

## ARTICLE



# Biallelic variants in genes previously associated with dominant inheritance: *CACNA1A*, *RET* and *SLC20A2*

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A subset of families with co-dominant or recessive inheritance has been described in several genes previously associated with dominant inheritance. Those recessive families displayed similar, more severe, or even completely different phenotypes to their dominant counterparts. We report the first patients harboring homozygous disease-related variants in three genes that were previously associated with dominant inheritance: a loss-of-function variant in the *CACNA1A* gene and two missense variants in the *RET* and *SLC20A2* genes, respectively. All patients presented with a more severe clinical phenotype than the corresponding typical dominant form. We suggest that co-dominant or recessive inheritance for these three genes could explain the phenotypic differences from those documented in their cognate dominant phenotypes. Our results reinforce that geneticists should be aware of the possible different forms of inheritance in genes when WES variant interpretation is performed. We also evidence the need to refine phenotypes and inheritance patterns associated with genes in order to avoid failures during WES analysis and thus, raising the WES diagnostic capacity in the benefit of patients.

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## INTRODUCTION

Mendelian genetics has by far provided the most medically actionable data on human DNA variation, and thus, curating this data is a priority to move forward to personalized medicine. Several factors complicate this curation, such as the high incidence of rare benign variants or the erroneous disease-gene associations [1]. In addition, DNA variation expresses itself with a wide spectrum of phenotypes in human populations, favored by the presence of genetic modifiers and by the autozygosity and genetic variations in non-related disease genes, among others [2]. The possibility of different inheritance patterns associated with a particular gene also influences phenotype heterogeneity [3].

Whole exome sequencing (WES) has improved the genetic diagnosis of rare diseases, with a diagnostic yield that ranges from 25 to 50% depending on the disorder and the examined series [4–8]. This diagnostic yield of WES strongly relies on the strategy used for the variant's prioritization and for the selection of genes to be analyzed [9]. Gaining insight into the different phenotypes and inheritance patterns associated with a gene will improve the curation of variants and genes linked to disease, and thus, should raise the WES diagnostics capacity.

Here, we report three consanguineous families with homozygous related disease variants in the *CACNA1A*, *RET*, and *SLC20A2* genes, which had been only previously associated with dominant inheritance. We also discuss the clinical implications for each case

and emphasize the importance of curating Mendelian genes to improve both variants interpretation and diagnostic yield of WES.

## METHODS

### Subjects

Family 1 was derived for a second opinion to the Gynecology Department of the University Hospital 12 de Octubre. Proband from family 2 and 3 were full clinically evaluated by the Pediatrics Department (Immunodeficiency Unit) and the Neurology Department of the Hospital, respectively. Since there was a suspicion of a genetic disorder, all patients were referred for analysis to the Clinical Genetics Department of the hospital.

Whole peripheral blood samples from the members of the three consanguineous families were collected. Written informed consent was obtained from each patient or their guardians. Genomic DNA extraction was performed following standard procedures, using the Maxwell 16 blood DNA purification (Promega).

The study was approved by the Ethics Committee of the University Hospital 12 Octubre, in accordance with the Declaration of Helsinki.

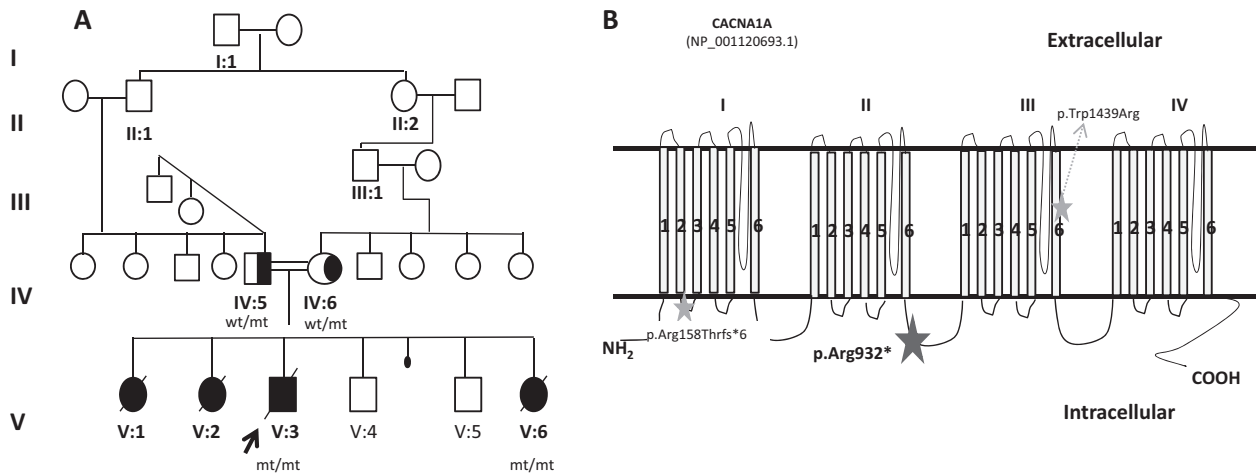
### WES and segregation analysis

WES was performed on the probands using the kit xGen Exome Panel v1.0 (IDT –Integrated DNA technologies–). Paired-end sequencing (2x75bp) was carried out on a NextSeq 550 (–Illumina–) and the Bioinformatics analysis was done using the custom pipeline *Karma*, validated according to Somak Roy et al. [10]. Reads were aligned to the reference human genome (hg19)

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**Fig. 1** **Family 1: CACNA1A gene.** **A** Family 1 pedigree and genotypes; wild type (wt); mutant allele (mt). **B** Schematic structure of the Cav2.1 channel (adapted from Damaj et al. [17]). The red star illustrates the p.(Arg932\*) variant detected in our family in the CACNA1A gene; the green stars indicate the variants described in Reinson et al. [31].

using BWA MEM (v0.7.17) [11] and Bowtie2 (v2.4.1) [12]. The variant calling process was performed using GATK (Haplotype Caller from Genome Analysis Toolkit, v.4.1) [13] and VarDict (AstraZeneca, v1.7.0) [14]. Annovar (v2018Apr16) [15] was used for the annotation of variants. Copy number variations were not assessed.

WES data was analyzed using custom panels targeted to the clinical suspicion. Panels were developed based on public databases (OMIM, DisGeNet, GTR, PanelApp, HPOs) and by reviewing the medical specific literature (see the panel content used for each case in Supplementary Table 1)

An extended analysis of the clinical exome panel (5686 genes) was performed in each proband in order to rule out other possible related disease genetic alterations. Deep intronic, UTR, and variants with non-OMIM associated genes were not analyzed (the list of unfiltered identified variants in each case is shown in Supplementary Tables 2.1, 2.2, and 2.3). Guided by the consanguinity of the family, a special focus was made on homozygous variants. Trio analysis could not be performed and thus, a de novo causal variant could not be ruled out in any of the cases. However, taking into account the family history, the occurrence of a de novo variant in the probands seemed not to be the most likely genetic cause underlying the clinical suspicion.

All variants were prioritized by filtering through different clinical and population databases (gnomAD, dbSNP). The main requirements for a good-quality variant to be analyzed and interpreted were: (1) read mapping quality, (2) depth coverage  $\geq 20\times$ ; (3) a variant fraction  $\geq 20\%$ , (4) a population frequency  $< 3\%$ , and (5) classification in the ClinVar database different from benign or likely benign by multiple suscriptors. According to these parameters, the list of all prioritized variants for each case are detailed in Supplementary Tables 2.1–2.3. Intronic, UTR variants and variants in genes not associated with OMIM are not shown.

All prioritized variants were classified following the ACMG criteria [16].

Family segregation analysis was carried out by Sanger sequencing using the kit BigDye Term v3.1 of Life Technologies, S.A (Applied Biosystems) and following standard procedures. The proband DNA was used as a positive control.

All variants reported here have been submitted to the LOVD database and can be now accessed using the corresponding ID or the following URLs:

CACNA1A\_000389: Variant #0000675114 (NC\_000019.9:g.13409683G > A, CACNA1A(NM\_001127221.1):c.2767C > T)—Global Variome shared LOVD

RET\_000288: Variant #0000675117 (NC\_000010.10:g.43608363G > A, RET (NM\_020975.4):c.1711G > A)—Global Variome shared LOVD

SLC20A2\_000056: Variant #0000675114 (NC\_000019.9:g.13409683G > A, CACNA1A(NM\_001127221.1):c.2767C > T)—Global Variome shared LOVD

## RESULTS AND DISCUSSION

### Family 1

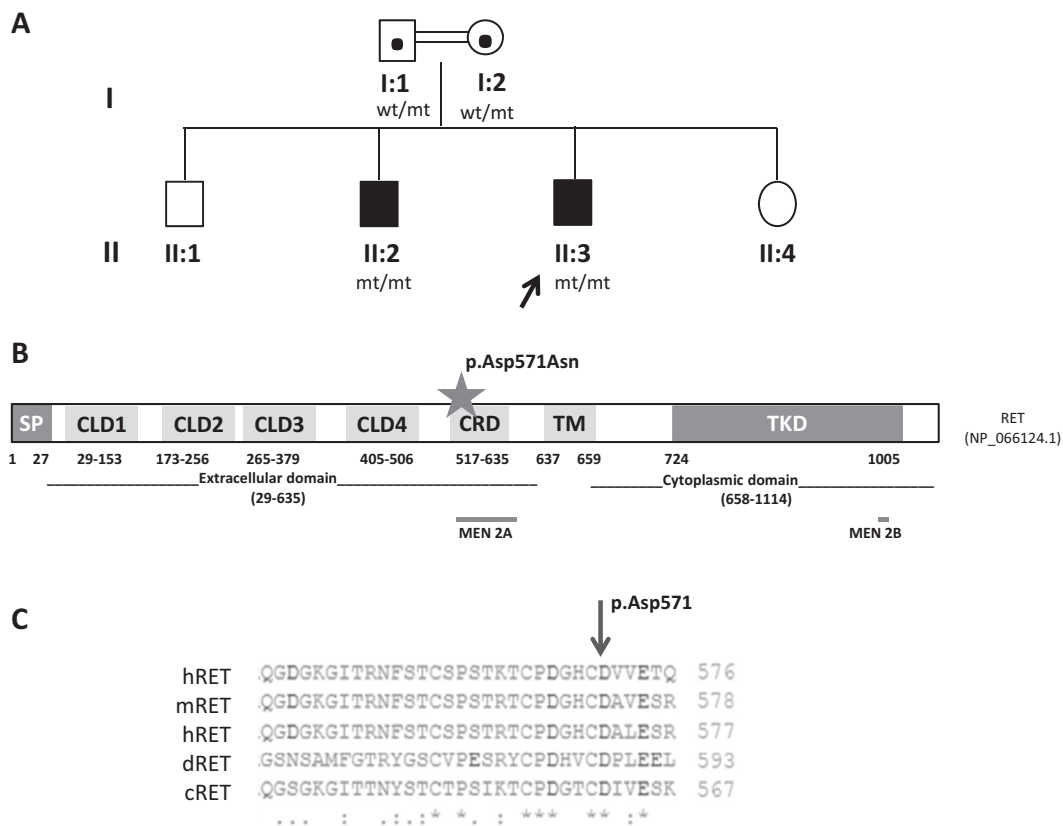
Four newborns, all presenting with hypotonia, mild facial dysmorphic features, encephalopathy, and seizures were born

from consanguineous parents of gipsy ethnicity. In all of them pregnancy and birth were normal, but they all died within the first 3–6 months of life. The couple had also two asymptomatic males and a spontaneous abortion in-between (Fig. 1A). Chromosome abnormalities in both parents were ruled out by conventional karyotype. In the proband (V:3), a mitochondrial disease was firstly suspected and thus, muscle mitochondrial DNA (mtDNA) copy number, whole mtDNA NGS and WES analysis of 305 nuclear-genes associated with mitochondrial disorders was performed. No genetic alterations were detected.

A second analysis of a panel containing 187 genes related to encephalopathies revealed the presence of the novel homozygous nonsense c.2767C>T p.(Arg932\*) variant in the exon 19 of the CACNA1A gene (NM\_001127221.1) where to our knowledge, only a heterozygous frameshift variant has been associated with Episodic ataxia type 2 (EA2) to date [17]. This variant is located in the intracytoplasmic loop between domain II and III of the protein, where most of the inherited loss-of-function (LOF) variants have been associated with encephalopathy epileptic and episodic ataxia [18] (Fig. 1B). The variant is not reported in the literature or in the clinical and population consulted databases (ClinVar, 1000 G, gnomAD, HGMD). Intriguingly and according to the gnomAD database, the CACNA1A gene is restricted for LOF variation and no homozygous LOF variants have been reported so far.

Segregation analysis showed that both parents were heterozygous carriers and the affected sister (V:2), who also died within the first 3 months of life with the same phenotype of the proband, was homozygous (Fig. 1A). Samples of the two affected sisters (V:1 and V:6) are not available and thus, segregation analysis could not be performed. The unaffected youngest and underage children (V:4 and V:5) were not genotyped but a clinical follow-up was recommended.

After genetic diagnosis, both apparently asymptomatic parents were referred to a Neurology consultation for clinical evaluation. The 44-year-old father referred frequent (2–3 per week) hours long ataxic episodes from the age of 17 to 30 that progressively decreased to completely disappear. The 39-year-old mother also referred to have similar episodes from the age of 30 to 37 years, that she defined as dizziness. Both parents had no clinical manifestations between episodes and remained asymptomatic since then. Therefore, they were diagnosed with EA2 due to the previous presence of ataxic episodes and the identification of the heterozygous apparently LOF variant in the CACNA1A gene. There were no other family members with similar background or phenotype to segregate the variant.



**Fig. 2 Family 2: *RET* gene.** **A** Family 2 pedigree and genotypes; wild type (wt); mutant allele (mt). **B** Schematic domain structure of the RET protein (adapted from Wang et al. [49] and Wu et al. 2019 [50]). The red star indicates the position of the variant p.(Asp571Asn) detected in the *RET* gene in our family. The regions were MEN2A and MEN2B causal variants cluster are indicated with blue bars. **C** Multiple sequence alignment of residues adjacent to amino acid 571 of the RET protein from human (h), mouse (m), rat (r), drosophila (d), and chicken (c). The tool Clustal Omega was used for multiple alignments (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). SP signal peptide, CLD cadherin-like domain, CRD Cysteine-rich domain, TM transmembrane, TKD tyrosine kinase domain.

The extended analysis of the clinical exome panel, revealed the presence of the three following homozygous rare variants: (1) c.55-2A>C in the *AMTN* gene (NM\_212557.3) associated with amelogenesis imperfecta IIIB (#617607); (2) c.1727C>T p.(Ser576-Leu) in the *ADGRE2* gene (NM\_013447.3) associated vibratory urticaria (#606100), and (3) c.622G>A p.(Val208Leu) in the *JAK3* gene (NM\_000215.3) associated with severe combined immunodeficiency (#600173). These three genes are associated with phenotypes not related to that of this family. No other relevant homozygous, heterozygous, or hemizygous variants related to the clinical suspicion were detected. In addition, the occurrence of a *de novo* causing-disease variant is unlikely considering the family history. Taken together, all these data allowed us to categorize the variant c.2767C>T p.(Arg932\*) in the *CACNA1A* gene as pathogenic following the ACMG criteria [16], being presumably the underlying cause of the clinical phenotype in this family.

Heterozygous variants in the *CACNA1A* gene (19p13.13, \*601011) are associated with clinically heterogeneous and occasionally overlapping autosomal dominant disorders. In general, missense gain of function variants are mainly associated with familial hemiplegic migraine (FHM1, #141500), LOF variants with EA2 (#108500) and the gain of toxicity expansions of CAG repeats with spinocerebellar ataxia (SCA6, #183086) [19–22]. However, both gain and LOF heterozygous variants have also been associated with cognitive/behavioral impairments [17, 21, 23, 24], with epileptic encephalopathy [18, 25–27] and with congenital ataxia [28], suggesting that the neuropsychological disturbances are caused by the channel dysfunction. Indeed, no major cognitive deficits have been described in SCA6 patients, probably because

polyglutamine expansions do not alter the calcium current of the channel [19].

To our knowledge, biallelic *CACNA1A* alterations have been described in a SCA6 patient homozygous for a 19-CAG repeats intermediate allele [29] and in two siblings with progressive myoclonus that were homozygous for a CAG insertion close to the C-terminal of the protein (c.6975\_6976insCAG; p.(A2326delinsQA)) [30]. To date, the only previous association of biallelic *CACNA1A* variants with early-onset epileptic encephalopathy has been reported in two compound heterozygous siblings (c.[4315T>A]; [472\_478del], p.([Trp1439Arg];[Ala158Thrfs\*6])) that presented with early-onset epileptic encephalopathy, progressive cerebral, cerebellar, and optic nerve atrophy. The elder sister suffered a significant clinical worsening that caused her death when she was 5 years old [31]. The authors described great intra-familial variability in the heterozygous reported relatives (parents and sister), showing a poor genotype-phenotype correlation that could be explained by the fact that the alteration of Cav2.1 channels function is differently compensated by the upregulation of other less efficient voltage-gated calcium channels (Cav2.2 and Cav1.2). In contrast to this reported genotype consisting of a missense and a presumably LOF frameshift variant in compound heterozygosis, the siblings we report were both homozygous for a nonsense presumably LOF variant, dying within the first months of life. We presume this genotype affects more severely the function of Cav2.1 channels causing a severe and lethal phenotype, as previously suggested in *CACNA1A* knock-out mice [32].

Herein, we describe the first lethal phenotype associated with a homozygous nonsense variant in the *CACNA1A* gene.

## Family 2

The proband was a 15-year-old boy born from healthy and consanguineous parents (Fig. 2A). At birth, he was diagnosed with Hirschsprung disease (HSCR) that required several surgical interventions due to complications that included severe enterocolitis and abdominal sepsis. Complete colonic removal with preservation of anal sphincter, continent thereafter. He was on monthly iron i.v. supplementation until the age of 10 years, due to patchy gut inflammation as seen by radiolabeled leukocytes. Intravenous iron is no longer needed. Histopathological analysis revealed the absence of ganglion cells in myenteric and submucosal plexus of intestine. Duodenal biopsy was normal, including normal villous architecture. The couple had also two asymptomatic siblings and an HSCR affected 24-year-old boy (II:2) who, like his brother, was also totally ileostomized during his first year of life.

The analysis of the panel containing 23 genes related to HSCR revealed the presence of the homozygous rare variant c.1711G>A p.(Asp571Asn) (rs750958377) in the exon 9 of the *RET* gene (NM\_020975.4). No homozygous, heterozygous, or hemizygous pathogenic or likely pathogenic neither candidate variants that could be related to the clinical suspicion of the patient and his brother were identified during the extended clinical exome analysis performed (see Supplementary Table 2.2).

Although there is not a mutational hot spot in HSCR, it seems to be a trend for HSCR-associated variants to cluster in the extracellular domain, particularly in the exon 3 of the gene [33]. The extracellular region of RET contains four atypical cadherin-like domains (CLD1–4) followed by a cysteine-rich domain (CRD) (Fig. 2), which are important for the RET receptor's interaction with its cognate ligands and co-receptors and thus, for its essential dimerization and activation [34, 35]. Intriguingly, the variant c.1711G>A p.(Asp571Asn) in exon 9 of *RET* gene is predicted to affect the protein in the extracellular CDR domain (Fig. 2B), where only two heterozygous de novo disease-causing variants have been described so far associated with total and short-HSCR respectively: p.(Glu595Gln) and p.(Gly605Asp) [36, 37]. The 571 Aspartic amino acid appears not to be one of the residues coordinating the calcium ion or the backbone carbonyl of the protein, based on RET cryo-electron microscopy [35]. However, the Aspartate (negatively charged amino acid) is highly conserved among species (Fig. 2C) and thus, its substitution for an Asparagine (a polar uncharged amino acid) may somehow affect the stability and normal function of the RET protein, which has been demonstrated to be a versatile receptor that can generate distinct signaling outputs depending on the ligand [35]. Accordingly, all the in silico prediction programs tested (MutationTaster, PolyPhen, SIFT) consider the variant to be damaging. This variant had already been reported in three heterozygotes in the gnomAD population database (MAF < 0.001%) but it has not been described in Clinvar or previously reported in the literature.

The *RET* proto-oncogene (10q11.21, \*164761) is the major susceptibility gene in HSCR (#142623) and encodes a transmembrane tyrosine kinase receptor that accounts for ~50% of familial and 15–20% of sporadic HSCR [38], a dominant congenital genetic disorder with reduced penetrance characterized by the absence of ganglion cells along with variable segments of the gut. Depending on the length of the affected segment, it is categorized as short-segment (up to 80%), long-segment (up to 20%) or total colonic aganglionosis (up to 8%). Most cases are sporadic (70–80%), though chromosomal abnormalities or congenital anomalies are associated in 12% and 18% of HSCR patients, respectively [38]. Germline LOF variants throughout the RET extracellular region have been associated with HSCR, but a specific hot spot has not been identified. Many of them are distributed in the co-receptor or the calcium-binding site, probably affecting the stability or co-receptor binding of RET, and thus impairing its activity [35]. It has been shown that causal variants located in the extracellular

cadherin-like domain (CDL1-3) interfere with the RET maturation and its translocation to the membrane, while intracellular mutations alter the RET kinase activity and signaling ability (Fig. 2) [36, 38–41].

Heterozygous germline gain of function RET variants are also associated with autosomal dominant multiple endocrine neoplasia type 2 syndromes (MEN2A and MEN2B), which predispose to medullary thyroid carcinoma, pheochromocytoma, parathyroid hyperplasia (MEN2A only) and mucosal neuromas and thickened corneal nerves (MEN2B only). MEN2A is mainly caused by activating missense variants clustered in the CRD domain that not dramatically affect the folding or cell surface localization of RET, leading to RET dimerization and constitutive activation [35]. MEN2B is caused by the almost unique M918T variant that accelerate RET autophosphorylation (Fig. 2).

Segregation analysis in this family revealed the brother (II:2) affected with the same phenotype of the proband (total colonic aganglionosis) was homozygous while both asymptomatic parents were heterozygous carriers (I:1 and I:2) for the c.1711G>A variant in the *RET* gene (Fig. 2B). Unfortunately, the analysis in the apparently asymptomatic siblings (II:1 and II:4) could not be performed.

The absence of associated phenotype in the parents could be explained by the already known reduce penetrance of RET dominant causal variants. However, in this family, the detected variant seems to have low-penetrance in heterozygous (there was no family history of HSCR) but full-penetrance when presented in both alleles, suggesting a possible co-dominant inheritance. Considering the high heterogeneity of HSCR, there is no evidence enough to postulate a recessive inheritance associated with a more severe phenotype (both siblings had total colonic aganglionosis) though it cannot be completely discarded. Further functional studies are warranted to get insight into these issues, whose results will have an impact on the genetic counseling of this disorder.

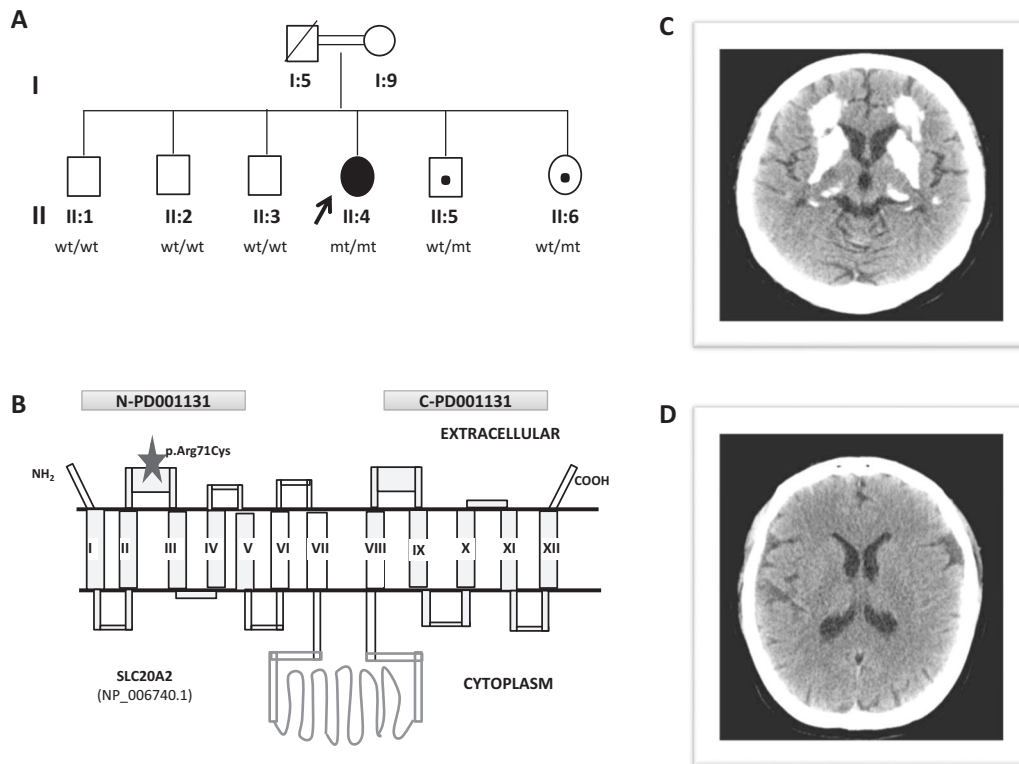
Taking into account all the abovementioned information, the variant was classified as likely pathogenic according to the ACMG criteria [16] and thus, we describe the first family harboring a homozygous missense variant in the *RET* gene (c.1711G>A p.(Asp571Asn)), leading to a non-lethal but severe form of HSCR.

## Family 3

The proband was a 55-year-old woman, the fourth of six siblings, born from healthy consanguineous parents (Fig. 3A). After an episode of vertigo, a computerized tomography scan (CT) that showed basal ganglia calcifications was performed when she was 20 years old. No endocrine or metabolic disorders related to brain calcifications were found. At the age of 28, she developed a psychotic disorder including delirium consistent with paranoia or delusional disorder that improved with aripiprazole. A CT scan showed extensive cortical bihemispheric calcifications in the basal ganglia, but also affecting the periventricular white matter, the posterior cortex and the cerebellum (Fig. 3B). In her forties, she started with motor clumsiness, balance disturbance, and severe dysarthria. In recent years, asymmetric Parkinsonism was observed. His father died at the age of 81 years, due to respiratory insufficiency.

The analysis of the panel containing 59 genes related to the presence of brain calcifications revealed the homozygous missense c.211C>T p.(Arg71Cys) variant (rs373139157; MAF < 0.0001%) in the exon 2 of the *SLC20A2* gene (NM\_006749.4) (Fig. 3C), where most of heterozygous missense disease-related variants have been described [42]. This variant has only been reported in 1 of 251,490 alleles in the gnomAD public population databases and, to our knowledge, it has not yet been associated with disease.

In the extended clinical exome analysis, no pathogenic or likely pathogenic neither candidate variants that could be related to the



**Fig. 3 Family 3: *SLC20A2* gene.** **A** Family 3 pedigree and genotypes; wild type (wt); mutant allele (mt). **B** Schematic representation of the *SLC20A2* protein (adapted from Hsu SC et al. 2013 [42]). Blue bars illustrate the N- and C-terminal ProDom domain (N-PD001131 and C-PD001131 respectively) of the protein. The red star indicates the position of the variant p.(Arg71Cys) in the *SLC20A2* gene detected in our family. **C** Cranial computed tomography (CT) scan of the homozygous patient showing extensive areas of calcification in the frontal white matter and basal ganglia. **D** Normal cranial CT scan of her asymptomatic heterozygous sister (II:6).

clinical suspicion of the patient were identified. Interestingly, we detected the rare homozygous c.476G>A p.(Arg159Lys) variant in the *NDUFA6* gene (NM\_152416.3). Defects in this gene have been associated with autosomal recessive Mitochondrial complex I deficiency type 17 (#618239) with onset during infancy or early childhood. However, the related phenotype is not consistent with the patient's phenotype, as she was diagnosed with Fahr's disease after a vertigo episode in her second decade of life. In addition, in this family, even though a trio analysis could not be performed, the occurrence of a causal variant is unlikely.

The *SLC20A2* gene encodes the type III sodium-dependent phosphate transporter 2, a Na/Pi transporter present in all kingdoms and ubiquitously expressed in various organs including the kidney, liver, and brain. The protein has 12 transmembrane regions and a large central intracytoplasmic domain (Fig. 3C). The two terminal transmembrane regions contain a unique sequence of ~150 amino acids with four highly conserved motifs known as ProDom domain 001132 (PD001131). This domain is characteristic of all PiTs family members and is essential for human Pi transport function [43–46]. The Arg71 residue is located in the ProDom domain and it is highly conserved among species. All in silico predictors indicate the p.(Arg71Cys) substitution to be damaging (MutationTaster, PolyPhen, SIFT). Interestingly, the substitution of the Arg71 residue for a Histidine (p.Arg71His)—both positively charged amino acids—has been previously reported in a heterozygous woman who developed clumsiness of her hands and gait unsteadiness at the age of 71, being diagnosed with Parkinson disease. She had calcification in her brain CT and an asymptomatic daughter also with minimal intracranial calcification. However, Parkinson disease is a common disorder in aged people, and thus, a coincidental presence of IBGC in this patient

cannot be discarded. Indeed, it is possible that IBGC would have been unnoticed if a CT were not performed in the patient [47, 48].

The *SLC20A2* gene (8p11.2, \*158378) is a main cause (41%) of familial idiopathic basal ganglia calcifications (IBGC; #213600)—also known as primary familial brain calcification or Fahr's disease—, a rare progressive neurodegenerative dominant disorder characterized by bilateral calcium deposits in the basal ganglia and other brain regions. Frequent clinical manifestations include psychiatric conditions and neurological symptoms although the severity and the age of onset are very diverse. Many cases are subclinical and incidentally diagnosed during image studies. The penetrance of the disease is reduced and might be as low as 70% [47].

Family segregation analysis in the five clinically asymptomatic siblings of the proband (both asymptomatic parents were not available) showed that only the two younger brothers (II:5 and II:6) were heterozygous for the c.211C>T variant (Fig. 3A). The CT performed in the elder asymptomatic sibling (II:6) was normal (Fig. 3D). Therefore, relying on all these findings, the variant was classified as likely pathogenic, according to the ACMG criteria.

Here, we describe the first patient harboring a homozygous variant in the *SLC20A2* gene associated with a severe IBGC phenotype. Based on these results, a co-dominant pattern for the variant in the *SLC20A2* gene cannot be excluded in this family.

#### CONCLUDING REMARKS

Biallelic disease-causing variants in genes in which only dominant alleles have been reported so far, as well as in genes with incomplete phenotypes, could be easily lost during variant analysis, and consequently lead to a failure in the genetic diagnosis. Identifying new forms of inheritance for particular

genes and their associated phenotype is essential to provide a reliable diagnosis through WES.

In this study, we expand the number of biallelic disease-causing variants reported in the *CACNA1A*, *RET*, and *SLC20A2* genes which were previously only associated with a dominant phenotype. We postulate that distinct inheritance forms in the same gene can contribute to phenotypic variability. Our results suggest that genetic analysts should be aware of this possibility when WES variant analysis is performed.

## REFERENCES

- Alkuraya FS. Discovery of mutations for Mendelian disorders. *Hum Genet.* 2016;135:615–23.
- Alsalem AB, Halees AS, Anazi S, Alshamekh S, Alkuraya FS. Autozygome sequencing expands the horizon of human knockout research and provides novel insights into human phenotypic variation. *PLoS Genet.* 2013;9:e1004030.
- Monies D, Maddirevula S, Kurdi W, Alanazy MH, Alkhalidi H, Al-Owain M, et al. Autozygosity reveals recessive mutations and novel mechanisms in dominant genes: implications in variant interpretation. *Genet Med.* 2017;19:1144–50.
- Dillon OJ, Lunke S, Stark Z, Yeung A, Thorne N, Gaff C, et al. Exome sequencing has higher diagnostic yield compared to simulated disease-specific panels in children with suspected monogenic disorders. *Eur J Hum Genet.* 2018;26:644–51.
- Lee H, Deignan JL, Dorrani N, Strom SP, Kantarci S, Quintero-Rivera F, et al. Clinical exome sequencing for genetic identification of rare mendelian disorders. *JAMA.* 2014;312:1880–7.
- Seo GH, Kim T, Choi IH, Park Jyoung, Lee J, Kim S, et al. Diagnostic yield and clinical utility of whole exome sequencing using an automated variant prioritization system, EVIDENCE. *Clin Genet.* 2020;98:562–70.
- Hu X, Guo R, Guo J, Qi Z, Li W, Hao C. Parallel tests of whole exome sequencing and copy number variant sequencing increase the diagnosis yields of rare pediatric disorders. *Front Genet.* 2020;11:473.
- Stefanski A, Calle-López Y, Leu C, Pérez-Palma E, Pestana-Knight E, Lal D. Clinical sequencing yield in epilepsy, autism spectrum disorder, and intellectual disability: a systematic review and meta-analysis. *Epilepsia.* 2021;62:143–51.
- Shamseldin HE, Maddirevula S, Faqeih E, Ibrahim N, Hashem M, Shaheen R, et al. Increasing the sensitivity of clinical exome sequencing through improved filtration strategy. *Genet Med.* 2017;19:593–8.
- Roy S, Coldren C, Karunamurthy A, Kip NS, Klee EW, Lincoln SE, et al. Standards and guidelines for validating next-generation sequencing bioinformatics pipelines: a joint recommendation of the association for molecular pathology and the college of american pathologists. *J Mol Diagn.* 2018;20:4–27.
- Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *ArXiv.* 2013; p. 1303.
- Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods.* 2012;9:357–9.
- Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Curr Protoc Bioinforma.* 2013;43:11.10.1–11.10.33.
- Lai Z, Markovets A, Ahdesmaki M, Chapman B, Hofmann B, McEwen R, et al. VarDict: a novel and versatile variant caller for next-generation sequencing in cancer research. *Nucleic Acids Res.* 2016;44:e108.
- Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* 2010;38:e164.
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17:405–23.
- Damaj L, Lupien-Meilleur A, Lortie A, Riou É, Ospina LH, Gagnon L, et al. *CACNA1A* haploinsufficiency causes cognitive impairment, autism and epileptic encephalopathy with mild cerebellar symptoms. *Eur J Hum Genet.* 2015;23:1505–12.
- Jiang X, Raju PK, D'Avanzo N, Lachance M, Pepin J, Dubeau F, et al. Both gain-of-function and loss-of-function de novo *CACNA1A* mutations cause severe developmental epileptic encephalopathies in the spectrum of Lennox-Gastaut syndrome. *Epilepsia.* 2019;60:1881–94.
- Indelicato E, Nachbauer W, Karner E, Eigentler A, Wagner M, Unterberger I, et al. The neuropsychiatric phenotype in *CACNA1A* mutations: a retrospective single center study and review of the literature. *Eur J Neurol.* 2019;26:66.
- Tottene A, Fellin T, Pagnutti S, Luvisetto S, Striessnig J, Fletcher C, et al. Familial hemiplegic migraine mutations increase Ca<sup>2+</sup> influx through single human Cav2.1 channels and decrease maximal Cav2.1 current density in neurons. *Proc Natl Acad Sci USA.* 2002;99(Oct):13284–9.
- Travaglini L, Nardella M, Bellacchio E, D'Amico A, Capuano A, Frusciantè R, et al. Missense mutations of *CACNA1A* are a frequent cause of autosomal dominant nonprogressive congenital ataxia. *Eur J Paediatr Neurol.* 2017;21:450–6.
- Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton DW, Amos C, et al. Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the  $\alpha(1A)$ -voltage-dependent calcium channel. *Nat Genet.* 1997;15:62–9.
- Blumkin L, Michelson M, Leshinsky-Silver E, Kivity S, Lev D, Lerman-Sagie T. Congenital ataxia, mental retardation, and dyskinesia associated with a novel *CACNA1A* mutation. *J Child Neurol.* 2010;25:892–7.
- García Segarra N, Gautschi I, Mittaz-Crettol L, Kallay Zetchi C, Al-Qusairi L, Van Bemmelen MX, et al. Congenital ataxia and hemiplegic migraine with cerebral edema associated with a novel gain of function mutation in the calcium channel *CACNA1A*. *J Neurol Sci.* 2014;342:69–78.
- Mammadova D, Kraus C, Leis T, Trollmann R. Severe Epileptic Encephalopathy in Siblings due to a Novel Heterozygous *CACNA1A* Gene Mutation. *Neuropediatrics* 2019;50(S 02):S1–S55
- Hayashida T, Saito Y, Ishii A, Yamada H, Itakura A, Minato T, et al. *CACNA1A*-related early-onset encephalopathy with myoclonic epilepsy: a case report. *Brain Dev.* 2018;40:130–3.
- Myers CTT, McMahon JMM, Schneider ALL, Petrovski S, Allen ASS, Carvill GLL, et al. De novo mutations in *SLC1A2* and *CACNA1A* are important causes of epileptic encephalopathies. *Am J Hum Genet.* 2016;99:287–98.
- Izquierdo-Serra M, Fernández-Fernández JM, Serrano M. Rare *CACNA1A* mutations leading to congenital ataxia. *Pflugers Arch.* 2020;472:791–809.
- Mariotti C, Gellera C, Grisoli M, Miner R, Castucci A, Di, Donato S. Pathogenic effect of an intermediate-size SCA-6 allele (CAG)19 in a homozygous patient. *Neurology.* 2001;57:1502–4.
- Lv Y, Wang Z, Liu C, Cui L. Identification of a novel *CACNA1A* mutation in a Chinese family with autosomal recessive progressive myoclonic epilepsy. *Neuropsychiatr Dis Treat.* 2017;13:2631.
- Reinson K, Öiglanc-Shlik E, Talvik I, Vaheer U, Öunapuu A, Ennok M, et al. Biallelic *CACNA1A* mutations cause early onset epileptic encephalopathy with progressive cerebral, cerebellar, and optic nerve atrophy. *Am J Med Genet Part A.* 2016;170:2173–6.
- Fletcher CF, Tottene A, Lennon VA, Wilson SM, Dubel SJ, Paylor R, et al. Dystonia and cerebellar atrophy in *Cacna1a* null mice lacking P/Q calcium channel activity. *FASEB J.* 2001;15:1288–90.
- Ngo DN, So MT, Gui H, Tran AQ, Bui DH, Cherny S, et al. Screening of the *RET* gene of Vietnamese Hirschsprung patients identifies 2 novel missense mutations. *J Pediatr Surg.* 2012;47:1859–64.
- Bigalke JM, Aibara S, Roth R, Dahl G, Gordon E, Dorbéus S, et al. Cryo-EM structure of the activated *RET* signaling complex reveals the importance of its cysteine-rich domain. *Sci Adv.* 2019;5(7):eaau4202.
- Li J, Shang G, Chen Y-J, Brautigam CA, Liou J, Zhang X, et al. Cryo-EM analyses reveal the common mechanism and diversification in the activation of *RET* by different ligands. *Elife.* 2019;8:e47650.
- So M-T, Leon TY-Y, Cheng G, Tang CS-M, Miao X-P, Cornes BK, et al. *RET* mutational spectrum in Hirschsprung disease: evaluation of 601 Chinese patients. *PLoS One.* 2011;6:e28986.
- Jiang Q, Wang Y, Li Q, Zhang Z, Xiao P, Wang H, et al. Sequence characterization of *RET* in 117 Chinese Hirschsprung disease families identifies a large burden of de novo and parental mosaic mutations. *Orphanet J Rare Dis.* 2019;14:237.
- Amiel J, Sproat-Emison E, Garcia-Barcelo M, Lantieri F, Burzynski G, Borrego S, et al. Hirschsprung disease, associated syndromes and genetics: a review. *J Med Genet.* 2008;45:1–14.
- De Falco V, Carlomagno F, Li H yu, Santoro M. The molecular basis for *RET* tyrosine-kinase inhibitors in thyroid cancer. Vol. 31, *Best Prac Res Clin Endocrinol Metabol.* 2017;31:307–18.
- Iwashita T, Murakami H, Asai N, Takahashi M. Mechanism of *Ret* dysfunction by Hirschsprung mutations affecting its extracellular domain. *Hum Mol Genet.* 1996;5:1577–80.
- Iwashita T, Kurokawa K, Qiao S, Murakami H, Asai N, Kawai K, et al. Functional analysis of *RET* with Hirschsprung mutations affecting its kinase domain. *Gastroenterology.* 2001;121:24–33.
- Hsu SC, Sears RL, Lemos RR, Quintáns B, Huang A, Spiteri E, et al. Mutations in *SLC20A2* are a major cause of familial idiopathic basal ganglia calcification. *Neurogenetics.* 2013;14:11–22.
- Tsai JY, Chu CH, Lin MG, Chou YH, Hong RY, Yen CY, et al. Structure of the sodium-dependent phosphate transporter reveals insights into human solute carrier *SLC20*. *Sci Adv.* 2020;6(32):eabb4024.
- Salaiún C, Maréchal V, Heard JM. Transport-deficient Pit2 phosphate transporters still modify cell surface oligomers structure in response to inorganic phosphate. *J Mol Biol.* 2004;340:39–47.
- Böttger P, Pedersen L. Mapping of the minimal inorganic phosphate transporting unit of human *PIT2* suggests a structure universal to *PIT*-related proteins from all kingdoms of life. *BMC Biochem.* 2011;12(1):21.

46. Travaglini L, Nardella M, Bellacchio E, D'Amico A, Capuano A, Frusciante R, et al. Evolutionary and experimental analyses of inorganic phosphate transporter PiT family reveals two related signature sequences harboring highly conserved aspartic acids critical for sodium-dependent phosphate transport function of human PiT2. *FEBS J.* 2005;272:3060–74.
47. Westenberger A, Klein C. The genetics of primary familial brain calcifications. *Curr Neurol Neurosci Rep.* 2014;14:490.
48. Yamada M, Tanaka M, Takagi M, Kobayashi S, Taguchi Y, Takashima S, et al. Evaluation of SLC20A2 mutations that cause idiopathic basal ganglia calcification in Japan. *Neurology.* 2014;82:705–12.
49. Wang XJ, Camilleri M. Hirschsprung disease: Insights on genes, penetrance, and prenatal diagnosis. *Neurogastroenterol Motil.* 2019;31(11):e13732.
50. Wu W, Lu L, Xu W, Liu J, Sun J, Zheng L, et al. Whole exome sequencing identifies a novel pathogenic RET variant in Hirschsprung disease. *Front Genet.* 2019;14(9):752.

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## COMPETING INTERESTS

All authors declare no competing interests.

## ADDITIONAL INFORMATION

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