

# The –308 G>A SNP of *TNFA* is a factor predisposing to chronic rhinosinusitis associated with nasal polyposis in aspirin-sensitive Hungarian individuals: conclusions of a genetic study with multiple stratifications

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## Abstract

Single nucleotide polymorphisms (SNPs) of the tumour necrosis factor alpha (TNF $\alpha$ ) gene (*TNFA*) have been extensively studied and shown to be associated with an increased risk of the development of various chronic inflammatory diseases. Inflammation has been demonstrated to play a central role in the pathogenesis of chronic rhinosinusitis (CRS), and TNF $\alpha$  is a key pro-inflammatory cytokine with important functions in these processes. In order to determine whether the well-known *TNFA* –308 G>A SNP has a role in a genetic predisposition to CRS in the Hungarian population, we analyzed our genomic collection containing control and CRS patient samples in a case–control study, and compared the genotype and allele frequencies. There was no significant difference in the observed genotype or allele frequencies between the controls and the total CRS group. However, after careful stratification of the patient group on the basis of the observed clinical symptoms, we found a significantly higher carriage rate of the rare A allele-containing genotypes among the CRS patients with nasal polyposis (NP) who also exhibited sensitivity to aspirin (acetylsalicylic acid, ASA<sup>+</sup>). It is concluded that genetic variants of the *TNFA* gene may affect the risk of CRS in a clinically well-defined group of CRSNP<sup>+</sup>ASA<sup>+</sup> patients in the Hungarian population. Our results also emphasize that the group of CRS patients is not homogenous in that patients exhibiting different clinical symptoms exist. Their carried genetic predisposing factors, and as a result, the exact molecular events leading to the development of various forms of CRS, may also differ.

**Keywords:** chronic rhinosinusitis, nasal polyposis, single nucleotide polymorphism, *TNFA* –308 G>A

## Introduction

Chronic rhinosinusitis (CRS) is characterized by persistent inflammation of the nasal and paranasal mucosa (1). CRS is present when any combination of the major symptoms, including

nasal congestion or blockage, loss of smell, rhinorrhoea, post-nasal drip and facial pain or pressure occurs for an extended period of time (over 12 weeks) despite various treatment

attempts. It is a clinically heterogeneous disease frequently associated with other airway diseases, such as asthma, allergy, chronic obstructive pulmonary disease and bronchiectasis (2–6).

Nasal polyposis (NP) is characterized by non-granulomatous inflammatory tissue extensions of the mucosal surface lining the sinonasal cavities (7). CRS can occur either with (CRSNP<sup>+</sup>) or without (CRSNP<sup>-</sup>) the appearance of NP. A special group of CRSNP<sup>+</sup> patients exhibits a marked sensitivity to aspirin (acetylsalicylic acid, ASA) or other non-steroidal anti-inflammatory drugs. In such sensitive individuals (ASA<sup>+</sup>), consumption of even a small dose of ASA can cause bronchiolar constriction, rhinorrhoea and shock symptoms related to a non-IgE-mediated pharmacological hypersensitivity reaction (8–10).

The pathogenesis of CRS is a complex process in which several self and environmental factors have been implicated (1, 11). Anatomic features of the upper airway that cause mucous stagnation inside the sinuses, bacterial and fungal infections and abnormal immune regulation have been shown to play an important role in the pathogenesis of the disease (12–14). Apart from these, individual genetic factors have a significant role in making certain individuals susceptible to the development of CRS (15, 16).

Aberrant immune regulation often results in chronic inflammation, thus the different molecules (cytokines, chemokines and anti-microbial peptides) that are playing an important role in these processes have been suggested to be involved in the pathogenesis of CRS, too. One such candidate is the pro-inflammatory cytokine tumour necrosis factor alpha (TNF $\alpha$ ), the presence of which has already been detected in various cellular components of the polypoid tissues of CRS patients (17, 18). This has led to the suggestion that genetic polymorphisms affecting the regulation and/or function of the gene encoding the TNF $\alpha$  cytokine (*TNFA*) may play important roles in the genetic predisposition to CRS (19, 20).

The *TNFA* locus is located within the highly polymorphic major histocompatibility III region on chromosome 6 (6p21.3). There are many single nucleotide polymorphisms (SNPs) within this gene, especially in its 5' regulatory region, often exhibiting associations with the pathogenesis of certain chronic inflammatory and immune-mediated diseases (21). One of them, the *TNFA* –308 G>A (rs1800629) is a relatively frequent SNP in Caucasian populations, which have already been investigated in the context of CRS (22–25). The results of these studies led to the postulation of a correlation between the carriers of the rare A allele and CRS, and also CRSNP<sup>+</sup>, in Turkish and US Caucasian populations, whereas the same association was not detected in Canadian individuals.

In order to establish whether the *TNFA* –308 G>A SNP is involved in a genetic predisposition to CRS in the Hungarian population, we performed case–control genetic studies. Our results indicate that the rare A allele, or alternatively, a linkage group carrying this allele may be a genetic predisposing factor in the Hungarian population, but this association is restricted to the ASA<sup>+</sup> group of CRSNP<sup>+</sup> patients.

## Methods

### Study population and ethics

Buccal swab samples were obtained from a total of 544 Caucasian individuals (169 controls and 375 patients) from

various otorhinolaryngology centres in Hungary (University of Szeged; Semmelweis University, Budapest; Pécs University; Borsod-Abaúj-Zemplén County Hospital and University Teaching Hospital, Miskolc; and St John's and North-Buda Hospitals of the Municipality of Budapest). The ages of the recruited individuals were between 18 and 72 years. Patients were selected after trained medical professionals had established the diagnosis of CRS. Exclusion criteria included the presence of signs of acute upper airway infection, known malignancy or any other general disease, infectious disease, primary or acquired immune deficiency, dialysis, any chronic autoimmune diseases, rhinitis medicamentosa or odontogenic sinusitis. The control group comprised individuals without any medical history of CRS and with none of the above-described diseases and conditions.

The study was approved by the Hungarian Research Ethics Committee. All participating subjects gave their written consent before sample collection. The study was performed in accordance with the principles stated in the Declaration of Helsinki and its later revisions.

### Polymorphism analysis

Genomic DNA was obtained from buccal swab samples by using the QIAGEN EZ1 DNA Investigator Kit (QIAGEN, Germany).

Patient and control samples were genotyped for the *TNFA* –308 G>A (rs1800629) SNP by the PCR-RFLP (Restriction Fragment Length Polymorphism) method, as described previously (26). Briefly, a short 106-bp genomic sequence around the –308 position of the *TNFA* promoter was amplified by using the following primers: 5'-GAG GCA ATA GGT TTT GAG GGG CAT-3' and 5'-GGG ACA CAC AAG CAT CAA GG-3'. Restriction analysis of the resulting fragments was performed with an *Nco*I enzyme (Fermentas, Vilnius, Lithuania). Electrophoresis of the digested PCR products was performed on a 2% agarose gel (Lonza, Rockland, ME, USA). To visualize DNA fragments, gels were stained with GelRed (Biotium, Inc., Hayward, CA, USA).

### Statistical analysis

Statistical analysis was carried out on the various groups of patients and controls according to the rules of case–control allelic association study designs. Genotype and allele frequencies were calculated by determining the percentage of individuals carrying the different genotypes and the percentage of carried alleles in each group. For the statistical analyses, the dominant genotype model was applied, where the group of rare-allele carriers (GA + AA) was compared with the group of non-carriers (GG). Statistical significance of the associations, odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated using the SPSS software (Version 17, SPSS, Chicago, IL, USA).

Hardy–Weinberg equilibrium (HWE) was calculated using a web-based calculator (<http://www.oege.org/software/hwe-mr-calc.shtml>) (27).

### Literature search

A PubMed search was performed and all the currently available reports investigating the possible relationship between

CRS (with or without NP) and the *TNFA* -308 G>A SNP in different populations were reviewed.

## Results

### Demographic analysis

The clinical characteristics of the study participants are presented in Table 1. Altogether 169 controls (84 males and 85 females) and 375 CRS patients (204 males and 171 females) were genotyped for the *TNFA* -308 G>A SNP. The mean ages of the controls and the cases were 43.1 and 48.8 years, respectively. Aspirin sensitivity (ASA<sup>+</sup>) was detected in 18.4% ( $n = 60$ ) of the CRSNP<sup>+</sup> patients. None of the controls was aware of ASA sensitivity (Tables 1 and 2). We also analyzed whether our study population was in HWE for the studied genetic variant. We did not detect any deviation from the HWE neither in the controls nor in our patient group (Table 1).

### Genetic analysis of the *TNFA* -308 G>A SNP in the unstratified and stratified groups of CRS patients

The observed genotype and allele frequencies of the *TNFA* -308 G>A SNP are presented in Supplementary Table 1, available at *International Immunology* Online. The genotype

distribution of the SNP did not exhibit a statistically significant difference as compared with the unstratified group of CRS patients ( $n = 375$ ) and the controls ( $n = 169$ ) (Pearson chi-square test 2×2 table,  $P = 0.54$ ). Similar results were obtained by comparing the allele frequencies in the groups of controls and patients.

Next, we stratified the CRS group according to the presence or absence of NP (Fig. 1 and Supplementary Table 2, available at *International Immunology* Online). We did not detect a statistically significant difference as concerns the observed genotype and allele frequencies in either the CRSNP<sup>-</sup> ( $n = 49$ ) or the CRSNP<sup>+</sup> ( $n = 326$ ) cohort relative to the controls ( $n = 169$ ) (Pearson chi-square test 2×2 table,  $P = 0.45$  and  $P = 0.39$ , respectively).

Finally, we stratified the CRSNP<sup>+</sup> group to cohorts of patients on the basis of their ASA tolerance (CRSNP<sup>+</sup>ASA<sup>-</sup>,  $n = 266$ ; and CRSNP<sup>+</sup>ASA<sup>+</sup>,  $n = 60$ ) (Fig. 1). The analysis revealed statistically significant differences in the distributions of various genotype and allele frequencies between the CRSNP<sup>+</sup>ASA<sup>+</sup> and control groups (Pearson chi-square test 2×2 table,  $P = 0.03$  and  $P = 0.04$ , respectively) (Table 3). Thus, our data indicated that the rare A allele was positively associated exclusively with the development of CRS and NP in the ASA<sup>+</sup> patients as the OR for this complex disease was twice as high in individuals who carried the minor A allele in at least one copy (OR = 2.0, at 95% CI: 1.1–3.7 when the observed genotype frequencies were compared and OR = 1.7, at 95% CI: 1.0–2.9 when the allele frequency data were analysed).

### Comparison of the results of independent studies analysing the role of the *TNFA* -308 G>A SNP in various ethnic populations

Next, we performed a literature search and selected all the available reports that investigate the possible relationship between CRS (with or without NP) and the *TNFA* -308 G>A SNP in different populations (22–25), and compared our data with the results of such investigations (Table 4). There had been four previous analyses of the association of the *TNFA* -308 G>A SNP and CRSNP<sup>+</sup>. Three studies reported an association between these symptoms and the rare A allele of the analysed SNP, whereas a study on a Canadian population did not. In agreement with the latter, we did not detect statistically significant differences

**Table 1.** Demographic data of the study population

Demographic parameters	Controls ( $n = 169$ )	CRS ( $n = 375$ )
Mean age (years)	43.1 (18–69)	48.8 (18–78)
$\chi^2$ ( $P$ value) <sup>a</sup>	$P < 0.05$	
Sex (M/F)		
$n$ (%) / $n$ (%)	84 (49.7) / 85 (50.3)	204 (54.4) / 171 (45.6)
$\chi^2$ ( $P$ value) <sup>a</sup>	n.s.	
ASA sensitivity		
$n$ (%)	0 (0)	60 (16.0)
$\chi^2$ ( $P$ value) <sup>a</sup>	$P < 0.05$	
HWE <sup>b</sup> for the <i>TNFA</i> -308 G>A SNP	n.s.	n.s.

n.s., statistical comparison was not significant ( $P > 0.05$ ).

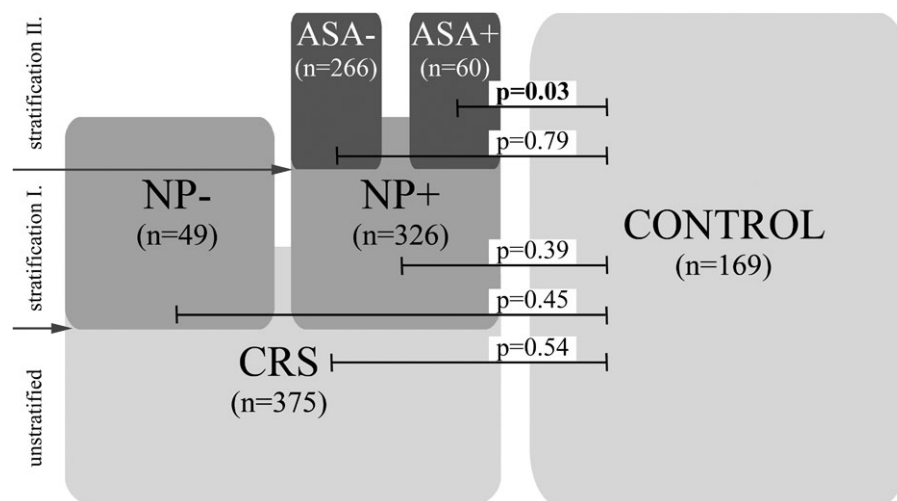
<sup>a</sup>Chi-square analysis with the Pearson correction. <sup>b</sup>Deviation from the Hardy–Weinberg equilibrium.

**Table 2.** Stratification of the CRS patient group

	CRSNP <sup>-</sup> ( $n = 49$ )	CRSNP <sup>+</sup> ( $n = 326$ )	CRSNP <sup>+</sup> ASA <sup>-</sup> ( $n = 266$ )	CRSNP <sup>+</sup> ASA <sup>+</sup> ( $n = 60$ )
Mean age (years)	48.2 (22–78)	49.1 (18–78)	49.0 (18–77)	46.7 (23–77)
$\chi^2$ ( $P$ value) <sup>a</sup>	n.s.		n.s.	
Sex (M/F)				
$n$ (%) / $n$ (%)	27 (55.1) / 22 (44.9)	182 (55.8) / 144 (44.1)	158 (59.4) / 108 (40.6)	24 (40.0) / 36 (60.0)
$\chi^2$ ( $P$ value) <sup>a</sup>	n.s.		$P < 0.05$	
ASA sensitivity				
$n$ (%)	0 (0)	60 (18.4)	0 (0)	60 (100)
$\chi^2$ ( $P$ value) <sup>a</sup>	$P < 0.05$		$P < 0.05$	

CRSNP<sup>+/−</sup>, chronic rhinosinuitis occurring with (+) or without (−) nasal polyposis, respectively; CRSNP<sup>+</sup>ASA<sup>+/−</sup>, chronic rhinosinuitis occurring with nasal polyposis and with (+) or without (−) aspirin sensitivity; n.s., the result of the statistical comparison is not significant ( $P > 0.05$ ).

<sup>a</sup>Chi-square analysis with the Pearson correction.



**Fig. 1.** Stratification of the CRS group ( $n = 375$ ) was performed on the basis of the clinical symptoms exhibited by the patients. At the first level, we grouped the patient cohort on the basis of the absence (NP<sup>-</sup>,  $n = 49$ ) or presence (NP<sup>+</sup>,  $n = 326$ ) of NP. The latter group was subsequently divided into groups of patients with (ASA<sup>+</sup>,  $n = 60$ ) or without (ASA<sup>-</sup>,  $n = 266$ ) aspirin sensitivity. A statistically significant difference was detected in the distribution of the rare A allele-containing genotypes only when the CRSNP<sup>+</sup>ASA<sup>+</sup> group of patients was compared with the controls.

when the distributions of various genotypes and alleles were compared in the control and the overall CRS patient groups, nor when we performed the analysis on the control and CRSNP<sup>+</sup> groups.

We also assessed the frequencies of ASA<sup>+</sup> patients within the CRSNP<sup>+</sup> cohorts in the previous studies. In the Canadian and one of the Turkish studies, this group was relatively numerous (38.8 and 34.0%, respectively), whereas in our case, the proportion of ASA<sup>+</sup> patients (18.4%) was more similar to the data reported on the US Caucasians (16.4%) and the other cohort of Turkish patients (14.6%). This may reflect differences in the inclusion criteria applied to select the patient and control cohorts from the different populations.

We also noted that the frequencies of rare A allele-containing genotypes varied greatly in the controls of the different ethnic

groups, which was in agreement with previous, similar case-control investigations. Moreover, one of the Turkish studies did not mention whether the studied cohort was in HWE (22), whereas the other Turkish study could not demonstrate HWE within their controls (24).

## Discussion

CRS is a multifactorial disease of unknown etiology (28) in which yet undefined external and internal factors initiate innate and adaptive immune responses in the sinonasal cavities (1, 14, 16). During these processes, elevated expressions of various cytokines (e.g. TNF $\alpha$  and IL-1) play important roles (17, 18), e.g. in the initiation of local inflammation, a process of great importance during the clearance of attacking pathogens (29, 30). However, the extensive activation of these mechanisms

**Table 3.** Genotype- and allele-frequencies of the TNFA -308 G>A SNP in the control individuals and the groups of CRSNP<sup>+</sup> patients (ASA<sup>+</sup> and ASA<sup>-</sup>)

	Controls <i>n</i> (%)	CRSNP <sup>+</sup> ASA <sup>-</sup> <i>n</i> (%)	$\chi^2$ ( <i>P</i> value) <sup>a</sup> OR <sup>b</sup> (95% CI)	CRSNP <sup>+</sup> ASA <sup>+</sup> <i>n</i> (%)	$\chi^2$ ( <i>P</i> value) <sup>a</sup> OR <sup>b</sup> (95% CI)	$\chi^2$ ( <i>P</i> value) <sup>a</sup> OR <sup>b</sup> (95% CI)
TNF $\alpha$ -308 G>A	169	266		60		
Genotype frequency						
GG	122 (72.2)	189 (71.0)	0.79 <sup>c</sup>	34 (56.7)	<b>0.03<sup>d</sup></b>	<b>0.03<sup>e</sup></b>
GA	41 (24.3)	68 (25.6)	1.1 (0.7–1.6)	23 (38.3)	2.0 (1.1–3.7)	1.9 (1.1–3.3)
AA	6 (3.5)	9 (3.4)		3 (5.0)		
Allele frequency						
G	285 (84.3)	446 (83.8)	0.84 <sup>c</sup>	91 (75.8)	<b>0.04<sup>d</sup></b>	<b>0.04<sup>e</sup></b>
A	53 (15.7)	86 (16.2)	1.0 (0.7–1.5)	29 (24.2)	1.7 (1.0–2.9)	1.7 (1.0–2.9)

CRSNP<sup>+</sup>ASA<sup>+</sup>/ASA<sup>-</sup>, chronic rhinosinusitis occurring with nasal polyposis and with (ASA<sup>+</sup>) or without (ASA<sup>-</sup>) aspirin sensitivity.

<sup>a</sup>Chi-square analysis with the Pearson correction. <sup>b</sup>The OR for the combination of both homozygote and heterozygote carriers of the minor alleles was determined in comparison with that for homozygote carriers of the common alleles. <sup>c</sup>Statistical analysis was done comparing the control and the CRSNP<sup>+</sup>ASA<sup>-</sup> groups. <sup>d</sup>Statistical analysis was done comparing the control and the CRS NP<sup>+</sup>ASA<sup>+</sup> groups. <sup>e</sup>Statistical analysis was done comparing the CRSNP<sup>+</sup>ASA<sup>-</sup> and CRSNP<sup>+</sup>ASA<sup>+</sup> groups. Bold text indicates values that are significant at the 0.05 level.



**Table 4.** Comparative analysis of the previously published genetic studies investigating the association of the *TNFA* –308 G>A SNP and CRS

Percent of GA+AA in controls	Percent of ASA+ in CRSNP+ patients	Association detected	Controls in HWE	Analysed population	Authors
21.7%	14.6%	Yes	No	Turkish	Erbek (2007) (24)
11.5%	16.4%	Yes	Yes	US Caucasian	Bernstein (2009) (23)
—	38.8% <sup>a</sup>	No	Yes	Canadian	Mfuna-Endam (2010) (25)
6.7%	34.0%	Yes	No data	Turkish	Batikhan (2010) (22)
27.8%	18.4%	Yes <sup>b</sup>	Yes	Hungarian	Present data

ASA+, aspirin sensitivity; CRSNP+, chronic rhinosinusitis occurring with nasal polyposis.

<sup>a</sup>Estimated value based on the reported proportions of NP+ and ASA+ patients in the overall CRS cohort. <sup>b</sup>Association was detected only between the rare A allele and the ASA+ group of CRSNP+ patients.

can also result in the establishment of chronic inflammation and the subsequent development of NP.

Hereditary genetic factors appear to be important in the genetic predisposition to CRS (15, 16), and it was also proposed in the literature that polymorphisms that participate in the regulation and/or function of pro-inflammatory cytokines may be involved in the pathogenesis of the disease. In this respect, polymorphisms of the promoter region of the *TNFA* locus have already been analyzed in Turkish (22, 24), Canadian (25) and US Caucasian (23) individuals, with special attention to the –308 G>A SNP. The results of these investigations are conflicting, however, for reasons that are currently unknown.

In order to investigate whether the well-known and relatively frequent *TNFA* –308 G>A promoter polymorphism predisposes carrier individuals to the development of CRS in the Hungarian population, we performed a case–control study to compare the frequencies of various genotypes and alleles in control individuals and in CRS patients. The results of the previous studies combined with our data led us to categorize our patients into clinically more homogeneous subgroups. Detailed analysis of these indicated that the proportion of individuals carrying the rare A allele differed between the controls and the CRSNP+ASA+ group ( $P = 0.03$  in the comparison of the genotypes and  $P = 0.04$  for the allele frequencies), and also comparing the CRSNP+ASA– versus the CRSNP+ASA+ groups ( $P = 0.03$  in the comparison of the genotypes and  $P = 0.04$  for the allele frequencies). These data suggest that the CRSNP+ASA+ subgroup is a genetically unique entity within the group of CRS patients, at least in the studied Hungarian population, in which the rare A allele of the studied *TNFA* –308 SNP can be regarded as a genetic susceptibility factor. This correlates well with the available clinical data, suggesting that this cohort of patients is a clinically distinct group that often demonstrates extreme resistance to therapy and in whom the incidence of relapses after surgical removal of the polypoid tissues is very high.

Next, we compared our results with the already published data, investigating the role of the *TNFA* –308 SNP in the genetic predisposition to CRS in different populations (22–25), but this turned out to be somewhat challenging. The reason, however, for the differences is not clear. Differences in study design and selection criteria utilized to recruit patients and controls can offer only a partial explanation. Another important factor to be considered is the ethnicity of the study populations. It has

been noted earlier both by ourselves and by other researchers that the frequency of the rare A allele-containing genotypes varies greatly already in the general population in different ethnic groups (31, 32). Discrepancies can also be observed with regard to whether a given –308 A allele predisposes the carrier individuals to the same diseases in different ethnic populations (31, 32). Thus, comparison of all these data with our current results strongly argues for the need of multicentric, systematic clinical and/or genetic investigations performed according to consensus patients' inclusion/exclusion criteria. Such investigations would help us to identify clear population-related differences, e.g. in the percentage of NP+ or ASA+ individuals among the CRS cohort or in the inherited genetic predisposing or protective factors.

The differences observed in various studies of CRS can formally also suggest that it is maybe not the A allele of the *TNFA* –308 G>A SNP itself that is responsible for the observed associations. Alternatively, an extended linkage group on the short arm of chromosome 6 around the *TNFA* locus may rather be responsible for the genetic predisposition to the investigated disease. This, together with the existence of population-specific linkage groups, could explain the observed population-related differences.

In our study, the gathered clinical and genetic data suggest that the overall group of CRS patients is indeed heterogeneous. Many differences have been described in the clinical characteristics of these patients (severity of inflammation, sensitivity to ASA), and severe cases often exhibit extreme treatment resistance and the recurrence of symptoms after the surgical resection of polypoid tissues. All these data suggest that not only the genetic predisposing factors but also the actual disease pathogenesis at the molecular level may differ in the various CRS subgroups.

Limitations of the current study arise from the moderate size of the cohort that was used for the analyses (169 controls and 375 CRS patients). We believe, however, that in the era of robust genome-wide association studies carefully designed classical case–control studies of such moderately sized study cohorts can still have their significance. Such targeted studies can investigate the relevance of the observed associations reported by large-scale analyses. Also, because of their relative ease and less cost-effective nature they can be used to detect population-related differences, as well as discover special cohorts within larger patient groups differ in terms of

the exact disease pathogenesis. Information gathered from such studies can be used to develop more sophisticated patient inclusion/exclusion criterion systems in order to generate homogenous study groups for future high-throughput analyses.

### Supplementary data

Supplementary data are available at *International Immunology* Online.

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### References

- Wang, X. and Cutting, G. R. 2011. Chronic rhinosinusitis. *Adv. Otorhinolaryngol.* 70:114.
- Boulet, L. P. 2009. Influence of comorbid conditions on asthma. *Eur. Respir. J.* 33:897.
- Guilemany, J. M., Mariño-Sánchez, F. S., Angrill, J. *et al.* 2011. The importance of smell in patients with bronchiectasis. *Respir. Med.* 105:44.
- Hellings, P. W. and Prokopakis, E. P. 2010. Global airway disease beyond allergy. *Curr. Allergy Asthma Rep.* 10:143.
- Joe, S. A. and Thakkar, K. 2008. Chronic rhinosinusitis and asthma. *Otolaryngol. Clin. North Am.* 41:297.
- Krause, H. F. 2003. Allergy and chronic rhinosinusitis. *Otolaryngol. Head Neck Surg.* 128:14.
- Huvenne, W., van Bruaene, N., Zhang, N. *et al.* 2009. Chronic rhinosinusitis with and without nasal polyposis: what is the difference? *Curr. Allergy Asthma Rep.* 9:213.
- Gosepath, J. and Mann, W. J. 2005. Current concepts in therapy of chronic rhinosinusitis and nasal polyposis. *ORL J. Otorhinolaryngol. Relat. Spec.* 67:125.
- Klimek, L. and Pfaar, O. 2009. Aspirin intolerance: does desensitization alter the course of the disease? *Immunol. Allergy Clin. North Am.* 29:669.
- Szczeklik, A., Gryglewski, R. J. and Czerniawska-Mysik, G. 1975. Relationship of inhibition of prostaglandin biosynthesis by analgesics to asthma attacks in aspirin-sensitive patients. *Br. Med. J.* 1:67.
- Wood, A. J. and Douglas, R. G. 2010. Pathogenesis and treatment of chronic rhinosinusitis. *Postgrad. Med. J.* 86:359.
- Fokkens, W. J., Ebbens, F. and van Drunen, C. M. 2009. Fungus: a role in pathophysiology of chronic rhinosinusitis, disease modifier, a treatment target, or no role at all? *Immunol. Allergy Clin. North Am.* 29:677.
- Orlandi, R. R. and Marple, B. F. 2010. The role of fungus in chronic rhinosinusitis. *Otolaryngol. Clin. North Am.* 43:531.
- Tieu, D. D., Kern, R. C. and Schleimer, R. P. 2009. Alterations in epithelial barrier function and host defense responses in chronic rhinosinusitis. *J. Allergy Clin. Immunol.* 124:37.
- Mfuna-Endam, L., Zhang, Y. and Desrosiers, M. Y. 2011. Genetics of rhinosinusitis. *Curr. Allergy Asthma Rep.* 11:236.
- Ooi, E. H., Psaltis, A. J., Witterick, I. J. and Wormald, P. J. 2010. Innate immunity. *Otolaryngol. Clin. North Am.* 43:473.
- Bernstein, J. M. 2001. The molecular biology of nasal polyposis. *Curr. Allergy Asthma Rep.* 1:262.
- Lennard, C. M., Mann, E. A., Sun, L. L., Chang, A. S. and Bolger, W. E. 2000. Interleukin-1 beta, interleukin-5, interleukin-6, interleukin-8, and tumor necrosis factor-alpha in chronic sinusitis: response to systemic corticosteroids. *Am. J. Rhinol.* 14:367.
- Knight, J. C. 2005. Regulatory polymorphisms underlying complex disease traits. *J. Mol. Med.* 83:97.
- Smith, A. J. and Humphries, S. E. 2009. Cytokine and cytokine receptor gene polymorphisms and their functionality. *Cytokine Growth Factor Rev.* 20:43.
- Waldron-Lynch, F., Adams, C., Shanahan, F., Molloy, M. G. and O'Gara, F. 1999. Genetic analysis of the 3' untranslated region of the tumour necrosis factor shows a highly conserved region in rheumatoid arthritis affected and unaffected subjects. *J. Med. Genet.* 36:214.
- Batikhan, H., Gokcan, M. K., Beder, E., Akar, N., Ozturk, A. and Gerceker, M. 2010. Association of the tumor necrosis factor-alpha -308 G/A polymorphism with nasal polyposis. *Eur. Arch. Otorhinolaryngol.* 267:903.
- Bernstein, J. M., Anon, J. B., Rontal, M., Conroy, J., Wang, C. and Sucheston, L. 2009. Genetic polymorphisms in chronic hyperplastic sinusitis with nasal polyposis. *Laryngoscope* 119:1258.
- Erbek, S. S., Yurtcu, E., Erbek, S., Atac, F. B., Sahin, F. I. and Cakmak, O. 2007. Proinflammatory cytokine single nucleotide polymorphisms in nasal polyposis. *Arch. Otolaryngol. Head Neck Surg.* 133:705.
- Mfuna-Endam, L., Cormier, C., Bossé, Y., Filali-Mouhim, A. and Desrosiers, M. 2010. Association of IL1A, IL1B, and TNF gene polymorphisms with chronic rhinosinusitis with and without nasal polyposis: a replication study. *Arch. Otolaryngol. Head Neck Surg.* 136:187.
- Wilson, A. G., di Giovine, F. S., Blakemore, A. I. and Duff, G. W. 1992. Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. *Hum. Mol. Genet.* 1:353.
- Rodriguez, S., Gaunt, T. R. and Day, I. N. 2009. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *Am. J. Epidemiol.* 169:505.
- Van Cauwenberge, P. and Watelet, J. B. 2000. Epidemiology of chronic rhinosinusitis. *Thorax* 55(Suppl. 2):S20.
- Kato, A. and Schleimer, R. P. 2007. Beyond inflammation: airway epithelial cells are at the interface of innate and adaptive immunity. *Curr. Opin. Immunol.* 19:711.
- Takeuchi, O. and Akira, S. 2010. Pattern recognition receptors and inflammation. *Cell* 140:805.
- Baz, K., Emin Erdal, M., Yazici, A. C. *et al.* 2008. Association between tumor necrosis factor-alpha gene promoter polymorphism at position -308 and acne in Turkish patients. *Arch. Dermatol. Res.* 300:371.
- Szabó, K., Tax, G., Teodorescu-Brinzeu, D., Koreck, A. and Kemény, L. 2011. TNFα gene polymorphisms in the pathogenesis of acne vulgaris. *Arch. Dermatol. Res.* 303:19.