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Impact of genetic and environmental factors on development of atopy and allergic diseases in Czech and Russian populations.

Dissertation Thesis for the degree

Philosophiae Doctor (Ph.D.)

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Prague, 2009

“What is the use of a book without pictures or conversations?”

Lewis Carroll

To my family (mam, dad, grandma and Slava), my friend Daria and all my teachers.

This thesis summarizes the results of my Ph.D. project devoted to investigation of impact of genetic and environmental factors on development of atopy and allergic diseases in humans. All experimental data were completed into five publications (three of which have been published in journal of *Allergy*, *JACI* and in journal of *Immunogenetics* and two are presented here as manuscripts) and one book chapter. A short summary statement preceding each article in the Results chapter indicates my contribution to these studies.

The experimental work was performed mostly in the Department of Molecular and Cellular Immunology, Institute of Molecular Genetics AS CR, v. v. i., Prague, Czech Republic and also in the Department of Medical Genetics, Siberian State Medical University, Tomsk, Russia.

The clinical material was collected in the Czech Republic by Department of Allergology and Clinical Immunology, University Hospital KV, Prague, Department of Immunology and Microbiology, Institute of Public Health, Ústí nad Labem and Allergologic Clinic of Dr. M. Zemanová, Trutnov, in Russia by Faculty of Pediatrics, Siberian State Medical University, Tomsk, General Hospital of Thumen State Medical Academy, Thumen and Department of Allergology and Pulmonology, Irkutsk State Institute for Post-graduate Medical Education, Irkutsk and in the Ukraine by Institute of Dermatology and Venerology, Academy of Medical Sciences of Ukraine, Kharkov.

Support for this work was provided by the European Commission (INTAS Genomics 05-1000004-7761), Academy of Sciences of the Czech Republic (project grant AVOZ50520514), Ministry of Education of the Czech Republic (project grant LC06009), and Grant Agency of the Czech Republic (grants 310/06/1745 and 310/03/H147).

List of abbreviations

IgE	immunoglobulin E
Specific IgEs to allergens	
m6	*Alternaria alternata*
m3	*Aspergillus fumigatus*
m2	*Cladosporium herbatum*
m1	*Penicillium notatum*
e3	horse
e2	dog
e1	cat
d2	*Dermatophagoides farinae*
d1	*Dermatophagoides pteronyssinus*
w9	plantain - *Plantago lanceolata*
w6	mugwort - *Artemisia vulgaris*
w1	common ragweed - *Ambrosia elatior*
t7	oak - *Quercus alba*
t4	hazel - *Corylus avellana*
t3	birch - *Betula verrucosa*
t2	alder - *Alnus incana*
g12	cultivated rye - *Secale cereale*
g6	timothy grass - *Phleum pratense*
g3	cock's foot - *Dactylis glomerata*
g1	sweet vernal grass - *Anthoxantum odoratum*
f23	crab
f3	codfish

f85	celery
f35	potato
f31	carrot
f25	tomato
f237	apricot
f84	kiwi
f49	apple
f20	almond
f17	hazelnut
f13	peanut
f14	soya bean
f9	rice
f5	rye flour
f4	wheat flour
f45	yeast
f2	cow's milk
f75	egg yolk
f1	egg white

Spirometric indeces

FEV1(%)	percentage of forced expiratory volume in 1 second
FVC(%)	percentage of forced vital capacity
FEV1/FVC(%)	Tiffenau index
BNR	bronchial hyperresponsiveness

PCR	polymerase chain reaction
STR	short tandem repeat markers
SNP	single nucleotide polymorphism
HapMap	haplotype map
p, q	chromosomal arms
CD	cytoplesmic domain
Th	T helper
IL	interleukin
IFN	interferon
PGE2	prostaglandin E2
TNF	tumor necrosis factor
CO2	carbon dioxide
NO	nitric oxide
DEPs	diesel exhausted particles

Content

I. Preamble

IgE overproduction, known as atopy, is usually associated with development of allergic diseases such as allergic asthma, allergic rhinitis, atopic dermatitis, conjunctivitis, hay fever and others. The level of serum IgE is a complex physiological trait with both environmental and genetic factors playing a role in its regulation. While the concept of allergy has been well accepted since asthma and IgE were first described in 1880 and 1921, respectively (Holgate and Holloway 2003), the progress in establishment of heredity of allergy and atopy and definition of crucial environmental factors that can contribute to their development has become considerable only in the last few decades. Integration of different approaches of human and mouse genetics has revealed a number of genetic loci and genes involved in regulation of the level of IgE and influencing development of allergic diseases. Investigation of environmental and life-style conditions in different human populations has enabled to distinguish important risk factors of development of allergy in different populations from all over the world. Despite this remarkable progress, the etiology of atopy and acute allergic disorders (asthma, rhinitis and dermatitis) has not been completely elucidated and there are much more questions in this field than answers.

II. Introduction

1 Atopy

Atopy is a complex trait characterized by an individual predisposition to hyperproduction of IgE against common environmental allergens. It is the major risk factor for development of allergic diseases, the most acute of which are asthma, rhinitis, and atopic dermatitis. Patients with allergy are usually sensitized to inhalant and/or food allergens and express high level of total and allergen-specific IgE. The levels of total IgE >100 kU/l and allergen-specific IgE > 3.5 kU/l are usually associated with clinical symptoms of allergy and characterized as atopy.

Normally IgE is produced to protect humans against extracellular parasites (helminthes, schistosomes etc.). However, in atopic individuals IgE overproduction is stimulated by innocuous antigenic proteins of food, plant, fungi, or animal epidermis. The production of IgE is a cascade process of activation of T lymphocytes by antigen presenting cells, differentiation of T lymphocytes into T helper (Th2) cells that produce cytokines for activation and proliferation of B lymphocytes that subsequently differentiate into plasma cells and start IgE secretion (Figure. 1). Cytokines IL-4, IL-13, IL-5, IL-6, IL-9 and TNFα augment IgE expression, whereas interferon (IFN)γ, IFNα, IL-8, IL-10, IL-12 and prostaglandin E2 (PGE2) inhibit this process (reviewed in Romagnani 1997). IgE receptors on mast cells FcεRI and FcεRII (CD23) influence IgE level by the feedback mechanism (Gould et al. 2003). The allergic inflammation and symptoms of allergy are caused by mediators of inflammation (histamine, leukotrienes

and prostaglandins) that are released by mast cells in response to repetitive allergen exposure and recognition of the allergens by IgE on the surface of mast cells (Figure 1). The initial allergic response leads to vasodilatation, vascular leakage, and smooth muscle spasm (in case of asthma). The late phase of allergic reaction is associated with activation and degranulation of mast cells and eosinophiles that cause mucosal edema, mucus secretion, leukocyte infiltration, epithelial damage, and bronchospasm (in case of asthma). Obviously, the symptoms can be very different depending on whether the allergen was injected, inhaled, or eaten and depending also on the dose of the allergen.

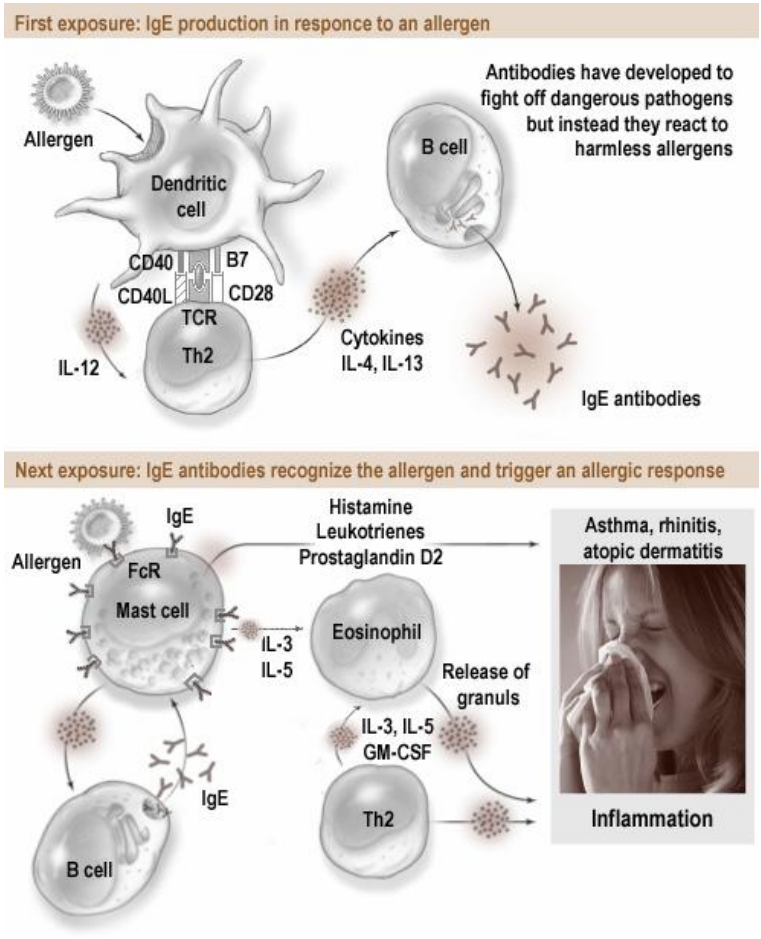

Figure 1. Type I hypersensitivity (allergic) reaction

2 Environmental risk factors of atopy in humans

The prevalence of atopy and atopic disorders varies in different geographical regions (Leonardi et al. 2002) and between urban and rural populations (Sozanska et al. 2007) and has a tendency to increase over the last few decades (Jarvis and Burney 1998). Since the natural mutation rate is low, altered environmental and life-style conditions most likely contribute to the increasing prevalence and incidence of atopy and allergic disorders. It is clear now that allergy is generally associated with the level of economical development, industrialization and life-style of a human population and it is the most prevalent in western and northern countries of Europe, USA, Australia, and New Zeeland (Jarvis and Burney 1998). Several factors have been found to be related to development of atopy: exposure to common allergens, deficiency in exposure to common viral and bacterial infections during early childhood, and exposure to irritants such as air pollutants and tobacco smoking (Figure 2).

Depending on socioeconomic habits (e.g. living conditions, profession, diet), prevalence of various allergen sources in a region and exposure to other environmental trigger factors, different allergens become the most harmful for development of atopy and manifestation of allergic disorders in human populations. For example, dust mites are potentially causative allergens for asthma in Australia (Peat et al. 1996), Central Virginia (Squillace et al. 1997), India (Chowgule et al. 1998), dust mites and cat in New Zealand (Sears et al. 1989), cat and dog in Scandinavian countries (Plaschke et al. 1999), cat in Siberia, Russia (Gusareva et al. 2006) and the mold *Alternaria* in Tuscon, Arizona (Halonen et al. 1997). In Puerto Rico atopic patients exhibited high sensitization to dust mites irrespectively of whether they suffered from dermatitis, rhinitis or asthma

(Montealegre et al. 2004). Dust mites, grass pollen and cat were the most prominent allergens in atopic children from the UK (Kurukulaaratchy et al. 2005), 74% and 45% atopic asthmatic children from Italy showed positive reaction to dust mites and grass pollen, respectively (Verini et al. 2001). Reactivity to cat, dog and house dust mite allergens exceeded 50% in patients with asthma and rhinitis from Canada (Boulet et al. 1997), whereas in China sensitization to silk allergens was significantly associated with allergic rhinitis (Celedon et al. 2001).

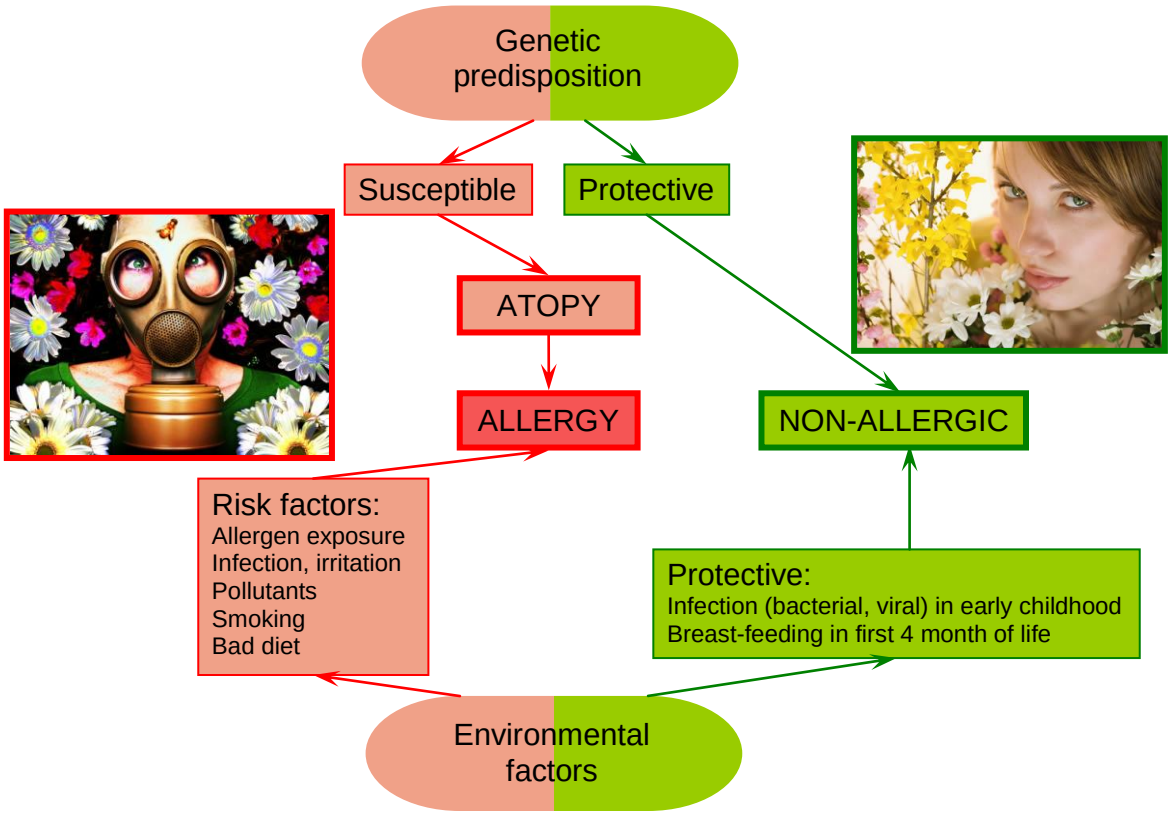

Figure 2. Hypothesis explaining the increase in the prevalence of atopy and allergic diseases. Risk factors (marked red) and protective factors (marked green) of atopy and allergic diseases.

Air pollution, particularly diesel fuel pollutants including volatile organic components, ozone, gases (CO_2, NO, aldehydes) and diesel exhausted particles (DEPs)

often exacerbate allergenicity of different inhalant allergens, especially pollens. Through physical contact with pollen particles, DEPs can disrupt it – thus facilitating their penetration to the human airways (Bartra et al. 2007). Accumulation of CO_2 and higher air temperature in a city versus suburb increases pollen output and makes it more aggressive to stimulate allergic reactions (Bartra et al. 2007).

However, the life-style conditions seem to have even stronger effect on the frequency of atopy and allergy in human populations. It has been consistently noted that in regions with higher pollution level the prevalence of atopy and allergic disease might be lower that in ecologically more clear places and that is strongly associated with "western type of life-style". Eventually, the comparison between East and West Germany give rise to "hygiene hypothesis". East Germany was much more polluted than West Germany before unification. However, the prevalence of allergic asthma was much lower in East than in West Germany, which is less polluted (Nicolai et al. 1997). It was also observed that children with a higher number of siblings have a reduced risk of allergic rhinitis than the older siblings (Strachan 1989). This observation was explained by the fact that younger children in the family acquire more respiratory infections at an early age, which are acquired from their older siblings. It was argued that infections in early life are protective against atopy and a decline in infections during early childhood has led to the increase in allergic disease in the industrialized world. Subsequently, studies showed a lower prevalence of atopy expression in children attended day care centers (Krämer et al. 1999), those living in farms (Braun-Fahrlander et al. 1999) and those with an anthroposophic life style (Alm et al. 1999), all of which are associated with an increased exposure to infections and microorganisms in early life.

However, the timing of the exposure to infection is critical for promoting

beneficial or harmful effects. The development of the immune system starts *in utero* and the initial priming of a fetus T cells against allergens intervenes during pregnancy. Allergens exposure of mother initiates Th2 immune response in fetus. In this time, the Th1 immune response is inhibited by IL-4, IL-10 and prostaglandin E2 because of the toxicity of Th1-cytokines (especially IFNγ) for placenta. The prenatal period and early childhood is considered to be critical for the establishment of the normal Th1/Th2 balance. All newborns have a Th2 immune response. During normal maturation through infancy, a Th1/Th2 balance is achieved with the induction of tolerance. However, atopic infants fail to achieve this balance continue to respond to allergens with a Th2-type response and the production of IgE antibodies. It was considered that bacterial and viral infection during early life directs the maturating of the immune system towards Th1 cells, which counterbalance to pro-allergic Th2 responses. A reduction in overall microbial exposure will result in weak Th1 cell stimulation that cause increased risk of atopy. However, when the infection occurs at the same site after the allergy has already developed and has become chronic it is considered to be more likely harmful. The presence of IL-4 during T cell priming may deviate the normally dominant Th1 response induced by infectious agent into a mixed Th1/Th2 response, resulting in exacerbation of the atopic disorder (Erb 1999). Thus, the development of the immune system is strongly dependent on the environmental factors that stimulate the immune system toward Th1 or Th2 response, whereas genetic factors determine individual predisposition to develop normally or to progress into pathology.

3 Genetic regulation of atopy and its impact on allergic disorders in humans

Atopy and allergic disorders show substantial familial aggregation. Although the model of IgE inheritance has not been completely elucidated (Los et al. 1999), studies of families, twins and mouse models of IgE overproduction have suggested that the level of IgE is a complex physiological trait with a high genetic heritability (36% - 82%) (Grundbacher 1975, Hopp et al. 1984, Hanson et al. 1991, Palmer et al. 2000) and is under control of multiple genes (Badalová et al. 2002), the effects of which can be strongly modified by environmental factors. Several approaches have been elaborated and applied to search for IgE-controlling genes and to investigate their biological role in development of allergies including candidate-gene approach, genome-wide linkage and association mapping in humans and genome-wide linkage analysis in mouse models with subsequent prediction of the trait loci/genes in humans (Figure 3).

The candidate-gene approach (hypothesis-driven) selects candidate genes on the basis of *a priori* knowledge of the trait/disease of interest (biological candidates) or of candidate-gene regions (positional) previously linked to the trait of interest. The method includes association studies that test the role of specific mutations (single nucleotide polymorphisms – SNPs and/or short tandem repeat – STR markers) in candidate genes. These can be either population-based (case-control) or family-based (transmission disequilibrium test). As an outcome of this approach, Th2 cytokines were a high priority of candidate genes in some of the earliest association studies on atopy and asthma (e.g. IL-4, IL-5, IL-13, CD14, ets.) and represent some of the most replicated associations to date (Wills-Karp and Ewart 2004). However, the complexity of IgE regulation makes

impossible to predict all genes that might be involved in predisposition to atopy.

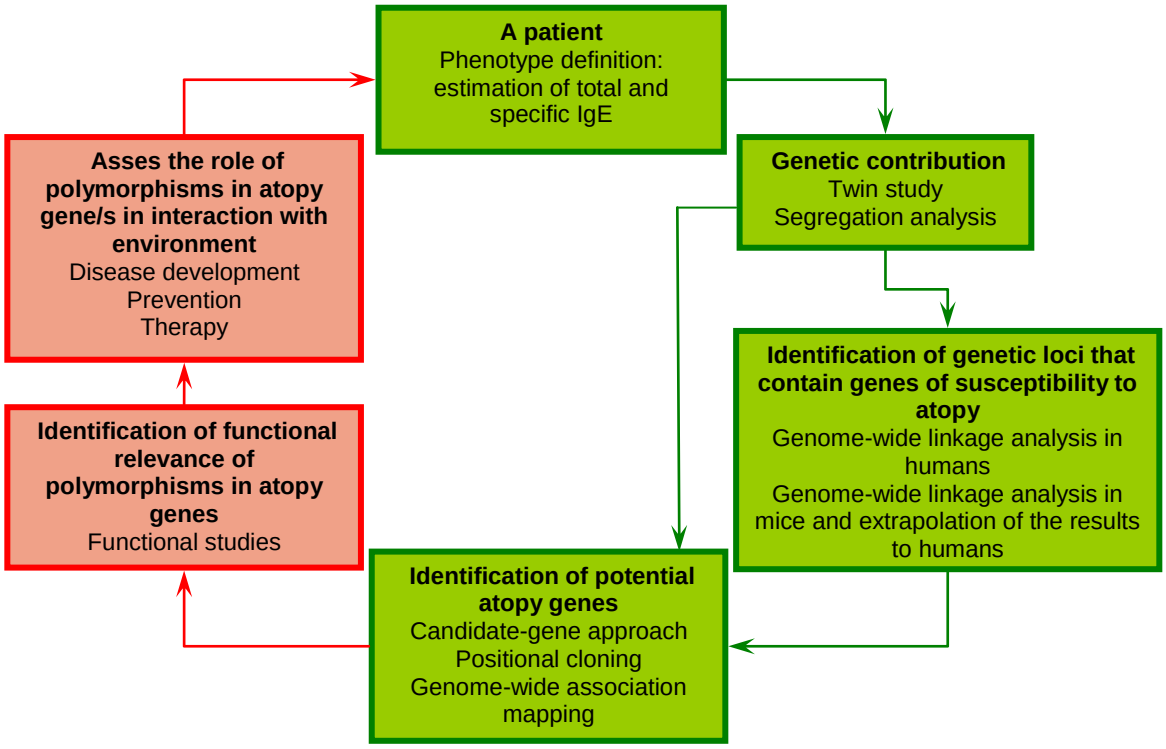

Figure 3. Strategies in genetic studies of atopy.

Strategies that have been successfully applied marked in green. Recent strategies that have just started applying are marked in red.

Alternatively, genome-wide screens allow previously unrecognized genes to be identified in an unbiased, hypothesis-independent manner. Genome-wide linkage studies, which might be model-based (inheritance pattern, extended family studies) or model-free (sibling pairs), attempt to identify patterns of co-segregation of the complex traits and polymorphic markers to identify loci and subsequently genes controlling these traits. In the genome-wide scans for IgE-controlling loci in humans the most often linkages were detected in chromosomal regions 5q (Xu et al. 2000; Yokoushi et al. 2000; Haagerup et al. 2002; Koppelman et al. 2002; Yokouchi et al. 2002), 6p (Daniels et al. 1996; Wjst et al. 1999; Haagerup et al. 2002; Ferreira et al. 2005), 7p (Daniels et al. 1996; Laitinen et

al. 2001; Shugart et al. 2001; Altmüller et al. 2005), 7q (Xu et al. 2000; Koppelman et al. 2002; Altmüller et al. 2005), 11q (Daniels et al. 1996; Shugart et al. 2001; Altmüller et al. 2005), 12q (Xu et al. 2000; Koppelman et al. 2002; Yokoushi et al. 2002) and 16q (Daniels et al. 1996; Ober et al. 2000; Kurz et al. 2005) (Table 1). Positional cloning approach (genome-wide scans for susceptibility loci and subsequent fine-mapping of the loci) indicated six genes at loci 2q14 (*DPP10* – dipeptidyl serine protease) (Allen et al. 2003), 2q33 (*CTLA4* – cytotoxic T-lymphocyte-associated-4 gene) (Howard et al. 2002), 5q32-33 (*PCDH1* – protocadherin-1) (Whittaker 2003), 7p14.3 (*GPRA* – G protein-coupled receptor) (Laitinen et al. 2004), 13q14 (*PHF11* – PHD finger protein 11) (Zhang et al. 2003), and 20p13 (*ADAM33* – Zn dependent metalloproteinase) (van Eerdewegh et al. 2002) predisposing to atopy or atopy-associated traits (Table 2).

In the last decade, the completion of the International HapMap Project makes the start of a new phase in human genetics (McVean et al. 2005). Characterization of patterns of genetic variation through typing over one million SNPs, calculation of linkage disequilibrium between them and construction of comprehensive map of SNP haplotypes provided an unprecedented view of human genetic diversity and become a wonderful tool for genome-wide association studies of complex traits in humans. In genetics of atopy and allergies genome-wide association mapping has led to identification of genes *ORMDL3* (an endoplasmic reticulum membrane protein) at locus 17q21 (Moffatt et al. 2007) and *CHI3L1* (chitinase 3-like 1) at locus 1q32.1 (Ober et al. 2008) that contribute to the risk of allergic asthma (Table 2).

Table 1. IgE-controlling loci described in genome-wide studies in humans

Chr.	Segment	cM	Marker	Population	Reference
1	1p31.1-13.2	138.69-146.53	D1S207 – D1S502	97 Caucasian families (83 German, 14 others)	Wjst1 et al. 999
	1q23-24	202.19	D1S518	533 Chinese families	Xu et al. 2001
2	2p21	65.94	D2S2298	97 Caucasian families (83 German, 14 others)	Wjst et al. 1999
	2q33.1	198.65	D2S116	97 Caucasian families (83 German, 14 others)	Wjst et al. 1999
3	3p24.1	52.6	D3S1266	48 Japanese families	Yokouchi et al. 2002
	3q24	161.04	D3S1744	100 Danish families	Haagerup et al. 2002
	3q29	224.88	D3S1311	200 Dutch families	Koppelman et al. 2002
4	4q23	104.94	D4S1647	38 Finnish pedigrees	Laitinen et al. 2001
	4q35.2	208.07	D4S2930	97 Caucasian families (83 German, 14 others)	Wjst et al. 1999
5	5q23.1-31.1	129.83-139.33	D5S1505 – D5S816	200 Dutch families	Koppelman et al. 2002
	5q23.2	127.29 (de code)	D5S818	100 Danish families	Haagerup et al. 2002
	5q33.2	156.47	D5S410	48 Japanese families	Yokouchi et al. 2002
	5q23-32	135.25-147.49	D5S666 – D5S402	200 Dutch families	Xu et al. 2000
6	6p23-21.3	34-60		USA Caucasians families	CSGA,1997
	6p23-21	35.0 – 60.9	D6S291	97 Caucasian families (83 German, 14 others)	Wjst et al. 1999
	6p22.2	44.41	D6S276	80 UK families	Daniels et al. 1996
	6p24.3	14.61	D6S277	100 Danish families	Haagerup et al. 2002
7	7p15.1-12.1	49.22 – 72.78	D7S526 – D7S1830	38 Finnish pedigrees	Laitinen et al. 2001
	7p14.1	57.79	D7S528	97 Caucasian families (83 German, 14 others)	Wjst et al. 1999
	7p14.3	54.11	D7S2250	80 UK families	Daniels et al. 1996
	7p14.1	61.0 – 63.67	D7S678 – D7S691	29 Karelian pedigrees	Laitinen et al. 2001
	7q21.11-22.3	98.44 – 109.12	D7S820 – D7S821	200 Dutch families	Xu et al. 2000
	7q21.11-22	98.44 – 109.12	D7S820 – D7S812	200 Dutch families	Koppelman et al. 2002
8	8p23.1	16.19	D8S503	107 French families	Dizier et al. 2003
9	9q31.1	111.99 – 129.74	D9S1784 – D9S195	97 Caucasian families (83 German, 14 others)	Wjst et al. 1999
11	11p13	42.55	D11S907	107 French families	Dizier et al. 2000
	11p15		D11S96	80 UK families	Daniels et al. 1996
	11q14.1	85.48	D11S901	80 UK families	Daniels et al. 1996
12	12p13.1	30.6	D12S364	48 Japanese families	Yokouchi et al. 2002
	12q24.23	134.54	D12S86	48 Japanese families	Yokouchi et al. 2002
	12q22-24.21	109.47 – 125.31	PAH – D12S2070	200 Dutch families	Xu et al. 2000
	12q22-24.21	109.47 – 125.31	PAH – D12S2070	200 Dutch families	Koppelman et al. 2002
13	13q13.2-13.3	25.80 – 32.90	D13S1493 – D13S218	200 Dutch families	Koppelman et al. 2002
15	15q26.1	86.81	D15S127	97 Caucasian families (83 German, 14 others)	Wjst et al. 1999
16	16q22.1-23.2	85.94 – 108.96	D16S421 – D16S505	80 UK families	Daniels et al. 1996
X	Xq23	70.91	DXS8081	97 Caucasian families (83 German, 14 others)	Wjst et al. 1999

Table 2. Genes controlling IgE, asthma and bronchial hyperresponsiveness (BHR)

Locus	Gene symbol	Gene name	Possible effects of the gene	Linkage to	Reference
1q32.1	***CHI3L1***	chitinase 3-like 1	remodeling lung tissue	asthma, BHR	Ober et al. 2008
2q33.2	***CTLA4***	cytotoxic T-lymphocyte-associated-4 gene	regulation of IgE level, costimulation and T-cell activation	**total IgE,** asthma, BHR	Hovard et al. 2002
2q14.1	***DPP10***	dipeptidyl serine proteases	cytokines and chemokines processing	asthma	Allen et al. 2003
5q31.3	**PCDH1**	protocadherin-1	Increase the susceptibility of epithelium, remodeling lung tissue	BHR	Whittaker et al. 2003
7p14.3	**GPRA**	G protein-coupled receptor	possible receptor for an unidentified ligand	asthma, BHR, **total IgE**	Laitinen et al. 2004
13q14.3	***PHF11***	PDH finger protein 11	regulation of IgE level	asthma, **total IgE**	Zhang et al. 2003
	SETDB2	histone H3 methyltransferase	regulation of IgE level	asthma, **total IgE**	Zhang et al. 2003
	RCBTB1	egulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 1	?	asthma, **total IgE**	Zhang et al. 2003
17q21	***ORMDL3***	endoplasmic reticulum transmembrane protein	?	asthma	Moffatt et al. 2007
20q13	***ADAM33***	Zn dependent metalloproteinase	airway hyperresponsiveness and remodeling	asthma, BHR	Van Eerdewegh et al. 2002

Despite this remarkable progress in identification of genes controlling atopy and asthma in humans, the complete elucidation of its genetics is hindered by many factors including sample size, genetic heterogeneity of human populations, gene interactions, low frequency and/or incomplete penentrance of trait-controlling alleles and a high variability of environmental factors (Lander and Schork 1994). Some limitations of human genetic studies can be overcome by the use of mouse models. The availability of genetically homogenous mouse strains, possibility to manipulate mouse genome through selective breading strategies and direct gene-targeting approaches and possibility to

control environment that reduces the phenotypic variance, affords mouse models considerable power to the study of complex genetic traits in human (Lipoldová and Demant 2006). Congenic mice strains are among the most useful tool for mapping of loci/genes of complex traits that simplify the genetic analysis because they allow characterization of a single locus without interference from multiple epistatic genes that also influence a phenotype (McIntire et al. 2001). Once the genetic regions of interest have been identified in mouse, the high level of synteny between many mouse and human chromosomal segments allows predicting their locations in human (DeBry and Seldin 1996) (Figure 4).

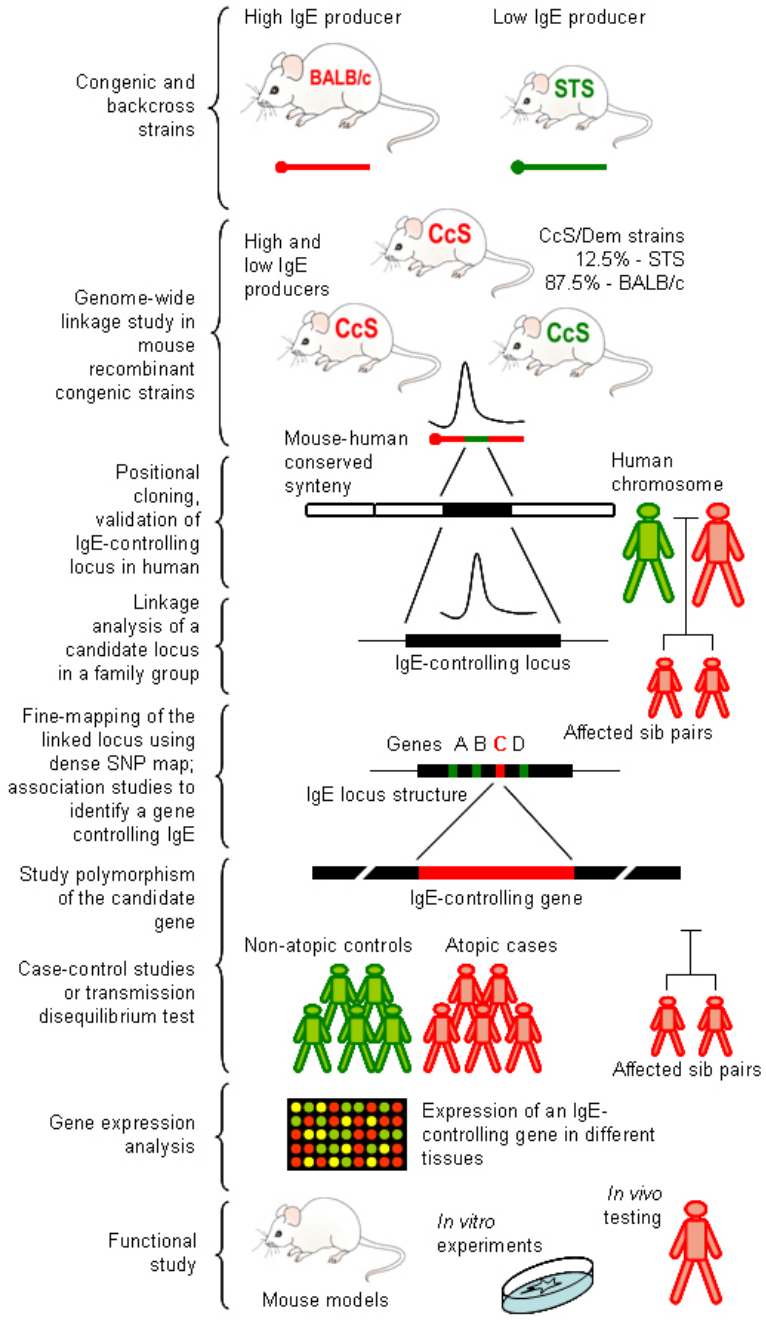

Figure 4. Combination of genome-wide scan for IgE-controlling loci in mice with candidate gene approach in humans.

Genome-wide scans in mouse models have been successfully applied for identification of human asthma genes on chromosome 2 (*C5* - complement component 5, human homolog 9q34) (Karp et al. 2000), chromosome 6 (*IL5RA* - interleukin 5 receptor, alpha, human homolog 3p26) (Daser et al. 2000), and chromosome 11 (*HAVCR1* - hepatitis A virus cellular receptor 1, human homolog 5q33.2) (McIntire et al. 2001) (Table 3).

Investigation of IgE regulation has been also conducted on mouse models of infection diseases caused by intracellular parasites (*Leishmania major*, *Heligmosomoides polygyrus* etc.) (Lipoldová et al. 2000, Badalová et al. 2002, Menge et al. 2003), the immune response to which is also characterized with IgE overproduction (Table 4). In the genome-wide search for IgE-controlling loci in mouse models of *Leishmania major* infection several chromosomal regions all over genome were identified (Table 4). From the conserved synteny between mouse and human genomes the homologous chromosomal segments were determined in humans. Majority of these segments (homologous to *Lmr3, Lmr5, Lmr8, Lmr10, Lmr11, Lmr13* and *Lmr14* in mouse) had been already described in genome-wide scans for atopy and asthma loci in humans (Koppelman et al. 2002; Xu et al. 2000; Yokoushi et al. 2002; Wjst et al. 1999; Howard et al. 2002). However, two chromosomal segments identified from the homology with mouse (*Lmr9* and *Lmr12*) have not shown evidence for linkage with IgE or some allergic disorder in the previous studies in humans, and therefore are good targets for further identification of novel human genes, which are relevant for atopy.

Table 3. Genes and loci of IgE and traits associated with allergic asthma: Mouse models of atopy and allergies

Chromosome / cM	Flanking markers	Locus	Candidate gene	Mouse cross	Phenotype	Reference	Orthologous locus in humans (MGI)	Orthologous locus in humans linked with IgE
6 / 45.5	D6Mit105		*Il5rα*	A/J ↑ C57BL/6 ↓	immediate cutaneous hypersensitivity in response to immunization with birch pollen	Daser et al. 2000	3p26	-
11 / 20.8 – 23.0	D11Mit271 –D11Mit22	*Tapr*	*Havcr1* (*Tim1*)	BALB/c ↑ DBA/2 ↓	Th2 cytokine production, BHR	McIntire et al. 2001	5q33.2	5q33.2 / Yokouchi et al. 2002
2 / 28.0 – 30.0		*Abhr2*	*C5*	A/J ↑ C3H/HeJ ↓	allergen induced BHR, IL-12 production	Karp et al. 2000	9q34	-

Note: ↑ - high responder; ↓ - low responder; CcS strains contains a different, random set of approximately 12.5% genes of the “donor” strain STS and approximately 87.5% genes of “background” strain BALB/c

In the genome-wide study in mouse models of *Heligmosomoides polygyrus* infection two loci on chromosome 12 (39.0 – 45.0 cM) and chromosome 17 (15.1 – 29.4 cM) were described to control IgE (Menge et al. 2003) (Table 4). Later genome-wide study conducted by another group confirmed the locus on chromosome 17 and even shortened it to 0.9 cM (18.4 – 19.1cM) (Behnke et al. 2006) (Table 4). This data corroborate the genome-wide study that earlier identified the homologous locus (6p21-22) to control total IgE in humans (Daniels et al. 1996; Wjst et al. 1999). This facts support efficiency and power of the genome-wide screening in mice in identification of loci/genes of complex traits such as IgE in humans.

Table 4. IgE-controlling loci: mice models of IgE regulation during parasite infection

Chromosome / cM	Flanking markers	Locus	Potential candidate gene	Mouse cross	Phenotype	Reference	Orthologous locus in humans (MGI)	Orthologous locus in humans linked with IgE during allergies
1 / 79.0 – 88.3	rs6181202 – gnf01.167.396	*Lmr8*	*Tnfsf4* *Tnfsf6*	CcS-20 ↓ BALB/c ↑	IgE induced by *Leishmania major* infection	Badalová et al. 2002	1q22-31.1	-
1 / 101.5 – 112.0	rs3693165 – gnf01.197.353	*Lmr8*	*?*	CcS-20 ↓ BALB/c ↑	IgE induced by *Leishmania major* infection	Badalová et al. 2002	1q32.2-41, 1q44	-
2 / 47.51–107.0	D2Mit272 – D2Mit74	*Lmr14*	*Traf6,* *Ltk* *Pla2g* *ADAM 33*	CcS-16 ↑ BALB/c ↑	IgE induced by *Leishmania major* infection	Badalová et al. 2002	2p13-q14, 2q31.2, 2q32 7p14-cen, 9q34 11p14-11 11q11-12 15q11-22.2 15q26 20q11-13.33 20pter-q12	7p14.1 / Wjst et al. 1999; Laitinen et al. 2001 7p14.3 / Daniels et al. 1996; Laitinen at al. 2004 15q26.1 / Wjst et al. 1999 20q13 / Van Eerdewegh at al. 2002
3 / 25.0 – 55.0	rs13477108 – rs13477365	*Lmr11*	*Il12a* *Il6ra*	CcS-20 ↓ BALB/c ↑	IgE induced by *Leishmania major* infection	Badalová et al. 2002	1p21-11.2 1p32-31 1q12, 1q21-23.1 4q21-25, 4q28-31.3, 4q32-35 13q13.1-13.3	4q23 / Laitinen et al. 2001 4q35.2 / Wjst et al. 1999
4 / 0 – 1.9	0 - D4Mit264	*Lmr9*	*Lyn*	CcS-20 ↓ BALB/c ↑	IgE induced by *Leishmania major* infection	Badalová et al. 2002	8q12	8q12 Gusareva et al. in press
5 / 36.0 – 44.0	CEL-5_56167948 – rs13478349	*Lmr3*	*?*	CcS-5 ↓ BALB/c ↑	IgE induced by *Leishmania major* infection	Lipoldová et al. 2000	4p15.1-12cen 4q11-13.1 13q12.2	-
5 / 28.0 – 64.0	D5Mit54 – D5Mit319	*Lmr3*	*?*	CcS-20 ↓ BALB/c ↑	IgE induced by *Leishmania major* infection	Badalová et al. 2002	1p22, 4p16-cen 4q11-13, 4q21, 4q24-25, 7q11, 12q24, 13q12 22cen-q12	4q23 / Laitinen et al. 2001 12q24.23 / Yokouchi et al. 2002 12q22-24.21 / Xu et al. 2000; Koppelman et al. 2002

Note: ↑ - high responder; ↓ - low responder; CcS strains contains a different, random set of approximately 12.5% genes of the “donor” strain STS and approximately 87.5% genes of “background” strain BALB/c.

Table 4. *Continuation*

Chromosome / cM	Flanking markers	Locus	Candidate gene	Mouse cross	Phenotype	Reference	Orthologous locus in humans (MGI)	Orthologous locus in humans linked with IgE during allergies
8 / 16.0 – 32.0	D8Mit64 – rs3717251	*Lmr10*	*Scvr* *Casp3*	CcS-20 ↓ BALB/c ↑	IgE induced by *Leishmania major* infection	Badalová et al. 2002	8p12-11.2 8p23.1-21.3 4q31-35.2	8p23.1 / Dizier et al. 2000 4q35.2 / Wjst et al. 1999
10 / 50.0 – 70.0	D10Mit161 – rs13480828	*Lmr5*	*Ifng* *Stat6*	CcS-5 ↓ BALB/c ↑	IgE indused by *Leishmania major* infection	Lipoldová et al. 2000	12cen -q24.1 19p13.3	12q22-24.21 / Xu et al. 2000; Koppelman et al. 2002
10 / 68.0 – 70.0	D10Mit25 – D10Mit269	*Lmr5*	*Stat6*	CcS-16 ↑ BALB/c ↑	IgE indused by *Leishmania major* infection	Badalová et al. 2002	12q13-15	-
12 / 39.0 – 45.0	D12Mit177 – D12Mit194		*?*	SWR ↑ CBA ↓	IgE induced by *Heligmosomoides polygyrus* infection	Menge et al. 2003	14q24 14q31-32	-
16 / 26.5 – 36.0	D16Mit167 – D16Mit184	*Lmr12*	*?*	CcS-16 ↑ BALB/c ↑	IgE induced by *Leishmania major* infection	Badalová et al. 2002	3q12-21 3q28-29	3q29 / Koppelman et al. 2002
17 / 15.1 – 29.4	D17Mit29 – D17Mit180		*?*	SWR ↑ CBA ↓	IgE induced by *Heligmosomoides polygyrus* infection	Menge et al. 2003	3p24, 6p12, 6p22-21 16p13 19p13 21q22	3p24.1 / Yokouchi et al. 2002 6p23-21.3 / CSGA 1997 6p23-21 / Wjst et al. 1999 6p22.2 / Daniels et al. 1996
17 / 18.4 – 19.1		*H2-D* *TNF*	*H2-D* *TNF*	SWR ↑ CBA ↓	IgE induced by L4 antigen (against worms)	Behnke et al. 2006	6p22-21	6p23-21.3 / CSGA 1997 6p23-21 / Wjst et al. 1999 6p22.2 / Daniels et al. 1996
18 / 21.0 – 49.9	D18Mit37 – D18Mit46	*Lmr13*	*Scf1r* *Cd14* *Adbr2*	CcS-16 ↑ BALB/c ↑	IgE induced by *Leishmania major* infection	Badalová et al. 2002	5q21-35 18p11.2 18q11-21	5q23.1-31.1 / Koppelman et al. 2002 5q23.2 / Haagerup et al. 2002 5q33.2 / Yokouchi et al. 2002 5q23-33 / Xu et al. 2000 5q31.3 / Whittaker et al. 2003

Note: ↑ - high responder; ↓ - low responder; CcS strains contains a different, random set of approximately 12.5% genes of the “donor” strain STS and approximately 87.5% genes of “background” strain BALB/c.

4 Concluding remarks

Over the past decades, using various new approaches, genetics of atopy and allergy progressed from broad linkage regions to gene identification. The range of candidate atopy and allergy genes includes not only immune response genes (such as *HAVCR1*, *C5*, genes of interleukins and its receptors) but also genes, the effects of which on the development of atopy and susceptibility to allergy have not been clearly understood (e.g. *PHF11*, *ADAM33*, *DPP10*). Some of the loci/genes were found to regulate susceptibility to atopy and/or atopic diseases in several populations, whereas others have been detected in one or a few populations only (reviewed in Ober and Hoffjan 2006). Since many of the identified loci contain several genes of unknown effects on development of atopy, it is possible that in different populations different genes from the same chromosomal region may be involved in control of susceptibility (reviewed in Ober and Hoffjan 2006). Although a number of genes were identified, it is highly improbable that all the genes or combinations of genes that underlie atopy have been identified, therefore the etiology of atopy is still hidden.

III. Aims of the study

Aim 1. Definition of the major allergens that trigger development of atopy and allergic disorders in different human populations.

1. Define the major allergens that trigger development of allergy in Czech, Russian and Ukrainian atopic patients.
2. Estimate the level of allergic sensitization in healthy blood donors from Prague.
3. Define the impact of specific IgE to particular allergens on the level of total IgE in Russian patients with asthma and in Prague blood donors.

Aim 2. Mapping genetic loci of atopy and atopy-associated traits in Czech and Russian populations using results of genome-wide search in mice (obtained in the Laboratory of Molecular and Cellular Immunology, Institute of Molecular Genetics, Prague, Czech Republic) and in humans (detected by other scientific groups).

1. Select the most important candidate chromosomal regions of atopy described in humans by others and define the candidate chromosomal regions from the conserved synteny between mouse and human genomes. Study the effect of the candidate loci on IgE regulation and development of atopy-associated traits in Czech and Russian family groups of atopic patients.

IV. Materials and Methods

1 Subjects and patients

The genetic studies were conducted using two groups of nuclear families of atopic patients and their relatives from the Czech Republic (67 families, n=276) and from the Russian Federation (152 families, n=602). For investigation of risk factors of atopy and prevalence of allergic sensitization in different human populations we used two groups of patients with atopy from the Czech Republic (n=75) and Ukraine (n=53) and a group of patients with asthma from the Russian Federation (n=110). We also used a group of Prague blood donors (n=476) to study prevalence of total and specific IgE in healthy population. All the analyzed groups are described in details in the chapter V. Results.

2 Estimation of total and specific IgE levels

The collection of blood samples of atopic individuals and their relatives from the Czech Republic and Ukraine was conducted from February 1999 to February 2001. Blood samples of donors from Prague (Czech Republic) were collected in December 2007 – January 2008. Blood samples of Russian patients with asthma and their relatives were collected in 2004 – 2007. No blood samples were collected during the summer months. The sera were stored at –70^{o}C before use. The total IgE level was estimated by CAP-FEIA (Phadia, Uppsala, SE) in the Czech and Ukrainian subjects and by IgE-EIA-BEST–strip (VECTOR-BEST, Novosibirsk, Russia) in Russian subjects. Specific IgE was measured by the *in vitro* test system EUROLINE (EUROIMMUN, Medizinische Labordiagnostika GmbH, Lübeck, Germany) according to the instructions of the

manufacturer. In this system allergen extracts were used for detection of specific IgEs. The lowest threshold of detection was 0.35 kU/l. Sensitization to moulds (m6 – *Alternaria alternata*, m3 – *Aspergillus fumigatus*, m2 – *Cladosporium herbatum*, m1 – *Penicillium notatum*), animals (e3 – horse, e2 – dog, e1 – cat), mites (d2 – *Dermatophagoides farinae*, d1 – *Dermatophagoides pteronyssinus*), weeds (w9 – *Plantago lanceolata*, w6 – *Artemisia vulgaris*, w1 – *Ambrosia elatior*), trees (t7 – *Quercus alba*, t4 – *Corylus avellana*, t3 – *Betula verrucosa*, t2 – *Alnus incana*), and grass (g12 – *Secale cereale*, g6 – *Phleum pratense*, g3 – *Dactylis glomerata*, g1 – *Anthoxantum odoratum*) was measured by Inhalation test-system in all studied groups. Sensitization to fish (f23 – crab, f3 – codfish), vegetables (f85 – celery, f35 – potato, f31 – carrot, f25 – tomato), fruits (f237 – apricot, f84 – kiwi, f49 – apple), nuts (f20 – almond, f17 – hazelnut, f13 – peanut), grains (f14 – soy bean, f9 – rice, f5 – rye flour, f4 - wheat flour), and food of animal origin (f45 – yeast, f2 – cow's milk, f75 – egg yolk, f1 – egg white) was measured by Food test-system only in Czech and Ukrainian patients. The inhalant and food atopy were defined as sensitization to at least one of the tested inhalant and food allergens, respectively. The major allergen and important allergen were defined as the allergen to which more than 50% and 30% of patients in a group were sensitized, respectively. The clinically important level of sensitization was defined as more than 3.5 kU/l, which is usually concomitant with symptoms of allergy (according to the EUROLINE protocol).

3 Genetic markers

Genetic studies were conducted in Czech and Russian family groups. Genetic markers are

described in details in the chapter V. Results. For the analysis, short tandem repeat (STR) markers located at different chromosomes/chromosomal regions were selected (Table 5) from the National Centre for Biotechnology Information (NCBI) database (http://www.ncbi.nih.gov). The STR markers are characterized in the Marshfield genetic map and show high heterozygosity.

Table 5. The list of markers tested for linkage and association with asthma, total IgE, and specific IgEs.

Marker	Chromosome	cM (Marshfield)	Reference
D2S308	2q14.1	124.03	Allen et al. 2003
D5S816	5q31.1	139.33	Koppelman et al. 2002
D5S1507	5q33.2	157.57	Yokoushi et al. 2000
D6S291	6p21.31	49.50	Wjst et al. 1999
D7S2250	7p14.3	54.11	Daniels et al. 1996; Shugartt et al. 2001; Laitinen et al. 2001
D7S821	7q21.3	109.12	Xu et al. 2000
D8S1828	8q12	71.00	Badalová et al. 2002
D8S285	8q12	71.00	Badalová et al. 2002
D8S1816	8q12	71.00	Badalová et al. 2002
D11S2006	11q12.1	59.24	Adra et al. 1999
D12S1298	12q14.1	75.17	
D12S379	12q21.31	93.69	Barnes et al. 1996, Nickel et al. 1997
D12S1059	12q23.1	105.18	
D12S338*	12q23.3	111.87	
D12S1645*	12q24.11	119.55	
D12S2082*	12q24.22	130.94	
D12S1282	12q24.3	136.82	
D12S1611*	12q24.3	140.17	
D12S1634*	12q24.3	148.24	
D13S165	13q14.2	45.55	Zhang et al. 2003
D16S3253	16q12.2	71.77	Daniels et al. 1996
D16S539	16q24.1	124.73	Ober et al. 2000
D19S601	19q13.33	83.19	Venanzi et al. 2001
D20S473	20p13	9.53	van Eerdewegh et al. 2002

Note: * - markers were tested in the Russian family group only.

All markers, with the exception of the markers on chromosome 8 (D8S1828, D8S285, D8S1816), are located in atopy candidate regions previously described in genome-wide studies of atopy and/or asthma (Daniels et al. 1996, Wjst et al. 1999, Ober et al. 2000, Xu2000, Yokoushi et al. 2000, Laitinen et al. 2001, Shugart et al. 2001, Haagerup et al. 2002, Koppelman et al. 2002, Yokouchi et al. 2002, Altmüller et al. 2005, Ferreira et al. 2005, Kurz et al. 2005). Markers selected in regions 2q14.1 (Allen2003), 5q31.1 (Koppelman et al. 2002), 5q33.2 (Yokoushi et al. 2000), 6p21.31 (Wjst et al. 1999), 7p14.3 (Daniels et al. 1996, Laitinen et al. 2001, Shugart et al. 2001), 7q21.3 (Xu et al. 2000), 12q21.31 (Nickel et al. 1997), 16q24.1 (Ober et al. 2000), and 19q13.33 (Venanzi et al. 2001) were exactly those that exhibited linkage, or in regions 11q12.1 (Adra et al. 1999), and 16q12.2 (Daniels et al. 1996, Kurz2000) were located in the close proximity. To test 13q14.2 and 20p13 we selected the nearest polymorphic STR marker to the genes *PHF11* (Zhang et al. 2003) and *ADAM33* (van Eerdewegh et al. 2002), respectively. Chromosome 12q harbors multiple genetic loci related to asthma and asthma-associated phenotypes, distinct peaks of linkage being observed in different populations (Raby et al. 2003). Four markers in regions 12q14.1, 12q21.31, 12q23.1, and 12q24.3 (D12S1298, D12S379 (Barnes et al. 1996, Nickel et al. 1997), D12S1059, and D12S1282, respectively) were selected in positions that would enable to test presence of described linkages in the studied population. After identification of a linkage of atopy to 12q24.3 at 136.82 cM in Russian family group, we selected five additional STRs from surrounding regions (D12S338 at 111.87 cM, D12S164 at 119.55 cM, D12S2082 at 130.94 cM, D12S1611 at 140.17 cM, and D12S1634 at 148.24 cM) to position the linkage map more precisely.

Markers on chromosome 8q12 (D8S1828, D8S285, D8S1816) were chosen on the basis of our previous whole-genome search for IgE-controlling loci in mouse (Badalová et al. 2002). The mouse locus *Lmr9* (represented by marker D4Mit149) was mapped to the centromeric part of the mouse chromosome 4 with the most likely and maximal lengths 3.58 and 9.32 Mb, respectively, and was shown to have linkage with IgE level (Badalová et al. 2002). The region homologous to the mouse *Lmr9* is located on human chromosome 8q12 (NCBI database) and the markers D8S1828, D8S285, D8S1816 were selected for the search for IgE-controlling loci in humans.

4 Genotyping

The primer sequences were obtained from the NCBI database. We used Cy5 carbocyanine dye 5'-end-labeled forward primers and unlabeled reverse primers synthesized by Generi-Biotech s.r.o., (Hradec Králové, Czech Republic) or Sigma-Genosys Ltd., (Steinheim, Germany). DNA was amplified in a 10-µl PCR reaction with 10 pmol/µl of forward and reverse primer, 0.2 mM concentration of each dNTP, 1.5 or 2.0 mM $MgCl_2$ (optimized for each STR), 50 mM KCl, 20 mM Tris-HCl (pH 8.4), and 0.1 U of *Taq* polymerase, recombinant (GIBCO, Grand Island, NY) and 5 ng/µl of template DNA. The PCR reaction was performed on 0.2 ml non-skirted 96-well PCR plate (ABgene, Epsom, UK) by MJ Research Thermal Cycler PTC 100 Model 96 (MJ Research, Watertown, MA). The universal program was used for DNA amplification: an initial hot start 5 min at 94 °C, followed by 39 cycles of 94 °C for 20 s for denaturing, 55 °C for 20 s for annealing, 74 °C for 20 s for elongation, and finally 10 min at 72°C for final extension. PCR products (0.25 µl) were separated by CEQ™ 8800 Genetic Analysis

System (Beckman Coulter Inc., Fullerton, CA, USA). All inconclusive genotypes were excluded (less than 2.2% for each marker).

5 Statistical analyses

5.1 Comparison of sensitization to the tested allergens in subgroups of patients and correlation between total and specific IgE.

Data were analyzed using the software Microsoft Excel 2000 (Microsoft Copyright 1985-1999) and STATISTICA 8.0 (StatSoft, Inc. 1984-2007, Tulsa, OK, USA). The level of sensitization in the groups was compared by Mann Whitney U test. All asymptotic *P* values were corrected for Bonferroni correction. Relationship between total and specific IgEs was evaluated by nonparametric Spearman correlation analysis.

5.2 Linkage and association analysis

We included all family members in statistical analysis regardless of affected status. Two different approaches were used for statistical analysis of data. The first approach included non-parametric linkage analysis for co-segregation of a chromosomal region and a trait of interest (qualitative and quantitative). The analysis is based on the calculation of LOD score using the Kong and Cox linear model (Kong and Cox 1997). This method allows using small nuclear families and calculation of linkage without assuming the normal distribution of the studied trait. We used the Whittemore and Halpern NPL pairs statistic (Whittemore and Halpern 1994) to test for allele sharing among affected individuals. The computer program MERLIN version 1.0.0 - © 2000 – 2005 (Abecasis et al. 2002) was used for calculation allele frequencies (across all individuals) and LOD scores.

The second approach used association analysis for quantitative and discrete traits (QTDT). The general models of QTDT described by Abecasis et al. (Abecasis et al. 2000a, Abecasis et al. 2000b) is applicable to the analysis of quantitative or discrete traits in nuclear families of any size and optionally uses parental phenotypes. We used the orthogonal model (Abecasis et al. 2002) to perform the association analysis of the markers as well as their allelic variants with total and specific IgEs. Calculation was conducted by QTDT program version 2.4.6 - © 1998 – 2004 (Abecasis et al. 2000b). Permutation framework (100 000 permutations) provided by QTDT program was used to obtain global *P* values that were subsequently corrected for multiple testing by Bonferroni correction for number of tested markers and numbers of alleles of the tested markers.

Sex and age were chosen as covariates in both non-parametric linkage and linkage disequilibrium analysis of the total and specific IgEs, and inhalant and food atopy. Correlation between phenotypes (sensitization to different allergens) was estimated by the Nonparametric Spearman Correlation analysis using STATISTICA for Windows version 8.0 (StatSoft, Inc. 1984-2008, Tulsa, OK, USA).

V. Results

1 Prevalence of total and specific IgE in different human populations

1.1 Cat is a major allergen in patients with asthma from the west Siberia, Russia.

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Allergy 2006; 61:509-510.

Summary statement

Specificity of the major allergen profiles characteristic for certain geographical area determines elaboration of prophylactic programs and immunotherapeutic methods of treatment. The aim of this study was to define major allergens for Russian patients with asthma from Siberia. Therefore, specificity and intensity of sensitization to 20 different inhalant allergens was estimated in patients with atopic bronchial asthma from Tomsk and Thumen, cities in the west Siberia, Russia. Our data indicated the importance of cat, and also dog, and dust mite allergens in maintaining the manifestation of asthma, while sensitization to outdoor allergens (molds and plant origin allergens) was low. The present work is the first report on allergic sensitization in Russian asthmatic patients from Siberian region and can serve as a baseline for future studies of risk factors of asthma development in Russia.

As a first author, I contributed to the study design, estimation of specific IgEs, statistical analysis and interpretation of data, and preparation of the manuscript.

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Cat is a major allergen in patients with asthma from west Siberia, Russia

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Key words: asthmatic patients; cat, dog and mite allergens; Russia; sensitization; specific IgE.

Asthma is the most severe allergic disease. The majority of cases are associated with atopy, which is characterized by hyperproduction of total and specific immunoglobulin E (IgE) against common environmental allergens (1, 2). Major allergens and prevalence of sensitization in atopic patients vary in different populations, providing cues about the pathogenic effects of environment and lifestyle. In Russia, measurement of sensitization to allergen mixtures is a common clinical practice in patients with severe symptoms of allergic disorders. The data on the less common tests of individual allergens have not been systematically collected nor published in international journals and they are not accessible to scientists and clinical allergologists.

We present the first data on allergic sensitization in asthma patients from Russia.

We therefore estimated the specificity and intensity of sensitization to 20 different airborne allergens in patients with atopic bronchial asthma from Tomsk (n = 67) and Thumen (n = 43), cities in west Siberia, Russia.

Participants were enrolled from databases of Tomsk and Thumen clinics. Nine (13.4%) and six (13.9%) patients from Tomsk and Thumen, respectively, refused to participate in the study for different reasons. Individuals aged 4–18 years were enrolled in the study on the basis of medical history of atopic bronchial asthma. Clinical specialists verified the diagnoses according to the Global Initiative for Asthma (GINA) protocol. A structured interview was performed with each participant (or his/her guardians), and a questionnaire about the clinical symptoms of asthma, nature of disorder manifestation and smoking habits was completed. The spirometric indexes were also taken into account when performing the diagnostics. All patients were noncognate and were not exposed to any intentional selection. The study was approved by the local Ethical Committee.

Blood samples were collected in April to May and in November to December of 2004 and in March 2005. Specific IgE was measured by the *in vitro* test system EUROLINE (EUROIMMUN, Medizinische Labordiagnostika GmbH, Lübeck, Germany), according to manufacturer's instructions. The groups were compared by Mann–Whitney U-test. The major allergen and important allergen were defined as those to which more than 50% and 30% of patients in a group were sensitized, respectively. The IgE distribution profiles and the level of sensitization were not significantly different in Tomsk and Thumen groups (P = 0.22–0.29); therefore, patients have been pooled in one group (n = 110).

Cat allergen (e1) was found to be the major allergen with 57.3% of Russian asthmatic patients sensitized to this allergen (Fig. 1). Other important allergens were *Dermatophagoides pteronyssinus* (d1), *D. farinae* (d2) and dog allergen (e2), with more than 30% of asthmatics sensitized.

Very high reactivity to indoor allergens has been observed, while sensitization to outdoor allergens (moulds and plant-origin allergens) has been very low. The inclement climate of Siberia with a long winter period and low air moisture prevents accumulation of pollen and mould spore allergens in the air and decreases the possibility of sensitization. Cats and dogs are present in many of the Russian houses. Moreover, the transportability of cat and dog allergens on clothing (3) facilitates sensitization of asthmatics irrespective of pet-keeping at home directly (P > 0.05). Poor ventilation of homes may also contribute to accumulation of mite allergens in beddings and

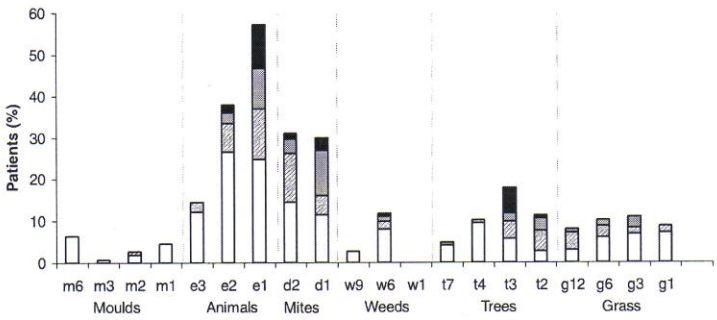

Figure 1. Proportion (%) of Tomsk and Thumen (n = 110) asthmatic patients sensitized to airborne allergens in relation to the level of circulating IgE. Sensitization to moulds (m6 – *Alternaria alternata*, m3 – *Aspergillus fumigatus*, m2 – *Cladosporium herbatum*, m1 – *Penicillium notatum*), animals (e3 – horse, e2 – dog, e1 – cat), mites (d2 – *Dermatophagoides farinae*, d1 – *D. pteronyssinus*), weeds (w9 – *Plantago lanceolata*, w6 – *Artemisia vulgaris*, w1 – *Ambrosia elatior*), trees (t7 – *Quercus alba*, t4 – *Corylus avellana*, t3 – *Bettula verrucosa*, t2 – *Alnus incana*), and grass (g12 – *Secale cereale*, g6 – *Phleum pratense*, g3 – *Dactylis glomerata*, g1 – *Anthoxantum odoratum*) is shown. □ 0.35–3.5 kU/l, ▨ 3.5–17.5 kU/l, ▩ 17.5–50 kU/l, ■ 50–100 kU/l.

upholstered furniture, triggering atopic sensitization (4). Thus, very high reactivity to indoor allergens of asthmatic patients from west Siberia (Russia) seems to be a man-made problem and could be partly avoided by reducing risk allergen concentration at homes by frequent ventilation and cleaning of flats.

This is the first report on allergic sensitization in asthmatic patients from Russia. It shows the importance of cat, dog and dust mite allergens in maintaining the manifestations of asthma. Our data can serve as a baseline for future studies of risk factors in asthma development in Russia.

This work was supported by grants GACR 310/03/H147 and AVOZ50520514.

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Accepted for publication 17 November 2005
Allergy 2006: 61:509–510

DOI: 10.1111/j.1398-9995.2006.01034.x

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No evidence of tumor necrosis factor-α release in blood of patients with chronic urticaria

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Key words: autologous serum skin test; chronic urticaria; tumor necrosis factor-α.

Mast cells play a key role in chronic urticaria as they release histamine and other inflammatory mediators and cytokines causing urticarial symptoms. In about 30% of chronic urticaria patients, mast cell activation is triggered by autoantibodies directed against the α-subunit of the high affinity IgE receptor; in the other patients the stimuli inducing mast cell activation have not been defined, but an immunological mechanism is also suspected. From a practical point of view, the presence of circulating histamine-releasing factors is demonstrated by *in vivo* autologous serum skin test (ASST), which is considered as a screening test for histamine-releasing autoantibodies (1). Positivity of ASST allows distinguishing patients with chronic autoimmune urticaria from patients with an idiopathic disorder. Tumor necrosis factor (TNF)-α is released by human skin mast cells and other inflammatory cells which can be found at the site of urticarial lesions (2). Therefore, TNF-α is a candidate mediator of urticaria. Tillie-Leblond et al. (3) found TNF-α release during a systemic reaction occurring after cold immersion test in two patients with cold urticaria, and an increased immunoreactivity for TNF-α and IL-3 was detected on endothelial and perivascular cells of the upper dermis in skin lesions from chronic urticaria patients (4). We measured serum TNF-α levels in patients with chronic autoimmune and chronic idiopathic urticaria. Sera were drawn from 62 adult patients diagnosed as having chronic urticaria on the basis of recurrent hives for more than 6 weeks. In all cases, known causes of chronic or recurrent urticaria were ruled out by appropriate investigations. Physical urticarias were excluded as well. All patients had active urticaria at the time of the study. Five days after anti-histamine therapy (cetirizine, loratadine, or fexofenadine in all cases) was stopped, an intradermal test with 0.05 ml of fresh autologous serum (ASST) was carried out. ASST was performed and read at 30 min following the method by Sabroe et al. (1). Intradermal injection of saline solution (0.9% weight/volume NaCl) was performed as negative control and skin prick test with 10 mg/ml histamine as positive control. Patients showing a wheal with a diameter at least 1.5 mm greater than the control saline solution were considered positive. Forty patients were strongly positive on ASST and 22 were negative. TNF-α was assayed by a sensitive immunoenzyme method (R & D Systems, Minneapolis, MN, USA) using the same serum samples employed for ASST. Sera from 12 normal adult subjects were used as control. TNF-α concentration was below the sensitivity of the assay (15.6 pg/ml) in all serum samples from chronic urticaria patients and normal subjects. These results indicate that TNF-α is not released in measurable amounts in blood of patients with chronic urticaria, either autoimmune or idiopathic. Although a role for TNF-α as mediator of urticarial lesions cannot be excluded, its release in the skin microenvironment does not lead to a substantial increase in blood concentration.

Tumor necrosis factor-α is undetectable in peripheral blood of patients with chronic urticaria.

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Accepted for publication 16 November 2005
Allergy 2006: 61:510–511

DOI: 10.1111/j.1398-9995.2006.01036.x

Additional results to the publication "Cat is a major allergen in patients with asthma from the west Siberia, Russia": High positive correlation was observed between sensitization to cat and dog allergens (R = 0.68 and R = 0.74, $P < 0.001$ for Tomsk and Thumen patients, respectively) and house dust mite allergens *D. pteronyssinus* and *D. farinae* (R = 0.90 and R = 0.84, $P < 0.001$ for Tomsk and Thumen patients, respectively).

1.2 Relationship between total and specific IgE in patients with asthma from Siberia, Russia.

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J Allergy Clin Immunol, 2008; 121:781.

Summary statement

The letter to the editor "Relationship between total and specific IgE in patients with asthma from Siberia, Russia" was reported in response to original paper of Erwin *et al.* (2007), which analyzed relationship between total and specific IgE to cat and dust mite in patients with wheezing from New Zealand and Sweden. The aim of this work was to study relationship between total IgE and specific IgE to cat, dog, birch, and dust mites (*D. pteronyssinus* and *D. farinae*) in Russian patients with asthma from Tomsk, Thumen and Irkutsk (Siberia, Russia) and compare our data with results of Erwin *et al.* (2007). In the present publication, we supported results of Erwin *et al.* and demonstrated that allergen-specific IgE might not contribute prominently to the level of total IgE. Similar to data from Sweden, cat-specific IgE did not correlate significantly to high total IgE even though cat is a major allergen in Siberia.

As a first author, I contributed to estimation of specific IgEs, statistical analysis and interpretation of data, and preparation of the manuscript.

Relationship between total and specific IgE in patients with asthma from Siberia

To the Editor:

Erwin et al[1] compared levels of total IgE and specific IgE to dust mite and cat, prevailing allergens in New Zealand and Sweden, respectively, in children with wheezing and found a significant correlation of mite-specific IgE with high total IgE (≥200 IU/mL) in New Zealand, whereas cat-specific IgE did not contribute significantly the high total IgE in either country.

Previously we demonstrated that cat is a major allergen in patients with asthma from Siberia, other important allergens being dog, dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), and birch.[2] Prevalence of wheezing (based on International Study of Asthma and Allergies in Childhood [ISAAC] questionnaires) and asthma (diagnosed according to Global Initiative for Asthma [GINA] guidelines) in children from Siberian cities is 11.4% and 3.1%, respectively,[3] similar to Sweden. Because of the importance of findings of Erwin et al,[1] we investigated the relationship of the prevailing specific IgEs and total IgE in patients with asthma from the cities Tomsk, Thumen, and Irkutsk (Siberia, Russia). We determined total IgE and specific IgEs to cat, dog, dust mites, and birch in 127 patients with bronchial asthma (mean age, 11.5; range, 4-18 years, male/female ratio, 0.72/0.28) by using the IgE-EIA-BEST–strip (VECTOR-BEST, Novosibirsk, Russia) and the EUROLINE system (EUROIMMUN; Medizinische Labordiagnostika GmbH, Lübeck, Germany),[2] respectively. Relationship between total and specific IgEs was evaluated by nonparametric Spearman correlation (STATISTIKA for Windows 5.0; StatSoft, Inc, Tulsa, Okla).

The geometric mean of the total IgE in patients with asthma from Siberia (193.0 kU/L) was lower than in New Zealand, but almost 3 times higher than in patients from northern Sweden.[1] In Russian patients with asthma, we observed a higher correlation between levels of total IgE and specific IgE to cat, birch, and dog allergens than to mite allergens (Table I). However, the subgroup of 70 patients with total IgE ≥200 IU/mL showed a weak tendency to correlation only between birch-specific and total IgE. Thus, similar to data from Sweden,[1] cat-specific IgE did not contribute significantly to high total IgE even though cat is a major allergen in Siberia. This might reflect the mechanism of sensitization to cat allergens or climatic similarities between Siberia and northern Sweden.

It would be interesting to compare levels of specific and total IgE in other regions where dust mites are dominant allergens in asthma such as Virginia[4] to see whether correlation of mite-specific IgE to high total IgE is a common or a region-specific phenomenon. Because IgE reactivity to individual birch pollen allergens varies between 6 European populations,[5] the response to individual dust mite allergens might also differ in different countries, causing a different contribution of mite-specific IgE to total IgE.

The data of Erwin et al[1] have an additional important implication not emphasized in the article. Genetic linkage or association studies of allergic diseases using total IgE levels might fail to reveal some of the critical genes, if levels of specific IgE to the major allergen do not correlate with total IgE. Information about the relationship between total IgE and specific IgE to the most prominent allergens can provide clues about operation of IgE-controlling genes in different human populations.

TABLE I. Correlation between total and specific IgE in patients with asthma from Siberia

		All patients (n = 127)		Total IgE >200 IU/mL (n = 70)	
Allergen		***r***	***P* value**	***r***	***P* value**
Cat	e1	0.36	.00003	0.18	.15
Dog	e2	0.26	.0037	0.05	.66
Birch	t3	0.28	.0015	0.23	.054
D pteronyssinus	d1	0.20	.028	−0.06	.61
D farinae	d2	0.19	.033	−0.06	.64

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Supported by the European Commission (International Association for the promotion of co-operation with scientists from the New Independent States of the former Soviet Union Genomics 05-1000004-7761), the Ministry of Education of the Czech Republic (project grant LC06009), and the Grant Agency of the Czech Republic (GACR 310/03/H147).

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

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doi:10.1016/j.jaci.2007.11.037

Reply

To the Editor:

We thank Gusareva et al[1] for sharing data relating specific IgE antibody measurements and total IgE among their patients with asthma in Siberia. It is interesting to compare their data with our own. The Russian cities where the children live, Tomsk, Thumen, and Irkutsk, are all south of the cities in northern Sweden (ie, below 60°N latitude). Although the climate of western Siberia would be similar to northern Sweden and not New Zealand, the prevalence of dust mite sensitivity was strikingly lower in northern Sweden. Gusareva et al[1] previously reported that among children with asthma living in the areas they studied, the prevalence of sensitization to cat allergen was highest (57%), followed by dog and dust mite (30% to 40%).[2] In Sweden, among wheezing

Related data: Relationship between total IgE and specific IgEs to the most important allergens in Czech and Ukrainian populations.

In both Czech (n=75) and Ukrainian (n=45) groups of atopic patients correlation between total IgE and allergen-specific IgEs to moulds, animal origin allergens, dust mites, weeds, trees, and grass was low (R=0.319 – -0.004 and R=0.359 – -0.016).

Table 6. Spearman Rank Order Correlations of total IgE and specific IgEs to inhalant allergens in Czech and Ukrainian atopic patients

Allergen		Czech patients n=75 R / *P*-level	Ukrainian patients n=45 R / *P*-level
m6	*Alternaria alternaria*	0.109 / 0.354	0.033 / 0.83
m3	*Aspergillius fumigatus*	**-0.004 / 0.971**	-0.038 / 0.803
m2	*Cladosporium herbatum*	0.151 / 0.195	0.264 / 0.080
m1	*Penicillium notatum*	0.041 / 0.727	0.046 / 0.762
e3	horse	0.100 / 0.394	0.186 / 0.221
e2	dog	0.147 / 0.210	0.281 / 0.061
e1	cat	0.103 / 0.379	**0.359 / 0.016**
d2	*D. farinae*	**0.319 / 0.005**	0.357 / 0.016
d1	*D. pteronyssinus*	0.158 / 0.176	0.291 / 0.052
w9	plantain	0.194 / 0.095	0.202 / 0.184
w6	mugwort	0.174 / 0.316	0.151 / 0.323
w1	common ragweed	0.105 / 0.370	0.123 / 0.420
t7	oak	0.048 / 0.685	**-0.016 / 0.918**
t4	hazel	0.087 / 0.459	0.059 / 0.698
t3	birch	0.096 / 0.413	0.192 / 0.206
t2	alder	0.140 / 0.231	0.221 / 0.145
g12	cultivated rye	0.259 / 0.025	0.208 / 0.170
g6	timothy grass	0.310 / 0.007	0.160 / 0.295
g3	cock's foot	0.224 / 0.054	0.112 / 0.464
g1	sweet vernal grass	0.251 / 0.030	0.102 / 0.504

1.3 Prevalence of allergic sensitization in the blood donors from Prague (Czech Republic).

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In progress

Summary statement

Blood transfusion is often associated with a risk of allergy manifestation in recipients due to hidden allergic sensitization of the blood donors that do not express symptoms of allergy but have increased levels of total and specific IgE to common allergens. Therefore, we performed a cross-sectional survey of sensitization to common inhalant allergens in blood donors form Prague (Czech Republic) and presented a retrospective changes in the level of total IgE in healthy members of the Prague population. The proportion of individuals declared been non-allergic but expressed elevated production of total IgE remains high and changed insignificantly from 1984 to 2008 years. These donors should be dismissed from blood transfusion to avoid passive transfer of IgE and priming of allergy in recipients. Grass allergens stimulate the highest IgE production and likely to be the most harmful for recipients.

As a first author I contributed to the study design, estimation of specific IgEs, statistical analysis and interpretation of data, and preparation of the manuscript.

Abstract

Background: Blood transfusion from asymptomatic sensitized donors that have high levels of total and specific IgEs to common allergens can lead to allergy manifestation in recipients. Estimation of atopic sensitization in healthy blood donors might therefore reveal a hidden risk factors of allergy reaction in recipients. In 1984 the level of total IgE>100 kU/l was detected in 31% of healthy blood donors from Prague.

Aim of the study: To assess present size and specificity of allergic sensitization in blood donors (n=476) from Prague.

Methods: Total IgE and specific IgEs to 20 inhalant allergens was estimated by *in vitro* test system CAP-FEIA (Phadia, Uppsala, SE) and EUROLINE (EUROIMMUN, Medizinische Labordiagnostika GmbH, Lübeck, Germany), respectively.

Results: None of the donors declared having active allergy in the current year, however some of them (n=105) had a medical history of allergy. Total IgE>100 kU/l was observed in 27% (n=129) of donors. 33.2% of the donors had IgE antibodies to at least one allergen; 12.6% exhibited specific IgEs>3.5 kU/l. The allergens most often causing sensitization were grass pollen, dog and dust mites. Male donors without and with a medical history of allergy differ in level of total IgE (P=0.027). Donors with allergy in the past exhibited significantly higher sensitization to grass allergens than non-allergic donors (P=0.0148 - 0.0059).

Conclusions: High level of total and specific IgE is common in blood donors from Prague and might potentially harm recipients. Grass allergens stimulate the highest IgE production and might be linked with a medical history of allergy.

Blood transfusion is often associated with risk of allergy manifestation in recipients due to hidden allergic sensitization of the blood donors that do not express symptomes of allergy but have increased levels of total and specific IgE to common allergens (Johansson2005a). Transferred allergy can be a serious medical problem since prevalence of hidden allergic sensitization may be high in healthy population. In Sweden and Norway the proportions of donors with potentially clinically important level of specific IgE to inhalant allergens (> 3.5kU/l) were 14.60%, and 10.6% and to common food allergens were 3.9% and 3.0%, respectively (Johansson2005b)). The most recent research on the range of total IgE in non-allergic donors from Prague was published in 1985 (Antošová1985). No information is available about present size and specificity of allergic sensitization of blood donors from the Czech Republic.

Therefore, we report a cross-sectional survey of sensitization to common inhalant allergens in blood donors from Prague (Czech Republic) and present a retrospective comparison of levels of total IgE in healthy members of the Prague population.

Material and Methods

Blood donors

In November 2007 – January 2008 the Department of Blood Transfusion of the University Hospital KV (Prague, Czech Republic) collected blood samples of 476 non-cognate blood donors aged between 18 and 63 years (male to female ratio - 0.73:0.27). All the donors reside in Prague, majority had Czech nationality (97.9%) and the others were Slovaks (2.1%). The volunteers provided information about their allergy status (no medical history of allergy, allergy in the past - before 2007 year), family history of allergy (no, yes, non-informed), smoking habits and pet ownership (45.0%). We stratified the group of donors depending on their allergy status into donors without and with a medical history of allergy (Table 1). None of the donors express any severe allergic disease, any serious infectious disease or diabetes (even in the past). The study was approved by the Ethical Committee of University Hospital KV.

Estimation of total and specific IgE levels

The total IgE level was estimated by CAP-FEIA (Phadia, Uppsala, SE). Specific IgE to moulds (*Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbatum*, *Penicillium notatum*), animals (horse, dog, cat), mites (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*), weeds pollen (*Plantago lanceolata*, *Artemisia vulgaris*, *Ambrosia elatior*), trees pollen (*Quercus alba*, *Corylus avellana*, *Betula verrucosa*, *Alnus incana*), and grass pollen (*Secale cereale*, *Phleum pratense*, *Dactylis glomerata*, *Anthoxantum odoratum*) was measured by *in vitro* test system EUROLINE (EUROIMMUN, Medizinische Labordiagnostika GmbH, Lübeck, Germany).

Statistical analysis

All data were analyzed using the software Microsoft Excel 2000 (Microsoft Copyright 1985-1999) and STATISTICA 8.0 (StatSoft, Inc. 1984-2007, Tulsa, OK, USA). The differences in total and specific IgE between subgroups of donors as well as contribution of sex, pet ownership, and family history of allergy were estimated by the Mann Whitney U test. Correlation was estimated by the Nonparametric Spearman Correlation analysis.

Results

Total IgE

We estimated total IgE in serum of all donors. Overproduction of total IgE (> 100 kU/l) was observed in 129 donors (27%), only 30 (6.3%) of them declared been allergic in the past. The geometric and arithmetic means of total IgE in non-allergic donors and donors with a medical history of allergy were 47.65 kU/l versus 60.60 kU/l and 120.6 kU/l versus 119.8 kU/l, respectively (the differences were insignificant - P=0.068) (Table 2). Males with a medical history of allergy had significantly higher level of total IgE than males without medical history of allergy (P=0.027), whereas total IgE in females without and with a medical history of allergy did not differ (P=0.752) (Table 2).

Specific IgE

We have studied circulating IgEs against 20 inhalant allergens in blood donors residing in Prague (n=476) (Table1). As many as 33.2% (n=158) of the donors had IgE to at least one of the 20 allergens tested. Sixty (12.6%) donors exhibited the level of one or more specific IgEs higher than 3.5 kU/l, which is usually concomitant with clinical symptoms of allergy. However, only one third of these donors (n=22) declared having allergy in the past.

The most prominent allergens were similar in both non-allergic donors and those having allergy in the past: dog (e2) (12.4% and 20.0%, respectively), dust mite (d2, *Dermatophagoides farinae*) (8.9% and 13.3%, respectively), cultivated rye (g12) (9.7% and 19.0%, respectively), timothy grass (g6) (9.2% and 19.0, respectively), cock's foot (g3) (10.0% and 20.0%, respectively), and sweet vernal grass (g1) (6.2% and 15.2%, respectively). Among them the most abundant were specific IgEs to grass allergens (1.3 –

2.6% and 5.7 – 9.5% of donors with the level of specific IgEs higher than 3.5 kU/l for non-allergic donors and donors with medical history of allergy, respectively).

Donors with and without medical history of allergy differed in the sensitization to cultivated rye (e2) (*P*=0.0148), timothy grass (g6) (*P*=0.0059), cock's foot (g3*)* (*P*=0.0101), and sweet vernal grass (g1*)* (*P*=0.0061) (Fig. 1). These two groups of donors differed also in sensitization to *Aspergillius fumigatus* (m3) (*P*=0.0017), *Penicillium notatum* (m1) (*P*=0.0103) and horse (e3) (*P*=0.0148), however the total number of donors sensitized to these allergens was small (Fig. 1).

We observed influence of family history of allergy on increased sensitization to *Dermatophagoides farinae* (d2) and sweet vernal grass (g1) in the subgroup of non-allergic donors (*P*=0.023 and *P*=0.041, respectively). However, the same effect was not found in the subgroup of donors with a medical history of allergy (*P*=0.116 and 0.819, respectively). Some donors (n=120) were not informed about allergy status of their relatives (Table 1).

We also observed dependency of mite (d1, *Dermatophagoides pteronyssinus*) sensitization on sex (only males (8.6%) were sensitized, *P*=0.014) in the subgroup of donors with a medical history of allergy. The same effect was not observed in the subgroup of non-allergic donors (*P*=0.148). Sensitization to other allergens did not depend on sex in both subgroups of donors (*P*=0.071 – 0.892). Ownership of pets such as dog or cat was not associated with sensitization to dog or cat allergens (*P*=0.76 and *P*=0.81, respectively).

Discussion

Our data demonstrate high level of total IgE and specific allergen sensitization in Prague blood donors that might be a crucial risk factor for priming allergy in recipients. High levels of total IgE and specific IgE to at least one inhalant allergen was detected in 26.7% and 28.8% of donors without medical history of allergy, respectively, and in 28.6% and 48.5% of donors that expressed some allergy in the past, respectively. These donors are of high risks and their exclusions as routine donors should be considered.

We compared distribution of total IgE in donors without medical history of allergy collected by us in 2007-2008 years with results of Antošová *et al.* (1985) describing total IgE in a group of Czech blood donors (n=320) collected in Prague in 1984 (Antošová1985) (Fig. 2) We observed only a weak tendency to increase of the proportion of donors with low level of total IgE (< 80 kU/l) collected by us in years 2007-2008 versus donors collected in 1984. There were no prominent differences in prevalence of donors with overproduction of total IgE (>100 kU/l) between the groups of donors. Thus, the proportion of individuals declared been non-allergic but expressed overproduction of total IgE remains high and changed insignificantly over the last 24 years.

The most frequently specific IgEs were detected to dog and grass allergens, intensity of sensitization being higher to the latter. 12.4% and 20% of non-allergic donors and donors with medical history of allergy, respectively, were sensitized to dog. High prevalence of reactivity to dog allergen is most likely explained by frequent dog ownership in Prague (27.7% of donors are dog owners). Although we did not found association between dog ownership, easy transportability of dog allergens on clothing

facilitates sensitization irrespectively of pet-keeping at home directly (Arbes2004).

Sensitization to grass allergens (g12, *Secale cereale,* cultivated rye; g6, *Phleum pretensem* timothy grass; g3, *Dactylis glomerata,* cock's foot; g1, *Anthoxantum odoratum,* sweet vernal grass*)* differed in non-allergic donors and donors with a medical history of allergy (Fig. 1). These data suggest that grass sensitization might be among the major risk factors for priming allergy manifestation in recipients. High level of air pollution due to high traffic and widespread individual residential heating using coal and other highly polluting fuels (Binková2003), which increases pollen output and makes it more aggressive to stimulate allergic reactions (Bartra2007) might contribute to the high sensitization to grass allergens.

However, besides unfavorable environment conditions, there seems to be also influence of genetic factors and sex since we found dependency of sensitization to some allergens on family history of allergy and sex of donors. It was described that boys are more prone to sensitization, the risk for allergy in adulthood is less evident (Govaere E2007). In our group, we found adult males more sensitized to at least some particular allergens, which can be explained by the modulation of the immune response by sex hormones.

In conclusion, high level of total and allergen-specific IgE is common in blood donors from Prague even though they do not express symptoms of allergy, which is similar to situation described in Norway and Sweden (Johanson,allergy 60: 1312, 2005). To avoid potential passive IgE sensitization in recipients it would be useful to test each donor for presence of specific IgE, especially to grass pollen, dog and dust mites allergens that have been found to be high in the blood donors from Prague.

Acknowledgments

We acknowledge the help of the staff of Department of Blood Transfusion, University Hospital KV, in recruitment and interviews of blood donor and Mrs. O. Jůzová and D. Kopelentová in estimation of total IgE levels. Most importantly, we thank all the donors without whom this study would have been impossible to undertake.

This investigation was supported by the European Commission (INTAS Genomics 05-1000004-7761), the Grant Agency of the Czech Republic (grant 310/06/1745), and Ministry of Education of the Czech Republic (program grant LC06009, Research Intention VZ MSM 0021620812).

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Table 1. Stratification of the group of Prague blood donors (n = 476).

Characteristics	**Subgroups of the Czech donors**	
Donors	No history of allergy	Allergy in the past
N of donors	371	105
Age		
mean ± SD	36.5 ± 9.87	33.2 ± 8.3
min - max	18 - 63	19 - 57
Sex ratio: male (%) / female (%)	284 (76.5) / 87 (23.5)	65 (61.9) / 40 (38.1)
Smokers		
active (%)	87 (23.5)	18 (17.1)
passive (%)	17 (4.6)	4 (3.8)
Family history of allergy		
yes (%)	115 (31.0)	45 (42.9)
no (%)	165 (44.5)	31 (29.5)
non-informed (%)	91 (24.5)	29 (27.6)
IgE > 100 kU/l, n (%)	99 (26.7)	30 (28.6)
Specific IgEs > 3.5 kU/l, n (%)	38 (10.2)	22 (21.0)
Sensitization to at least one inhalant allergen, n (%)	107 (28.8)	51 (48.5%)

Table 2. Total IgE (kU/l) in the Prague blood donors. Dependency of total IgE on sex.

	Total IgE		***P* - level of difference between donors with and without history of allergy**
Characteristics	**No history of allergy**	**Allergy in the past**	
Arithmetic mean ± SD	120.6 ± 204.8	119.8 ± 272.4	0.068
Geometric mean	47.65 (n=371)	60.60 (n=105)	
Sex differences in total IgE			
Geometric mean in males	49.05 (n=284)	70.57 (n=65)	0.027
Geometric mean in females	43.36 (n=87)	47.31 (n=40)	0.752
P - level of difference between males and females	0.458	0.056	

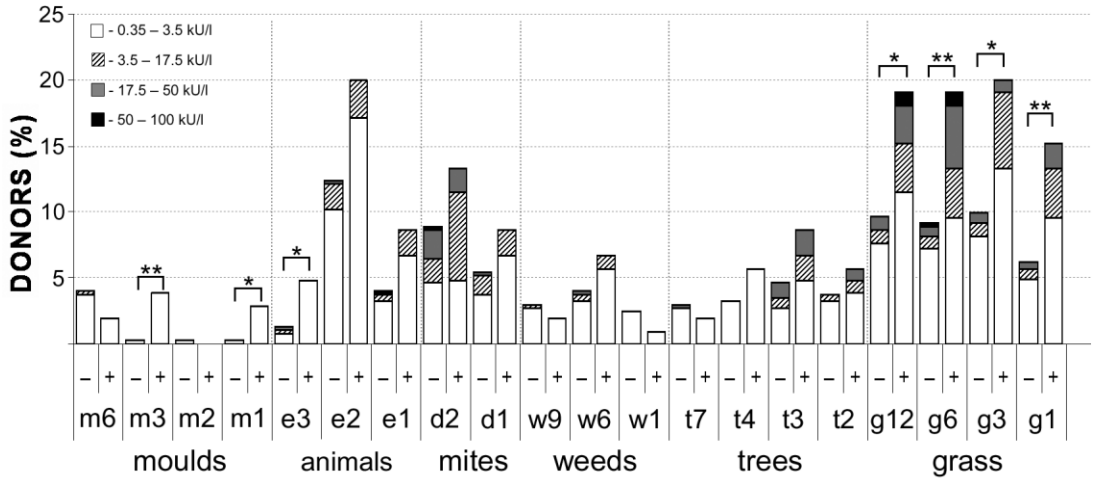

Figure 1. The profiles of allergic sensitization in Prague blood donors.

Proportion of the donors declared to be non-allergic (marked as +) (n=371) and the donors declared having allergy in the past (marked as –) (n=105) in relation to the level of circulating specific IgEs. Sensitization to moulds (m6 – *Alternaria alternata*, m3 – *Aspergillus fumigatus*, m2 – *Cladosporium herbatum*, m1 – *Penicillium notatum*), animals (e3 – horse, e2 – dog, e1 – cat), mites (d2 – *Dermatophagoides farinae*, d1 – *Dermatophagoides pteronyssinus*), weeds (w9 – *Plantago lanceolata*, w6 – *Artemisia vulgaris*, w1 – *Ambrosia elatior*), trees (t7 – *Quercus alba*, t4 – *Corylus avellana*, t3 – *Betula verrucosa*, t2 – *Alnus incana*), and grass (g12 – *Secale cereale*, g6 – *Phleum pratense*, g3 – *Dactylis glomerata*, g1 – *Anthoxantum odoratum*) is shown.

Significant differences between the subgroups of the donors are represented by asterisks (* - *P* – level < 0.02, ** - *P* – level < 0.002).

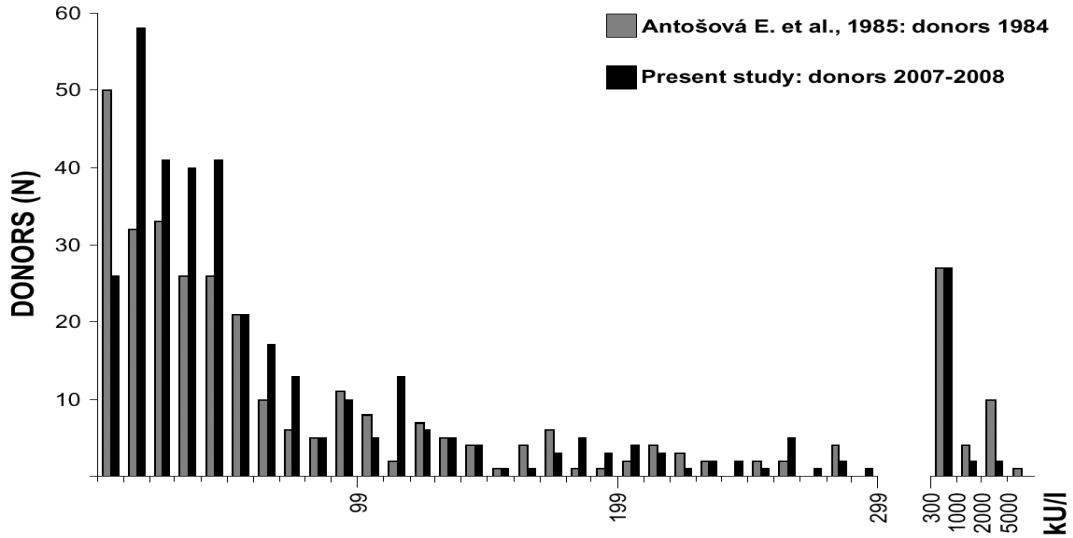

Figure 2. Distribution of the total IgE in the non-allergic blood donors from Prague collected in the year 1984 (n=320) (Antošová et al., 1985) and in 2007 – 2008 years (n = 371). Number of the donors in percentage in relation to the level of total IgE is shown.

1.4 Different environmental influences on etiology of atopic diseases in European populations as a basis for study of gene-environment interactions

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Genetic Predisposition to Disease, Nova Science Publishers, ISBN: 978-1-60456-835-6, accepted, pub. date: pp. 257-270, 2008.

Summary statement

The aim of this work was to study the differences of sensitization to a wide range of inhalant and food allergens between atopic patients from the Czech Republic and from Ukraine, representing two genetically not very distant populations, which live in different environmental conditions. Atopic patients from the Czech Republic and Ukraine differed in the level of sensitization to the tested allergens, proportion of sensitized persons, and in major allergens. The allergic sensitization was significantly higher in the Czech patients than in the patients from Ukraine. Ukrainians were mostly sensitized to cat and dust mite allergens. In the atopic patients from the Czech Republic, the level of sensitization to these allergens was similar, but the level of sensitization to outdoor allergens, grasses and trees was dramatically higher. The presented information about the specificity and intensity of allergic sensitization in a country at the very beginning of the process of westernization (Ukraine) and a country well progressed along this path (Czech Republic) is relevant in view of the high prevalence of atopy in Western industrial countries. Therefore, our study will form a baseline for monitoring of atopic diseases in these countries in the future and will likely contribute to the understanding of mechanisms by which social and environmental aspects of westernization contribute to these diseases. This study is also the first step in analysis of gene-environment interactions.

As a first author, I contributed to the estimation of specific IgEs, statistical analysis and interpretation of data, and preparation of the manuscript.

In: Genetic Predisposition to Disease
Editor: Sara L. Torres and Marta S. Marin

ISBN 978-1-60456-835-6

Chapter 9

DIFFERENT ENVIRONMENTAL INFLUENCES ON AETIOLOGY OF ATOPIC DISEASES IN EUROPEAN POPULATIONS AS A BASIS FOR STUDY OF GENE-ENVIRONMENT INTERACTIONS

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ABSTRACT

Atopy is a predisposition to hyperproduction of immunoglobulin E (IgE) against common environmental allergens. Sensitization to various airborne and food allergens contributes to different types of atopic diseases, including asthma, eczema, and allergic rhinitis. The development of these diseases is influenced by both genetic and environmental factors. Several loci and genes that control IgE level have been described in different chromosomal regions. Some of them have been detected in several

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populations, others only in one or a few populations. These differences might be caused by variations of genetic composition between populations, different lifestyles and/or by environmental variations in major allergens triggering development of atopic diseases. Thus, the environmental conditions may likely determine, which from the potential atopy-controlling genes will operate in a certain population.

As the first step in study of such gene-environment interactions we analyzed the specificity and intensity of sensitization to 40 different allergens in atopic patients from the Czech Republic and Ukraine, representing two genetically not very distant populations, which live in different environmental conditions. The atopic patients from both countries displayed a higher reactivity to inhalant than to food allergens. We found highly significant differences in sensitization to airborne allergens between patients from the two countries. The most pronounced allergens for the atopic patients from Ukraine were allergens from dust mites *Dermatophagoides pteronyssinus* (38.5%), *Dermatophagoides farinae* (48.1%) and cat (44.2%). In the atopic patients from the Czech Republic the level of sensitization to these allergens was similar, but the level of sensitization to outdoor allergens, grasses and trees was dramatically higher. More than 68% of the patients from the Czech Republic in comparison with less than 25% of the patients from Ukraine have been sensitized to cocksfoot, sweet vernal grass, timothy grass and cultivated rye (Bonferroni-corrected P values ranged from 0.0007 to 0.000000003). More than 50% and 60% of the patients from the Czech Republic but only 2% and 19.2% of the patients from Ukraine reacted to alder (corrected $P < 0.00009$) and birch (corrected $P < 0.002$), respectively. The higher sensitization to plant allergens of the patients from the Czech Republic was present in those with asthma and rhinitis, but not with dermatitis. The higher sensitization levels to outdoor allergens in the Czech Republic suggest an influence of westernization on development of allergic reactivity. Genetic analysis of atopic patients from these two countries will establish which gene-loci control development of atopy under different environmental conditions.

INTRODUCTION

Atopy is a predisposition to hyperproduction of immunoglobulin E (IgE) against common environmental allergens. It is the major risk factor for development of allergic diseases such as asthma, rhinitis, and atopic dermatitis. The prevalence of atopic disorders is rising in most regions [1,2]. Although several hypotheses have been proposed for this increase (hygienic [3,4], dietary [reviewed in refs. 5,6], air pollution [reviewed in refs. 7,8], and tobacco smoke [reviewed in refs. 9,10]), the aetiopathogenic process remains poorly understood [11]. Probably a combination of unfavorable lifestyle factors causes the disease development [reviewed in refs. 1,12].

The susceptibility to atopic diseases is influenced by both environmental and genetic factors and it varies in different populations [13,14].

Several loci and genes that control IgE level have been described on different chromosomal regions [reviewed in ref. 15]. Some of these genes or loci were found to regulate susceptibility to atopic diseases in several populations, whereas others have been detected in one or a few populations only. Moreover, as many of these loci contains a large number of genes and it is not known which of them is responsible for the observed effects, it is possible that in different populations different genes from the same chromosomal region

may be involved in control of susceptibility [reviewed in ref. 15]. These differences in susceptibility to atopic diseases might be caused by variations of genetic composition between populations, by different lifestyles and exposures [reviewed in ref. 16] and/or by environmental variations in major allergens triggering development of atopic diseases.

An additional factor that can modify specific immune responses to individual antigens or groups of related antigens are MHC-linked immune response genes, whose specificity is determined by antigen-binding groves of class I and class II MHC molecules. The presence of molecules capable to present peptide fragments of antigens to T cell receptors is one of the major factors modifying specificity and magnitude of the response. These processes are important also for development of atopic responses (for example ref. [17,18]).

The variations of genetic composition might cause either missing a controling variant (allele) of a certain gene, or missing an allele that plays role in epistatic interaction with a second, controling gene. As a consequence, the controlling gene (allele) cannot have the expected effect, even if it is present. Mouse is a useful model for investigation of these genetic regulations. Mouse model of recombinant congenic strains [19] developed for the analysis of complex traits were used to study the genetic control of serum IgE level. It was demonstrated that the loci on chromosomes 2 and 3 have no apparent effect on this trait on their own, but they interact with the loci on chromosomes 10 and 1, respectively [20]. This research also shown that the most important players (genes with the largest effect) varied depending on the genotype of the host [20,21], which is the situation similar to that observed in human genetic studies [15]. So, the recombinant congenic strains can serve as a model of different human populations [22].

The same allele can have opposite effect in different environment as was shown in the example of the polymorphism in promoter (-159 C/T) of gene encoding the monocyte receptor for endotoxin, CD14. The Tuscon children with the TT genotype had reduced serum levels of soluble CD14 and IgE [23], in the German children TT genotype was associated with elevated levels of soluble CD14, but had no effect on IgE levels [24], and in Barbados children the TT genotype was protective against asthma in environments with low house dust endotoxin levels, but was associated with risk for asthma in children from homes with high levels of endotoxin [25]. Although the influence of polymorphism in other genes in the studied populations that might modify the effect of CD14 gene cannot be excluded, similar differences in gene effects were observed also in mice with well defined genotypes. It was found that some regulatory genes modify cellular or local tissue behavior depending on the nature of stimulus or specific tissue where they operate. For example cancer susceptibility genes that seem increase development of colon and intestinal cancer seem to suppress development of mammary tumors and vice versa [26].

Another important cause of differences in genetic control in different populations might be caused by environmental variations in major allergens. It was found that the allergens triggering and supporting development of atopy vary in different parts of the world [27-29] providing cues about the pathogenic effects of environment and lifestyle. For example, dust mites are potentially causative allergens for asthma in Australia [30], Central Virginia [31], India [32], dust mites and cat in New Zealand [33], cat and dog in Scandinavian countries [34], cat in Siberia, Russia [29] and the mold *Alternaria* in Tuscon, Arizona [35]. In Puerto Rico atopic patients exhibited high sensitization to dust mites, no matter whether they

suffered with dermatitis, rhinitis or asthma [28]. Dust mites, grass pollen and cat were the most prominent allergens in atopic children from UK [36]. In Italy, 74% and 45% of atopic asthmatic children showed positive reaction to dust mites and grass pollen, respectively [37]. Reactivity to cat, dog and house dust mite allergens exceeded 50% in patients with asthma and rhinitis from Canada [38], whereas in China sensitization to silk allergens was significantly associated with allergic rhinitis [39]. Thus, the environmental conditions may likely determine, which from the potential atopy-controlling genes will operate in a certain population. Moreover, it was also found that individual allergens contribute differently to total IgE [40,41]. Genetic linkage or association studies of allergic diseases using total IgE levels might fail to reveal some of the critical genes, if levels of specific IgE to the major allergen do not correlate with total IgE.

As the first step in study of the gene-environment interactions we analyzed the specificity and intensity of sensitization to 40 different allergens in atopic patients from the Czech Republic and Ukraine, representing two genetically not very distant populations, which live in different environmental conditions. In the Czech Republic, measurement of sensitization to allergen mixtures is a common clinical practice in patients with symptoms of atopic disorders, but only prevalence of sensitization to ragweed has been reported [42]. Similarly, despite introduction of skin-prick testing of allergic sensitization, no systematic assessment of reactivity to individual allergens has been attempted in Ukraine.

We have therefore analyzed sensitization to 40 different allergens in patients from the Czech Republic (Prague, Northern and Eastern Bohemia) and from Ukraine (Kharkov city and Kharkov region). The presented data on the prevalence and distribution of specific IgEs in patients with asthma, rhinitis and dermatitis define the most prominent allergens in the studied areas and demonstrate striking differences between Czech and Ukrainian patients in both the level of sensitization and in profiles of specific IgE antibodies. The rapidly changing living conditions and health care patterns in these countries generate a unique opportunity to investigate the effects of changes in natural and social environment on patterns of sensitization and atopy and on gene-environment interactions.

METHODS

Patients

Atopic patients 4 – 33 years old from the Czech Republic, including Prague (n = 53), Northern Bohemia (n = 15), and Eastern Bohemia (n = 7), and from Ukraine (Kharkov city and Kharkov region, n = 53) were randomly recruited from databases of local clinics. Participants were enrolled in the study through the medical history of atopic disease by a Board certified physician (Table 1). All patients were non-cognate. Specialists performed a structured interview with each participant (or his/her guardians), verified the diagnosis of asthma, rhinitis or dermatitis according to EAACI (European Academy of Allergology and Clinical Immunology) guidelines, and completed a questionnaire about the disorder manifestation and smoking status.

A full explanation of the study design was given to all participants, and subsequently a written consent was obtained. The study was carried out according to a clinical protocol approved by local Ethical Committees. Eight percent and 16% of the patients from the Czech Republic and Ukraine, respectively, refused to participate in the study for different reasons.

Table 1. Description of patients' characteristics

Origin of population	Patients number	Age (years)		Sex	Smokers
		mean ± SD	(min - max)	male (%) / female (%)	number (%)
Czech Republic	75	15 ± 6.3	4 - 33	40 (53.3) / 35 (46,7)	1 (1.3)
Ukraine	53 *	12 ± 6.5	4 - 32	24 (45.3) / 29 (54.7)	1 (1.9)

* - Due to limited amount of sera, specific IgEs for inhalant and food allergens were measured in different 52 and 48 patients of the total Ukrainian group (n=53), respectively.

Determination of Total and Allergen-Specific Serum IgE Levels

The collection of blood samples was conducted from February 1999 to February 2001 (Czech Republic) and from March 2000 to April 2003 (Ukraine). No blood samples were collected during the summer months. The sera from Ukraine were transported to Prague in a dry ice in order to perform all IgE analyses in a single laboratory. The total IgE level was estimated by CAP-FEIA (Phadia , Uppsala, Sweden). Specific IgE levels were measured using *in vitro* test system EUROLINE (EUROIMMUN, Medizinische Labordiagnostika GmbH, Lübbeck, Germany), according to instructions of the manufacturer. In this system allergen extracts are used for detection of specific IgE. Positive and negative controls were included in the analysis. The lowest level of detection was 0.35 kU/l. We have used an assay for 20 inhalant and 20 food allergens (Table 2).

Statistical Analysis

All data analyses were performed using the software Microsoft Excel 2000 (Microsoft Copyright 1985-1999) and STATISTICA for Windows version 8.0 (StatSoft, Inc. 1984-2008, Tulsa, OK, USA). The level of sensitization in the groups was compared by Mann Whitney U test. Bonferroni correction for multiple testing was applied to all *P* values.

RESULTS

Specific IgE to Inhalant and Food Allergens

We have compared circulating IgE antibodies against 20 inhalant and 20 food allergens in atopic patients from the Czech Republic and Ukraine (Figure 1, Figure 2).

The IgE profiles and the level of sensitization were not significantly different in the Czech patients from different areas as well as in the Ukrainian patients who came from Kharkov city and Kharkov region, therefore patients were pooled into two groups (n = 75 and n = 53, Czech and Ukrainian patients, respectively).

Table 2. List of tested allergens

Inhalant allergens	**Food allergens**
molds	**fishes**
m6 *Alternaria alternata*	f23 crab
m3 *Aspergillius fumigatus*	f3 codfish
m2 *Cladosporium herbatum*	**vegetables**
m1 *Penicillium notatum*	f85 celery
animal allergens	f35 potato
e3 horse	f31 carrot
e2 dog	f25 tomato
e1 cat	**fruits**
mites	f237 apricot
d2 *Dermatophagoides farinae*	f84 kiwi
d1 *Dermatophagoides pteronyssinus*	f49 apple
weeds	**nuts**
w9 plantain	f20 almond
w6 mugwort	f17 hazelnut
w1 common ragweed	f13 peanut
trees	**grains**
t7 oak	f14 soya bean
t4 hazel	f9 rice
t3 birch	f5 rye flour
t2 alder	f4 wheat flour
grass	**food of animal origin**
g12 cultivated rye	f45 yeast
g6 timothy grass	f2 cow's milk
g3 cock's foot	f75 egg yolk
g1 sweet vernal grass	f1 egg white

Highly significant differences in sensitization to inhalant allergens between patients from the two countries have been found (Figure 1, Table 3). Indoor inhalant allergens such as dust mites *Dermatophagoides farinae* (d2) (38.5%), *Dermatophagoides pteronyssinus* (d1) (48.1%), and cat (e1) (44.2%) were the most pronounced for the Ukrainian atopic patients. In the Czech atopic patients the level of sensitization to these allergens was similar (Figure 1, Table 3), but there was a dramatically higher level of sensitization to individual outdoor allergens. The highest differences have been observed in sensitization to grasses cocksfoot (g3, *Dactylis glomerata*), sweet vernal grass (g1, *Anthoxantum odoratum*), timothy grass (g6,

Phleum pratense) and cultivated rye (g12, *Secale cereale*), where more than 68% of the Czech patients in comparison with less than 25% of the Ukrainian patients have been sensitized (Figure 1). These differences between the two groups were significant (corrected P ranged 0.0007 - 0.000000003) (Table 3). Significant differences were also found in sensitization to trees alder (t2, *Alnus glutinosa*) (corrected $P < 0.00009$) and birch (t3, *Betula verrucosa*) (corrected $P < 0.002$), to which reacted more than 50% and 60% of the Czech patients compared with 2% and 19.2% of the Ukrainian patients, respectively (Table 3). The observed differences were due to higher sensitization to plant allergens of the Czech patients with asthma and rhinitis, but not with dermatitis (Table 3).

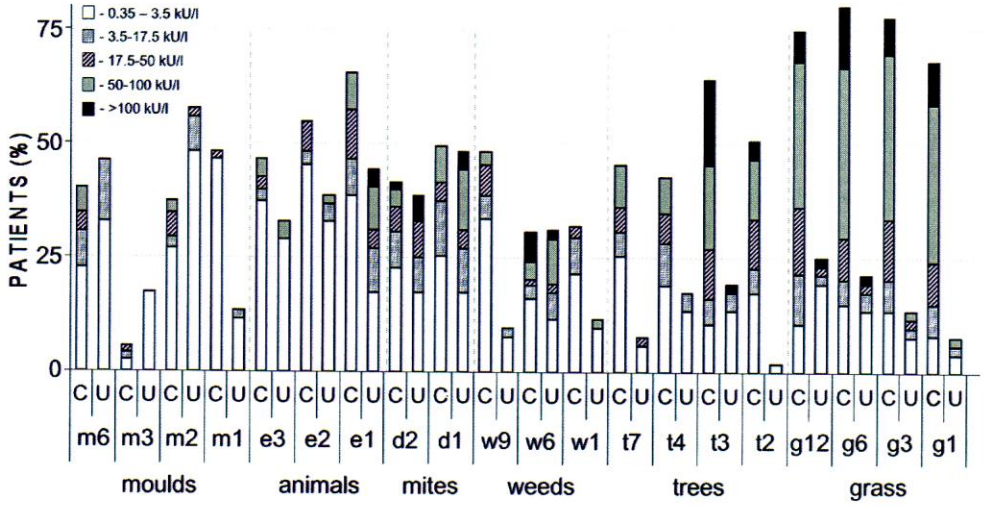

Figure 1. Proportion (%) of Czech (C) (n=75) and Ukrainian (U) (n=52) atopic patients sensitized to inhalant allergens in relation to the level of circulating IgE. Sensitization to moulds (m6 – *Alternaria alternata*, m3 – *Aspergillus fumigatus*, m2 – *Cladosporium herbatum*, m1 – *Penicillium notatum*), animals (e3 – horse, e2 – dog, e1 – cat), mites (d2 – *Dermatophagoides farinae*, d1 – *Dermatophagoides pteronyssinus*), weeds (w9 – *Plantago lanceolata*, w6 – *Artemisia vulgaris*, w1 – *Ambrosia elatior*), trees (t7 – *Quercus alba*, t4 – *Corylus avellana*, t3 – *Betula verrucosa*, t2 – *Alnus incana*), and grasses (g12 – *Secale cereale*, g6 – *Phleum pratense*, g3 – *Dactylis glomerata*, g1 – *Anthoxantum odoratum*) is shown. According to EUROLINE protocol, the clinically important level of sensitization was defined as more than 3.5 kU/l, which is usually accompanied by symptoms of allergy.

There was a tendency to higher sensitization to food allergens in the Czech patients than in the patients from Ukraine, but the differences were not significant (Figure 2). The Czech atopic patients were mostly sensitized to potato (f35) (52.0%), hazelnut (f17) (49.3%), rice (f9) (45.3%) and cow's milk (f2) (42.7%). More than 30% of the patients also reacted to celery (f85), almond (f20) and rye flour (f5). For the Ukrainian atopic patients the most prominent allergens were cow's milk (f2) (43.8%), rye flour (f5) (35.4%) and rice (f9) (31.3%).

There was no influence of age and sex on the level of sensitization neither in Czech nor in Ukrainian group ($P > 0.05$). The number of smokers in both groups of patients was low (Table 1) and therefore the influence of this factor could not be established.

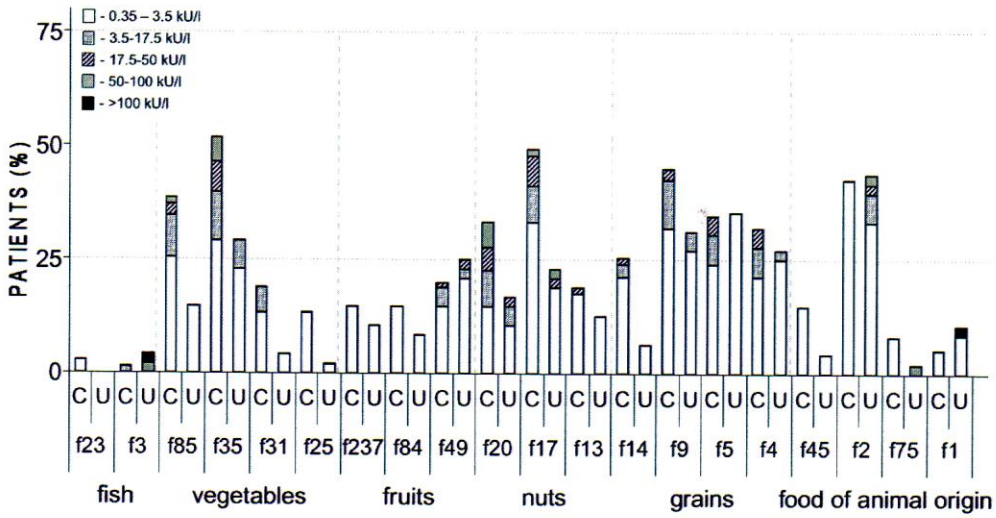

Figure 2. Proportion (%) of Czech (C) (n=75) and Ukrainian (U) (n=48) atopic patients sensitized to food allergens in relation to the level of circulating IgE. Sensitization to fishes (f23 – crab, f3 – codfish), vegetables (f85 – celery, f35 – potato, f31 – carrot, f25 – tomato), fruits (f237 – apricot, f84 – kiwi, f49 – apple), nuts (f20 – almond, f17 – hazelnut, f13 – peanut), grains (f14 – soya bean, f9 – rice, f5 – rye flour, f4 – wheat flour), food of animal origin (f45 – yeast, f2 – cow's milk, f75 – egg yolk, f1 – egg white) is shown. According to EUROLINE protocol, the clinically important level of sensitization was defined as more than 3.5 kU/l, which is usually accompanied by symptoms of allergy.

CONCLUSION

Atopic patients from the Czech Republic and Ukraine differed in the level of sensitization to tested allergens, proportion of sensitized persons, and in major allergens. The allergic sensitization was significantly higher in the Czech patients than in the patients from Ukraine, although the prevalence of childhood asthma based on ISAAC (International Study of Asthma and Allergies in Childhood) questionnaires seems to be similar in both countries. In Ukraine, one study reported 6.1-8.1% [43], whereas the other found 12.9 and 20.9 % prevalence of asthma symptoms in two succesive time intervals [2]. In the Czech Republic two independent cross-sectional studies reported 15.9% [44] and 17.5% [45] prevalence of 12 - month asthma symptoms in children and adolescents, respectively. This high prevalence of asthma symptoms is similar to that reported in West European countries [2]. Although the reported asthma and atopy symptoms prevalence seems to be similar in both countries, the degree of atopic sensitization is higher in the patients from the Czech Republic, thus supporting data about specific IgE sensitization. The total IgE level measured in all the Czech patients (n = 75) and in a representative sample of the Ukrainian patients (n = 46) was also higher in the Czech than in the Ukrainian group (P = 0.0008) (mean ± SD was 661.3 ± 1061.1 kU/l and 267.6 ± 407.2 kU/l in Czech and Ukraine group, respectively).

High levels of atopic sensitization in the Czech Republic population have been already observed before the introduction of market economy. The measurement of total IgE levels of 320 healthy blood donors performed in 1984 revealed that 29% of donors had the total IgE level higher than 100 U/ml and 15% higher than 300 U/ml [46].

Table 3. Differences in sensitization of atopic patients from the Czech Republic and Ukraine

Allergens	**Atopic patients** CZ n = 75 UKR n = 52	**Asthma patients** CZ n = 29 UKR n = 27	**Patients with atopic rhinitis** CZ n = 63 UKR n = 28	**Patients with dermatitis** CZ n = 23 UKR n = 38
moulds				
Alternaria alternata	NS	NS	NS	NS
Aspergillus fumigatus	NS	NS	NS	NS
Cladosporium herbatum	**0.02**	NS	NS	NS
Penicillium notatum	NS	NS	NS	NS
animals				
horse	NS	NS	NS	NS
dog	NS	NS	NS	NS
cat	NS	NS	NS	NS
mites				
D. farinae	NS	NS	NS	NS
D. pteronyssinus	NS	NS	NS	NS
weeds				
plantain *Plantago lanceolata*	**0.02**	NS	NS	NS
mugwort *Artemisia vulgaris*	NS	NS	NS	NS
common ragweed *Ambrosia elatior*	NS	NS	NS	NS
trees				
oak *Quercus alba*	**0.01**	NS	**0.03**	NS
hazel *Corylus avellana*	NS	NS	NS	NS
birch *Betula verrucosa*	**0.002**	NS	NS	NS
alder *Alnus glutinosa*	**0.00009**	**0.01**	**0.005**	NS
grass				
cultivated rye *Secale cereale*	**0.0007**	0.07	**0.005**	NS
timothy grass *Phleum pratense*	**0.000006**	**0.01**	**0.0003**	NS
cock's foot *Dactylis glomerata*	**0.00000003**	**0.0002**	**0.0000002**	NS
sweet vernal grass *Anthoxantum odoratum*	**0.000001**	**0.0008**	**0.000003**	NS

The P - value of differences was estimated by the Mann-Whitney U test. The P -values were corrected for multiple comparisons (number of tested allergens). Significant differences (corrected $P < 0.05$) are shown in bold.

All tested allergens are common for both the Czech Republic and Ukraine and the prevailing allergenic pollens are rather similar in these countries [47,48]. Only two Ukrainian patients showed no sensitization to the tested set of allergens despite symptoms of disease

and increased total IgE level, probably due to missing of an allergen for these patients in the EUROLINE allergen set. There were no patients without any sensitization in the Czech group.

Stratification of the patients depending on clinical symptoms of asthma, rhinitis or dermatitis has shown the same differences in allergen sensitization between the Czech and Ukrainian patients with asthma and rhinitis; while, no differences in reactivity to grass allergens has been observed between the Czech and Ukrainian patients with dermatitis (Table 3). Thus, allergen sensitization is associated not only with an allergic status as reported in ref. [28], but also with organ affected by allergen exposure. The high pollution level [49,50] together with the more westernized lifestyle and less favorable climatic factors may cause the differences in specificity and degree of sensitization between the Czech Republic and Ukraine. Czech Republic has a dense network of highways that are the main sources of air pollution and could be a critical predisposing factor for induction of sensitization to inhalant allergens in the Czech Republic [50,51]. Moreover, Prague appears today to be one of the most polluted residential areas in the Czech Republic because it is situated in a deep, insufficiently ventilated valley and because of a large intensity of traffic and widespread individual residential heating using coal and other highly polluting fuels [49].

The presented information about the specificity and intensity of sensitization in a country at the very beginning of the process of westernization (Ukraine) and a country well progressed along this path (Czech Republic) is relevant in view of the high prevalence of atopy in Western industrial countries. Therefore, our study will form a baseline for monitoring of atopic diseases in these countries in the future and will likely contribute to the understanding of mechanisms by which social and environmental aspects of westernization contribute to these diseases. This study is also the first step in analysis of gene-environment interactions.

ACKNOWLEDGMENTS

We thank Dr. Christine Ambrosone, Chair, Department of Epidemiology, Roswell Park Cancer Institute, Buffalo, NY, USA for the critical reading of manuscript and suggestions on statistical procedures. Support for this work was provided by the European Commission (INTAS Genomics 05-1000004-7761), Academy of Sciences of the Czech Republic (project grant AVOZ50520514) and Ministry of Education of the Czech Republic (project grant LC06009). This investigation was also supported by Research Themes ϕ-02 and MSM0021620812.

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2 Genetic regulation of IgE in Czech and Russian populations

2.1 Mouse to human comparative genetics reveals a novel immunoglobulin E - controlling locus on Hsa8q12

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Immunogenetics, in press

Summary statement

Mouse to human comparative genetics can significantly improve understanding of etiology of complex traits and diseases in humans. The aim of this study was to study genetic regulation of IgE level in humans and its impact on development of allergic diseases. Therefore, we used information on previously reported IgE-controlling locus in mice to test their orthologous region for regulation of total IgE and a numbers of specific IgEs in humans (Czech atopic patients). Using this approach, a new IgE-controlling locus was identified on human chromosome 8q12 that influences the sensitization to a number of allergens. The finding of this linkage shows the precision and predictive power of mouse models in investigation of the complex traits in humans. In order to define other loci that may control IgE in Czech atopic patients we also selected markers in additional 16 candidate chromosomal regions for linkage and association testing with the atopic phenotypes. By the candidate gene approach, we confirmed the role of the previously reported loci 5q33.3, 7p14.1, 12q13 and 13q14 in control of IgE and development of atopy.

As a first author, I contributed to the study design, genotyping of DNA samples, estimation of specific IgEs, statistical analysis and interpretation of data, and preparation of the manuscript.

Immunogenetics
DOI 10.1007/s00251-008-0343-x

ORIGINAL PAPER

Mouse to human comparative genetics reveals a novel immunoglobulin E-controlling locus on Hsa8q12

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Received: 29 May 2008 / Accepted: 23 October 2008

Abstract Atopy is a predisposition to hyperproduction of immunoglobulin E (IgE) against common environmental allergens. It is often associated with development of allergic diseases such as asthma, rhinitis, and dermatitis. Production of IgE is influenced by genetic and environmental factors. In spite of progress in the study of heredity of atopy, the genetic mechanisms of IgE regulation have not yet been completely elucidated. The analysis of complex traits can benefit considerably from integration of human and mouse genetics. Previously, we mapped a mouse IgE-controlling locus *Lmr9* on chromosome 4 to a segment of <9 Mb. In this study, we tested levels of total IgE and 25 specific IgEs against inhalant and food allergens in 67 Czech atopic families. In the position homologous to *Lmr9* on chromosome 8q12 marked by D8S285, we demonstrated a novel human IgE-controlling locus exhibiting suggestive linkage to composite inhalant allergic sensitization (limit of detection, LOD=2.11, P=0.0009) and to nine specific IgEs, with maximum LOD (LOD=2.42, P=0.0004) to plantain. We also tested 16 markers at previously reported chromosomal regions of atopy. Linkage to plant allergens exceeding the LOD>2.0 was detected at 5q33 (D5S1507, LOD=2.11, P=0.0009) and 13q14 (D13S165, LOD=2.74, P=0.0002). The significant association with plant allergens (quantitative and discrete traits) was found at 7p14 (D7S2250, corrected P=0.026) and 12q13 (D12S1298, corrected P=0.043). Thus, the finding of linkage on chromosome 8q12 shows precision and predictive power of mouse models in the investigation of complex traits in humans. Our results also confirm the role of loci at 5q33, 7p14, 12q13, and 13q14 in control of IgE.

Keywords Atopy · Specific IgE · Genetic loci · Mouse–human homology · Czech population · 8q12

Electronic supplementary material The online version of this article (doi:10.1007/s00251-008-0343-x) contains supplementary material, which is available to authorized users.

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Introduction

Atopy is a complex trait characterized by predisposition to hyperproduction of immunoglobulin E (IgE) against common environmental allergens. It is a major risk factor for the development of allergic diseases such as asthma,

rhinitis, and dermatitis. The susceptibility to atopic diseases has an important hereditary component. In the past 12 years, a number of genome-wide studies of atopy in humans identified several IgE-controlling loci and genes on different chromosomes (reviewed in Ober and Hoffjan 2006; Vercelli 2008). Some of these genes or loci were found to regulate susceptibility to atopic diseases in several populations, whereas others have been detected in one or a few populations only (reviewed in Ober and Hoffjan 2006). These controlling loci in most cases contain a number of genes, and it is not known which of them is responsible for the observed effects. It was suggested that in different populations, different genes from the same chromosomal region may be involved in control of susceptibility (reviewed in Ober and Hoffjan 2006; Zhang et al. 2008).

The most often detected linkages were to chromosomal regions 5q (Xu et al. 2000; Yokouchi et al. 2000, 2002; Haagerup et al. 2002; Koppelman et al. 2002), 6p (Daniels et al. 1996; Wjst et al. 1999; Haagerup et al. 2002; Fereira et al. 2005), 7p (Daniels et al. 1996; Laitinen et al. 2001; Shugart et al. 2001; Altmüller et al. 2005), 7q (Xu et al. 2000; Koppelman et al. 2002; Altmüller et al. 2005), 11q (Daniels et al. 1996; Shugart et al. 2001; Altmüller et al. 2005), 12q (Xu et al. 2000; Koppelman et al. 2002; Yokouchi et al. 2002), and 16q (Daniels et al. 1996; Ober et al. 2000; Kurz et al. 2005). Positional cloning indicated six genes at loci 2q14 (*DPP10*—dipeptidyl serine protease; Allen et al. 2003), 2q33 (*CTLA4*—cytotoxic T-lymphocyte-associated-4 gene; Howard et al. 2002), 5q32-33 (*PCDH1*—protocadherin-1; Whittaker 2003), 7p14.3 (*GPRA*—G-protein-coupled receptor; Laitinen et al. 2004), 13q14 (*PHF11*—PHD finger protein 11; Zhang et al. 2003), and 20p13 (*ADAM33*—Zn-dependent metalloproteinase; van Eerdewegh et al. 2002) predisposing for atopy or atopy-associated traits. Genome-wide association mapping led to the identification of the genes *ORMDL3* (an endoplasmic reticulum membrane protein) at locus 17q21 (Moffatt et al. 2007) and *CHI3L1* (chitinase 3-like 1) at locus 1q32.1 (Ober et al. 2008) that contribute to the risk of asthma.

Despite this remarkable progress in the identification of genes controlling atopy and asthma in humans, the complete elucidation of its genetics is hindered by many factors including sample size, genetic heterogeneity of human populations, gene interactions, low frequency and/or incomplete penentrance of trait-controlling alleles, and a high variability of environmental factors (Lander and Schork 1994).

Some limitations of human genetic studies could be overcome by the use of mouse models. The availability of genetically homogenous mouse strains and possibility of testing large numbers of F_2 and backcross mice in a controlled environment that reduces the phenotypic variance makes the mouse a useful model for study of the complex traits in human (Lipoldová and Demant 2006). Once the genetic regions of interest have been identified in mouse, the high level of synteny between many mouse and human chromosomal segments allows predicting their locations in human (DeBry and Seldin 1996). A mouse model has been successfully applied for the identification of human homologues of mouse asthma genes *Tim1* and *Tim3* in *Tapr* (an airway hyperreactivity regulatory) locus at chromosomes 5q33.2 and 5q33.3, respectively (McIntire et al. 2001).

In our previous genome-wide search performed in mouse, we described nine genetic loci on chromosomes 1, 2, 3, 4, 5, 8, 10, 16, and 18 that control IgE level (Lipoldová et al. 2000; Badalová et al. 2002). This search did not target certain genes throughout the genome, but a set of genetic regions of total length of about 360 Mb. These regions are distributed randomly in the tested recombinant congenic (RC) strains. It must be emphasized that this occurred without any previous selection and without any prior knowledge about the gene content of these regions. The description of the principle of construction of RC strains is described in Demant and Hart (1986) and Lipoldová and Demant (2006). Subsequently, we used the homology between genetic maps of mouse and human to identify the corresponding orthologous regions on human chromosomes and found that the loci *Lmr3*, *Lmr5*, *Lmr8*, *Lmr10*, *Lmr11*, *Lmr13*, and *Lmr14* (Lipoldová et al. 2000; Badalová et al. 2002) are located in the regions homologous with the human chromosomal segments known to control serum IgE in human atopic diseases (Wjst et al. 1999; Dizier et al. 2000; Xu at al. 2000, 2001; Yokouchi et al. 2000, 2002; Koppelman et al. 2002), indicating a likely relatedness of IgE-controlling genes in the two species. However, for two loci (*Lmr9* and *Lmr12*; Badalová et al. 2002) described by us, the homologous human regions have not been connected with atopy. These two loci may point to hitherto undetected human genes that are relevant for atopy. As *Lmr12* maps to a broad segment, we used for further study the locus *Lmr9*, which is rather precisely mapped to a segment with the most likely length of 3.58 Mb and maximal possible length of 9.32 Mb on chromosome 4 in the strain CcS-20. The mice homozygous for BALB/c (high IgE responder) and STS/A (low IgE responder) alleles at this locus differed 1.6 times in IgE level (corrected P value 0.00313; Badalová et al. 2002). In the orthologous region on human chromosome 8q12, we selected three short tandem repeat (STR) markers, D8S1828 (56.96 Mb), D8S285 (57.22 Mb), and D8S1816 (57.52 Mb; Fig. 1), and tested their non-parametric linkage and association (quantitative and discrete traits, QTDT) with inhalant and food atopy and with levels of total IgE and specific IgEs to 20 inhalant and five food allergens in the 67 Czech atopic nuclear families comprising 276 subjects. In order to define other loci that may control IgE

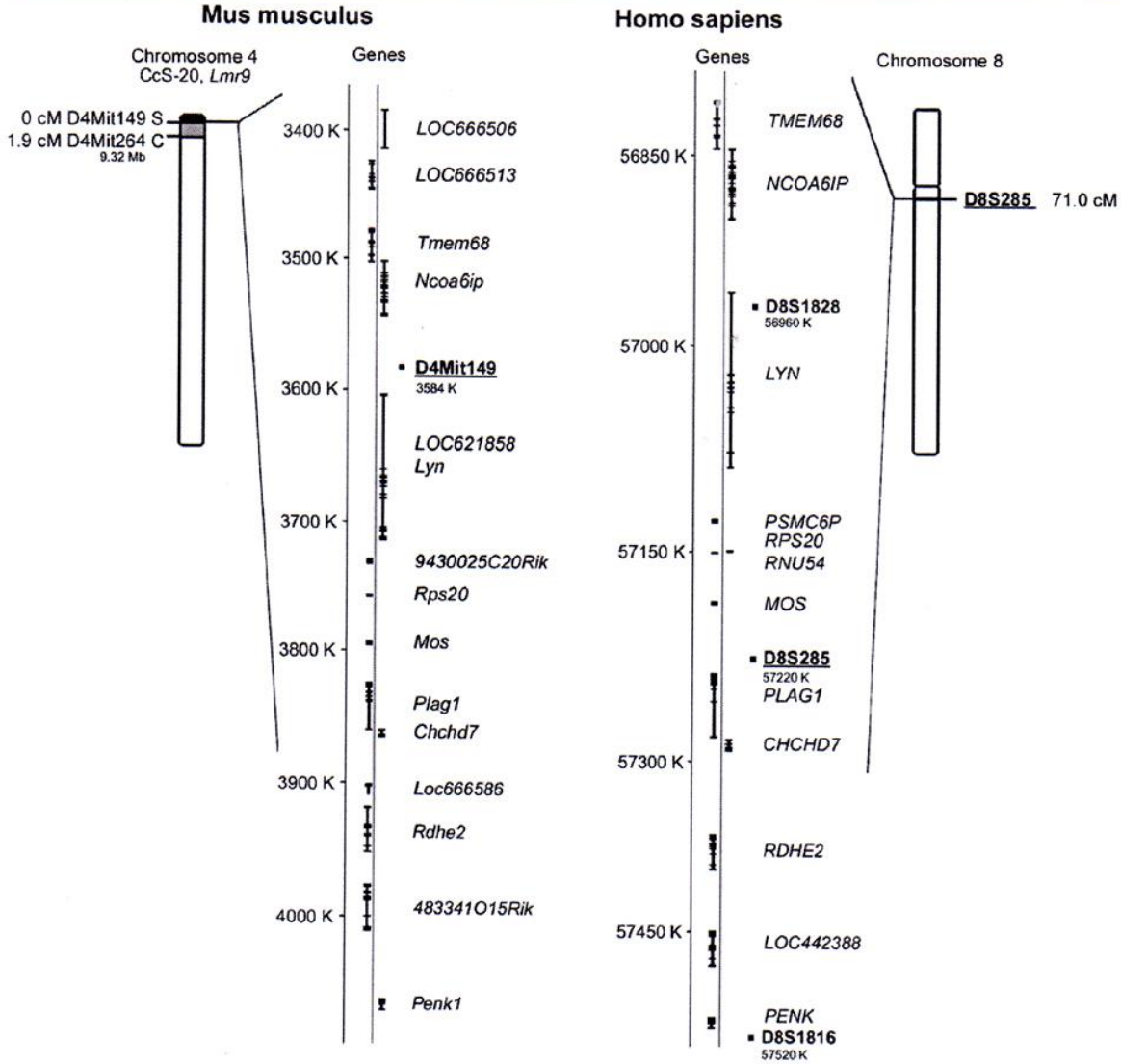

Fig. 1 Conserved synteny between the mouse and human genome regions. The region on recombinant mouse chromosome 4 (*Lmr9* locus) is homologous to the region on human chromosome 8q12. In the mouse chromosomal map, the segments of BALB/c origin, where the *Lmr9* locus is excluded, is marked *open*. The segment of STS origin, containing the *Lmr9* gene, is marked *closed*. The *gray* segment indicates region of undetermined origin. The most likely and the maximal lengths of *Lmr9* locus in mouse are 3.58 and 9.32 Mb, respectively. The 530 Kb region shown in detail encompasses genes in the close vicinity of the marker D4Mit149. The position of the marker is given as 0 cM because it has not yet been separated from the centromere by recombination (http://www.informatics.jax.org/, August 25, 2008). The markers in the segment on human chromosome 8 were selected so that one is located in the center of the ±5 Mb region orthologous to *Lmr9* (D8S285) and the two other markers are approximately equidistant centromerically (D8S1828, 260 kb) and telomerically (D8S1816, distance 300 kb; NCBI Homology Maps page—http://www.ncbi.nlm.nih.gov/projects/homology/maps/)

in Czech atopic patients, we also selected markers in additional 16 candidate chromosomal regions for linkage and association testing with the atopic phenotypes (Table 1). These additional regions were previously shown to be linked or associated with atopy and/or asthma (Daniels et al. 1996; Wjst et al. 1999; Ober et al. 2000; Xu et al. 2000; Yokouchi et al. 2000, 2002; Laitinen et al. 2001; Shugart at al. 2001; Haagerup at al. 2002; Koppelman et al. 2002; van Eerdewegh et al. 2002; Allen et al. 2003; Altmüller et al. 2005; Ferreira at al. 2005; Kurz et al. 2005).

Hence, in the present study, instead of performing total genome scans in the analyzed families, we tested only the markers in the chromosomal regions that were either previously shown to define linkages in humans or repre-

Table 1 List of markers tested for linkage and association with asthma, rhinitis and dermatitis, total IgE, and specific IgE to 20 inhalant and five food allergens

Marker	Chromosome[a]	cM (Marshfield)	Reference
D2S308	2q14.3	124.03	Allen et al. 2003
D5S816	5q31.1	139.33	Koppelman et al. 2002
D5S1507	5q33.3	157.57	Yokouchi et al. 2000
D6S291	6p21	49.50	Wjst et al. 1999
D7S2250	7p14.1	54.11	Daniels et al. 1996; Shugartt et al. 2001; Laitinen et al. 2001
D7S821	7q22.1	109.12	Xu et al. 2000
D8S1828	8q12	71.00	Badalová et al. 2002
D8S285	8q12	71.00	Badalová et al. 2002
D8S1816	8q12	71.00	Badalová et al. 2002
D11S2006	11q12	59.24	Adra et al. 1999
D12S1298	12q13	75.17	Barnes et al. 1996
D12S379	12q21.31	93.69	Nickel et al. 1997
D12S1059	12q22	105.18	Barnes et al. 1996
D12S1282	12q24.31	136.82	Barnes et al. 1996
D13S165	13q14	45.55	Zhang et al. 2003
D16S3253	16q21	71.77	Daniels et al. 1996
D16S539	16q23.2	124.73	Ober et al. 2000
D19S601	19q13.32	83.19	Venanzi et al. 2001
D20S473	20p13	9.53	Van Eerdewegh et al. 2002

[a] The chromosomal regions were found in the GDB Human Genome Database (http://www.gdb.org, May 23, 2008)

sentative markers in the human chromosomal region that is homologous to the mouse *Lmr9* locus that controls IgE levels (Table 1).

Materials and methods

Subjects and families

Nuclear families from the Czech Republic (67 families, n=276) originated from Prague (47 families, n=192) and from towns Ústí nad Labem, Teplice and Most (13 families, n=55) and Trutnov (7 families, n=29), all located within less than 120 km from Prague. The families were collected through probands registered in local clinics as patients with a medical history of atopic disease. All available families were recruited into the study with the exception of 8% of families, which declined to participate for different reasons. The probands were not ascertained for another disorder.

The 67 nuclear atopic families contained 276 subjects, 138 of whom were offspring (Table 2, Electronic supplementary material Table 1). There was no stated relatedness between families. The mean and median age of the parents were 43.4 and 42.0 years, respectively, and that of the offspring were 15.9 and 14.0, respectively. The sex ratio of the offspring was 0.53:0.47 (male to female). The percentage of the allergen-sensitized subjects among parents was 84.8% and among offspring 87.7%.

All participants (offspring and spouses) have undergone clinical examination under the protocol approved by the Ethical Committee of the Third Faculty of Medicine, Charles University, Prague, Czech Republic. A full explanation of the study design was given to all participants, and subsequently, a written consent was obtained. Clinical specialists performed a structured interview with each participant (or his/her guardians), verified or newly established the diagnosis of asthma, rhinitis, dermatitis, conjunctivitis and/or urticaria according to EAACI guidelines (Johansson et al. 2001), and completed a questionnaire about the disease manifestations and smoking status.

Estimation of total and specific IgE levels

The collection of blood samples was conducted from February 1999 to February 2001. No blood samples were collected during the summer months. The sera were stored at −70°C before use. The total IgE level was estimated in by CAP-FEIA (Pharmacia, Uppsala, Sweden). Specific IgE was measured by the in vitro test system EUROLINE (EUROIMMUN, Medizinische Labordiagnostika GmbH, Lübeck, Germany) according to the instructions of the manufacturer. In this system, allergen extracts were used for the detection of specific IgEs. The lowest threshold of detection was 0.35 kU/l. We have tested 20 inhalant and five food allergens. Sensitization to moulds (m6—*Alternaria alternata*, m3—*Aspergillus fumigatus*, m2—*Cladosporium*

Table 2 Characteristics of group of 276 participants from 67 families

Group characteristics	Number (%)
Nuclear atopic families	67
Subjects	276
Parents	138
Age, mean±SD, median	43.4±10.7, 42.0
Smokers among parents, *n* (%)	9 (6.5%)
Total IgE>100 kU/l, *n* (%)	60 (43.5%)
Parents with inhalant allergic sensitization	112 (81.2%)
Food-sensitized parents	52 (37.7%)
Parents with allergic asthma	27 (19.6%)
Parents with allergic rhinitis	69 (50%)
Parents with atopic dermatitis	14 (10.1%)
Offspring/Children	138
Age, mean±SD, median	15.9±8.53, 14.0
Sex, male/female	0.53:0.47
Smokers among children, *n* (%)	0
Children with total IgE≥100 kU/l	89 (64.5% of all children)
Four affected sibs	1
Affected sib trios	2
Affected sib pairs	23
Affected half-sibs	33
Children with inhalant allergic sensitization	109 (79.0% of all children)
Four affected sibs	3
Affected sib trios	3
Affected sib pairs	30
Affected half-sibs	28
Food-sensitized children	77 (55.8% of all children)
Affected sib trios	2
Affected sib pairs	20
Affected half-sibs	31
Children with allergic asthma	37 (26.8% of all children)
Affected sib pairs	6
Affected half-sibs	25
Children with allergic rhinitis	87 (63.0% of all children)
Four affected sibs	2
Affected sib pairs	23
Affected half-sibs	33
Children with atopic dermatitis	39 (28.3% of al children)
Affected sib pairs	8
Affected half-sibs	23

herbatum, m1—*Penicillium notatum*), animals (e3—horse, e2—dog, e1—cat), mites (d2—*Dermatophagoides farinae*, d1—*Dermatophagoides pteronyssinus*), weeds (w9—*Plantago lanceolata*, w6—*Artemisia vulgaris*, w1—*Ambrosia elatior*), trees (t7—*Quercus alba*, t4—*Corylus avellana*, t3—*Betula verrucosa*, t2—*Alnus incana*), and grasses (g12—*Secale cereale*, g6—*Phleum pratense*, g3—*Dactylis glomerata*, g1—*Anthoxantum odoratum*) was measured by inhalation test system. We also measured reactivity to celery (f85), potato (f35), almond (f20), hazelnut (f17), and rice (f9). The inhalant and food atopy were defined as sensitization to at least one of the tested inhalant and food allergens, respectively.

Genetic markers

For the analysis, 19 STR markers located at different chromosomes/chromosomal regions were selected (Table 1) from the National Centre for Biotechnology Information (NCBI) database (http://www.ncbi.nih.gov). The STR markers are characterized in the Marshfield genetic map and show high heterozygosity. All markers, with the exception of the markers on chromosome 8 (D8S1828, D8S285, and D8S1816), are located in atopy candidate regions previously described in genome-wide studies of atopy and/or asthma (Daniels et al. 1996; Wjst et al. 1999; Ober et al. 2000; Xu et al. 2000; Yokouchi et al. 2000,

2002; Laitinen et al. 2001; Shugart et al. 2001; Haagerup et al. 2002; Koppelman et al. 2002; Altmüller et al. 2005; Ferreira et al. 2005; Kurz et al. 2005) by other groups. Markers selected in regions 2q14.3 (Allen et al. 2003), 5q31.1 (Koppelman et al. 2002), 5q33.3 (Yokouchi et al. 2000), 6p21 (Wjst et al. 1999), 7p14.1 (Daniels et al. 1996, Laitinen et al. 2001; Shugart et al. 2001), 7q22.1 (Xu et al. 2000), 12q21.31 (Nickel et al. 1997), 16q23.2 (Ober et al. 2000), and 19q13.32 (Venanzi et al. 2001) were exactly those that exhibited linkage or, in region 11q12 (Adra et al. 1999), were located in close proximity. Chromosome 12q harbors multiple genetic loci related to asthma and asthma-related phenotypes including atopy, distinct peaks of linkage being observed in different populations (Raby et al. 2003). Markers in regions 12q13 and 12q24 were selected in positions that would enable to test presence of these linkages in the studied population. Similar approach was used in the selection of marker in 16q21 in the vicinity of linkages detected by (Daniels et al. 1996) and (Kurz et al. 2005). In tests of 13q14 and 20p13, we selected the nearest STR marker to the genes *PHF11* (Zhang et al. 2003) and *ADAM33* (van Eerdewegh et al. 2002), respectively.

Markers on chromosome 8q12 (D8S1828, D8S285, and D8S1816) were chosen on the basis of our previous whole genome search for IgE-controlling loci in mouse (Badalová et al. 2002). The mouse locus *Lmr9* (represented by marker D4Mit149) was mapped to the centromeric part of the mouse chromosome 4 with the most likely and maximal lengths 3.58 and 9.32 Mb, respectively, and was shown to have linkage with IgE level (Badalová et al. 2002). The region homologous to the mouse *Lmr9* is located on human chromosome 8q12 (NCBI database), and the markers D8S1828, D8S285, and D8S1816 were chosen for the search for IgE-controlling loci in human. There is no LD between the regions carrying the three STRs (UCSC Genome Browser Assembly March 2006).

Genotyping

The primer sequences were obtained from the NCBI database. We used Cy5 carbocyanine dye 5′-end-labeled forward primers and unlabeled reverse primers synthesized by Generi-Biotech s.r.o. (Hradec Králové, Czech Republic) or Sigma-Genosys, (Steinheim, Germany). DNA was amplified in a 10-μl polymerase chain reaction (PCR) reaction with 10 pmol/μl of forward and reverse primer, 0.2 mM concentration of each dNTP, 1.5 or 2.0 mM $MgCl_2$ (optimized for each STR), 50 mM KCl, 20 mM Tris–HCl (pH 8.4), and 0.1 U of *Taq* polymerase, recombinant (GIBCO, Grand Island, NY, USA) and 5 ng/μl of template DNA. The PCR reaction was performed on 0.2 ml non-skirted 96-well U-bottom microtiter plate (ABgene, Epsom, UK) by MJ Research Thermal Cycler PTC 100 model 96 (MJ Research, Watertown, MA, USA). The universal program was used for DNA amplification: an initial hot start 5 min at 94°C, followed by 39 cycles of 94°C for 20 s for denaturing, 55°C for 20 s for annealing, 74°C for 20 s for elongation, and finally 10 min at 72°C for final extension. PCR products (0.25 μl) were separated by CEQ™ 8800 Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA). All inconclusive genotypes were excluded (less than 2.2% for each marker).

Statistical analysis

The statistical analysis included all family members (also probands) regardless of affected status. Two different approaches were used for statistical analysis of data. The first approach included non-parametric linkage analysis for co-segregation of a chromosomal region and a trait of interest (qualitative and quantitative). The analysis is based on the calculation of LOD score using the linear model of Kong and Cox (1997). This method allows using small nuclear families and calculation of linkage without assuming the normal distribution of the studied trait. We used the Whittemore and Halpern NPL pair statistics (Whittemore and Halpern 1994) to test for allele sharing among affected individuals. The computer program MERLIN version 1.0.0-© 2000–2005 (Abecasis et al. 2002) was used for the calculation of identical-by-descent, allele frequencies (across all individuals), and LOD scores.

The second approach used association analysis for QTDT. The general model of QTDT described by Abecasis et al. (2000a, b) is applicable to the analysis of quantitative or discrete traits in nuclear families of any size and optionally uses parental phenotypes. We used the orthogonal model (Abecasis et al. 2002) to perform the association analysis of the markers as well as their allelic variants with total and specific IgEs. Calculation was conducted by QTDT program version 2.4.6-© 1998–2004 (Abecasis et al. 2000b). Permutation framework (100,000 permutations) provided by QTDT program was used to obtain global *P* values. These were subsequently corrected for multiple testing by Bonferroni correction for number of tested markers and numbers of alleles of the tested markers.

Sex and age were chosen as covariates in both non-parametric linkage and linkage disequilibrium analysis of the total and specific IgEs and inhalant and food atopy. Correlation between phenotypes (sensitization to different allergens) was estimated by the nonparametric Spearman's correlation analysis using STATISTICA for Windows version 8.0 (StatSoft 1984–2008, Tulsa, OK, USA).

Results

8q12 is the human genetic homologue of the IgE-controling mouse locus *Lmr9*

Marker D8S285, located in the human homologue of *Lmr9*, showed a suggestive linkage with IgE to *P. lanceolata* (w9) allergen (LOD=2.42, P=0.0004) and with a composite phenotype–inhalant allergic sensitization (LOD=2.11, P=0.0009; Table 3). The locus 8q12 has not been previously reported in connection with atopy in humans. Potential linkage with LOD>1 was also suggested to specific IgE against moulds (*A. fumigatus*—m3 and *C. herbatum*—m2), animal origin allergens (dog—e2 and cat—e1), mites (*D. farinae*—d2 and *D. pteronyssinus*—d1), *A. elatior* (w1), and potato (f35) allergens (Table 3). The association QTDT analysis of the D8S285 with IgE to *P. lanceolata* (w9) revealed association with the marker (P=0.0317) and with the certain alleles of the marker (allele 112 bp P=0.0056 and allele 114 bp P=0.0184), supporting the linkage and suggesting that a gene controlling atopy in humans is localized close to D8S285. However, we did not find the significant association after adjusting for number of comparisons by Bonferroni correction.

We found also some evidence of linkage to the markers D8S1828 and D8S116 on chromosome 8q12 that are flanking D8S285. D8S1828 that is located 260 Kb centromerically from the marker D8S285 exhibited potential linkage with alder allergens (*A. incana*—t2; LOD=1.16, P=0.011) and with allergen of cultivated rye (*S. cereale*—g12; LOD=1.11, P=0.012). The marker D8S116, located 300 Kb telomerically from the marker D8S285, showed a weak linkage with allergens of cultivated rye (LOD=0.68, P=0.04).

Testing of previously reported atopy-controlling regions in the Czech population

We also tested STR markers at the human chromosomal regions that were previously described in genome-wide studies to control atopy in order to determine whether these genetic loci influence IgE level in the Czech population (Table 1).

Two loci on chromosomes 13q14 and 5q33.3 showed the strongest linkages with IgE to plant allergens (Table 3). We found a suggestive linkage of marker D13S165 (Zhang et al. 2003) with IgE to *A. elatior* (w1) allergen (LOD=2.74, P=0.0002). We also detected a suggestive linkage for marker D5S1507 (Yokouchi et al. 2000) with IgE to *S. cereale* (g12; LOD=2.11, P=0.0009). Finally, markers on chromosomes 7p14.1 (D7S2250; Daniels et al. 1996; Laitinen et al. 2001; Shugart et al. 2001) and 12q13 (D12S1298; Barnes et al. 1996) were found by QTDT assay to be significantly associated with *P. lanceolata* (w9; marker D7S2250, corrected P=0.026; allele 147bp of marker D7S2250, corrected P=0.034) and *A. vulgaris* (w6; allele 199bp of marker D12S1298, corrected P=0.043), respectively.

Thus, two markers (D13S165 and D5D1507) were found to have suggestive linkages, and markers D7S2250 and D12S1298 were significantly associated with a number of specific IgEs (Table 3). These data support the results that have been previously published by others (see references in Table 1 and in "Introduction").

There was also some evidence for linkage (LOD>1) with a number of inhalant and food allergens to the markers D5S816 (5q31.1), D12S1059 (12q22), D16S3253 (16q21), and D20S473 (20p13; Table 3). We did not find any association with and any linkage exceeding the level of a LOD>1 to the markers D2S308 (2q14.3), D6S291 (6p21), D7S821 (7q22.1), D11S2006 (11q12), D12S379 (12q21.31), D12S1282 (12q24.31), D16S539 (16q23.2), and D19S601 (19q13.32).

No significant linkage was found with asthma, rhinitis, dermatitis, and total IgE. Markers D19S601 (LOD=0.70, P=0.04), D16S539 (LOD=0.69, P=0.04), D7S2250 (LOD=0.83, P=0.03), and D8S285 (LOD=0.38, P=0.09) showed the highest LOD score with asthma, rhinitis, dermatitis, and total IgE, respectively.

Discussion

Although several genome-wide linkage studies of IgE-controlling loci in humans were conducted, our data for the first time indicate a locus on chromosome 8q12 that could influence development of atopy. The finding of this linkage shows the precision and predictive power of mouse models in investigation of the complex traits in humans.

There are no obvious candidate genes in 8q12 chromosomal region. In the near proximity of the marker D8S285 are localized two oncogenes: *MOS* (V-MOS Moloney murine sarcoma viral oncogene homolog) and *PLAG1* (Pleiomorphic adenoma gene 1). *MOS* exerts many cellular functions; however, its described impact on B cell functions is limited to B cell malignancies caused by chromosomal translocations of this chromosomal segment (Kirsch et al. 1982). *PLAG1* encodes a developmentally regulated, SUMOylated, and phosphorylated zinc finger transcription factor which recognizes a specific bipartite DNA consensus sequence regulating expression of a spectrum of target genes (Van Dyck et al. 2007). One of the target genes of

Table 3 Specific IgE-controlling loci in the Czech atopic families

Locus	cM (Marshfield)	Marker	LOD[a]/P level	Association (corrected *P* level[b])		Allergen-specific IgE[e]
				Marker[c]	Alleles[d]	
5q31.1	139.33	D5S816	1.77/0.002			g12—Cultivated rye
			1.35/0.006			g3—Cock's foot
			1.27/0.008			g1—Sweet vernal grass
5q33.3	157.57	D5S1507	1.17/0.01			w9—Plantain
			2.11/0.0009			g12—Cultivated rye
			1.47/0.005			g6—Timothy grass
			1.41/0.005			g3—cock's foot
			1.95/0.0014			g1—Sweet vernal grass
7p14.1	54.65	D7S2250		0.026	147 bp/0.034	w9—Plantain
8q12	71.0	D8S1828	1.16/0.011			t2—Alder
			1.11/0.012			g12—Cultivated rye
8q12	71.0	D8S285	**2.11/0.0009**			Inhalant atopy[f]
			1.22/0.009			m3—Mould
			1.36/0.006			m2—Mould
			1.11/0.012			e2—Dog
			1.21/0.009			e1—Cat
			1.05/0.014			d2—Dust mite
			1.04/0.014			d1—Dust mite
			2.42/0.0004			w9—Plantain
			1.14/0.011			w1—Common ragweed
			1.59/0.003			f35—Potato
12q13	75.17	D12S1298			199 bp/0.043	w6—Mugwort
12q22	105.18	D12S1059	1.12/0.012			e1—Cat
			1.00/0.02			f20—Almond
13q14	45.55	D13S165	1.80/0.002			w9—Plantain
			2.74/0.0002			w1—Common ragweed
16q21	71.77	D16S3253	1.33/0.007			Inhalant atopy[f]
			1.32/0.007			Food atopy[f]
			1.72/0.002			g3—Cock's foot
20p13	9.53	D20S473	1.30/0.007			e3—Horse

[a] Only LOD scores>1.0 are shown, LOD scores >2 are shown in bold
[b] *P* values obtained by QTDT program were corrected by Bonferroni correction (see "Materials and methods")
[c] Association with markers
[d] Association with specific allele(s)
[e] Phenotype is described in detail in "Materials and methods"
[f] Phenotypes of inhalant and food atopy are defined as sensitization to at least one of the inhalant and food allergens, respectively

PLAG1 is insulin-like growth factor-2 (*IGF2*; Van Dyck et al. 2007), which has pleiotropic functions in immunity. It was shown that $Igf2^{-/-}$ mice had decreased numbers of $B220^{+}$ dendritic cells in spleen (Hansenne et al. 2006). Adoptive transfer of dendritic $B220^{-}$ cells from allergic mice induces specific immunoglobulin E antibody against food allergens in naïve recipients (Chambers et al. 2004), thus showing possible pathway how could *PLAG1* influence atopy.

Another promising target for future research of this locus is LYN kinase gene (*LYN*), which is mapped near marker D8S1828 and 150 Kb proximally of the marker D8S285. The Src tyrosine kinase Lyn is an important modulator in the high affinity receptor for IgE (FcεRI) signaling (reviewed in Rivera and Olivera 2007). Lyn-deficient mice exhibit increased serum levels of IgE, increased numbers of mast cells, increased expression of FcεRI on mast cells, and other allergy-associated traits (Odom et al. 2004). Although the etiology of the allergy-like phenotype of Lyn deficiency is not completely understood, the regulatory role of Lyn kinase in the development of allergy is strongly indicated by these results. Further, dense SNP coverage, fine mapping, expression studies, and re-sequencing of the 8q12 region are required to define the gene(s) affecting disease susceptibility.

In pooled groups of Caucasian families recruited from Minnesota and from 11 clinical centers in Europe, Australia, and USA, a suggestive linkage was detected with total serum IgE with a peak of linkage near the marker D8S2324 (94.08 cM—Marshfield; Webb et al. 2007). This

marker is located outside the <9 Mb region homologous to mouse IgE-controlling locus *Lmr9* (Fig. 1), and also 23 cM distally, and therefore is distinct from the locus at 8q12 described here. These data suggest that two loci controlling human IgE might be localized on chromosome 8q.

Linkage of locus 13q14 with atopy was initially detected in atopic families from Busselton, Western Australia (Daniels et al. 1996). Subsequent association mapping using dense SNP map of this region postulated PDH finger protein 11 gene (*PHF11*) as a gene predisposing to atopy (Zhang et al. 2003). In our study, the marker D13S165 that maps within 1 Kb from *PHF11* gene is linked to IgE to *A. elatior* (w1; Table 3), suggesting the effect of this gene on IgE regulation in the Czech atopic patients.

Chromosomal region 5q31-33 seems to be one of the most attractive targets for the investigation of the IgE control in humans. This region encompasses a cluster of pro-inflammatory cytokine genes [*IL-4*, *IL-5*, *IL-9*, *IL-13*, *IRF-1* (interferon releasing factor 1) and *CSF-1R* (receptor for colony stimulating factor 1], the protein products of which are directly involved in immune regulation. The linkage to this locus was corroborated in multiple studies (Marsh et al. 1994; Ober et al. 2000; Xu et al. 2000; Haagerup et al. 2002; Koppelman et al. 2002; Yokouchi et al. 2002). This locus also contains genes *TIM1/HAVCR/KIM1* (5q33.2) and *TIM3* (5q33.3) that were shown to control asthma and airway hyperreactivity (McIntire et al. 2001). In the present study, a marker on chromosome 5q33.3 (D5S1507) had a suggestive linkage with IgE to allergens of *S. cereale* (g12) and showed a potential linkage to allergens of *Phleum pretense* (g6), *D. glomerata* (g3), and *A. odoratum* (g1; Table 3). All grass sensitization phenotypes (g12, g6, g3, and g1) showed very high positive correlation with each other (R=0.937–0.988) and therefore showed very similar LOD scores with D5S1507.

QTDT analysis also revealed two atopy-associated markers on chromosomes 7p14.1 (D7S2250) and 12q13 (D12S1298) that did not show genetic linkage with atopy phenotype. Linkage and QTDT association analysis [Kong and Cox linear model (Kong and Cox 1997) and Abecasis orthogonal model (Abecasis et al. 2000a, b), respectively] exploit two different aspects of genetic information. Thus, the results of association and linkage analysis may, but need not, coincide (Wills-Karp and Ewart 2004).

Remarkably, none of the loci showed a significant linkage or association with total IgE. This might be partly due to a high overall sensitization of Czech atopic families that reached nearly 90% in both parents and offspring. However, we observed that various types of the specific IgE were controlled by different genetic loci. Thus, sensitization to different allergens seems to be determined by different genes. This might also partly explain differences in results obtained by different laboratories that postulated loci controlling total IgE in humans (Hoffjan and Ober 2002).

We did not find any linkage or association with asthma, rhinitis, and dermatitis. This is probably due to the low number of affected sib pairs with asthma and dermatitis in the tested sample (please see details in "Materials and methods", Table 2). Moreover, asthma (Hoffjan and Ober 2002), and probably also rhinitis and dermatitis, may comprise groups of several disorders. In the mouse model, the various components of the pathogenetic pathway of allergic asthma are under separate genetic control (Piavaux et al. 2007); this may explain why in this and other similar studies, asthma is not necessarily linked to the genes that control one out of the multiple sets of pathogenetic components. The analysis of less complex and more exactly defined phenotypes such as levels of total and specific IgE is therefore an important part of genetics of atopic diseases.

The present work demonstrates the power of the genome-wide screening in mice in finding new loci determining complex traits such as IgE levels in humans. Using this approach, a new IgE-controlling locus has been identified on chromosome 8q12 that influences the sensitization to a number of allergens. Our data also confirm the role of the previously reported loci 13q14, 5q33.3, 7p14.1, and 12q13 in control of IgE and development of atopy.

Acknowledgments We acknowledge the help of E. Antošová, MD, A. Voříšková, H. Vávrová, MD, E. Vernerová, MD, M. Heroldová, MD, M. Cvačková, MD (University Hospital Royal Vinyards, Prague), E. Škvorová, MD, D. Mudruňková, MD (Department of Immunology and Microbiology, Institute of Public Health, Ústí nad Labem), and M. Zemanová, MD (Allergology practice, Trutnov) and many other clinical specialists involved in recruitment, interviews and clinical description of patients. Most importantly, we thank all the patients without whom this study would have been impossible to undertake. We thank Prof. Peter Demant, MD, PhD, Roswell Park Cancer Institute, Buffalo, U.S.A., and Jasper Manning Jr., M.Sc., Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic for critical reading of the manuscript and useful suggestions. This investigation was supported by the European Commission (INTAS Genomics 05-1000004-7761), the Grant Agency of the Czech Republic (grant 310/06/1745), Academy of Sciences of the Czech Republic (program grant AVOZ50520514), Ministry of Education of the Czech Republic (program grant LC06009) and by Research Theme MSM0021620812.

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2.1 Chromosome 12q24.3 controls sensitization to cat allergen in patients with asthma from Siberia, Russia

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Immunology Letters, on revision

Summary statement

Definition of the genetic component of asthma-associated traits such as total IgE, specific IgE to the most prominent allergens and pulmonary function is important for understanding of etiology of asthma and atopy in humans. To define loci contributing asthma and atopy in Russian patients from Siberia, we have selected a set of microsatellite markers from the most promising candidate chromosomal regions reported by others and have tested them for linkage with asthma, and traits often related to asthma in Siberian population (total IgE, specific IgE to cat (e1), dog (e2) and dust mite (d1 and d2) and pulmonary function represented by spirometric indeces: FEV1(%) – percentage of forced expiratory volume in 1 second, FVC(%) – percentage of forced vital capacity, and FEV1/FVC(%) – Tiffenau index. The most significant results were obtained for the candidate region on chromosome 12. Cat-specific IgE, which is the most abundant in asthma patients from Siberian population, showed suggestive linkage with a segment of 136-140 cM on chromosome 12q24.3. This data indicate that the locus 12q24.3 is the most promising candidate for identification of genes of cat allergic sensitization and asthma in Siberian population of Russian patients.

As a first author, I contributed to the study design, DNA isolation and genotyping, estimation of specific IgEs, statistical analysis and interpretation of data, and preparation of the manuscript.

Abstract

In Russian population of Siberia asthma is usually concomitant with high sensitization to indoor allergens (cat, dog and house dust mite), overproduction of total IgE and airway hyperreactivity. Definition of genes that predispose to development of various subcomponents of the asthma phenotype is important for understanding of etiology of this disease. To map genes predisposing to asthma, we tested 21 microsatellite markers from candidate chromosomal regions in 152 Russian nuclear families from Siberia. We performed non-parametric analysis for linkage with asthma, total IgE, specific IgE to cat, dog, and dust mites, and spirometric indices (FEV1(%) – percentage of forced expiratory volume in 1 s, FVC(%) – percentage of forced vital capacity, and FEV1/FVC(%) - Tiffenau index). The most significant linkage was to the candidate region on chromosome 12. Cat-specific IgE, which is the most abundant in asthma patients from Siberian population, showed suggestive linkage with the interval between 136 and 140 cM on chromosome 12q24.3, near markers D12S1282 (LOD=2.0, *P*=0.0012) and D12S1611 (LOD=2.15, *P*=0.0008). Total IgE was also linked to this region (D12S1611 – LOD=1.22, *P*=0.009). FEV1(%) and FVC(%) exceeded LOD>1 threshold for significance with the same locus 12q24.3 but with the peak at a more proximal region at 111.87 cM (D12S338 – LOD=1.19, *P*=0.01 and LOD=1.16, *P*=0.01, respectively). Some evidence of linkage (LOD>1.0) was also detected for asthma at 6p21.31 (D6S291), total IgE at 13q14.2 (D13S165), and FEV1(%) at 5q31.1 (D5S816). These data indicate that the locus 12q24.3 is the most promising candidate for identification of asthma genes in Russian population of Siberia.

Introduction

Atopy is an inherited predisposition to overproduction of immunoglobulin E (IgE) in response to common environmental allergens. It is the major risk factor for development of allergic diseases, the most severe of which is allergic bronchial asthma, a chronic inflammatory disease of airways characterized by air-flow limitations that cause recurrent episodes of wheezing, breathlessness, chest tightness, and cought. The reduced pulmonary function during asthma is usually caused by allergic reaction with elevated production of allergen-specific IgE and T helper 2 type cytokines, reversible bronchial obstruction, mucus hypersecrecion, and bronchial hyperreactivity [1]. Both atopy and allergic asthma are complex traits that are under control of multiple genes, the effects of which can be modulated by environmental factors. In the last decades a number of asthma and atopy associated trait loci have been identified. Several loci including 5q [2–6], 6p [4,7–9], 7p [7,10–12], 7q [2,5,12], 11q [7,11,12], 12q [2,5,6,13–17] and 16q [7,18,19] were corroborated in different human populations. Positional cloning indicated genes at six loci 2q14 (*DPP10* – dipeptidyl serine protease) [20], 2q33 (*CTLA4* – cytotoxic T-lymphocyte-associated-4 gene) [21], 5q32-33 (*PCDH1* – protocadherin-1) [22], 7p14.3 (*GPRA* – G protein-coupled receptor) [23], 13q14 (*PHF11* – PHD finger protein 11) [24], and 20p13 (*ADAM33* – Zn dependent metalloproteinase) [25] predisposing to asthma, atopy and airway responsiveness. Genome-wide association mapping led to identification of the genes *ORMDL3* (an endoplasmic reticulum membrane protein) at locus 17q21 [26] and *CHI3L1* (chitinase 3-like 1) at locus 1q32.1 [27] that contribute to the risk of asthma.

Genetic heterogeneity of human populations and variety of environmental factors

cause diversity in allergic sensitization in different geographical areas. Our previous study revealed that cat (e1) and also dog (e2) and dust mites (d1, d2) are the most prominent allergens in patients with allergic bronchial asthma from the Russian population of Siberia [28]. Later study provided evidence that early-life exposure to cats increases the risk of asthma in Russians [29]. Definition of the genetic component of sensitization to the most prominent allergens and other asthma associated traits (total IgE, pulmonary function) in Russian patients with asthma is therefore important for understanding of etiology of this disease in Siberian population. To define loci that contribute to susceptibility to asthma and atopy in Russian population from Siberia, we have selected a set of short tandem repeat (STR) markers (Table 1) from the most promising candidate chromosomal regions reported by others and have tested them for linkage with asthma, and traits often related to asthma: total IgE, specific IgE to cat (e1), dog (e2) and dust mite (d1 and d2) allergens and pulmonary function represented by spirometric indices FEV1(%) – percentage of forced expiratory volume in 1 second, FVC(%) – percentage of forced vital capacity, and Tiffenau index - FEV1/FVC(%).

Material and Methods

Families

Nuclear families (152 families, n=602) of Russian ethnicity from Siberian cities Tomsk, Thumen and Irkutsk (Russia) were collected through probands registered in local clinics as patients with allergic bronchial asthma (136 families) or with allergic rhinitis and/or atopic dermatitis (16 families) (Table 2). Asthma was diagnosed according to criteria of the Global Initiative for Asthma (GINA 2002 - http://ginaasthma.org). Allergic rhinitis and dermatitis were diagnosed according to EAACI guidelines [30]. All participants received a full explanation of the study design. The clinical examination was approved by the local Ethical Committees.

Estimation of total and specific IgE

Collection of blood samples was conducted in 2004 – 2007 excluding summer months (pollination season). The total IgE level was measured by IgE-EIA-BEST–strip (VECTOR-BEST, Novosibirsk, Russia). Specific IgEs to cat (e1), dog (e2), and dust mite *Dermatophagoides pteronyssinus* (d1) and *D. farinae* (d2) allergens were estimated by *in vitro* test system EUROLINE (EUROIMMUN, Medizinische Labordiagnostika GmbH, Lübeck, Germany). In this system allergen extracts are used for detection of specific IgE. The lowest threshold of detection was 0.35 kU/l.

Genetic markers

For the analysis, 21 STR markers located at different chromosomes/chromosomal regions

were selected (Table 1) from the National Centre for Biotechnology Information (NCBI) database (http://www.ncbi.nih.gov). The STR markers are characterized in the Marshfield genetic map and show high heterozygosity. All markers are located in atopy candidate regions previously described in genome-wide studies of atopy and/or asthma [2-12,19,18] (Table 1). Markers selected in regions 2q14.1 [20], 5q31.1 [5], 5q33.2 [3], 6p21.31 [8], 7p14.3 [7,10,11], 7q21.3 [2], 12q21.31 [14], 16q24.1 [18], and 19q13.33 [32] were exactly those that exhibited linkage, or in regions 11q12.1 [31], and 16q12.2 [7,19] were located in the close proximity. To test 13q14.2 and 20p13 we selected the nearest polymorphic STR marker to the genes *PHF11* [24] and *ADAM33* [25], respectively. Chromosome 12q harbors multiple genetic loci related to asthma and asthma-associated phenotypes, distinct peaks of linkage being observed in different populations [33]. Four markers in regions 12q14.1, 12q21.31, 12q23.1, and 12q24.3 (D12S1298, D12S379 [13,14], D12S1059, and D12S1282, respectively) were chosen in positions that would enable to test presence of described linkages in the studied population. After identification of a linkage with atopy to 12q24.3 at 136.82 cM, we selected five additional STRs from surrounding regions (D12S338 at 111.87 cM, D12S164 at 119.55 cM, D12S2082 at 130.94 cM, D12S1611 at 140.17 cM, and D12S1634 at 148.24 cM) to map position of the linkage more precisely.

Genotyping

The primer sequences were obtained from the NCBI database. We used Cy5 carbocyanine dye 5'-end-labeled forward primers and unlabeled reverse primers synthesized by Generi-Biotech s.r.o. (Hradec Králové, Czech Republic) or Sigma-

Genosys Ltd. (Steinheim, Germany). DNA was amplified in a 10-µl PCR reaction using universal program described in detail elsewhere [34]. PCR products were separated by CEQ™ 8800 Genetic Analysis System (Beckman Coulter Inc., Fullerton, CA, USA). All inconclusive genotypes were excluded (less than 2.2% for each marker).

Statistical analysis

The statistical analysis included all family members (also probands) regardless of affected status. Non-parametric linkage analysis for co-segregation of a chromosomal region and a trait of interest (qualitative and quantitative) was performed. The analysis is based on the calculation of LOD score using the Kong and Cox linear model [35]. This method allows using small nuclear families and calculation of linkage without assuming the normal distribution of the studied trait. We used the Whittemore and Halpern NPL pairs statistics [36] to test for allele sharing among affected individuals. The computer program MERLIN version 1.0.0 - © 2000 – 2005 [37] was used for calculation of allele frequencies (across all individuals) and LOD scores. Sex, age and smoking were covariates in all calculations.

Results

The most significant results were obtained for the candidate region on chromosome 12. Initially we tested four markers in regions 12q14.1, 12q21.31, 12q23.1, and 12q24.3 (D12S1298, D12S379, D12S1059, and D12S1282, respectively). Multipoint linkage analysis showed suggestive linkage of cat-specific IgE with marker D12S1282 at the position 136.82 cM in 12q24.3 chromosomal region (LOD=2.13, *P*=0.0009). To localize the locus of linkage more precisely we have tested five additional STRs (D12S338 at 111.87 cM, D12S1645 at 119.55 cM, D12S2082 at 130.94 cM, D12S1611 at 140.17 cM, and D12S1634 at 148.24 cM) from the regions surrounding the marker D12S1282. This analysis revealed suggestive linkage of cat-specific IgE with a peak of linkage in an interval between 136 and 140 cM (markers D12S1282 – LOD=2.0, *P*=0.0012 and D12S1611 – LOD=2.15, *P*=0.0008) in 12q24.3 (Table 3) (Fig. 1). The linkage of the adjacent markers D12S2082 (130.94 cM) and D12S1634 (148.24 cM) exceeded LOD>1.0 (LOD=1.15, *P*=0.011 and LOD=1.28, *P*=0.008, respectively). After elimination of the families collected though probands without asthma, the linkage of cat-specific IgE to D12S1611 increased (LOD=2.23, *P*=0.0007). Total IgE showed evidence of linkage at 12q24.3 with marker D12S1611 (LOD=1.22, *P*=0.009) (Table 3) (Fig. 1), the peak of linkage being the same as for cat-specific IgE. On the other hand, peak of linkage with spirometric indices FEV1(%) and FVC(%) was observed at the more proximal position of 111.87 cM to the marker D12S338 – LOD=1.19, *P*=0.01 and LOD=1.16, *P*=0.01, respectively (Table 3). Some evidence of linkage (LOD>1.0) was also detected to asthma at 6p21.31 (D6S291 – LOD=1.04, *P*=0.014), total IgE at 13q14.2 (D13S165 – LOD=1.20, *P*=0.009), and FEV1(%) at 5q31.1 (D5S816 – LOD=1.12,

P=0.012) (Table 3). We did not detect linkage of specific IgE to dog and dust mites, and to Tiffenau index to any of the tested markers.

Discussion

In the present study we have replicated evidence for an atopy susceptibility locus at the chromosomal region 12q24 in Russian patients with asthma from Siberia. We have observed a peak of a suggestive linkage at the positions of 136-140 cM for cat-specific IgE and some evidence of linkage for total IgE at the same position. The linkage of the region 12q24.3 (125-134 cM) with total IgE, asthma and wheeze, eosinophil count and airway responsiveness, was reported in Dutch [2,5] and Japanese [6], British [15], French [38] and Costa Rica Hispanic [17] population, respectively, supporting the important role of this locus in development of asthma and atopy related traits (Fig. 2). The locus at 136-140 cM linked to cat-specific IgE detected in the present study is located in a close proximity (within 9 cM) to the loci described above (Fig. 2) and confirms the presence of atopy related loci reported by others.

We have not found a significant or a suggestive linkage of the locus on 12q24.3 with asthma. This difference in susceptibility in different human populations might be caused by variation of genetic composition, by different lifestyles and exposures and/or by environmental variations in major allergens triggering development of asthma. This supports the importance of analysis of disease susceptibility in different human populations.

Although the peak of linkage for cat-specific IgE and total IgE was at the same position (marker D12S1611), linkage of a total IgE was weaker than linkage for cat-specific IgE. This can be explained by the fact that total IgE and cat-specific IgE correlate only partly (R=0.36, *P*=0.00003) [42]. These data also show importance of studies of different subphenotypes of complex traits because they are often under a distinct genetic

control.

The locus 12q24.3 encompasses in the interval 136-140 cM a large gene cluster with a several potential candidate genes (Fig. 2). The most promising target for a future research is a gene for interleukin 31 (*IL31*). IL-31 is a pro-inflammatory cytokine expressed preferentially by $CD4^+$ T helper (Th) type cells, which has been recently implicated as a good marker for allergic skin inflammation during atopic dermatitis [43] and bronchial inflammation during allergic asthma [44]. Increased mRNA levels of IL-31 mRNA were observed in biopsy specimens taken from patients with atopic dermatitis [43]. Similarly, mRNA levels of IL-31 were significantly higher in peripheral blood mononuclear cells of patients with asthma versus non-asthmatic healthy individuals [44] and correlated with the serum concentration of this cytokine [44]. It was reported that IL-31 mediates activation of bronchial epithelial cells, thereby contributing to bronchial inflammation during asthma [45]. All this data strongly indicate *IL31* gene as the main target from the locus 12q24.3 (136-140 cM) for atopy and allergy development.

Three other candidate genes previously suggested in this locus include *PLA2G1B* (phospholipase A2, group IB) [46], *NCOR2* (nuclear receptor co-repressor 2) [17], and *UBC* (ubiquitin C) [17]. Phospholipase A2 cleaves phospholipids releasing lysophosphatidylcholine plus free fatty acid, most commonly arachidonic acid, which can be metabolized to prostaglandins and leukotrienes. These molecules could be important mediators of the early phase of the asthmatic response to inhaled allergens and can also regulate T-cell trafficking that occurs in allergic pulmonary inflammation [47]. Nuclear receptor co-repressor (NCOR) is involved in control of broad subsets of AP-1 (activator protein 1) and NF-κB (nuclear factor kappa-B)-dependent gene networks that regulate

diverse biological processes including inflammation and cell migration [48]. The attachment of ubiquitin chains targets proteins for proteosomal degradation and thus has ability to modulate immune responses, such as NF-κB activation and differentiation of $CD4^+$ T cells into T helper 2 cells [49].

This is a first report indicating the role of the locus 12q24.3 in control of sensitization to cat allergens. Previous studies detected some evidence of linkage to cat-specific IgE to chromosomal region 11q13 in African American families with asthma [50] and to 12q22 in Czech atopic families [34]. Association studies indicated polymorphic variants in genes *HLA-DRB1* (6p21.3*)* in Australian population [51], interleukin 4 receptor, alpha - *IL4RA*, polymorphism Gln551Arg (16p12) in German population [52], and chemokine (C-C motif) ligand 5 – *CCL5/RANTES*, polymorphism G401A (17q11.2-q12) and thromboxane A2 receptor – *TBXA2R*, polymorphism T924C (19p13.3) in Chinese population [53,54] that predispose to cat allergic sensitization in patients with asthma. The relatively limited phenotypic effects of the detected loci and genes suggest that they represent only a part of an extensive polygenic inheritance. The genetic and environmental variation in different geographical areas might explain different genetic control of sensitization to cat allergens in different populations.

The present data describe the first genetic linkage to asthma related traits in the Russian population. They will also contribute to understanding of genetic control of sensitization to cat allergens.

Acknowledgments

We acknowledge the help of Evgenija V. Deeva, Olga S. Fedorova and Ivan A. Deev from Siberian State Medical University, Faculty of Pediatrics, Tomsk, Russia and Nadezhda V. Kazakevich from General Hospital of Thumen State Medical Academy, Thumen, Russia and many other clinical specialists in recruitment, interviews and clinical description of patients. Most important, we thank all the patients without whom this study would have been impossible to undertake.

This investigation was supported by the European Commission (INTAS Genomics 05-1000004-7761), Academy of Sciences of the Czech Republic (program grant AVOZ50520514), Grant Agency of the Czech republic (grant GAČR 310/0631745) and Ministry of Education of the Czech Republic (program grant LC06009).

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Table 1. The list of markers tested for multipoint linkage with asthma, total IgE, specific IgE to cat (e1), dog (e2) and dust mites (d1 and d2) and spirometric indices (FEV1(%), FVC (%) and FEV1/FVC(%).

Marker	Chromosome	cM (Marshfield)	Reference
D2S308	2q14.1	124.03	20
D5S816	5q31.1	139.33	5
D5S1507	5q33.2	157.57	3
D6S291	6p21.31	49.50	8
D7S2250	7p14.3	54.11	7, 10, 11
D7S821	7q21.3	109.12	
D11S2006	11q12.1	59.24	31
D12S1298	12q14.1	75.17	
D12S379	12q21.31	93.69	13, 14
D12S1059	12q23.1	105.18	
D12S338	12q23.3	111.87	
D12S1645	12q24.11	119.55	
D12S2082	12q24.22	130.94	
D12S1282	12q24.3	136.82	
D12S1611	12q24.3	140.17	
D12S1634	12q24.3	148.24	
D13S165	13q14.2	45.55	24
D16S3253	16q12.2	71.77	7
D16S539	16q24.1	124.73	18
D19S601	19q13.33	83.19	32
D20S473	20p13	9.53	25

[a] The chromosomal regions were found in the Ensembl Database (http://www.ensembl.org/index.html, November 13, 2008)

Table 2. Characterization of the Russian nuclear family group.

Russian family group characteristics	number (percentage)
Nuclear families with atopy	152
Subjects	602
Parents	305
Age, mean ± SD, median	39.51 ± 6.69, 39.0
Smokers among parents, n (%)	
active	76 (24.92 %)
passive	81 (26.56 %)
non-smokers	148 (48.52 %)
Parents with allergic bronchial asthma	56 (18.36 %)
Parents with total IgE ≥ 100 kU/l, n (%)	135 (44.26 %)
Parents with sensitization to cat (e1)	70 (22.95 %)
Parents with sensitization to dog (e2)	68 (22.30 %)
Parents with sensitization to *D. pteronyssinus* (d1)	40 (13.11 %)
Parents with sensitization to *D. farinae* (d2)	41 (13.44%)
Children	297
Age, mean ± SD, median	12.95 ± 5.67, 13.0
Sex, male : female	0.65 : 0.35
Children with allergic bronchial asthma	169 (56.90 %)
affected sib trios	2
affected sib pairs	32
affected half-sibs	99
Smokers among children, n (%)	
active	32 (10.77 %)
passive	94 (31.65 %)
non-smokers	171 (57.58 %)
Children with total IgE ≥ 100 kU/l, n (%)	175 (58.92 %)
affected sib trios	6
affected sib pairs	41
affected half-sibs	76
Children with sensitization to cat (e1)	127 (42.76 %)
affected sib trios	4
affected sib pairs	27
affected half-sibs	62
Children with sensitization to dog (e2)	103 (34.68 %)
affected sib trios	1
affected sib pairs	26
affected half-sibs	48
Children with sensitization to *D. pteronyssinus* (d1)	63 (21.21%)
affected sib pairs	9
affected half-sibs	45
Children with sensitization to *D. farinae* (d2)	56 (18.86 %)
affected sib pairs	5
affected half-sibs	46

Table 3. Linkage results for asthma, total and specific IgE, and spirometric indices.

Locus	cM (Marshfield)	Marker	Linkage in all family group LOD_a / P – level	Linkage in families with asthma LOD_a / P – level	Phenotype
5q31.1	139.33	D5S816	1.12 / 0.012	-	FEV1(%)
6p21.31	49.5	D6S291	1.04 / 0.014	1.04 / 0.014	asthma
12q23.1	105.18	D12S1059	-	1.01 / 0.02	cat-specific IgE
12q23.3	111.87	D12S338	-	1.28 / 0.008	cat-specific IgE
			1.19 / 0.01	1.21 / 0.009	FEV1(%)
			1.16 / 0.01	-	FVC(%)
12q24.22	130.94	D12S2082	1.15 / 0.011	1.01 / 0.02	cat-specific IgE
12q24.3	136.82	D12S1282	2.0 / 0.0012	1.76 / 0.002	cat-specific IgE
	140.17	D12S1611	2.15 / 0.0008	2.23 / 0.0007	cat-specific IgE
			1.22 / 0.009	1.12 / 0.012	total IgE
	148.24	D12S1634	1.28 / 0.008	-	cat-specific IgE
13q14.2	45.55	D13S165	1.20 / 0.009	1.05 / 0.014	total IgE

[a]Only LOD scores > 1.0 are shown

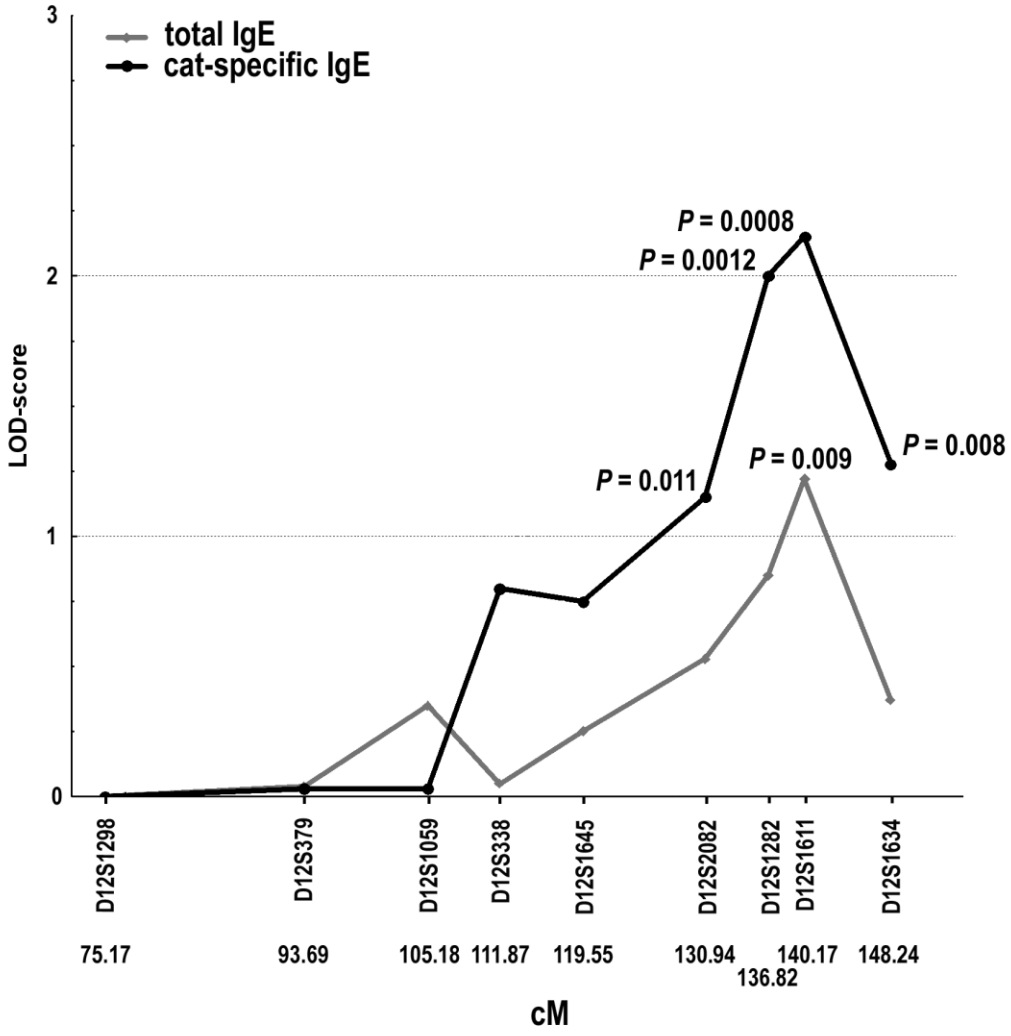

Fig. 1. Linkage of total IgE and cat-specific IgE to markers on chromosome 12q24.

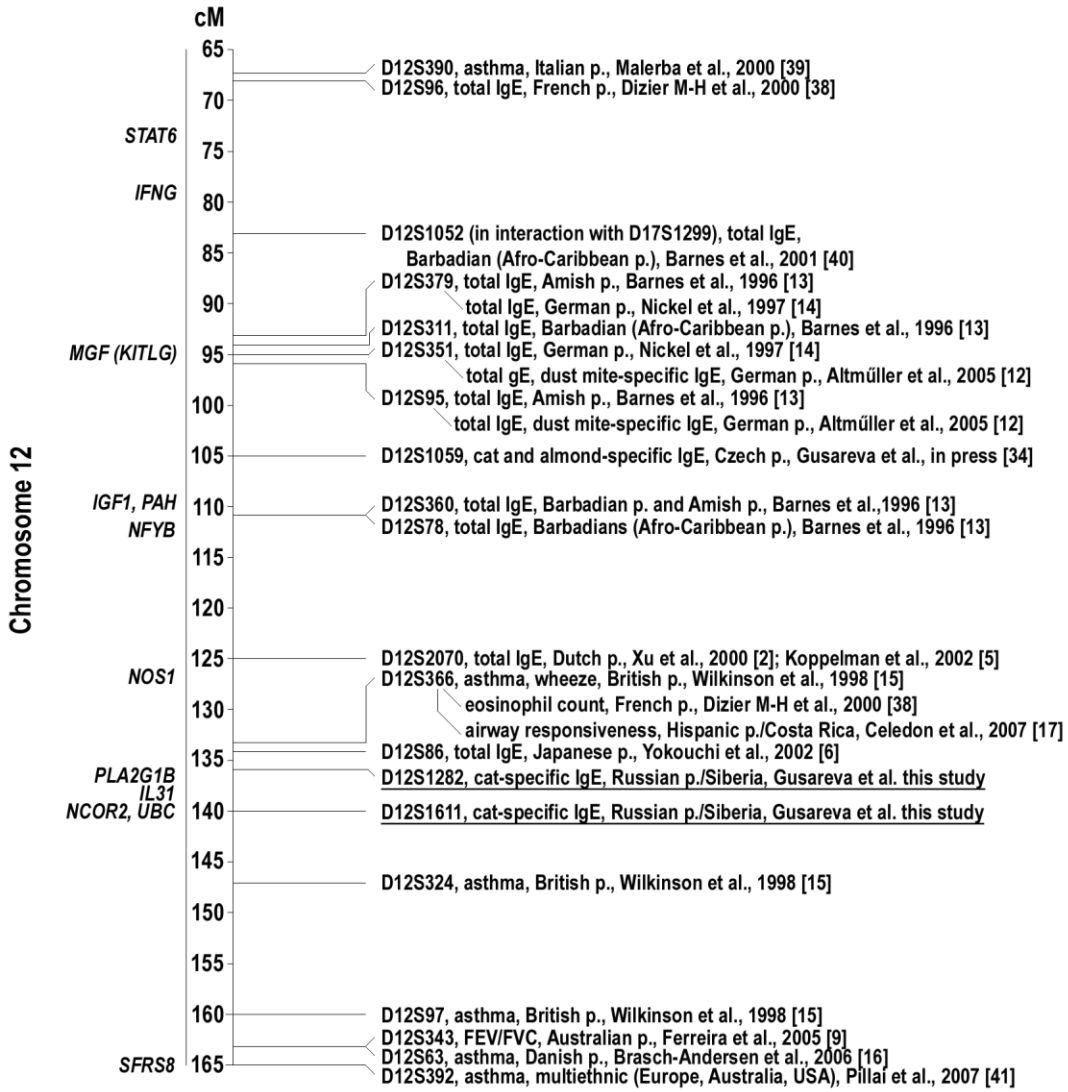

Fig. 2. Results of linkage analysis of chromosomal region 12q13-q24.3 for asthma, total and specific IgE, and spirometric indices in different human populations. Candidate genes in the locus 12q are shown: *STAT6* - signal transducer and activator of transcription 6, *IFNG* - interferon gamma, *MGF* (*KITLG*) - mast cell growth factor, *IGF1* - insulin-like growth factor 1 (somatomedin C), *PAH* - phenylalanine hydroxylase, *NFYB* - nuclear transcription factor Y, beta, *NOS1* - nitric oxide synthase 1, *PLA2G1B* - phospholipase A2, group IB, *IL31* - interleukin 31, *NCOR2* - nuclear receptor co-repressor 2, *UBC* - ubiquitin C, *SFRS8* - splicing factor, arginine/serine-rich 8.

VI. Discussion and future perspectives

The level of IgE is a complex trait, which is under control of multiple genes and various environmental factors. In the present work, both genetic and environmental components were studied in Caucasian populations from the Czech Republic, Russia and Ukraine. The detailed discussion of all the results is given in our publications and manuscripts, please see chapter V. Results. In this chapter, the discussion of the most important data and conclusions are presented.

The first part of the Results chapter is devoted to study prevalence of sensitization to a wide number of inhalant and food allergens, distribution of total IgE and relationship between total and specific IgE. Several important results were obtained. First, major allergens for atopic patients from the Czech Republic, Russia and Ukraine were identified. Reactivity to food allergens was low in both Czech and Ukrainian patients (sensitization to food allergens in Russians was not estimated, because a pilot study had shown that it is very low). Russian and Ukrainian patients were mostly sensitized to indoor allergens such as cat, dog and dust mites. In the Czech patients with atopy, there was approximately the same level of sensitization to indoor allergens but they were dramatically higher sensitized to outdoor allergens such as trees and most prominently grass pollens. Interestingly, the level of allergic sensitization to inhalant allergens was also high in the healthy Czech blood donors. Thus, the profiles of the allergic sensitization in different populations can vary significantly and most probably reflect differences in environmental conditions and life-style. The major allergens seem to contribute most significantly to priming allergy in atopic patients of a particular population.

Second, the retrospective changes in the levels of total IgE in the healthy Czech blood donors were studied (the results of Dr. Antošová *et al.* (1985) were used for comparison). The high level of total IgE was found to be common in healthy blood donors from Prague and it changed insignificantly from 1984 to 2008 years. This might be potentially harmful for blood recipients because passive transfer of IgE can increase the risk of priming allergy.

Finally, the relationship between total and specific IgE was studied in Russian patients with asthma from Siberia and in atopic patients from the Czech Republic and Ukraine. In Russians, we observed a weak correlation between the high levels of total IgE and specific IgE to cat. Thus, cat-specific IgE contributes only partly to the total IgE even though cat is a major allergen for patients with asthma from Siberia. Similarly, major allergens for the Czech (grass, trees, dust mites and cat) and Ukrainian (cat and dust mites) atopic patients did not correlate significantly with the levels of total IgE in these groups. This may have an important implementation to genetic studies of atopy in this populations and provide clues on operation of IgE-controlling genes. In particular, the low correlation between total and the allergen-specific IgEs can indicate that these phenotypes might be partly under control of different genes. These hypothesises were tested by a genetic analysis (please see the next section).

The second part of the Results chapter is devoted to study genetic regulation of IgE (total and allergen-specific) and mapping of genetic loci that contribute to atopy and are involved in development of allergic diseases. Two approaches were used for identification of genetic loci regulating IgE level: the candidate loci approach in humans and genome-wide search for IgE-controlling loci in mouse with subsequent verification

of these loci in human (the detailed description of the used approaches is given in chapter II. Introduction). In the previous genome-wide search performed in mouse recombinant congenic strains (Badalova et al. 2002, Lipoldová et al. 2000) several regions on chromosomes 1, 2, 3, 4, 5, 8, 10, 16 and 18 were identified to control IgE level. Subsequently, the homology between genetic maps of mouse and human was used to identify the corresponding orthologous regions on human chromosomes. Most of the orthologous regions in humans have been already found to be linked with regulation of IgE level, thus supporting the precision and predictive power of mouse models in investigation of complex traits in humans. However, the human region 8q12 orthologous to the shortest IgE locus *Lmr9* (chromosome 4) in mouse has not been described in human studies of atopy. Therefore, we suggested that the 8q12 locus might point to hitherto undetected human genes, which are relevant for regulation of IgE level. In the 8q12 region, we selected three microsatellite markers D8S1828, D8S285, and D8S1816, and tested them for non-parametric linkage and association (QTDT) with inhalant and food atopy and with levels of total IgE and specific IgEs to 20 inhalant and to 5 food allergens in the Czech atopic nuclear families. In the position marked by D8S285 (57.22 Mb, 71cM) we demonstrated a novel human IgE-controlling locus exhibiting suggestive linkage to composite inhalant allergic sensitization and to nine specific IgEs (Table 7). Further dense SNP coverage, fine-mapping, expression studies and resequencing of the 8q12 region are required to define the gene/s affecting atopy.

Table 7. IgE-controlling loci in the Czech and Russian atopic families.

Locus	cM (Marshfield)	LOD[a] / P - level	Association (corr. *P* – level[b])		Group[g]	Allergen-specific IgE[e]
			Marker[c]	Alleles[d]		
5q31.1	139.33	1.77 / 0.002			CZ	g12 - cultivated rye
		1.35 / 0.006			CZ	g3 - cock's foot
		1.27 / 0.008			CZ	g1 - sweet vernal grass
5q33.3	157.57	1.17 / 0.01			CZ	w9 - plantain
		2.11 / 0.0009			CZ	g12 - cultivated rye
		1.47 / 0.005			CZ	g6 - timothy grass
		1.41 / 0.005			CZ	g3 - cock's foot
		1.95 / 0.0014			CZ	g1 - sweet vernal grass
7p14.1	54.65		0.026	147 bp / 0.034	CZ	w9 - plantain
8q12	71.0	1.16 / 0.011			CZ	t2 - alder
		1.11 / 0.012			CZ	g12 - cultivated rye
8q12	71.0	**2.11 / 0.0009**			CZ	inhalant atopy[f]
		1.22 / 0.009			CZ	m3 - mould
		1.36 / 0.006			CZ	m2 - mould
		1.11 / 0.012			CZ	e2 - dog
		1.21 / 0.009			CZ	e1 - cat
		1.05 / 0.014			CZ	d2 - dust mite
		1.04 / 0.014			CZ	d1 - dust mite
		2.42 / 0.0004			CZ	w9 - plantain
		1.14 / 0.011			CZ	w1 - common ragweed
		1.59 / 0.003			CZ	f35 - potato
12q13	75.17			199 bp / 0.043	CZ	w6 - mugwort
12q22	105.18	1.12 / 0.012			CZ	e1 - cat
		1.00 / 0.02			CZ	f20 - almond
12q24.22	130.94	1.15 / 0.011			RUS	e1 - cat
12q24.3	136.82	**2.0 / 0.0012**			RUS	e1 - cat
	140.17	**2.15 / 0.0008**			RUS	e1 - cat
		1.22 / 0.009			RUS	total IgE
	148.24	1.28 / 0.008			RUS	e1 - cat
13q14.2	45.55	1.80 / 0.002			CZ	w9 - plantain
		2.74 / 0.0002			CZ	w1 - common ragweed
		1.20 / 0.009			RUS	total IgE
16q21	71.77	1.33 / 0.007			CZ	inhalant atopy[f]
		1.32 / 0.007			CZ	food atopy[f]
		1.72 / 0.002			CZ	g3 - cock's foot
20p13	9.53	1.30 / 0.007			CZ	e3 - horse

[a]Only LOD scores > 1.0 are shown, LOD scores >2 are shown in bold; [b]*P*-values were corrected for multiple testing by the permutation framework (100 000 permutations) provided by QTDT program and by Bonferroni correction; [c]association with markers; [d]association with specific allele(s); [e]phenotype is described in detail in methods; [f]the phenotypes of inhalant and food atopy are defined as sensitization to at least one of the inhalant and food allergens, respectively; [g]Czech (CZ) and Russian (RUS) nuclear family groups were analyzed.

To define loci that control total IgE and sensitization to the most prominent allergens in Czech and Russian atopic patients a set of microsatellite markers from the most promising candidate chromosomal regions was tested for linkage and association (QTDT) with the levels of specific IgEs to inhalant and food (only in Czech family group) allergens. Linkage and association to plant-specific IgEs were identified at loci 5q33 (157.57 cM), 7p14 (54.65 cM), 12q13 (75.17 cM) and 13q14 (45.55 cM) in the Czech atopic patients (Table 7). In Russian family group, cat-specific IgE (which is the most abundant in Russian patients with asthma from Siberia) showed suggestive linkage with a segment of 136-140 cM on chromosome 12q24.3 (Table 7). We also found some evidence of linkage for total IgE at the same position. Identification of the candidate genes from these loci using fine-mapping approach and subsequent study of the polymorphism of the potential candidate genes and functional studies are required to prove the role of the genes from the identified loci in predisposition to atopy.

This is a first report presenting genetic loci linked to different allergen-specific IgEs in Czech and Russian populations. Before the present work, the association studies had only been reported. In the Czech population, polymorphic variants of nitric oxide synthase 1 (*NOS1,* locus 12q24.2-q24.31, 128.05 cM) and *CD14* (locus 5q31.1, 142.92 cM) genes were associated with high total IgE (Hollá et al. 2004) and specific IgE to moulds and mites (Bucková at al. 2006), respectively. In Russian population, polymorphic variants of genes for interleukin (IL) 4 and IL5 (locus 5q31.1) were associated with allergic asthma (Freidin et al. 2003). In our study, we observed a weak linkage to 5q31.1 locus in Czech population and no linkage to 12q24.2-q24.31 locus. That can indicate the low effect of the gene/s on atopy, so we were able to detect only a

weak linkage or no linkage.

Sensitization to different allergens was found to be under control of different sets of loci/genes that only partly overlap (Table 7). Therefore, elevated production of particular specific IgE is most likely under control of a few genetic loci/genes (e.g. weeds-specific IgE) that might be the same for some allergens (e.g. loci on chromosome 5q control sensitization to different grass allergens, 8q12 locus controls sensitization to moulds, dust mites, weeds and animal origin allergens) and different for others. Moreover, the related phenotypes (such as specific IgEs to grasses, dust mites, animal origin allergens, etc.) might be under control of the same gene/s, whereas different allelic variants of the gene/s may predispose to these phenotypes.

The present work also gives evidence for distinct genetic regulation of specific IgE level in Czech and Russian populations. Different genetic loci showed linkage with the same allergen-specific IgEs in the studied family groups. In the Czech population cat-specific IgE is controlled by two loci on chromosomes 8q12 and 12q22, whereas in Russian population cat sensitization was linked to the single locus on chromosome 12q24. The level of sensitization to cat allergen was found to be similar in both Czech and Russian populations. The different genetic background in Czech and Russian populations may modify the effects of the majority of genes and the same allele can have opposite effects in different environments. These data are in agreement with literature and support the importance of mapping of genes controlling pathological phenotype/s in different populations.

In the present work, we found multiple loci controlling sensitization to various allergens. Total IgE only showed some linkage with loci 12q24.3 and 13q14.2 in Russian

population and no linkage in Czech population (Table 7). Indeed, total IgE is an attractive parameter for genetic study of allergies, as it has well-established values and in large population surveys correlates with the presence of allergic asthma. However, total IgE and the most prevalent specific IgEs correlate only partly that was shown in the present work in Russian, Czech and Ukrainian populations (please see the previous section). This finding is also in agreement with data from Sweden, where cat-specific IgE (one of the most important for patients with allergic wheezing) did not correlate with the high level of total IgE (Erwin et al. 2007). In the other study, only about 45% of the variation in the total serum IgE is attributable to the specific IgE to house dust mite or grass pollen, that were found to be of the important allergens in British population (Cookson at al. 1991). After specific IgE was taken into account, the residual total IgE did not correlate with the presence of asthma (Cookson at al. 1991). These data show importance of studies of different subphenotypes of complex traits (such as specific IgEs) because they are often under a distinct genetic control and are less complex physiological traits.

The present work contributes understanding of etiology of atopy in humans and gives new information about prevalence of allergic sensitization in different geographical areas.

VII. Conclusions

Major allergens and relationship between total and specific IgE in atopic patients from different human populations

1. Cat was found to be the major allergen in patients with asthma from the west Siberia, Russia and was the most important allergen in maintaining the manifestation of asthma. Sensitization to outdoor allergens (molds and plant origin allergens) was insignificant in patients with asthma from the west Siberia, Russia.
2. Cat-specific IgE did not correlate significantly to the high level of total IgE even though cat is a major allergen in Siberia.
3. Cat and dust mites were found to be the major allergens in atopic patients from Ukraine.
4. In atopic patients from the Czech Republic, outdoor allergens (grasses and trees) and indoor allergens (cat and dust mites) were the major allergens.
5. Sensitization to food allergens was low in both Czech and Ukrainian atopic patients.
6. The level of total and allergen-specific IgE was found to be constantly high in blood donors from Prague even though they do not express symptoms of allergy. Grass allergens stimulated the highest IgE production in Czech population.

Genetic regulation of IgE in Czech and Russian populations

1. From the homology with mice, a new IgE-controlling locus has been identified on human chromosome 8q12 that influences specific IgE level to a number of allergens in the Czech atopic patients.

2. We confirmed the role of the previously reported loci 5q33.3, 7p14.1, 12q13, and 13q14 in the control of IgE and the development of atopy in Czech atopic patients.
3. Cat-specific IgE showed suggestive linkage with a segment of 136-140 cM on chromosome 12q24.3 in patients with asthma from Siberia, Russia. According to our data, chromosomal region 12q24.3 is the most promising candidate for the identification of asthma genes in the Siberian population of Russian patients.
4. Genetic regulation of allergen-specific IgEs is different in Czech and Russian populations.
5. Specific IgEs are more likely under the control of several genetic loci than a single locus/gene that is in agreement with the data of others.

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2002 – 2009 PhD student, Third Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

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