Angioedema-induced by nonsteroidal anti-inflammatory drugs: a genotype-phenotype correlation in a Brazilian population

Short running title: Genotype-phenotype in NSAIDs induced angioedema

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0382
Non-steroidal anti-inflammatory drugs (NSAIDs) are the leading cause of hypersensitivity reactions in Latin America, both in adults and children. Among the different types of NSAIDs-induced hypersensitivity reactions, urticaria and angioedema are the most common. It is characterized by wheals and/or angioedema in healthy patients after taking at least two NSAIDs with a different chemical structure on different occasions. Interestingly, some of these patients will present only angioedema. Despite not totally clear, the mechanism involved in these reactions are likely to be related to cyclooxygenase (COX) inhibition, affecting the arachidonic acid metabolism. Different genes polymorphisms have been associated with NSAIDs induced hypersensitivity, including those related to prostaglandin, leukotriene, Ca2+, cAMP and P53 signaling pathways. Therefore, this study aimed to detect possible genetic markers that determine a hypersensitivity response in patients with NSAIDs induced isolated angioedema (NIA) by using the Whole Exome Sequencing (WES) approach.

The Ethic Committee of the institution 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 and is demonstrated in Table S1. In this study, NSAIDs hypersensitivity was characterized according to the criteria pointed out by Laidlaw and Calil (2017), and subjects with a history of angioedema within six hours after taking two different NSAIDs in at least two different episodes were selected. In our cohort, angioedema was observed in the eyelid region in all subjects, although two individuals also referred to lip angioedema. Although angioedema location apparently has no direct relationship with the mechanism of reaction, eyelid involvement in all cases studied characterized a homogeneously clinical profile and appears to be the most frequent pattern.
in angioedema cases related to NSAIDs. All the patients have positive skin tests or in vitro IgE to house dust mites.

WES was performed on Ion ProtonTM platform according to manufacturer recommendations. DNA samples were extracted from collected peripheral blood using the QIAmp DNA Blood Mini Kit (Qiagen), and sequencing occurred in the Ion P1TM 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 regarding its quality to assure that the obtained data could be used to the genotype-phenotype association proposed (Table S2). Nine genes related to the COX and 5-LO signaling pathways (ALOX5, PTGS2, CYSLTR1, CYSLTR2, LTC4S, PTGER1, PTGER2, TBXA2R, TXBAS1) were filtered in the VCF file according to the variant segregation in order to propose the genotype-phenotype association. A cohort analysis was performed in PhenoDB (https://phenodb.org), and files obtained in 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 allele (lower than 25% for each allele) were ruled out. Remaining variants were then prioritized based on their relevance to the studied phenotype.

The cohort analysis was aimed to determine a causative gene shared at least within 50% of the studied families. Each mode of inheritance was combined to generate two-analysis report, one for autosome dominance (AD) and another for autosome recessiveness (AR) (Table 1). Loss of function (LOF) variants found in each mode of inheritance in all the four families are available in the Supplementary material (supplementary material - Tables S3, S4, S5 and S6). Both analyses return no genes mutated in at least two families.

Using the WES we could analyze all the genes involved in arachidonic acid pathway. In a study comprising 15 genes related to the arachidonic acid pathway, Cornejo-Garcia et al. (2013) observed a positive association of 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 (Table S7).

It is still unknown why certain individuals manifest urticaria while others have angioedema or anaphylaxis, or even why the manifestations could be different in each episode in the same patient. More intriguing is to understand why some patients react to
some NSAIDs, but not to others of similar potency. It might be that COX inhibition is part of the mechanism, but not the most determinant.

One of the WES advantages is the possibility to evaluate different polymorphisms at the same time, as those related to mast cell activation, histamine release and IgE receptors. In the only genetic study performed in a Brazilian population with non-selective hypersensitivity by NSAIDs, an association with higher risk of reaction was found for the IL-10 gene, whereas an association with a protection factor was found for DAO gene. We found no relevant variant associated with these previous described genes.

Notwithstanding, three different mutations in MUC5B gene were found in families 1, 2 and 3: c.13157_13158del, c.13245_13246insC, and c.13154dupC, respectively. These three mutations cause frameshift change in protein sequence and are associated with pulmonary fibrosis (OMIM # 600770). Although MUC5B gene is recognized as one of the most mutation tolerated gene 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 viscoelastic of whole saliva, normal lung mucus and cervical mucus. This finding suggests the possible participation of other pathways in NIA pathophysiology.

Taking our data together, we can conclude that there is probably no unique genetic marker determining NIA in our cohort of patients. A combination of genetic alterations most likely determines NIA. Following this reasoning, large whole exome and genome sequencing studies will certainly contribute to determine and establish the variants that together build this complex trait. In addition, further approaches such as transcriptome, proteomics and metabolomics should be addressed to better explore the genetic footprints of NSAIDs hypersensitivity. Furthermore, a better understanding of NSAIDs signaling pathway and its relationship with other systems will probably enlighten the specificity of NSAID and NIA.

Acknowledgements

This work was supported by the São Paulo Research Foundation - FAPESP (grant number 2014/27198-8).
References


Table 1. Variants found in each mode of inheritance.

<table>
<thead>
<tr>
<th>Families</th>
<th>AD</th>
<th>AR-H</th>
<th>AR-CH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total variants (Final)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>158 (33)</td>
<td>99 (17)</td>
<td>160 (10)</td>
</tr>
<tr>
<td>2</td>
<td>241 (64)</td>
<td>45 (9)</td>
<td>140 (23)</td>
</tr>
<tr>
<td>3</td>
<td>153 (37)</td>
<td>39 (4)</td>
<td>128 (28)</td>
</tr>
<tr>
<td>4</td>
<td>283 (55)</td>
<td>64 (14)</td>
<td>147 (24)</td>
</tr>
</tbody>
</table>

AD – autosome dominant; AR-H – autosome recessive homozygous; AR-CH – autosome recessive compound heterozygous. *Total of variants found in each mode of inheritance before filtering. Final variants found before filtering present between brackets.