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SERPING1 mutations in 59 families with hereditary angioedema

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ABSTRACT

Hereditary angioedema due to C1 Inhibitor (C1Inh) deficiency (HAE types I and II) is a rare, life-threatening disease causing spontaneous edema of the submucosal layers.

A cohort of 127 individuals with symptoms of recurrent familial angioedema from 59 non-related families was studied. All the patients included fulfilled the diagnostic and biochemical criteria of HAE, including low C1Inh function and/or concentration. Genetic studies were carried out by PCR and sequencing of the *C1NH* locus followed, in the negative cases, by MLPA, long-range PCR and restriction enzyme analysis of genomic DNA to detect potential large rearrangements. Mutations located in consensus splicing sequences or nearby positions were studied by RT-PCR.

The study identified 52 different mutations (25 missense, 15 frameshift, 7 splicing defects and 5 large deletions) responsible for the disease in 56 HAE families. In the remaining three families no molecular alteration could be detected. Twenty-seven of the mutations in this cohort are novel and 10 are confirmed *de novo* cases. The pathologic effect of the 5 splicing defects first reported here was assessed at the RNA and protein levels. Large deletions affecting exons 4 and 7, ranging from approximately 1500 to 2500 bp, were partially characterized by their altered restriction patterns upon long-range amplification.

These results highlight the heterogeneity of mutations in the *C1NH* gene causing C1Inh deficiency and HAE. An approach to the molecular effects associated to each of the mutations reported here was made when possible based on the available data of pathological variants of serpins.

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1. Introduction

Hereditary angioedema (HAE, MIM#106100) is a primary immunodeficiency with an autosomal dominant pattern of inheritance that affects approximately 1 in 50,000 in the population. Patients of HAE have spontaneous episodic attacks of non-pitting, non-pruritic, self-limiting swelling due to a transient increase of endothelial permeability in the capillaries of the deep cutaneous and mucosal layers. It typically affects the skin and upper respiratory and gastrointestinal tracts (Frank, 2010; Grigoriadou and Longhurst, 2009). Laryngeal, nasal, and sinus edema in these patients may lead to respiratory tract compromise and death from suffocation if not promptly and properly treated. In most of the cases, the bases of the disease are mutations in the C1-Inhibitor (C1Inh) gene that alter its protein synthesis (HAE type I, low antigenic and functional levels) or function (HAE type II) (Bowen et al., 2008).

C1Inh is a serine protease inhibitor (Serpin) that plays a central role in vascular homeostasis. Its main function is preventing unnecessary activation of the classical pathway of complement by acting on C1s and C1r proteases. It also participates in the control of MASP proteases of the lectin pathway of complement and acts as an important regulator of coagulation, fibrinolytic and contact (kinin-realising) cascades (Gaboriaud et al., 2004; Caliezi et al., 2000). C1Inh regulates the production of kinins by preventing the activation of kallikrein and coagulation factor XII (FXII) in the contact system. In the context of a C1Inh deficiency, this step runs uncontrolled, which in turn results in an episodic increase of bradikynin production and in the consistent rise in vascular permeability and edema (Nussberger et al., 1999; Cugno et al., 2003; Davis, 2008).

Serpins are globular proteins comprised of nine α helices, three β sheets and an exposed, flexible reactive center loop (RCL). They function as suicide substrates of serin-proteases by displaying a bait sequence in the RCL that mimics the normal enzyme substrate. Upon docking, the protease cleaves the RCL in the so-called P1–P1′ scissile reactive center bond and forms a complex with the serpin that ultimately leads to the irreversible insertion of the RCL into the β sheet A and the protease being trapped (Lomas et al., 2005).

Abbreviations: C1Inh, C1 inhibitor protein; *C1NH*, C1 inhibitor locus; HAE, hereditary angioedema; RCL, reactive center loop.

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C1Inh is the largest member of the serpin superfamily and, besides the serpin domain it presents a large, highly glycosylated and non-conserved N-terminal domain that is dispensable for inhibitory capacity (Bos, 2003).

The *C1NH* gene (SERPING1 MIM#606860; GenBank NM_000062.1) extends over a genomic region of 17 kb located on chromosome 11q12-q13.1. It is comprised of 8 exons, the first one containing 38 bp of non coding sequence and the second one having a 22 bp-long signal peptide before the first methionine. The 7 introns present a high density of interspersed repetitive Alu elements (Carter et al., 1991), making the gene prone to large deletions and duplications that are responsible for approximately 15% of all HAE cases.

HAE type I is due to impairment in C1Inh's protein folding or secretion and is caused by mutations that can be found over the entire gene. In HAE type II patients, C1Inh is secreted normally but the protein is dysfunctional as a result of mutations located within the serpin's reactive center or in the proximal hinge region (both encoded by exon 8).

Systematic mutational analysis of the *C1NH* locus has been performed in several cohorts and accounts for more than two hundred different mutations distributed across the entire gene (Bissler et al., 1997; Verpy et al., 1996; Bowen et al., 2001; Zuraw and Herschbach, 2000; Blanch et al., 2002; Cumming et al., 2003; Roche et al., 2005; Pappalardo et al., 2008; Bygum et al., 2011). Mutation screening has proven useful for establishing structure–function correlations and for providing diagnostic criteria in serpinopathies (Gooptu and Lomas, 2008). Moreover, genetic studies have recently allowed the first case of successful pre-implantational diagnosis in a HAE patient (Bautista-Llácer et al., 2010).

In the present report, we describe 52 different disease-causing mutations (10 of which are novel) in 56 families affected of HAE from the Spanish population and attempt to establish the molecular mechanisms involved in the lack of functional C1Inh.

2. Materials and methods

2.1. Subjects of the study

One hundred and twenty seven patients affected of HAE (113 with HAE type I and 14 with HAE type II) from 59 unrelated families were included in this study. In order to investigate genotype–phenotype co-segregation, additional relatives from each family were also included when possible, for a total number of 166 individuals analyzed. Fifty unrelated healthy donors, previously screened for eventual polymorphic variants in the *C1INH* gene, were used as controls. All participants gave informed consent for this study.

2.2. Sampling and complement studies

Blood samples were obtained and centrifuged at 4 °C. Complement screening for the diagnosis of HAE was carried out with fresh serum and/or plasma samples stored at −80 °C. Briefly, C3, C4 and C1inh levels were measured by nephelometry (Siemens, Marburg, Germany), C1 inhibitor function was quantified by using a Berichrom chromogenic test (Siemens) and C1q was measured by radial immunodiffusion using the C1q Binarid Radial Immunodiffusion kit (The Binding Site, Birmingham, UK). Western blot of fresh plasma samples was performed as described before (López-Lera et al., 2010).

DNA and RNA samples were obtained from peripheral blood mononuclear cells using Gentra Puregene BloodCore and RNeasy Midi kits from Qiagen (Valencia, CA), respectively, following the manufacturer's instructions. Once the diagnosis of HAE was established, a Family Code (A, B,. . .AB, AC. . .) was assigned to each family. In all cases, the index patients of each family were referred to with their Family Code (patient A, B. . .AB, AC....) while their relatives were assigned with additional numbers.

2.3. Genetic profile

Genetic study of *C1NH* was performed following a protocol modified from that previously described. Briefly, PCRs of 0.5 μg genomic DNA were set up by using primers and cycling conditions as described (Roche et al., 2005). Amplified products were purified from a 2% low melting agarose gel using the QIAquick gel extraction kit (Qiagen Inc., Valencia, CA). The sequencing reactions were performed in 10 μl, with 5–20 pmol of primer and using Big Dye terminator cycle sequencing v.3.1 (Applied Biosystems, Foster City, CA). Sequencing products were precipitated in 65% ethanol, washed twice in 70% ethanol and analyzed on an automatic sequencing device (ABI-PRISM 3130, Applied Biosystems).

2.4. RT-PCR

Patients harbouring sequence substitutions in canonical or nearby conserved splicing positions were analyzed for potential alterations in RNA processing. Total cDNA from patients and controls was synthesized from 1 μg RNA in 20 μl reactions with 0.5 μg Oligo$(dT)_{12-18}$, 0.2 mM dNTPs, 1 mM $MgCl_2$ and 50 units of SuperScript II RT, included in the SuperScript™ First-Strand kit (Invitrogen, Barcelona, Spain) as recommended by the manufacturer. Two cDNA overlapping fragments ranging from exons 1 to 5 and 3 to 8 of the *C1NH* locus were amplified with sequence-specific primers previously described (Roche et al., 2005). The resulting fragments were separated by electrophoresis on 2% low melting agarose gels, purified as described above and sequenced using primers and conditions described by Roche et al.

Besides the full-length transcript, alternative splicing in the *C1NH* locus gives rise to an additional variant lacking exon 3 (ENST00000531133) that is constitutively expressed in PBMCs. Due to our RT-PCR experimental design, this exon3-skipped variant is also amplified in the exon 1 to exon 5 amplicon.

2.5. Multiplex ligation-dependent probe amplification (MLPA)

In order to study large deletions/duplications affecting the *C1NH* locus, MLPA was performed with the MLPA kit P243 SERPING1 (MRC-Holland, Amsterdam, The Netherlands) following kit's protocol instructions. MLPA is a high throughput, sensitive technique for detecting copy number variations in genomic sequences (Schouten et al., 2002). The MLPA kit P243 contains probes for each of the 8 exons of SERPING1, 1 flanking probe for *APLNR* (located approximately 364 kb upstream of SERPING1) and several reference probes for other chromosomal locations. Data normalization and analysis were carried out using the Coffalyser v9.4 MLPA data analysis software (MCR Holland). The results were confirmed by long range PCR (XL-PCR) comprising the affected regions of the SERPING1 locus followed by restriction analysis of the obtained fragments. Amplification was done with the GeneAmp XL PCR kit (Applied Biosystems, Madrid, Spain) following manufacturer instructions. Restriction analysis of the PCR products was developed with the suitable enzymes (BglII and BamH1 from Applied Biosystems) during 12 h at 37 °C, stopped by incubation at 65 °C for 20 min and resolved in 0.7% ultrapure agarose gels.

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