MICRO REPORT

Rare functional missense variants in CACNA1H: What can we learn from Writer’s cramp?

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Abstract

Writer’s cramp (WC) is a task-specific focal dystonia that occurs selectively in the hand and arm during writing. Previous studies have shown a role for genetics in the pathology of task-specific focal dystonia. However, to date, no causal gene has been reported for task-specific focal dystonia, including WC. In this study, we investigated the genetic background of a large Dutch family with autosomal dominant–inherited WC that was negative for mutations in known dystonia genes. Whole exome sequencing identified 4 rare variants of unknown significance that segregated in the family. One candidate gene was selected for follow-up, Calcium Voltage-Gated Channel Subunit Alpha1 H, CACNA1H, due to its links with the known dystonia gene Potassium Channel Tetramerization Domain Containing 17, KCTD17, and with paroxysmal movement disorders. Targeted resequencing of CACNA1H in 82 WC cases identified another rare, putative damaging variant in a familial WC case that did not segregate. Using structural modelling and functional studies in vitro, we show that both the segregating p.Arg481Cys variant and the non-segregating p.Glu1881Lys variant very likely cause structural changes to the Cav3.2 protein and lead to similar gains of function, as seen in an accelerated recovery from inactivation. Both mutant channels are thus available for re-activation earlier, which may lead to an increase in intracellular calcium and increased neuronal excitability. Overall, we conclude that rare functional variants in CACNA1H need to be interpreted very carefully, and additional studies are needed to prove that the p.Arg481Cys variant is the cause of WC in the large Dutch family.

Keywords: Writer’s cramp, Focal dystonia, CACNA1H, Rare variants, Structural and functional analysis

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writing. The daughter of patient II-3 is also reported to have difficulties with writing but has not been examined nor included in the genetic analysis.

After performing whole exome sequencing (WES) in II:3 and III:7, as described before [5], we discovered several rare missense variants shared between the two affected cases, but only 4 variants segregated with disease phenotype after Sanger sequencing (Table 1). All 4 variants exhibited Combined Annotation Dependent Depletion (CADD) Phred scores higher than 10 and were predicted to be probably damaging by Mutation Taster and/or Polyphen 2.0. Based on this data, these variants are classified as variants of unknown significance, and thus we could not define any of them as likely benign or likely pathogenic.

Notably, an association between $\text{CACNA1H}$, which encodes a subunit of the neuronal voltage-gated T-type calcium channel Calcium Voltage-Gated Channel Subunit Alpha1 H, and dystonia has been proposed because a weighted dystonia gene co-expression network [6] directly connected $\text{CACNA1H}$ to the known dystonia gene $\text{KCTD17}$, which encodes the protein Potassium Channel Tetramerization Domain Containing 17, leading to the assumption that both proteins function in the same signalling pathway. This was not the case for the other three candidate genes. Additionally, novel and rare variants in $\text{CACNA1H}$ have been linked to childhood absence and idiopathic generalized epilepsy, familial...
Table 1  Variants in genes co-segregating with the disease phenotype

| Gene       | Transcript | Transcript variant | Protein variant | gnomAD v3.1 (MAF) | CADD Phred score | Mutation Taster | Poly-Phen   |
|------------|------------|--------------------|----------------|------------------|-----------------|----------------|-------------|
| CACNA1H    | NM_021098  | c.1441C>T         | p.R481C        | 8/143316         | 18.2            | PM            | PrD         |
| GPER1      | NM_001039966 | c.505C>T       | p.R160C        | 2/143370         | 26.2            | N.A           | N.A         |
| SPTBN5     | NM_016642  | c.8572C>T         | p.H2858Y       | Absent; present in dbSNP rs887835041 | 13.9            | PM            | PrD         |
| NUBP2      | NM_012225  | c.296C>T          | p.P99L         | 2/143346         | 22.9            | DC            | PoD         |

MAF minor allele frequency, PM polymorphism, DC disease-causing, PrD Probably damaging, PoD Possibly damaging. N.A. Not analysed. gnomAD browser accessed March 2020

hyperaldosteronism, amyotrophic lateral sclerosis and severe congenital amyotrophy [7–10]. Given that epilepsy overlaps with paroxysmal movement disorders such as focal dystonia [11], and the observation that CACNA1H functions in similar biological pathways as other known dystonia genes, we attempted to validate a role for CACNA1H in WC by screening the complete coding region of CACNA1H using a targeted array in a cohort of 82 genetically undiagnosed WC cases (both sporadic and familial). We identified 3 additional rare missense variants in CACNA1H in 3 WC cases: the c.5989G>A p.Ala1997Tyr variant predicted to be benign by various programs, the c.314T>G p.Val105Gly variant that was also detected in a patient with spinocerebellar ataxia type 3, and variant c.5641G>A p.Glu1881Lys, which was predicted to be damaging but did not segregate (Fig. 1b). This data reinforces that CACNA1H is relatively tolerant for rare missense variants, as confirmed by its gene constraint score of 1.17 (gnomADv3.1) [12].

To further investigate the consequence of rare missense variants in CACNA1H, we performed structural and functional analysis of the two putative damaging variants, p.Arg481Cys and p.Glu1881Lys. Structural analysis using the Protein Data Bank (PDB) entry 5GJW, showed that the p.Arg481Cys caused a likely loss of stability of an α-helix bundle and likely affects the α-helix bundle interactions in the interface with the main domain (Fig. 1c). Additionally, the presence of a cysteine at position 481 could lead to the formation of a disulphide bond with a native cysteine at position 847, which is located within the bundle, and this may cause conformational restraints that influence protein folding, stability and function. The introduction of the positively charged lysine at position 1881 due to the p.Glu1881Lys variant is likely to cause movement of the positively charged arginines at positions 1596 and 1597, changing the protein structure in this interface (Fig. 1d). Furthermore, we performed functional analysis of the mutant and wild type (WT) Cav3.2 channels in transiently transfected HEK tsA-201 cells, as done before [13]. Both variants did not change the conductance of the channel, as we observed a similar current density compared to WT Cav3.2 (Fig. 1e, f). However, the p.Glu1881Lys variant did cause a small, significant shift in the mean half-activation potential toward more positive potentials, and both variants led to an accelerated recovery from inactivation compared to WT Cav3.2 (Fig. 1g–j). This implies that Cav3.2 channels carrying the p.Arg481Cys and p.Glu1881Lys variants are less likely to inactivate and are available for re-activation earlier. This gain of function may lead to an increase in intracellular calcium and increased neuronal excitability [14, 15].

In summary, using WES, we identified 4 rare variants of unknown significance that segregated with the WC in the family. Given the established link between CACNA1H and the previously reported dystonia gene KCTD17 and its link with paroxysmal movement disorders, we focused our additional studies on a putative role of CACAN1H in WC. Our follow-up work highlights that the need for caution in interpreting in silico predictions of rare missense variants in large genes like CACNA1H as damaging. We show that both the segregating p.Arg481Cys variant and the non-segregating p.Glu1881Lys variant very likely cause structural changes to the protein and lead to a similar gain of function of the Cav3.2 channel. Whether the p.Arg481Cys variant is the cause of disease in the large Dutch family remains to be proven, but our study corroborates that rare, functional missense variants in CACNA1H are quite common and may associate with numerous disorders, including WC.

Abbreviations
WC: Writer's cramp; ADDS: Arm Dystonia Disability Scale; WES: Whole exome sequencing; CADD: Combined Annotation Dependent Depletion; PDB: Protein Data Bank.

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Authors' contributions
EARN and MH performed the experiments, analysed the data and wrote the paper. JL performed phenotypic analysis. IAS performed the experiments and analysed the data. F-XZ performed experiments. JHTMK performed phenotypic analysis. NA conceived, designed and performed the structural modelling experiments and wrote the paper. RJS conceived and designed the experiments. GWZ conceived and designed the experiments, analysed the data and wrote the paper. DSV conceived and designed the experiments and performed phenotypic analysis. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article. The WES data is available upon request.

Ethics approval and consent to participate
The Medical Ethical Committee of the Academic Medical Center (Amsterdam, the Netherlands; METC protocol 05/030 #05.17.0239) gave study approval, and the Medical Ethical Committee of the Academic Medical Center (Amsterdam, the Netherlands; METC protocol 05/030 #05.17.0239) gave study approval, and the Medical Ethical Committee of the Academic Medical Center (Amsterdam, the Netherlands; METC protocol 05/030 #05.17.0239) gave study approval. All participants gave written informed consent. All in vitro experiments were performed in accordance with the guidelines of the Hotchkiss Brain Institute, University of Calgary (Calgary, Alberta, Canada).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interest.

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