Angioedema-Induced by Nonsteroidal Anti-inflammatory Drugs: A Genotype-Phenotype Correlation in A Brazilian Population

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are the leading cause of hypersensitivity reactions in Latin America, both in adults and children. Among the different types of NSAID-induced hypersensitivity reactions, urticaria and angioedema are the most common [1]. These generally take the form of wheals and/or angioedema in healthy patients after at least 2 NSAIDs with a different chemical structure on separate occasions. Interestingly, some of these patients only present angioedema. The mechanism involved in these reactions is unclear, although it is likely associated with inhibition of cyclooxygenase (COX), which affects the metabolism of arachidonic acid [2]. Different gene polymorphisms have been associated with NSAID-induced hypersensitivity, including those related to prostaglandin, leukotriene, $Ca2^{+}$, cAMP, and P53 signaling pathways [3]. Therefore, in this study, we used whole-exome sequencing (WES) to detect genetic markers that determine a hypersensitivity response in patients with NSAID-induced isolated angioedema (NIA).

The local ethics committee approved the study. Four unrelated patients with NIA and their parents were selected and agreed to participate in the study. Clinical data were collected using a specific questionnaire (Supplementary Table 1) [4]. In this study, hypersensitivity to NSAIDs was characterized according to the criteria of Laidlaw and Cahill [5], and patients with a history of angioedema within 6 hours of taking 2 different NSAIDs in at least 2 different episodes were selected. Angioedema affected the eyelid in all cases, although 2 individuals also reported lip angioedema. While the location of angioedema does not seem to have a direct relationship with the mechanism of reaction, eyelid involvement in all of the cases studied indicated a homogeneous clinical profile and

appears to be the most frequent pattern in angioedema related to NSAIDs. All the patients had positive skin test or in vitro IgE results to house dust mite.

WES was performed on the Ion ProtonTM platform according to the manufacturer's recommendations. DNA samples were extracted from collected peripheral blood using the QIAmp DNA Blood Mini Kit (Qiagen), and sequencing was performed using the Ion PITM Chip v3 at Ion ProtonTM Sequencer. Sequencing data were analyzed in Torrent Suite v5.x.x, and all sequenced reads were mapped to human genome reference (hg19/GRC37). WES was assessed in terms of its quality to ensure that the data obtained could be used for the genotype-phenotype association proposed (Supplementary Table 2). Nine genes related to the COX and 5-LO signaling pathways (*ALOX5, PTGS2, CYSLTR1, CYSLTR2, LTC4S, PTGER1, PTGER2, TBXA2R, TBXAS1*) were filtered in the VCF file according to variant segregation analysis in order to propose the genotype-phenotype association. A cohort analysis was performed in PhenoDB (https://phenodb.org), and files obtained by each approach were then filtered based on Annovar annotations and multiple computational pathogenicity predictors. Variants were analyzed on Integrative Genomics Viewer (IGV ver. 2.3.92, Broad Institute) to evaluate alignment and variant calling. Variants presenting low mapping quality and unbalanced alleles (lower than 25% for each allele) were ruled out. The remaining variants were then prioritized based on their relevance to the studied phenotype.

The cohort analysis aimed to determine a causative gene shared by at least 50% of the families studied. Each mode of inheritance was combined to generate 2 reports, one for autosomal dominance and another for autosomal recessiveness (Table). Loss of function variants found in each mode of inheritance in all 4 families are available in the supplementary material (Supplementary Tables 3-6). Both analyses return no genes mutated in at least 2 families.

Using WES, we analyzed all the genes involved in the arachidonic acid pathway. In a study comprising 15 genes related to the arachidonic acid pathway, Cornejo-Garcia et al [6] observed a positive association for the *ALOX5AP, ALOX15, PTGDR, PTGER1, PTGER2*, and *CYSLTR1* genes in the Spanish population. However, the segregation analysis in our cohort of COX/5-LO pathway genes revealed no variant of biological relevance (Supplementary Table 7).

Table. Variants Found in Each Mode of Inheritance

Family	AD	AR-H	AR-CH
		Total Variants (Final)[a]	
1	158 (33)	99 (17)	160 (10)
2	241 (64)	45 (9)	140 (23)
3	153 (37)	39 (4)	128 (28)
4	283 (55)	64 (14)	147 (24)

Abbreviations: AD, autosomal dominant; AR-H, autosomal recessive homozygous; AR-CH, autosomal recessive compound heterozygous.
[a]Total of variants found in each mode of inheritance before filtering. Final variants found before filtering are shown in parentheses.

It is still unknown why certain individuals manifest urticaria while others have angioedema or anaphylaxis, or even why the manifestations are different in each episode in the same patient. It is even more intriguing that some patients react to some NSAIDs, but not to others of similar potency [7]. It might be that COX inhibition is part of the mechanism, but not a major factor.

One of the advantages of WES is the possibility of simultaneous evaluation of different polymorphisms, such as those related to mast cell activation, histamine release, and IgE receptors. In the only genetic study performed in a Brazilian population with nonselective hypersensitivity to NSAIDs, an association with a higher risk of reaction was found for the IL10 gene, whereas an association with a protection factor was found for the DAO gene [8]. We found no relevant variant associated with these genes.

Notwithstanding, 3 different mutations in MUC5B were found in families 1, 2, and 3, namely, c.13157_13158del, c.13245_13246insC, and c.13154dupC, respectively. These 3 mutations cause a frameshift change in protein sequence and are associated with pulmonary fibrosis (OMIM # 600770). Although MUC5B is recognized as one of the most mutation-tolerant genes in the human genome, it encodes a protein member of the mucin family, which is a major gel-forming mucin in mucus, contributing to the lubrication and viscoelasticity of whole saliva, normal lung mucus, and cervical mucus. This finding suggests the possible participation of other pathways in the pathophysiology of NIA.

Taken together, our data enable us to conclude that there is probably no single genetic marker of NIA in our cohort of patients. NIA is most likely determined by a combination of genetic alterations. Following this reasoning, large WES and genome sequencing studies will certainly help to determine and establish the variants that together build this complex trait. In addition, further approaches such as transcriptomics, proteomics, and metabolomics should be addressed to better explore the genetic footprints of hypersensitivity to NSAIDs. Furthermore, a better understanding of the NSAID signaling pathway and its relationship with other systems will probably clarify the specificity of NSAIDs and NIA.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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