Brief Report

Whole Exome Sequencing in Acquired Angioedema by Angiotensin-Converting Enzyme Inhibitors: A Pilot Study in Five Patients

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Whole Exome Sequencing in Acquired Angioedema by Angiotensin-Converting Enzyme Inhibitors: A Pilot Study in Five Patients

Running title: Utility of the WES in AAE-IECAs

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Abstract: Background/Objectives: One of the most common causes of bradykinergic angioedema (Bk-AE) is related to reduced Bk breakdown after the use of certain medications. This is the case of forms of Acquired Angioedema (AAE) due to the use of angiotensin-converting enzyme inhibitors (ACEi), which are used for the treatment of cardiovascular conditions. The AE causes is not clear in these patients. Given the limited number of AAE-ACEi genetic loci identified by genome-wide association studies, here we opted for assessing the utility of NGS of a panel of relevant genes to aim to identify candidate genetic risk factors in severely affected patients. Methods: Five Hypertensive patients from unrelated families with clinical AAE-ACEi were included in the study. Whole-exome sequencing, variant calling, and annotation techniques were used. ANNOVAR was used to annotate the variant calls . These variant were processed for each patient by Hereditary Angioedema Database Annotation tool and Franklin genomic platform for variant prioritization and clinical impact interpretation. Results: The genetic variant rs6025 in F5 gene was identified in all recruited samples, which has been associated with an increase in blood clotting in AAE-ACEi patients. In two patients, a common synonymous genetic variant of ACE gene was found (rs4343). Finally, we identified the ACE genetic variant rs142947404 in only one patient. This variant has not been assessed in AAE-ACEi. Conclusions: More studies will be needed to clarify the genetics involved in acquired forms of Bk-AE. In this way, we will be able to try to predict future episodes of angioedema due to use of ACEi.

Keywords: acquired angioedema; bradykinin; ACEi; NGS; exome

Introduction

Bradykinin (Bk)-induced angioedema (Bk-AE) is a medical condition often associated with reduced Bk degradation following the use of specific medications. A prominent example is acquired angioedema (AAE) triggered by angiotensin-converting enzyme inhibitors (ACEi) used as a treatment for cardiovascular conditions, referred to as AAE-ACEi. These medications interfere with the function of angiotensin-converting enzyme, leading to an excessive accumulation of Bk in some

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patients.[1] AAE-ACEi symptoms can appear at any point during treatment, even after years of otherwise safe use.

Genetic testing in Bk-AE mostly focuses on rare hereditary forms, relying on conventional methods such as Sanger sequencing, Multiplex Ligation-dependent Probe Amplification (MLPA), and PCR. However, these genetic tests are rapidly evolving towards the use of Next-Generation Sequencing (NGS), enabling highly efficient and holistic high-throughput genetic assessments. This transformation has paved the way for the identification of disease-causing variants, even in conditions with small genetic contributions. We have exposed the nuances of Bk-AE, shedding light on the acquired forms, their connection to medication use, and the evolving methods for genetic testing, including Whole Exome Sequencing (WES), as a powerful transformative tool to better understand and precisely manage this multifaceted condition in the patients.[1] Given the limited number of AAE-ACEi genetic loci identified by genome-wide association studies, here we opted for assessing the utility of NGS of a panel of relevant genes to aim to identify candidate genetic risk factors in severely affected patients.

Materials and Methods

Patient description

Five Hypertensive patients from unrelated families with clinical AAE-ACEi suspicion residing in the Canary Islands were included in the study (**Table 1**). The most commonly used drug was enalapril. These patients maintained treatment with ACEi for more than 15 months. The patients had, at least, one life-threatening episode of angioedema. The oropharynx was the most common location. All patients required assistance in the critical care unit. Complement studies were normal in all patients.

Table 1. Patients data collection.

ID	Sex	Age	C4 mg/dl	C1q mg/dl	Drug	HTA Diagnosis	Onset of ACEI	Onset of AE (months)	Localization
AM_2450	Male	64	25,5	18	Enalapril	2014	2014	47	Oropharynx
AM_2419	Male	43	26,1	19	Enalapril	2016	2016	27	Oropharynx
AM_2498	Male	62	24,3	21	Enalapril	2010	2010	82	Oropharynx, lips
AM_2569	Male	49	28	17	Ramipril	2019	2020	16	Oropharynx, lips
AM_2712	Female	90	27,2	ND	Perindopril	2010	2012	78	Oropharynx

ID: Identification number; ND: No data; ACEI: Angiotensin-converting enzyme inhibitors; AE: Angioedema.

Whole-exome sequencing, variant calling, and annotation

DNA was extracted from 4 mL of peripheral blood with Illustra™ blood genomicPrep kit (GE Healthcare; Chicago, IL). DNA concentration was evaluated using the dsDNA BroadRange Assay Kit for the Qubit® 3.0 Fluorometer (Termo Fisher Scientific, Waltham, MA). Libraries were prepared using the DNA Prep with Exome 2.0 Plus Enrichment Kit (Illumina, San Francisco, CA), with fragment sizes and concentrations assessed on a TapeStation 4200 (Agilent Technologies, Santa Clara, CA) and sequencing obtained with a NovaSeq 6000 Sequencing System (Illumina, San Francisco, CA) with paired-end 101-base reads. PhiX was loaded and sequenced at 1% as an internal control of the experiments.

Sequencing reads were preprocessed with bcl2fastq v2.18 and mapped to hg19/GRCh37 reference genome with Burrows-Wheeler Aligner v0.7.15, [2] and BAM files were processed with Qualimap v2.2.1, [3] SAMtools v1.3, [4] BEDTools, [5] and Picard v2.10.10 (http://broadinstitute.github.io/picard) for quality control steps. Variant calling of small germline

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variants was performed using the Genome Analysis Toolkit (GATK) v.3.8 for the detection of nucleotide substitutions (SNVs) and small indels (<50 bp) following the best practices. [6] The pipeline description is publicly available (https://github.com/genomicsITER/benchmarking/tree/master/WES).

The identified genetic variation was filtered by means of SAMtools and VCFtools based on "PASS" filter, depth of coverage per position (≥ 20×), genotype quality (≥ 100), and mapping quality (≥ 50).

ANNOVAR [7] was used to annotate the variant calls by including the allele frequency in reference populations, gene location, known functional consequences, links with disease based on ClinVar [8] and The Human Gene Mutation Database, [9] and several pathogenicity scores including the Combined Annotation-Dependent Depletion (CADD) in the context of the Mutation Significance Cutoff (MSC), among others. The classification of pathogenic potential of variants was obtained and annotated using InterVar software following the American College of Medical Genetics and Genomics (ACMG) guidelines. [10]

The analysis was carried out at the Teide-HPC Supercomputing facility (http://teidehpc.iter.es/en).

Prioritization of potential deleterious variants

The annotated variant calls were processed for each patient by Hereditary Angioedema (HAE) Database Annotation tool (HADA, http://hada.hpc.iter.es/), to rule out undiagnosed HAE. This tool facilitates the identification of the variants affecting function as well as other accompanying information from the literature. [11]

We used the Franklin genomic platform for variant prioritization and clinical impact interpretation as a second tier for identifying genetic factors that could be responsible of AAE-ACEi symptoms. For this approach, we extracted from scientific literature the most common affected genes in AAE-ACEi and designed a virtual gene panel composed by *ACE*, *BDKRB2*, *XPEPNP2*, *MME*, *F5*, *ETV6*, *DENND1B*, and *CRB1*. [12] This assessment was done combining the search with a list of phenotypic abnormalities related with Bk-AE (HP:0100665, HP:0025018, HP:0100540, HP:0002098, HP:0040315, HP:0031244, HP:0010783, HP:0002781, HP:0012027, HP:0011855).

Patient population and sequencing summary

WES of the five patients yielded an average of 6.58 Gb sequence, with an average of 100% of on-target reads and a median depth of 139.8×, and a transition/transversion ratio in the range of the expected (3.1 to 3.3).

Results and Discussion

HADA did not reveal previously reported HAE causal variants present in these patients, thus reducing the possibility that the symptoms could be due to an undiagnosed HAE. Franklin was able to prioritize interesting likely deleterious variants (**Table 2**).

Table 2. Prioritized genetic variants in patients with AAE-ACEi.

Individual ID	Gene	RS ID	Chr	Position start-end	Reference allele	Alternative allele	Total depth (ref/alt)	HGVS coding	HGVS protein	ACMG class	ACMG criteria	GnomAD	CADD Phred score	MSC 95% HGMD	Predicted effect*
AM_2419	*F5*	rs6025	1	169,519,049 - 169,519,049	C	T	156 (0 / 156)	c.1601C>T	p.Gln534Arg	Likely pathogenic	PM1 (moderate), PM5 (moderate), PM2 (supporting)	Absent	0.255	12.286	Increased blood clotting[13]
AM_2450							124 (0 / 124)								
AM_2498							145 (75 / 70)								
AM_2569							121 (0 / 121)								
AM_2712							125 (0 / 125)								
AM_2419	*CRB1*	*rs2786098*	1	197,325,908 -	T	G	101 (0 / 101)			Benign	BA1 (stan	0.7967	0.446	11.653	Higher risk of

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AM_2450			197,325,908			117 (0 / 117)								ACEi induced angioedema[12]
AM_2498						96 (1 / 95)								
AM_2569						87 (47 / 40)	c.989-53T>G	none (intronic)						
AM_2712						102 (0 / 102)								
AM_2498						213 (138 / 75)								
AM_2712	*ACE*	rs4343	17	61,566,031 - 61,566,031	G	A	206 (136 / 70)	c.2328G>A	p.Thr776=	Benign	BA1 (standalone), BP6 (very strong), BP4 (strong), BP7 (supporting)	0.4727	0.083	0.177	Increased ACE activity[14]
AM_2450	*ACE*	rs142947404	17	61,570,992 - 61,570,992	C	A	88 (39 / 49)	c.3108C>A	p.Asn1036Lys	Uncertain significance	BP4 (strong), BP1 (supporting), PM2 (supporting)	0.0009597	22.7	0.177	Not reported in the scientific literature[15]

ACMG: American College of Medical Genetics and Genomics; CADD: Combined Annotation Dependent Depletion; GnomAD: Genome Aggregation Database; HGDM: Human Gene Mutation Database; HGVS: Human Genome Variation Society; MSC: Mutation Significance Cutoff.

The genetic variant rs6025 in *F5* gene was identified in all recruited samples, which has been associated with an increase in blood clotting in AAE-ACEi patients. [13] Franklin also prioritized the intronic variant rs2786098 in *CRB1* gene, likely due to the association between the increased risk of angioedema attacks and ACEi intake in the ONTARGET dataset.[12]

In two patients, AM_2498 and AM_2712, a common synonymous genetic variant of *ACE* gene was found (rs4343). This variant was previously assessed to determine the association with AAE-ACEi, specifically with captopril. [14] In addition, *ACE* gene is one of the mainly components that inactivate the Bk activity. However, this variant has not been associated with AAE-ACEi, and the different pathogenic scores and allele frequency support it as benign.

Finally, we identified the *ACE* genetic variant rs142947404 in AM_2450. This variant has not been assessed in AAE-ACEi despite it is included in ClinVar as variant of uncertain significance. [15] Some of the prediction scores such as SIFT and PROVEAN suggest a deleterious effect. The allele frequency in European populations is <0.1%.

More studies will be needed to clarify the genetics involved in acquired forms of Bk-AE. In this way, we will be able to try to predict future episodes of angioedema due to use of ACEi.

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Author Contributions: AMA, IMR, and ACa wrote the first draft of the manuscript and designed the table. AMA and IMR performed the statistical analysis. JAMT, EPR, JBR, and ACa contributed to the sample and clinical data collection. JMLS, ACo, and RGM performed the experiments. CF, ACa, and JCGR designed the project. CF, ACa, and IMR obtained funding. All the authors revised and approved the final version of the manuscript.

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Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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