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REPORT

De novo variants in *PAK1* lead to intellectual disability with macrocephaly and seizures

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Using trio exome sequencing, we identified *de novo* heterozygous missense variants in *PAK1* in four unrelated individuals with intellectual disability, macrocephaly and seizures. *PAK1* encodes the p21-activated kinase, a major driver of neuronal development in humans and other organisms. In normal neurons, PAK1 dimers reside in a trans-inhibited conformation, where each auto-inhibitory domain covers the kinase domain of the other monomer. Upon GTPase binding via CDC42 or RAC1, the PAK1 dimers dissociate and become activated. All identified variants are located within or close to the autoinhibitory switch domain that is necessary for trans-inhibition of resting PAK1 dimers. Protein modelling supports a model of reduced ability of regular autoinhibition, suggesting a gain of function mechanism for the identified missense variants. Alleviated dissociation into monomers, autophosphorylation and activation of PAK1 influences the actin dynamics of neurite outgrowth. Based on our clinical and genetic data, as well as the role of *PAK1* in brain development, we suggest that gain of function pathogenic *de novo* missense variants in *PAK1* lead to moderate-to-severe intellectual disability, macrocephaly caused by the presence of megalencephaly and ventriculomegaly, (febrile) seizures and autism-like behaviour.

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Introduction

With a prevalence of about 1–3%, intellectual disability is a major health and socioeconomic issue (Maulik *et al.*, 2011). Intellectual disability is genetically highly heterogeneous, with each recognized cause affecting only a small fraction of all patients (Vissers *et al.*, 2016). Services for people with intellectual disability increasingly focus on innovative and personalized therapies. Major research efforts have used efficient molecular diagnostic tools for rare diseases, especially exome sequencing given its high diagnostic yield (Ligt *et al.*, 2012; Boycott *et al.*, 2017; Trujillano *et al.*, 2017), thus providing knowledge of specific genetic variants as a prerequisite for personalized therapy in cases where mechanistic insights on the affected genes and proteins exist.

At four centres in Germany, the Netherlands, the USA and Israel, we ascertained four unrelated individuals with a neurodevelopmental disorder, including intellectual disability, seizures and macrocephaly. We performed exome sequencing to identify the underlying genetic cause of the disease. Here we present data that indicate a pathogenic mechanism of *PAK1* mutations by a dominant gain-of-function effect. *De novo PAK1* missense pathogenic variants have also recently been described in two cases with intellectual disability, macrocephaly, seizures and speech delay (MIM: 618158) (Harms *et al.*, 2018). Human PAK1 is a 545-amino acid (aa) protein with two major domains, an autoregulatory domain and a protein kinase domain (Fig. 1). In its inactive form, it homodimerizes by interacting and binding at a region within the autoregulatory domain (74–132 aa) masking the active site of the kinase. Therefore the autoregulatory domain is important for the autoinhibition of this kinase, and this mechanism has been well described for PAK1 (Lei *et al.*, 2000; Parrini *et al.*, 2002; Pirruccello *et al.*, 2006). In the inhibited state, the inhibitory-switch domain of one homodimer overlaps the GTPase binding region (75–105 aa) of the other homodimer and a polypeptide segment covers the kinase cleft (Parrini *et al.*, 2002). During activation, GTPase binding triggers refolding of the inhibitory-switch domain, disrupting the PAK1 dimer and leading to rearrangement of the kinase active site into a catalytically competent state.

We propose that PAK1 disease-causing variants result in activation of the PAK1-LIMK1 pathway, a mechanism that is discussed in fragile X syndrome (FXS) (Hayashi *et al.*, 2007; Pyronneau *et al.*, 2017).

Materials and methods

Subjects

Informed consent of all examined individuals and/or their guardians was obtained according to Institutional Review Board approved research protocols, including consent for publishing of the clinical as well as the genetic and genomic results. In Proband 4, genetic testing was carried out as part of routine clinical care and therefore institutional ethics approval was not required. Informed consent has been obtained for the published photo.

Exome sequencing

The study design comprised whole exome-sequencing (WES), genotype calling and the comparison of parent-offspring trios for the identification of rare variants in coding sequence associated with the observed human phenotype. We performed trio or family exome sequencing for all four probands and their biological parents according to standard methods (Supplementary material). Reported variants for all individuals were confirmed with Sanger dideoxynucleotide sequencing.

Protein modelling and analysis of gene expression

Energetic calculations of the observed protein changes were carried out using BindProfX (Xiong *et al.*, 2017) and visualized using UCSC Chimera v.1.12 (Pettersen *et al.*, 2004). To determine an unbiased set of relevant PAK1 interacting genes for comparisons, we analysed gene expression in normal brain development using the R2 Genomics analysis and visualization platform (dataset brspv10rs). Interacting genes were predicted by using GENEMANIA (https://genemania.org).

Limitations of the study

Limitations of the retrospective case series are to be noted as there is limited information on the seizure types of the patients, with respect to detailed descriptions of seizures and post-ictal symptoms to classify them in more detail.
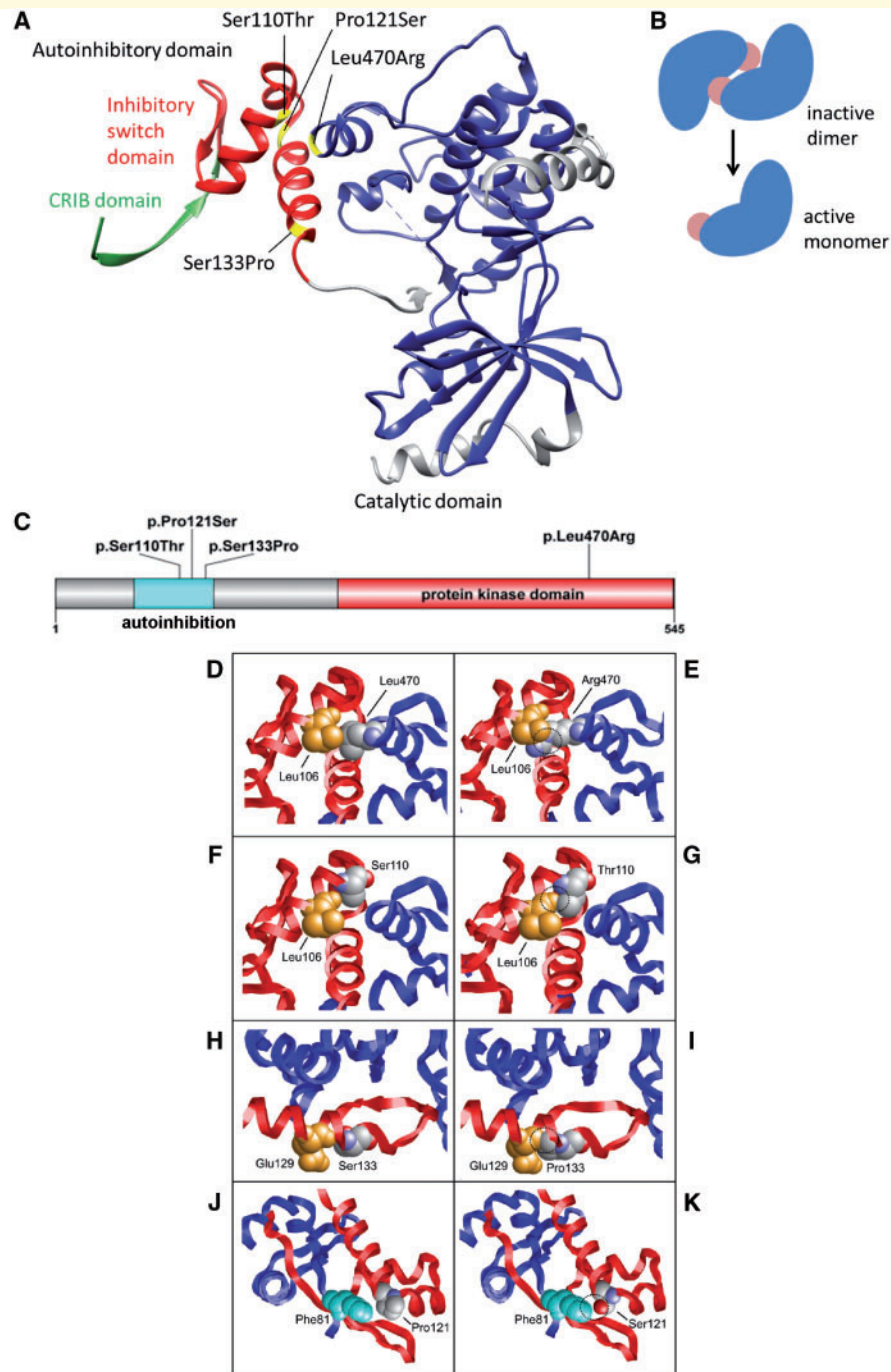
Figure 1 Structural effects of the PAK1 variants. (**A**) Protein structure of PAK1 with the catalytic domain (270–521 aa, blue) and the autoinhibitory domain (70–140 aa), comprising the Cdc42 Rac Interactive Binding (CRIB) domain (75–86 aa, green) and the inhibitory switch domain (87–136 aa, red, https://www.uniprot.org). Variants Leu470Arg, Ser133Pro and Ser110Thr affect the contact zone of the catalytic and inhibitory switch domains. Pro121Ser is located in the interface between both PAK1 monomers (see also Supplementary Fig. 1). The CRIB domain (green) mediates activation of PAK1 by binding of RAC1 and CDC42. (**B**) Autoinhibiton of PAK1. Autoinhibitory domains (light red) of inactive dimers cover the active site *in-trans*. After CDC42 or RAC1 binding, monomers dissociate, PAK1 autophosphorylation creates the active form of the protein. (**C**) Distribution of PAK1 variants across the coding region. (**D** and **E**) Leu470 forms tight hydrophobic interactions with Leu106, whereas the longer and charged Arg470 sidechain results in clashes with Leu106. In all panels the site of mutation is shown in space-filled presentation and coloured according to the atom types. Key interacting residues are shown in space-fill and are coloured orange or cyan. The two subunits of PAK1 are shown as red and blue ribbon, respectively. Sites of unfavourable interactions in the variants are highlighted by dashed circles. (**F** and **G**) Ser110 interacts with Leu106, whereas the additional methyl group present in the Thr110 sidechain forms clashes with Leu106. (**H** and **I**) Ser133 forms a backbone hydrogen bond with Glu129 within an α-helix. The presence of a cyclic sidechain in Pro133 results in a loss of this hydrogen bond and additionally causes steric clashes with Glu129. (**J** and **K**) Pro121 is located at a kink between two α-helices and near Phe81 of the second subunit. Replacement by a more flexible serine is expected to destabilize the kink and additionally creates an unfavourable interaction by placing the polar sidechain hydroxyl group in close proximity to the hydrophobic Phe81 sidechain.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and/or its Supplementary material.

Results

The probands presented with moderate to profound intellectual disability, seizures, macrocephaly, ventriculomegaly and other abnormalities in brain MRI, muscular hypotonia, and abnormal gait (Table 1, see detailed clinical descriptions in the Supplementary material). The analyses revealed *de novo* missense variants in *PAK1* in the four probands, c.1409T>G in exon 13 of 16 leading to p.(Leu470Arg) in Proband 1, and three changes in exon 4: c.397T>C; p.(Ser133Pro) in Proband 2, c.361C>T; p.(Pro121Ser) in Proband 3, and c.328T>A; p.(Ser110Thr) in Proband 4 (Table 1 and Supplementary Table 1). None of the variants in *PAK1* have been observed in the gnomAD database (accessed in September 2018) or in internal databases. All of the variants are predicted to be pathogenic by *in silico* bioinformatics prediction algorithms and reaching between the top 1% and 0.1% CADD scores (Kircher *et al.*, 2014). In addition, all variants affect highly conserved amino acid residues and nucleotides, except for a moderately conserved nucleotide in Proband 4. *PAK1* shows fewer missense variants than expected by chance with a z-score of 4.16 (Lek *et al.*, 2016), suggesting that heterozygous missense variants are less tolerated. In addition, *in silico* modelling of protein structures of wild-type and altered PAK1 revealed that all four identified variants similarly lead to a disturbance of the autoinhibition mechanism (Fig. 1).

Variants Leu470Arg, Ser133Pro and Ser110Thr are located in the interface between the inhibitory switch domain and the catalytic domain (Fig. 1). In each variant, the wild-type residue is replaced by a bulkier amino acid resulting in steric problems ('clashes') with adjacent amino acids, which destabilize the autoinhibitory domain and its interaction with the catalytic domain. Introducing arginine at p.Leu470, proline at p.Ser133, and threonine at p.Ser110 reduces binding by 1.76 kcal/mol, 1.26 kcal/mol, and 0.99 kcal/mol, respectively. The fourth variant, Pro121Ser, is located in the PAK1 dimer interface formed by the inhibitory switch domains of both subunits (Fig. 1 and Supplementary Fig. 1) and weakens binding by 2.30 kcal/mol. As a reference, an energy change of 1.36 kcal/mol corresponds to a reduction in binding affinity by one order of magnitude indicating that all three variants significantly destabilize the interaction between the inhibitory switch and the catalytic domain. In addition, the Pro121Ser variant is also expected to destabilize the autoinhibitory conformation of the switch domain itself: the rigid Pro121 with its cyclic sidechain is involved in the formation of a kink between two α-helices (Fig. 2K), which is expected to become more flexible in the Ser121

variant. The inhibitory switch domain has been described as the core of the autoregulatory fragment, which appears to inhibit the kinase with one surface and anchor the dimer contact with another. Conformational changes in this highly conserved protein domain are likely to affect protein function. A loss of stability in the autoinhibitory domain, thereby reducing autoinhibition would be supportive of a gain-of-function mode of the observed genetic variants. A *PAK1* variant in the inhibitory switch domain, Leu107Phe, is already known to prevent the interaction between the N-terminal regulatory portion and catalytic domain, leading to kinase activation (Brown *et al.*, 1996).

Discussion

PAK1 is a family member of the serine/threonine p21-activating (PAK) kinases composed of six known members in humans, PAK1–6. These proteins are critical effectors that link RhoGTPases to cytoskeleton reorganization and nuclear signalling and other intracellular processes (Manser *et al.*, 1998; Zhao and Manser, 2012; Rane and Minden, 2014). Both PAK1 and PAK3 have been reported to control brain size through coordinating neuronal complexity and synaptic properties (Huang *et al.*, 2011). Nonsense and missense variants that are thought to decrease autophosphorylation and activation of PAK3 have been described in males with X-linked recessive developmental delay (MIM: 300558). Recently, two probands with a neurodevelopmental disorder and *de novo* missense variants in *PAK1* have been described. Their phenotype is similar to the phenotype presented by our probands with developmental delay, macrocephaly, seizures, and ataxic gait (Harms *et al.*, 2018). The described variants p.(Tyr131Cys) and p.(Tyr429Cys) are located in the autoinhibitory and kinase domains of PAK1 similarly to the *de novo* variants presented in our study. Harms *et al.* (2018) furthermore showed that both variants lead to significantly reduced dimerization, providing evidence for a gain-of-function pathomechanism. Our results on the location of variants and the predicted effects on the protein provide additional evidence for this pathomechanism.

PAK1 is central to a well-described signalling pathway and an involvement in neurodevelopmental disorders was also described for other members of the pathway. Interacting partners of PAK1, especially the PAK1 activators RAC1 and CDC42, have been associated with developmental syndromes (Fig. 3 and Supplementary Table 2). *De novo* pathogenic missense *RAC1* variants are associated with varying degrees of developmental delay, brain malformations, and additional phenotypes in autosomal dominant mental retardation 48 (MIM: 617751). Notably, of the seven reported affected individuals, two were macrocephalic; however, without a clear effect of protein activation. Pathogenic, heterozygous *de novo* or familial variants in *CDC42* cause a highly heterogeneous developmental

Table 1 Clinical features of individuals with predicted missense variants in *PAK1*

	Proband 1	Proband 2	Proband 3	Proband 4
cDNA change	c.1409T>G	c.397T>C	c.361C>T	c.328T>A
Amino acid change	p.(Leu470Arg)	(p.Ser133Pro)	p.(Pro121Ser)	p.(Ser110Thr)
Ethnicity	Caucasian	Caucasian	Morocco	Sephardi Jew
Age at last exam/Sex	4 y/Female	17 y/Male	20 y/Male	8 y/Male
HC last examination	55.5 cm (>P99 +3.80 SD)	59.8 cm (P98 +2.07 SD)	61.5 cm (>P99 +2.95 SD)	59.5 cm (>P99 +4.93 SD)
BMI	13.9 (P14 −1.09 SD)	Not measured	24.7 (P84 +0.98 SD)	15.3 (P33 −0.45 SD)
Pregnancy	Maternal gestational diabetes, pre-eclampsia	No anomalies	No anomalies	Choroid plexus cyst
Birth at gestational week	30 weeks	32 weeks	34 weeks	40 weeks
Birth parameters	Weight 1120 g (P25 −0.69 SD), Length 41 cm (P67 +0.45 SD), HC 27.5 cm (P43 −0.18 SD), APGAR 8/9/10	Weight 2268 g (P86 +1.07 SD), Length NA, HC NA, APGAR NA	Weight 2100 g (P34 −0.4 SD), Length NA, HC NA, APGAR 6/8/9	Weight 2800 g (P3 −1.87SD), Length NA, HC 34.5 cm (P20 −0.85 SD), APGAR NA
Neonatal period	Primary C-section; NICU	Spontaneous uncomplicated premature vaginal delivery NICU 4 weeks	IRDS, 4 days artificial respiration (CPAP and IPPV); Neonatal jaundice (phototherapy for 3 days)	NA
Congenital anomalies	Partial 2–4 toe syndactyly; long fingers	Partial 2–3 toe syndactyly; macrocephaly	No anomalies	No anomalies
Postnatal growth (Strauss et al., 2018), HC	At 3 months: HC 37 cm (P89 +1.2 SD), At 6 months: HC 43.5 cm (P97 +1.9 SD), At 12 months: HC 48.3 cm (>P97 +2.75 SD)	At 13 months: HC 52 cm (>P99 +3.96 SD), length 76 cm (P26 −0.65SD), weight 10.84 kg (P62 +0.31 SD)	At 12y: HC 59 cm (>P99 +3.27 SD), length 140 cm (P7 −1.49 SD)	At 18 months: HC 58 cm (>P99 +7.76 SD)
Developmental delay	Yes	Yes	Yes	Yes
Intellectual disability	Moderate to severe	Profound	Moderate to severe	Profound
Speech and language	Delayed, no active speech	Non-verbal	Delayed, sentences at age of 10y	Non-verbal
Mental health disorders	Autistic traits	Autism	ADHD	Autism
Facial dysmorphism/physical signs	Macrocephaly, frontal bossing, thin upper vermillion, broad nasal bridge, low-set posteriorly rotated ears	Craniofacial disproportion	Macrocephaly	Macrocephaly, strabismus
Muscular tone	Hypotonia	Initial hypotonia, later spastic quadriplegia	First year hypotonia	Hypotonia
Walking abilities	Unstable gait	No walking	Walking, mild ataxia	Ataxic, unstable gait
Brain/MRI	MRI: thin corpus callosum, ventriculomegaly, globular and mildly amorphous hippocampi bilaterally	MRI: thin corpus callosum, ventriculomegaly with widening of the sulci, megalencephaly, ventriculomegaly, mildly small and amorphous hippocampi bilaterally	MRI: thick corpus callosum, non-specific white matter anomalies of the deep white matter, bilateral and parietal, and of splenium of corpus callosum. MRS (spectroscopy): in white matter strongly increased total NAA (not in cortex)	MRI: thick corpus callosum, high bilateral signal intensity in frontal subcortical region
Seizures	At age 19–21 months three febrile seizures, seizures with awareness impairment and myoclonic seizures	Starting age 6y, atonic, tonic-clonic seizures	One typical febrile seizure (age not known)	Starting age 1.5y, focal epilepsy
EEG	Irregular basal activity at 6–8 Hz, intermittent beta wave activity, focus in right hemispheric region centroparietal and frontotemporal, spike waves occipital left, focal morphology, without generalization	High-amplitude paroxysmal discharges at the vertex	NA	Sharp waves in frontotemporal regions
Additional features/notes	NA	NA	CSF normal, tremor onset at 10y, positional tremor and tremor of tongue, no intention tremor, progressive tremor, no pyramidal signs	NA

Percentiles and standard deviations (SD) were calculated using https://www.pedz.de. CPAP = continuous positive airway pressure; HC = head circumference; IPPV = intermittent positive pressure ventilation; IRDS = infant respiratory distress syndrome; MRS = magnetic resonance spectroscopy; NA = not available; NAA = N-acetyl aspartate; NICU = neonatal intensive care unit.
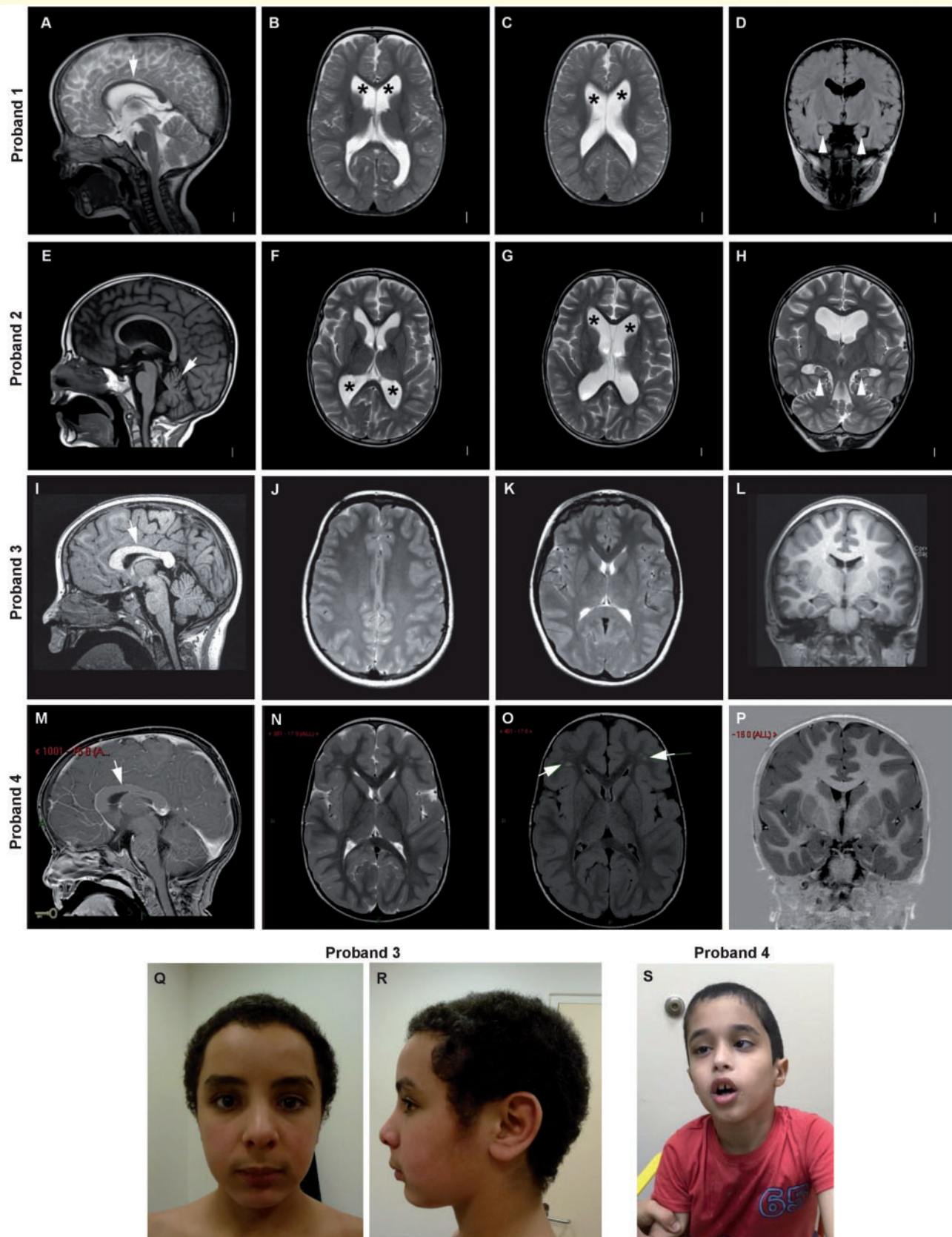
Figure 2 Brain MRI and facial features of individuals with heterozygous *PAK1* mutation. Brain MRIs of Proband 1 at age 2 years (**A–D**), Proband 2 at age 14 years (**E–H**), Proband 3 at age 12 years (**I–L**) and Proband 4 at age 8 years (**M–P**). Macrocephaly was caused by megalencephaly with or without accompanying ventriculomegaly. (**A**) T_2 mid-sagittal image showing a thin corpus callosum (arrow). (**B** and **C**) T_2 axial images showing ventriculomegaly (asterisks). (**D**) T_1 coronal image showing globular and mildly amorphous hippocampi bilaterally. (**E**) T_1
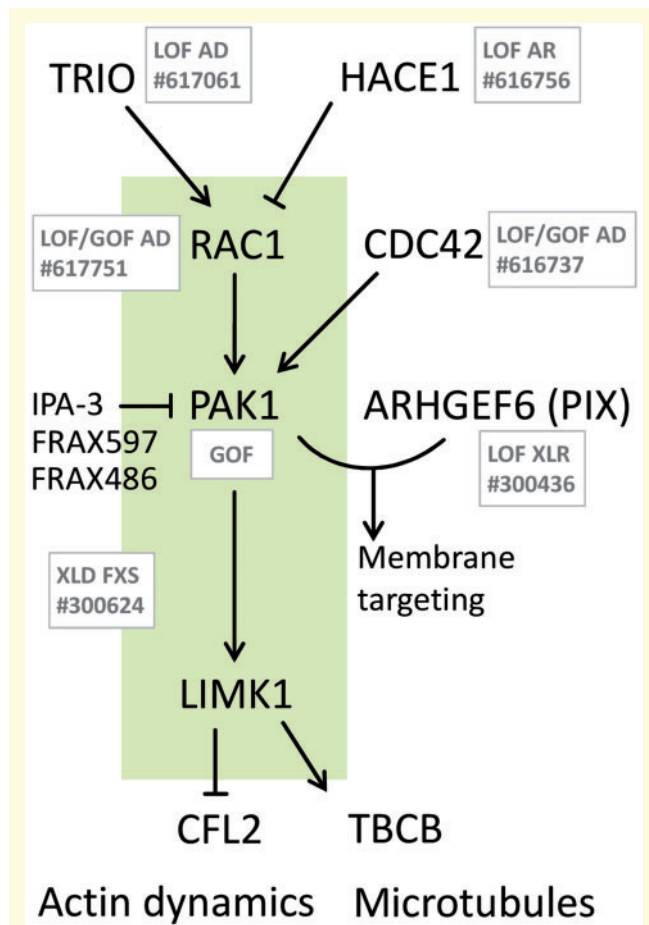
Figure 3 Involvement of the PAK1 pathway in developmental phenotypes. RAC1 and CDC42 are direct activators, TRIO and HACE1 are indirect (via RAC1) regulators of PAK1. ARHGEF6 binds PAK1 for regulation of neurite outgrowth. PAK1 activates LIMK1, the regulator of PAK1 downstream effectors cofilin (CFL2) and tubulin cofactor B. Disorders associated with deactivating and/or activating variants for each gene are indicated in grey with the respective mode of inheritance given (see Supplementary Table 2 for details and phenotypic overlap). Green shaded box indicates activation of RAC1-PAK1-LIMK1 pathway in FXS, where macrocephaly also occurs. Currently available inhibitors of PAK1 are indicated.

disorder (MIM: 616737). Some individuals with *CDC42* variants showed a broad forehead; however again, carrying both activating and deactivating variants.

Variants in *TRIO* and *HACE1*, known interactors of RAC1, are mainly reported as loss-of-function variants (MIM: 617061 and MIM: 616756). Notably, the PAK1-pathway is presumably activated in *HACE1* deficiency neurodevelopmental syndrome (Hollstein *et al.*, 2015) and the disorder resembles many of the symptoms described in this study, excluding macrocephaly, though one *HACE1* patient had a large head circumference at birth (Supplementary Table 2). To determine other interactors of PAK1 we identified genes that show an expression associated with that of *PAK1* and that code for proteins that physically interact with PAK1 (Supplementary Fig. 2). Additionally *ARHGEF6* has been associated with a neurodevelopmental disorder (MIM: 300436). *ARHGEF6* encodes the Rac/Cdc42 guanine nucleotide exchange factor 6 (PIX) (Manser *et al.*, 1998), which can bind to PAK1 for the mediation of neurite outgrowth (Rane and Minden, 2014). A role of PAK1 in neuronal growth is also supported by MRI data of our probands. *N*-acetyl aspartate (NAA) is a key metabolite and marker of intact neurons. Increased total levels of NAA were detected in the MRI of Proband 3 and could possibly be related to the increased growth of neurons as a cause of megalencephaly in *PAK1*-related disorders. Amorphous hippocampi were present in two of four probands (Probands 1 and 2). It has been shown previously that PAK1 is critical in hippocampal synaptic plasticity by the regulation of cofilin activity and the actin cytoskeleton (Asrar *et al.*, 2009). Hence, altered PAK1 activity may result in morphological changes of hippocampi, a feature that could be tested in future patients.

PAK1 is highly expressed in the human cerebellum and cortex and studies have described its role in neuronal migration by activating its downstream targets: LIMK1, cofilins and tubulin cofactor B (Sells *et al.*, 1997; Delorme *et al.*, 2007; Martinelli *et al.*, 2018). PAK1 phosphorylation of tubulin cofactor B is essential for the polymerization of new microtubules, which is important for building and rebuilding neuronal structures (Edwards *et al.*, 1999; Vadlamudi *et al.*, 2005). PAK1 activates LIMK1 that plays a critical role in dendritic spine morphogenesis and brain function by phosphorylation and deactivation of cofilin (Meng *et al.*, 2002). Cofilin can directly bind to actin filaments and promote their disassembly, needed for the reorganization of actin networks during neuronal growth (Chen *et al.*, 2011). Consequently, increased activity of

Figure 2 Continued
mid-sagittal image showing a thin corpus callosum and cerebellar atrophy with widening of the sulci (arrow). (**F** and **G**) T_2 axial images showing ventriculomegaly (asterisks). (**H**) T_2 coronal image showing mildly small and amorphous hippocampi bilaterally. (**I**) T_1 mid-sagittal image showing a thick corpus callosum (arrow). (**J** and **K**) T_2 axial images showing normal lateral ventricles. The white matter signal is slightly hyperintense in the posterior white matter, including the splenium (also present on FLAIR images, not shown). (**L**) T_1 coronal image showing normal volume and position of the hippocampi. At proton MR spectroscopy, *N*-acetyl aspartate was elevated (not shown). These findings were unchanged 9 months later. (**M**) T_1 mid-sagittal image showing macrocephaly and a thick corpus callosum (arrow). (**N**) T_2 axial image showing normal size of the lateral ventricles. (**O**) FLAIR axial image showing small foci of abnormal signal in frontal white matter may be related to old insult. (**P**) T_1 coronal image showing normal anatomy of the hippocampi. (**Q** and **R**) Photographs of Proband 3 at age 14 years with macrocephaly. (**S**) Photograph of Proband 4 at age 8 years with macrocephaly, facial muscular hypotonia and strabismus.

PAK1 in our probands would lead to increased activity of LIMK and thereby reduced actin dynamics via cofilin.

Recently, it was shown that the inherited intellectual disability and autism-associated FXS (MIM: 300624) is characterized by activated PAK1 (Pyronneau *et al.*, 2017). Intriguingly, the characteristic loss of the mRNA-binding protein FMR1 increased the abundance and activity of Rac1 in mice. Rac1 activated the kinases Pak1 and Limk1, which inactivated cofilin, thus preventing actin de-polymerization dynamics (Hayashi *et al.*, 2007; Pyronneau *et al.*, 2017). Both macrocephaly and seizures are also typ-ically present in FXS, underscoring a similarity of pheno-types in patients with mutations in *PAK1* and FXS.

Another piece of evidence supporting a gain-of-function mechanism for the identified variants in our patients is that *PAK1* homozygous knockout ($Pak1^{-/-}$) mice appear to be viable with no gross abnormalities. Human and mouse *PAK1* genes are extremely well conserved, being 98% iden-tical. $Pak1^{-/-}$ mice have some metabolic abnormal pheno-types, mainly increased blood urea levels, increased triglycerides, decreased lean body mass and decreased bone mineral content, and decreased neutrophils; however, no neurodevelopmental abnormalities or abnormal head size were reported (http://www.mousephenotype.org/data/genes/MGI:1339975). The fact that loss-of-function vari-ants of *PAK1* are tolerated in human populations (pLI = 0.67) (Karczewski *et al.*, 2016) also indicates that this may not represent a prevailing pathomechanism of PAK1-associated disease.

Several inhibitors of PAK1 are currently under develop-ment and comprise ATP-competitive inhibitors, such as FRAX597 and FRAX486, which block ATP binding at the catalytic domain of PAK1 (Sampat and Minden, 2018). Dibenzodiazepines inhibit PAK1 by preventing its autophosphorylation (Karpov *et al.*, 2015). If *PAK1*-asso-ciated disease proves to exhibit a gain-of-function mechan-ism, novel options for the treatment of affected patients could arise.

The importance of *PAK1* in neuronal growth and struc-ture, the positions and amino acids affected by the four identified genetic variants and their predicted effects on the protein, the strongly overlapping phenotypes of the af-fected individuals, as well as previously published data on other members of the *PAK1*-associated pathway allow us to consider that the *de novo* variants in *PAK1* discovered in this study are the cause of a neurodevelopmental disorder characterized by intellectual disability with macrocephaly and seizures.

Web resources

Genematcher, https://genematcher.org/
GnomAD, http://gnomad.broadinstitute.org/
VARVIS, https://www.limbus-medtec.com/
UCSC Genome Browser, https://genome.ucsc.edu/

HGMD, https://portal.biobase-international.com/cgi-bin/portal/login.cgi?redirect_url=/hgmd/pro/start.php?
PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/
MutationTaster, http://www.mutationtaster.org/
CADD, http://cadd.gs.washington.edu/score
R2, https://hgserver1.amc.nl/cgi-bin/r2/main.cgi
NCBI Pubmed, https://www.ncbi.nlm.nih.gov/pubmed/
Pedz, https://www.pedz.de
Online Mendelian Inheritance in Man, http://www.omim.org/
Genemania, https://genemania.org

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Competing interests

C.G-J. is a full-time employee of the Regeneron Genetics Center from Regeneron Pharmaceuticals Inc. and receives stock options as part of compensation. A.C. and C.M. are employed by and receive a salary from ARUP Laboratories. P.B-T. is a consultant to ARUP Laboratories. The other authors declare no conflict of interest.

Supplementary material

Supplementary material is available at *Brain* online.

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