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Impacts of different cytokine and chemokine polymorphisms in Pakistani asthmatics a case control study

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Abstract

Background: Asthma is a chronic disease of the airways. Its symptoms are caused by inflammation and constriction of the bronchial muscles. During asthma there are changes in immunological pathways in which various cytokines and chemokines are involved directly or indirectly. The present study was conducted to explore the involvement of 15 Single nucleotide polymorphisms (SNPs) in 10 candidate cytokine and chemokine genes with asthma in Pakistani population.

Methods: We conducted this study in 333 asthmatic cases and 220 healthy controls.Genotyping was performed using the Sequenom Mass ARRAY iPLEX platform (10 SNPs) and TaqMan assay (5 SNPs).

Results: The minor allele at two SNPs have shown evidence of association with risk for asthma, rs1800896 in the interleukin 10 (*IL10)* (OR 1.38, 95% CI 1.01–1.88, $P = 0.04$) and rs1800925 in the interleukin 13 (*IL13)* (OR 1.45, 95% CI 1.04–2.02, $P = 0.03$).

Conclusion: Variations at the *IL10* and *IL13* genes are found to be associated with asthma susceptibility in the Pakistani population.

Keywords: Asthma, Genetic polymorphisms, Pakistan, Asthma association with cytokines

Background

Asthma is a disease of the lower airways that is remarkably heterogeneous between affected individuals [1]. Asthma symptoms are caused by inflammation, which results in narrowing of the airways, mucous secretion, and are characterized by recurrent attacks of paroxysmal dyspnea, with wheezing due to spasmodic contraction of the bronchi [2, 3]. Asthma is very common, with approximately 10% of people in the western world diagnosed with asthma at some stage in their life. The causes of asthma are not fully understood. Both genetic and environmental factors are involved, but how these factors interact to confer risk is still largely unknown.

Many biological pathways, and genes in those pathways, have been implicated in asthma pathogenesis. Variants in over 100 genes have been associated with asthma, but all with small individual effect sizes. It is likely that many genes act in concert to determine individual-specific risks for asthma [4]. Genes involved in immunological pathways are important in asthma pathogenesis. Therefore, based on results of previous candidate gene and genome-wide studies, we selected 15 single nucleotide polymorphisms (SNPs) in 10 cytokine and chemokine genes for genotyping in Pakistani asthma cases and controls. The selection of genes is purely rely on the immunological pathways.

Methods

Patient population and study design

Ethical statement

The ethical review committee of the parent organization approved this project (ERC-08-01).

Informed consent

Written informed consent was obtained from all participants.

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Asthmatic subjects were recruited from cities of Islamabad and Lahore of Pakistan. These all subjects belong to ethnic groups from Pakistan. Cases and controls all are from same ethnicity. There are total 533 samples included in the present study in which 333 Pakistani adult subjects were with an asthma diagnosis provided by a pulmonologist. Cases of asthma were selected for sample collection from the outpatient clinics of Rawalpindi, Islamabad and Lahore. Chest specialists based on clinical examination diagnosed the patients. Both patients and controls were from a similar ethnic background, and belonged to various castes and tribes from northern Punjab and the Northwest Frontier Province of Pakistan. Normal subjects, as control, were selected from general healthy population. Two hundred non-asthmatic healthy controls were recruited from the general population to be similar to the cases with respect to ethnicity and proportions of males and females.

Blood sample collection, DNA extraction, and genotyping

A venous blood sample was obtained from each study participant, and genomic DNA was extracted from whole blood using a standard phenol chloroform extraction protocol [5]. We selected SNPs in genes that are involved in the immune system and implicated in asthma risk, as reported in previous studies. Ten SNPs were genotyped using a Sequenom iPLEX assay and five SNPs were genotyped using TaqMan assays and analyzed on an ABI 7900 HT Fast Real Time PCR (Applied Biosystems, USA). All genotyping was performed at the University of Chicago USA.

Quality checks and statistical analyses

Hardy-Weinberg equilibrium (HWE) was determined in the entire sample and separately in the cases and controls. All SNPs were in HWE. Multiple logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for each SNP.

Results

Demographic and genotyping characteristics

The asthma cases included 148 (44.5%) males and 185 (55.5%) females; the mean age was 40 ± SE = 0.93 years. The controls included 88 males (44.0%) males and 112 (56.0%)) females with mean age of 30 ± SE = 0.97 years.

Of the 15 SNPs included in our study, all were in Hardy-Weinberg equilibrium. The genotyping methods, call rates, minor allele frequencies, and Hardy-Weinberg *P*-value calculated as a group are shown for all SNPs in Table 1.

Allelic and genotypic associations

Four SNPs showed evidence for an association with asthma at a $P < 0.05$. The allele and genotype frequencies in cases and controls for these four SNPs and results of all analyses are shown in Table 2.

Homozygosity for the minor alleles at SNPs in two other genes, *IL10* and *IL13*, was associated with asthma risk. There were more GG homozygotes at rs1800896 in *IL10* in cases compared to controls (recessive risk model $p = 0.04$) and more TT homozygotes at rs1800925 in *IL13*in cases compared to controls (recessive risk model, $P = 0.009$).

Table 1 Single Nucleotide Polymorphisms (SNPs) Genotyped in Asthma cases and controls

Gene	Chr location	rs #	Literature cited (Sup Ref)	Genotyping technique	Call Rate	Allele (minor/major)	MAF	HWE *P*-value
IL10	1	rs1800871	[36–38]	iPLEX	0.969	T/C	0.403	0.229
IL10	1	rs1800896	[36–39]	iPLEX	0.968	G/A	0.257	0.216
IL1R1	2	rs10173081	[44]	Taqman	0.986	T/C	0.060	0.997
IL13	5	rs1295685	[42]	iPLEX	0.969	T/C	0.311	0.155
IL13	5	rs1800925	[40, 42]	iPLEX	0.962	T/C	0.215	0.382
IL13	5	rs20541	[40, 41]	iPLEX	0.960	T/C	0.299	0.050
TSLP	5	rs1837253	[50, 51]	Taqman	0.993	T/C	0.319	0.556
TSLP	5	rs2289278	[52, 53]	Taqman	0.915	G/C	0.092	0.355
IL33	9	rs1342326	[43]	iPLEX	0.969	G/T	0.165	0.090
TLR4	9	rs4986790	[56]	Taqman	1.007	G/A	0.106	0.216
CC16	11	rs3741240	[54, 55]	Taqman	0.977	A/G	0.404	0.623
IL4RA	16	rs1801275	[48, 49]	iPLEX	0.971	G/A	0.201	0.246
IL4RA	16	rs1805011	[42, 48]	iPLEX	0.968	C/A	0.049	0.010
CCL11	17	rs17809012	[45–47]	iPLEX	0.957	G/A	0.454	0.615
CCL5	17	rs1800825	[57]	iPLEX	0.973	C/T	0.030	0.432

Literature cited is attached as a separate supplementary references file
MAF minor allele frequency, *HWE* Hardy-Weinberg equilibrium

Table 2 Association of Genotype and Allele Frequencies with Asthma Among Cases and Controls

SNP	sample	Minor Allele	Major Allele	Homozygotes Minor	Heterozygotes	Homozygotes Major	odds Ratio	95%CI
		T	C	TT	CT	CC		
rs1800871	case	252	396	55	142	127	0.81	(0.62–1.05)
	control	155	197	33	89	54		
		G	A	GG	AG	AA		
rs1800896	case	179	473	30	119	177	1.38	(1.01–1.88)
	control	74	270	7	60	105		
		T	C	TT	CT	CC		
rs10173081	case	36	624	1	34	295	0.68	(0.41–1.14)
	control	28	332	1	26	153		
		T	C	TT	CT	CC		
rs1295685	case	206	450	36	134	158	1.06	(0.80–1.41)
	control	103	239	16	71	84		
		T	C	TT	CT	CC		
rs1800925	case	149	497	21	107	195	1.45	(1.04–2.02)
	control	59	285	3	53	116		
		T	C	TT	CT	CC		
rs1837253	case	199	455	28	143	156	0.85	(0.65–1.11)
	control	126	244	20	86	79		
		T	C	TT	CT	CC		
rs20541	case	191	449	35	121	164	0.97	(0.73–1.28)
	control	107	243	17	73	85		
		G	C	GG	CG	CC		
rs2289278	case	54	560	1	52	254	0.98	(0.61–1.56)
	control	30	304	4	22	141		
		G	T	GG	GT	TT		
rs1342326	case	104	546	14	76	235	0.92	(0.65–1.30)
	control	60	290	5	50	120		
		G	A	GG	AG	AA		
rs4986790	case	70	604	7	56	274	0.91	(0.60–1.36)
	control	42	328	1	40	144		
		A	G	AA	AG	GG		
rs3741240	case	259	393	53	153	120	0.94	(0.72–1.22)
	control	148	210	31	86	62		
		G	A	GG	AG	AA		
rs1801275	case	134	522	15	104	209	1.08	(0.78–1.50)
	control	66	278	10	46	116		
		C	A	CC	AC	AA		
rs1805011	case	39	605	3	33	286	1.68	(0.88–3.19)
	control	13	339	1	11	164		
		G	A	GG	AG	AA		
rs17809012	case	298	344	69	160	92	1.12	(0.86–1.46)
	control	149	193	34	81	56		

Table 2 Association of Genotype and Allele Frequencies with Asthma Among Cases and Controls *(Continued)*

SNP	sample	Minor Allele	Major Allele	Homozygotes Minor	Heterozygotes	Homozygotes Major	odds Ratio	95%CI
		C	T	CC	CT	TT		
rs1800825	case	22	636	1	20	308	1.29	(0.59–2.83)
	control	9	335	0	9	163		

Discussion

This candidate gene study was conducted in Pakistani asthma patients and healthy controls. This study was carried out to validate in our population various cytokines and chemokines associations with asthma. This is the first study to report associations between these SNPs and asthma in Pakistani population to see the effect of these variations in pathogenesis if we look at the immunological pathways.

In the present study, we investigated the association between 15SNPs in 10 candidate genes (Table 1) for asthma in the Pakistani population. All of the genes included in the present study were either directly or indirectly involved in pathways affecting the immunological process. The lack of very strong associations in our data could be due to the relatively small size of the study sample and the fact that the subjects in our study were adults whereas most of the previous genetic studies were performed largely in children with asthma [3, 6–8]. The present data is a helpful in future genetic studies of adult asthmatics.

Asthma is caused by interaction of multiple genes, some of which have a protective effect and others contribute to the pathogenesis of the disease, with each gene having its own tendency to be influenced by the environment. In asthmatic individuals, antigen presentation is thought to result in the polarization of T-cell differentiation towards a Th2 pattern, whereas T-cells from non-atopic, non-asthmatic individuals show the opposing Th1 (interferon-γ and *IL-2*) pattern of cytokine secretion [9]. Activated Th2cells secrete cytokines such as *IL-4*, *IL-13* and *IL-5*. *IL-13* has a pivotal role in asthma pathogenesis: it activates a receptor complex that is composed of the *IL-4Rα* and *IL-13Rα1* on many cell types in the airway wall and is thought to mediate many processes that are relevant to asthma pathology as a result of activation of this receptor complex [10]. Th17 lymphocytes also play a role in the pathogenesis of several autoimmune and inflammatory diseases [11]. Studies have also demonstrated that the proportion of Th17/Th2 cells is extremely low in healthy subjects, whereas their numbers appeared to be significantly higher in the circulation of patients with chronic severe asthma [12].

Homozygosity for the minor alleles at *IL13* was associated with asthma risk. There were more TT homozygotes at rs1800925 in *IL13*in cases compared to controls (recessive risk model, $P = 0.009$). Interleukin *(IL)-13* is a critical mediator in the pathogenesis of allergic inflammation [13]. This cytokine upregulates major histocompatibility complex class II expression and promotes IgEisotype switching. This cytokine is found to be critical to the pathogenesis of allergen-induced asthma but operates through mechanisms independent of IgE [14]. Multiple genetic variants in the promoter (C-1112 T: rs1800925; A-1521C: rs1881457) and coding regions (G2044A: rs20541) have been associated with atopic asthma and non-atopic asthma, increased risk of sensitization to food and outdoor allergens, and bronchial hypersensitiveness in multiple studies [15, 16]. It has also been reported that the rs1800925 (–1112C/T) polymorphism resulted in enhanced promoter activity [17]. In spite of the importance of *IL-13* in asthma [18–20], some studies failed to show an association between *IL13* polymorphisms and asthma phenotypes [21, 22], possibly because of different exposures to environmental risk factors such as tobacco smoke exposure. Beghe [23] reported associations of rs1800925, rs1295685 and rs20541 in *IL13* with both atopy and asthma. These are different SNPs which are located in *IL13*, some or which are not in LD. All three SNPs were included in our study of Pakistani cases and controls, but only rs1800925 showed evidence of association with asthma. In all studies, including ours, the T allele was associated with susceptibility. Previous functional study on SNP rs1800925 showed that -1112 T allele enhanced *IL13* promoter activity in CD4+ Th2 lymphocytes. Increased expression of *IL13* -1112 T in Th2 cells was associated with attenuated *STAT6*-mediated transcription repression [24]. This offers a possible reason for the significant *P*-value for the T allele of rs18000925 in our Pakistani subject population.

Homozygosity for the minor alleles at SNPs *IL10* was associated with asthma risk. There were more GG homozygotes at rs1800896 in *IL10* in cases compared to controls (recessive risk model $P = 0.04$). *IL-10* is important in immuno-regulation and considered to be an immunosuppressive factor. Low levels of *IL-10* expression have been reported to play a role inthe pathogenesis of asthma [25, 26]. In contrast, high levels of *IL-10* from regulatory T-cells have a protective effect against airway hyperreactivity and inflammation [27]. In our study, we replicated the association between asthma and a SNP (rs1800896) in the promoter region of *IL10*. This polymorphism conferred susceptibility to

asthma in East Asians and adult asthmatics [28]. It lies within a putative ETS-like transcription factor binding site, and it has been suggested that a G allele at this position results in higher expression levels of *IL-10* transcript [29, 30].The association with the G allele in the Pakistani population is consistent with results of studies in Indian [31], Egyptian [6] and other populations [32–34], although the opposite allele was reported in a Korean population [35].

Conclusion

The GG genotype at rs1800896 (*IL10*) and the TT genotype at rs1800925 (*IL13*) are susceptibility genotypes for asthma in the Pakistani population. These results are consistent with previous results on Caucasians and other related population. This adds up new information of these cytokines in Pakistani population as this is not reported in our population previously and this data can be helpful infuture prospects of genetic studies in other world. Further functional genomicsstudies on large number of samples will be needed to replicate these associations and determine the influence of these genes on asthma pathogenesis in Pakistani population, and their roles in gene-gene and gene-environment interactions.

Summary at glance

Asthma is a chronic disease of the airways. Its causes are not understood. Both genetic and environmental factors are involved. In the present study15 SNPs in 10cytokine and chemokine genes were genotyped in Pakistani asthmatic cases and controls. For genotyping the Sequenom Mass ARRAY iPLEX platform and TaqMan assay were used. Polymorphism in *IL10* and *IL 13* are associated with asthma susceptibility in Pakistani population.

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Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analysed during the current study. All the data published here can be reproduced by the corresponding author if asked.

Authors' contributions

NS carried out the molecular genetic studies, participated in the data analysis and drafted the manuscript. GR participated in the design of the study and supervised all the work done. OY diagnosed the cases of asthma and helped in sample collection of cases and controls. SR participated in the study design and helped to draft the manuscript. SM participated in the design of the study and helped in molecular genetic studies. AM participated in the design of the study and guided in the statistical analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The ethical review committee of the parent organization approved this project (ERC-08-01). Written informed consent was obtained from all participants.

Consent for publication

Informed consent for publication was obtained from all participants.

Competing interests

All authors declare that they have no competing interests.

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