J Mol Med (2005) 83: 700–710
DOI 10.1007/s00109-005-0660-6

ORIGINAL ARTICLE

G. F. Korytina · D. G. Yanbaeva · L. I. Babenkova · E. I. Etkina · T. V. Victorova

Genetic polymorphisms in the cytochromes P-450 (1A1, 2E1), microsomal epoxide hydrolase and glutathione *S*-transferase M1, T1, and P1 genes, and their relationship with chronic bronchitis and relapsing pneumonia in children

Received: 12 October 2004 / Accepted: 28 February 2005 / Published online: 1 June 2005

Abstract The purpose of this study was to investigate the possible roles of the genes functioning in xenobiotic metabolism and antioxidant pathways in the development of severe chronic lung disease in children. Polymorphisms in the genes encoding CYP1A1, CYP2E1, EPHX1, GSTM1, GSTT1, and GSTP1 were investigated in cases of Tatar children with chronic bronchitis (n=129) and relapsing pneumonia (n=50) and in cases of ethnically matched healthy individuals (n=227) living in the city of Ufa, the Republic of Bashkortostan (South Ural region of Russia), by polymerase chain reaction–restriction fragment length polymorphism (PCR-RLFP) method. The frequency of the **2C* allele of the *CYP1A1* gene was significantly higher in patients than in the healthy control group (χ^2= 15.02, P=0.0007, P_{cor}=0.0021). This allele was associated with a higher risk of chronic bronchitis in children (OR 4.14, 95% CI 1.83–9.53; P_{cor}=0.0024). Similar results were obtained in cases of patients with relapsing pneumonia (OR 3.86, 95% CI 1.34–10.95; P_{cor}=0.027 for the **2C* allele of the *CYP1A1* gene). The frequency of the **5B* allele of the *CYP2E1* gene was higher in the relapsing pneumonia patients (7.0 vs 1.98% in the control group; χ^2=5.68, P=0.018, P_{cor}=0.054; OR 3.72, 95% CI 1.21–11.24). The increase in the *GSTT1* gene deletion was significant only in cases of chronic bronchitis (39.53 compared to 21.15% in the control group; χ^2=12.96, P=0.001, P_{cor}=0.003; OR 2.44, 95% CI 1.48–4.04). Our results show that the presence of the *GSTM1* gene deletion is unfavorable for the development of chronic lung disease in females (χ^2=9.57; P=0.0029, P_{cor}=0.0116) and was associated with increased risk (OR 2.44, 95% CI 1.36–4.38). The distribution of *EPHX1* and *GSTP1* gene genotypes was similar in the

G. F. Korytina (✉) · D. G. Yanbaeva · T. V. Victorova
Institute of Biochemistry and Genetics,
Ufa Scientific Center,
Pr. Oktyabrya, 69,
Ufa, 450054, Russia
e-mail: Guly_Kory@mail.ru
Tel.: +7-3472-355255
Fax: +7-3472-356088

L. I. Babenkova · E. I. Etkina
Department of Pediatrics,
Bashkortostan State Medical University,
Lenina st., 3,
Ufa, 450000, Russia

T. V. Victorova
Department of Biology,
Bashkortostan State Medical University,
Lenina st., 3,
Ufa, 450000, Russia

Gulnaz F. Korytina received her Ph.D. in genetics at the Institute of Biochemistry and Genetics at the Ufa Scientific Center, Russia. She is presently senior research scientist at the Laboratory of Human Ecological Genetics at the Institute of Biochemistry and Genetics. Her research interests include the molecular genetics of complex respiratory diseases (chronic obstructive pulmonary disease, lung diseases in children) and the genetic basis of cystic fibrosis.

Tatyana V. Victorova received her Dr. Sci. degree in genetics at the Federal Medical Genetic Centre in Moscow, Russia. She is presently chief of the Laboratory of Human Ecological Genetics at the Institute of Biochemistry and Genetics and Head of the Biology Department at the Medical University, Ufa, Russia. Her research interests include the genetics of complex diseases and identification of gene-environmental interaction for occupation diseases, chronic respiratory diseases, blood cancer, and reproductive disorders.

control and patient groups. Our findings indicate that the polymorphisms of the *CYP1A1*, *CYP2E1*, and *GSTT1* genes probably play a substantial part in susceptibility to severe airway and lung injury in cases of children with chronic bronchitis and relapsing pneumonia.

Keywords Chronic bronchitis · Relapsing pneumonia · Oxidative stress · Cytochrome P-450 · Glutathione *S*-transferases

Abbreviations CYP1A1: cytochrome P-4501A1 · CYP2E1: cytochrome P-4502E1 · GSTM1: Glutathione *S*-transferase · GSTT1: Glutathione S-transferase T1 · GSTP1: Glutathione *S*-transferase · EPHX1: Microsomal epoxide hydrolase

Introduction

Chronic respiratory diseases are increasingly becoming widespread throughout the world because of anthropogenic environmental pollution. Note that environmental pollution promotes predisposition to respiratory system diseases in children [1]. Respiratory diseases are the most frequent pathology of childhood. Benign respiratory diseases are usually followed by complete recovery, but still, 20% of children develop chronic lung disease, chronic bronchitis, and relapsing pneumonia [1, 2]. Chronic lung disease is a chronic inflammatory nonspecific process, having in its basis irreversible morphological changes accompanied by relapses of inflammation in the lungs and airway. In chronic bronchitis, airway obstruction results from chronic and excessive secretion of abnormal airway mucus, inflammation, bronchospasm, and infections. Chronic bronchitis lasts for a long period and recurs over several years [3]. Pneumonia, or inflammation of the lungs, is the most common type of infectious disease of the lung. Infectious pneumonias are usually identified by naming the cause of the infection or the pattern of the infection in the respiratory tract. Irreversible morphological changes, accompanied by relapses of inflammation in the lungs and airways, are typical in cases of relapsing pneumonia [1–3].

Children with chronic lung disease develop chronic respiratory disorders at a mature age because respiratory infections in childhood impair the lungs' functioning and increase the risks in adulthood of acute symptoms from exacerbations of asthma or of chronic disease such as chronic obstructive pulmonary disease and disseminated bronchiectasis [1–5]. An understanding of the true relationships between respiratory infections in early life and lung diseases in adult life will allow a better understanding of the disease mechanisms underlying some adult lung diseases [5].

Among the numerous factors implicated in airway damage, heredity plays an important role [6]. Recently, the role of genetic factors in chronic respiratory diseases has been extensively studied [6–18]. Because the lungs and other respiratory organs are at the interface of the internal and external environment of the body, they are permanently affected by adverse atmospheric air-polluting agents [1, 9]. The complex system of coordinated defense mechanisms counteracts the effect of various pollutants of the inhaled air. This system includes the metabolism of toxic products and detoxification. There exists published evidence that patients homozygous for abnormal alleles of genes encoding enzymes involved in the biotransformation of xenobiotics and endobiotics are extremely sensitive to airway damage [8, 10, 12, 15, 17, 18]. Recent data suggest that free radical oxidation plays the key role in the pathogenesis of many pulmonary diseases. Oxidative stress causes various types of damage to almost all lung structures; the degree of the damage depends on the antioxidant system activity [7, 11].

The cytochrome P-450 system, which is also known as the monooxygenase system, is located mostly in membranes of the endoplasmic reticulum [15, 19, 20]. Members of the cytochrome P-450 superfamily of hemoproteins catalyze the oxidative metabolism of exogenous chemicals such as drugs, carcinogens, and toxins, as well as endogenous substances such as steroids, fatty acids, and vitamins [19, 21]. Some CYPs activate their substrates into carcinogenic, mutagenic, and reactive intermediates that are significantly more toxic than their parent compounds and therefore may initiate lung injury [15, 19, 21].

The *CYP1A1* gene belongs to the CYP1 subfamily and encodes for the enzyme aryl hydrocarbon hydroxylase (AHH), which catalyzes the first step in the metabolism of polycyclic aromatic hydrocarbons such as those found in cigarette smoke, transforming them into carcinogens. The *CYP1A1* gene is located in chromosome 15, band 15q22–24 [22], and various patterns in restriction fragment length polymorphism (RFLP) for this gene have been reported. An *Msp*I RFLP (3801 T→C) was identified in the 3′-flanking region of the *CYP1A1* gene [23]. This mutation determines three different genotypes, called **1A*1A*, which are homozygotes for the wild-type allele and do not have the restriction site for *Msp*I, **1A*2A*, and **2A*2A*, which are, respectively, the heterozygotes and the homozygotes for the mutant allele and which have the site for *Msp*I. A second point mutation, a transversion in position 2455 A→G in exon 7 in the heme-binding region, is in complete linkage disequilibrium in Caucasians with the CYP1A 3801 T→C mutation. 2455 A→G mutation leads to an isoleucine/valine substitution and is known as Ile462Val or exon 7 polymorphism [23]. The **1A*1A* genotype corresponds to the wild type, and **1A*2C* and **2C*2C* to the heterozygous and homozygous genotypes for the mutant allele, respectively [23]. Its function has still not been completely defined and may depend on its link to the *Msp*I polymorphism or to other polymorphisms, for example, in the regulatory region important for *CYP1A1* inducibility that can affect *CYP1A1* transcription levels such as polymorphisms for promoter genes, *AHR* (Ah receptor) genes, or other metabolic genes [24].

The *CYP2E1* gene encodes the enzyme that catalyzes the oxidation of many low molecular weight procarcinogens like benzene, styrene, and the nitrosamines [19, 25]. This enzyme is also involved in the metabolism of ethanol and

acetone and can be induced by isoniazide and ethanol [25]. It is present in various tissues, including the brain and lung, but its greatest expression occurs in the liver. *CYP2E1* has a clear toxicological role because it activates procarcinogens, organic solvents, and drugs, converting them into cytotoxic or carcinogenic products and potentiating the toxicity of solvents and drugs. The *CYP2E1* gene was mapped in region 10q24.3 of chromosome 10 [27, 28]. Restriction fragment length polymorphism analysis revealed detectable polymorphisms through endonucleases *Taq*I, *Dra*I, *Rsa*I, *Xmn*I, and *Msp*I, and two principal sites were studied in correlation with diseases. These sites are located in region 5′ (−1293 G→C), in which RFLP analysis revealed alleles **1A* and **5B*. The wild allele **1A* presents a restriction site for *Rsa*I. Allele **5B* results from a mutation, losing the restriction site to *Rsa*I and creating a site for the enzyme *Pst*I (1053 C→T) [26]. These sites are in linkage disequilibrium. Interest in these sites stemmed from observations on the possible association with the induction of the gene. The polymorphisms affect its link to a transcription factor, altering its transcriptional regulation and leading to interindividual differences in the microsomal oxidation activity for drugs and other xenobiotics [26]. The **5B* allele form binds the transcription factors to the mutation region less efficiently, suggesting that the gene is less expressed in vivo in individuals carrying this allele [26].

Microsomal epoxide hydrolase (EPHX1) catalyzes the hydrolysis of xenobiotic epoxides [benzene, benzo(*a*)pyrene, etc.] into diols and thereby plays an important role in protecting the lungs from highly reactive epoxide derivatives [19]. Combinations of *EPHX1* mutations result in several phenotypes differing in enzyme activity [12].

Glutathione *S*-transferases form a multigenic family of enzymes that detoxify a large number of electrophilic xenobiotics via conjugation with glutathione. This is the primary method of protection against chemical toxins and carcinogens (xenobiotics). Glutathione *S*-transferases are expressed in various tissues; the highest level of expression is characteristic of the liver and lungs [19]. Glutathione *S*-transferase genes encode for five families of cytosolic enzymes: glutathione *S*-transferases alpha (GSTA), mu (GSTM), pi (GSTP), theta (GSTT), and sigma (GSTS). They may also function as disease-modifying enzymes when oxidative stress is contributory [10, 14, 17]. Apart from detoxification, glutathione *S*-transferases perform other functions such as isomerization of steroids and prostaglandins and participation in the biosynthesis and metabolism of leukotriene C4 and prostaglandin E2, which are anti-inflammatory mediators of chemotactic effect on neutrophils [15, 19]. Therefore, changes in glutathione *S*-transferase activity may influence the inflammatory process in bronchial and lung systems at various stages of its development. An extended deletion accounts for the *GSTM1* and *GSTT1* gene polymorphism [15]. This mutation leads to the lack of synthesis of the corresponding protein products. Numerous data testify to an enhanced risk of some diseases associated with the *GSTM1* and *GSTT1* null genotype [5, 8, 11, 12, 15, 19]. Two diallelic polymorphisms of the *GSTP1* gene are described: Ile105Val (exon 5) and Ala114Val (exon 6) [14, 17]. When isoleucine is substituted by valine in position 105 of *GSTP1* gene, the enzyme catalytic activity, with respect to polycyclic aromatic compounds, is increased sevenfold, although it is reduced threefold with respect to 1-chloro-2,4-dinitrobenzene [17].

We hypothesized that polymorphic variants of the genes functioning in biotransformation and antioxidant pathways that modulate oxidative stress are associated with chronic bronchitis and relapsing pneumonia in children.

Therefore, we studied the frequencies of the genetic polymorphisms of the *CYP1A1*, *CYP2E1*, *EPHX1*, *GSTM1*, *GSTT1*, and *GSTP1* genes in cases of children with chronic lung disease and in healthy subjects to determine whether multiple polymorphisms of these genes are linked to the development of chronic bronchitis and relapsing pneumonia of children.

Materials and methods

Patients and controls

The study group consisted of 179 children with chronic bronchitis (n=129) and relapsing pneumonia (n=50) recruited from the Republican Children's Hospital (Ufa) (see Table 1). All of the patients' chronic bronchitis and relapsing pneumonia were diagnosed by the hospital specialists on the basis of the medical histories and the results of general, clinical, and special tests (chest X-ray, spirometry measures, and fibrobronchoscopy), physical examination, and laboratory approaches.

Children with a diagnosis of chronic bronchitis had disseminated defeats of bronchi accompanied by productive cough (more than 3 months a year), constant heterogeneous wheezes in lungs (during several months) in the presence of two or three exacerbations a year in the course of 2 years [J41, according to the *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision* (ICD-10)] [29].

Irreversible morphological changes (deformation of bronchi and pneumosclerosis in one or several segments of the lung) accompanied by relapses of inflammation in the lung

Table 1 Characteristics of the study groups

	Diseased subjects			Healthy subjects
	All patients	Chronic bronchitis	Relapsing pneumonia	
Sex				
Female	99 (55.3%)	66 (51.2%)	33 (66.0%)	133 (58.6%)
Male	80 (44.7%)	63 (48.8%)	17 (34.0%)	94 (41.4%)
Age (years) ±SD	11.7±2.1	10.6±0.4	11.4±1.7	12.5±1.3
Ethnicity	White, Tatar	White, Tatar	White, Tatar	White, Tatar
Total no. of cases	179	129	50	227

tissue and airways (three or four exacerbations in a year) are revealed in cases with patients having frequently recurring (relapsing) pneumonia (J18, according to the ICD-10) [29]. The duration of disease in cases of children with chronic bronchitis and relapsing pneumonia was from 5 to 11 years.

Subjects were excluded if they had a history of asthma (according to the GINA project) [4], atopy (skin-prick testing and a high level of total serum IgE), cystic fibrosis [based on clinical features, a positive result for sweat test (CI >60 mmol/L), and detection of *CFTR* gene mutations], and tuberculosis (based on clinical features and a positive result for the test for *M. tuberculosis*).

In the control group, DNA samples from 227 unrelated, healthy, ethnically age- and sex-matched individuals inhabiting Ufa were used. All were subjects who visited Republican Children's Hospital for medical examination. These people had no respiratory or other chronic diseases in their medical records. The Ethics Committee of Ufa approved the study, and all subjects gave their informed consent.

PCR and PCR-RLFP analysis

Genomic DNA was isolated from peripheral blood lymphocytes by phenol–chloroform extraction [30]. Polymerase chain reaction (PCR) with *Thermus aquaticus* DNA polymerase (SibEnzym, Russia) was run on a DNA-Technology thermal cycler. The reaction mixture (30 μl) contained 67 mM Tris–HCl (pH 8.8), 16.6 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.01% Tween 20, 0.1 mg of genomic DNA, 200 μM each dNTP, 1 unit of Taq DNA polymerase, and 10 pM each primer specific for *CYP1A1* [15], *CYP2E1* [25], *EPHX1* [12], *GSTM1* [15], *GSTT1* [15], and *GSTP1* [17] (see Table 2). The cycling condition is given in Table 2. Polymorphic alleles were identified by restriction enzyme analysis. Point mutation 2455 A→G, which arises in *CYP1A1* exon 7 and results in substitution Ile462Val, was detected by a gain of a *Hin*cII site [15, 31]. Point mutation −1053 C→T in the 5′ region of *CYP2E1* was detected by a gain of a *Pst*I site [25, 31]. The *EPHX1* gene was tested for mutations 337 T→C (Tyr113His) and 451 A→C (His139Arg), which correlate with the EPHX1 enzymic activity. Substitution Tyr113His is detectable by a loss of the *Eco*RV [12] site and decreases the enzymic activity of the protein by 50% in homozygotes His/His and by 25% in heterozygotes His/Tyr. Substitution His139Arg is detectable by a gain of an *Rsa*I site [12] and increases the enzymic activity of the protein by 25% in homozygotes Arg/Arg. Combinations of *EPHX1* mutations result in several phenotypes differing in enzyme activity: a "fast" phenotype corresponds to homozygosity or heterozygosity for exon 4 mutation and the absence of exon 3 mutation; a "slow" phenotype, to heterozygosity for exon 3 mutation and the absence of exon 4 mutation; a "very slow" phenotype, to homozygosity for exon 3 mutation and the absence of exon 4 mutation; and a "normal" phenotype, to

Table 2 Sequences of the amplification primers and PCR–RFLP conditions

No.	Polymorphic state/gene	Primer sequences, 5′→3′	Method/ restriction enzyme	Genotype PCR–RFLP product (bp)	Annealing temperature (°C)	References
1	2455 A→G (Ile462Val)/ *CYP1A1*	GAACTGCCACTTCAGCTGTCT GAAAGACCTCCCAGCGGTCA	PCR–RFLP/ *Hin*cII	**1A*1A* (144+48) **1A*2C* (144+125+ 48+19) **2C*2C* (125+48+ 19)	55	[15]
2	−1053 C→T/ *CYP2E1*	CAGTCGAGTCTACATTGTC TTCATTCTGTCTTCTAACTG	PCR–RFLP/ *Pst*I	**1A*1A* (410) **1A*2B* (410+290+ 120) **2B*2B* (290+120)	54	[25]
3	T337C/*EPHX1*	GATCGATAAGTTCCGTTT CACC ATCCTTAGTCTTGAAGTGAGGAT	PCR–RFLP/ *Eco*RV	*TT* (140+22) *TC* (162+140+22) *CC* (162)	53	[12]
4	A415G/*EPHX1*	ACATCCACTTCATCCACG T ATGCCTCTGAGAAGCCAT	PCR–RFLP/ *Rsa*I	*AA* (164+46) *AG* (210+164+46) *GG* (210)	57	[12]
5	Del/*GSTM1*	GAACTCCCTGAAAAGCTAAAGC GTTGGGCTCAAATATACGGTGG	PCR	*N* (271) *Del* (0)	55	[15]
6	Del/*GSTT1*	TTCCTTACTGGTCCTCACATCTC TCACCGGATCATGGCCAG CA	PCR	*N* (480) *Del* (0)	59	[15]
7	A313G/*GSTP1*	ACCCCAGGGCTCTATGGGAA TGAGGGCACAAGAAGCCCCT	PCR–RFLP/ *Bso*MAI	*AA* (176) *AG* (176+91+85) *GG* (91+85)	54	[17]

the absence of both mutations or to heterozygosity for both mutations [12].

GSTM1 and *GSTT1* gene fragments were amplified using primers described in [15]. Homozygous and heterozygous carriers of normal alleles were determined due to the presence of an amplification product on electropherograms, whereas the lack of this fragment indicated a deletion in the homozygous state [15]. Amplification with corresponding primers of a fragment in *CYP1A1* gene exon 7 was used as the internal control. To determine 313 A→G (Ile105Val) polymorphism in the *GSTP1* gene, the product obtained with primers described in [17] was treated with *Bso*MAI enzyme (SibEnzym).

The amplified fragments were digested with restriction enzymes obtained from SibEnzym under standard conditions. The amplificate (5 ml) was supplemented with 5 units of an enzyme and incubated at 37 or 55°C for no less than 12 h. The results of amplification and restriction were evaluated using vertical electrophoresis in 6–8% polyacrylamide gel in 1×TBE buffer at 200–300 V. Gels were stained with 0.1 mg/ml ethidium bromide for 15 min and photographed in transmitted UV light.

Statistical analysis

Estimation of the allelic and genotype frequencies was carried out using standard procedures as implemented in the BIOSYS-2 software program [32]. The observed genotype frequencies were compared with the expected Hardy-Weinberg distributions by χ^2 analyses using the BIOSYS-2 software program [32].

Differences in allele or genotype frequency between patients and controls were tested for significance by the χ^2 test with Yates correction. P values of <0.05 were considered statistically significant. To avoid type I errors, multiple comparisons were corrected by multiplying the statistically significant P values by the number of pairwise comparisons being made. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to assess the relative disease risk conferred by a particular allele and genotype [33]. Statistical analysis was carried out with the Statistica v. 6.0 program (StatSoft Inc., USA) [34].

Results

Hardy–Weinberg equilibrium was tested for all polymorphisms, and no obvious deviations were found in patients and controls. First, we compared the frequencies of one particular allele or genotype in all patients with the healthy controls. Then, we tested the differences of allele or genotype frequency between chronic bronchitis patient controls and relapsing pneumonia patient controls. Table 3 shows the distribution of *CYP1A1*, *CYP2E1*, and *EPHX1* genotypes and alleles in healthy controls and in the patients.

The CYP1A1 gene polymorphism

The frequencies of the **1A*2C* genotype and the **2C* allele of the *CYP1A1* gene were significantly higher in diseased subjects than in healthy controls (χ^2=14.38, P=0.0008, P_{cor}=0.0024 and χ^2=15.02, P=0.0007, P_{cor}= 0.0021, respectively).

This genotype and allele were found with significantly high frequencies in chronic bronchitis patients: 15.5 vs 3.52% in the control group for the **1A*2C* genotype (χ^2=14.68, P=0.0007, P_{cor}=0.0021) and 8.53 vs 2.2% in the control group for the **2C* allele (χ^2=13.89, P=0.0008, P_{cor}=0.0024), and were associated with a higher risk of this airway disease in children (OR 5.02, 95% CI 2.01–12.87 and OR 4.14, 95% CI 1.83–9.53, respectively).

Similar results were obtained in cases of relapsing pneumonia. The frequency of the **1A*2C* genotype in patients with relapsing pneumonia increased to 12.0% compared to those in the healthy control group (3.52%) (χ^2=4.68, P=0.031, P_{cor}=0.093; OR 3.73, 95% CI 1.08–12.63), and the frequency of the **2C* allele in patients with relapsing pneumonia was 8.0 vs 2.2% in the control group (χ^2=7.02, P=0.009, P_{cor}=0.027; OR 3.86, 95% CI 1.34–10.95).

The *CYP2E1* gene polymorphism

For the *CYP2E1* gene, the frequency of the **5B* allele increased from 1.98 to 4.75%, comparing the healthy control group with all diseased children (χ^2=4.09, P=0.043, P_{cor}= 0.13; OR 2.46, 95% CI 1.02–6.5). However, the frequency of the **5B* allele was higher only in the patients with relapsing pneumonia (7.0 vs 1.98% in the control group, χ^2=5.68, P=0.018, P_{cor}=0.054; OR 3.72, 95% CI 1.21–11.24), and there were no differences in the frequency of the **5B* allele of the *CYP2E1* gene between patients with chronic bronchitis and the healthy controls (χ^2=1.64, P=0.2).

The *EPHX1* gene polymorphisms

We did not find any significant differences in the genotype and allele distributions of the 337 T→C and 451 A→C polymorphisms of the *EPHX1* gene between patients and healthy controls (χ^2=0.56, P=0.46; χ^2=0.99, P=0.36, respectively). The proportion of individuals with T allele of the 337 T→C polymorphism is increased to 90.0% only in the group of patients with relapsing pneumonia compared with those in the control group (80.62%) (χ^2=4.33, P=0.037, P_{cor}=0.11). Table 4 shows the distribution of the *GSTM1*, *GSTT1*, and *GSTP1* genotypes in the patients and healthy controls.

Table 3 Allele and genotype frequencies of the *CYP1A1*, *CYP2E1*, and *EPHX1* genes polymorphisms in diseased and healthy subjects

Genotype and allele	Diseased subjects						Healthy subjects (*n*=227)	
	All patients (*n*=179)		Chronic bronchitis (*n*=129)		Relapsing pneumonia (*n*=50)		No.	%
	No.	%	No.	%	No.	%		
CYP1A1 gene (2455 A→G)								
**1A*1A*	151	84.36	108	83.72	43	86.0	218	96.04
**1A*2C*	26	14.53[a]	20	15.5[b]	6	12.0[c]	8	3.52
**2C*2C*	2	1.12	1	0.78	1	2.0	1	0.44
**1A*	328	91.62	236	91.47	92	92.0	444	97.8
**2C*	30	8.38[d]	22	8.53[e]	8	8.0[f]	10	2.2
CYP2E1 gene (−1053 C→T)								
**1A*1A*	162	90.5	119	92.25	43	86.0	218	96.04
**1A*5B*	17	9.5	10	7.75	7	14.0	9	3.96
**5B*5B*	0	0	0	0	0	0	0	0
**1A*	341	95.25	248	96.12	93	93.0	445	98.02
**5B*	17	4.75	10	3.88	7	7.0[g]	9	1.98
337 T→C *EPHX1* gene (Tyr113His)								
TT	119	66.48	79	61.24	40	80.0	142	62.56
TC	58	32.4	48	37.21	10	20.0	82	36.12
CC	2	1.12	2	1.55	0	0	3	1.32
T	296	82.68	206	79.84	90	90.0	366	80.62
C	62	17.32	52	20.16	10	10.0	88	19.38
415 A→G *EPHX1* gene (His139Arg)								
AA	132	73.74	92	71.32	40	80.0	175	77.09
AG	37	20.67	29	22.48	8	16.0	43	18.94
GG	10	5.59	8	6.20	2	4.0	9	3.96
A	301	84.08	213	82.56	88	88.0	393	86.56
G	57	15.92	45	17.44	12	12.0	61	13.44
Phenotype *EPHX1*								
Normal (N)	110	61.45	76	58.91	34	68.0	129	56.83
Fast (F)	28	15.64	20	15.50	8	16.0	33	14.54
Slow (S)	39	21.79	31	24.04	8	16.0	62	27.31
Very slow (SS)	2	1.12	2	1.55	0	0	3	1.32

[a] Diseased subjects vs healthy controls (χ^2=14.38, P=0.0008, P_{cor}=0.0024)
[b] Chronic bronchitis patients vs controls (χ^2=14.68, P=0.0007, P_{cor}=0.0021)
[c] Relapsing pneumonia vs controls (χ^2=4.68, P=0.031, P_{cor}=0.093)
[d] Diseased subjects vs healthy controls (χ^2=15.02, P=0.0007, P_{cor}=0.0021)
[e] Chronic bronchitis patients vs controls (χ^2=13.89, P=0.0008, P_{cor}=0.0024)
[f] Relapsing pneumonia vs controls (χ^2=7.02, P=0.009, P_{cor}=0.027)
[g] Relapsing pneumonia vs controls (χ^2=5.68, P=0.018, P_{cor}=0.054)

The *GSTM1* gene polymorphism

The frequency of the *GSTM1* gene deletion genotype was higher in the patients (46.37%) compared to controls (36.12%) but without statistical significance (χ^2=3.94, P=0.047, P_{cor}=0.14). Analyzing children with relapsing pneumonia, we found that the frequency of the *GSTM1* gene deletion was rather high (52.0 vs 36.12% in the healthy controls; χ^2=3.7, P=0.054, P_{cor}=0.162).

The *GSTT1* gene polymorphism

Patients showed significantly elevated frequencies of the *GSTT1* gene deletion (36.31 vs 21.15% in controls; χ^2=10.72, P=0.0019, P_{cor}=0.0057; OR 2.13, 95% CI 1.34–3.39). The increase in the *GSTT1* gene deletion was significant only in the case of chronic bronchitis (39.53 compared to 21.15% in controls; χ^2=12.96, P=0.001, P_{cor}=0.003). Patients with relapsing pneumonia did not differ significantly in deletion genotype frequency from the control group (χ^2=0.75, P=0.39). Thus, the *GSTT1* deletion genotype was associated with a more than twofold increased risk of developing chronic bronchitis in children (OR 2.44, 95% CI 1.48–4.04).

The GSTP1 gene polymorphism

The distribution of the *GSTP1* gene alleles did not significantly differ among patients and controls (χ^2=0.87, P=0.35). However, there was a trend toward a higher fre-

Table 4 Allele and genotype frequencies of the *GSTM1*, *GSTT1*, and *GSTP1* genes polymorphisms in diseased and healthy subjects

Genotype and allele	Diseased subjects						Healthy subjects (*n*=227)	
	All patients (*n*=179)		Chronic bronchitis (*n*=129)		Relapsing pneumonia (*n*=50)		No.	%
	No.	%	No.	%	No.	%		
GSTM1 gene								
Normal (n)	96	53.63	72	55.81	24	48.0	145	63.88
Deletion (del)	83	46.37	57	44.19	26	52.0	82	36.12
GSTT1 gene								
Normal (n)	114	63.69	78	60.47	36	72.0	179	78.85
Deletion (del)	65	36.31[a]	51	39.53[b]	14	28.0	48	21.15
313 A→G *GSTP1* gene (Ile105Val)								
AA	137	76.54	95	73.64	42	84.0	157	69.16
AG	35	19.55	27	20.93	8	16.0	66	29.07
GG	7	3.91	7	5.43	0	0	4	1.76
A	309	86.31	217	84.11	92	92.0	380	83.7
G	49	13.69	41	15.89	8	8.0	74	16.3

[a]Diseased subjects vs healthy controls (χ^2=10.72, P=0.0019, P_{cor}=0.0057)
[b]Chronic bronchitis patients vs controls (χ^2=12.96, P=0.001, P_{cor}=0.003)

Table 5 Allele and genotype frequencies of the *CYP1A1*, *CYP2E1*, and *EPHX1* gene polymorphisms in female and male subjects

Genotype and allele	Diseased subjects, no. (%)		Healthy subjects, no. (%)	
	F (*n*=99)	M (*n*=80)	F (*n*=133)	M (*n*=94)
CYP1A1 gene (2455 A→G)				
**1A*1A*	82 (82.83)	69 (86.25)	127 (95.49)	91 (96.81)
**1A*2C*	15 (15.15)	11 (13.75)	6 (4.51)	2 (2.13)
**2C*2C*	2 (2.02)	0 (0)	0	1 (1.06)
**1A*	179 (90.4)	149 (93.13)	260 (97.74)	184 (97.87)
**2C*	19 (9.6)[a]	11 (6.88)	6 (2.26)	4 (2.13)
CYP2E1 gene (−1053 C→T)				
**1A*1A*	87 (87.88)	74 (92.5)	126 (94.74)	92 (97.87)
**1A*5B*	12 (12.12)	6 (7.5)	7 (5.26)	2 (2.13)
**5B*5B*	0	0	0	0
**1A*	186 (93.9)	154 (96.25)	259 (97.4)	186 (98.9)
**5B*	12 (6.1)	6 (3.75)	7 (2.6)	2 (1.1)
337 T→C *EPHX1* gene (Tyr113His)				
TT	68 (68.69)	51 (63.75)	85 (63.91)	57 (60.64)
TC	30 (30.3)	28 (35.0)	46 (34.59)	36 (38.3)
CC	1 (1.01)	1 (1.25)	2 (1.5)	1 (1.06)
T	166 (83.8)	130 (81.3)	216 (81.2)	150 (79.8)
C	32 (16.2)	30 (18.7)	50 (18.8)	38 (20.2)
415 A→G *EPHX1* gene (His139Arg)				
AA	72 (72.73)	60 (75.0)	109 (81.95)	66 (70.21)
AG	21 (21.21)	16 (20.0)	19 (14.29)	24 (25.53)
GG	6 (6.06)	4 (5.0)	5 (3.76)	4 (4.26)
A	165 (83.3)	136 (85.0)	237 (89.1)	156 (83.0)
G	33 (16.7)	24 (15.0)	29 (10.9)	32 (17.0)
Phenotype EPHX1				
Normal (N)	61 (61.6)	49 (61.25)	74 (55.64)	55 (58.51)
Fast (F)	17 (17.2)	11 (13.75)	18 (13.54)	15 (15.96)
Slow (S)	20 (20.2)	19 (23.75)	39 (29.32)	23 (24.47)
Very slow (SS)	1 (1.0)	1 (1.25)	2 (1.5)	1 (1.06)

[a]Diseased vs healthy females (χ^2=10.6, P=0.002, and P_{cor}=0.008)

Table 6 Allele and genotype frequencies of the *GSTM1*, *GSTT1*, and *GSTP1* genes polymorphisms in female and male subjects

Genotype and allele	Diseased subjects, no. (%)		Healthy subjects, no. (%)	
	F (*n*=99)	M (*n*=80)	F (*n*=133)	M (*n*=94)
GSTM1 gene				
Normal (n)	51 (51.52)	45 (56.25)	96 (72.18)	49 (52.13)
Deletion (del)	48 (48.48)[a]	35 (43.75)	37 (27.82)	45 (47.87)[b]
GSTT1 gene				
Normal (n)	69 (69.7)	45 (56.25)	109 (81.95)	70 (74.47)
Deletion (del)	30 (30.3)	35 (43.75)	24 (18.05)	24 (25.53)
313 A→G *GSTP1* gene (Ile105Val)				
AA	79 (79.8)	58 (72.5)	85 (63.9)	72 (76.6)
AG	17 (17.17)	18 (22.5)	45 (33.8)	21 (22.34)
GG	3 (3.03)	4 (5.0)	3 (2.3)	1 (1.06)
A	175 (88.38)	134 (83.75)	215 (80.8)	165 (87.8)
G	23 (11.62)	26 (16.25)	51 (19.2)	23 (12.2)

[a]Diseased vs healthy females (χ^2=9.57, *P*=0.0029, P_{cor}=0.0116)
[b]Male vs female controls (χ^2=9.59, *P*=0.003, P_{cor}=0.012)

quency of the A allele in the relapsing pneumonia group than in healthy controls (92.0 vs 83.7%; χ^2=3.84, *P*=0.05, P_{cor}=0.15). The *GSTP1* gene *GG* genotype is very uncommon among Tatars from Ufa, and in the present study only seven individuals with this genotype were found in the patients and four individuals in healthy subjects.

Analysis of the distribution of CYP1A1, *CYP2E1*, *EPHX1*, *GSTM1*, *GSTT1*, and GSTP1 genotypes and alleles in patients and healthy subjects according to gender

The number of the **2C* allele of the *CYP1A1* gene was significantly higher in diseased (9.6%) than in healthy females (2.26%) (χ^2=10.6, *P*=0.002, P_{cor}=0.008) and were associated with a higher risk of chronic lung disease in female patients (OR 4.6, 95% CI 1.69–13.14) (see Table 5). The frequency of the **2C* allele of the *CYP1A1* gene was also increased in male patients (6.88 vs 2.13% in healthy males) but without statistical significance (χ^2=3.6, P=0.056 compared to healthy males). At the same time, we did not find any gender differences in the genotype and allele distributions of the *CYP1A1* gene within the patients and controls.

The *GSTM1* deletion genotype occurs with a significantly higher frequency in the healthy male group (47.87%) compared to healthy female subjects (27.82%) (χ^2=9.59, *P*=0.003, P_{cor}=0.012) (see Table 6). Our results show that the presence of the *GSTM1* gene deletion is unfavorable for the development of chronic lung disease in females (48.8 in diseased girls compared to 27.82% in healthy girls; χ^2=9.57, *P*=0.0029, P_{cor}=0.0116) and was associated with an increased risk (OR 2.44, 95% CI 1.36–4.38). There were no significant gender differences in the frequency of the *CYP2E1*, *EPHX1*, *GSTT1*, and *GSTP1* gene polymorphisms (Tables 5 and 6).

Discussion

In this work, we studied the link between polymorphisms at *CYP1A1*, *CYP2E1*, *EPHX1*, *GSTM1*, *GSTT1*, and *GSTP1* gene loci and susceptibility to chronic bronchitis and relapsing pneumonia in cases of Tatar children from Ufa and identified the alleles and genotypes that were associated with a higher risk of childhood chronic bronchitis and relapsing pneumonia.

We focused this study on *CYP*, *EPXH1*, and *GST* genotypes because these genes are expressed in the lung, are involved in the biotransformation of xenobiotics and endobiotics and in antioxidant defense pathways, and have common functional variant alleles [12–17, 19, 20]. As recent studies have shown, free radical oxidation plays a key role in various lung diseases [7]. The lung is exposed to a substantial burden of endogenously produced and inhaled oxidants and pro-oxidants, including O_2 and a variety of toxic aerosols. If antioxidant defenses are inadequate, substantial oxidative stress can occur that may interfere with normal lung growth and may contribute to increased incidence, prevalence, and severity of respiratory diseases such as chronic obstructive pulmonary disease, asthma, and viral infections [8, 9, 14, 16].

One of the direct consequences of oxidative stress is higher viscosity of bronchial mucus, which reduces the mucociliary clearance rate and expedites lung disease aggravation and progression [7].

Our results showed that the *EPHX1* and *GSTP1* genotypes did not influence susceptibility to chronic bronchitis and relapsing pneumonia in cases of children, whereas the **2C* allele of the *CYP1A1* gene was associated with a significant risk of chronic bronchitis and relapsing pneumonia (see Fig. 1). *CYP1A1* probably plays an appreciable role in the development of chronic lung disease or acute respiratory diseases in cases of children. An increase in the frequency of the *CYP1A1* genotype **1A*2C* has been

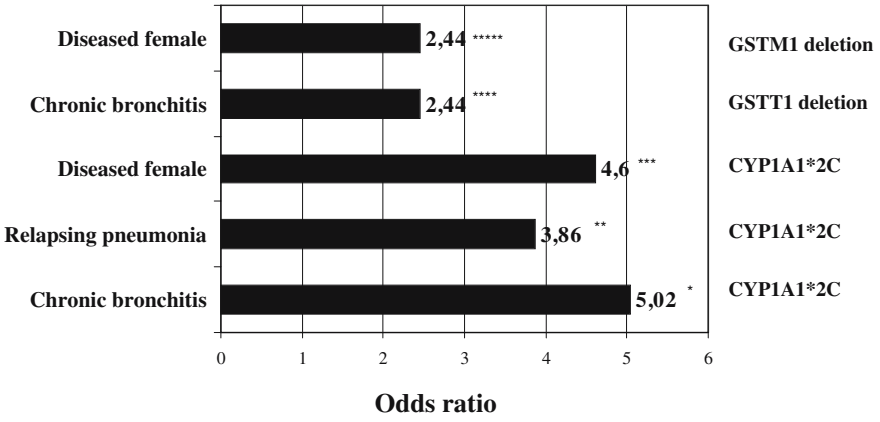

Fig. 1 Relationship between the *CYP1A1*, *GSTT1*, and *GSTM1* genotypes and risk of chronic bronchitis and relapsing pneumonia in cases of children. *, chronic bronchitis vs control subjects (95% CI 2.01–12.87; P_{cor}=0.0021); **, relapsing pneumonia vs control subjects (95% CI 1.34–10.95; P_{cor}=0.027); ***, diseased female subjects vs healthy females (95% CI 1.69–13.14; P_{cor}=0.008). ****, chronic bronchitis vs control subjects (95% CI 1.48–4.04; P_{cor}= 0.003); *****, diseased female subjects vs healthy females (95% CI 1.36–4.38; P_{cor}=0.0116)

observed in cases of patients with cystic fibrosis [35], lung cancer [18, 19], or pulmonary emphysema [20]. The phase I enzyme *CYP1A1* is known to catalyze the oxygenation of exogenous and endogenous chemicals, thereby producing certain metabolites that are highly reactive. This increases the contents of toxic metabolites, including reactive oxygen species, and leads to greater tissue damage by oxidative stress [19].

It was already reported that there was a significantly higher expression level of *CYP1A1* enzyme in the lungs of women than in men [36]. Mollerup et al. hypothesized that these sex differences can be related to circulating female steroid hormones [36].

Therefore, we tested a gender-dependent difference in the genotype and allele distribution of polymorphism at the *CYP1A1* gene. Our results showed that the number of **2C* alleles of the *CYP1A1* gene was significantly higher among female patients than in the healthy females. Analysis of *CYP2E1* gene polymorphism indicated that the frequency of the **5B* allele of *CYP2E1* was higher in the relapsing pneumonia patients. Probably, the **5B* allele of the *CYP2E1* gene plays a substantial part in predisposition to lung parenchymal injury resulting from long-term pulmonary infections in children.

The results of our study demonstrate an increase in the frequency of the *GSTM1* deletion among healthy males as compared to healthy females. However, Gilliland et al. did not find any differences in the frequency of *GSTM1* gene deletion in male and female children's groups from California, USA (The Children's Health Study) [14]. Dresler et al. also demonstrated that the distribution of the *GSTM1* gene genotypes is similar in healthy male and female controls [37]. It is very difficult to explain these findings, and various hypotheses may be suggested. It is possible that an increase in the frequency of *GSTM1* deletion genotype in healthy girls (mean age 12.5±1.3) living in Ufa is connected with selection against the deletion genotype of the *GSTM1* gene that specifically concerns female embryos in the given age group. It is known that one of the functions of glutathione *S*-transferases concerns the binding and transportation of steroid hormones, and the absence of *GSTM1* activity leads to impairments of the transportation of steroid hormones [15]. On the other hand, evidence exists for hormonal regulation of glutathione *S*-transferases in humans, with transferase levels generally being higher in females than in males [19]. However, the sample size in our study is not large, and gender differences in the genotype distribution of *GSTM1* gene polymorphism should be studied on a large sample and in different age groups.

We also found that the presence of the *GSTM1* gene deletion was associated with increased risk of chronic lung disease in females. Karagas et al. found an increased risk of bladder cancer associated with *GSTM1* null genotype only among women (OR 1.7, 95% CI 1.0–3.0) [38]. Dresler et al. found that female patients with lung cancer were significantly more likely than female controls to have both the *CYP 1A1* mutation and *GSTM1* null genotype and suggested that polymorphisms in *CYP 1A1* and *GSTM1* contribute to the increased risk of females for lung cancer [37]. Our results indicate that there is a tendency for an increased frequency of the *GSTM1* deletion genotype in patients with relapsing pneumonia.

We suggest that *GSTT1* deletion plays a role in chronic bronchitis pathogenesis in children. The *GSTT1* deletion genotype was associated with a more than twofold increased risk of developing chronic bronchitis in children from Ufa. Ivashenko et al. [15] have previously found a significant increase in the frequency of deletion in the *GSTT1* gene in cases of patients with atopic bronchial asthma. Kabesch et al. found the strongest effect on the development of childhood asthma and asthma symptoms in *GSTT1*-deficient individuals [8].

In adults cases, the *CYP1A1*, *GSTM1*, and *GSTP1* variant alleles have been associated with chronic obstructive pulmonary disease and asthma, suggesting a role for these loci in the pathogenesis of airflow limitation at older ages [10, 17, 18, 20].

In contrast to *CYP1A1*, phase II enzymes (GSTs) generally detoxify highly reactive metabolites. This is a major pathway of protection against chemical toxins and carcinogens.

Glutathione *S*-transferases are important antioxidant enzymes in the lung that function as antioxidants in xenobiotic, peroxide, and hydroperoxide metabolism pathways to reduce oxidative stress [19].

Chronic airway and lung inflammation results from long-term oxidative stress and infections. Probably, the mechanisms of central airway injury may be different from those of pulmonary parenchymal injury. Our findings indicate that polymorphisms of the *CYP1A1* and *GSTT1* genes play a substantial part in the susceptibility to severe airway injury in cases of children with chronic bronchitis, and the polymorphic variants of the *CYP1A1* and *CYP2E1* genes are associated with relapsing pneumonia in children.

In addition, the results of our study suggest that it is important to investigate the gender-dependent differences for susceptibility to complex respiratory diseases, especially in association studies of the biotransformation enzyme genes.

Because of the small number of subjects with chronic bronchitis and relapsing pneumonia investigated in the given work, it is necessary to further study the polymorphisms of the biotransformation genes within the framework of the extensive sample group of children with chronic lung disease.

Acknowledgements This work was supported by the Russian Foundation for Basic Research (grant 04-04-48318a), the Russian Scientific Foundation for Humanities (04-06-00016a) and the Russian Federation President Program (grant Мк-2755.2004). We would like to express our gratitude to the families of patients who participated in this study.

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