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HUMAN GENETICS

Analysis of Gene Complexes Predisposing to Coronary Atherosclerosis

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Abstract—The following seven polymorphic marker loci of genes responsible for predisposition to coronary atherosclerosis (CAS) were studied: the *ACE* locus responsible for angiotensin-converting enzyme insertion/deletion polymorphism for the presence or absence of the *Alu* insertion in the gene; the *F13*, *PLAT*, and *APOA1* loci, controlling the clotting factor 13, plasminogen-activating tissue factor, and apolipoprotein A, respectively; the *MTHFR* and *AGT* polymorphic loci responsible for point mutations in methylenetetrahydrofolate reductase and those in angiotensinogen, respectively, and the *NOS3* locus controlling the number of tandem repeats in the nitric oxide synthase gene. These loci are located on different chromosomes and encode products involved into various metabolic pathways leading to CAS. In the populations studied, significant differences between healthy subjects and patients predisposed to cardiovascular diseases were revealed with regard to the above seven markers. The *174M* allele (T174M polymorphism in the *AGT* gene) was significantly associated with coronary atherosclerosis. It was found that specific gene combinations are involved in the CAS development and determine variation in the pathogenetically important quantitative traits.

INTRODUCTION

The contribution of various genetic factors into coronary atherosclerosis and the degree of interaction between genetic and environmental factors are different in each individual case. Various approaches were used to identify the genetic component of atherosclerosis. In this study, an association between the genetic marker systems, coronary atherosclerosis (CAS), and the quantitative CAS risk factors of pathogenetic importance have been examined. We analyzed relatively little studied marker gene loci of various metabolic systems, which may play a role in development of atherosclerosis and other cardiovascular diseases (CVD), such as hypertension and cardiomyopathy. These were the genes of angiotensin–renin system, those of angiotensin-converting enzyme (*ACE*) and angiotensinogen (*AGT*); the gene controlling nitric oxide metabolism, which encodes the constitutive endothelial nitric oxide synthase (*NOS3*); the apolipoprotein A1 gene (*APOA1*) contributing to the system responsible for lipid metabolism; the genes of the homeostasis system, namely those of the clotting factor 13 (*F13B*) and plasminogen activator (*PLAT*); and a gene of the homocysteine metabolism, which encodes methylenetetrahydrofolate reductase (*MTHFR*). These marker genes were chosen because their protein products are involved in etiopathogenesis of the cardiovascular diseases, as judged from the published data.

Briefly, the following evidence is available of the candidate genes predisposing to coronary atherosclerosis and of their protein products.

The angiotensin-converting enzyme gene (*ACE*) located on chromosome 17q23 consists of 26 exons (4.3 kb in overall length) and encodes a protein containing 1306 amino acid residues including a signal peptide of 29 amino acids. ACE is metallopeptidase whose major function is hydrolysis and conversion of angiotensin I to angiotensin II, which is a potent vasoconstrictor and the growth-regulating agent of the vessel smooth muscle cells and cardiomyocyts. In addition, ACE inhibits bradykinin by splitting off two carboxy-terminal dipeptides from its molecule. The ACE-induced angiotensin II production and bradykinin degradation promote vasoconstriction effect, vascular cell proliferation, and changes in arterial tone [1–5]. In the present work, we studied the diallelic insertion/deletion (I/D) polymorphism of the *ACE* gene (Table 1).

The angiotensionogen gene (*AGT*) is located on chromosome 1q42-43. Angiotensiongen is a serum globulin, the first component in the reaction sequence of renin-angiotensin system, and a precursor of the biologically active hormone angiotensin II, which is a potent vasoconstrictor [6–9]. In our study we analyzed diallelic polymorphism for the presence or absence of a restriction site at position 174 of the *AGT* gene (Table 1).

The 21-kb gene of nitric oxide synthase (*NOS3*) located on chromosome 7q-36 contains 26 exons. Nitric oxide synthase (NOS) catalyzes formation of nitric oxide from L-arginine in vascular endothelium, which leads to activation of guanylyl cyclase and an increase in cGMP concentration in cells. Accumulation of cGMP leads to activation of cGMP-dependent pro-

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Table 1. Amplified DNA regions and conditions for PCR and restriction endonuclease treatment

Locus	Polymorphism	PCR and restriction conditions
ACE	The presence or absence of 287-bp *Alu* repeat in intron 16 of the gene	PCR: initial denaturation at 95°C for 5 min; 33 cycles of treatment at 94°C for 1 min, at 57°C for 1 min, and at 72°C for 1 min; elongation at 72°C for 5 min [24]
NOS3	Polymorphism for the repeat number of a 27-mer nucleotide in intron 4 of the gene, variation in the 27-mer nucleotide repeat number in intron 4 of the gene	PCR: initial denaturation at 94°C for 5 min; 30 cycles of treatment at 94°C for 1 min; at 56°C for 1 min, and at 72°C for 1 min; elongation at 72°C for 5 min [11]
APOA1	The presence/absence of a 287-bp *Alu*-repeat	PCR: initial denaturation at 95°C for 5 min; 33 cycles of treatment at 94°C for 1 min, at 50°C for 1.5 min, and 72°C for 1.5 min; elongation at 72°C for 5 min [25]
F13	The presence/absence of a 287-bp *Alu*-repeat	PCR: initial denaturation at 94°C for 5 min; 33 cycles of treatment at 94°C for 1 min, at 56°C for 1.5 min, and at 72°C for 1.5 min; elongation at 72°C for 5 min [26]
PLAT	The presence/absence of a 287-bp *Alu*-repeat	PCR: initial denaturation at 95°C for 5 min; 33 cycles at 94°C for 1 min, at 58°C for 1.5 min, and at 72°C for 1.5 min; elongation at 72°C for 5 min [27]
AGT	A point mutation in exon 2 of codon 174 of the gene, which led to T for M amino acid substitution	PCR: initial denaturation at 95°C for 5 min; 35 cycles of treatment at 94°C for 2.5 min, at 58°C for 40 s, and at 72°C for 2 min; elongation at 72°C for 10 min. Restriction: 12 h at 37°C, *Bsp*191 [7]
MTHFR	A point mutation at position 677 (cytosine for thymine substitution, C677T), which led to alanine for valine substitution in the amino acid sequence	PCR: initial denaturation at 96°C for 5 min; 35 cycles of treatment at 93°C for 50 s, at 55°C for 50 s, and at 72°C for 30 s; elongation at 72°C for 7 min. Restriction: 12 h at 37°C, *Hinf*I [17]

tein kinase and Ca^{2+}-ATPase involved in dephosphorylation of myosin light chains, which in turn results in Ca^{2+} release from the smooth muscle cells and eventually in vasodilatation. There are three isoforms of nitric oxide synthase: constitutive neuronal (nNOS, or NOS1), inducible (iNOS, or NOS2), and constitutive endothelial (ecNOS, or NOS3). Another form, NOS4, belonging to iNOS also has been described [5, 10–12]. In the present work, we studied diallelic polymorphism for the number of tandem repeats in intron 4 of the *NOS3* gene (Table 1).

The apolipoprotein A gene (*APOAI*) (1863-bp overall length) is located on chromosome 11q23 and consists of four exons (76, 63, 157, and 658 bp) and three introns (197, 186, and 588 bp). The apoA1 protein activates an enzyme involved in esterification of cholesterol and, hence, participates in lipoprotein metabolism. HDLPs may also constitute a system that doubles cell cholesterol regulation. When cell receptor system is incapable of reducing the high level of cholesterol in a cell, HDLPs promote the outflow and transporting of free cholesterol into the liver, from where it is excreted, and mediate esterification of blood cholesterol. The low level of HDLPs decreases their regulating role, and cholesterol is accumulated in tissues including that the vessel wall [13, 14]. In the present study, we analyzed diallelic insertion/deletion (I/D) polymorphism in the *APOA1* gene (Table 1).

The clotting factor gene (*F13B*) is located on chromosome 1q31.2-q32.3. The FXIII factor is a proenzyme of fibrinoligase; its conversion into the active form is mediated by thrombin and calcium ions. Factor XIII consist of two subunits, A and B. The A component of the factor is associated with major histocompatibility complex, whereas the B subunit seems to display nonenzymatic activity and may transfer the plasma molecules. In this work, we studied diallelic insertion/deletion (I/D) polymorphism in the *F13B* gene (Table 1).

The gene of plasminogen tissue activator, *PLAT*, is located on chromosome 8p12-q11.2. PLAT is a serine-protease, which activates proenzyme plasminogen, to convert it into plasmin responsible for the fibrinolytic activity. PLAT is synthesized in endothelial cells of vessels as a continuous polypeptide chain. PLAT inhibits growth of already formed thrombus and causes its lysis. In this study, we analyzed diallelic insertion/deletion (I/D) polymorphism of the *PLAT* gene (Table 1).

The gene of methylenetetrahydrofolate reductase, *MTHFR*, is located on chromosome 1p36.3. A thermolabile enzyme 5,10-methylenetetrahydrofolate reductase is a cytosolic flavoprotein that catalyzes the reduc-

tion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The latter is a predominant circulatory form of folate and a carbon donor for remethylation of homocysteine to methionine, which is a final stage in the sulfur cycle that yields methionine. Impaired activity of thermolabile MTHFR decelerates the 5-methyltetrahydrofolate-homocysteine-methyltransferase reaction, which disturbs homocysteine metabolism, increases the level of homocysteinic acid, and leads to hyperhomocysteinemia or hypomethioninemia. An increase in the homocysteine level results in thrombogenesis, lipid plaque formation, changes in nitric oxide production by endothelium, and disturbed vasoconstriction [15–20]. In the present work, we studied the allelic polymorphism for the presence or absence of a restriction site at position 677 of the *MTHFR* gene (Table 1).

The present study is an extension of a large-scale investigation of genes predisposing to development of cardiovascular diseases, which is performed in Research Institute of Medical Genetics, Tomsk Research Center, Siberian Division, Russian Academy of Medical Sciences [5, 9, 14, 20, 21].

MATERIALS AND METHODS

We examined a group of unrelated male patients ($n = 94$) from the Department of Ischemic Heart Disease, Research Institutes of Cardiology, Tomsk Research Center, Russian Academy of Medical Sciences. In these patients atherosclerosis of coronary arteries was verified angiographically. The average age of the patients was 48.7 ± 8.9 years. The control group comprised the healthy unrelated subjects ($n = 122$) of similar to patients aged 41.4 ± 7.9 without clinical signs of cardiovascular failure, which was confirmed by electrocardiography and by case records. A sample of the rural population contained 117 unrelated subjects, both males and females, from the town of Kargala, Tomsk rayon. This sample was not differentiated with regard to CAS.

In subjects of the control group, the following parameters were measured: total concentrations of cholesterol and triglycerides in the blood plasma (TCC and TG, respectively); the level of cholesterol in different subclasses of lipoprotein particles, those of high, low, and extremely low density (CC-HDLP, CC-LDLP, and CC-ELDLP, respectively); the level of arterial pressure, both diastolic and systolic (DP and SP, respectively); and the body weight index (BWI). Both TCC and TG concentration was determined by the enzymatic methods.

DNA was isolated from peripheral blood lymphocytes by a conventional method [22, 23]. DNA regions containing polymorphic fragments and restriction sites were amplified by using polymerase chain reaction (PCR). The amplified DNA regions, as well as conditions for PCR and digestion with restriction endonuclease, are indicated in Table 1.

PCR was conducted in 25 μl of a reaction mixture containing about 1 μg of the DNA sample, 2.5 μl of the $(NH_4)_2SO_4$-containing incubation buffer ×10; 0.2 mM of each deoxynucleoside triphosphate, 1 mM $MgCl_2$, 1 unit of *Taq*-polymerase, and 0.01 optical unit of each primer. Restriction endonuclease digestion was conducted in 10 μl of a mixture containing 5 μl of the amplified products, 1 μl of the buffer for restriction endonuclease digestion, 10 to 15 units of *Hinf*I and 2 units of *Bsp*191 restriction endonucleases (for the *MTHFR* and *AGT* loci, respectively). Both the amplified products and those obtained by treatment with restriction endonucleases were analyzed by electrophoresis in 2% agarose and 8% polyacrilamide gels, respectively, and visualized by staining with ethidium bromide.

The χ^2 analysis was used to determine the allele and genotype conformity to Hardy–Weinberg proportions. The allelic frequencies in patients were compared with those in control and a population sample by pairwise comparisons using χ^2 test. Prior to statistical treatment, the blood pressure estimates and quantitative lipid parameters were adjusted for the body weight index, age, and sex using multiple regression. In all statistical tests, the level of significance was taken to be 5%. Estimates of gametic disequilibrium between the marker loci were obtained by Hill's method [28] in Thompson's modification [29]. The multiway ANOVA (MANOVA) was used to determine association between the gene complexes and variation of lipid spectrum, blood pressure, and body weight index. Statistical analysis was performed using STATISTICA 5.0.

RESULTS AND DISCUSSION

Table 2 shows the genotype distribution, estimates of conformity to Hardy–Weinberg proportions, and allelic frequencies for all loci examined in the populations. The allelic frequencies of these genetic markers were comparable to those previously studied in Caucasoid and Asian populations [1, 2, 30, 31]. The distribution of seven loci examined was consistent with that expected at Hardy–Weinberg equilibrium. In the rural population, the *F13B* and PLAT loci deviated from Hardy–Weinberg proportions, which may have several explanations. However, in both cases, the deviations might reflect either specific genetic processes in the rural sample or the functional importance of these loci (the gene of the clotting factor XIII and that of plasminogen tissue activator are involved in pathogenesis of cardiovascular diseases (CVD). Hence, it is unlikely that variations in the genetic markers linked to these genes represent neutral polymorphism.

Pairwise comparison for allelic frequencies of each of the genes studied showed significant differences between patients with CAS and the control sample with regard to a genetic marker T174M ($P < 0.001$). The frequency of the *174M* allele was significantly higher in patients than in subjects of the control group, which suggest a linkage between the allele and CAS.

Table 2. Genotype distribution, assessment of conformity to Hardy–Weinberg proportions and the frequencies of alleles of all loci studied in the populations

Locus		Genotypes			Allelic frequencies	
		II	ID	DD	I	D
ACE	Control group, $n = 122$	24(20%)	60(49%)	38(31%)	0.5107	0.4893
		$\chi^2 = 0.0115$ ($P > 0.5$)				
	Rural population, $n = 117$	34(29%)	61(52%)	22(19%)	0.5420	0.4580
		$\chi^2 = 0.1257$ ($P > 0.5$)				
	Patients, $n = 94$	24(26%)	46(49%)	24(25%)	0.5107	0.4893
NOS3		*AA*	*AB*	*BB*	*A*	*B*
	Control group, $n = 122$	5(4%)	29(24%)	88(72%)	0.1598	0.8402
		$\chi^2 = 1.6120$ ($P > 0.5$)				
	Patients, $n = 94$	0(0%)	27(29%)	67(71%)	0.1436	0.8564
APOA1		+ +	+ −	− −	+	−
	Control group, $n = 122$	98(81%)	22(18%)	2(1%)	0.8968	0.1032
		$\chi^2 = 0.4022$ ($P > 0.5$)				
	Rural population, $n = 117$	96(82%)	12(10%)	1(8%)	0.9357	0.0643
		$\chi^2 = 0.7697$ ($P < 0.01$)				
	Patients, $n = 94$	77(82%)	13(14%)	4(4%)	0.8883	0.1117
F13B		+ +	+ −	− −	+	−
	Control group, $n = 122$	23(19%)	68(56%)	31(25%)	0.4419	0.5581
		$\chi^2 = 0.6091$ ($P > 0.5$)				
	Rural population, $n = 117$	26(23%)	41(36%)	48(41%)	0.4043	0.5957
		$\chi^2 = 7.7662$ ($P < 0.01$)				
	Patients, $n = 94$	17(18%)	49(52%)	28(30%)	0.4415	0.5585
PLAT		+ +	+ −	− −	+	−
	Control group, $n = 122$	37(30%)	63(52%)	22(18%)	0.5586	0.4414
		$\chi^2 = 0.1134$ ($P > 0.5$)				
	Rural population, $n = 117$	20(17%)	76(65%)	21(18%)	0.4957	0.5043
		$\chi^2 = 10.4767$ ($P < 0.01$)				
	Patients, $n = 94$	35(37%)	40(43%)	19(20%)	0.5851	0.4149
AGT		*TT*	*TM*	*MM*	*T174*	*M174*
	Control group, $n = 122$	112(92%)	20(8%)	0(0%)	0.9242	0.0758
		$\chi^2 = 0.8869$ ($P > 0.5$)				
	Rural population, $n = 117$	78(73%)	27(26%)	1(1%)	0.8679	0.1321
		$\chi^2 = 0.6546$ ($P > 0.5$)				
	Patients, $n = 94$	55(59%)	36(38%)	3(3%)	0.7766	0.2234
MTHFR		*CC*	*CT*	*TT*	*C677*	*T677*
	Control group, $n = 122$	64(53%)	48(39%)	10(8%)	0.6822	0.3178
		$\chi^2 = 2.5977$ ($P > 0.5$)				
	Rural population, $n = 117$	56(48%)	44(38%)	17(14%)	0.6667	0.3333
		$\chi^2 = 2.7692$ ($P > 0.5$)				
	Patients, $n = 94$	44(47%)	41(43%)	9(10%)	0.6882	0.3118

Table 3. Genetic association between different gene complexes and variation in the quantitative parameters of coronary atherosclerosis

Quantitative parameter	Gene ensembles	*F* test	Level of significance, *P*	Determination coefficient R^2, %	Genotypic combinations correlated with	
					maximum value of quantitative parameter	minimum value of quantitative parameter
TCC	*ACE–MTHFR*	2.448	0.018	1.6	IICT	IDCT
	ACE–F13	5.183	0.00002	0.7	II––	DD––
	ACE–AGT–MTHFR	1.886	0.030	1.9	IITTCT	DDTTTT
TG	*ACE–APOA1*	2.933	0.007	2.8	II––	DD+–
	APOA1–MTHFR	2.330	0.037	0.2	CT––	TT+–
CC-HDLP	*NOS3–APOA1*	2.194	0.048	0.2	BB+–	AA++
	NOS3–AGT	2.753	0.022	0.3	ABTM	AATT
	PLAT–AGT	2.582	0.030	0.3	ABTM	AATT
	ACE–NOS3	2.054	0.055	1.9	DDAB	IDAA
CC-LDLP	*ACE–MTHFR*	2.305	0.025	11.7	IICT	IDCT
	ACE–F13	4.982	0.00003	9.0	II––	II++
	ACE–AGT–MTHFR	1.875	0.034	12.3	IITTCT	IITMCC
CC-ELDLP	*ACE–APOA1*	2.933	0.007	2.8	II––	DD+–
	APOA1–MTHFR	2.334	0.036	0.2	––CC	+–TT
DP	*F13–AGT*	3.476	0.006	0.8	++TM	––TM
CP	*F13–AGT*	4.044	0.001	0.08	+–TM	––TM
BWI	*ACE–PLAT*	3.674	0.0008	5.2	II+–	DD––
	ACE–AGT–MTHFR	1.729	0.055	5.0	IITMCC	DDTMTT

Assessment of Linkage between the Polymorphic Variants of the Predisposing Genes and the Risk Factors for Coronary Atherosclerosis

Table 3 shows the association of the quantitative parameters of blood pressure and lipid metabolism with gene complexes as determined by MANOVA, as well as specific combinations of genotypes that were correlated with maximum and minimum values of the quantitative parameters.

The same gene combinations contribute to the variance of the total cholesterol and the cholesterol in low-density lipoproteins. These gene ensembles are *ACE–MTHFR*, *ACE–F13B*, and *ACE–AGT–MTHFR*. Combined influence of the *ACE* and *MTHFR* genes may be explained by the effect of methylenetetrahydrofolate reductase and participation of angiotensin-converting enzyme in blood pressure regulation by means of the angiotensin II protein, which has a vasoconstriction effect. Shortage of the MTHFR-product, 5-methyltetrahydrofolate, leads to the low level of plasma folate, methionine, and high level of homocysteine [32], which promotes thrombogenesis, formation of lipid plaques, changes in endothelial production of nitric oxide, and vasoconstriction [33].

A precursor of the vasoconstriction hormone angiotensin II, angiotensinogen, causing a potent vasoconstriction, enhances the co-influence of the *ACE* and *MTHFR* marker loci. There is no apparent explanation for the role of the *ACE–F13B* combination of polymorphic marker variants. Some unknown events probably occur in pathogenesis of coronary atherosclerosis. Note that the same combinations of the genetic markers *ACE*, *APOA1*, *MTHFR*, *F13B*, and *AGT* are associated with such atherogenic factors as TCC, CC-LDLP, CC-ELDLP, TG, and BWI. Moreover, a gene ensemble *ACE–AGT–MTHFR* is associated to the variance of

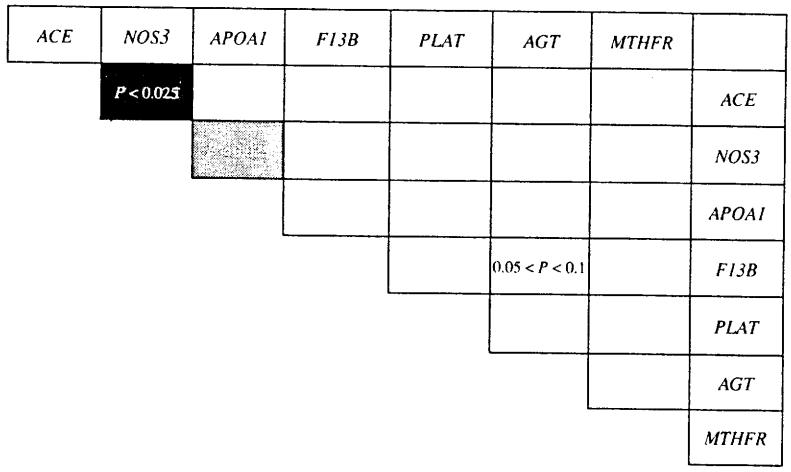

Fig. 1. Gametic disequilibrium between pairs of loci in the control sample.

three atherogenic quantitative parameters (CC-LDLP, BWI, and TCC), which additionally confirms the importance of this gene complex for atherosclerosis development. This complex of loci may be considered a substantial genetic element involved in CAS etiopathogenesis.

Conversely, gene ensembles linked to such an antiatherogenic factor as CC-HDLP, consist of other genes: *NOS3*, which is encountered in three out of four combinations, *AGT*, *PLAT*, and *APOA1*.

Variation in systolic and diastolic pressure was associated to the same gene ensemble, which suggests a significant effect of the *AGT* and *F13B* on the level of blood pressure. The protein product of the *AGT* gene, angiotensinogen, is involved into the reaction sequence of the renin-angiotensin system, directly controlling the synthesis of a precursor for hormone angiotensin II which causes a strong vasoconstriction. The biological implication of a combination including *AGT* and thrombogenesis-controlling locus still remains unclear, but this very gene combination may extend the range of the effect of each of the genes.

Analysis of a group of markers associated with cholesterol variation in low-density lipoproteins revealed an important contribution of the gene complexes in the trait variance (from 9 to 12%) (Table 3), which is another supporting evidence of a large contribution of gene ensembles *ACE–MTHFR*, *ACE–F13*, and *ACE–AGT–MTHFR* to atherogenesis. The contributions of gene ensembles *ACE–PLAT* and *ACE–AGT–MTHFR* to variation of such an atherogenic factor as body weight were also significant (about 5%). The contribution of gene complexes *NOS3–APOA1*, *NOS3–AGT*, *PLAT–AGT*, and *F13B–AGT* to variation of CC-HDLP, SP, and DP did not exceed 1%.

Analysis of Gametic Disequilibrium between DNA-Markers of the Genes Studied

Associations between different traits may be caused by either the pleiotropic effect of alleles of the same locus or by nonrandom association of alleles of different loci. We have analyzed gametic disequilibrium between pairs of loci of all candidate genes examined in the two populations. Since the examined loci were located on different chromosomes (except for syntenic genes *APOA1* and *F13B*), the high degree of gametic disequilibrium was not expected.

Figure 1 shows the results obtained by analysis of gametic disequilibrium between pairs of loci in the control population with regard to all candidate genetic markers studied (*ACE*, *NOS3*, *APOA1*, *F13B*, *PLAT* and *MTHFR*). The three pairs of loci were in gametic disequilibrium, which probably was not an accidental result. The two of these gene pairs were in gametic disequilibrium with high degree of significance: *NOS3* and *ACE* ($\chi^2 = 6.54$, $P < 0.025$); *NOS3* and *APOA1* ($\chi^2 = 5.04$, $P < 0.025$). One pair of loci, *AGT* and *F13B*, showed disequilibrium at a marginal level of significance ($\chi^2 = 3.34$, $0.05 < P < 0.1$). Gametic disequilibrium of genetic markers *NOS3* and *ACE* located on different chromosomes (7q35-q36 and 17q22-q24, respectively) suggests selection for definite allelic combinations in the population studied and, probably, a role of this gene complex in resistance to CVD, because this gene combination was observed in the groups of subjects without clinical signs of cardiovascular failures. The same appears to be true of the two-locus gametic disequilibrium *NOS3* and *APOA1* (the genes are located on chromosomes 7q35-q36 and 11q23, respectively).

Figure 2 shows data on the two-locus gametic disequilibrium among the genes studied in the rural sample from Kargala town. Only one pair of loci was in significant gametic disequilibrium. Hence, in this population,

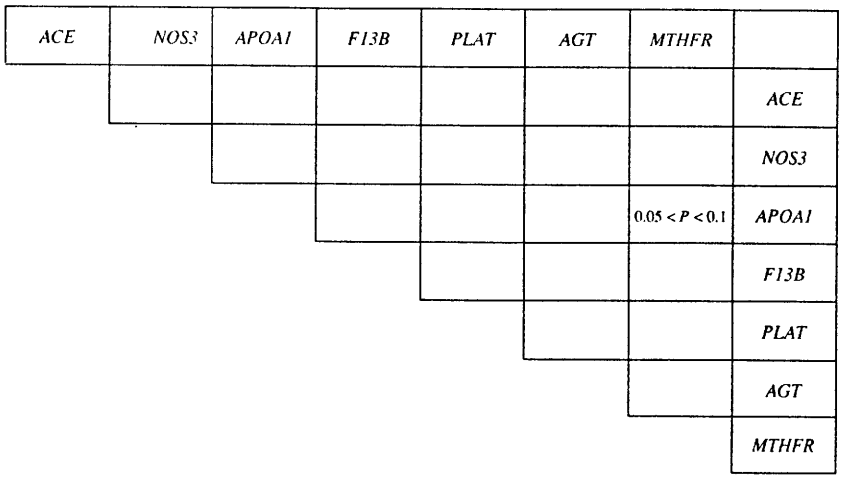

Fig. 2. Gametic disequilibrium between pairs of loci in the rural population from Kargaly.

gametic disequilibrium was not observed more frequently than expected from accidental causes. The absence of any significant diallelic gametic disequilibrium may indirectly suggest that this sample was neutral with respect to the studied disease (this group may comprise healthy subjects and patients with CAS of different severity and at different stages of the disease onset).

Directly opposed opinions exist on the origin of gametic disequilibrium. In Vogel's view, many common cases of gametic disequilibrium can be explained by selection, whereas the effect of recent population mixing is of no importance [34]. Some haplotypes and allelic combinations of nonlinked loci have probably a selective advantage, and, therefore, occur more frequently in a population than the others. The selective advantage may be directly associated with the disease studied. Conversely, Zebra *et al.* [35], who revealed many cases of gametic disequilibrium, believe that this phenomenon is a result of population expansion and mixing affecting the population genetic structure.

Numerous polymorphic markers revealed at the DNA level can play an extremely important role in analysis of multifactorial human diseases. Most of these studies aimed at identifying and characterization of functional mutations. Gametic disequilibrium at the loci that we have examined was never previously described. Zebra *et al.* have studied 16 polymorphic loci of seven CAS candidate genes. In 19 cases, these authors detected statistically significant disequilibrium for 101 pairs of marker loci from different nonlinked regions. The common opinion that disequilibrium can not prove conclusively physical linkage between the loci in the DNA region examined was confirmed by Zebra's studies [29, 36]. All polymorphic variants were suggested to be of equal age, and the recombination was assumed to be the only cause of disequilibrium between the loci.

Thus, the data on gametic disequilibrium suggest that in the control group, marker systems *NOS3–ACE* and *NOS3–APOA1* are significantly linked. The linkage of *AGT–F13B* pair of loci was at the marginal level of significance. These associations were most probably caused by either the demographic processes leading to population mixing, or some other processes resulting in selection for particular gene complexes.

The same combinations of the candidate genes were revealed by analysis of gametic disequilibrium and MANOVA. In patients with CAS, in particular, an the *NOS3* and *APOA1* gene ensemble was in gametic disequilibrium and at the same time it was linked to CC-HDLP variations.

Thus, in this study, the complexes of genetic markers were revealed among the candidate CAS genes. Some of these gene combinations proved to be associated with the quantitative risk factors of the coronary atherosclerosis. We believe that the ensembles of genetic markers may be responsible for genetic resistance–predisposition to CAS in the population studied due to their pleiotropic effects. A search for the marker complexes may be helpful for constituting the groups with high genetic predisposition to CAS.

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