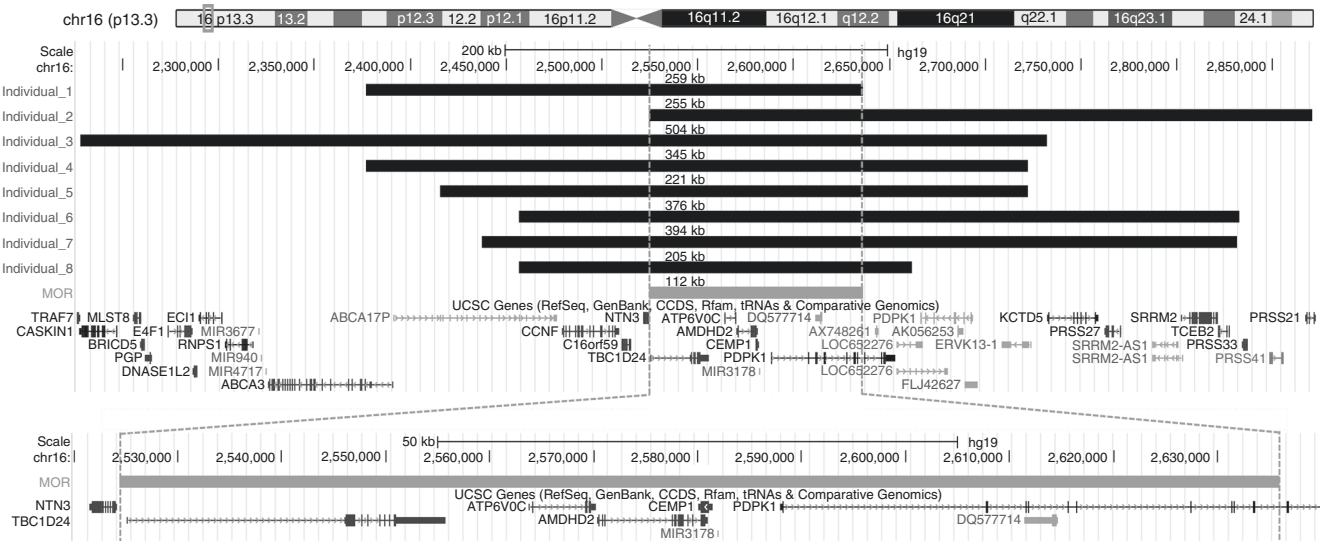


Fig. 2 Schematic of microdeletions observed in the cohort. The borders of the minimal overlapping region (MOR) are demarcated by dotted lines encompassing seven genes.

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

See text for explanation.
NA not available, *P(AD)* probability of autosomal dominant inheritance, *pLI* probability of loss-of-function intolerance.

microdeletion was absent in two additional cohorts, 1 of 5531 cases that were sent to a diagnostic laboratory for clinical testing[14] and 1 including 1133 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* ortholog 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 16 kDa, V0 subunit C) is a component of vacuolar ATPase (V-ATPase), a multisubunit 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 signaling pathways (reviewed in ref. [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 (ref. [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 pathogenic variants 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* pathogenic variants 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 [ref. [30]]) and the ExAC data set 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 1 individual from a cohort of 4293 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 pathogenic variants. One proband in a cohort of 1133 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 ref. [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.

ELECTRONIC SUPPLEMENTARY MATERIAL

The online version of this article (https://doi.org/10.1038/s41436-018-0290-3) contains supplementary material, which is available to authorized users.

DISCLOSURE

The authors declare no conflicts of interest.

REFERENCES

1. Campeau PM, Kasperaviciute D, Lu JT, et al. The genetic basis of DOORS syndrome: an exome-sequencing study. Lancet Neurol. 2014;13:44–58.
2. Banuelos E, Ramsey K, Belnap N. et al. Case report: novel mutations in TBC1D24 are associated with autosomal dominant tonic-clonic and myoclonic epilepsy and recessive Parkinsonism, psychosis, and intellectual disability. F1000Res. 2017;6:553.
3. Kent WJ, Sugnet CW, Furey TS, et al. The human genome browser at UCSC. Genome Res. 2002;12:996–1006.
4. Huang N, Lee I, Marcotte EM, Hurles ME. Characterising and predicting haploinsufficiency in the human genome. PLoS Genet. 2010;6: e1001154.
5. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536:285.
6. Quinodoz M, Royer-Bertrand B, Cisarova K, Di Gioia SA, Superti-Furga A, Rivolta C. DOMINO: using machine learning to predict genes associated with dominant disorders. Am J Hum Genet. 2017;101:623–629.
7. Bartsch O, Rasi S, Delicado A, et al. Evidence for a new contiguous gene syndrome, the chromosome 16p13.3 deletion syndrome alias severe Rubinstein-Taybi syndrome. Hum Genet. 2006;120:179–186.
8. Nelson M, Quinonez S, Ackley T, Iyer RK, Innis JW. Multiple congenital anomalies and developmental delay in a boy associated with a de novo 16p13.3 deletion. Am J Med Genet A. 2011;155A:612–617.
9. Ghebranious N, Giampietro PF, Wesbrook FP, Rezkalla SH. A novel microdeletion at 16p11. 2 harbors candidate genes for aortic valve development, seizure disorder, and mild mental retardation. Am J Med Genet A. 2007;143:1462–1471.
10. Bijlsma E, Gijsbers A, Schuurs-Hoeijmakers J, et al. Extending the phenotype of recurrent rearrangements of 16p11. 2: deletions in mentally retarded patients without autism and in normal individuals. Eur J Med Genet. 2009;52:77–87.
11. Cooper GM, Coe BP, Girirajan S, et al. A copy number variation morbidity map of developmental delay. Nat Genet. 2011;43:838.
12. Coe BP, Witherspoon K, Rosenfeld JA, et al. Refining analyses of copy number variation identifies specific genes associated with developmental delay. Nat Genet. 2014;46:1063.
13. Männik K, Mägi R, Macé A, et al. Copy number variations and cognitive phenotypes in unselected populations. JAMA. 2015;313:2044–2054.
14. Vulto-van Silfhout AT, Hehir-Kwa JY, Bon BW, et al. Clinical significance of de novo and inherited copy-number variation. Hum Mutat. 2013;34:1679–1687.
15. Fitzgerald T, Gerety S, Jones W, et al. Large-scale discovery of novel genetic causes of developmental disorders. Nature. 2015;519:223.
16. Fischer B, Lüthy K, Paesmans J, et al. Skywalker-TBC1D24 has a lipid-binding pocket mutated in epilepsy and required for synaptic function. Nat Struct Mol Biol. 2016;23:965.
17. Mangieri LR, Mader BJ, Thomas CE, et al. ATP6V0C knockdown in neuroblastoma cells alters autophagy-lysosome pathway function and

metabolism of proteins that accumulate in neurodegenerative disease. PLoS ONE. 2014;9:e93257.
18. Chung AY, Kim MJ, Kim D, et al. Neuron-specific expression of atp6v0c2 in zebrafish CNS. Dev Dyn. 2010;239:2501–2508.
19. Gagliardi PA, di Blasio L, Primo L. PDK1: A signaling hub for cell migration and tumor invasion. Biochim Biophys Acta. 2015;1856:178–188.
20. di Blasio L, Gagliardi PA, Puliafito A, et al. PDK1 regulates focal adhesion disassembly by modulating endocytosis of αvβ3 integrin. J Cell Sci. 2015;128:863–877.
21. Lawlor MA, Mora A, Ashby PR, et al. Essential role of PDK1 in regulating cell size and development in mice. EMBO J. 2002;21:3728–3738.
22. Bayascas JR, Wullschleger S, Sakamoto K, et al. Mutation of the PDK1 PH domain inhibits protein kinase B/Akt, leading to small size and insulin resistance. Mol Cell Biol. 2008;28:3258–3272.
23. Zurashvili T, Cordón-Barris L, Ruiz-Babot G, et al. Interaction of PDK1 with phosphoinositides is essential for neuronal differentiation but dispensable for neuronal survival. Mol Cell Biol. 2013;33:1027–1040.
24. Bergfeld AK, Pearce OM, Diaz SL, Pham T, Varki A. Metabolism of vertebrate amino sugars with N-glycolyl groups: elucidating the intracellular fate of the non-human sialic acid N-glycolylneuraminic acid. J Biol Chem. 2012;287:28865–28881.
25. Diaz SL, Padler-Karavani V, Ghaderi D, et al. Sensitive and specific detection of the non-human sialic acid N-glycolylneuraminic acid in human tissues and biotherapeutic products. PLoS ONE. 2009;4:e4241.
26. Alvarez-Pérez MA, Narayanan S, Zeichner-David M, Carmona BR, Arzate H. Molecular cloning, expression and immunolocalization of a novel human cementum-derived protein (CP-23). Bone. 2006;38:409–419.
27. Li W, Shen S, Wu S, Chen Z, Hu C, Yan R. Regulation of tumorigenesis and metastasis of hepatocellular carcinoma tumor endothelial cells by microRNA-3178 and underlying mechanism. Biochem Biophys Res Commun. 2015;464:881–887.
28. Zara F, Gennaro E, Stabile M, et al. Mapping of a locus for a familial autosomal recessive idiopathic myoclonic epilepsy of infancy to chromosome 16p13. Am J Hum Genet. 2000;66:1552–1557.
29. Balestrini S, Milh M, Castiglioni C, et al. TBC1D24 genotype–phenotype correlation: epilepsies and other neurologic features. Neurology. 2016;87:77–85.
30. Hirtz D, Thurman D, Gwinn-Hardy K, Mohamed M, Chaudhuri A, Zalutsky R. How common are the "common" neurologic disorders? Neurology. 2007;68:326–337.
31. Falace A, Buhler E, Fadda M, et al. TBC1D24 regulates neuronal migration and maturation through modulation of the ARF6-dependent pathway. Proc Natl Acad Sci U S A. 2014;111:2337–2342.
32. Inoue H, Noumi T, Nagata M, Murakami H, Kanazawa H. Targeted disruption of the gene encoding the proteolipid subunit of mouse vacuolar H+-ATPase leads to early embryonic lethality. Biochim Biophys Acta. 1999;1413:130–138.
33. Sun-Wada G-H, Murata Y, Yamamoto A, Kanazawa H, Wada Y, Futai M. Acidic endomembrane organelles are required for mouse postimplantation development. Dev Biol. 2000;228:315–325.
34. Carvill GL, Weckhuysen S, McMahon JM, et al. GABRA1 and STXBP1: novel genetic causes of Dravet syndrome. Neurology. 2014; 82:1245–1253.
35. Gilissen C, Hehir-Kwa JY, Thung DT, et al. Genome sequencing identifies major causes of severe intellectual disability. Nature. 2014; 511:344.
36. Helbig KL, Hagman KDF, Shinde DN, et al. Diagnostic exome sequencing provides a molecular diagnosis for a significant proportion of patients with epilepsy. Genet Med. 2016;18:898.
37. Bowling KM, Thompson ML, Amaral MD, et al. Genomic diagnosis for children with intellectual disability and/or developmental delay. Genome Med. 2017;9:43.
38. Hamdan FF, Myers CT, Cossette P, et al. High rate of recurrent de novo mutations in developmental and epileptic encephalopathies. Am J Hum Genet. 2017;101:664–685.
39. Deciphering Developmental Disorders Study. Prevalence and architecture of de novo mutations in developmental disorders. Nature. 2017;542:433.

[1]Division of Medical Genetics, Department of Specialized Medicine, McGill University Health Centre, Montreal, QC, Canada. [2]Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, M13 9PL Manchester, UK. [3]Centre de Recherche du CHU Sainte-Justine, Montreal, QC, Canada. [4]Department of Psychiatry, McGill University, Montreal, QC, Canada. [5]Department of Pediatrics, Division of Child & Adolescent Neurology, The University of Texas McGovern Medical School, Houston, TX, USA. [6]Service de Génétique CHU de Lyon-GH Est, Lyon, France. [7]Department of Pediatrics, Flinders Medical Centre, Bedford Park, SA, Australia. [8]Department of Medical Genetics, University of Calgary, Calgary, AB, Canada. [9]Department of Pediatrics, Université de Montréal, Montreal, QC, Canada. [10]Department of Pediatrics, CHU Laval, Quebec City, QC, Canada. [11]Division of Medical Genetics, Department of Pediatrics, Massachusetts General Hospital, Boston, MA, USA. [12]Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA. [13]Adelaide Medical School and Robinson Research Institute, The University of Adelaide, Adelaide, Australia. [14]South Australian Clinical Genetics Service, SA Pathology, Women's and Children's Hospital, Adelaide, Australia. [15]Klinik für Neuropädiatrie und Stoffwechselerkrankungen, Zentrum für Kinder- und Jugendmedizin, Oldenburg, Germany. [16]Department of Clinical and Experimental Epilepsy, Institute of Neurology, University College London, London, United Kingdom. [17]Chalfont Centre for Epilepsy, Bucks, United Kingdom. [18]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

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).

Bibliography for supplementary material

Bayascas JR, Wullschleger S, Sakamoto K, García-Martínez JM, Clacher C, Komander D, *et al.* Mutation of the PDK1 PH domain inhibits protein kinase B/Akt, leading to small size and insulin resistance. Mol Cell Biol. 2008;28(10):3258-72.

Cho, K.S., Lee, J.H., Kim, S., Kim, D., Koh, H., Lee, J., *et al*. Drosophila phosphoinositide-dependent kinase-1 regulates apoptosis and growth via the phosphoinositide 3-kinase-dependent signaling pathway. Proc Nat Acad Sci. 2001;98(11):6144-6149.

Chung AY, Kim MJ, Kim D, Bang S, Hwang SW, Lim CS, *et al*. Neuron-specific expression of atp6v0c2 in zebrafish CNS. Dev Dyn. 2010;239(9):2501-8.

di Blasio L, Gagliardi PA, Puliafito A, Sessa R, Seano G, Bussolino F, *et al*. PDK1 regulates focal adhesion disassembly by modulating endocytosis of αvβ3 integrin. J Cell Sci, 12. 2015;(5):863-77.

Ellis K, Bagwell J, Bagnat M. Notochord vacuoles are lysosome-related organelles that function in axis and spine morphogenesis. J Cell Biol. 2013;200(5):667-79.

Falace A, Filipello F, La Padula V, Vanni N, Madia F, Tonelli DD, *et al*. TBC1D24, an ARF6-interacting protein, is mutated in familial infantile myoclonic epilepsy. Am J Hum Genet. 2010;87(3):365-70.

Falace A, Buhler E, Fadda M, Watrin F, Lippiello P, Pallesi-Pocachard E, *et al*. TBC1D24 regulates neuronal migration and maturation through modulation of the ARF6-dependent pathway. P Natl A Sci. 2014;111(6):2337-42.

Fischer B, Lüthy K, Paesmans J, De Koninck C, Maes I, Swerts J, *et al*. Skywalker-TBC1D24 has a lipid-binding pocket mutated in epilepsy and required for synaptic function. Nat Struct Mol Biol. 2016;23(11),:965-73.

Gagliardi PA, di Blasio L, Primo L. PDK1: A signaling hub for cell migration and tumor invasion. BBA-Rev Cancer. 2105;1856(2):178-88.
Huang N, Lee I, Marcotte EM, Hurles ME. Characterising and predicting haploinsufficiency in the human genome. PLoS Genet. 2010;6(10):e1001154.

Inoue H, Noumi T, Nagata M, Murakami H, Kanazawa H. Targeted disruption of the gene encoding the proteolipid subunit of mouse vacuolar H+-ATPase leads to early embryonic lethality. BBA-Bioenergetics. 1999;1413(3):130-38.

Lawlor MA, Mora A, Ashby PR, Williams MR, Murray-Tait V, Malone L, *et al*. Essential role of PDK1 in regulating cell size and development in mice. EMBO J. 2002;21(14):3728-38.

Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, *et al*. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536(7616):285-91.

Mangieri LR, Mader BJ, Thomas CE, Taylor CA, Luker AM, Tonia ET, *et al*. ATP6V0C knockdown in neuroblastoma cells alters autophagy-lysosome pathway function and metabolism of proteins that accumulate in neurodegenerative disease. PloS one. 2014;9(4):e93257.

Murphy CT, Hu PJ. Insulin/insulin-like growth factor signaling in C. elegans. WormBook. 2013, ed. The C. elegans Research Community

Nuckels RJ, Ng A, Darland T, Gross JM. The vacuolar-ATPase complex regulates retinoblast proliferation and survival, photoreceptor morphogenesis, and pigmentation in the zebrafish eye. Invest Ophthalmol Vis Sci. 2009;50(2):893-905.

Paradis S, Ailion M, Toker A, Thomas JH, Ruvkun G. A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in Caenorhabditis elegans. Genes Dev. 1999;13(11):1438-52.

Quinodoz M, Royer-Bertrand B, Cisarova K, Di Gioia SA, Superti-Furga A, Rivolta C. DOMINO: Using Machine Learning to Predict Genes Associated with Dominant Disorders. Am J Med Genet. 2017;101(4):623-9.

Rintelen, F., Stocker, H., Thomas, G. Hafen, E. PDK1 regulates growth through Akt and S6K in Drosophila. Proc Nat Acad Sci. 2001;98(26):15020-15025.

Sun-Wada GH, Murata Y, Yamamoto A, Kanazawa H, Wada, Y, Futai M. Acidic endomembrane organelles are required for mouse postimplantation development. Dev Biol. 2000;228(2):315-25.

Tognon E, Kobia F, Busi I, Fumagalli A, De Masi F, Vaccari T. Control of lysosomal biogenesis and Notch-dependent tissue patterning by components of the TFEB-V-ATPase axis in Drosophila melanogaster. Autophagy. 2016;12(3):499-514.

Uytterhoeven V, Kuenen S, Kasprowicz J, Miskiewicz K, Verstreken P. Loss of skywalker reveals synaptic endosomes as sorting stations for synaptic vesicle proteins. Cell. 2011;145(1):117-32.

Zurashvili T, Cordón-Barris L, Ruiz-Babot G, Zhou X, Lizcano JM, Gómez N, *et al*. Interaction of PDK1 with phosphoinositides is essential for neuronal differentiation but dispensable for neuronal survival. Mol Cell Biol. 2013 33(5):1027-40.