Article

PCR-based Approach for Determining the Genetic Risk Factors of Antipsychotic-Induced Metabolic Disorders

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Abstract: The antipsychotic-induced metabolic disorders (AIMD) are common side effects during the treatment of schizophrenia. Single nucleotide polymorphisms in the genes associated with AIMD, in particular in the leptin and neuropeptide Y genes were explored. The aim of this study was to develop a real-time PCR technique for SNP allele discrimination and allele frequency estimation in the Russian population. A total of 9 real-time PCR tests for rs7799039, rs1137101, rs8179183, rs16147, rs6837793, rs11100494, rs1414334, rs3813929 and rs518147 SNPs were developed and examined using 106 DNA samples. The revealed allele frequencies did not show any statistically significant differences with ones for the Caucasian population from Ensembl data base. Thus, our results are in accordance with the allele frequencies for the studied populations and allow using published data on the risk alleles for the development of new diagnostics PCR kits for the complex diagnostics of AIMD.

Keywords: *antipsychotic; leptin; neuropeptide Y; single nucleotide polymorphism; pharmacogenetics; pyrosequencing; real-time PCR.*

Introduction

Antipsychotics are widely used in the treatment of schizophrenia and other psychiatric disorders. A significant proportion of patients with schizophrenia cause antipsychotic-induced metabolic disorders (AIMD), which can reduce compliance. The risk of side effects including AIMD can be genetically determined [2, 4, 7]. The accumulation of data about genetic factors that determine the efficacy and safety of drugs ensure the rapid progress of pharmacogenetics [2], the main task of which is to search for predictors of response to therapy, as well as to determine the probability of side effects and adverse reactions of prescribing drugs. In this regard, the identification of risk factors for the occurrence of adverse events, in particular, AIMD, is a standard test in psychiatric practice.

Recently, significant advances have been made in the study of the AIMD pharmacogenetics, which designate genetic risk factors, most of which are represented as single nucleotide polymorphisms (SNPs) in genes associated with individual metabolism characteristics. This made it possible to form a separate area of pharmacogenetics – the pharmacogenetics of metabolic disorders [3, 4, 8]. The inclusion of a pharmacogenetic approach in clinical guidelines is necessary, but currently it is difficult due to the insufficient level of evidence of the studied genetic factors, ambiguous and contradictory data for

different populations [8]. To increase the reliability of pharmacogenetic data and their further introduction into clinical practice, it is necessary to develop available laboratory methods for routine determination of SNPs that potentially determine the risk of AIMD.

Objective

The objective of this study is to develop the PCR based approaches for determining alleles of genetic polymorphisms associated with the risk of antipsychotic-induced metabolic disorders.

Materials and Methods

The choice of SNP for the developing was carried out on the basis of information analyzed and published in monographs [4,7].

To determine SNP alleles, we used the DNA samples isolated from blood cells using the RIBO-prep kit. Determination of SNP alleles was carried out by real-time PCR with LNA probes [4]. The design of primers and probes was carried out similarly to those developed earlier [1,6]. To verify the specific determination of SNP alleles the PCR products were examined by pyrosequencing [4,5] using equipment and reagents from Qiagen (Germany). All reagents for DNA extraction, real-time PCR and preparation for pyrosequencing were produced at the Central Research Institute of Epidemiology.

To determine the SNP allele and genotype frequencies we have used 106 samples of genomic DNA from residents of the Moscow region randomly distributed by sex and age. The data obtained were compared with allele frequencies for Caucasian (EUR population) from the Ensembl data base [9].

The SNP's linkage disequilibrium was estimated using coefficient of determination calculated by the LDlinkR software [10].

Statistical analysis of allele and genotype frequencies in the samples was carried out in the R environment using standard functions for the Pearson $\chi2$ and Fisher's exact tests. The null hypothesis that the samples belonged to the same general population and that there were no differences between them was rejected if the p-value was less than 0.05.

Results

Based on the published data the 12 SNPs associated with AIMD were selected [3,4,7]. The SNPs were found in the genes of the leptin system, neuropeptide Y, and serotonin receptor 5-HT2. Gene products, nucleotide substitutions and risk alleles are shown in the table.

The studies analyzed in monographs [4] and [7] provide data on the association of various SNPs in the same genes with AIMD. Therefore, it becomes necessary to test SNP alleles for linkage disequilibrium. The performed calculation of the coefficients of determination (the table shows the values of $R2$) allows us to conclude that some SNPs are linked and probably their effect – the AIMD association – is realized by similar pathogenetic mechanisms. For closely linked SNPs rs3828942 (linked to rs7799039), rs5573, and rs5574 (linked to rs16147), no allele frequencies were determined, while information on the allele frequencies of these SNPs can be used to assess the risk of AIMD.

The developed real-time PCR approaches for determining SNP alleles make it possible to determine alleles and genotypes for all biological samples included in the study in an unambiguous manner. An example of the determination of alleles and genotypes of the rs1414334 (G>C) polymorphism and confirmation of certain genotypes using pyrosequencing is shown in the figure **(Figure 1).**

Comparison of the allele frequencies of the samples with the frequencies presented for the Caucasian population in the Ensembl database did not reveal statistically

significant differences for all SNPs, which is confirmed by χ2 and p-values. For SNPs presented in the table statistically significant differences in the alleles and genotypes frequencies between men and women, assessed by the population of Caucasians, were not observed.

Published data on population frequencies and risks of AIMD development for SNPs included in this study are comparable with data for the observed population which allows them to be used to choose or correct antipsychotic therapy. Since the minimum frequency of a rare allele in the studied sample is 9%, it is possible to use SNP genotypes to identify individual characteristics of patients both based on the determination of individual SNP genotypes, and in combinations, depending on clinical parameters (**Table 1).**
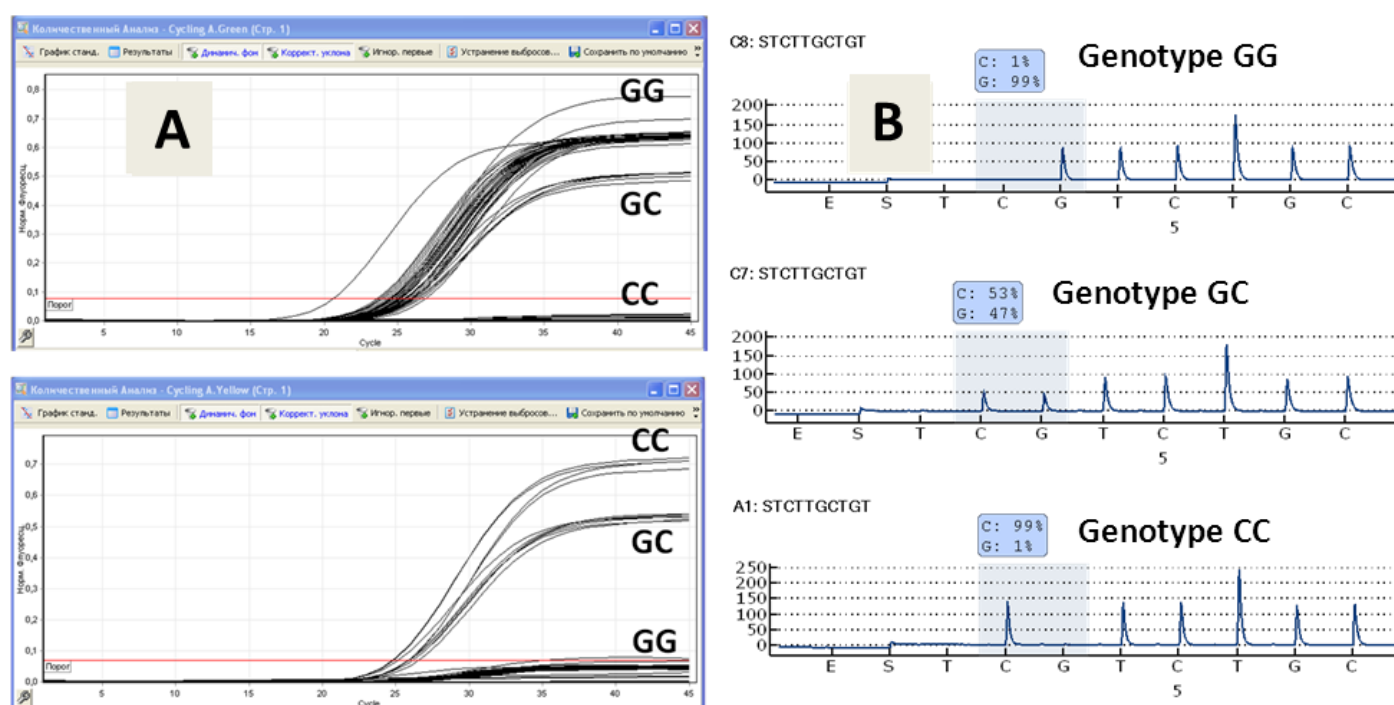

Figure 1. The rs1414334 (G>C) alleles detection using real-time PCR and pyrosequencing-based genotype confirmation.
A. Real-time PCR curves detected by «Rotor-Gene Q» («Qiagen») instrument. The curves for GG genotypes samples are cross the threshold on «Green» channel (upper graph) and do not cross or cross at late cycles on «Yellow» channel (lower graph). The curves for CC genotypes are cross the threshold on «Yellow» channel and do not cross the «Green» channel threshold. The curves for heterozygous samples are cross the threshold lines on both channels.
B. Pyrograms performed by «PyroMark Q24» («Qiagen») instrument. The ordinate axis is the signal level detected by the instrument; the signal height is proportional to the number of sequenced nucleotides. The abscissa axis contains the nucleotide dispensation order.

Table 1. The SNPs associated with antipsychotic-induced metabolic disorders

Gene	Protein	rs*	SNP**	Genotype frequencies (N=106)	Allele	Allele frequencies, %	Allele frequencies EUR (Ensembl), %	χ2 frequencies (p-value)***
LEP	Leptin	**rs7799039**	G>A	GG (31), GA (55), AA (20)	G A	55 45	56 44	0,04 (0,84)
		rs3828942 (R²=0,84)	<u>G</u>>A	–	G A	– 	57 43	–
LEPR	Leptin receptor	**rs1137101**	A><u>G</u>	AA (29), AG (49), GG (28)	A G	50 50	53 47	0,37 (0,55)
		rs8179183 (R²=0,15)	G><u>C</u>	GG (74), GC (28), CC (4)	G C	83 17	85 15	0,26 (0,61)
NPY	Neuropeptide Y	**rs16147**	T><u>C</u>	TT (26), TC (57), CC (23)	T C	51 49	49 51	0,1 (0,75)
		rs5573 (R²=0,99)	G><u>A</u>	-	G A	- -	49 51	-
		rs5574 (R2=0,85)	C><u>T</u>	-	C T	- 	53 47	-
NPY5R	Neuropeptide Y receptor	**rs6837793**	<u>G</u>>A	GG (88), GA (17), AA (1)	G A	91 9	91 9	0,06 (0,81)
		rs11100494	C>A	CC (87), CA (19)	C A	91 9	94 6	1,35 (0,25)
HTR2C	Serotonin 5-HT2 receptor	**rs1414334**	G><u>C</u>	GG (86), GC (14), CC (6)	G C	88 12	85 15	0,61 (0,43)
		rs3813929	<u>C</u>>T	CC (80), CT (15), TT (11)	C T	83 17	84 16	0,12 (0,73)
		rs518147	<u>G</u>>C	GG (58), GC (30), CC (18)	G C	69 31	68 32	0,03 (0,86)

* – The squared coefficient of correlation between SNPs is indicated in parentheses (it was not calculated for HTR2B gene), the allele frequencies were performed only for SNPs highlighted in bold.

** – The risk alleles are underscore marked; for ambiguous SNPs (rs7799039 и rs11100494) the risk alleles are not detected.

*** – Comparison of the allele frequencies revealed in this study and frequencies in the «EUR» population of Ensembl database.

Discussion

The organizational, ethical and pharmacoeconomic aspects of pharmacogenetic testing, as well as the advantages and prospects of individualized approaches in practical medicine have been discussed earlier [2, 4, 8, 11]. The development of practical tools – molecular biological techniques and kits for the implementation of such tasks is an important area of laboratory medicine. Since real-time PCR is available on the basis of many laboratories, there is a possibility of widespread use of the developed approaches in scientific and clinical institutions. The created complex of methods after extended clinical testing is supposed to be used for pharmacogenetic testing in practice with the aim of effective and safe prescription of antipsychotics in the patients with risk of AIMD [12, 13].

Conclusions

The data presented in the work show the prospects for kits of reagents which can be produced in Russia and can be used in routine practice to predict the AIMD risk. The use of pharmacogenetic testing will improve the quality of life of patients with mental disorders and reduce the financial burden on the treatment of this category of patients, and a personalized approach to the prescription of antipsychotics will increase adherence to treatment. In the field of laboratory diagnostics, an urgent direction is the development and clinical trials of a medical device for the determination of SNPs in the genes of the leptin and neuropeptide Y system, which in the future will make it possible to include it in the recommendations and standards of care for patients with mental disorders.

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