Mutations analysis of C1 inhibitor coding sequence gene among Portuguese patients with hereditary angioedema

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A R T I C L E   I N F O
Article history:
Received 4 August 2012
Accepted 5 September 2012
Available online 31 October 2012

Keywords:
HAE
C1-INH
Mutations
SERPING1 gene

A B S T R A C T
Mutations that modify the amino acid sequence of C1-INH (except Val458Met) are associated with HAE. More than 200 different mutations scattering the entire C1-INH gene have been reported. The main objective of this study was to report the mutational findings in a HAE cohort of 138 Portuguese patients followed in specialized consultation all over the country.

DNA was extracted from peripheral blood with QiAmp Blood Kit (Qiagen Portugal). The sequence reactions were performed by using a DNA sequencing kit (Big Dye terminator cycle sequencing v1.1 from Applied Biosystems) and sequencing products were immediately submitted to direct sequencing in an Applied Biosystem 3130 DNA Analyser.

DNA sequences were analyzed at four different stages. Raw data and sequence alignments of all 8 exons and intron–exon boundaries were performed for each patient individually with SeqScape software and using SERPING1 gene NG_0009625 of 24,300 bp (12-March-2011) as reference sequence. Sequence comparisons among patients and controls were performed with software CodonCode Aligner v.3.7 from CodonCode Corp and with Geneious 4.5 from Biomatters Ltd.

A total of 94 mutations were observed among patients, and 67% of them were located on exon 8. In addition, we noticed one not described stop codon at position c.1459 C>T in three different patients. Translation termination was also found on exon 3 and 7, as a result of mutations at positions c.481A>T, c.1174C>T. In this population, the prevalence of the missense mutation p.Arg444Cys was 39 out of 42.

Mutational analysis revealed 22 different pathogenic mutations, of which 64% were not described on HAE database. Although identification of disease causing mutations is not necessary to establish HAE diagnosis, studies on gene expression and characterization of rearrangements in SERPING1 gene are suggested in order to get new insights on function and genetic tests of C1 inhibitor.

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1. Introduction

HAE is an autosomal dominant disease characterized by recurrent episodes of potentially life-threatening angioedema. This rare disease is caused by a deficient production of the active protein C1 esterase inhibitor (C1-INH) and it is identified for the capacity to inhibit the esterase activity of the first component of the complement system (Gompels et al., 2005; Kaplan et al., 2002). C1-INH belongs to a family of serine proteases inhibitors termed serpins and controls complement pathways (classical, lectin and alternative) and the kinin-forming system (Bossi et al., 2009). Mutations that modify the amino acid sequence of C1-INH (except Val458Met) (Kalmár et al., 2003) are associated with HAE (Online Mendelian Inheritance in Man, OMIM106100), the most common human disease due to the genetic defect of a complement protein.

In HAE associated with C1-INH gene mutations, protein synthesis or function are impaired leading to low antigenic and functional C1-INH plasma levels (HAE type I) or normal/elevated antigenic plasma levels but low C1-INH function (HAE type II) (Pappalardo et al., 2008).

C1-INH gene, also called SERPING1 (OMIM606880), is located on chromosome 11q12–q13.1 and more than 200 different mutations scattering the entire gene, have been reported and collected in an online HAE database (URL http://www.hae.ensim.hu – assessed.
1st July 2011) (Pappalardo et al., 2008). Efforts have been made, in published studies, to get a correlation between genotype, C1-INH expression and phenotype but without success (Gompels et al., 2005; Kalmár et al., 2005).

Although the variety and number of mutations reported, there are only a few previous published studies focused on the epidemiology of HAE in well defined populations (Kalmár et al., 2005).

In this study we aim to report the mutational findings in a HAE cohort of 138 Portuguese patients followed in specialized consultation all over the country. Furthermore, this is the first report from a nationwide study and an effort was made to bring together, in further publications, genetic and genomic data supported by clinical findings.

2. Materials and methods

2.1. Patients and controls

Patients were recruited from the HAE case lists of Portuguese HAE specialized consultation. A total of 138 patients (23% from the north, 24% from the center and 53% from south part of Portugal), previously diagnosed with HAE type I and type II, were involved in this study, representing most Portuguese patients with HAE diagnosis. The diagnosis was based on functional or antigen levels of C1-INH below 50% of the mean value of a healthy donor reference population at two separate determinations in combination with clinical symptoms (Gompels et al., 2005).

Relevant clinical data, including family history was collected from individual clinical process.

Healthy blood donors (n = 30) recruited from a selected panel, with no individual and familiar clinical history of angioedema, were included as control group.

All participants signed their informed consent and the study was approved by the Ethical Committee of each hospital according to national and international laws. Biological material including DNA, RNA, serum and plasma, was labeled, selectively aliquoted and stored, according to the OECD biobanking guidelines.

2.2. Mutation detection by sequencing

DNA was extracted from peripheral blood with QiaSymphony BioRobot (QIAGEN Portugal) and exons 1–8 were amplified with primers chosen for the intron–exon boundaries using Primer Express software, v2.0 (Applied Biosystems). Genomic DNA was subjected to 35 cycles of PCR touch-down amplification, 95 °C – 5 min; 10 cycles at 95 °C – 20 s, 65 °C – 1 min; 15 cycles at 95 °C – 20 s, 60 °C – 1 min and 10 cycles 95 °C – 20 s, 55 °C – 30 s. 72 °C – 30 s. in a final volume of 50 μl using AmpliTaq DNA polymerase (LifeTechnologies, Inc.).

The specific length of each amplicon was confirmed by 2% agarose gel electrophoresis and sequencing reactions for each exon were performed in both directions (forward and reverse specific primers) after ExoSap purification (GE Healthcare life-sciences). The sequence reactions were performed by using a DNA sequencing kit (Big Dye terminator cycle sequencing v1.1/v3.1 from Applied Biosystems) and sequencing products were immediately submitted to direct sequencing on an Applied Biosystem 3130 DNA Analyser (LifeTechnologies, Inc.) after being submitted to a second purification using Multiscreen-HV plates (Millipore) filled with Sephadex G50 Superfine beads (Amersham Biosciences).

Integrity and specificity of PCR products was also checked by melting curves analysis in a LightCycler® 480 with SYBR Green 1 Master (Roche Diagnostics).

2.3. Analysis of nucleotide and amino acid variations

DNA sequences were analyzed at four different stages. Raw data and sequence alignments of all 8 exons and intron–exon boundaries were performed for each patient individually with SeqScape software and using SERPING1 gene NG_009625 of 24,300bp (12–March–2011) as reference sequence. Variations in DNA sequence were numbered and identified using cDNA sequence as reference (#NM_000626 Genbank accession number) and according to guidelines of Human Genomic Variation Society (HGVS). For amino acid alterations we used the mature protein sequence without signal peptide (22N terminal residues). Sequence comparisons among patients and controls were performed with software CodonCode Aligner v.3.7 from CodonCode Corp. and with Geneious 4.5 from Biomatters Ltd.

2.4. Theory/calculation

The knowledge of the HAE genetic background of a specific population is of interest not only to get new insights on the diversity of the disease, but also on the correlation of specific mutational map with the variability of the frequency and severity of such disease. The contribution of this first HAE Portuguese population reinforces the idea of the extreme diversity of mutations and the absence of correlation between gene mutations, Cl inhibitor levels and the phenotypic expression of HAE. Although we have only presented mutation frequencies, this study could be a starting point to evaluate alterations in the mRNA transcription pathway, to establish the degree of involvement of specific transcription factors, to assess the influence of the mutational profile and its correlation with phenotypic manifestations.

3. Results

One hundred and thirty eight HAE patients (type I and type II HAE), composed by 42% male and 58% female, were included in the study. The age range from 10 to 75 years (average 41 years). Assuming that this population is representative of Portuguese HAE patients we could affirm that the frequency of the disease is 1:72,000 inhabitants. Considering the type of HAE, 60% of the patients were classified as HAE type I, a lower frequency when compared with other reported populations (Gompels et al., 2005; Kalmár et al., 2003, 2005; Pappalardo et al., 2008).

Although exon 1 and exon 2 have been sequenced, only the cDNA sequence correspondent to the mature protein was analyzed for establishment of the mutational profile of patients. Furthermore, we noticed that the frequency of mutations in these two exons was very low or even absent (Bossi et al., 2009). For this reason we did not take into account the occurrence of c.-21T>C polymorphism in exon 2 that could be associated with a more severe phenotype (Bygum et al., 2011; Cumming et al., 2003; Stoppa-Lyonnet et al., 1990, 1991).

A total of 94 point mutations were observed among patients. As represented in Table A1, 63 out of 94 (67%) of the mutations are located on exon 8. For this, largely contributes the well reported missense mutation p.Arg444Cys. However, in addition, we also noticed one not described stop codon at position c.1459 C–T in three different patients. Translation termination was also found on exon 3 and 7, as a result of mutations at positions c.481A>T, c.1174C>T.

It is interesting to highlight, in this population, the great prevalence of the missense mutation p.Arg444Cys, characteristic of type II HAE patients (39 out of 42) (Skriver et al., 1989). However, apart from this, no particular mutational profile was characteristic of each type of HAE.
The incidence of large deletions is evident, as expected (Bygum et al., 2011; Cumming et al., 2003), on exons 4 and 5 (Table A.2) but point mutations, not reported in HAE database were also detected (Table A.1) within this exons.

4. Discussion

Identification of mutations is of interest to establish the causality of C1-INH deficiency. For the diagnosis of HAE, the mutation analysis of SERPING1 gene establishes the genetic determinant of the low C1-INH function. Predictive genetic counseling requires knowledge of the disease-causing mutation (Bygum et al., 2011). The Portuguese cohort consisted of 138 HAE patients, 60% type I and 40% type II. The HAE type II frequency is higher when compared with others previously reported (85–90% type I and 15–10% type II).

The technical approach used in this work allows us to sequence and compare the coding region of the SERPING1 gene. Confirmation of the specific length of PCR products submitted to sequencing reactions by high resolution agarose gel electrophoresis and melting calculation assay by RealTime PCR reveals to be a good approach for the evidence of large deletions or insertions within exons.

Mutational analysis revealed 22 different pathogenic mutations, of which 64% were not described in HAE database.

Large deletions were found on exons 4 and 5. The high density of Alu rich repeats (Stoppa-Lyonnet et al., 1990, 1991) in most introns of the gene, mainly in introns 3 and 4, gives rise to the occurrence of recombinations that can cause this kind of instability (Table A.2).

5. Conclusions

Although we did not analyze, in this work, the impact of mutational profile in C1-INH levels, functional activity and the severity of the disease, seems to us to be relevant for the contribution for the knowledge and identification of mutations in HAE Portuguese population. In this work we were able to verify earlier reported mutations and to identify new ones which allow us to extend the HAE database with this new population. Although identification of disease causing mutations is not necessary to establish HAE diagnosis, studies on mRNA expression and characterization of rearrangements in SERPING1 gene are suggested in order to get new insights on function and genetics of C1-INH.

Disclosure statement

Authors received an unrestricted educational grant from Shire Human Genetic Therapies, Inc., for the development of the manuscript.

Role of the funding source

Shire Human Genetic Therapies, Inc. was responsible for reviewing the manuscript.

Acknowledgments

We thank Shire Human Genetic Therapies, Inc. for supporting this study via an unrestricted educational grant.

We also thank all the collaboration and support given by R. Silva, A. Leblanc from Serviço de Imunoallergologia, Centro Hospitalar de S. João EPE and Paiva M., Gaspar A. from Imunoallergology

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### Table A.1

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<td>p.Lys162Ser</td>
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<tr>
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<td>p.Gly186Ser</td>
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<tr>
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### Table A.2

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Department, Hospital D. Estefânia, Centro Hospitalar de Lisboa Central.

Editorial support was provided by Tiago Monteiro at Novexem Portugal and was supported by Shire Human Genetic Therapies, Inc. Responsibility for opinions, conclusions, and interpretation of data lies with the authors, and such perspectives do not reflect those of Shire Human Genetic Therapies Inc.

Appendix A.

Tables A.1 and A.2.

References


