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Genome-wide study of Almaty region people for a long time living in areas of pesticide contamination

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Abstract. The purpose of this study is to analyze the associations of gene polymorphisms involved in the pathogenesis of chronic diseases that develop against the background of the toxic effects of pollutants in the environment. The study sample for the research was a cohort of people who had been exposed to pesticide pollution for a long time, taken from villages in the Almaty region, on the territory of which destroyed warehouses of pesticides prohibited for use were discovered. Analysis were performed using Genome-wide association study (GWAS) method. A total of 297,397 SNPs were analyzed, 6 of which were found to be significantly associated with the negative effects of chronic exposure to pesticides.

1 Introduction

The problem of the impact of pesticide pollution on public health is still acute in many countries, including the Republic of Kazakhstan. According to the monitoring data from 2019, about 68.443 kg of unutilized pesticides still remain in the Almaty region. Chemicals that belong to the class of persistent organic pollutants (POPs) are common among them. Even in small doses, POPs pose a threat to humans and nature - they are poorly soluble in water and highly soluble in fats and oils, which makes POPs able to accumulate in the fatty tissues of living organisms. In the presence of an active source of pollution, the concentration of these substances both in the human body and in the environment can increase by thousands and tens of thousands of times [1-3].

It is known that pesticides are highly toxic and have a negative impact on human health [4]. The long-term effects of pesticides, especially at low doses, and their possible synergy with other environmental pollutants and disease vectors are poorly understood due to the relative novelty of most pesticides. However, pesticide metabolites remaining in food may not have toxic or lethal effects, but reduce resistance to disease and gradually accumulate in the body to dangerous levels [5-7]. Functional changes in some enzymes caused by polymorphism of genes involved in the metabolism of toxic substances aggravate the negative effect of these substances, leading to the development of a number of acute and

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chronic diseases [8]. Therefore, in studies devoted to the toxic effects of pesticides, it is important to identify marker polymorphisms that are reliably associated with a high risk of developing diseases.

This study identified highly reliable single nucleotide variations (SNP) of genes with $P<9{,}5\cdot10^{-6}$, which significantly increase the risk of developing diseases in humans against the background of long-term pesticide pollution.

2 Materials and Methods

The study group included 164 people from the villages of Kyzylkairat, Belbulak, Amangeldy, Beskainar, Enbekshi, Karakastek and Umbetaly. Biomaterial in the form of peripheral blood was collected from 2018 to 2021. 206 people from the villages of Basshi and Usharal were selected as a control, on whose territory no traces of pesticide contamination were found. For the purity of the experiment, selection into the experimental and control groups was carried out taking into account homogeneity according to key criteria for the population (average age, ethnic and gender composition).

DNA extraction was performed using a commercial Wizard genomic DNA purification kit (Promega, WI, USA). Qualitative and quantitative characteristics of DNA samples were assessed using a NanoDrop OneC spectrophotometer (ThermoScientific, MA, USA) and a Qubit fluorimeter (ThermoScientific, MA, USA).

Microarray SNP genotyping was performed on the Infinium Global Screening Array 24 v3.0 platform on the iScan system (Illumina, CA, USA) using the automated Infinium HTS protocol [9, 10]. Detailed results of annotation of 654,270 SNP markers are presented in a previously published study [11]. Quality control was performed using PLINK v1.9 [12] to according to the standard algorithm. Furthermore, SNPs on sex chromosomes (X and Y) and the mitochondrial DNA were also excluded. Finally, 297,397 SNPs remained for further analyses. The genetic association of the study population was visualization as Manhattan plot in program R v3.6.0 (http://www.r-project.org). To annotate and interpret genetic variants obtained using iScan, several databases were used: 1000 Genomes database (https://www.internationalgenome.org/1000-genomes-browsers).

3 Results and Discussion

Bioinformatics analysis revealed a high significance association of the risk of developing functional disorders induced by long-term exposure to pesticide pollution for the genes *NLRP3* (rs7525979), *ABCB5* (rs1011559), *MAN2A1* (rs2269197), *PTPRD* (rs10733553), *GRM5* (rs10765819), *CFAP46* (rs10870351), *NCOR2* (rs4765147). The analysis also showed a high degree of reliability for some markers localized in non-coding regions of the genome in an interval less than $p = 1\times10^{-4}$.

Table 1. Significant markers on the analyzed chromosomes (threshold 9.5×10^{-6})

Gene	Consequence	SNP ID	Base Change	Chr	Position	MAF (Database)	Effect Allele
-	-	rs1116294	C>A/ C>T	2	49764298	0.46(T)	C/A,T
NLRP3	Synonymous Variant	rs7525979	C>G/ C>T	1	247587408	0.12(T)	C/G,T
-	-	rs6442441	A>G	3	14359685	0.49(G)	A/G
-	-	rs1349084	G>A/ G>T	4	33030781	0.42(A)	G/A,T
ABCB5	Intron Variant	rs1011559	A>C/ A>T	7	20703948	0.16(C)	A/C,T
-	-	rs2838411	G>A/ G>C	21	45249474	0.16(A)	G/A,C
MAN2A1	Intron Variant	rs2269197	T>C	5	109037077	0.06(C)	T/C

Continuation of Table 1

-	-	rs4457133	C>A	6	92537385	0.09(A)	C/A
-	-	rs16889174	C>A	8	40187492	0.07(A)	C/A
PTPRD	Intron Variant	rs10733553	A>C/ A>G/ A>T	9	9709405	0.45(G)	A/C, G,T
GRM5	Intron Variant	rs10765819	G>A/ G>T	11	88731228	0.44(A)	G/A,T
NCOR2	Intron Variant	rs4765147	G>A	12	124857274	0.33(A)	G/A
CFAP46	Intron Variant	rs10870351	A>G	10	134718123	0.33(A)	A/G
RNASE6	Downstream/ Intron Variant	rs7150888	G>A/ G>C/ G>T	14	21250676	0.24(G) 0.75(A)	G/A
-	-	rs75104951	G>A	16	55578510	0.0003(A)	G/A
-	-	rs28419703	A>C/ A>G	18	22456791	0.16(C)	A/C,G
RNF225	Downstream Variant	rs11666216	A>G/ A>T	19	58908540	0.08 (G)	A/G,T
-	Non Coding Transcript	rs4778909	C>T	15	79155088	0.33(C)	C/T

Visualization of the obtained association results were plotted by a Manhattan plot, –log10 (p-value) against the chromosome position (Fig. 1).

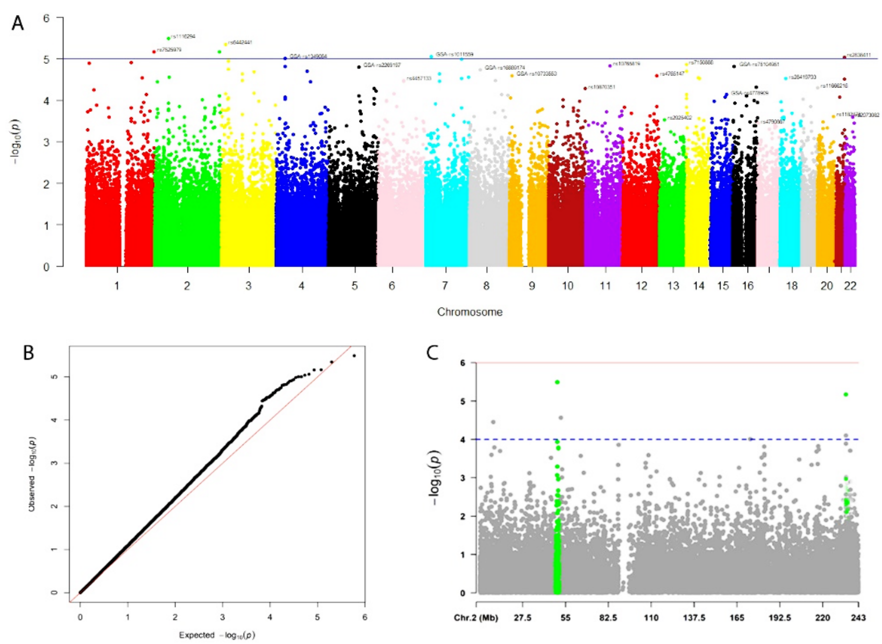

Fig. 1. Visualization of the obtained association results (**A**) Manhattan plot of the association results ($\log_{10}(p)$) for 164 cases and 206 control samples. The x-axis includes the genomic coordinates of analyzed polymorphisms (SNPs) for each chromosome. Horizontal border shows the GWAS threshold at $p=9{,}5\times10^{-6}$ ($-\log_{10}$ P=5). (**B**) QQ-plot showing distribution of expected P values under a null model of no significance versus observed P values. (**C**) Manhattan plot of chromosome 2

The figure shows 297,397 SNPs that passed the filter and QC. Of these, 6 passed the log10(p) threshold, which reflects the high p-values of these polymorphisms. The highest p value was observed for rs1116294 on the second chromosome

4 Conclusion

The genes identified during the analysis can help in marking risk groups for developing chronic and acute diseases against the background of pesticide pollution. Among them, the *NLRP3* gene can be marked as one of the most influensial polymorphism. In various studies it is noted as a key factor in the development of chronic diseases that can be caused by exposure to various toxicants, including pesticides [13]. In this study, this gene also showed a highly significant association with diseases resulting from long-term exposure to toxicants through the food chain. This only confirms the need for a more detailed study of the mechanisms of excessive activation of inflamasomes (INF), coded by the *NLRP3* gene under the influence of toxicants. Presumabely, targeted therapy of this reaction may be able to prevent the development of a number of diseases induced by the action of pesticides.

The *ABCB5* gene, which also showed a high association in this study, being a regulator of the metabolism of carbohydrates and lipids, in case of mutation is also capable to negatively influence the result of the toxic effect of chemical substances on internal organs. The study on zebrafish demonstrates abnormal development of the liver, gonads and heart in fish exposed to trifloxystrobin, along with activation of ABCB family genes [14]. A more detailed study of the effect of ABCB family genes on chronic pesticide pollution is required.

Other GWAS studies have shown that many of the common association signals may result from non-coding variations that alter gene expression [15, 16]. It is worth noting that the associations identified in the results of our study for non-coding regions and regulatory elements (rs1116294, rs6442441, rs1349084, rs2838411) showed possible participation in pathogenetic processes caused by the toxic effects of pesticides. However, the mechanisms of interaction of these regions with the development of diseases remains unclear, and requires additional research.

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