

DR JOSÉ ANTONIO CORNEJO-GARCÍA (Orcid ID : 0000-0003-3465-8735)

Article type : Original Article

Polymorphisms in eicosanoid-related biosynthesis enzymes associated with acute urticaria/angioedema induced by nonsteroidal anti-inflammatory drug hypersensitivity

Running title: NSAID-cutaneous hypersensitivity genetics.

R. Jurado-Escobar,^{1,2} I. Doña,^{1,3,4} J.R. Perkins,^{5,6,7} J.J. Laguna,^{4,8} R. Muñoz-Cano,^{4,9} A. García-Sánchez,^{4,10} P. Ayuso,^{4,11} M.J. Torres,^{1,2,3,4,12} C. Mayorga^{1,3,4,12} and J.A. Cornejo-García^{1,4}

¹Allergy Research Group, Instituto de Investigación Biomédica de Málaga-IBIMA, ARADyAL, Malaga, Spain.

²Departamento de Medicina, Universidad de Málaga, Malaga, Spain.

³Allergy Unit, Hospital Regional Universitario de Málaga, Malaga, Spain.

⁴ARADyAL Network. Instituto de Salud Carlos III, Madrid, Spain.

⁵Department of Molecular Biology and Biochemistry, University of Malaga, Malaga, Spain.

⁶CIBER de Enfermedades Raras (CIBERER), ISCIII, Madrid, Spain.

⁷The Biomedical Research Institute of Malaga (IBIMA), Malaga, Spain.

⁸Unidad de Alergia, Hospital Central de la Cruz Roja, Madrid, Spain.

⁹Allergy Section, Pneumology Department, Hospital Clinic, Universitat de Barcelona, ARADyAL, Barcelona, Spain.

¹⁰Department of Clinical Biochemistry, Pharmacogenetics Unit, University Hospital of Salamanca, Salamanca, Spain.

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: 10.1111/BJD.20440](#)

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¹¹Department of Pharmacology, University of Extremadura, Caceres, Spain

¹²Nanostructures for Diagnosing and Treatment of Allergic Diseases Laboratory, Andalusian Center for Nanomedicine and Biotechnology-BIONAND, Malaga, Spain.

Departamento de Medicina, Universidad de Málaga, Malaga, Spain.

Corresponding author: Dr. José A. Cornejo-García

Email: josea.cornejo@gmail.com, josea.cornejo@ibima.eu

Funding sources: This work was supported by Instituto de Salud Carlos III (ISCIII, Spanish Ministry of Science and Innovation), co-funded by Fondo Europeo de Desarrollo Regional-FEDER for the Thematic Networks and Co-operative Research Centres: ARADyAL (RD16/0006/0001) and Research Projects (PI17/01593 and PI20/01540), and from the Sociedad Española de Alergología e Inmunología Clínica (SEAIC; Ref. Convocatoria Ayudas 2016 and Convocatoria Ayudas 2018 Ref. 18 B02). R Jurado-Escobar holds a P-FIS PhD student grant (Ref FI18/00133), RMC is a researcher from the Juan Rodes Program (JR16/00016), and JA Cornejo-García is a senior researcher from the Miguel Servet Program II (Ref CPII19/00006), all of them from the ISCIII. I Doña is a Clinical Investigator (B-0001-2017) and C Mayorga is a senior researcher from the Nicolas Monardes Program (RC-0004-2016), both from Consejería de Salud of Junta de Andalucía.

Conflict of interest: The authors declare that they have no conflict of interest.

What is already known about this topic?

NSAIDs are the main triggers of drug hypersensitivity reactions, being skin the most frequently affected organ (NIUA). Their underlying mechanism is linked to COX-1 inhibition and to the imbalance in eicosanoid metabolism (mainly PGs and CysLTs).

The idiosyncratic nature of NSAID-hypersensitivity supports the involvement of genetic factors; however, no study to date has evaluated simultaneously the potential role of genetic variants in main eicosanoid synthesis enzymes in these reactions.

What does this study add?

The variants rs10306194 (*PTGS1*) and rs28395868 (*ALOX5*) were associated with the risk of NIUA in two independent populations, with the latter being also associated with NERD. The first polymorphism could help to discriminate NIUA patients from those whose respiratory symptoms are exacerbated after NSAID intake.

Our results not only give consistency to the COX-1 inhibition hypothesis in NSAID-hypersensitivity, but could also help to differentiate its clinical phenotypes.

What is the translational message?

NSAID-hypersensitivity diagnosis represents a daily challenge in clinical settings as there is a lack of *in vivo* and *in vitro* tests, and drug provocation is not a risk-free procedure. Genetic research can provide substantial information not only to fully understand the underlying mechanism but also to identify potential biomarkers for diagnosis and to develop targeted precision therapies.

SUMMARY

Background

Nonsteroidal anti-inflammatory drugs (NSAIDs), probably the most consumed medicines, are the main triggers of drug hypersensitivity, being NSAID-induced acute urticaria/angioedema (NIUA) the most frequent phenotype. NSAID-hypersensitivity is bred by cyclooxygenase (COX)-1 inhibition, which leads to an imbalance in prostaglandin (PG) and cysteinyl-leukotriene (CysLT) synthesis. As only susceptible individuals develop NSAID-hypersensitivity, genetic factors are believed to participate; however, no study has assessed the overall genetic variability of key enzymes in PG and CysLT synthesis in NSAID-hypersensitivity.

Objectives

We aimed to simultaneously evaluate genetic variants in main genes involved in PG and CysLT biosynthesis in NIUA.

Methods

Two independent cohorts of patients were recruited in Spain, alongside NSAID-tolerant controls. The discovery cohort only included NIUA patients; the replication one also included NSAID-exacerbated respiratory disease (NERD) patients. A set of tagging single nucleotide polymorphisms (tagSNPs) in *PTGS1*, *PTGS2*, *ALOX-5* and *LTC4S* was genotyped using mass spectrometry coupled with end point PCR.

Results

The study included 1272 individuals. Thirty-five tagSNPs were successfully genotyped in the discovery cohort, being three significantly associated after Bonferroni correction (rs10306194 and rs1330344 in *PTGS1*, and rs28395868 in *ALOX5*). These polymorphisms were genotyped in the replication cohort: rs10306194 and rs28395868 remained associated with NIUA, and rs28395868 marginally with NERD. Effect sizes in the combined analysis (discovery and replication NIUA populations) were: rs10306194 (OR=1.7, 95%CI=1.34-2.14; corrected p-value=2.44E-5) and rs28395868 (OR=2.19, 95%CI=1.43-3.36, corrected p-value=2.17E-4).

Conclusions

PTGS1 and *ALOX5* gene variants may play a role in NIUA and NERD, supporting the proposed mechanisms of NSAID-hypersensitivity and shedding light into their genetic basis.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most used drugs because of their utility to treat pain and inflammatory processes.¹⁻³ However, they are also

responsible for 21-25% of adverse drug reactions, including drug hypersensitivity.⁴ The most common reactions in NSAID-hypersensitivity are due to cross-hypersensitivity, and NSAID-induced acute urticaria/angioedema (NIUA), a phenotype characterised by wheals and/or angioedema development in patients without chronic spontaneous urticaria (CSU), is the most frequent clinical entity.^{5,6,7,8.}

Despite NSAID-cross-hypersensitivity frequency, there is a lack of *in vivo* and *in vitro* diagnostic tests, and drug provocation, the gold standard for drug-hypersensitivity diagnosis, is not a risk-free procedure.^{5,6}

These reactions are not immunologically-mediated but associated with a pharmacological mechanism linked to cyclooxygenase-1 (COX-1) inhibition.^{4,7,8} This inhibition shunts the arachidonic acid (AA) metabolism from prostaglandin (PG) towards cysteinyl-leukotriene (CysLT) synthesis, eliciting a reaction in susceptible individuals.⁹⁻¹¹

COX-1 and its inducible isozyme, COX-2, which are encoded by the prostaglandin-endoperoxide synthase 1 (*PTGS1*) and 2 (*PTGS2*) genes, catalyse the initial step in PG biosynthesis, leading to the formation of PGG₂ and PGH₂, which are further metabolised into PGE₂, prostacyclins or thromboxanes. PG pathway inhibition through NSAID-mediated COX-1 inhibition leads to the increased transformation of AA into LTA₄ by arachidonate 5-lipoxygenase (ALOX-5), which is transformed by leukotriene C₄ synthase (LTC₄S) into LTC₄, the first step in CysLT biosynthesis.¹²

Susceptibility for developing NSAID-cross-hypersensitivity is thought to be influenced by genetic factors, which is supported by some studies showing familial aggregation; however, inheritance does not follow a classical Mendelian pattern.^{13,14} Most genetic association studies have followed a candidate gene approach, largely focusing on NSAID-exacerbated respiratory disease (NERD).¹⁵ However, despite NIUA being the most frequent entity, it has been less studied.¹⁵

Some genetic associations have been found for enzyme and receptor from the AA pathway and NSAID-cross-hypersensitivity,^{15,16} as well as variants outside eicosanoid biosynthesis.¹⁷⁻²⁰ However, these studies have usually included a limited number of single nucleotide polymorphisms (SNPs), without considering the overall variability in genes of interest. Moreover, most studies reporting genetic associations did not include a second population for replication.^{15,16} Despite their role in NSAID-cross-hypersensitivity, no

previous study has evaluated genetic variation in the main enzymes involved in the synthesis of PGs and CysLTs simultaneously.¹⁵

In this case-control study we have analysed the overall genetic variability in the key enzymes involved in PG (*PTGS1* and *PTGS2*) and CysLT (*ALOX-5* and *LTC4S*) biosynthesis in terms of predisposition to NIUA. For this, we have evaluated a set of tagging SNPs (tagSNPs), which are representative of their overall variability, in a population of NIUA patients and NSAID-tolerant controls. In addition, we have included a second population for replication purposes, comprising not only NIUA patients and controls but also NERD patients. To our knowledge, this is the first time that such an approach has been conducted in NSAID-cross-hypersensitivity. Our findings help to shed light into their underlying molecular mechanisms and into the identification of potential biomarkers.

MATERIAL AND METHODS

Subjects

The study included two consecutively-recruited, independent cohorts of unrelated cases and controls (identified as discovery and replication cohorts), aged 18-60 years, and all of them self-reporting Spanish ancestry. The discovery cohort included 264 NIUA patients and 298 controls, diagnosed in the Allergy Unit of the Malaga Regional University Hospital (Malaga, Spain) from June 2016 to December 2017. The replication cohort included 120 NIUA patients and 379 controls recruited from January 2018 to December 2019 in the Allergy Units of the Cruz Roja Central Hospital (Madrid, Spain) and Clinic Hospital (Barcelona, Spain). In addition, the latter also included a group of 211 NERD Malaga patients diagnosed from June 2016-December 2019 (Table I).

Only patients reporting at least two episodes of acute urticaria (NIUA) or exacerbation of their underlying rhinitis/asthma (NERD), induced by NSAIDs belonging to at least two different chemical groups, were included. For all NIUA patients, the presence of CSU (reoccurrence of urticaria for more than 6 weeks with no external trigger) was excluded by clinical history.

In NIUA patients, NSAID-cross-hypersensitivity was confirmed by drug provocation test with acetylsalicylic acid (DPT-ASA), and in NERD by nasal provocation test with Lys-ASA (NPT-LASA), according to a previous clinical algorithm.²¹

We also included age- and sex-matched control groups of individuals who reported regularly taking NSAIDs without developing a clinical reaction, and had no history of CSU, drug hypersensitivity, rhinitis and/or asthma, or nasal polyposis.

All participants gave informed consent. The study was approved by the Ethics Committee of the participant centres and conducted according to the principles of the Declaration of Helsinki.

DPT-ASA and NPT-LASA

DPT-ASA was performed in a single-blind manner giving placebo capsules at different times on the 1st day, as reported.²¹ ASA and placebo were given in opaque capsules prepared by the hospital pharmacy services. Other medications were withheld before testing, according to international guidelines.²²

For DPT-ASA, two doses were administered orally with an interval of 3 hours (50 and 100 mg) on the 2nd day. If negative, two larger doses of ASA (250 and 500 mg) were administered on the 3rd day, with a 3-hour interval. The procedure was stopped if cutaneous and/or respiratory symptoms or changes in vital signs (cardiac rhythm alterations, decrease in peak expiratory flow or hypotension) appeared, and symptoms were evaluated and treated.²¹ If no symptoms appeared during these periods, this was followed by a 2-day/8-hour course of the therapeutic dose (500 mg) after a gap of 24 hours.²¹

NPT-LASA was carried out as described,^{21,23} and results were considered positive if an increase $\geq 30\%$ in the total nasal symptoms and a decrease $\geq 30\%$ in the total volume of both nasal cavities from 2 to 6 cm (vol 2–6 cm) was observed, measured by acoustic rhinometry.

Genotype determination

Data from the 1000 Genome Project (phase 3) (<https://www.internationalgenome.org/data-portal/data-collection/phase-3>) for individuals of European ancestry were downloaded from the project website. The regions corresponding to *PTGS1*, *PTGS2*, *ALOX5*, and *LTC4S* genes plus 2kb up and downstream from the gene start and end positions, respectively, were extracted using Tabix²⁴ and VCFtools²⁵ (Table II). The resulting file was converted to plink format to be loaded into Haploview.²⁶ Non-biallelic and indel markers were discarded.

tagSNPs were selected based on linkage disequilibrium using Tagger.²⁷ Pairwise tagging was used, with an r^2 threshold of 0.8. The SNP rs730012 in *LTC4S* was included by force because of previous evidence of potential association. Assuming a disease prevalence of 0.01, a risk allele frequency of 0.2, and a relative risk of >1.5, the discovery phase of this study has a 80% power to detect an associated tagSNP with $\alpha=0.05$ (additive model).²⁸

Genomic DNA was isolated from peripheral blood using the FlexiGene DNA Kit system (QIAGEN). Genotyping during the discovery phase was performed using the iPLEX Sequenom MassArray technology, whereas the iPLEX Gold system from Agena Bioscience was used for the replication cohort. To ensure the different technologies gave identical results, 10 samples from each population were genotyped using both systems, finding 100% coincidence.

Statistical analysis

The Mann-Whitney U test was used to compare continuous variables between groups, and the χ^2 test for categorical variables. Hardy-Weinberg equilibrium and individual SNP association tests with NIUA and NERD were performed using SNPAssoc (additive model).²⁹ The latter was done using logistic regressions to calculate tagSNP effects as odd ratios with 95% confidence intervals. A p-value ≤ 0.05 after Bonferroni correction for multiple testing was considered statistically significant. Corrected p-values were obtained taking into account 35 tagSNPs in the discovery phase and in the meta-analysis (discovery+replication), and 3 tagSNPs in the replication phase.

RESULTS

Demographic and clinical data

In total, 1272 individuals were genotyped, representing patients and controls from two non-overlapping consecutively recruited populations. The distribution of individuals between groups is showed in Table I. These groups are referred to as discovery and replication cohorts. No significant differences in sex or age between patients and controls were found in neither of the considered groups (Table I).

Although more than 80% of patients had suffered at least three episodes of NSAID-cross-hypersensitivity according to their clinical history, in all instances diagnosis was established by drug provocation, as described elsewhere²¹. No statistical differences were found in the number of previous episodes when comparing NIUA and NERD patients from

the replication cohort ($p=0.088$), or between NIUA patients from both discovery and replication cohorts ($p=0.861$) (Table I).

Concerning the culprit drugs inducing hypersensitivity episodes, no statistically significant differences were found between NIUA patients from the two cohorts ($p=0.748$) (Table I). Similarly, no significant differences were found in terms of culprits when comparing NIUA and NERD patients within the replication cohort ($p=0.174$). For all three patient groups, propionic derivatives were the most frequent triggers, followed by ASA and pyrazolones (Table I).

Genetic association study

A total of 36 tagSNPs were selected: 10 in *PTGS1*, 9 in *PTGS2*, 15 in *ALOX5*, and 2 in *LTC4S* (Table II). However, rs115693689 in *PTGS1* was monomorphic in our population, and rs3776944 in *LTC4S* could not be genotyped due to technical limitations; consequently, they were not further considered. None of the remaining tagSNPs deviated significantly from HWE in the control group (threshold for significance after considering the multiple comparisons performed was $p\text{-value}=0.001$). As such, they were all retained for association analyses. The genotyping call rate for all tagSNPs was $>99\%$ across all individuals.

The results of genetic association testing in the discovery phase are shown in Table III. Concerning COX-1 and COX-2 genes, we found marginal associations for the variants rs10306194, rs1330344, and rs76942325 in *PTGS1*, as well as for rs689467 in *PTGS2*. For *ALOX5*, marginal associations were found for rs28395868 and rs3780901, whereas the polymorphism rs730012 in *LTC4S* was not associated with NIUA risk in our study. However, of all these associations, only three remained statistically significant after Bonferroni multiple testing correction (Table III). In more detail, these were: rs10306194 in *PTGS1*, which was associated with an increased risk of NIUA ($OR=1.72$, $95\%CI=1.25\text{-}2.36$, corrected $p\text{-value}=2.4E\text{-}4$), rs1330344, which was linked to reduced NIUA risk ($OR=0.61$, $95\%CI=0.45\text{-}0.82$, corrected $p\text{-value}=0.032$), and rs28395868 in *ALOX5*, which was associated with increased risk ($OR=2.61$, $95\%CI=1.37\text{-}4.98$, corrected $p\text{-value}=0.024$) (Table III).

To validate these findings, all three nominally associated tagSNPs in the NIUA discovery cohort were further genotyped in an independent case-control population (replication cohort), which also included a group of NERD patients, and tested for association after verifying that none of them departed significantly from HWE in the control

group. The rs10306194 *PTGS1* variant was associated with an increased risk of NIUA after Bonferroni correction (OR=1.74, 95%CI=1.2-2.52; corrected p-value=0.011). However, the association between NIUA and the rs1330344 *PTGS1* polymorphism in the discovery cohort could not be replicated in this second cohort. Finally, the *ALOX5* rs28395868 tagSNP was also associated with increased risk for NIUA (OR=2.2, 95%CI=1.18-4.1, corrected p-value=0.035) (Table III).

Regarding the NERD patients included in the replication stage, we did not find the rs10306194 and rs1330344 *PTGS1* variants to be linked to risk. Although the rs28395868 polymorphism in *ALOX5* did not survive multiple testing correction, it was also marginally associated with NERD (OR=1.88, 95%CI=1.08-3.27, p-value=0.018; corrected p-value=0.052) (Table III).

Concerning the combined analysis of associated data variants from NIUA patients in the discovery and replication populations, effect size was OR=1.7 (95%CI=1.34-2.14; corrected p-value=2.44E-5) for rs10306194, and OR=2.19 (95%CI=1.43-3.36, corrected p-value=2.17E-4) for rs28395868 (Table III). The variant rs1330344 did not surpass multiple testing analysis in the joint analysis (Table III).

DISCUSSION

NSAIDs are currently considered the main triggers of drug hypersensitivity, with the most frequent mechanism being linked to their pharmacological property of PG biosynthesis inhibition through COX-1 blockade (NSAID-cross-hypersensitivity). Such inhibition shunts the AA metabolism towards the CysLT pathway, leading to a pathological condition in susceptible subjects.⁹⁻¹¹ Individual susceptibility to NSAID-cross-hypersensitivity appears to involve a complex interaction of factors, including a genetic component. Although the familial inheritance of hypersensitivity to NSAIDs has not been extensively evaluated, data from patients with skin involvement support this. In a seminal study, Mastalerz *et al.* evaluated two patients with NIUA and a familial history of NSAID-cross-hypersensitivity showing that in these families the disease did not follow a similar inheritance pattern, with no clear Mendelian segregation.¹³ More recently, another study reported the development of cutaneous NSAID-cross-hypersensitivity in two twins.¹⁴ Nevertheless, most studies on the genetics of NSAID-cross-hypersensitivity have been conducted in NERD patients,¹⁵ despite NIUA being the most frequent clinical entity induced by drug hypersensitivity.^{5,6,8} Although

most studies have focused on SNPs from genes related to the AA pathway, they have not evaluated the overall genetic variability in such genes. Moreover, they have not included a second population to validate their findings, and results have been variable and often not replicable.

Given that both PG and CysLT pathways are critical players in modifying inflammatory responses, it is not surprising that variants in *PTGS1* and *ALOX5* are important markers for inter-individual differences in diseases for which these pathways play a key role, such as allergy and a variety of inflammatory conditions,^{30,31} including NSAID-cross-hypersensitivity.⁹⁻¹¹ In this study we have evaluated the overall genetic variability in the genes encoding the initial enzymes for both PG (*PTGS1* and *PTGS2*) and CysLT (*ALOX5* and *LTC4S*) synthesis in NIUA patients, using a tagging strategy. We have also included a second independent population for validation purposes which comprised not only NIUA but also NERD patients. We have found that the variant rs10306194 in the 3'-untranslated region (3'-UTR) of *PTGS1* was associated with an increased risk of NIUA in the two cohorts considered, as well as the intronic rs28395868 polymorphism in *ALOX5*, which was also associated with NERD.

In general, there is not much information about the variants we have found to be associated with NSAID-hypersensitivity in other human diseases. Several variants in *PTGS1* have been clinically associated with different inflammatory pathologies, including cardiovascular diseases,³² ischemic stroke,³³ multiple cancers,³⁴⁻³⁶ and antiplatelet therapy resistance.³⁷ The rs10306194 polymorphism in *PTGS1* has been associated with breast cancer³⁸, and more recently it has been described as part of a haplotype block associated with acute myeloid leukaemia risk;³⁹ however, its functional relevance needs to be clarified. Although several SNPs in the 3'-UTR of *PTGS1* have been identified, appearing at high frequencies in some cases, very few of them have been evaluated with regards to potential clinical associations.⁴⁰ However, at the molecular level, the sequence and, consequently, the structure of 3'-UTRs are crucial for the stability, localisation, and expression of mRNAs through their interactions with different factors such as miRNAs and RNA-binding proteins. These interactions can lead to various effector processes, which include translational control, deadenylation, and decay of mRNA, and to their localisation in a particular cellular area through active transport along the cytoskeleton and/or asymmetric anchoring.⁴¹ 3'-

UTRs may also determine the fate of proteins through the regulation of protein-protein interactions and facilitate co-translational protein complex formation. Most of these 3'-UTR roles involve effector proteins that are recruited by 3'-UTR cis-element RNA-binding proteins.⁴² Thus, 3'-UTR SNPs may affect gene regulation and, consequently, influence cellular function and determine phenotypic diversity, including specific pathological conditions.

There is a lack of information concerning the clinical significance of the intronic variant rs28395868, unsurprisingly when we consider that information about *ALOX5* SNPs and human diseases is scarce. However, data from the Open Target Genetic Portal (<https://genetics.opentargets.org/>), which combines information from the UK Biobank and the NHGRI GWAS Catalogue repositories, indicate this polymorphism may be linked to different traits, including immature reticulocyte fraction, mean corpuscular haemoglobin and red cell distribution width, among others. Intronic regions are long stretches of noncoding DNA found between coding regions (exons) that were initially considered "junk DNA".⁴³ However, more recent studies have suggested that actually the majority of the genome is biochemically active.⁴⁴ Although biochemical activity does not necessarily imply functionality, it is widely accepted that introns regulate gene expression and alternative splicing, not only by functioning as transcription factor binding sites but also by affecting transcription rate, nuclear export, and transcript stability.⁴⁵ Such regulation may also play a key role in the development of particular clinical phenotypes. Thus, intronic variants have been associated with different types of cancers,⁴⁶⁻⁴⁹ cardiovascular diseases,^{50,51} and autoimmune disorders such as systemic lupus erythematosus, Hashimoto thyroiditis, and rheumatoid arthritis.⁵² In fact, we have recently reported several variants in a guanine nucleotide-binding protein to be associated with NIUA.²⁰ Moreover, next-generation sequencing development has allowed the identification of deep intronic mutations in over 75 disease-associated genes that can be deleterious by activating non-canonical splicing, modifying splicing regulatory elements, or disrupting transcription regulatory motifs or non-coding RNA genes.^{53,54} A non-coding variant in *ALOX5* has been recently associated with type 2 diabetes and the effects of vitamin D on monocytes.⁵⁵ Data from two clinical trials have revealed that other variants in *ALOX5* may affect the clinical response to montelukast.

⁵⁶ Some polymorphisms in this gene have also been associated with NERD in a Korean population. ^{57,58}

Finally, SNPs in noncoding sequences, including 3'-UTR and introns, may induce methylation changes that could affect gene expression, as it has been also reported in various human pathologies. ⁵⁹⁻⁶³

In summary, we have found several variants in genes encoding the key enzymes in the synthesis of PGs and CysLTs to be associated with NSAID-cross-hypersensitivity (particularly with NIUA), which is consistent with the proposed triggering mechanism. To our knowledge, this is the first study simultaneously addressing genetic variability in these genes in NIUA and NERD patients. Moreover, it represents the largest sample population study in patients with NSAID-cross-hypersensitivity to date.

In spite of the similarities observed in our study between NIUA and NERD, they appear to show also some particular differences as the 3'-UTR variant rs10306194 was not associated in the latter. At present the differences at the molecular level supporting these associations are unknown.

This study suffers from several limitations. We have considered only the additive model of inheritance as there was a strong correlation with the dominant and recessive models (data not shown), and all significant associated variants resulted from the additive model. Moreover, genetic association studies are usually performed considering this model, which is known to be sensitive and may only lead to a decrease in power when there is a departure from additivity. ⁶⁴⁻⁶⁵ Potential relationships of associated variants and quantitative markers have not been explored. There may be variants in other genes or in intergenic regions that could interact with those we have evaluated here or affect gene expression and eQTLs. Unfortunately, we were unable to assess such variation, which would require a more agnostic approach, such as GWAS. In addition to functional studies, further research expanding the sample size to cover rare variants, and evaluating other populations could provide a better understanding of the genetics underlying NSAID-cross-hypersensitivity and its different phenotypes. This would help us to shed light into potential biomarkers for prognostic and diagnosis purposes.

Acknowledgements: We thank Ms. Claudia Corazza for her help with the English version of the manuscript. We also thank Dr. Maria Torres from the Spanish National Genotyping Center (CEGEN), Dr. Tatiana Diaz, from the Malaga Regional Biobank (Andalusian Public Health System Biobank), and Dr. Juan Miguel Gómez Zumaquero, from the Genotyping and Sequencing Unit of IBIMA, for technical support.

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Table I. Demographic and clinical data for patients and controls.

Variable	Discovery cohort			Replication cohort					
	NIUA	Controls	p-value	NIUA	NERD	Controls	p-value	p-value	p-value
	(n=264)	(n=298)		(n=120)	(n=211)	(n=379)	(NIUA vs controls)	(NERD vs controls)	(NIUA vs NERD)
Age, years (mean±SD)	41.0±15.0	42.6±13.6	0.191	41.4±15.4	42.3±12.4	42.8±11.6	0.392	0.655	0.580
Female counts, n (%)	148 (55.0)	181 (60.7)	0.169	63 (52.9)	129 (61.1)	201 (53.0)	0.986	0.058	0.149
No. of episodes (mean±SD)*	3.8±1.7	NA	NA	3.8±1.0	3.5±1.9	NA	-	-	0.088
Pharmacological culprit drug, No. of episodes (%)**		NA	-			NA	-	-	0.312
<i>Propionic acid derivatives</i>	434 (41.9)			195 (42.5)	299 (40.6)				
<i>Acetylsalicylic acid</i>	208 (20.1)			93 (20.3)	177 (24.0)				
<i>Pyrazolones</i>	202 (19.5)			89 (19.4)	159 (21.6)				
<i>Arylacetic acid derivatives</i>	98 (9.5)			45 (9.8)	62 (8.4)				
<i>Others</i>	95 (9.0)			37 (8.0)	40 (5.4)				

Abbreviations: NIUA, NSAIDs-induced acute urticaria/angioedema; NERD, NSAIDs-exacerbated respiratory disease.

*There were no statistically significant differences in terms of the number of episodes between discovery and replication NIUA patient cohorts ($p=0.861$).

*There were no statistically significant differences in terms of the culprit pharmacological group inducing the different episodes between discovery and replication NIUA patient cohorts ($p=0.748$).

Table II. Selected tagSNPs.

Gene	Chromosome	Start-2Kb	Start	End	End+2Kb	tagSNPs		
<i>PTGS1</i>	9	125131284	125133284	125157982	125159982	Reference	Position	Alleles (M/m)
						rs10306122	125135489	T/C
						rs10306194	125157198	C/A
						rs12238505	125151884	T/A
						rs1238420	125144145	A/G
						rs1330344	125131688	C/T
						rs3119773	125135254	G/A
						rs4273915	125145329	G/C
						rs5788	125143792	C/A

						rs76942325	125151375	T/G
						rs7866582	125138695	A/C
<i>PTGS2</i>	1	186638944	186640944	186649559	186651559	rs2066826	186645927	C/T
						rs2206593	186642429	A/G
						rs4648268	186647138	C/T
						rs5275	186643058	A/G
						rs5277	186648197	C/G
						rs4648308	186640617	C/T
						rs689467	186641273	A/C
						rs2745557	186649221	A/G
						rs689466	186650751	T/C
<i>ALOX5</i>	10	45867624	45869624	45941567	45943567	rs11239515	45902055	A/G
						rs11239526	45918380	C/T
						rs12264801	45914744	G/A
						rs17157798	45942712	T/G
						rs1864412	45870690	A/G
						rs28395868	45938677	G/A
						rs3780901	45917376	T/C
						rs41502051	45936698	A/G

						rs4948672	45914527	G/C
						rs4949001	45906573	G/A
						rs61854091	45917542	T/C
						rs702365	45934331	C/G
						rs7099684	45897155	A/T
						rs74911239	45922801	C/A
						rs892690	45870688	C/T
<i>LTC4S</i>	5	179218986	179220986	179223513	179225513	rs730012	179220638	A/C
						rs3776944	179220010	G/A

Abbreviations: *ALOX-5*, arachidonate 5-lipoxygenase; *LTC4*, leukotriene C4 synthase; *PTGS1*, prostaglandin-endoperoxide synthase 1; *PTGS2*, prostaglandin-endoperoxide synthase 2.

Table III. Association study with the selected tagSNPs in the discovery cohort comprising patients with the NIUA phenotype and controls.

			Discovery population (NIUA)			Replication population NIUA NERD					Discovery+Replication (NIUA)		
Gene	tagSNPs	OR referred allele*	OR (95%CI)	p- value	Corrected p-value	OR (95%CI)	p- value	Corrected p-value	OR (95%CI)	p- value	OR (95%CI)	p- value	Corrected p-value
PTGS1	rs10306122	T/C	0.60 (0.37-1.05)	0.091									
	rs10306194	C/A	1.72 (1.25-2.36)	6.96E-5	2.4E-3	1.74 (1.2-2.52)	0.003	0.011	1.32 (0.97-1.8)	0.076	1.7 (1.34-2.14)	8.1E-6	2.83E-5
	rs12238505	T/A	1.14 (0.72-1.79)	0.576									
	rs1238420	A/G	0.98 (0.62-1.55)	0.934									
	rs1330344	C/T	0.61 (0.45-0.82)	9.24E-4	0.032	1.1 (0.79-1.56)	0.551		1.13 (0.85-1.51)	0.404	0.78 (0.62-0.97)	0.023	

PTGS2	rs3119773	G/A	0.69 (0.47-1.00)	0.051
	rs4273915	G/C	0.85 (0.63-1.15)	0.299
	rs5788	C/A	0.87 (0.63-1.21)	0.405
	rs76942325	T/G	1.91 (1.24-2.94)	0.003
	rs7866582	A/C	0.89 (0.65-1.20)	0.438
	rs2066826	C/T	1.12 (0.82-1.53)	0.475
PTGS2	rs2206593	A/G	1.39 (0.91-2.13)	0.128
	rs4648268	C/T	1.23 (0.86-1.76)	0.247
	rs5275	A/G	0.98 (0.75-1.26)	0.848
	rs5277	C/G	0.82	0.192

ALOX5	rs4648308	C/T	1.05 (0.77-1.43)	0.747
	rs689466	T/C	1.00 (0.75-1.32)	0.978
	rs2745557	A/G	1.10 (0.82- 1.47)	0.522
	rs689467	A/C	0.57 (0.27- 1.22)	0.041
	rs11239515	A/G	1.01 (0.75-1.36)	0.954
ALOX5	rs11239526	C/T	1.14 (0.71-1.83)	0.589
	rs12264801	G/A	1.15 (0.91-1.46)	0.235
	rs17157798	T/G	1.36 (0.88-2.11)	0.163
	rs1864412	A/G	1.05 (0.79-1.40)	0.714

rs28395868	G/A	2.61 (1.37-4.98)	6.9E-4	0.024	2.2 (1.18-4.1)	0.012	0.035	1.88 (1.08-3.27)	0.018	2.19 (1.43-3.36)	5.75E-5	0.002
rs3780901	T/C	1.29 (1.00-1.66)	0.048									
rs41502051	A/G	0.91 (0.47-1.75)	1									
rs4948672	G/C	1.10 (0.86-1.40)	0.447									
rs4949001	G/A	1.08 (0.83-1.41)	0.567									
rs61854091	T/C	0.95 (0.59-1.55)	0.607									
rs702365	C/G	1.15 (0.90-1.47)	0.256									
rs7099684	A/T	0.74 (0.54-1.03)	0.074									
rs74911239	C/A	1.47 (0.83-2.61)	0.258									

	rs892690	C/ T	1.07 (0.85-1.36)	0.566
<i>LTC4S</i>	rs730012	A /C	0.99 (0.75-1.32)	0.962

Abbreviations: *ALOX-5*, arachidonate 5-lipoxygenase; *LTC4*, leukotriene C4 synthase; *PTGS1*, prostaglandin-endoperoxide synthase 1; *PTGS2*, prostaglandin-endoperoxide synthase 2. *OR referred allele is shown in bold.