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ORIGINAL ARTICLE

First Analysis of *SERPING1* Gene in Patients with Hereditary Angioedema in Colombia Reveals Two Genotypic Variants in a Highly Symptomatic Individual

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Abstract

Hereditary angioedema (HAE) is a heterogeneous genetic disease caused by a deficit in C1 inhibitor (C1-INH) and clinically characterized by sudden events of edema, swelling, and pruritus. Here, we describe the first *SERPING1* genotyping in 22 subjects from 4 non-related families, all from southern Colombia. The previously reported heterozygous gene mutations, c.1081C>T (p.Gln361*), c.1396C>G (p.Arg466Gly), c.1029+84G>A, or c.106_107del (p.Ser36Phefs*21), were found in 12 patients. Of note, a single patient clinically characterized as severe HAE type 2 expressed mutations in exon 8 and intron 6, whereas all the others have type 1 HAE and expressed one pathogenic variant. One of the subjects, a 5-year-old girl was discovered to have a pathogenic variant, and she is still asymptomatic. This is the first report focused on HAE genetic analysis in a Colombian population.

Keywords Hereditary angioedema · C1-inhibitor · *SERPING1* · Complement · C4

Introduction

Hereditary angioedema (HAE) (Online Mendelian Inheritance in Man -OMIM # 106100) is an autosomal dominant disease mainly associated with a C1 inhibitor (C1-INH) deficiency. It is considered an uncommon primary immunodeficiency (PID) with a frequency of 1:10,000 to 1:50,000 in the general population [1]. Clinically, this disease is characterized by the sudden onset of swelling in the face, neck, and arms; painful abdominal edema that simulates a surgical condition is frequently found [1]. Symptoms last up to 5 days and most events are reversible under appropriate treatments; however, HAE might be a life-threatening condition when there is laryngeal edema. It is accepted that HAE is a heterogeneous disorder currently classified as one of the following types depending of the pathophysiology: (i) Type 1 has decreased plasma C1-INH levels and is responsible for 85% of reported HAE cases; (ii) type 2 is due to a non-functional secreted protein (in the remain 15% of cases); and (iii) some patients have other genetic defects, such as mutations in the gene encoding coagulation factor XII. In a small number of patients, C1-INH is normal and HAE pathogenesis is unknown; these patients are classified as U-HAE [2].

C1-INH is a member of the serine protease inhibitor (serpin) superfamily that regulates the vascular permeability through the inhibition of enzymes producing vasoactive peptides called kinins. Its gene (*SERPING1*, OMIM # 606860; GenBank NM_000062.2) is located in chromosome 11 in the q12-q13.1 region and has 8 exons and 7 introns. Introns 3, 4, 6, and 7 are highly rich in Alu elements, which represent "hotspots" for non-homologous recombination, leading to partial deletions or duplications in this gene [3]. These rearrangements in *SERPING1* explain approximately 20% of the genetic defects in HAE patients [4, 5]. Exon 8 contains many CpG sequences that are prone to spontaneous deamination; the portion of the gene that encodes the reactive center of the protein, including an arginine at position 444 (HUGO:

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466), is a target for recurrent amino acid substitutions. Mutations at this amino acid position make C1-INH dysfunctional [6].

Over 450 different *SERPING1* mutations have been detected, particularly in exons 5, 6, and 8, most of which are found in an HAE-specific database (HAEdb, hae.enzim.hu). Most of the *SERPING1* mutations are missense mutations (34%), followed by frameshift alterations, and small indels (31%), large gene rearrangements (17%) splice-site defects (10%), non-sense mutations (7%), and regulatory mutations (1%) [7]. Mutations resulting in the expression of truncated or misfolded proteins that are not efficiently secreted occur throughout the gene and are responsible for type 1 C1-INH-HAE. This gene contains a number of single nucleotide polymorphisms (SNPs), which be associated with different diseases and have made genotyping analysis difficult [7].

C1-INH regulates the classical and lectin pathways associated with the complement system by binding to C1r and C1s in the former or MASP1 and MASP2 in the latter. In addition to its action on complement pathways, C1-INH modulates several other serine proteinases including plasmin, kallikrein (enzyme responsible for bradykinin synthesis), and coagulation factors XIa and XIIa. Of note, bradykinin is one of the most powerful mediators able to induce vascular permeability and non-pruritic edema. If there is a decrease in or a non-functional C1-INH, the production of bradykinin is higher, which generates painful edema episodes that may be initiated by trauma, infections, stress, surgical procedures (such as tonsillectomy), menstruation, or the use of certain medications (particularly angiotensin-converting enzyme inhibitors) [8].

To diagnose HAE, serum concentrations of C4 and C1-INH are complemented with a functional test for C1-INH. If both, C4 and C1-INH are present in low concentrations, type 1 HAE is diagnosed. Low circulating C4 with a normal concentration but dysfunction in C1-INH is characteristic of type 2 HAE [9]. Although previous clinical and quality of life local analyses have been realized [10], to date, no mutations in the *SERPING1* gene have been reported from a Colombian population. Here, we analyzed the sequence of the *SERPING1* gene from 22 individuals (including symptomatic and asymptomatic subjects) from 4 families non-related from southern Colombia.

Material and Methods

Patients and Samples

This study was approved by the Ethics Committee of the Facultad de Salud Universidad Surcolombiana and followed all principles expressed in the Declaration of Helsinki. Twenty-two subjects from 4 unrelated families were included in this study performed from January to October in 2016 at the Laboratorio de Infección & Inmunidad, Facultad de Salud – Universidad Surcolombiana, Neiva in Southern Colombia. Most of the included patients attended the allergy/immunology clinic at the Hospital Universitario de Neiva. Study population was constituted by 16 subjects (family A), a father and two children (family B), a grandmother and her grandson (family C), and one independent patient (family D). Pedigree analysis of the family A (most of the patients including here belong to this Family) is shown in the supplementary Fig. 1. From whole study population, 11 subjects reported symptoms compatible with HAE, starting between 1 and 62 years ago, such as episodes of edema in different parts of the body, including the face, arms, and legs, and recurrent abdominal pain. Therefore, most of these individuals have consulted the emergency room because of the severity of the episodes. Additionally to clinical symptoms, patients fulfilling the criteria of decreased concentrations of C4 and C1-INH or dysfunctional C1-INH [11] were included in the study. Eight related asymptomatic subjects from the same family A, two from family B, and one from family C, were also analyzed for *SERPING1* mutations as controls.

Detection of Circulating C4 and C1-INH Levels and Functional Analysis of C1-INH

To determine the levels of C4 and C1-INH antigens, at least 2 mL of peripheral venous blood was obtained by venipuncture from the patients and collected into tubes containing coagulation activator (BD Vacutainer®, SST™, catalog number 367814, NJ, USA). The C4 levels in serum were evaluated with an immunoturbidimetric assay (Abbott, catalog number 9D97-21, IL, USA). The reported detection limit of the C4 assay was of 1 mg/dL.

Circulating C1-INH protein levels were determined in serum by nephelometry using a commercial kit (catalog number OQEY09, Siemens, Marburg, Germany) and a BNII nephelometer (Siemens, Marburg, Germany).

The function of circulating C1-INH was evaluated in plasma (isolated from sodium citrated tubes) with the commercially available chromogenic kit Berichrom (catalog number OUIA155, Siemens). This assay allows the detection of changes in C1-INH function as small as 5%. In all cases, the manufacturers' recommendations were followed while performing the tests. The values of reference for the evaluated parameters are presented in the legend of Table 1.

SERPING1 Gene Analysis

Whole venous blood samples from all the subjects were applied to filter paper (CentoCards®, Centogene, Germany) containing circles, which were filled with 50 μL of whole blood, left to dry at room temperature for 2 h, covered, and

Table 1 Demographic, clinical, genetic, and laboratory characteristics of the subjects included in the study

Subject - Family	Age (Y)	Gender	C4 mg/dL	C1-INH mg/dL	C1-INH function (%)	# episodes/month	Treatment	Identified mutation	Ref	Type of disease
1 – A	21	M	ND	ND	ND	Asymptomatic	–	None	–	–
2 - A	44	M	ND	ND	ND	Asymptomatic	–	None	–	–
3 – A	21	M	ND	ND	ND	Asymptomatic	–	None	–	–
4 –A	1	F	ND	ND	ND	Asymptomatic	–	None	–	–
5- A	2	F	ND	ND	ND	Asymptomatic	–	None	–	–
6 – A	62	F	8	10	30	6	Ica, pdC1-INH	c.1081C>T (p.Gln361*)	[12]	*HAE* 1
7 – A	37	F	ND	ND	ND	Asymptomatic	–	None	–	–
8 – A	47	F	ND	ND	ND	Asymptomatic	–	None	–	–
9 – A	70	F	8	8	28	4	D, pdC1-INH	c.1081C>T (p.Gln361*)	[12]	*HAE* 1
10 – A	5	F	7	8	15	Asymptomatic	NT	c.1081C>T (p.Gln361*)	[12]	*HAE* 1
11- A	52	F	6	9	22	4	AT, Ica	c.1081C>T (p.Gln361*)	[12]	*HAE* 1
12 – A	31	F	3	7	23	6	Ica, pdC1-INH	c.1081C>T (p.Gln361*)	[12]	*HAE* 1
13 – A	73	F	5	8	3	6	Ica, D	c.1081C>T (p.Gln361*)	[12]	*HAE* 1
14 – A	28	F	9	10	50	3	D	c.1081C>T (p.Gln361*)	[12]	*HAE* 1
15 – A	9	M	ND	ND	ND	Asymptomatic	–	None	–	–
16 – A	35	M	4	4	8	4	D, Ica	c.1081C>T (p.Gln361*)	[12]	*HAE* 1
17 – B	4	M	ND	ND	ND	–		None	–	–
18 – B	7	F	ND	ND	ND	–		None	–	–
19 – B	35	M	2	37	30	8	Ica, pdC1-INH	c.1396C>G (p.Arg466Gly), c.1029+84G>A	[13, 14]	*HAE* 2
20 – C	15	M	4	6	26	1[#]	NT	c.106_107del (p.Ser36Phefs*21)	[4]	*HAE* 1
21 – C	65	F	25	11	70	0.5	D	c.106_107del (p.Ser36Phefs*21)	[4]	*HAE* 1
22 - D	34	F	1	< 3	< 10	4	Ica	c.1081C>T (p.Gln361*)	[12]	*HAE* 1

Reference values for circulating C4 (10–40 mg/dL). Reference value of the circulating C1-INH (17–37 mg/dL). Reference values for functional C1-INH test (70–130%)

y years old, *ND* not done, *Ica* icatibant, *pdC1-INH* plasma-derived concentrate C1-INH, *D* danazol, *NT* no treatment, *HAE* hereditary angioedema

[#] Patient reported only one mild episode that occurred in May/2016 and did not need medical attention

then sent under appropriate conditions to external reference laboratory company (CENTOGEN, Rostock, Germany), for genetic analysis.

According to the external reference laboratory, the *SERPING1* gene was initially amplified by polymerase chain reaction (PCR). Then, the complete coding region, as well as the joint exon-intron highly conserved sequences in genomic DNA, was sequenced and analyzed. All the obtained sequences were compared to the *SERPING1* reference gene NM_000062.2. Mutations were confirmed by analyzing a second pared independent sample.

Results

Characteristics of the Included Subjects

In this study, 22 subjects from 4 different families were analyzed. As shown in supplementary Fig. 1, a clear autosomal dominant pattern was observed in the family tree of family A (most of the included patients belong to this family). Fourteen of the 22 subjects were female. The median (range) age of the subjects was 32.5 years (1–73) (Table 1). Clinical symptoms and laboratory tests (C4, C1-INH and functional C1-INH) compatible with HAE were reported for 11 subjects.

The treatment for this group of subjects is heterogeneous; some of them are controlled with danazol, whereas others require plasma-derived concentrate C1-INH (pdC1-INH) to reduce the frequency and severity of the symptoms and icatibant to control acute episodes (Table 1). Close to 50% of the symptomatic patients were receiving danazol at the time of the study. Although other efficient therapies are now available, the low cost of danazol allows this drug to be a local alternative. As also shown in Table 1, all patients expressing pathogenic mutation were usually highly symptomatic, except a young girl in whom no event has occurred at the moment of the study (patient 10-A).

Genotyping Analysis of SERPING1

The *SERPING1* gene was sequenced in all 22 subjects, and the resulting sequences were compared with a typified reference sequence (*SERPING1* reference gene NM_000062.2). As shown in Table 1, at least one type of mutation was found in 12 of the included subjects (54%).

Four different mutations were found in this Colombian cohort (Table 1); three of them were pathogenic (c.1081C>T [p.Gln361*], c.1396C>G [p.Arg466Gly], and c.106_107del [p.Ser36Phefs*21]), and the remaining was recognized as a variant of uncertain significance (VUS) (c.1029+84G>A). The previous reported pathogenic variant c.1081C>T (p.Gln361*) was the most frequent within this study, as two unrelated families (family A and family D) were found to have this mutation (Table 1).

Members analyzed from family C were affected by a deletion in exon 3 (c.106_107 del) that produced a frameshift p.Glu35fs alteration [4]; this mutation is classified as a pathogenic variant responsible of Type 1 C1-INH–HAE. As shown in Table 1, subjects with this variant had fewer and milder disease episodes compared with family A. One of these patients was well controlled with danazol and the other member from family C is still mildly symptomatic (Table 1).

Interestingly, one patient from family B (subject 19-B) expressed two mutations, including the pathogenic variant c.1396C>G (pArg466Gly) in exon 8 and a. C.1029+84G>A affecting intron 6 [13]. Of note, this last variant has been previously nominated to be a VUS. As shown in Table 1, this patient was highly symptomatic and suffered from 8 crisis episodes per month starting at 13 years of age. The patient's disease was characterized by edema affecting the face and airway-causing dyspnea, which frequently forces him to consult the emergency room. Despite these two variants, the patient's C1-INH concentrations remain around normal levels, whereas its function was low (only 30% of normal), as is expected for HAE type 2 (Table 1). Applications of pdC1-INH have been used to control his severe symptoms; additionally, the bradykinin B2 receptor antagonist (icatibant) is also being prescribed for acute episodes.

Discussion

In this study, we analyzed for the first time the *SERPING1* gene in 22 Colombian subjects from 4 non-related families, each one included patients clinical and laboratory compatible with HAE. We found 4 variants in this gene, all of which have been previously reported. The c.1081C>T (p.Gln361*), c.1396C>G (p.Arg466Gly), c.1029+84G>A, and c.106_107del (p.Ser36Phefs*21) variants were identified. Of note, two different mutations, c.1396C>G (p.Arg466Gly) and c.1029+84G>A were expressed in same patient (Table 1).

Deficiencies in complement proteins are considered rare among PID; however, more laboratory centers around the world are reporting regularly C1-INH–HAE cases and genotypes (HAEdb, hae.enzim.hu). A registry of patients with a C1-INH-HAE diagnosis has been established in Colombia, and approximately 50 patients have been included, but specific mutations in the *SERPING1* gene in the local patients have not been established yet [15].

As shown in Table 1, c.1081C>T (p.Gln361*) was the most frequently detected variant in symptomatic patients, as this variant affected members in A and D families. This mutation affecting exon 7 changes the codon for Gln361 to a termination codon (TAG), truncating C1-INH to 140 amino acids, which therefore affects protein expression [12].

In members of family C, another rare and previously reported variant was noted (Table 1). The c.106_107del (p.Ser36Phefs*21) variant is a 2-bp deletion that affects exon 3 resulting in a classical frameshift mutation. Similar to how that c.1081C>T (p.Gln361*) variant affects A and D families, the c.106_107del (p.Ser36Phefs*21) variant has been clinically associated with HEA type 1 [4].

Of note, one member (child) in family A as well as another (adolescent) in family C who are currently asymptomatic and mildly symptomatic, respectively, were detected as carriers of their family's respective mutations (Table 1). Thus, is well-known that other factors, such as stress, hormonal changes, and diseases, can influence expressed phenotype in different patients [2].

One patient in family B (19-B, Table 1), had the following two mutations: (i) c.1396C>G (p.Arg466Gly) and ii. c.1029+84G>A. The first of them is considered a pathogenic variant in exon 8, which affects the C1-INH reactive center and is classically associated with type 2 C1-INH—HAE [13]. The second one is a variant considered as a VUS, which might generate a splicing defect as it is located 82 bp from a consensus GT site as has been reported earlier [14] (Table 1). This patient was the most clinically affected to date, as he suffers from 8 edema events per month (Table 1). Therefore, the synergic effects of both mutations on the disease phenotype could be considered. Additionally, mutations in intron 6, such as rs2511989, have also been related to diseases, such as macular degeneration, since *SERPING1* mRNA expression is also found in retina and choroid layers in the eyes [16]. However, eye defects were not observed in this patient.

Few reports have been published focused on *SERPING1* gene mutations in Latin America. Recently, 5 new mutations were found in 30 subjects from 16 unrelated families in Brazil [17], and none of the mutations reported are similar to the ones found here. The reasons could be the different genetic backgrounds among Brazilian and Colombian populations. Strikingly, one report in individuals from Spain affected by C1–INH-HAE has shown the same pathogenic variant found in subject 19-B [18].

In summary, four different mutations that have been previously reported were found in patients suffering from C1-INH-HAE types 1 and 2 in Neiva, Southern Colombia. To our knowledge, this is the first report focused on genetic alterations in HAE patients in Colombia. Hopefully, these results will contribute to the design of strategies for the better recognition of HAE in Colombia and will help provide all patients with better and more accessible local treatment.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest

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