THE ASSOCIATION OF INTERLEUKIN-1 GENE POLYMORPHISM WITH CHRONIC RHINOSINUSITIS WITH AND WITHOUT NASAL POLYP

DR SAKINAH BINTI MOHAMAD

Dissertation Submitted in Partial Fulfillment Of the Requirements of the Degree of Master of Medicine (Otorhinolaryngology – Head and Neck Surgery)

UNIVERSITI SAINS MALAYSIA

2018

ACKNOWLEDGEMENT

In the Name of Allah, the Most Compassionate, the Most Merciful.

Thank you Allah, The Almighty for your guidance, grace and mercy.

A sincere and deepest gratitude to my supervisor, Prof. Dr Suzina Sheikh Ab Hamid, a Senior Consultant in our department, Department of Otorhinolaryngology – Head and Neck Surgery, for her utmost guidance and unfailing support. Also greatest thank you to my most respected co-supervisor, Dr Azlina Ahmad, who is a senior Molecular Biologist in School of Dental Sciences for her continuous guidance and patience. Also a special thank you to Dr Norasnieda Md Shukri as my co-supervisor for her invaluable advices.

To my beloved husband, Dr Muhammad Rajaei Ahmad @ Mohd Zain, my wonderful parents, Dr Mohamad Hamzah and Ustazah Tempawan Ishak, and my lovely children, Raniyah Zihni and Muhammad Zayyan, thank you so much for being very understanding and giving me utmost continuous support at all times. Also not to forget, special thanks to all those who contributed towards the completion of this thesis manuscript, namely Liu Kien Ting (for his guidance in statistical analysis), Hidayah Nazzran (for giving extra hand in laboratory works) and other respected staff in Craniofacial Lab in School of Dental Sciences, for their guidance and co-operation.

Thank you very much from the bottom of my heart..

TABLE OF CONTENTS

		PAGE
TITLE		i
ACKNOWLEDGEMENT		ii
TABLE OF CONTENTS		iii
ABSTRAK (BAHASA MELAYU)		v
ABSTRACT (ENGLISH)		vii
CHAPTER 1: INTRODUCTION		2
CHAPTER 2: OBJECTIVES OF THE STUDY		
2.1	General Objectives	6
2.2	Specific Objectives	6
CHAPTER 3: MANUSCRIPT		8
CHAPTER 4: STUDY PROTOCOL		
4.1	Study Protocol Submitted for Ethical Approval	43
4.2	Consent Form Submitted for Ethical Approval	61
4.3	Ethical Approval Letter	75
CHAPTER 5: APPENDICES		
5.1	List of Abbreviations	79
5.2	Elaboration of Methodology	81

5.3	Additional Results	93
5.4	Additional Figures/Attachments/Tables	94
5.5	Additional Reference	108
5.5	Raw Data on SPSS Softcopy	109

ABSTRAK (BAHASA MELAYU)

Pengenalan: Rhinosinusitis kronik (CRS) adalah merupakan salah satu penyakit keradangan yang seringkali berlaku dan kompleks, yang melibatkan mukosa hidung dan paranasal sinus. Walaupun patogenesis CRS adalah multifaktorial dan masih tidak jelas, peranan sitokin terutamanya interleukin-1 (IL-1) sedang diselidiki di seluruh dunia dalam populasi yang berbeza kerana keputusan yang berbeza-beza diperolehi.

Objektif: Untuk mengkaji hubungan antara polimorfisme genetik *IL-1* (*A* dan *B*) dengan rhinosinusitis kronik dengan polip hidung (CRSwNP) dan tanpa polip hidung (CRSsNP), dan faktor lain yang berkaitan.

Kaedah: Ini adalah kajian terkawal yang melibatkan sejumlah 138 subjek yang direkrut dari klinik Otorinolaringologi- Pembedahan Kepala dan Leher (ORL-HNS) di Hospital Universiti Sains Malaysia (HUSM). Genotyping *IL-1A* (+4845G, +4845T) dan *IL-1B* (-511C, -511T) dilakukan dengan analisa panjang pecahan polimorfisme (RFLP).

Keputusan: Dari 138 peserta, terdapat 61 lelaki (44.2%) dan 77 (55.8%) wanita. Umur purata [SD] pada diagnosis adalah 46.6 [13.70] dan 34.41 [12.37] tahun bagi CRSwNP dan CRSsNP, masing-masing. Majoriti subjek adalah asal Melayu. Sejarah merokok dikaitkan dengan pesakit CRSwNP dan CRSsNP (p-value <0.001). Terdapat hubungan penting statistik antara *IL-1B* (-511C, -511T) polimorfisme dengan CRSwNP dan CRSsNP (*p*-value <0.001). Genotip CT dalam *IL-1 B* adalah tinggi dengan ketara dalam subjek CRSwNP. Walaubagaimanapun, tiada hubungan yang signifikan antara *IL-1A* (+4845G, +4845T) dan CRSwNP dan CRSsNP (*p*-value = 0.093). Tiada hubungan yang penting dijumpai dalam

faktor-faktor yang berkaitan dengan CRS, termasuk asma, atopy, alergi, sensitiviti kepada aspirin dan sejarah keluarga polip hidung (*p*-value 0.382, 0.382, 0.144, >0.95 dan 0.254 masing-masing).

Kesimpulan: Kajian ini menunjukkan terdapat hubungan antara *IL-1B* (-511C, -511T) polimorfisme dengan CRSwNP dan CRSsNP dalam populasi kita, oleh itu terdapat kemungkinan penglibatan *IL-1B* dalam memodulasi patogenesis CRS. Tiada hubungan signifikan *IL-1A* (+4845G, +4845T) polimorfisme dengan CRSwNP dan CRSsNP, dan faktor lain yang berkaitan.

ABSTRACT (ENGLIGH)

Background: Chronic rhinosinusitis (CRS) is one of the most common and complex chronic inflammatory disease of sinonasal mucosa. Eventhough the pathogenesis of CRS is multifactorial and still unclear, the role of cytokines especially interleukin-1 (IL-1) is being investigated worldwide in different population because of varying results obtained.

Objective: To study the association of *IL-1 (A* and *B)* gene polymorphisms with chronic rhinosinusitis with nasal polyp (CRSwNP) and without NP (CRSsNP), and other factors related.

Methods: This is a case controlled study which include a total of 138 subjects recruited from Otorhinolaryngology-Head and Neck Surgery (ORL-HNS) clinic in Hospital Universiti Sains Malaysia (HUSM). Genotyping of the *IL-1A* (+4845G, +4845T) and *IL-1B* (-511C, -511T) was performed with restriction fragment length polymorphism (RFLP) analysis.

Results: From 138 participants, there were 61 males (44.2%) and 77 (55.8%) females. The mean [SD] age at diagnosis was 46.6 [13.70] and 34.41 [12.37] years for CRSwNP and CRSsNP, respectively. Majority of the subjects was Malay in origin. Cigarette smoking was significantly associated with CRSwNP and CRSsNP patients (p-value < 0.001). There was a statistical significant association between *IL-1B* (-511C, -511T) gene polymorphism with CRSwNP and CRSsNP (p-value < 0.001). The CT genotype of *IL-1B* was markedly increased in CRSwNP subjects. However, there was no significant association found between *IL-1A* (+4845G, +4845T) and CRSwNP and CRSsNP (p-value = 0.093). No association was found in factors related to CRS, which included asthma, atopy, allergy, aspirin sensitivity and

family history of nasal polyp (NP) (*p*-value of 0.382, 0.382, 0.144, >0.95 and 0.254, respectively).

Conclusion: This study indicates an association of *IL-1B* (-511C, -511T) polymorphism with CRSwNP and CRSsNP in our population, hence there is a possibility of *IL-1B* involvement in modulating pathogenesis of CRS. There was no significant association of *IL-1A* (+4845G, +4845T) polymorphism with CRSwNP and CRSsNP, and other factors related.

Chapter 1

INTRODUCTION

CHAPTER 1: INTRODUCTION

Chronic rhinosinusitis (CRS) is one of the most common chronic inflammatory disease of sinonasal mucosa, affecting 15.5% of the total population in United States (US), making it the second most common condition of all chronic conditions [1]. The Global Allergy and Asthma Network of Excellence (GA^2LEN) study revealed that the overall prevalance of CRS in the Europe countries was 10.9% [2], whereas the prevalence in the Asian countries was reported to be around 6.9% to 8.0% [3].

CRS can be further classified into two phenotypes: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP) [4]. Histopathologically, nasal polyp is categorized by proliferation of the epithelial layer, thickening of the basement membrane, focal fibrosis, glandular hyperplasia, oedema, cellular infiltration of stromal layer and presence of inflammatory cells [5-6]. It is usually bilateral and described as peeled grape-like, glistening, pale-grey, smooth, semitransparent mass with a pedicle arising from the osteomeatal-complex [7-8]. Apart from the presence of polyp in the nasal cavity for CRSwNP, these patients were reported to have a higher frequency of nasal discharge, nasal obstruction and change in smell, as compared to CRSsNP patients who complain more of facial pain or headache [9].

CRS is a complex disease whereby the actual pathogenesis is still under active investigation and is believed to be of a multifactorial. The inflammatory reaction of the sinonasal mucosal lining causing mucosal oedema which obstructs the sinus ostia, leading to mucus retention and infection, hence development of CRS [10]. Among the predisposing factors associated with this disease are asthma, aspirin sensitivity, allergy, atopy, cigarette smoking and genetic

factor [1]. It is also believed that CRS is affected by multiple genes that may interact with undetermined environmental factors and potentially cause disease expression [1].

Interleukin-1 (IL-1) is one of the most important proinflammatory cytokines as well as a potent transmitter between cells which modulates early in the cascade of inflammatory response in CRSwNP [11]. IL-1 plays a role in activating T lymphocytes and monocytes, and also upregulating expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [6, 12]. *IL-1* exists in three forms namely *IL-1A*, *IL-1B* and IL-1 receptor antagonist (*IL-1 Ra*), which are located on the long arm of chromosome 2 [6].

Several studies conducted in different countries had proven that genetic polymorphisms of IL-1 have been contributed to development of CRS. Genetic polymorphism is defined as multiple alleles occur at a single locus, whereby at least two alleles present with a frequency greater than 1 percent [13]. Initially, Karjalainen et al. [14] demonstrated an association of *IL-1A* (+4845G, +4845T) with nasal polyp in asthmatic adults in Finnish population. Similar finding was subsequently found in CRS patients in Canadian population [6]. A Turkish study successfully reported association of both *IL-1A* (+4845G, +4845T) and *IL-1B* (-511C, -511T) polymorphisms with CRSwNP patients [5]. In contrast, Bernstein et al. [15] in United State of America (USA) showed no significant association of *IL-1A* and *IL-1B* in their CRSwNP patients. The contradictory results may suggest that variation between ethnic groups affecting frequency of many genetic alleles [16].

Besides CRS, studies shown that IL-1 genetic polymorphisms is also associated with other inflammatory disease such as periodontitis, rheumatoid athritis, inflammatory bowel disease

and gout [16-19]. Examples of IL-1 inhibitor available in clinical use are anakinra, canakinumab and rilonacept which are effective in the advanced treatment of gout [19].

To date, there is no such study done in Southeast population, the present study aimed to study the association of *IL-1A* and *IL-1B* genetic polymorphisms with CRSwNP and CRSsNP. Besides that, we also attempted to determine the association of other factors (asthma, atopy, allergy, aspirin sensitivity and family history of nasal polyp) related to CRSwNP and CRSsNP.

Chapter 2

OBJECTIVES OF THE STUDY

CHAPTER 2: OBJECTIVES OF THE STUDY

2.1 GENERAL OBJECTIVE

The general objective for this study was to determine the association of Interleukin-1 (IL-1) gene polymorphisms with chronic rhinosinusitis (CRS) with (CRSwNP) and without nasal polyp (CRSsNP).

2.2 SPECIFIC OBJECTIVES

The specific objectives for this study were as the following:

i) To profile the CRS patient population in Hospital Universiti Sains Malaysia (HUSM).

ii) To determine the association of *IL-1A* and *IL-1B* gene polymorphisms with CRSwNP, CRSsNP and control.

iii) To determine the association of other factors (asthma, atopy, allergy, aspirin sensitivity, and family history of nasal polyp) related to CRSwNP and CRSsNP.

Chapter 3

MANUSCRIPT

(Page 8-41)

TITLE PAGE

Association of interleukin-1 gene polymorphisms with chronic rhinosinusitis with and without nasal polyp

Sakinah Mohamad[1,*], Suzina Sheikh Ab Hamid[1], Ahmad Azlina[2], and Norasnieda Md Shukri[1]

[1]Department of Otorhinolaryngology-Head & Neck Surgery, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia.

[2]Basic Science and Oral Biology Unit, School of Dental Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia.

***Corresponce to:**

Sakinah Mohamad

Department of Otorhinolaryngology-Head & Neck Surgery, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia.

Tel: +609-7676420

Fax: +609-7676424

E-mail: tr_kmkstuds03@yahoo.com

Financial Disclosures/Conflicts of Interest:

This study was funded by Short Term Grant from Universiti Sains Malaysia (Project number: 304/PPSP/61313180).

The authors have no conflicts of interest to declare pertaining to this article.

ABSTRACT

Background: Chronic rhinosinusitis (CRS) is one of the most common and complex chronic inflammatory disease of sinonasal mucosa. Eventhough the pathogenesis of CRS is multifactorial and still unclear, the role of cytokines especially interleukin-1 (IL-1) is being investigated worldwide in different population because of varying results obtained.

Objective: To study the association of *IL-1 (A* and *B)* gene polymorphisms with chronic rhinosinusitis with nasal polyp (CRSwNP) and without NP (CRSsNP), and other factors related.

Methods: This is a case controlled study which include a total of 138 subjects recruited from Otorhinolaryngology-Head and Neck Surgery (ORL-HNS) clinic in Hospital Universiti Sains Malaysia (HUSM). Genotyping of the *IL-1A* (+4845G, +4845T) and *IL-1B* (-511C, -511T) were performed with restriction fragment length polymorphism (RFLP) analysis.

Results: There was a statistical significant association between *IL-1B* (-511C, -511T) polymorphism with CRSwNP and CRSsNP (p-value <0.001). The CT genotype of *IL-1B* was markedly increased in CRSwNP subjects (52.2%). However, there was no significant association found between *IL-1A* (+4845G, +4845T) with CRSwNP and CRSsNP (p-value = 0.093). No association was found in factors related to CRS, which included asthma, atopy, allergy, aspirin sensitivity and family history of nasal polyp (NP) (p-value of 0.382, 0.382, 0.144, >0.95 and 0.254, respectively).

Conclusion: This study indicates an association of *IL-1B* (-511C, -511T) polymorphism with CRSwNP and CRSsNP in our population, hence there is a possibility of *IL-1B* involvement in modulating pathogenesis of CRS. There was no significant association of *IL-1A* (+4845G, +4845T) polymorphism with CRSwNP and CRSsNP, and other factors related.

Keywords: *Rhinosinusitis; Nasal Polyposis; Interleukin-1; Single Nucleotide Polymorphism*

INTRODUCTION

Chronic rhinosinusitis (CRS) is one of the most common chronic inflammatory disease of sinonasal mucosa, affecting 15.5% of the total population in United States (US), making it the second most common condition of all chronic conditions [1]. The Global Allergy and Asthma Network of Excellence (GA^2LEN) study revealed that the overall prevalance of CRS in the Europe countries was 10.9% [2], whereas the prevalence in the Asian countries was reported to be around 6.9% to 8.0% [3].

CRS can be further classified into two phenotypes: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP) [4]. Histopathologically, nasal polyp is categorized by proliferation of the epithelial layer, thickening of the basement membrane, focal fibrosis, glandular hyperplasia, oedema, cellular infiltration of stromal layer and presence of inflammatory cells [5-6]. It is usually bilateral and described as peeled grape-like, glistening, pale-grey, smooth, semitransparent mass with a pedicle arising from the osteomeatal-complex [7-8]. Apart from the presence of polyp in the nasal cavity for CRSwNP, these patients were reported to have a higher frequency of nasal discharge, nasal obstruction and change in smell, as compared to CRSsNP patients who complain more of facial pain or headache [9].

CRS is a complex disease whereby the actual pathogenesis is still under active investigation and is believed to be of a multifactorial. The inflammatory reaction of the sinonasal mucosal lining causing mucosal oedema which obstructs the sinus ostia, leading to mucus retention and infection, hence development of CRS [10]. Among the predisposing factors associated with this disease are asthma, aspirin sensitivity, allergy, atopy, cigarette smoking and genetic

97 factor [1]. It is also believed that CRS is affected by multiple genes that may interact with
98 undetermined environmental factors and potentially cause disease expression [1].
99
100 Interleukin-1 (IL-1) is one of the most important proinflammatory cytokines as well as a
101 potent transmitter between cells which modulates early in the cascade of inflammatory
102 response in CRSwNP [11]. IL-1 plays a role in activating T lymphocytes and monocytes, and
103 also upregulating expression of adhesion molecules such as intercellular adhesion molecule-
104 1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [6, 12]. *IL-1* exists in three
105 forms namely *IL-1A*, *IL-1B* and IL-1 receptor antagonist (*IL-1 Ra*), which are located on the
106 long arm of chromosome 2 [6].
107
108 Several studies conducted in different countries had proven that genetic polymorphisms of
109 IL-1 have been contributed to development of CRS. Genetic polymorphism is defined as
110 multiple alleles occur at a single locus, whereby at least two alleles present with a frequency
111 greater than 1 percent [13]. Initially, Karjalainen et al. [14] demonstrated an association of
112 *IL-1A* (+4845G, +4845T) with nasal polyp in asthmatic adults in Finnish population. Similar
113 finding was subsequently found in CRS patients in Canadian population [6]. A Turkish study
114 successfully reported association of both *IL-1A* (+4845G, +4845T) and *IL-1B* (-511C, -511T)
115 polymorphisms with CRSwNP patients [5]. In contrast, Bernstein et al. [15] in United State
116 of America (USA) showed no significant association of *IL-1A* and *IL-1B* in their CRSwNP
117 patients. The contradictory results may suggest that variation between ethnic groups affecting
118 frequency of many genetic alleles [16].
119
120 Besides CRS, studies shown that IL-1 genetic polymorphisms is also associated with other
121 inflammatory disease such as periodontitis, rheumatoid athritis, inflammatory bowel disease

and gout [16-19]. Examples of IL-1 inhibitor available in clinical use are anakinra, canakinumab and rilonacept which are effective in the advanced treatment of gout [19].

To date, there is no such study done in Southeast population, the present study aimed to study the association of *IL-1A* and *IL-1B* genetic polymorphisms with CRSwNP and CRSsNP. Besides that, we also attempted to determine the association of other factors (asthma, atopy, allergy, aspirin sensitivity and family history of nasal polyp) related to CRSwNP and CRSsNP.

MATERIAL AND METHODS

Sample size calculation

Sample size calculation was determined by using Power and Sample software (Version 3.0.43) based on previous literature by Karjalainen et al. [14] and Berstein et al. [15]. The power of study used was 0.80 with level of statistical significant (α) of 0.05, meanwhile the probablitity of exposure among controls and cases were 0.40 and 0.70. 10% drop out was added to the largest sample size calculated making it 138 subjects in total (46 subjects in each group: CRSwNP, CRSsNP and control).

Subjects

A case controlled study was conducted with a total of 92 patients (46 CRSwNP patients and 46 CRSsNP patients) and 46 controls aged more than 18 years old were recruited from Otorhinolaryngology-Head and Neck Surgery (ORL-HNS) clinic in Hospital Universiti Sains Malaysia (HUSM). The diagnosis of CRS was based on clinical history and confirmed by direct visualisation via nasal endoscopy as proposed by the European Position Paper on Rhinosinusitis and Nasal Polyp (EPOS) [1] or those with history of polypectomy confirmed with pathology reports. Those with cystic fibrosis, Kartagener's syndrome, Young syndrome, antrochoanal polyp, inverted papilloma or any malignancy were excluded from the cases. The control group consisted of healthy individuals those who volunteered. They were not blood-related to the cases and living in the same district areas with the cases to minimise the environmental bias. They did not have any history of nasal symptoms, allergy, family history of allergy and any chronic inflammatory disorders.

A standardised questionnaire comprised of demographic characteristics (e.g. age at diagnosis, gender, ethnicity, and smoking history), duration of symptoms, nasal symptoms (according to EPOS 2012) [1], history of previous sinus surgery, predisposing factors of CRS (e.g. the presence of asthma, atopy, allergy, aspirin intolerance, and family history of nasal polyps) and nasoendoscopic findings were obtained from all subjects. During nasoendoscopy, presence of any polyp, grading of nasal polyp (according to Lund [20]), mucopurulent discharge or obstruction in the middle meatus, and mucosal oedema were recorded.

Deoxyribonucleic acid (DNA) collection and extraction

Each patient's DNA was collected using a buccal swab and then stored at -20 degree celcius until DNA extraction was done. Then, the DNA extraction was performed using Exgene^{TM} Blood SV Mini Kit (GeneAll®, Korea) by following the manufacturer's protocol.

Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP)

The genotype of *IL-1A* (+4845G, +4845T) and *IL-1B* (-511C, -511T) were determined by PCR amplification by using Master Cycler Vapo Protect (Eppendorf, Germany) with the primers as identified by Karjalainen et al. [14], then followed by RFLP. The primers used for *IL1A* and *IL1B* were as following: 5'-ATG GTT TTA GAA ATC ATC AAG CCT AGG GCA-3' (forward primer) and 5'-AAT GAA AGG AGG GGA GGA TGA CAG AAA TGT-3' (reverse primer); and 5'-TGGCATTGATCTGGTTCATC-3' (forward primer) and 5'-GTTTAGGAATCTTCCCACTT-3' (reverse primer), respectively. Then, for both genes, 1 μL forward primer, 1 μL reverse primer, 3 μL genomic DNA, 5 μL Dnase free water and 10 μL Phusion® High-Fidelity PCR Master Mix with HF Buffer (New England Biolabs, USA) were mixed together. The PCR cycling conditions were as follows: (a) For *IL-1A* (+4845G,

196 +4845T) initial denaturation at 94 °C for 3 minutes (min), 30 cycles of denaturation at 98 °C
197 for for 60 seconds (s), annealing at 54 °C for 60 s, extension at 72 °C for 2 min and final
198 extension at 72 °C at 5 min; (b) For *IL-1B* (-511C, -511T): initial denaturation at 94 °C for 10
199 min, 30 cycles of denaturation at 94 °C for for 45 s, annealing at 55 °C for 45 s, extension at
200 72 °C for 60 s and final extension at 72 °C at 10 min.
201
202 Subsequently, to detect *IL-1A* (+4845G, +4845T), digestion with restriction enzyme *Sat*I
203 (New England Biolabs®, UK) was performed after amplification to yield 124-,76-, and 29-
204 base pair (bp) bands in the presence of allele G, and 153-bp and 76-bp bands in the presence
205 of allele T [5]. Whilst, to detect *IL-1B* (-511C, -511T), digestion with restriction enzyme
206 *Ava*I (New England Biolabs®, UK) is performed to yield 305-bp bands in the presence of
207 allele C, and 190-bp and 115-bp bands in the presence of allele T [5]. Therefore, a 4.0 μL
208 PCR product were digested with 0.5 μL respective restriction enzyme together with 18.0 μL
209 Dnase free water and 2.5 μL CutSmart® buffer. Then, the mixture was spun down for a few
210 seconds and incubated at 37 °C for 20 minutes. The PCR product was also sent for DNA
211 sequencing for validation.
212
213 **Electrophoresis**
214 The 10-12 μL digested DNA was added with 1-2 μL BlueJuice Gel Loading buffer
215 (Invitrogen, USA) and loaded into the 2.5% Agarose gel for *IL-1A* and 2% Agarose gel for
216 *IL-1B*. Following that, the digested DNA was separated on the Agarose gel and stained with
217 SYBR® Safe DNA gel stain at 75 volts (V) for 90 min and 70 V for 60 min for *IL-1A* and *IL-*
218 *1B*, respectively. The image on the Agarose gel was then visualised under ultraviolet light
219 and captured using an image analyser i.e. Quantity One, 1-D Analysis Software (Bio-Rad
220 Laboratories, USA).

Statistical analysis

The statistical calculation and evaluation were performed with IBM SPSS version 22.0 (IBM, Armonk, NY, USA). The data analysis was derived descriptively and the inferential statistics mainly used *Pearson chi square test, Fisher-Exact test* and *simple logistic regression.* A *p* value of less than 0.05 is considered significant.

Ethical approval

The study protocol was approved by Human Research Ethics Committee of Universiti Sains Malaysia (Federalwide Assurance Registration No. 00007718; Instituitional Review Board No. 00004494) and the written informed consent was gained from all participants.

RESULTS

From 138 participants, there were 61 males (44.2%) and 77 (55.8%) females. The mean [SD] age at diagnosis was 46.6 [13.70] and 34.41 [12.37] years for CRSwNP and CRSsNP, respectively. Meanwhile, the mean [SD] age for cases and control was 40.52 [14.36] and 42.41 [12.26] years. Majority of the subjects was Malay in origin followed by Chinese, Indian and others. Cigarette smoking was significantly associated with CRSwNP and CRSsNP patient (p-value < 0.001).

The SNPs for *IL-1A* (+4845G, +4845T) and *IL-1B* (-511C, -511T) were successfully genotyped. For *IL-1A* (+4845G, +4845T), homozygous wild-type (GG) expected to produce three fragments of 124 bp, 76 bp and 29 bp. However, the 29 bp was too small to be captured in the 2.5% Agarose gel electrophoresis. Homozygous mutant-type (TT) produced two fragments of 153 bp and 76 bp, and therefore, heterozygous mutant-type (GT) was seen to yield 153 bp, 124 bp, 76 bp and 29 bp fragments as shown in **Fig. 1**. On the other hand, the uncut fragment of 305 bp represented the homozygous wild-type (CC) for *IL-1B* (-511C, -511T). Heterozygous mutant-type (CT) yielded three fragments of 305 bp, 190 bp and 115 bp, and homozygous mutant-type (TT) produced two fragments of 190 bp and 115 bp as shown in **Fig. 2**.

Table 1 illustrates the genotype distributions and allele frequencies of both *IL-1A* and *IL-1B* in CRSwNP, CRSsNP and controls. The GT genotype of *IL-1A* was common in patients with CRSwNP and CRSsNP but not amongst controls. Whereas, TT was a common genotype in controls. Thus, these findings contributed to significantly higher frequency of T allele (p-value = 0.021). However, there was no statistical significant

differences found between *IL-1A* (+4845G, +4845T) genotype distributions against CRSwNP, CRSsNP and controls (*p*-value = 0.093).

Indeed, our study showed a significant association of *IL-1B* (-511C, -511T) polymorphism with both CRSwNP and CRSsNP patients (*p*-value <0.001). A slightly different trend of genotype frequencies was observed in *IL-1B* (-511C, -511T) polymorphism. The frequency of CC genotype of *IL-1B* was significantly higher in CRSsNP and controls (*p*-value <0.001). However, in patients with CRSwNP, CT genotype was markedly increased in *IL-1B*. In terms of allele frequency, allele T was found to be highly associated with CRSwNP compared to CRSsNP and control groups (*p*-value <0.001).

No significant association was found in all factors related to CRS, which includes asthma, atopy, allergy, aspirin sensitivity and family history of NP with respective *p*-values (all *p*-value >0.05), as shown in **Table 2**.

296 **DISCUSSION**

297

298 This study demonstrated that there was a statistically significant association between *IL-1B* (-
299 511C, -511T) polymorphism with both CRSwNP and CRSsNP patients (*p*-value <0.001)
300 which is consistent with a study by Erbek et al. [5] in a Turkish population. This proves that
301 single nucleotide polymorphism (SNP) of *IL-1B* within the promoter region at locus -511 in
302 chromosome 2 maybe one of the key players in the inflammatory cascade of CRSwNP as
303 well as CRSsNP. However, the means by which this gene results in the clinical progression
304 of the disease is unknown. The *IL-1B* (-511C, -511T) polymorphism may affect or alter the
305 transcription of other cytokine genes involved in the disease process [21]. Perhaps further
306 studies of this gene can be conducted in an even larger sample size to reduce the risk of
307 random association between SNPs and CRS patients.

308

309 Our finding of CT genotype of *IL-1B* as a significantly common genotype (*p*-value <0.01) in
310 CRSwNP population is also similar to other studies [5, 12, 14]. However, studies by Cheng et
311 al. [5] and Erbek et al. [12] showed that CT genotype as a common genotype in CRSsNP
312 population. This may imply that CT genotype of *IL-1B* gene is an important genotype for
313 development of both CRSwNP and CRSsNP in Asian population. In contrary, other studies
314 [12, 15] also investigated the association of genetic polymorphism of *IL-1B* at position -511
315 and at different polymorphism site such as at exon 5 for *IL-1B* (+3953C, +3953T). However,
316 no statistical difference were found between those genes and subjects tested. **Table 3** showed
317 various studies performed investigating *IL-1A* and *IL-1B* polymorphism with nasal polyposis
318 in different populations.

319

Even though our study did not find any association between *IL-1A* (+4845G, +4845T) genotype distributions against CRS patients and controls (*p*-value = 0.093), but the role of IL-1A gene family still cannot be ruled out as other studies [5-6, 14] had shown significant association as been summarised in **Table 3**. This leads to a possibility that variation exists between the ethnicity affecting frequency of many genetic alleles [16]. Besides that, this lack of association between *IL-1A* (+4845G, +4845T) gene polymorphism and CRSwNP or CRSsNP, maybe contributed by epigenetic factors such as environmental factor interacting within the genome and immunologic process modified by immunomodulator prescribed to the patient [10, 21]. For example, macrolides used in treatment of CRSwNP, may inhibit neutrophilic rather than eosinophilic activity and macrophage activation, and lower the IL-1B concentration [22].

No significant association found in this study for the factors related to CRSwNP and CRSsNP patients with regards to asthma, atopy, allergy, aspirin sensitivity and family history of nasal polyp (all *p*-values > 0.05). However, our demographic data revealed that there was an association of environmental factor such as smoking history with CRS patients (*p*-value = 0.017) which is consistent with other studies [2, 23].

There was a well-established association between aspirin sensitivity, asthma and CRSwNP termed “aspirin-exacerbated respiratory disease” (AERD) or Samter’s triad [24-25]. Failure in obtaining association with aspirin sensitivity in the present study could be due to the low number of patients (only two patients out of total 138 subjects) who had consumed aspirin. This low incidence maybe due to an underestimation of aspirin sensitivity as the data was again based on patient’s history and majority of our patients may have not taken aspirin before. Aspirin is commonly used as a prophylaxis for cardiovascular diseases worldwide. It

345 was underutilised in Asian countries compared to Western population maybe due to
346 overestimation of bleeding risks by the physicians [26].
347
348 The relationship of CRSwNP and positive family history of nasal polyp has been well-
349 established [1, 27-28]. Nevertheless, an identical twin study showed that both siblings did not
350 always develop nasal polyps and this discordance proposed the role of environmental factors
351 that may affect disease expression [1, 29]. The lack of association of family history of nasal
352 polyp and CRS in our study was obtained maybe contributed by underestimation of family
353 history of nasal polyp. Some of our patients claimed that their family member had not seek
354 any medical check-up as they were asymptomatic, thus assuming that their family member
355 does not have any nasal polyp.
356
357 In terms of study limitation, the subjects were not homogenously distributed between the
358 subgroups. The subjects should be equally matched for age, gender and ethnicity to reduce
359 the bias by the confounders. We attempted to reduce the environmental bias by matching the
360 subjects geographically. In terms of sample size, the number of cases in our study (n = 92) is
361 comparable to other studies (a total number of 35-179 cases) [5, 12, 14-15]. This study might
362 not represent the general population in Malaysia because the distribution of ethnic groups in
363 Kelantan differs from other states of Malaysia. Therefore, a larger sample size and multi-
364 centre study would be more representative of CRS population in Malaysia.
365
366 In conclusion, this study indicates an association of *IL-1B* (-511C, -511T) polymorphism
367 with CRSwNP and CRSsNP in our population, hence there is a possibility of *IL-1B*
368 involvement in modulating pathogenesis of CRS. Therefore, it can be a potential new target
369 for treatment of CRS. We hope that this finding added a significant value in contributing to

370 understanding of genetics and pathogenesis of CRS in our population. Perhaps future
371 research can improvise this study to use *IL-1B* as a genetic marker for disease susceptibility
372 and risk stratification especially in patients with CRSwNP so that we can predict which
373 patients are predisposed to recurrence of nasal polyp and may need revision nasal surgery.
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394

ACKNOWLEDMENTS

Dr Sakinah Mohamad received funding of Short Term Grant from Universiti Sains Malaysia (Project number: 304/PPSP/61313180) to support this project.

The authors have no conflicts of interest to declare pertaining to this article.

REFERENCES

1. Fokkens WJ, Lund VJ, Mullol J, Bachert C, Alobid I, Baroody F, Cohen N, Cervin A, Douglas R, Gevaert P, Georgalas C. EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. Rhinology supplement 23 2012;50:1-198.
2. Hastan DF, Fokkens WJ, Bachert C, Newson RB, Bislimovska J, Bockelbrink A, Bousquet PJ, Brozek G, Bruno A, Dahlén SE, Forsberg B. Chronic rhinosinusitis in Europe–an underestimated disease. A GA2LEN study. Allergy 2011;66:1216-23.
3. Shi JB, Fu QL, Zhang H, Cheng L, Wang YJ, Zhu DD, Lv W, Liu SX, Li PZ, Ou CQ, Xu G. Epidemiology of chronic rhinosinusitis: results from a cross-sectional survey in seven Chinese cities. Allergy 2015;70:533-9.
4. Lam M, Hull L, Imrie A, Snidvongs K, Chin D, Pratt E, Kalish L, Sacks R, Earls P, Sewell W, Harvey RJ. Interleukin-25 and interleukin-33 as mediators of eosinophilic inflammation in chronic rhinosinusitis. Am J Rhinol Allergy 2015;29:175-81.
5. Erbek SS, Yurtcu E, Erbek S, Atac FB, Sahin FI, Cakmak O. Proinflammatory cytokine single nucleotide polymorphisms in nasal polyposis. Arch Otolaryngology Head Neck Surg 2007;133:705-9.
6. Endam LM, Cormier C, Bossé Y, Filali-Mouhim A, Desrosiers M. Association of IL1A, IL1B, and TNF gene polymorphisms with chronic rhinosinusitis with and without nasal polyposis: a replication study. Arch Otolaryngology Head Neck Surg 2010;136:187-92.
7. Bachert C, Zhang N, Van Zele T, Gevaert P. Chronic rhinosinusitis: from one disease to different phenotypes. Ped Allergy Immunol 2012;23:2-4.