Brain calcifications and PCDH12 variants.

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Nicolas, Gaël
Sanchez-Contreras, Monica
Ramos, Eliana Marisa
et al.

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ABSTRACT

Objective: To assess the potential connection between PCDH12 and brain calcifications in a patient carrying a homozygous nonsense variant in PCDH12 and in adult patients with brain calcifications.

Methods: We performed a CT scan in 1 child with a homozygous PCDH12 nonsense variant. We screened DNA samples from 53 patients with primary familial brain calcification (PFBC) and 26 patients with brain calcification of unknown cause (BCUC).

Results: We identified brain calcifications in subcortical and perithalamic regions in the patient with a homozygous PCDH12 nonsense variant. The calcification pattern was different from what has been observed in PFBC and more similar to what is described in in utero infections. In patients with PFBC or BCUC, we found no protein-truncating variant and 3 rare (minor allele frequency <0.001) PCDH12 predicted damaging missense heterozygous variants in 3 unrelated patients, albeit with no segregation data available.

Conclusions: Brain calcifications should be added to the phenotypic spectrum associated with PCDH12 biallelic loss of function, in the context of severe cerebral developmental abnormalities. A putative role for PCDH12 variants remains to be determined in PFBC. *Neurol Genet* 2017;3:e166; doi: 10.1212/NXG.0000000000000166

GLOSSARY

BCUC = brain calcification of unknown cause; ExAC = Exome Aggregation Consortium; PFBC = primary familial brain calcification.

A homozygous nonsense PCDH12 variant has recently been reported in consanguineous families, where the affected children had congenital microcephaly, epilepsy, and profound global developmental disability.\(^1\) Fetal MRI and USG showed dysplastic elongated masses in the midbrain-hypothalamus-optic tract area and hyperechogenic perithalamic foci. PCDH12 encodes a protocadherin associated with membrane physical stability, adhesion, and vasculature maintenance and has recently been pointed out as a candidate gene for primary familial brain calcification (PFBC). PFBC is characterized by the presence of calcifications affecting primarily the basal ganglia, in the absence of secondary cause.\(^2\) Clinical manifestations include movement disorders, cognitive impairment, psychiatric disturbances, and headache, most frequently beginning during adulthood.\(^2,3\) Heterozygous variants causing autosomal dominant PFBC in up to 50% of the families were identified in 4 genes: SLC20A2, PDGFRB, PDGFβ, and XPR1.\(^4–8\) We previously searched for genes with a cerebral expression pattern similar to the PFBC major...
causative gene SLC20A2 using the Allen Brain Atlas (brain-map.org),9,10 observing a higher SLC20A2 expression in regions affected by calcifications in PFBC. PCDH12 was singled out with the highest significant correlation,10 and a follow-up analysis with additional brains still shows PCDH12 as the most similar pattern to SLC20A2, even when compared with the other known PFBC causative genes (table e-1 at Neurology.org/ng).

To evaluate the potential link between PCDH12 and brain calcifications, (1) we performed a CT scan in a patient reported to carry a homozygous nonsense PCDH12 variant and (2) we screened DNA samples from patients with PFBC or brain calcifications of unknown cause (BCUC).

**METHODS** CT imaging in PCDH12 homozygous variant carriers. In the original report, patients with symmetric intrauterine growth retardation, severe microcephaly, visual impairment, dystonia, epilepsy, and profound developmental disability were shown to carry a PCDH12 c.995T>A, p.R839X homozygous variant.1 This variant is considered to be pathogenic when carried at the homozygous state following the American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations.11 Brain imaging revealed midbrain hypothalamus dysplasia and significant periventricular and/or periventricular hyperechogenicity. Fetal USG and MRI did not enable to determine whether these foci are eventually calcifications. Therefore, we performed a brain CT scan in individual III-1, family B from the original pedigree.1

**PCDH12 screening in patients with brain calcification.** We included a total of 79 worldwide adult cases with brain calcifications that were referred to 5 centers of expertise, negatively screened for the known PFBC causative genes (supplemental data). Of these, 53 cases matched the clinical inclusion criteria for PFBC (detailed previously in reference 3). Briefly, these cases exhibited at least bilateral basal ganglia calcifications and no secondary cause. The remaining 26 patients were included on a neuropathologic basis if they presented moderate-to-severe basal ganglia calcifications. Note that calcifications also involved other brain regions in almost all cases and that other causes of brain calcifications could not be excluded in these patients, thereafter referred as having BCUC. All patients were screened for pathogenic variants by sequencing all coding exons of PCDH12 (reference transcript: NM_016085.3). Bioinformatics predictions were performed using direct access to Polyphen2 HumDiv,12 SIFT,13 and Mutation Taster14 tools, and the minor allele frequency (MAF) was checked at the Exome Aggregation Consortium (ExAC) website accessed in August 2016 (exac.broadinstitute.org/).15 Detailed inclusion criteria and sequencing methods are provided in supplemental data.

**RESULTS** CT of a PCDH12 homozygous variant carrier. CT is the reference imaging to identify brain calcification, so we used it to determine the nature of the hyperechogenic foci identified in a patient with a homozygous nonsense p.R839X PCDH12 variant.1 We identified spots of perithalamic calcification located in the posterior arms of the internal capsules and in juxtacortical right white matter (figure).
function. DNA from relatives was not available for segregation analysis. This variant is located in the second cadherin tandem repeat domain (EC2) (NCBI accession cd11304) and, therefore, could affect homophilic adhesive behavior and calcium-dependent cell adhesion.16

The c.995T>A, p.(I332N) and c.3271G>A, p.(G1091S) variants are both predicted damaging by all 3 in silico tools. The p.I332N variant was reported with an overall MAF of 0.0001 in ExAC, found in 12 individuals of East Asian ancestry (the patient was born in Southeastern Asia) and 1 individual of European non-Finnish ancestry. The p.G1091S variant has an overall MAF of 3.3e-05, found in 1 individual of European non-Finnish ancestry (same as the patient) and 3 individuals of South Asian ancestry. DNA from relatives was not available for segregation analysis of any variant. Variant p.I332N is also located in a cadherin tandem repeat domain, namely EC3. However, p.G1091S variant is located in a highly conserved site in the cytoplasmic domain, which has a unique sequence among the cadherin family. Unlike the other cadherins, the cytoplasmic domain of PCDH12 does not interact with catenins, and it is involved in cellular processes other than cell junction, such as regulation of gene expression and signaling pathways.17 Clinical details of all 3 predicted damaging variant carriers are provided in the supplemental data.

**DISCUSSION**

We show here that a homozygous nonsense PCDH12 variant, detected in patients with severe developmental delay and microcephaly,1 is associated with brain calcifications. This feature should therefore be added to the phenotypic spectrum of this rare disorder. The pattern of calcifications is, however, different from the typical findings in PFBC, where calcifications always affect at least both pallidum,3 and resembled to those observed in various neuroinfectious prenatal conditions, such as TORCH infections.18 Brain calcification is a highly informative feature on brain imaging of children with neurodevelopmental disorders.19 Although CT is the reference imaging tool for detecting and assessing calcifications, MRI is the primary imaging tool for the detection of all other brain abnormalities in the absence of radiation. T2* or susceptibility-weighted images increase the diagnostic performance of MRI for calcification compared with the other sequences. However, they can sometimes miss small calcifications, and they are still complementary with CT to describe precise shape and intensity and to definitely conclude on the differential identification with iron deposits.19,20 In our patient, neither T2* nor susceptibility-weighted images were available.

In the original report, the efficiency of nonsense-mediated decay has been measured as 84%, suggesting a strong loss of function. The patients carrying the nonsense PCDH12 variant in a homozygous state may still express little amount of the truncated protein, but no full-length PCDH12. This supports the hypothesis that loss of function of PCDH12 is the mechanism leading to the patient’s phenotype, including brain calcification.

In a candidate gene approach, we searched for rare PCDH12 variants in PFBC and BCUC patients and found no protein-truncating variants. Three heterozygous missense variants, predicted damaging by at least one of the tools, were identified in 2 patients with PFBC and 1 patient with BCUC. Given the fact that biallelic loss of PCDH12 function leads to a severe neurodevelopmental phenotype, it is unlikely that these variants have a dominant-negative effect. However, as they are missense variants, their putative effect on protein function is hard to predict, and it remains possible that they are responsible for loss of function, gain of function, or have a neutral effect on protein function. The frequencies of these variants in the patients’ respective populations as estimated in ExAC are not inconsistent with a causative effect, as they are in the same frequency ranges as other disease-causing variants in SLC20A2.8 Because neither segregation nor functional data are available, it is not possible to conclude about their pathogenicity at this stage.

Besides PFBC, brain calcifications can be detected in other numerous distinct conditions, such as

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### Table 1 Rare PCDH12 variants identified in a series of 79 patients with PFBC or BCUC

<table>
<thead>
<tr>
<th>Location (Ghrc37)</th>
<th>cDNA change*</th>
<th>Protein change*</th>
<th>ExAC frequency*</th>
<th>SIFT prediction</th>
<th>Polyphen2 HumDiv prediction</th>
<th>Mutation Taster prediction</th>
<th>PhyloP</th>
</tr>
</thead>
<tbody>
<tr>
<td>chr5:141337254</td>
<td>c.163C&gt;G</td>
<td>p.(R55G)</td>
<td>6.1e-04</td>
<td>Tolerated</td>
<td>Benign</td>
<td>Polyorphism</td>
<td>–1.01</td>
</tr>
<tr>
<td>chr5:141336977</td>
<td>c.440G&gt;T</td>
<td>p.(S147V)</td>
<td>2.5e-05</td>
<td>Tolerated</td>
<td>Possibly damaging</td>
<td>Disease causing</td>
<td>2.14</td>
</tr>
<tr>
<td>chr5:141336422</td>
<td>c.995T&gt;A</td>
<td>p.(I332N)</td>
<td>1e-04</td>
<td>Deleterious*</td>
<td>Probably damaging</td>
<td>Disease causing</td>
<td>4.48</td>
</tr>
<tr>
<td>chr5:141325230</td>
<td>c.3271G&gt;A</td>
<td>p.(G1091S)</td>
<td>3.3e-05</td>
<td>Deleterious*</td>
<td>Probably damaging</td>
<td>Disease causing</td>
<td>4.81</td>
</tr>
</tbody>
</table>

Abbreviations: BCUC = brain calcification of unknown cause; cDNA = complementary DNA; ExAC = Exome Aggregation Consortium; PFBC = primary familial brain calcification.

*Accession number: NM_016085.3.

ExAC minor allele frequency assessed in August 2016.15

Values are above each threshold.
systemic phosphocalcic metabolism disorders of inherited or acquired cause, in utero or postnatal infections, interferonopathies, inborn errors of metabolism, and other rare inherited diseases.\textsuperscript{20} Calculifications are believed to be related to increased type-I interferon response in both in utero viral infections and interferonopathies.\textsuperscript{22} Several of these clinical presentations, including TORCH in utero infections and typical Aicardi-Goutières syndrome, are similar to the ones observed in the \textit{PCDH12} homozygous carriers. In other conditions, mutations in \textit{OCLN} and \textit{JAM3} genes, encoding endothelial cell adhesion proteins, result in microangiopathy associated with calcifications.\textsuperscript{18,23,24} Given the known function of \textit{PCDH12}, we postulate that similar mechanisms could be associated with the calcifications observed in the \textit{PCDH12} homozygous loss-of-function carriers.

\textit{PCDH12} is a protocadherin associated with membrane physical stability and adhesion.\textsuperscript{25} A \textit{Pcdh12} knockout mouse model revealed several age-independent vessel impairments, such as rami-
fications of medial elastic lamellae and increased inner diameter and circumferential mid-wall stress.\textsuperscript{26} \textit{PCDH12} has been widely studied as a key-player cadherin involved in placental maintenance and also a preeclampsia biomarker; however, little is known about its involvement in brain phys-
ology. It is conceivable that mutations in \textit{PCDH12} and \textit{SLC20A2}, which share similar expression patterns in the brain, might lead to similar phenotypes. Of interest, \textit{Slc20a2} knockout mice developed not only brain calcifications but also fetal growth restriction, lower birth viability, and placental calcification associated with thickened basement membranes.\textsuperscript{27} In both mouse models, the placental phenotype and the vascular impairment are additional putative links between \textit{SLC20A2} and \textit{PCDH12}, which deserve additional studies on mouse models.

\textit{PCDH12} biallelic loss of function causes a severe neurodevelopmental phenotype associated with brain calcifications. Rare predicted damaging heterozygous \textit{PCDH12} variants were identified in patients with PFBC or BCUC here, but whether they are associated with brain calcification or not remains to be determined. To address this question, follow-up studies will be necessary including screening other series, assessing the segregation of rare variants and functional consequences.

\section*{AUTHOR CONTRIBUTIONS}


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