MUTATION ANALYSIS OF CACNA1A AND ATP1A2 GENES IN BRAZILIAN FHM FAMILIES

Luciana R. Lopes¹, Mario Fernando Prieto Peres², Kaate R.J. Vanmolkot³, Patricia R. Tobo¹, Eliova Zukerman⁴, Rune R. Frants⁵, Arn M.J.M. van den Maagdenberg⁵, Carlos Alberto Moreira-Filho⁶

ABSTRACT - Familial hemiplegic migraine (FHM) is a rare autosomal dominant form of migraine with aura. This disease has been associated with missense mutations in the CACNA1A and ATP1A2 genes. The aim of this study was to identify whether CACNA1A and ATP1A2 are or not related to Brazilian FHM. Here we screened four Brazilian FHM families (total of 26 individuals - 13 affected and 13 asymptomatic or normal) for mutations in both genes. We found an amino acid change in a member of family FHM-D (Arg2206Gly). However since this alteration is not present in all affected individuals and is present in one asymptomatic individual it should be considered a polymorphism. Further studies with additional families will be necessary to reveal the importance of both CACNA1A and ATP1A2 genes on the pathogeneses of FHM in Brazil and to test the third gene (SCN1A) in these FHM families.

KEY WORDS: familial hemiplegic migraine, CACNA1A, ATP1A2, SCN1a, gene, mutation.

Análise de mutações dos genes CACNA1A e ATP1A2 em famílias brasileiras afetadas por enxaqueca hemiplégica familiar

RESUMO - A enxaqueca hemiplégica familiar (EHF) é uma forma rara de enxaqueca com aura e apresenta herança autossômica dominante. Esta doença está associada com mutações do tipo missense nos genes CACNA1A e ATP1A2. O objetivo deste estudo foi identificar se os genes CACNA1A e ATP1A2 estão ou não relacionados com a enxaqueca hemiplégica familiar em famílias brasileiras. Os genes citados acima foram analisados em quatro famílias brasileiras (total de 26 indivíduos - 13 afetados e 13 assintomáticos ou normais) e uma troca de aminoácido em um membro da família FHM-D (Arg2206Gly) foi observada. Porém, esta alteração não foi identificada em todos os indivíduos afetados e está presente em um indivíduo assintomático, devendo, portanto, ser considerada um polimorfismo. Estudos adicionais nas famílias já estudadas e em outras famílias brasileiras afetadas por enxaqueca hemiplégica familiar serão necessários para esclarecer a importância dos genes CACNA1A e ATP1A2 na patogênese da EHF no Brasil, bem como para testar o terceiro gene (SCN1A) relacionado à EHF.

PALAVRAS-CHAVE: enxaqueca hemiplégica familiar, gene CACNA1A, gene ATP1A2, gene SCN1a, mutação.
A missense Gln1489Lys mutation was identified in three German families with common ancestry. The functional analyses revealed that mutation causes a two-fold to four-fold accelerated recovery from fast inactivation.

In this report, we screened for mutations in the \textit{CACNA1A} and \textit{ATP1A2} genes in four Brazilian FHM families (Fig 1).

**METHOD**

**Subjects** – The present study was approved by the local Ethical Commitee in Research (Albert Einstein Hospital - São Paulo / Brazil) in May/2003. All subjects provided written informed consent, as required by appropriate local (and national) committees on the protection of research subjects, and were interviewed and examined by one of the neurologist of the Albert Einstein Hospital (Peres, MFP; Zuckerman, E). Diagnostic criteria of the International Headache Society were used to define familial hemiplegic migraine (IHS, 2004). Four Brazilian FHM families without cerebellar signs were analyzed (FHM-A, FHM-B, FHM-C and FHM-D - Fig 1) and selected from June/2003 to December/2004.

**Clinical features** – A total of 26 individuals (16 females and 10 males) were selected, where 13 were affected and 13 were asymptomatic or normal. The middle age was 29 years. All four probands have other family members with episodes of hemiplegia (Table). The age of onset of hemiplegic episodes varied from 3 to 30 years, and the typical duration of episodes varied from minutes to days.

**Genomic DNA samples** – Blood samples of all patients were collected and genomic DNA was isolated from leukocytes as described by22.

**Mutation screening** – We screened the probands from families FHM-A, FHM-B, FHM-C and FHM-D for mutations in the \textit{CACNA1A} (47 exons) and \textit{ATP1A2} (23 exons) genes. Mutation analysis was performed by direct sequencing of all exons and flanking introns.

**RESULTS**

Mutation analysis of the \textit{CACNA1A} and \textit{ATP1A2} genes in probands of four Brazilian FHM families revealed several polymorphisms, but no mutations were identified in either gene. In the individual FHM-D II.5 (Fig 2) an amino acid change was identified substituting a glycine for an arginine (Arg2206Gly) in exon 46 of the \textit{CACNA1A} gene (Fig 2). Her mother (FHM-D I.2), affected by FHM, presented the same alteration, but her brother (FHM-D II-3), an asymptomatic individual, has the same aminoacid change. This result shows this alteration is a polymorphism not related to the disease. No other amino acids changes were identified in the \textit{CACNA1A} or \textit{ATP1A2} genes of the FHM probands. There remain always the possibility of deletions and promoter mutations that remain undetected with direct sequencing.
DISCUSSION

No mutations were identified in \textit{CACNA1A} or \textit{ATP1A2} genes of probands of four Brazilian FHM families, only one novel amino acid change (Arg2206Gly) that is, probably, a polymorphism. This amino acid change was present in two affected (FHM-D I.2 and FHM-D II.5) and one asymptomatic individual (FHM-D II.3), excluding that this variation is causative in this family. The location of this alteration occurred in an important part of the gene (cytoplasmatic domain), so functional studies could be important to reveal whether this alteration is or not related to the phenotype in this patient (FHM-D II.5). The present functional tests are designed to look at the currents of the channel. For this, the mutations need to be in the transmembrane domains or in short loops (P-loops). A mutation in the cytoplasmatic domain might be located in a binding domain of the associated, regulatory proteins. Test whether the mutation affects binding of these proteins has never been tried for a pathogenic mutation.

This is the first report of Brazilian FHM families and these data suggest that maybe both genes are not involved in these Brazilian FHM families. None of the patients in our FHM families had interictal ataxia, nystagmus or seizures.

Future studies will be important to understand the role of both genes in our population. Of course it is possible that other Brazilian FHM families may have mutations on the \textit{CACNA1A} or \textit{ATP1A2} genes, especially FHM families presenting with ataxia and/or seizures. Besides, the investigation of the third FHM gene (\textit{SCN1A}) will be performed to investigate the role of this gene in our FHM families.

In conclusion, in our four FHM families no mutations in either FHM gene were identified and we have no evidence for involvement of these genes in these families. This may be an indication that \textit{SCN1A} gene is causing FHM in these families.

\begin{table}
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\begin{tabular}{|l|c|c|c|c|c|c|c|c|}
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Patient Identification & Sex & Age (years) & Age at onset & Aura & Hemiplegia & Photofobia & Phonofobia & Ataxia & Familial history \\
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FHMA-I.2 & F & 63 & 15 & Y & Y & Y & N & Y \\
FHMA-II11 & F & 43 & 3 & Y & Y & N & Y \\
FHMA-II12 & M & 38 & 8 & Y & Y & N & Y \\
FHMA-II18 & F & 33 & 8 & Y & Y & N & Y \\
FHMA-III.1 & F & 21 & 14 & Y & Y & N & Y \\
FHMB-II.2 & F & 60 & 30 & Y & Y & N & Y \\
FHMB-II.3 & F & 31 & 16 & Y & Y & Y & N & Y \\
FHMC-I.1 & F & 53 & 10 & Y & Y & Y & N & Y \\
FHMC-II.1 & M & 23 & 7 & Y & Y & N & Y \\
FHMD-I.2 & F & 60 & 10 & Y & Y & Y & N & Y \\
FHMD-II.1 & F & 40 & 12 & Y & Y & N & Y \\
FHMD-II.5 & F & 38 & 15 & Y & Y & N & Y \\
FHMD-III.1 & F & 10 & 7 & Y & Y & Y & N & Y \\
\hline
\end{tabular}
\caption{Clinical signs of Brazilian FHM index patients.}
\end{table}

\textit{F}, female; \textit{M}, male; \textit{Y}, yes; \textit{N}, no.
REFERENCES