Short Communication

For reprint orders, please contact: reprints@futuremedicine.com

Personalized Medicine

Promoter region single nucleotide polymorphism of *siglec-8* gene associates with susceptibility to allergic asthma

Mohammad Sajay-asbaghi[‡,1], Mahnaz Sadeghi-shabestrai[‡,2], Amir Monfaredan[3], Narges Seyfizadeh[4,5], Alireza Razavi*[,1] & Tohid Kazemi**[,2]

[1]Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[2]Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[3]Research Division of Tabriz International Hospital, Tabriz, Iran
[4]Department of Medical Oncology, National Center for Tumor Diseases, Heidelberg University Hospital, Heidelberg, Germany
[5]Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
*Author for correspondence: Fax: +98 21 6646 2267; razavial@tums.ac.ir
**Author for correspondence: Tel.: +41 33 36 46 65; Fax: +41 33 36 46 65; kazemit@tbzmed.ac.ir
‡ Authors contributed equally

Aim: Siglec-8 is exclusively expressed on mast cells, eosinophils and basophils. Possible association of six *siglec-8* single nucleotide polymorphisms (SNPs) with susceptibility to allergic asthma in the Azeri population of Iran was investigated in this study. **Materials & methods:** A total of 194 patients and 190 normal subjects were enrolled. PCR single strand conformation polymorphism (PCR-SSCP) was used to determine the genotypes of the studied SNPs. **Results:** The rs36498 showed significant association with allergic asthma (odds ratio [OR]: 0.65; p = 0.022) and the T allele was found as a protective allele (OR: 0.61; p = 0.008). Also, eosinophil count in the CC genotype was significantly higher than that in the other genotypes (p = 0.026). **Conclusion:** The rs36498 is thought to influence the expression level of *siglec-8*. Siglec-8 could be a potential therapeutic target for allergic asthma.

First draft submitted: 28 July 2018; Accepted for publication: 17 May 2019; Published online: 20 February 2020

Keywords: allele • allergy • asthma • basophil • eosinophil • genotype • PCR-SSCP • polymorphism • Siglec-8 • SNP

Asthma is a complex immunologic disorder mediated by immune cells including mast cells, basophils and eosinophils. Type 2 helper T (Th2)-mediated cytokines, in addition, play major roles in production and recruiting the cells into the inflamed bronchial tissues. The main immunoglobulin isotype involved in activation of mast cells and basophils is IgE which is produced in response to an allergen challenge and production of IL-4 and IL-13 released mainly by Th2 cells. Cross-linking allergen-specific IgE antibodies bound to the high affinity receptor for IgE (FcεRI) on mast cells and basophils result in degranulation and releasing different mediators with variety of functions. The late activation of eosinophils completes the inflammatory processes and leads to clinical manifestations characterized by bronchial hyperresponsiveness, airway inflammation and remodeling. Collectively, multiple genetic and environmental factors determine the susceptibility of an individual to asthma [1–4]. Also, there is a clear role for epithelial and other stromal cells in asthma with direct effects of cytokines on these cells. Whether granulocytes are required for airway hyperresponsivenes (AHR) is also still debated [5,6].

Siglecs are immunoglobulin superfamily members which bind to sialic acid-containing moieties by their N-terminal lectin domain. Siglecs have intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and a potential to transduce inhibitory signals. By recognizing mammalian glycans that are different from prokaryotic structures, these molecules may participate in immune discrimination between self and nonself, and also in immune regulation in diverse clinical conditions [7–9]. They are expressed on different immune cells, but the expression of Siglec-8 is unique for mast cells and eosinophils, as well as basophils. It has been shown that engagement of siglec-8 induces apoptosis in eosinophils, and inhibits FcεRI-mediated activation and degranulation of mast cells [10–15]. Eosinophilic inflammation could be triggered by lung epithelial ligands for siglec-8 [16,17]. Recently, the role for

Table 1. Demographic features of study populations.

Characteristics	Patients (n = 194)	Controls (n = 190)	p-value
Mean age ± standard deviation (years)	25.84 ± 15.08	27.43 ± 15.64	0.802
Gender			
– Female	115 (59%)	106 (55.8%)	0.247
– Male	70 (41%)	84 (44.2%)	0.125
Eosinophil count per μl (%)	363.15 ± 61.25 (4.24%)	116.26 ± 36.45 (1.5%)	0.028†
History of allergic reactions			
– Allergic rhinitis	57 (29.4%)	–	
– Conjunctivitis	45 (23.2%)	–	
– Atopic dermatitis	23 (17.8)	–	
– Other	69 (35.6%)	–	

†Statistically significant ($p < 0.05$).

siglec-8 as an activation receptor for IL-5-primed eosinophils was confirmed [18]. Therefore, siglec-8 is an emerging new target for immunotherapy of asthma and all clinical conditions in which these cells have a major role [19]. Genetic variations in *siglec-8* gene could exert functional differences and effects on immunopathogenesis of asthma, as it has been shown that *siglec-8* gene is located in 19q13.33–q13.41 region, which contains genes involved in susceptibility to asthma [20–23].

According to the literature and previous studies, and also *in silico* studies of *siglec-8* gene in NCBI (gene ID: 27181) and SNPedia databases, we aimed in this study to investigate the possible association of six *siglec-8* SNPs including rs36498, rs10409962, rs36495 and rs3829659, and for the first time – rs36491 and rs36493 – with susceptibility to allergic asthma in the Azeri population of Iran.

Materials & methods

Patients & controls

A total of 194 asthmatic patients and 190 normal subjects were included in this case-control study. Patients were diagnosed by specialists at Allergy clinic, Tabriz University of Medical Sciences according to the International Study of Asthma and Allergies in Childhood (ISAAC) guideline and Global Initiative for Asthma (GINA). Also clinical signs and paraclinical findings were considered and patients with no other disease were included in the study. Therefore, having any clinical or paraclinical evidence showing any other inflammatory disease, autoimmunity and infectious disease were led to the exclusion of the patient from this study. Age, weight and gender were matched in both groups, and normal individuals without any history of autoimmunity, asthma and allergy diseases were selected. Consent form was taken from all the participants. Informed consent letter was signed by all participants and the study was approved by The Ethic Committee of Tehran University of Medical Sciences. Demographic features of studied groups have been shown in Table 1. The number and percentage of eosinophils in all asthmatic patients and normal subjects were determined by Sysmex XS-800i (Kobe, Japan) using 50 μl vein whole blood.

PCR single strand conformation polymorphism for genotyping of *siglec-8* SNPs

Whole peripheral blood samples were taken from each individual and the DNA was extracted using the salting-out method [24]. Briefly, 800 μl whole blood was mixed with 650 μl cell lysis buffer (CLB). The mixture was centrifuged and the supernatant was harvested. This procedure was repeated three-times. At the last step, nucleus lysis buffer (NLB) was added to the pellet and homogenized. After incubation for 15 min at room temperature, 100 μl NaCl 6M and 400 μl chloroform were added and centrifuged for 10 min. DNA was precipitated by adding absolute ethanol and after washing by ethanol 70%, was solublized in tris-EDTA (TE) buffer.

The frequencies of genotypes and alleles of six SNPs of *siglec-8* gene including rs36498, rs36491, rs10409962, rs36495, rs36493 and rs3829659 were determined using PCR single strand conformation polymorphism (PCR-SSCP) method. At the first step, flanking regions of studied SNPs were amplified by conventional PCR method using specific primers (Table 2), and specific bands with expected sizes were seen by standard agarose gel electrophoresis. The PCR conditions were as follows: initial denaturation at 94°C for 5 min, 33 cycles of denaturation at 94°C for 15 s, annealing at 63°C for 20 s and an extension at 72°C for 25 s. Reactions were completed at 72°C for 5 min as final extension step. Double stranded PCR products were denatured to single stranded DNAs by

Table 2. Primer sequences and the size of amplified regions for *siglec-8* SNPs.

SNPs	Primers sequence (5′→ 3′)	PCR product length (bp)
rs10409962	F: TGGACCAGAGCTTGAGCTTCC R: ACTGAGGAGCGGGCAGTAGT	245
rs36498	F: TCTGGAGTGTGAGGGTCCTGG R: ATGTTGGCCATGCCTGAGTCT	148
rs36491	F: GGAATCACAACCCCTCCAGCA R: GCTAGAGGCAGGGATGGGTC	173
rs36493	F: TCTGTGCTCAGCTGTGAGGC R: CGCTTGTCCCTCCAAACACAC	155
rs36495	F:CGTGACCATCTGAGCCCCTG R: TGAATGTCACCCTGTCCTTG	100
rs3829659	F: AATACAGGGCAGGGGTTGGTC R: TGGATGCTCGGTGTGGAGAAG	209

bp: Base pair; F: Forward primer; R: Reverse primer; SNP: Single nucleotide polymorphism.

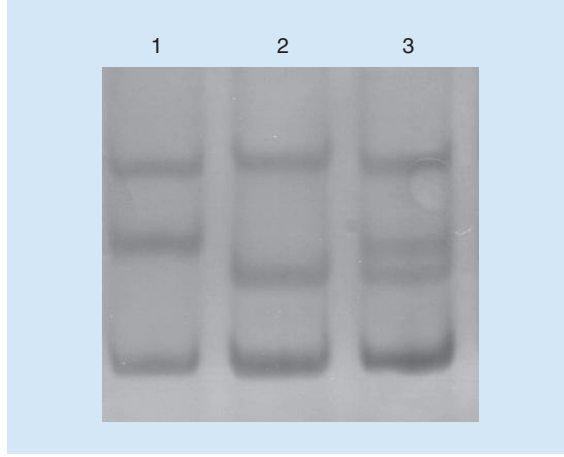

Figure 1. Representative result for PCR single strand conformation polymorphism analysis of rs36498. After DNA extraction and amplification of flanking regions of three independent samples, PCR products were underwent polyacrylamide gel electrophoresis and silver nitrate staining. After the direct sequencing of random samples, three corresponding patterns of electrophoresis for three possible genotypes where revealed as lane 1 for TT, lane 2 for CC and lane 3 for CT.

adding formamide-loading buffer (FLB) and incubating at 95°C for 10 min. Different electrophoretic patterns for denatured PCR products were determined by polyacrylamide gel electrophoresis (PAGE) and silver nitrate staining. Genotyping of all patterns for SNPs were determined and verified by the direct sequencing of some random samples.

Statistical analysis

Statistical analyses were made by STATA 11. Frequency of genotypes and alleles, and odds ratio (OR) for alleles and genotypes were analyzed by χ^2 and logistic regression tests, respectively. Unbalanced completely randomized design was used to evaluate associations between genotype and eosinophil count in whole peripheral blood using SAS 9.1 software. The p-values less than 0.05 were considered statistically significant.

Results

This study was performed on 194 asthmatic patients and 190 control subjects. PCR reactions for amplification of flanking regions of rs36498, rs36491, rs10409962, rs36495, rs36493 and rs3829659 SNPs resulted in specific bands whose sizes have been shown in Table 2. Genotyping of DNAs form patients and controls using single strand conformation polymorphism method revealed different electrophoretic patterns (Figure 1), reflecting variable frequencies of genotypes and alleles in five studied SNPs, except of rs36491 that showed no polymorphism in our population (Table 3). Statistical analysis showed a significant difference in frequency of genotypes in rs36498 between asthmatic patients and normal controls (OR: 0.65; p = 0.022). No other significant difference was seen in frequency of the studied SNPs and T allele of rs36498 also showed significant different frequency between patient (n = 58; 14.94%) and control (n = 85; 22.36%) groups (p = 0.008), and was found as protective allele (OR: 0.61). In addition, the A allele of rs36495 slightly was shown as a risk allele (OR: 1.12), but it was not significantly different from G allele (p = 0.628). Allele frequencies in other SNPs showed no significant differences between two studied groups.

10.2217/pme-2018-0080

Table 3. Genotype and allele frequencies of *siglec-8* SNPs in both studied populations.

SNPs	Genotype/allele	Patients (n = 194)	Controls (n = 190)	p-value	OR (95% CI)
rs36498	**Genotype**			**0.022**	0.65
	CC	140 (72.16%)	119 (62.63%)		
	CT	50 (25.77%)	57 (30%)		
	TT	4 (2%)	14 (7.36%)		
	Allele			**0.008**	0.61
	C	330 (85.05%)	295 (77.63%)		
	T	58 (14.94%)	85 (22.36%)		
rs36491	**Genotype**				
	AA	194 (100%)	190 (100%)		NA
	AG	0 (0%)	0 (0%)		
	GG	0 (0%)	0 (0%)		
	Allele				
	A	388 (100%)	380 (100%)		NA
	G	0 (0%)	0 (0%)		
rs10409962	**Genotype**			0.127	
	AA	128 (65.97%)	111 (58.42%)		
	AG	66 (34.02%)	79 (41.57%)		
	GG	0 (0%)	0 (0%)		
	Allele			0.18	0.85
	A	322 (82.98%)	301 (79.01%)		
	G	66 (17.01%)	70 (20.78%)		
rs3829659	**Genotype**			0.433	
	AA	116 (59.79%)	121 (63.68%)		
	AG	78 (40.20%)	69 (26.31%)		
	GG	0 (0%)	0 (0%)		
	Allele			0.493	1.15
	A	310 (79.89%)	311 (81.84%)		
	G	78 (20.10%)	69 (18.15%)		
rs36493	**Genotype**			0.473	
	CC	74 (38.14%)	68 (35.78%)		
	TC	56 (28.86%)	52 (27.36%)		
	TT	64 (32.98%)	70 (36.84%)		
	Allele			0.575	1.10
	C	204 (52.57%)	188 (49.47%)		
	T	184 (47.42%)	192 (50.52%)		
rs36495	**Genotype**			0.575	
	AA	0 (0%)	0 (0%)		
	AG	81 (41.75%)	74 (38.94%)		
	GG	113 (58.24%)	116 (61.05%)		
	Allele			0.628	1.11
	A	81 (20.87%)	74 (19.07%)		
	G	307 (79.12%)	306 (78.86%)		

Bold p-values are statistically significant.
NA: Not applicable; OR: Odds ratio; SNP: Single nucleotide polymorphism.

Unbalanced completely randomized design analysis showed significant difference in eosinophils count between asthmatic patients and control subjects (p = 0.028). Also there was a significant difference between the genotypes of rs36498 SNP in asthmatic patients. Eosinophil count in CC genotype was significantly higher than that in the other genotypes (p = 0.026; Table 4).

Table 4. Eosinophil count in the different genotype of rs36498 SNP in asmathic patients.

Factor	CC	CT	TT	p-value
Eos count	410.16 ± 104.47	247.51 ± 66.12	154.02 ± 72.34	0.026

Eos: Eosinophil; SNP: Single nucleotide polymorphism.

Discussion

The Siglec family are a sialic acid-binding lectins-containing immunoglobulin-like domains. To date, 14 members of this family have been identified and upon binding to yet unknown ligand, siglec-8 has been known to cause apoptosis in eosinophils [8,12]. SNPs in *siglec-8* gene have been therefore interested as regulatory elements for expression and function of siglec-8 molecule. Here, we studied genotype and allele frequencies of *siglec-8* SNPs in asthmatic patients and control subjects in the Azeri population of Iran and found significant association between rs36498 (-4354C/T) and susceptibility to allergic asthma, and the T allele was associated with reduced risk of asthma and revealed as a protective allele.

Asthma is a chronic inflammation of airways whose development relies on multiple known and yet unidentified environmental and genetic factors. Recently, a large number of genes has been studied for a possible implication in susceptibility of individuals for asthma. Among them, genes coding for key elements of immune mechanism, for example, IL-4, IL-5, IL-13, and FcεRI have been interested more due to pivotal roles of immune cells in the development of asthma. Major players in asthma are mast cells, basophils, eosinophils and all genetic factors influencing their development and functions can determine the predisposition of individuals to asthma [2].

To the best of our acknowledge, there is only one similar study regarding association of *siglec-8* SNPs and asthma. Gao *et al.* [25] studied 14 different SNPs of the *siglec-8* gene in three African-American, Brazilian and Japanese populations. They found significant association between SNP rs36498 T allele and asthma among African-American ($p = 8.8 \times 10^{-5}$; OR: 0.69) and Brazilian ($p = 0.013$) populations. In agreement with this study, we also found statistically significant association between SNP rs36498 and susceptibility to asthma ($p = 0.22$; OR: 0.65), and the T allele was found as a protective allele ($p = 0.008$; OR: 0.61). It might be concluded that the substitution of C by T allele in this SNP located in promoter of *siglec-8* gene results in a firmer binding of transcription machinery and higher expression of siglec-8 on eosinophils. This in turn may result in more susceptibility of eosinophils to apoptosis and slower progression of asthma. Consistent with this finding, we also showed significant association between rs36498 SNP and total eosinophil count ($p = 0.026$). Also, in the Gao *et al.* [25] study, significant association was also found for SNP rs10409962 A/G (Ser170Pro) in the Japanese population ($p = 0.019$; OR: 0.69), and a similar but not significant trend was seen in the African-American population ($p = 0.072$; OR: 0.82). This SNP is located in exon 2 and codes for the glycan-binding extracellular domain of siglec-8. According to their study, mutant protective G allele of nonsynonymous coding SNP rs10409962 could therefore increase the affinity of ligand binding of siglec-8 by replacing serine by proline at position 170. This in turn could give rise to more susceptibility of eosinophils for apoptosis and consequently lower predisposition of individuals to asthma. Our study; however, failed to show significant association between SNP rs10409962 and asthma ($p = 0.127$), but a trend in OR was seen in G allele (OR: 0.85), and this allele was revealed as a protective allele. No significant association between rs10409962 and susceptibility to asthma in our study might be due to a lower number of our study populations in comparison with the Gao *et al.* study [25], or the ethnic difference between two studies.

For the first time, we also studied two other SNPs – rs36491 and rs36493 – located in intron and 3′-UTR of *siglec-8* gene, respectively. The rs36491 showed no polymorphism in both studied groups, and statistical analysis revealed no significant association between rs36493 and susceptibility to asthma ($p = 0.473$), as rs3829659 and rs36495 ($p = 0.433$ and $p = 0.575$, respectively).

Conclusion

Siglec-8 gene polymorphisms – especially rs36498 – might be considered as important factors influencing susceptibility to asthma. On the other hand, rs36498 mutant T allele was revealed as protective allele and individuals carrying T allele might have a slower or no progression to asthma in the presence of other predisposing factors. However, more studies in diverse ethnicities with higher number of attendance need to be conducted for a more precise conclusion. There were some limitations in this study, for example, categorizing the patients according to the severity of disease and investigation of the association of *siglec-8* SNPs with different stages of the asthma, which was not considered in our study, would be more indicative. These findings – in concomitance with other studies –

could shed more light on the important role of siglec-8 in all clinical conditions in which eosinophils play pivotal roles. Siglec-8 could be used in targeted therapy of asthma and allergic conditions, and a precise role of *siglec-8* SNPs in asthma would be considered in personalized medicine of the patients to avoid their 'one-size-fits-all' treatment.

Summary points

- Mast cells, basophils and eosinophils have major roles in immunopathogenesis of asthma.
- Siglec-8 has an exclusive expression on mast cells, basophils and eosinophils.
- Engagement of siglec-8 leads to apoptosis of eosinophils and inhibition of degranulation of mast cells.
- Polymorphisms in *siglec-8* gene may influence susceptibility to allergic asthma.
- In this study, the rs36498 single nucleotide polymorphism of *siglec-8* was found to be associated with susceptibility to asthma in the Azeri population of Iran, and the T allele was showed as a protective allele.

Acknowledgements

The authors would like to thank all patients and healthy control groups for their coordination in this investigation.

Financial & competing interests disclosure

This study was carried out by a grant from Tehran University of Medical Sciences (22992). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Beasley R, Semprini A, Mitchell EA. Risk factors for asthma: is prevention possible? *Lancet* 386(9998), 1075–1085 (2015).
2. March ME, Sleiman PM, Hakonarson H. Genetic polymorphisms and associated susceptibility to asthma. *Int. J. Gen. Med.* 6, 253–265 (2013).
3. Komi DE, Kazemi T, Bussink AP. New insights into the relationship between chitinase-3-like-1 and asthma. *Curr. Allergy Asthma Rep.* 16(8), 57 (2016).
4. Scadding GK. Allergens, germs and asthma. *Clin. Respir. J.* 9(2), 153–156 (2015).
5. Bartemes KR, Kita H. Dynamic role of epithelium-derived cytokines in asthma. *Clin. Immunol.* 143(3), 222–235 (2012).
6. Tliba O, Panettieri RA Jr. Paucigranulocytic asthma: uncoupling of airway obstruction from inflammation. *J. Allergy Clin. Immunol.* 143(4), 1287–1294 (2019).
7. Bochner BS, Zimmermann N. Role of siglecs and related glycan-binding proteins in immune responses and immunoregulation. *J. Allergy Clin. Immunol.* 135(3), 598–608 (2015).
8. Macauley MS, Crocker PR, Paulson JC. Siglec regulation of immune cell function in disease. *Nat. Rev. Immunol.* 14(10), 653–666 (2014).

•• **Reviews the siglec family of molecules and their role in immunologic and immunopathologic conditions.**

9. Von Gunten S, Bochner BS. Basic and clinical immunology of siglecs. *Ann. NY Acad. Sci.* 1143(1), 61–82 (2008).
10. Bochner BS. Siglec-8 on human eosinophils and mast cells, and siglec-F on murine eosinophils, are functionally related inhibitory receptors. *Clin. Exp. Allergy* 39(3), 317–324 (2009).
11. Floyd H, Ni J, Cornish AL, Zeng Z *et al.* Siglec-8 A novel eosinophil-specific member of the immunoglobulin superfamily. *J. Biol. Chem.* 275(2), 861–866 (2000).
12. Kano G, Almanan M, Bochner BS *et al.* Mechanism of siglec-8 mediated cell death in IL-5 activated eosinophils: role for reactive oxygen species–enhanced MEK/ERK activation. *J. Allergy Clin. Immunol.* 132(2), 437–445 (2013).
13. Nutku E, Aizawa H, Hudson SA *et al.* Ligation of siglec-8: a selective mechanism for induction of human eosinophil apoptosis. *Blood* 101(12), 5014–5020 (2003).

•• **One of the most original research regarding the function and/or importance of the expression of siglec-8 in eosinophils.**

14. Nutku E, Hudson SA, Bochner BS. Mechanism of siglec-8-induced human eosinophil apoptosis: role of caspases and mitochondrial injury. *Biochem. Biophys. Res. Commun.* 336(3), 918–924 (2005).

15. Feng Y, Mao H. Specific regulator of eosinophil apoptosis: siglec-8-new hope for bronchial asthma treatment. *Chin. Med. J.* 125(11), 2048–2052 (2012).

16. Kiwamoto T, Katoh T, Tiemeyer M, Bochner BS. The role of lung epithelial ligands for siglec-8 and siglec-F in eosinophilic inflammation. *Curr. Opin. Allergy Clin. Immunol.* 13(1), 106–111 (2013).

17. Schleimer RP, Schnaar RL, Bochner BS. Regulation of airway inflammation by siglec-8 and siglec-9 sialoglycan ligand expression. *Curr. Opin. Allergy Clin. Immunol.* 16(1), 24–30 (2016).

18. Carroll DJ, O'Sullivan JA, Nix DB, Cao Y, Tiemeyer M, Bochner BS. Sialic acid-binding immunoglobulin-like lectin 8 (Siglec-8) is an activating receptor mediating β2-integrin-dependent function in human eosinophils. *J. Allergy Clin. Immunol.* 141(6), 2196–2207 (2018).

19. Kiwamoto T, Kawasaki N, Paulson JC *et al.* Siglec-8 as a drugable target to treat eosinophil and mast cell-associated conditions. *Pharmacol. Ther.* 135(3), 327–336 (2012).

20. Banks-Schlegel S. A genome-wide search for asthma susceptibility loci in ethnically diverse populations. *Nat. Genet.* 15, 389–392 (1997).

21. Ober C, Cox NJ, Abney M *et al.* Genome-wide search for asthma susceptibility loci in a founder population. *Hum. Mol. Genet.* 7(9), 1393–1398 (1998).

22. Venanzi S, Malerba G, Galavotti R *et al.* Linkage to atopy on chromosome 19 in north-eastern Italian families with allergic asthma. *Clin. Exp. Allergy* 31(8), 1220–1224 (2001).

23. Angata T. Associations of genetic polymorphisms of Siglecs with human diseases. *Glycobiology* 24(9), 785–793 (2014).

24. Tajik N, Kazemi T, Delbandi A *et al.* The predictive value of HLA-DR matching and cytokine gene polymorphisms in renal allograft acute rejection: a living-unrelated donor (LURD) study. *Iran J. Immunol.* 3(4), 150–156 (2006).

25. Gao P-S, Shimizu K, Grant AV *et al.* Polymorphisms in the sialic acid-binding immunoglobulin-like lectin-8 (*Siglec-8*) gene are associated with susceptibility to asthma. *Eur. J. Hum. Genet.* 18(6), 713–719 (2010).

- **Regards the association of *siglec-8* gene single nucleotide polymorphisms with predisposition to asthma.**