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

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ABSTRACT

In Russian population of Siberia asthma is usually concomitant with high sensitization to indoor allergens (cat, dog and house dust mites), overproduction of total immunoglobulin E (IgE) and airway hyperreactivity. Definition of genes that predispose to development of various sub-components 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 136 Russian nuclear families with asthma 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 predicted forced expiratory volume in 1 s, FVC (%) – percentage of predicted forced vital capacity, and FEV1/FVC (%) – Tiffenau index). The most significant linkage was to the candidate region on chromosome 12. Locus controlling cat-specific IgE, which is the most abundant in asthma patients from Siberian population, mapped within the interval between 136 and 140 cM on chromosome 12q24.3, with the suggestive linkage at the marker D12S1611 (LOD = 2.23, $P = 0.0007$). Total IgE was also linked to this region (D12S1611 – LOD = 1.12, $P = 0.012$). FEV1 (%) 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 = 1.21, $P = 0.009$). Some evidence of linkage (LOD > 1.0) was also detected for asthma at 6p21.31 (D6S291) and total IgE at 13q14.2 (D13S165). These data indicate that the locus 12q24.3 is the most promising candidate for identification of asthma genes in Russian population of Siberia.



## 1. 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 cough. 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, hypersecretion of mucus, and hyper-responsiveness of the airways to non-specific bronchospasmogenic stimuli [1]. Allergic asthma is a complex trait that is under control of multiple genes, the effects of which can be modulated by environmental factors. In the last decades a number of loci controlling traits associated with asthma and atopy 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 hyper-responsiveness. Genome-wide association mapping led to identification of the genes *ORMDL3* (an endoplasmic reticulum membrane protein) at

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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 variability in 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]. A 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 predicted forced expiratory volume in 1 s, FVC (%) – percentage of predicted forced vital capacity, and Tiffenau index – FEV1/FVC (%).

## 2. Material and methods

### 2.1. Families

Nuclear families (136 families, $n = 540$) 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 (Table 2). Asthma was diagnosed according to criteria of the Global Initiative for Asthma (GINA 2002 – http://ginaasthma.org). All participants received a full explanation of the study design. Spirometric indices were measured according to ref. [30]. The clinical examination was approved by the local Ethical Committees.

**Table 1**
The list of markers tested for 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[a] | 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 asthma[a] | 136 |
| Subjects | 540 |
| Parents | 273 |
| Age, mean ± SD, median | 39.37 ± 6.51, 39.0 |
| Smokers among parents, *n* (%) | |
| Active | 71 (26.01%) |
| Passive | 75 (27.47%) |
| Non-smokers | 127 (46.52%) |
| Parents with allergic bronchial asthma | 56 (20.51%) |
| Parents with total IgE ≥ 100 kU/l, *n* (%) | 118 (43.22%) |
| Parents with sensitization to cat (e1) | 64 (23.44%) |
| Parents with sensitization to dog (e2) | 60 (21.98%) |
| Parents with sensitization to *D. pteronyssinus* (d1) | 35 (12.82%) |
| Parents with sensitization to *D. farinae* (d2) | 38 (13.92%) |
| Children | 267 |
| Age, mean ± SD, median | 12.92 ± 5.58, 13.0 |
| Sex, male: female | 0.66: 0.34 |
| Children with allergic bronchial asthma | 169 (63.30% of all children) |
| Affected sib trios | 2 |
| Affected sib pairs | 31 |
| Affected half-sibs | 101 |
| Smokers among children, *n* (%) | |
| Active | 30 (11.24% of all children) |
| Passive | 86 (32.21% of all children) |
| Non-smokers | 151 (56.55% of all children) |
| Children with total IgE ≥ 100 kU/l, *n* (%) | 153 (57.30% of all children) |
| Affected sib trios | 5 |
| Affected sib pairs | 34 |
| Affected half-sibs | 70 |
| Children with sensitization to cat (e1) | 115 (43.07% of all children) |
| Affected sib trios | 2 |
| Affected sib pairs | 28 |
| Affected half-sibs | 53 |
| Children with sensitization to dog (e2) | 89 (33.33% of all children) |
| Affected sib pairs | 25 |
| Affected half-sibs | 39 |
| Children with sensitization to *D. pteronyssinus* (d1) | 55 (20.60% of all children) |
| Affected sib pairs | 8 |
| Affected half-sibs | 39 |
| Children with sensitization to *D. farinae* (d2) | 50 (18.73% of all children) |
| Affected sib pairs | 5 |
| Affected half-sibs | 40 |

[a] At least one member of the family was diagnosed with asthma (according to GINA 2002).

### 2.2. Estimation of total and specific IgE

Collection of blood samples was conducted in 2004–2007 excluding summer months (pollination season). Total IgE levels were measured by ELISA using the kit IgE-EIA-BEST–strip (Cat. no. A-8660) (VECTOR-BEST, Novosibirsk, Russia). Total IgE levels were determined using the ELISA reader Uniplan (PICON, Moscow, Russia). Specific IgEs to cat (e1), dog (e2), and dust mite *Dermatophagoides pteronyssinus* (d1) and *Dermatophagoides farinae* (d2) allergens were estimated by *in vitro* test system (Cat. no. DP-3110-1609 E) EUROLINE (EUROIMMUN AG, Medizinische Labordiagnostika GmbH, Lübeck, Germany). In this system allergen extracts on membrane strips and three control bands of different color intensity are used for detection and quantification of specific IgEs. We used the conditions recommended by the manufacturer with slight modifications. In brief, the strips were blocked in the kit "universal buffer" for 5 min at the room temperature. After blocking, each strip was incubated overnight in the cold room in 1 ml of serum diluted 1:10 in universal buffer (100 μl of serum per 1 ml of universal buffer) on rocking shaker 3D

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Rocking Platform STR9 (Stuart Scientific/Keison Products, Essex, UK). After the incubation, strips were washed by the 1 ml of kit universal buffer on rocking shaker 3 times for 5 min. Further, strips with attached antibodies were incubated with enzyme conjugate (alkaline–phosphatase-labeled anti-human IgE antibodies) on rocking shaker for 1 h and then washed by the kit universal buffer 4 times for 5 min. Subsequently the strips were incubated 10 min with 1 ml of NBT/BCIP (nitrobluetetrazoliumchloride/5-bromo-4-chloro-3-indolylphosphate) to promote color reaction. The color reaction was stopped after 10 min by strip washing in distilled water. Damp strips were attached to the adhesive foil on the green protocol paper sheet (EUROIMMUN AG, Lübeck, Germany). After the strips were completely dry, they were scanned using USB flatbed scanner CanoScan LIDE 30 (Canon, Newport News, VA, USA) and EUROLINE Scan software for quantitative evaluation of the EUROLINE test strips. The levels of specific IgEs were determined using EUROLINE Scan software and three standard color bands. The lowest threshold of detection was 0.35 kU/l.

Manufacturer's tests have shown that IgE levels obtained by EUROLINE system correlate with levels obtained by CAP system from Phadia (Uppsala, Sweden) (http://www.euroimmun.de/file-admin/template/images/pdf/Allergie_6S_EN.pdf). We performed our own parallel comparative study of the two methods and confirmed this information. We measured levels of IgEs to dust mites (d2) and timothy grass (g6) in 112 samples by EUROIMMUN and Phadia CAP system and compared the obtained values using the non-parametric Spearman Correlation analysis (STATISTICA 8.0, StatSoft, Inc. 1984–2007, Tulsa, OK, USA). We found high correlation in IgE concentrations estimated by these two systems $R = 0.87$, $P < 0.009$ and $R = 0.92$, $P < 0.009$ for d2 and g6, respectively (data not shown).

### 2.3. 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 those that detected linkage in previous studies, and in regions 11q12.1 [31] and 16q12.2 [7,19] in close proximity of such markers. 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 establish the position of the susceptibility locus more precisely.

### 2.4. 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 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).

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

| Locus | cM (Marshfield) | Marker | Linkage in families with asthma LOD[a]/*P*-level | Phenotype |
|---|---|---|---|---|
| 6p21.31 | 49.5 | D6S291 | 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 |
| | | | 1.21/0.009 | FEV1 (%) |
| 12q24.22 | 130.94 | D12S2082 | 1.01/0.02 | Cat-specific IgE |
| 12q24.3 | 136.82 | D12S1282 | 1.76/0.002 | Cat-specific IgE |
| | 140.17 | D12S1611 | 2.23/0.0007 | Cat-specific IgE |
| | | | 1.12/0.012 | Total IgE |
| 13q14.2 | 45.55 | D13S165 | 1.05/0.014 | Total IgE |

[a] Only LOD scores > 1.0 are shown.

### 2.5. 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.

## 3. Results

The most significant results were obtained for the candidate region on chromosome 12 (Table 3). 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 some evidence of linkage of cat-specific IgE with marker D12S1282 at the position 136.82 cM in 12q24.3 chromosomal region (LOD = 1.46, $P = 0.005$). 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 a locus controlling cat-specific IgE with a peak of linkage in an interval between 136 and 140 cM (markers D12S1282 – LOD = 1.76, $P = 0.002$ and D12S1611 – LOD = 2.23, $P = 0.0007$) in 12q24.3 (Table 3) (Fig. 1). The linkage of the adjacent marker D12S2082 (130.94 cM) exceeded LOD > 1.0 (LOD = 1.01, $P = 0.02$). Total IgE showed indications of linkage at 12q24.3 with marker D12S1611 (LOD = 1.12, $P = 0.012$) (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 index FEV1 (%) was observed at the more proximal position of 111.87 cM to the marker D12S338 – LOD = 1.21, $P = 0.009$. The linkage of cat-specific IgE to this marker also exceeded LOD > 1 (Table 3). Some evidence of linkage (LOD > 1.0) was also detected to asthma at 6p21.31 (D6S291 – LOD = 1.04, $P = 0.014$) and to total IgE at 13q14.2 (D13S165 – LOD = 1.05, $P = 0.014$) (Table 3).

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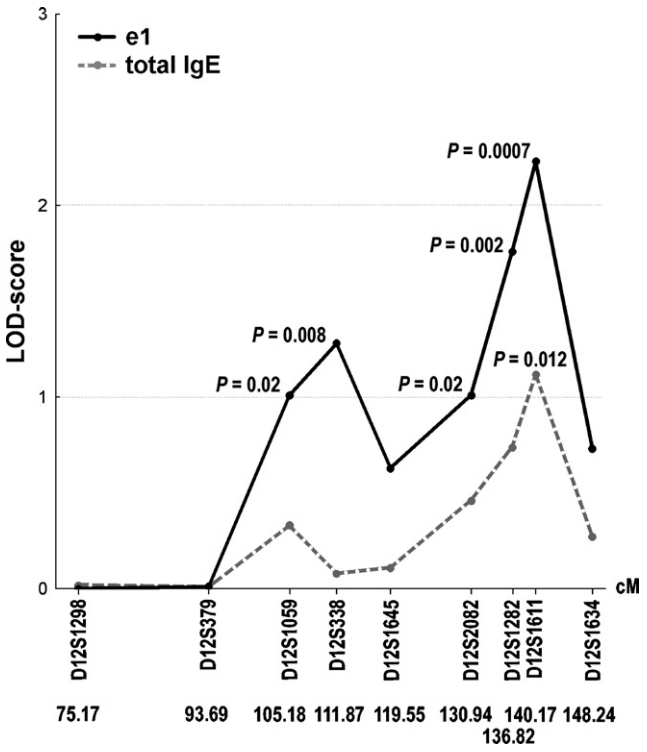


**Fig. 1.** Linkage of total IgE and cat-specific IgE to markers on chromosome 12q24.

We did not detect linkage of specific IgE to dog and dust mites, and of FVC (%) and Tiffenau index to any of the tested markers.

## 4. 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 previous reports of atopy related loci at this site [2,5,6,15,17,38].

We have not found a significant or a suggestive linkage of the locus on 12q24.3 with asthma. We report that asthma and different asthma sub-components are controlled by sets of loci that partly overlap, but are not identical (Table 3). Similar results were also obtained by others [4,7–9,41]. The difference in susceptibility to a specific sub-component of a complex trait in different human populations might be caused by different genetic composition, by different lifestyles and exposures and/or by environmental variations in major allergens triggering development of asthma. In the mouse model, studying genetically different mice in controlled environment, the various components of the pathogenetic pathway of allergic asthma (airway reactivity, serum IgE levels, cellular and cytokine composition of the broncho-alveolar lavage fluid) are under separate genetic control [43]. This may explain why in the present study and in other similar studies asthma is not necessarily linked to the genes that control one out of its several distinct 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 asthma and other atopic diseases. Therefore analysis of susceptibility to asthma in different human populations may elucidate the prevalent population-specific pathogenic pathways and indicate the optimal preventive measures.

The peaks of linkage for cat-specific IgE and total IgE are at the same position (marker D12S1611), but 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 sub-phenotypes 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 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 [44] and bronchial inflammation during allergic asthma [44]. Increased levels of IL-31 mRNA were observed in biopsy specimens taken from patients with atopic dermatitis [44]. Similarly, mRNA levels of IL-31 were significantly higher in peripheral blood mononuclear cells of patients with asthma than in non-asthmatic healthy individuals [45] and correlated with the serum concentration of this cytokine [45]. It was reported that IL-31 mediates activation of bronchial epithelial cells, thereby contributing to bronchial inflammation during asthma [46]. All these data strongly indicate *IL31* gene as the main candidate 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) [47], *NCOR2* (nuclear receptor co-repressor 2) [17], and *UBC* (ubiquitin C) [17]. Phospholipase A2 cleaves phospholipids and releases 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 [48]. 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 [49]. 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 [50].

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 [51] and to 12q22 in Czech atopic families [34]. Association studies indicated polymorphic variants in genes *HLA-DRB1* (6p21.3) in Australian population [52], interleukin 4 receptor, alpha – *IL4RA*, polymorphism Gln551Arg (16p12) in German population [53], 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 [54,55] 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 the extensive multigenic control of this trait. The genetic and environmental variation in different geographical areas might explain different genetic control of sensitization to cat allergens in different populations.

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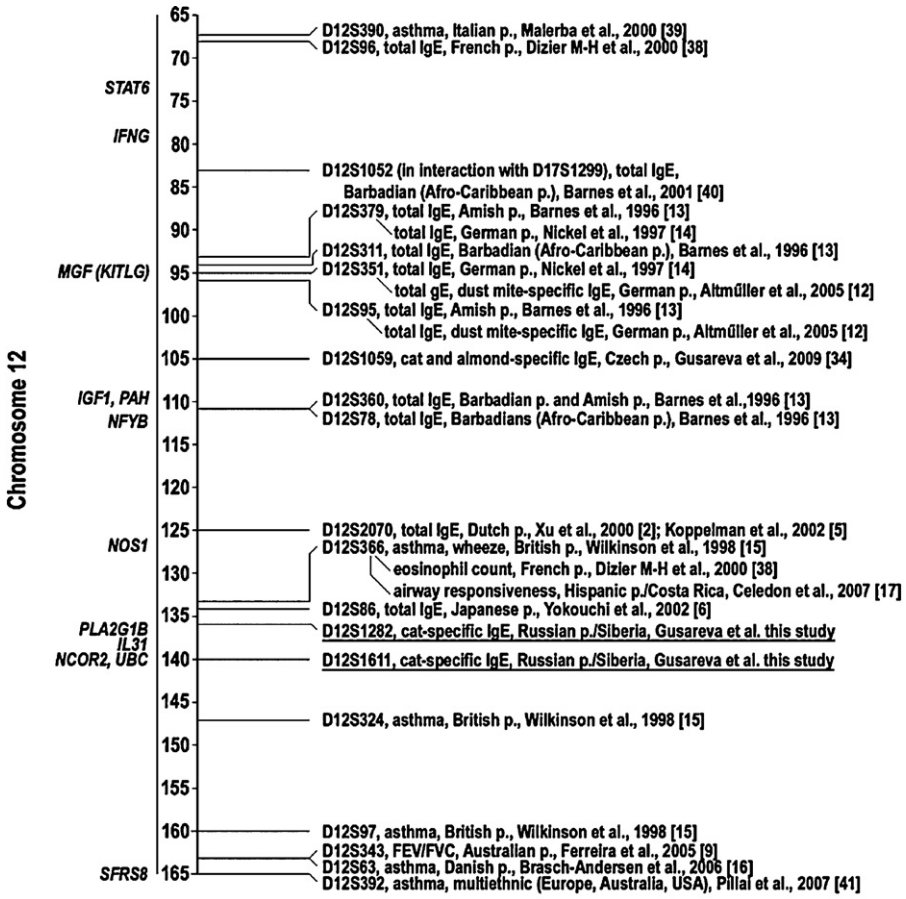


**Fig. 2.** Results of linkage analysis of chromosomal region 12q13-q24.3 for asthma, total and specific IgE, and spirometric indices in different human populations. Candidate genes in the locus 12q are shown: *STAT6* – signal transducer and activator of transcription 6, *IFNG* – interferon gamma, *MGF* (*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.

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

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