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Article type : Letter to the Editor

Editor : María José Torres

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Genotype-phenotype correlations in Brazilian patients with Hereditary Angioedema due to C1-inhibitor deficiency

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Short title: **Genotype-phenotype correlations in Hereditary Angioedema**

Key words: Bradykinin, C1 Inhibitor, Complement, Hereditary angioedema, *SERPING1*

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To the Editor,

Hereditary Angioedema (HAE) is a rare disease with autosomal dominant inheritance that affects 1 in every 50,000 individuals.[1] Patients with HAE present recurrent episodes of edema of subcutaneous tissue and submucosa that mainly affects the skin, gastrointestinal tract, and upper airways. In most cases, the disease results from the deficiency of C1 inhibitor (C1-INH) owing to mutations in *SERPING1,* the gene encoding C1-INH protein. The decrease in C1-INH activity leads to uncontrolled activation of the kallikrein-kinin system and increased formation of bradykinin, resulting in angioedema. HAE with normal C1-INH has also been characterized.[2] Diagnosis of C1-INH-HAE is based on clinical symptoms, positive family history, low levels and/or functional activity of C1-INH, and decreased C4.[1] Whether genetic analysis should be performed in routine clinical practice is yet debated. Variability in clinical presentation of HAE has prompted researchers to look for novel biomarkers. Kaplan and Maas have recently discussed the role of potential biomarkers, including blood levels of bradykinin or the pentapeptide Arg–Pro–Pro–Gly–Phe derived from bradykinin degradation and cleaved High Molecular Weight Kininogen, in assessing HAE severity and response to therapeutic agents.[3] The type of mutation in *SERPING1* could account for clinical phenotypes. Patients with missense mutations have been shown to present symptoms at later ages, milder clinical course, and lesser need for prophylactic medications than those with mutations that cause more profound changes in the molecule.[4,5] Grouping patients with missense mutations in *SERPING1* affecting Arg466 at the reactive center with those carrying mutations leading to more significant changes in C1-INH molecule revealed association with more severe disease.[6,7] In the present study, we aimed to identify and characterize mutations in *SERPING1* in Brazilian patients with C1-INH-HAE and investigate the impact of the type of mutation on clinical features of the disease.

Sixty patients with C1-INH-HAE from 16 distinct families were characterized based on mutations in *SERPING1*. Diagnosis was established according to consensus criteria (Supporting Information, Data S1).[1] One of the families (Family 7) has been previously reported by our group; we provide follow-up information, including five new members with HAE-1.[8] Patients were divided into the following groups: group 1 comprising patients with deletion, insertion, duplication, or nonsense mutation and mutations affecting Arg466 at the reactive center (n = 48); and group 2 comprising patients with missense mutations, with the exception of mutations at Arg466 (n = 12). The rationale for dividing patients into these groups was the fact that mutations causing more profound alterations in the structure of the protein could lead to more severe disease, as previously reported.[6,7]

Genomic DNA was extracted from whole blood or oral mucosa material using the DNA Wizard Genomic DNA Purification Kit (Promega, Madison, WI). PCR was conducted using *SERPING1* primers (Data S2, Table S1), and DNA sequences were analyzed using the SeqMan software™ (Lasergene; DNA Star, Inc., Madison, WI). Sequence variations were identified by comparison with GenBank accession number NM_000062.2, X54486. Protein effect was defined using the mature protein sequence, including signal peptide. Criteria of the American College of Medical Genetics and Genomics (ACMG) were applied for classifying sequence variants. Predicted functional analysis of missense mutations was performed using the bioinformatics tools SIFT (http://sift.jcvi.org/www/SIFT_enst_submit.html), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) and MutationTaster, which could also assess nonsense mutations (http://www.mutationtaster.org/). Two proposed scores, though non-validated, were applied to classify the disease severity.[8,9] Statistical analysis is described in Data S3. For regression models, a random effect was introduced to consider variability in the individuals from the same family.

Among the 16 index patients, information on family members was available for 14 families (Figure S1). Sixty patients (33F:27M), from 10 months to 79 years of age, were diagnosed with C1-INH-HAE; of these, 55 were symptomatic. Fifty-eight patients presented HAE-1, whereas two had

HAE-2 (Table S2). Time from onset of symptoms to diagnosis of HAE was examined in Family 7, comprising 33 patients carrying the c.351delC mutation (Figure S2). Mean time for diagnosis was 15.7 years (SD 13.3 years) and 1.06 years (SD 7.03 years) in the groups of patients receiving care before and after genetic testing became available, respectively ($p < 0.01$).

A total of 205 individuals (110F:95M) underwent genetic analysis. *SERPING1* mutations included 10 missense and 6 non-missense mutations (structural mutations leading to frameshift or nonsense mutations). Six mutations were novel and two were *de novo* mutations (Table 1). According to ACMG guidelines, 15/16 variants were classified as pathogenic and 1/16 as likely pathogenic. With SIFT, 9/10 missense mutations were characterized as damaging, while PolyPhen-2 characterized 7/10 missense mutations as probably damaging. MutationTaster deemed 10/11 missense/nonsense mutations as disease-causing (data not shown). Clinical parameters and severity of disease were not significantly different among patients from groups 1 and 2 (Table 2).

Patients with C1-INH-HAE from our cohort presented similar clinical features as well as some common genetic alterations previously reported in patients from other areas. Although Brazilian population is a mixture of native, European, and African ancestry, European ancestry is predominant, ranging from 60.6% to 77.7% (Pena et al. 2011, Supporting Information). The mean age of 14.3 years at onset of symptoms was in agreement with previous reports.[1] The majority of families presented HAE-1; only 12.5% presented HAE-2. The majority of our patients (91.6%) were symptomatic, and 81% required prophylactic treatment at a rate higher than that previously reported,[6] owing to the limited availability of the specific on-demand HAE medications in Brazil. Common symptoms were angioedema of subcutaneous and submucosal tissues, with the frequent involvement of face and lips, and abdominal pain attacks, in accordance with published studies.[1] Frequency of *de novo* mutations was slightly lower in the present study (12.5%) than that previously reported (approximately 25%). These results indicate that Brazilian patients with C1-INH-HAE share common features with those from other areas, allowing for genotype-phenotype comparisons.

Our results showed that clinical severity of C1-INH-HAE may not be strictly related to the type of mutation. On the other hand, genetic testing was helpful in substantially decreasing the time for diagnosis to allow precise diagnosis, which was key to dealing with concerns and providing guidance. Moreover, recent reports using next-generation sequencing technologies for C1-INH-HAE diagnosis point to a breakthrough strategy in clinical practice. Our approach is in keeping with the current consensus recommendation of testing children from C1-INH-HAE-affected families as soon as possible, ideally before the onset of clinical manifestations[1].

In conclusion, our results suggest that factors including epigenetic elements and gene-gene or gene-environment interactions may play a role in shaping the clinical course of C1-INH-HAE. Although our study may be limited by the small number of participating families, it has the strength that patients were well characterized clinically and followed over time by physicians experienced with diagnosis and management of HAE, allowing for accurate evaluation of severity. Furthermore, our data add contributions on genetic and clinical aspects of C1-INH-HAE to the few reports in patients from Latin America. Our clinical experience with families with C1-INH-HAE prompts us to suggest that genetic testing should be considered a valuable tool for improving diagnostic accuracy and, ultimately, excellence of care for patients with HAE.

Table 1. Characteristics of *SERPING1* mutations identified in patients with C1-INH-HAE

Location (exon)	cDNA numbering (CDS)	Protein effect (HUGO)	Type of mutation	Classification according to ACMS[†]	Reference
3	c. 249delT	p.Asp84Metfs*64	Deletion	Pathogenic	Cimbollek S and Garcia-Lozano R – HAEdbase
3	**c.314_317dupCAAC**	**p.Thr107Asnfs*27**	**Duplication**	**Pathogenic**	**Novel**
3	c.351delC	p. Pro117fsx	Deletion	Pathogenic	Ferraro et al. 2011[8]
3	c.550G>A	p.Gly184Arg	Missense	Pathogenic	Verpy et al. 1996
5	c.689T>C	p.Leu230Pro	Missense	Pathogenic	Xu et al. 2012
5	c.728T>C	p.Leu243Pro	Missense	Pathogenic	Pappalardo et al. 2008
5	**c.730T>G**	**p.Tyr244Asp**	**Missense**	**Likely pathogenic**	**Novel**
5	**c.871A>T**	**p.Asn291Tyr**	**Missense**	**Pathogenic**	**Novel**
6	**c.939T>G**	**p.Phe313Leu**	**Missense**	**Pathogenic**	**Novel, described at protein level Siddique et al. 1995**
6	**c.995delT**	**p.Val332Glyfs*9**	**Deletion**	**Pathogenic**	**Novel**
6	c.1010A>G	p.Asp337Gly	Missense	Pathogenic	Pappalardo et al. 2008
8	**c.1334_1335insA**	**p.Val446Serfs*27**	**Insertion**	**Pathogenic**	**Novel**
8	c.1396C>T	p.Arg466Cys	Missense[‡]	Pathogenic	Skriver et al. 1989
8	c.1397G>A	p.Arg466His	Missense[‡]	Pathogenic	Skriver et al. 1989
8	c.1431C>G	p.Phe477Leu	Missense	Pathogenic	Guarino et. al.
8	c.1480C>T	p.Arg494*	Nonsense	Pathogenic	Verpy et al. 1995

[†]Classification was performed according to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines.

[‡]Missense mutations at Arg466 were included within Group 1 together with deletion, insertion, duplication, and nonsense mutation for the analysis of genotype-phenotype correlations.

Novel mutations are marked in bold. *De novo* mutations were c. 939T>G (Family 17) and c.689T>C (Family 28), identified among patients without family history of HAE and confirmed by the absence of mutation upon sequencing of the parental DNA.

Full references are presented as online Supporting Information.

Table 2. Genotype-phenotype associations among patients with C1-INH-HAE according to the type of mutations

		Group 1[†] n = 48	Group 2[‡] n = 12
Age median, range (year)		33.0 (1-79)	30.5 (3-52)
Age at onset of symptoms median, range (year)[§]		14.0 (0.2-46)	13.5 (1-19)
Gender	F	26/48 (54.2%)	7/12 (58.3%)
	M	22/48 (45.8%)	5/12 (41.7%)
Estrogen sensitivity[¶]	Yes	15/24 (62.5%)	4/7 (57.1%)
	No	9/24 (37.5 %)	3/7 (42.9%)
Prophylaxis	Yes	37/45 (82.2%)	8/10 (80%)
	No	8/45 (17.8%)	2/10 (20%)
Angioedema sites			
Subcutaneous edema		41/45 (91.1%)	10/10 (100%)
Abdominal attacks		42/45 (93.3%)	9/10 (90%)
Facial swelling		34/45 (75.5%)	8/10 (80%)
Laryngeal attacks		24/45 (53.3%)	6/10 (60%)
Severity score by Ferraro et al.[8#]	Asymptomatic	3/48 (6.2%)	2/12 (16.6%)
	Mild	7/48 (14.5%)	1/12 (8.3%)
	Moderate	8/48 (16.6%)	1/12 (8.3%)
	Severe	30/48 (62.5%)	8/12 (66.6%)
Severity score by Bygum et al.[9], median[#]		6.0	7.0

[†]Group 1: Deletion, insertion, duplication, or nonsense mutation and mutations affecting Arg466 at the reactive center in exon 8.

[‡]Group 2: All missense mutations with the exception of those at Arg466.

[§]Forty-five and ten symptomatic patients in groups 1 and 2, respectively. Five C1-INH-HAE patients were asymptomatic.

[¶]Estrogen sensitivity was defined as triggering and/or worsening of symptoms in association with

estrogen exposure owing to the use of an estrogen-containing oral contraceptive or a hormone replacement therapy, or during pregnancy, among female patients only.

[#]In the score by Ferraro et al.[8], disease severity is classified based on symptom frequency and intensity. In the score by Bygum et al.[9], the parameters used are age at disease onset, clinical manifestations, and need for long-term prophylaxis, with values ranging from 0 to 10, the latter being the most severe clinical presentation.

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Funding sources

This study was supported by the São Paulo Research Foundation (FAPESP) and the Institute of Investigation in Immunology, National Institutes of Science and Technology, Brazilian National Council for Scientific and Technological Development (iii-INCT-CNPq).

Acknowledgements

We thank Janaina Michelle Lima Melo, MD, PhD and Thais Mendonça Nociti, MD for helping with the care of the HAE patients; Wagner Narciso de Campos, PhD for his assistance with the sequencing analysis, and Luciana Rodrigues Roberti, BSc for her technical assistance. We are particularly grateful to the São Paulo Research Foundation (FAPESP) for issuing a Doctoral scholarship to Luana Sella Motta Maia, MSc, which includes support for her current research at Prof. Sven Cichon's laboratory in Basel, Switzerland (BEPE Program). We also thank the Brazilian Association of Hereditary Angioedema (ABRANGHE) for referring patients to our study.

Conflict of Interests

LSM Maia is a recipient of a Doctoral scholarship from the São Paulo Research Foundation (FAPESP) (grant numbers 2014/26693-5 and 2017/18669-5). She also received research support from the Coordination of Superior Level Staff Improvement (CAPES) and the Brazilian National Council for Scientific and Technological Development (CNPq), in addition to receiving travel support from Shire.

AS Moreno is a recipient of Post-Doctoral Fellowships from the Coordination of Superior Level Staff Improvement (CAPES) and São Paulo Research Foundation (FAPESP grant number 2011/23439-2). She also received travel support from Shire.

MPL Ferriani has received lecture fees from Shire.

FL Nunes has received lecture fees from Shire, and is a recipient of an Investigator-Initiated Research Grant from Shire.

P Roxo-Junior has received lecture fees from Shire and CSL Behring.

SOR Valle is on the board for Novartis, Sanofi, and CSL Behring, and has received lecture fees from Novartis and Takeda.

FS Serpa has received travel support and lecture fees from Shire, Novartis, and AstraZeneca.

LK Arruda is a recipient of a Brazilian National Council for Scientific and Technological Development (CNPq) Research Productivity Grant, and has received travel support and lecture fees from Shire, Novartis, and Sanofi.

MF Ferraro; MM Dias; FC Dias; S Levy; MLO Alonso; AT França; AA Motta ; WA Silva Jr.; W Sarti; DC Aragon; FGM Maia; S Cichon; and K Bork have no relevant conflict of interests to declare.

Author Contributions

LMS Maia participated in acquisition of patients' data, conducted the sequencing experiments and analysis, and prepared the data for the manuscript.

LK Arruda and AS Moreno designed the study, analyzed the data, prepared the first draft of the manuscript and revised the manuscript to its final version.

MPL Ferriani, FL Nunes, MF Ferraro, P Roxo Jr, SOR Valle, S Levy, MLO Alonso, A França, FS Serpa, AA Motta and W Sarti contributed with recruitment of patients and acquisition of patients' data. MPL Ferriani prepared the complete set of clinical data for analysis and critically revised the manuscript. MM Dias, FC Dias and FGM Maia assisted with acquisition and analysis of sequencing data. WA Silva Jr. supervised the final analysis of sequencing data, and critically revised the manuscript. DC Aragon conducted the statistical analysis. S Cichon and K Bork contributed to the interpretation of the data and critically revised the manuscript.

All authors contributed to the interpretation of the data, revised the manuscript and approved the final version.