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Chronic rhinosinusitis in Asia

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Current perspectives: Chronic rhinosinusitis in Asia

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Abstract

Chronic rhinosinusitis (CRS), although possibly overdiagnosed, in those truly affected, is associated with a high burden of disease and often difficult to treat. Recent research has demonstrated that inflammatory signatures of CRS vary around the world, with less eosinophilic and more neutrophilic inflammation found in Asia compared to Europe and North America. Whereas in the Western world, about 80% of nasal polyps carry a type-2 signature, this may be between 20% and 60% in China and Korea or Thailand, respectively. These differences are associated with a lower asthma co-morbidity and risk of disease recurrence after surgery in the Asian population. As a hallmark of severe type-2 inflammation, eosinophils attacking *Staphylococcus aureus* at the epithelial barrier have recently been described; they also can be found in a subgroup of Asian patients with nasal polyps. Furthermore, the percentage of type-2 signature disease in CRS is dramatically increasing ("eosinophilic shift") in several Asian countries over the last 20 years. Establishing an accurate diagnosis along with considering the current and shifting patterns of inflammation seen in Asia will enable more effective selection of appropriate pharmacotherapy, surgical therapy, and eventually biotherapy. Determining the causes and pathophysiology for this "eosinophilic shift" will require additional research.

Keywords

Chronic rhinosinusitis, nasal polyps, pheno- and endotypes, asthma co-morbidity, type-2 inflammation, EETs

52 **Abbreviations**

53	AERD	Aspirin-exacerbated respiratory disease
54	CRS	Chronic rhinosinusitis
55	CRSsNP	Chronic rhinosinusitis without nasal polyps
56	CRSwNP	Chronic rhinosinusitis with nasal polyps
57	ECP	Eosinophilic cationic protein
58	EETs	Eosinophilic extracellular traps
59	GATA-3	Th2-cell transcription factor
60	GWAS	genome-wide association studies
61	HRQoL	Health-related Quality of life
62	MPO	Myeloperoxidase
63	NETs	Neutrophil extracellular traps
64	SE-IgE	Staphylococcus aureus enterotoxin-specific IgE

Epidemiology and burden of disease of CRS

Chronic rhinosinusitis (CRS), an inflammatory condition of the nose and sinuses, is a common medical condition that affects about 11% of adults in Europe (1) and about 12% of adults in the United States (2). CRS has a significant impact on health-related quality of life (HRQoL) (3, 4) and is associated with substantial healthcare (5-7) and productivity costs (8). The prevalence of CRSwNP in Europe is estimated between 2.1% (France) (9) to 4.4% (Finland) (10), and 4.2% in the USA (11).

CRS is one of the most commonly diagnosed diseases of the upper airways not only in western countries, but increasingly also in Asia. Overall, studies have indicated wide variations in the prevalence of CRSsNP and CRSwNP between Asian and European countries (Table 1). Using telephone (12) or face-to-face interview (13), multicenter studies from China reported that the prevalence of CRS by European Position Paper on Rhinosinusitis and nasal Polyps (EP³OS) diagnostic criteria was 2.2% (14) or 8% (15), respectively. The patients perceived themselves as having impaired quality of life (16). Since the CRS definitions of those population-based surveys were solely based on symptomatic criteria, the self-reported prevalence of CRS may be overestimated (17). Specifically, headache or migraine not related to sinusitis may be wrongly taken for sinusitis symptoms in epidemiological studies, or misdiagnosed in clinical settings. The diagnostic accuracy of CRS therefore needs to be increased by the addition of objective findings, either direct visualization or sinus imaging ; accurately identifying individuals with CRS requires direct visualization of polyps or purulent secretions coming from paranasal sinus ostia by nasal endoscopy and/or polyps, air/fluid levels, and osteomeatal complex obstruction on sinus CT. In two Korean nationwide surveys, the prevalence of CRS, defined by symptoms plus positive endoscopic findings, were 6.95% (18) and 8.4% (19). The prevalence of CRS with nasal polyps (CRSwNP) diagnosed by endoscopy in the general population was 2.5% (20) or 2.6% (21) in Korea, very similar to the 2.7% reported in Sweden (19). In China, a prevalence of 1.1% was reported (13).

95 CRS is often associated with lower airway disease, and comorbid diseases have also
 96 been recognized in Asia (22-24). However, the incidences of concomitant asthma and
 97 aspirin-exacerbated respiratory disease (AERD) in Chinese patients with CRS were
 98 low compared to Caucasian patients (25,26) associated with differences in the
 99 inflammatory cytokine profile (27). Over a period of 12 years, the prevalence of
 100 comorbid asthma increased from 8 to 18% in a CRSwNP population in Bangkok,
 101 together with an increase in the incidence of eosinophilic polyps and *Staphylococcus*
 102 *aureus* colonization (28).

103

104 There are limited data evaluating the disease burden of CRS in Asia (29). A
 105 multi-country, cross-sectional, observational study which examined the economic
 106 burden of respiratory diseases in the Asia-Pacific region including allergic rhinitis,
 107 CRS, asthma and COPD, demonstrated that on average, patients were impaired for
 108 one-third of their time at work and 5% of their work time missed, which resulted in a
 109 36% reduction in productivity. CRS patients most frequently visited a specialist (29).
 110 Also, CRS patients reported higher work productivity loss than other respiratory
 111 diseases in Korea, Taiwan (30) and Thailand (31).

112

113 **Genetic and epigenetic considerations**

114 Chronic rhinosinusitis (CRS) is a complex disease; both genetic and environmental
 115 components are likely to contribute to its pathogenesis (32). The presence of nasal
 116 polyposis in cystic fibrosis, a common genetic disorder characterized by mutations in
 117 the CFTR gene, provides an important example how genetically determined
 118 alterations may lead to the development of CRS (33). Like for other common diseases,
 119 candidate gene or genome-wide association studies (GWAS) have been applied in the
 120 genetic exploration of CRS. A variety of cytokines, cytokine receptors, immunity and
 121 mucosal airway remodeling related molecules have been associated with CRS in
 122 multi-ethnic populations. Among them, only two polymorphisms in the IL1alpha
 123 (rs17561) and tumor necrosis factor-alpha (rs1800629) genes have been replicated
 124 (34). Representative genetic and epigenetic studies conducted in CRS in the Asian

125 population are summarized in Table 2. Relatively few single gene association studies
126 in CRS were performed in Asia, and no susceptibility genes and loci reported to be
127 associated in the Asian population (HLA, TGFB1, TSLP, IL1RN, IL4, TLR2, EBI3,
128 MMP9, MMP2 (35-44) could be replicated in another cohort. Concerning GWAS, to
129 date there is only one DNA pool-based GWAS performed in Caucasian CRS patients
130 and control subjects (45). Zhang et al (46) tried to replicate 17 CRS susceptibility
131 genes obtained from this study in a Han Chinese population, and could only confirm
132 one corresponding SNP locus (rs4504543) in the acyloxyacyl hydroxylase (AOAH)
133 gene. This revealed the potential of a common as well as a different genetic basis in
134 the development of CRS in Chinese and Caucasian populations.

135 Other than genetics, epigenetic mechanisms offer yet another possibly more plausible
136 explanation, particularly focusing on the ability of environmental factors to shape
137 health and disease (47, 48). A recent Korean study examined genome-wide DNA
138 methylation levels in CRSwNP tissues and peripheral blood cells collected from
139 aspirin-intolerant (AIA) and aspirin-tolerant asthma (ATA) patients and demonstrated
140 characteristic methylation patterns affecting 337 genes in AIA patients (49). The
141 promoter hypermethylation of COL18A1 was confirmed in CRSwNP tissues in a
142 Chinese cohort (50). Overexpression of miR-125b was reported in eosinophilic
143 CRSwNP individuals and involved in the regulation of innate immunity, affecting the
144 IFN pathway (51). Furthermore, candidate miRNAs that might regulate dendritic cells
145 were identified (52).

146 A very recent study demonstrated a distinct transcriptome in eosinophilic vs.
147 non-eosinophilic CRSwNP (53), a possible reason for the heterogeneity and poor
148 repeatability of the genetic findings among different ethnic groups with different
149 distributions of those subgroups. It is evident that the differentiation of CRS into
150 endotypes rather than phenotypes will offer further opportunities to discover genetic
151 and epigenetic patterns in CRS across the Western world and Asia (54, 55), and by
152 comparing those findings we might be able to identify and confirm key molecules.

153

154 **Cytokine profiles in CRS**

Before 2005, our knowledge on inflammatory patterns in CRS nearly exclusively came from studies in Western patients, and mostly was based on mean values in either CRS without or with nasal polyps (56, 57). Those studies indicated that nasal polyps were “eosinophilic” and characterized by the expression of interleukin (IL)-5 and other Th2 cytokines, whereas CRSsNP resembled a Th1 disease with expression of IFN-gamma. However, the above immunological patterns of CRS were not universal in all ethnic population; a first study in South China indicated less activated eosinophils in Chinese nasal polyps compared to European polyps, as well as a less prominent increase in IgE (58). About a third of polyp samples, but none of the controls, contained IgE antibodies to staphylococcal enterotoxins (SE-IgE). An extended analysis of transcription factors, cytokines and cellular infiltrates in South Chinese vs. Belgian polyp samples later clarified that tissue from Caucasian patients with CRSwNP displayed a significant increase in the Th2 cytokine IL-5 and the Th2 transcription factor GATA-3 with eosinophilic inflammation (ECP/MPO ratio > 1) versus control subjects, but Chinese patients with CRSwNP showed a Th1/Th17 cell pattern with neutrophilic inflammation (ECP/MPO ratio < 1) versus control tissue (59). Both CRSwNP groups demonstrated a significant downregulation of Foxp3 expression and TGF- β 1 protein content versus their respective control groups. Similarly, Cao et al found Central South Chinese patients with CRSsNP had higher levels of IFN- γ expression, whereas only a subpopulation of eosinophilic CRSwNP demonstrated an enhanced expression of GATA-3 and IL-5 (60). The above studies clearly indicated immunological heterogeneity among different regions within the same disease phenotype. This offered another opportunity to learn from the differences: 83% of the Belgian, but only 16% of the polyp samples from Sichuan province were IL-5 protein positive, and 34% vs 9% ($P < .01$) of the subjects suffered from asthma comorbidity, respectively. Statistical methods allowed recognizing SE-IgE and total IgE as the most important predictors for asthma using categorical and continuous classifying determinants in the European, and IL-5 for categorical and continuous classifying determinants in the Chinese population (61). For the first time, the central role of the type-2 immune reaction and IgE for asthma comorbidity was

unravelling, and the difference in asthma comorbidity between these ethnic groups understood.

187

188 **CRS endotypes and asthma comorbidity**

189 Using cluster analysis in CRS samples from 11 European centers, Tomassen et al (54)
 190 recently described 3 endotypes of CRS with and without nasal polyps with different
 191 expression patterns of Th cytokines, inflammatory biomarkers and IgE. According to
 192 this investigation, CRS can be differentiated in a non-type-2, a moderate and a severe
 193 type-2 inflammation, with a clear increase in nasal polyp phenotype (from less than
 194 15% to more than 90%) and asthma co-morbidity from the first (about 5%) to the last
 195 (60 - 70%). Based on this analysis, Wang et al (55) described Th cytokine and marker
 196 profiles in CRS patients of three Continents: Asia (China and Japan), Europe (Benelux
 197 and Germany) and Australia and demonstrated a remarkable diversity in TH cytokine
 198 signatures between regions, showing that CRSwNP tissues in Europe and Australia
 199 were characterized by a stronger expression of type-2 inflammation than in Asia;
 200 however, within Asia, the expression patterns varied from low type-2 expression in
 201 Chengdu/China to moderate expression in Beijing and Japan. These differences were
 202 also reflected in CRSsNP tissues, but to a lower degree; also the presence of SE-IgE
 203 antibodies within the polyp tissues showed significant variation, in parallel to the
 204 type-2 inflammation signatures. These observations may be impacted by air pollution,
 205 as reported from large Asian cities, with a variety of pollutants acting on the nasal
 206 mucosa; however, a longitudinal cohort study of schoolchildren indicated that
 207 specifically exposure to particulate matter PM(2.5) might induce neutrophilic nasal
 208 inflammation (62) under real-life conditions.

209

210 The question arises whether these differences in type-2 signatures all over the world
 211 would stay over time or would be matter to change; in fact, there are several
 212 publications showing a dramatic change in the expression of eosinophilic disease
 213 within the CRSwNP population (28, 63, 64). A first report from Thailand made use of
 214 CRSwNP patients visiting the same hospital in Bangkok in 1999 and 2011, and

demonstrated that in the tissue samples eosinophils, IgE positive cells, and also the concentrations of IL-5 and other inflammatory markers increased significantly; this “eosinophilic shift” was accompanied by *higher Staphylococcus aureus* carriage, as well as higher frequencies of *S. aureus* invasion into the tissue (29). Patients with asthma were more likely to have higher SA carriage rates compared with non-asthmatic patients. This observation of an increasing eosinophilic signature of CRSwNP was also reported in South Korea over 10 and 17 years (63, 64) and was unraveled in Beijing recently (unpublished data). In Korea, the number of eosinophilic cells tripled and the prevalence of eosinophilic polyps doubled within 17 years, accompanied by basement membrane thickening and glandular hyperplasia (64). As type-2 inflammatory reactions are not only associated with co-morbid asthma, as discussed above, but also recurrence of nasal polyp disease, the “eosinophilic shift” in Asia could lead to a significantly larger burden of disease in terms of need for pharmacological treatment for the upper and lower airways, as well as sinus surgery. This latter association has been described in Europe (65), but also Japan (66) and China (67), with tissue eosinophil counts $\geq 54.5\%$ showing the highest polyp recurrence rate (68).

Taking these observations together, it is evident that there is a difference in the inflammatory signatures of both, CRSsNP and CRSwNP, with less type-2 inflammation in parts of Asia. E.g., the expression of IL-5 as a type-2 marker cytokine is as low as 16% in CRSwNP in central China vs. 83% in the Benelux, and in a greater proportion of the patients, none of the principal Th2, 2 or 17 cytokines is expressed in the tissues at all. This is remarkable, as these differences are associated with a lower asthma co-morbidity and risk for recurrence in the Asian population. However, the endotypes described in a European population recently (55) are reflected in the Asian population, and it is rather the distribution of the endotypes within a phenotype than the signatures of the endotypes themselves, which are different between Asia and Europe, and between specific regions within Asia. Whereas in the Western world, about 80% of nasal polyps carry a type-2 signature, this may be between 20% and 60% in China and Korea or Thailand, respectively;

245 furthermore, this percentage is dramatically increasing (“eosinophilic shift”) in
246 several Asian countries over the last 20 years, associated with a higher disease burden
247 (28, 63, 64).

248

249

250 Extracellular eosinophilic traps and *S. aureus*

251 Recently it was reported that eosinophils generate so-called extracellular eosinophilic
252 traps (EETs) at epithelial barrier defects of patients with severe CRSwNP in
253 Caucasian populations. In these patients, the amount of EET formation was linked to
254 elevated levels of IL-5 and correlated with *S. aureus* colonization (69); in an ex-vivo
255 mucosal challenge model, it was also demonstrated that *S. aureus* was a triggering
256 factor for EET formation. One hour exposure of tissue fragments from patients with
257 CRSwNP to the germs enhanced EET formation markedly (3.5-fold increase) using
258 an in vitro migration assay, with up to 60% of eosinophils contributing to EETs
259 compared with non-exposed controls (69). The presence of eosinophils releasing
260 EETs was focused at sites where *S. aureus* was observed and concentrated in the
261 subepithelial region, entrapping the germ specifically at sites of epithelial damage.
262 This effect was *S. aureus*-specific and could not be observed with *S. epidermidis*.
263 Isolated cells from nasal secretions of patients with CRSwNP were also shown to be
264 extremely reactive, with massive EET formation upon ex vivo challenge with *S.*
265 *aureus* for 15 minutes without any priming. The bacteria were finally found to be
266 entrapped in the massive EET network. Ueki and coworkers for the first time
267 described the presence of eosinophilic DNA traps in secretions of eosinophilic polyps
268 in a Japanese population (70). In analogy to our previous studies, showing the
269 presence of the type-2 endotype in Asian populations of CRS patients (56) and the
270 formation of EETs in the European counterparts (1), our group has investigated EET
271 formation in polyp tissues of Chinese patients. Unpublished data show that Chinese
272 CRSwNP patients of the type-2 endotype (characterized by IL5 positive polyps)
273 displayed similar patterns as the European polyps, with subepithelial recruitment of
274 eosinophils and extracellular eosinophilic DNA trap formation ($n = 7$, mean $18.7 \pm$
275 12.1 % EET forming eosinophils, figure 1) resulting from both EET formation
276 (extracellular traps generated by viable and intact cells) and cytolysis (extracellular
277 traps generated by lysis of the eosinophils), in all IL-5 positive polyps. This finding
278 showed strong similarities with the European polyps described before (69);
279 furthermore, no extracellular eosinophilic DNA traps were found in healthy controls

(inferior turbinates, n=7) or IL-5 negative polyp tissues (n=13) and in none of the groups, the neutrophils present were found to form neutrophil extracellular traps (NETs) in the tissues.

In addition, 4 out of 7 IL-5 positive Chinese polyps stained positive for *S. aureus*, and all stained negative for *P. aeruginosa* (determined by Fluorescence in situ hybridization using peptide nucleic acid probes (PNA FISH)). None of the individuals (controls/patients) stained positive for Panfungus (covering detection of all fungal species) or *E. coli* using PNA FISH (unpublished data). In addition, the amount of eosinophilic trap formation correlated positively with *S. aureus* presence.

These data confirm the presence of eosinophilic DNA traps as a strong type-2 associated phenomenon in severe CRSwNP in the European but also Chinese population. In analogy with the Caucasian population, *S. aureus* is likely to be involved in the presence of eosinophilic DNA traps in eosinophilic Chinese polyps indicating that, regardless of the studied population, the same mechanisms play in CRSwNP patients with a dominant Th2 profile (Figure 2).

Hypothetical therapeutic consequences

Whereas the differences in inflammatory signatures between Asian and European countries would indicate that optimal therapeutic approaches should differ between continents, the lately observed “eosinophilic shift” would however point to an adjustment of treatment approaches within the near to midterm future also in Asia. For example, a study in China showed that the therapeutic response to oral glucocorticosteroids very much depends on the degree of eosinophilia in polyp disease (71). In contrast, the clinical response to clarithromycin is more favorable in neutrophilic disease (72). Thus, the knowledge of the inflammatory endotypes prevalent within a region or country may be decisive to prescribe an adequate pharmacotherapy for the majority of patients.

Surgical techniques in CRS are very much aligned to the “functional endoscopic sinus surgery” concept since its introduction in the 80’s by Messerklinger and Stammberger, Wigand and Hosemann and Kennedy in the US (73-75); however, it turned out that

310 this approach does not serve all situations, and specifically in severe nasal polyposis
311 of the sinuses, recurrence rates are unacceptably high (76 - 78); studies showed that an
312 “extended approach (“nasalization, full house FESS”) could possible deliver better
313 results (77, 78). Studies in recurrent polyposis showed that type-2 inflammatory
314 markers substantially increase the risk for recurrence, and possibly ask for less
315 “minimally invasive” approaches compared to type-1 inflammatory signatures in
316 CRSSNP or CRSwNP (79). We therefore recently proposed to also differentiate
317 surgical approaches on the basis of the inflammatory endotypes (80), with minimally
318 invasive “functional” approaches in non-type 2 inflammation and a total removal of
319 the diseased mucosa from all sinuses (including the frontal and sphenoid sinuses)
320 together with the resident microbiome in the severe type-2 inflammation (“reboot”)
321 and regrowth of healthy mucosa, based on the cluster analysis of Tomassen et al (54).
322 Once this approach has been confirmed in appropriate studies, it would have to be
323 adapted in Asia, with a currently better prognosis compared to Europe after minimally
324 invasive surgery in CRSwNP, which may deteriorate over time due to the above
325 discussed “eosinophilic shift”.

326
327 Finally, T helper cell (Th) cytokines and IgE have been demonstrated to play key
328 regulatory roles in the pathomechanisms of CRS, which have been convincingly
329 confirmed by the effects of therapeutic utilization of monoclonal antibodies, such as
330 anti-IL5, anti-IL-4/IL-13, and anti-IgE (81-83). These innovative treatment
331 approaches do more than any other approach rely on the inflammatory signature of the
332 sinus disease, and an appropriate indication for any of these biotherapeutics is
333 mandatory. Dupilumab, Mepolizumab and Omalizumab have demonstrated clinical
334 efficacy and safety in proof-of-concept studies in carefully selected populations, and
335 are currently approaching registration studies (81-83). So far, no studies using these
336 monoclonal antibodies have been carried out in CRSwNP in Asia, and it can be
337 estimated that the percentage of CRSwNP patients in Asia eligible for treatment will
338 be smaller than in Europe or the US at the moment; however, also this percentage
339 might change in the future, and the need and eligibility for monoclonal antibodies

may considerably increase.

Unmet needs and future directions

Precision medicine has just started to be appreciated for the upper airways in Europe and the US, and biotherapeutics will soon reach the market and will further speed up this development. Studies on inflammatory endotypes and their relevance for recurrence of disease and asthma co-morbidity and the verification and mapping of the “eosinophilic shift” are of great importance to optimize care pathways in Asia as well, but also offer a unique chance to better understand disease pathomechanisms and possibly causal relationships (Table 3). It also needs clarification whether similar differences in the inflammatory endotypes and changes over time apply to other diseases such as asthma. These observations may finally optimize the management of airway diseases in terms of timing of interventions, optimal approaches in pharmacotherapy and surgery, and use of biologicals in Asia, but also world-wide.

358 Table 1. Studies documenting variation in prevalence of CRSs/wNP between Asian
 359 and European countries.

<u>Asia/ Europe</u>	<u>Publication year</u>	<u>Region/ Country</u>	<u>Population characteristics</u>	<u>Study design/ method for assessing prevalence</u>	<u>% Pre of CR CRSw</u>
<u>Asia</u>	<u>2016</u>	<u>18 major cities in mainland China</u>	<u>36,577 respondents</u>	<u>Telephone interview</u>	<u>2.1%</u>
	<u>2015</u>	<u>7 Chinese cities</u>	<u>10,636 respondents</u>	<u>Face-to-face interview</u>	<u>8% C CRSw</u>
	<u>2011</u>	<u>KNHANES</u>	<u>4,098 participants</u>	<u>Interview regarding nasal symptoms and endoscopic examination</u>	<u>6.95%</u>
	<u>2016</u>	<u>KNHANES (2008-2012)</u>	<u>28,912 adults (aged ≥ 20 years)</u>	<u>Interview regarding nasal symptoms and endoscopic examination</u>	<u>8.4% 2.6%</u>
	<u>2015</u>	<u>KNHANES (2009-2011)</u>	<u>19,152 participants (aged ≥ 20 years)</u>	<u>Interview regarding physical examinations and endoscopic examination</u>	<u>2.5%</u>
<u>Europe</u>	<u>2011</u>	<u>19 centers in 12 countries in Europe</u>	<u>57,128 adults aged 15-75 years</u>	<u>Postal questionnaire</u>	<u>10.9%</u>
	<u>2005</u>	<u>France</u>	<u>10,033 subjects (aged ≥ 18 years)</u>	<u>A kind of validated questionnaire/algorithm (90% specificity and sensitivity)</u>	<u>2.1%</u>
	<u>1999</u>	<u>Southern Finland</u>	<u>4,300 of adults aged 18-65 years</u>	<u>Postal questionnaire</u>	<u>4.4%</u>
	<u>2003</u>	<u>Skövde, Sweden</u>	<u>1,387 subjects (aged > 20 years)</u>	<u>Interview regarding questions and endoscopic examination</u>	<u>2.7%</u>

360 CRS: Chronic rhinosinusitis

361 CRSwNP: Chronic rhinosinusitis with nasal polyps

362 KNHANES: Korean National Health and Nutrition Examination Survey

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366 Table 2. Summary of genetic and epigenetic studies conducted in chronic
 367 rhinosinusitis in Asia

<u>Genetic /epigenetic studies</u>	<u>Publication year</u>	<u>Population</u>	<u>Size of study</u>	<u>Study strategy or methods</u>	<u>Associated phenotype</u>	<u>Associated Variant and gene / methylation or miRNA</u>	<u>Reference</u>
<u>Genetic</u>	<u>2006</u>	<u>Korea</u>	<u>106 CRS patients and 70 controls</u>	<u>Candidate gene association study</u>	<u>CRSw NP</u>	<u>Interleukin-4 C-590T</u>	<u>34</u>
	<u>2006</u>	<u>Taiwan</u>	<u>61 CRSwNP patients, 27 CRSsNP patients and 103 controls</u>	<u>Candidate gene association study</u>	<u>CRSw NP</u>	<u>IL1RN polymorphism in intron 2</u>	<u>35</u>
	<u>2007</u>	<u>Chinese</u>	<u>30 CRSwNP patients and 81 controls</u>	<u>Candidate gene association study</u>	<u>CRSw NP</u>	<u>HLA-DR16, HLA-DQ8, and HLA-DQ9</u>	<u>36</u>
	<u>2007</u>	<u>Korea</u>	<u>203 AIA patients, 324 ATA patients, and 456 controls</u>	<u>Candidate gene association study</u>	<u>AIA with CRS</u>	<u>TGFbeta1-5'09C>T</u>	<u>37</u>
	<u>2009</u>	<u>Turkey</u>	<u>93 CRSwNP patients and 115 controls</u>	<u>Candidate gene association study</u>	<u>CRSw NP</u>	<u>MMP-9 -1562CT</u>	<u>38</u>
	<u>2010</u>	<u>Taiwan</u>	<u>203 CRSwNP</u>	<u>Candidate gene</u>	<u>CRSw NP</u>	<u>rs3918242 and</u>	<u>39</u>

			<u>patients</u> <u>and 730</u> <u>controls</u>	<u>associati</u> <u>on study</u>	<u>rs2274756</u> <u>in MMP-9</u>	
	<u>2011</u>	<u>Korea</u> <u>n</u>	<u>106 CRS</u> <u>patients</u> <u>and 108</u> <u>controls</u>	<u>Candidat</u> <u>e gene</u> <u>associati</u> <u>on study</u>	<u>CRS</u> <u>and</u> <u>rs3804099</u> <u>rs3804100</u> <u>in TLR2</u>	<u>40</u>
	<u>2012</u>	<u>Chines</u> <u>e</u>	<u>306</u> <u>CRSwNP</u> <u>patients,</u> <u>332</u> <u>CRSsNP</u> <u>patients</u> <u>and</u> <u>315control</u> <u>s</u>	<u>Candidat</u> <u>e gene</u> <u>associati</u> <u>on study</u>	<u>CRSw</u> <u>NP and</u> <u>CRSsN</u> <u>P</u> <u>in</u> <u>CRSwNP;</u> <u>AOAH rs45</u> <u>04543 and</u> <u>RYBP rs45</u> <u>32099 in</u> <u>CRSsNP</u>	<u>44</u>
	<u>2013</u>	<u>Chines</u> <u>e</u>	<u>306</u> <u>CRSwNP</u> <u>patientsts,</u> <u>332</u> <u>CRSsNP</u> <u>patients</u> <u>and 325</u> <u>controls</u>	<u>Candidat</u> <u>e gene</u> <u>associati</u> <u>on study</u>	<u>CRSw</u> <u>NP</u> <u>and</u> <u>rs764917 in</u> <u>TSLP</u>	<u>41</u>
	<u>2013</u>	<u>Chines</u> <u>e</u>	<u>360</u> <u>CRSwNP</u> <u>patients,</u> <u>306</u> <u>CRSsNP</u> <u>patients</u> <u>and 330</u> <u>controls</u>	<u>Candidat</u> <u>e gene</u> <u>associati</u> <u>on study</u>	<u>CRSw</u> <u>NP and</u> <u>CRSsN</u> <u>P</u> <u>rs428253 in</u> <u>EBI3</u>	<u>42</u>
<u>Epigen</u> <u>etic</u>	<u>2011</u>	<u>Korea</u> <u>n</u>	<u>5</u> <u>CRSwNP</u> <u>with AIA</u> <u>patients</u> <u>and 4</u> <u>CRSwNP</u>	<u>Genome-</u> <u>wide</u> <u>DNA</u> <u>methylati</u> <u>on by</u> <u>microarr</u>	<u>CRSw</u> <u>NP</u> <u>with</u> <u>AIA</u> <u>hypermethyl</u> <u>ation or</u> <u>hypomethyl</u> <u>ation in</u> <u>337 genes</u>	<u>47</u>

		<u>with ATA</u> <u>patients</u>	<u>ay</u> <u>analysis</u>			
<u>2015</u>	<u>Chines</u> <u>e</u>	<u>32</u> <u>CRSwNP</u> <u>patients</u> <u>and 18</u> <u>controls</u>	<u>DNA</u> <u>methylation</u> <u>on</u> <u>microarray</u>	<u>CRSw</u> <u>NP</u>	<u>Promoter</u> <u>methylation</u> <u>of</u> <u>COL18A1</u>	<u>48</u>
<u>2012</u>	<u>Chines</u> <u>e</u>	<u>43</u> <u>CRSsNP</u> <u>patients,</u> <u>46</u> <u>eosinophili</u> <u>c</u> <u>CRSwNP</u> <u>patients,</u> <u>31</u> <u>non-eosino</u> <u>philic</u> <u>CRSwNP</u> <u>patients</u> <u>and 50</u> <u>controls</u>	<u>miRNA</u> <u>microarray</u> <u>ays</u>	<u>eosinop</u> <u>hilic</u> <u>CRSw</u> <u>NP</u>	<u>up-regulated</u> <u>expression</u> <u>of miR-125b</u>	<u>49</u>
<u>2015</u>	<u>Chines</u> <u>e</u>	<u>7 CRSsNP</u> <u>patients, 8</u> <u>atopic</u> <u>CRSwNP</u> <u>patients, 7</u> <u>Non-topic</u> <u>CRSwNP</u> <u>patients</u> <u>and 7</u> <u>controls</u>	<u>MiRNA</u> <u>microarray</u> <u>ays</u>	<u>CRSsN</u> <u>P,</u> <u>atopic</u> <u>CRSw</u> <u>NP and</u> <u>Non-to</u> <u>pic</u> <u>CRSw</u> <u>NP</u>	<u>MiR-1290</u> <u>was</u> <u>down-regula</u> <u>ted</u> <u>in CRSsNP;</u> <u>up-regulated</u> <u>in both</u> <u>atopic</u> <u>CRSwNP</u> <u>and</u> <u>non-atopic</u> <u>CRSwNP</u>	<u>50</u>

368 Abbreviations used:

369 AIA: Aspirin-intolerant asthma

370 ATA: Aspirin-tolerant asthma

- 371 AOAH: Acyloxyacyl hydroxylase
- 372 COL18A1: Collagen, type XVIII, alpha 1
- 373 CRS: Chronic rhinosinusitis
- 374 CRSsNP: Chronic rhinosinusitis without nasal polyps
- 375 CRSwNP: Chronic rhinosinusitis with nasal polyps
- 376 EBI3: Epstein-Barr virus-induced gene 3
- 377 MMP-9: Matrix metalloproteinase-9
- 378 RYBP: Ring1A and YY1 binding protein
- 379 TGF: Transforming growth factor
- 380 TLR2: Toll-like receptor 2
- 381 TSLP: Thymic stromal lymphopoietin

382 **Table 3: Unmet needs: Studies to be performed in Asia, results to be compared to**
383 **other parts of the world**

384 Studies on inflammatory endotypes and their relevance for clinical parameters such as
385 recurrence of disease, asthma co-morbidity, in Asia

386 Optimization of patient care pathways including pharmacotherapy, surgical
387 approaches and eventl. biotherapeutics according to inflammatory endotypes

388 Identification of biomarkers suitable for endotyping and eventl. prediction of response
389 to biotherapeutics in Asia

390 Identification of relevant genetic findings, microbiome patterns and tissue gene
391 expressions according to inflammatory endotyping

392 Identification of relevant environmental factors impacting on mucosal inflammation

393 Verification and mapping of the “eosinophilic shift” in upper airway disease and
394 eventl. lower airway disease in various Asian countries

395 Studies to identify the causes for the “eosinophil shift” and eventl. measures to
396 prevent or reduce it

397 Studies on inflammatory endotypes in related diseases such as asthma and atopic
398 dermatitis and eventl. changes over time

399

400 **Fig. 1 Subepithelial eosinophils in IL-5 positive Chinese polyps.** Paraffin slides of
 401 IL-5 positive Chinese polyps were stained and analyzed using confocal microscopy.
 402 Eosinophils and EETs were stained with an anti-Major Basic Protein (MBP, green)
 403 antibody and DNA with propidium Iodide (DNA, red) as described in (69). **(A)** The
 404 epithelium (basal membrane indicated by a solid line and epithelial cells indicated by
 405 EC) shows frequent defects in nasal polyps. Eosinophils (MBP + cells, green) are
 406 mostly found near these defects (indicated by arrows). *Scale bar 50 μ m* **(B)** A
 407 magnification of the recruited eosinophils showed that groups of eosinophils produce
 408 extracellular traps (indicated by dashed line) near the defect as a result of both,
 409 cytolysis (lysed cells, dashed line) and extracellular eosinophilic trap (EET) formation
 410 (viable non lysed cells, indicated by arrowhead). *Scale bar 25 μ m*

411

412 **Fig. 2:** *S. aureus* and its products can evoke the release of epithelial cytokines like
 413 interleukin-33 (IL-33), Thymic stromal lymphopoietin (TSLP) and eotaxin, which
 414 directly or indirectly can cause extracellular eosinophilic trap (EET) formation
 415 (84-87). In addition, *S. aureus* and staphylococcal proteins can directly activate
 416 T-cells to release IL-5 resulting in EET formation in the eosinophils (88). Direct
 417 contact between eosinophils and *S. aureus* evokes EET formation, resulting in the
 418 killing of the bacteria, but also likely in epithelial damage (86). As a consequence, the
 419 barrier dysfunction remains or is worsened, resulting in more invasion and a cyclic
 420 enhancement of the Th2 inflammation. Type 2 Innate lymphoid cells (ILC2), T helper
 421 2 cells (Th2).

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