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Targeted next generation sequencing identifies a genetic spectrum of DNA variants in patients with hemiplegic migraine

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Abstract

Objective: Hemiplegic migraine in both familial (FHM) and sporadic (SHM) forms is a rare subtype of migraine with aura that can be traced to mutations in the CACNA1A, ATP1A2 and SCN1A genes. It is characterised by severe attacks of typical migraine accompanied by hemiparesis, as well as episodes of complex aura that vary significantly between individuals.

Methods: Using a targeted next generation sequencing (NGS) multigene panel, we have sequenced the genomic DNA of 172 suspected hemiplegic migraine cases, in whom no mutation had previously been found by Sanger sequencing (SS) of a limited number of exons with high mutation frequency in FHM genes.

Results: Genetic screening identified 29 variants, 10 of which were novel, in 35 cases in the three FHM genes (CACNA1A, ATP1A2 and SCN1A). Interestingly, in this suspected HM cohort, the ATP1A2 gene harboured the highest number of variants with 24/35 cases (68.6%), while CACNA1A ranked the second gene, with 5 variants identified in 7/35 cases (20%). All detected variants were confirmed by SS and were absent in 100 non-migraine healthy control individuals. Assessment of variants with the American College of Medical Genetics and Genomics guidelines classified 8 variants as pathogenic, 3 as likely pathogenic and 18 as variants of unknown significance. Targeted NGS gene panel increased the diagnostic yield by fourfold over iterative SS in our diagnostics facility.

Conclusion: We have identified 29 potentially causative variants in an Australian and New Zealand cohort of suspected HM cases and found that the ATP1A2 gene was the most commonly mutated gene. Our results suggest that screening using NGS multigene panels to investigate ATP1A2 alongside CACNA1A and SCN1A is a clinically useful and efficient method.

Keywords

ATP1A2, CACNA1A, custom gene panel, familial hemiplegic migraine, migraine with aura, next generation sequencing, SCN1A

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Introduction

Hemiplegic migraine (HM) is a rare subtype of migraine with aura (MA) that is characterised by severe attacks of typical migraine accompanied by hemiparesis, as well as episodes of complex aura, the effects of which vary significantly and include sensory effects, speech difficulties, confusion, coma and other symptoms.1,2 HM attacks can occur spontaneously or can be triggered by events such as exercise, emotional stress or minor head trauma. The latter trigger can sometimes precipitate quite severe attacks, including coma in some cases and even recurrent ischemic strokes which have been reported after minor head trauma in some individuals with HM.1,3 The sporadic type of HM (SHM) differs from familial hemiplegic migraine (FHM) by the absence of family history.4,5 FHM and SHM can both be traced to mutations in the CACNA1A, ATP1A2 and SCN1A genes, which are responsible for causing FHM1, FHM2 and FHM3, respectively.6–8 All three FHM genes are involved in ion translocation in cells, and mutations can lead to increased cortical excitability due to high concentrations of synaptic glutamate and/or extracellular potassium ions.9,10

There is additional complexity involved in the aetiology of FHM, however. In addition to familial hemiplegic migraine type 1 (FHM1), mutations in the CACNA1A gene are responsible for two other allelic disorders with autosomal dominant inheritance: episodic ataxia type 2 (EA2) and spinocerebellar ataxia type 6 (SCA6).11 A wide spectrum of phenotypic variability for CACNA1A mutations has been previously observed, even within the same family, indicating that FHM causing mutations may have phenotypical outcomes dependent on other genetic variations or environmental effects.12 The overlapping clinical features and phenotypic variability can thus make it difficult for clinicians to diagnose the specific disorders present in a patient. The current diagnostic protocol for FHM or SHM diagnoses involves analysis of a patient’s symptom manifestation by neurologists and determining whether they fulfill the criteria specified by the Headache Classification Subcommittee of the International Headache Society (IHS).13 The clinically diagnosed HM cases are then referred for molecular genetic analysis to support the clinical diagnosis. This has important clinical implications, as each of these disorders is treated differently, so it is important to select the correct gene(s) for genetic screening. In addition, testing for FHM1-3, EA2 or SCA6 by traditional Sanger sequencing (SS) is both difficult and expensive to obtain a definitive diagnosis due to the size of the genes. This study details a spectrum of variants for a suspected HM population referred to our laboratory for diagnosis in Australia between 1999 and 2013. We also demonstrate an improved molecular diagnostic capacity for HM using an efficient strategy of comprehensive exonic sequencing of the known FHM genes through the use of a targeted next generation sequencing (NGS) custom panel.14

Materials and methods

Ethical approval

This study was approved by the Queensland University of Technology (QUT) Ethics Committee (approval number: 1800000611). All cases consented to genetic testing with their doctors, as required under current regulations.

Study design and participants

Between 1999 and 2013, 183 cases clinically diagnosed as having HM by their neurologists were referred from Australia and New Zealand to our laboratory for molecular diagnostic screening of the CACNA1A, ATP1A2 and SCN1A genes. Of the 183 cases, only 9 patients (4.9%) had mutations in CACNA1A (exon 4, 5, 16 and 17)15 while no mutations were detected in ATP1A2 and SCN1A in a limited SS screen of exons in CACNA1A (exon 4, 5, 16, 17 and 36), ATP1A2 (exon 17 and 19) and/or SCN1A (exon 23), as part of a regular diagnostic service. Of the remaining 174 suspected HM cases without a detected mutation, two cases declined to participate in this NGS screening study. The remaining 172 suspected HM cases were included in this study based on the following criteria: (i) they fulfilled the IHS criteria for HM by their neurologists, (ii) molecular screening using SS was negative in our previous diagnostic work in our patient cohort. While participants fulfilled the criteria for HM by initial neurologist assessment, not all referring neurologists were headache specialists and information on detailed follow-up and assessment was not available for all cases.

The 172 suspected HM cases (99 females and 73 males) with a mean age of 30.5 ± 19.9 years displayed various accompanying clinical phenotypes, including seizures, ataxia, headache, migraine with or without aura, white matter abnormalities, alternating hemiplegia of childhood, developmental delay, autism, stroke, and confusional migraine and concussion after minor head trauma. These additional symptoms may represent the existence of patients with non-HM related causes for their conditions in this cohort, but they are consistent with known migraine comorbidities and symptoms arising from characterised mutations in HM causing genes. All participants were screened for variants in the CACNA1A, ATP1A2 and SCN1A genes using a targeted NGS gene panel, 47 patients were reported with a family history of classical HM symptoms, 8 cases were diagnosed with SHM, having no family history of similar symptoms as the index case, and the remaining patients had no family information available. The study included three pairs of related participants. These were cases 49 and 131 (sisters), cases 12 and 13 (son and father) and 51 and 110 (siblings). No other participants were known to be related. All 172 cases were screened using the targeted NGS panel previously described by Maksemous et al.14,16 to perform variant detection in regions.
not covered by traditional SS and in an attempt to improve the positive diagnosis rate.

One hundred Caucasian healthy (non-migraine) adult control individuals from our laboratory’s research populations were genotyped for the presence of the variants identified by the NGS panel. In addition, the available population databases such as 1000 genome project, Exome Aggregation Consortium and the genome aggregation database were used to obtain the minor allele frequency (MAF) for previously observed variants (Table 1).

**NGS FHM gene panel design.** The targeted NGS 5-gene panel comprises the three FHM genes (*CACNA1A, ATP1A2* and *SCN1A*), as well as two genes involved in migraine-related disorders: *NOTCH3* in which mutations can cause Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) and the *KCNK18* gene, which has previously implicated in familial migraine with aura. The AmpliSeq automated primer design tool was able to design primers covering 92.41% of the targeted exons, flanking introns and 3’ and 5’ untranslated regions (25,346 bp) of the three FHM genes, according to the reference human genome 19 (hg19). The missing regions comprised only 1924 bp (7.59%) of coding regions and 3’ and 5’ untranslated regions in *CACNA1A, ATP1A2* and *SCN1A* (Online Supplementary Table 1).

**Procedures**

DNA samples for 172 patients were previously isolated for diagnostic work from blood using standard techniques. A NGS panel which includes the three known FHM genes, previously described by Maksemous et al., encompassing the exons, flanking introns and 3’ and 5’ untranslated regions of the *CACNA1A, ATP1A2* and *SCN1A* genes was designed through AmpliSeq for Library construction, emulsion PCR, enrichment and sequencing were performed according to the manufacturers’ instructions as previously described. Sequencing was performed using the Ion Torrent Personal Genome Machine (PGM) system (Thermo Fisher Scientific, Scoresby, Victoria, Australia). All variants detected were validated by SS. Primer sequences and conditions are available upon request. Confirmed variants were also genotyped in the cohort of 100 non-migraine healthy control individuals via the Agena MassARRAY platform (Agena Bioscience, San Diego, California, USA), according to the manufacturers’ procedures (iPlex design and data are available upon request).

**Bioinformatics analyses**

Raw sequence reads were aligned to the hg19 human genome with the Ion Torrent Suite server V3.6. For all samples sequenced, the average read depth across the target region was >500×, while the average percentage of target bases covered at 20× or greater was 96% and the average uniformity of coverage was 91%. Variant annotation was performed via locally hosted Ion Reporter (IR) software V4.6 (Thermo Fisher Scientific, Scoresby, Victoria, Australia).

**Variant filtering**

Variants were filtered and prioritised in IR software (V4.6) according to the following criteria: MAF of <0.01; variant effect (missense, stop gain, stop loss, in-frame insertion, in-frame deletions or frameshift, and splicing). Variant causality was interpreted using three in silico prediction tools (MutationTaster, SIFT and Polyphen-2). Variant nomenclature was assigned according to HGVS guidelines, against the following transcripts: *CACNA1A* (NM_001127221); *ATP1A2* (NM_000702); and *SCN1A* (NM_001165963). The pathogenicity of the identified variants in the three FHM genes was assessed according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

**Results**

**Genetic findings**

A comprehensive screening of the FHM genes *CACNA1A, ATP1A2* and *SCN1A* using targeted NGS led to the identification of 29 variants which were predicted to change the amino acid, cause a frameshift or interfere with a canonical splice site. Of these, 19 known and 10 novel variants that had not been previously reported in any published source before were identified in 35 of the 172 cases referred for HM testing (Table 1). In total, 20 variants were identified in *ATP1A2* in 24 cases, 5 variants in *CACNA1A* in 7 cases and 4 variants in *SCN1A* in 4 cases.

**ATP1A2 variants (NM_000702).** The 20 variants identified in *ATP1A2* in 24 patients, scattered over 11 different exons (2, 4, 7, 8, 9, 12, 13, 16, 17, 18 and 20) (Table 1). Of these, 18 were non-synonymous variants, one was an insertion of a C nucleotide within exon 2 (p.Ala13fsTer32 c.34_35insC) and one was a canonical splice site variant leading to a novel single nucleotide substitution within intron 4, c.381+1T>A. Nine variants were novel and not previously reported in the ExAC, gnomAD and 1000 genome databases or in the available literature (Table 1; Figure 1(a)).

**CACNA1A variants (NM_001127221).** A total of five non-synonymous variants were identified in seven unrelated cases in *CACNA1A* (Table 1). Of these, a new likely pathogenic variant (Arg1346Pro) was identified in three unrelated patients (7, 25 and 128).

**SCN1A variants (NM_001165963).** A total of four non-synonymous variants were detected in four patients in *SCN1A* (Table 1). Three of these had previously been reported in FHM patients; p.Lys1936Glu, p.Arg1928Cys and p.Thr1174Ser, while the p.Arg187Gln variant found...
| Sample ID# | Age | Gender | Gene | Inheritance | Exon | Protein | Coding | Mutation | Taster | Sift | Polyphen-2 | ACMG classification | Allele frequency | Ref. |
|-----------|-----|--------|------|-------------|------|---------|--------|----------|--------|------|------------|---------------------|-----------------|-----|
| 105       | 9   | F      | ATP1A2 | Unknown     | 2    | p.Ala13fs | c.34_35insC | D        | –      | –    | –          | Pathogenic          | –               |     |
| 80        | 14  | M      | ATP1A2 | Unknown     | 2    | p.Met39Val | c.115A>G   | D        | T      | B    | –          | VOUS                | –               |     |
| 49, 131   | 49, 53 | F, F | ATP1A2 | Familial    | 4    | p.Arg65Leu | c.194G>T  | D        | T      | B    | –          | VOUS                | –               |     |
| 144       | 59  | F      | ATP1A2 | Unknown     | 4    | p.Gly14Ser | c.340G>A  | D        | T      | B    | –          | VOUS                | –               |     |
| 130       | 29  | M      | ATP1A2 | Familial    |       | –         | c.381+1T>A | D        | –      | –    | –          | Pathogenic          | –               |     |
| 164       | 15  | M      | ATP1A2 | Familial    | 7    | p.Thr217Ile | c.650C>T  | D        | D      | D    | –          | VOUS                | –               |     |
| 24        | 11  | M      | ATP1A2 | Familial    | 9    | p.Val340Leu | c.1018G>T | D        | D      | D    | –          | Pathogenic          | –               |     |
| 33        | 14  | F      | ATP1A2 | Unknown     | 9    | p.Thr368Met | c.1103C>T | D        | D      | D    | –          | VOUS                | –               |     |
| 104       | 24  | F      | ATP1A2 | Unknown     | 12   | p.Glu599Gln | c.1525G>C | D        | D      | D    | –          | VOUS                | –               |     |
| 111, 140  | 11, 25 | M, M | ATP1A2 | Unknown/unknown | 12   | p.Arg548His | c.1643G>A | D        | D      | D    | –          | Likely pathogenic   | –               |     |
| 31, 48    | 14, 31 | F, F | ATP1A2 | Familial/unknown | 13   | p.Ala606Thr | c.1816G>A | D        | D      | D    | –          | Pathogenic          | –               |     |
| 81        | 10  | F      | ATP1A2 | Sporadic    | 16   | p.Ala717Thr | c.2161G>A | D        | D      | D    | –          | Pathogenic          | –               |     |
| 60        | 36  | M      | ATP1A2 | Unknown     | 16   | p.Gly762Ser | c.2289G>A | D        | D      | D    | –          | Pathogenic          | –               |     |
| 103       | 20  | M      | ATP1A2 | Unknown     | 17   | p.Arg763His | c.2289G>A | D        | D      | D    | –          | Pathogenic          | –               |     |
| 132       | 3   | F      | ATP1A2 | Unknown     | 17   | p.Pro768Ser | c.2386G>A | D        | D      | D    | –          | Pathogenic          | –               |     |
| 15        | 3   | F      | ATP1A2 | Unknown     | 18   | p.Leu195Ser | c.2456T>C | D        | D      | D    | –          | VOUS                | –               |     |
| 172       | 9   | M      | ATP1A2 | Unknown     | 18   | p.Glu822Val | c.2465A>T | D        | D      | D    | –          | VOUS                | –               |     |
| 16, 166   | 14, 14 | M, M | ATP1A2 | Unknown/familial | 18   | p.Gly855Arg | c.2563G>A | D        | D      | D    | –          | Pathogenic          | –               |     |
| 71        | 14  | F      | ATP1A2 | Unknown     | 20   | p.Val924Met | c.2770G>A | D        | D      | D    | –          | VOUS                | –               |     |
| 84        | 32  | F      | SCN1A  | Unknown     | 4    | p.Arg187Gln | c.560G>T  | D        | D      | D    | –          | VOUS                | –               |     |
| 75        | 44  | M      | SCN1A  | Unknown     | 17   | p.Thr174Ser | c.521G>C  | T        | T      | T    | B          | VOUS                | –               |     |
| 26        | 34  | F      | SCN1A  | Familial    | 26   | p.Arg1928Cys | c.5872G>A | D        | T      | D    | –          | VOUS                | –               |     |
| 112       | 42  | F      | SCN1A  | unknown     | 26   | p.Lys136Glu | c.506T>C  | D        | D      | D    | –          | VOUS                | –               |     |
| 173       | 14  | M      | CACNA1A | Unknown     | 14   | p.Leu608Phe | c.1822G>A | D        | D      | D    | –          | Pathogenic          | –               |     |
| 142       | 5   | F      | CACNA1A | Unknown     | 20   | p.Arg1049Trp | c.3145G>A | D        | D      | D    | –          | Pathogenic          | –               |     |
| 143       | 36  | F      | CACNA1A | Unknown     | 20   | p.Gly1090Cys | c.3268C>A | T        | D      | D    | –          | VOUS                | –               |     |
| 7, 25, 128 | 59, 32, 38 | F, M, F | CACNA1A | Familial/familial/unknown | 25   | p.Arg1346Pro | c.4037G>C | D        | D      | D    | –          | Likely pathogenic   | –               |     |
| 85        | 5   | F      | CACNA1A | Unknown     | 25   | p.Arg1349Gln | c.4046C>T | D        | D      | D    | –          | Pathogenic          | –               |     |

D: damaging or deleterious; P: possibly damaging; T: tolerated; B: benign; NGS: next generation sequencing; ACMG: American College of Medical Genetics and Genomics; VOUS: variants of uncertain significance.

Transcript, ATP1A2 (NM_000702); SCN1A (NM_001165963); and CACNA1A (NM_001127221).

Patient previously reported.
in patient 84 is present in the gnomAD population in a very low frequency (0.000004).

Validation and pathogenicity

All 29 variants were confirmed through SS (Online Supplementary Figure 1) and were absent in a cohort of 100 non-migraine control individuals. The pathogenicity of all variants identified in this study in the three FHM genes screened was determined according to the ACMG guidelines21 (Table 1). In total, 11 variants were classified as likely pathogenic or pathogenic (class 4 and 5) including the three novel variants (frameshift and canonical +1 or 2 splice sites) identified in ATP1A2, c.34_35insC, c.381+1T>A and p.Val340Leu in cases 130, 105 and 24, respectively. The other 18 non-
synonymous variants identified in this study were classified as variants of uncertain significance (VOUS), including the novel non-synonymous variants identified in cases 15, 52, 80, 104, 164 and 172. Further family segregation or functional investigations may cause the classification of these variants to change.

**Rare variants.** Several rare (minor allele frequencies of <0.001) and novel variants \((n = 10)\) in the three FHM genes were identified in 14 cases (Online Supplementary Table 2).

Finally, we also screened for the pathological CAG repeat expansion region responsible for causing the SCA6 disorder that is found in the carboxyl terminal region of the CACNA1A protein. All patients had CAG repeats within the normal range of 4–18.

**Discussion**

In this study, we screened the three FHM genes in a cohort of 172 patients referred to us with a diagnosis of HM. Of the 29 variants identified, 11 variants were classified to be

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**Table 2.** Variants detected in ATP1A2 by targeted gene panel that noted to be involved in mTBI/concussion phenotype using NGS approach.\(^a\)

| Sample ID# | Age | Sex | Locus | Exon | Protein | Coding | Reference | TBI | FHx | Clinical characterisation |
|------------|-----|-----|-------|------|---------|--------|-----------|-----|-----|--------------------------|
| 80         | 14  | M   | chr1:160090798 | 2 | p.Met39Val | c.115A>G | Novel | Yes | No | Patient has right facial weakness and hemiparesis after a relatively minor head trauma. Severe concussion-related symptoms have been noted including persisting headaches and personality changes since that time. |
| 49, 131    | 49, 53 | F, F | chr1:160093019 | 4 | p.Arg65Leu | c.194G>T | rs187733403 | Yes | Yes | Migraine headaches (hemicranial, severe, throbbing and associated with vomiting). Has confusion, memory loss after a mTBI, episodes are less like transient global amnesia than sister (case 131). |
| 24         | 11  | M   | chr1:160098442 | 9 | p.Val340Leu | c.1018G>T | Novel | Yes | Yes | Concussion has been noted in father after minor head injury and the son had attacks of concussion-related symptoms including acute confusional migraine. |
| 33         | 14  | F   | chr1:160098527 | 9 | p.Thr368Met | c.1103C>T | rs746383817 | Yes | Yes | Unusual responses to mild head injury, confusion for about 48 h, vomiting and agitation. |
| 48         | 31  | F   | chr1:160100376 | 13 | p.Ala606Thr | c.1816G>A | rs1414742926 | Yes | No | Age of onset was 6 years. Attacks have been triggered by minor head trauma. Episodes of concussion-related symptoms associated with aphasia, hemiplegia, grey matter oedema and speech disturbance. |
| 15         | 3   | F   | chr1:160106053 | 18 | p.Leu819Ser | c.2456T>C | Novel | Yes | Yes | Recurrent episodes of transient hemiparesis in association with trivial head injuries. |
| 71         | 14  | F   | chr1:160106751 | 20 | p.Val924Met | c.2770G>A | rs373276446 | Yes | Yes | Recurrent episodes of transient hemiparesis, loss of consciousness, slurred speech, headache and vomiting in association with trivial head injuries. |

M: Male; F: Female; TBI: traumatic brain injury; FHx: family history; NGS: next generation sequencing.

\(^a\)Transcript, ATP1A2 (NM_000702).
likely pathogenic or pathogenic (class 4 or 5) in CACNA1A, ATP1A2 and SCN1A (Table 1). All other variants were classified as VOUS. Although the appearance of these variants in genes known to be involved in HM pathophysiology is suggestive of a potential role in the symptoms suffered by these patients, additional data, such as segregation analysis and/or functional evaluation is required for a definitive assessment of pathogenicity. Accordingly, we report these VOUS variants along with their available clinical data to enable future evaluation and interpretation if these variants can be observed independently. For instance, case 15 (p.Leu819Ser) had recurrent episodes of transient hemiparesis in association with trivial head injuries; case 52 (p.Ile293Phe) had a family history of migraine; case 80 (p.Met39Val) reported to have right facial weakness and hemiparesis after a relatively minor head trauma; and case 172 (p.Glu822Val) who experienced a right sided hemiplegia and recurrent attacks of seizures, developmental delay.

In our cohort of suspected HM cases (n = 183), which includes 9 mutations that were identified in 9 HM cases in CACNA1A in our previous study using SS,15 known or novel variants were present in 44 cases (24%). A Danish population study showed that 14% of 42 studied FHM families had a mutation in CACNA1A or ATP1A2, while a recent study by Hiekkala and colleagues showed that only 9% (4/44) of the studied Finnish FHM families have mutations in the known FHM genes.33,34 In contrast, a French study by Riant and colleagues identified a significantly high proportion of de novo mutations (76%) in CACNA1A and ATP1A2 among SHM patients (n = 25). The high rate of mutation detection in this study may be attributable to the selection of SHM cases with an early age of onset (before 16 years of age) and severe phenotype, which might favour detection of new mutations in the classical FHM genes.35 We identified a higher percentage of potentially pathogenic variants in our population, than that observed by Hiekkala et al. in n = 131 FHM and n = 275 SHM patients, but in many of our cases family members were unavailable for segregation analysis to provide further evidence of pathogenicity. It is possible that in selecting patients referred directly by neurologists for FHM gene testing rather than families, our study has many more probands with a bias towards more severe HM related phenotypes, similar to the study by Riant et al.24 Alternatively, our population may simply represent a different mix of ethnicities, with patients carrying founder variants more concentrated in the classical FHM genes.

In contrast to other studies and cohorts, in which the majority of FHM gene mutations were found in CACNA1A (FHM1), we found a high proportion of variants (n = 20) in ATP1A2 (FHM2) in our cohort, comprising 24/44 cases (54.5%). CACNA1A (FHM1) ranked the second most likely to potentially cause HM in our cohort, with 14 variants identified in 16/44 cases (36.4%). SCN1A (FHM3) harboured the least number of variants with 4/44 cases (9.1%). Loss of function (LOF) heterozygous frameshift variants in ATP1A2 have previously been reported to segregate with FHM2 phenotypes,44 however LOF heterozygous variants have also been reported in individuals who never suffered from typical HM45 suggesting an incomplete penetrance.24 This may be a consequence of the specific functional effects of the frameshifts, or that mRNA degradation due to nonsense mediated decay may be able to be compensated for in some individuals, perhaps via compensatory increases in the expression of the functional allele. Functional analysis to determine the consequences for biallelic and monoallelic loss of function variants is thus needed to properly assess their pathogenicity.

Around 40% of the ATP1A2 variants identified in this study were located within the intracytoplasmic loop between M4 and M5, which harbours the majority of known FHM2 mutations and is critical for the function of the Na, K-ATPase pump, containing the phosphorylation site and ATP-binding domains.37,38 Functional consequences of different heterozygous FHM2 mutations in HeLa cells and Xenopus oocytes on the activity of the Na/K-ATPase α2 subunits have shown a partial or a complete loss of function of the ion pump activity.6,39-41 Haploinsufficiency of ATP1A2 or having one wildtype allele only is not sufficient to maintain the ion pump activity in these cells.6 The remaining variants in ATP1A2 exist in transmembrane regions and were classified as VOUS.

It is also interesting to note that while most of the ATP1A2 variant carriers in our population had relatively young ages at diagnosis (mean age of 16.3 years), patients who bore the p.Arg655Leu and p.Gly114Ser variants were referred for genetic testing between the ages of 49 and 59. The two variants are localised in the cytoplasmic N-terminal and extracellular M1–M2 of the ATPase α2 subunit, respectively (Figure 1(a)). The p.Arg655Leu variant which segregates with FHM phenotype in two sisters shares the same N-terminal location as previously identified FHM variant p.Arg65Trp in patients who were in their mid/late 40s on diagnosis.42 This may indicate that variants in part of the protein cause less serious disruptions to function, that only show later in life.

In this study, seven amino-acid changing variants in ATP1A2 (p.Met39Val, p.Arg655Leu, p.Val340Leu, p.Thr368Met, p.Arg606Thr, p.Leu819Ser and p.Val924Met) were detected in individuals noted to have incidence of concussion like symptoms after mild head trauma. This represents 35% of ATP1A2 variants, found in eight suspected HM cases (Table 2). This increased susceptibility to concussion could represent a similar effect caused by the CACNA1A p.Ser218Leu mutation, where minor head impacts have been known to cause severe oedema, seizures and coma.1,2,43 The locations of the ATP1A2 variants associated with this phenotype are diverse, as can be seen when they are shown on the crystal structure of the ATP1A2 protein based on the Sus scrofa version (Figure 1(b)).
of these ATP1A2 variants on the expression and activity of the human Na\(^+\), K\(^+\)-ATPase α2 subunit will provide further relevant information on these variants.

Finally, a likely pathogenic missense variant was identified in CACNA1A. The novel p.Arg1346Pro variant is a highly preserved amino acid in the transmembrane region found in three unrelated patients, who displayed variable symptoms, including cerebellar syndrome, migraine hemiplegia, paraesthesia and stroke. Ischemic stroke was also reported in patient 143 who carries the p.Gly1090Cys variant.

Interestingly, after comprehensive screening for variants in the known FHM causing genes, 75% of patients still do not have a molecular diagnosis. This suggests that mutations in other ion channel or other genes may play a role in causing HM. The next step would be to screen the whole exome to increase the probability of identifying new variants/mutations or genes causing HM, allowing improvement of diagnosis rates. Our targeted gene panel has been designed to cover exonic regions of FHM implicated genes but the coverage is not complete with approximately 93% coverage (Online Supplementary Table 1). Variants in the missing exonic parts in the three FHM genes as well as variants in other genes, including PRRT2, PNKD, SLC2A1, SLC4A4 and SLC1A3 that have been previously associated with HM, but do not appear to be major loci and usually display additional symptoms and signs, will be prioritised in planned future analysis. Finally, further evaluation and functional analysis using in vitro methods for all variants identified in this study might help to confirm phenotype–genotype correlations.

In conclusion, from this study, it is clear that in this Australian and New Zealand suspected HM cohort the ATP1A2 gene harboured the most likely causal variants for HM with 22/44 cases in which a variant was identified being in ATP1A2 (i.e. FHM2). Our results also show a strong link between ion channel gene variants found in ATP1A2 and increased concussion susceptibility in 35% of suspected HM patients with variants in this gene. Finally, we demonstrate that using a NGS multigene panel to screen full exonic sequence of ATP1A2 alongside CACNA1A and SCNA1 is a clinically useful and efficient method to identify variants that potentially cause HM.

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Supplemental material

Supplemental material for this article is available online.

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