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The Study of the Role Polymorphism of the P2RY12 Gene rs 2046934 in the Pathogenesis of Disaggregation Thrombocytopathy

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ABSTRACT

Background: Thrombocytopathy (TP) is one of the dangerous pathologies related to the platelet's function; widespread of them is disaggregation thrombocytopathy. Authors aimed to study the role of polymorphism of the P2RY12 and rs2046934 genes in the formation of individual accident of Uzbek nationality. **Methods:** The study was conducted (n=71) patients with disaggregation thrombocytopathy, which was observed in consultative outpatient clinic of Institute of Hematology and blood transfusion Ministry of health Uzbekistan. **Results:** the study showed that the unfavorable allele A association with risk of development of hemorrhagic syndrome in disaggregation thrombocytopathy. **Conclusions:** The frequency of A/A and G/A genotypes of the P2RY12 gene in our sample was somewhat comparable with the world data and is consistent with the assumption that they affect the development of hemorrhagic states in disaggregation thrombocytopathy.

Keywords: Disaggregation thrombocytopathy, gene, polymorphism, allele, hemorrhagic syndrome.

INTRODUCTION

Thrombocytopathy (TP) are one of the pathologies of the disorder in the function of platelets, widespread of them is disaggregation thrombocytopathy. Disaggregation thrombocytopathy (DTP) is divided into hereditary and acquired groups, characterized by stable, long-lasting functional, biochemical and morphological changes in platelets with normal or slightly reduced their number. The main clinical feature, which is the nasal, gingival, uterine and other bleeding, petechial bruising hemorrhages on the body.[1,2,4,9,14]

The prevalence of hereditary forms of thrombocytopathies is approximately 3-5 per 100 000 population. Acquired platelet dysfunction observed much more often, and the incidence in recent years has increase, what is primarily due to the uncontrolled use of drugs, especially when trying to self-medication.[3,5,17]

Literature data suggests that the cause of the symptoms of disaggregation thrombocytopathy diver: genetic predisposition; previous infections: viral and bacterial; vaccinations, etc.[1,7]

Studies of genetic polymorphisms in various diseases are becoming increasingly important in modern medicine, due to the improvement of diagnostic methods, since it is violations at the molecular level are the trigger mechanism of the Genesis of multifactorial diseases. A number of foreign scientists have studied various genetic polymorphisms in road accidents that determine a particular role in the Genesis of the disease.[6,8,10,11,16]

Data for the study of genetic changes in hereditary disaggregation thrombocytopathy in the available literature is rather scarce.[15,18]

In particular there are a number of research in the world for the study of the involvement of polymorphism of the P2RY12 gene rs2046934 in the pathogenesis of thrombocytopathy.[4,12,13]

In this regard, the aim of our work is to study the role of polymorphism of the P2RY12 gene rs2046934 the formation of the accident in individuals of Uzbek nationality.

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MATERIALS & METHODS

For carrying out the detection of the genetic polymorphism of the P2RY12 gene rs2046934 in the study were included patients with DTP (n=71) Uzbek nationality (of these, hereditary accident (HTP) n=39 and acquired accident (ATP) n=32), which were observed in the consultative polyclinic of the Research Institute of Hematology and blood Transfusion of the Ministry of Health of the Republic of Uzbekistan. The control group consisted of 48 healthy unrelated persons of Uzbek nationality, without pathology of haemostasis. Testing of

polymorphism of the P2RY12 gene rs2046934 was carried out on programmable thermal cycler of "Applied Biosystems" company 2720 (USA), using test systems of the company "Liteh" (Russia), according to the manufacturer's instructions. Statistical analysis of the results was carried out using the package of statistical programs "Open Epi, Version 9.3".

Electrophoregram detection of polymorphism of the P2RY12 gene rs2046934 in the control group and in patients with road accidents. [Figure 1]

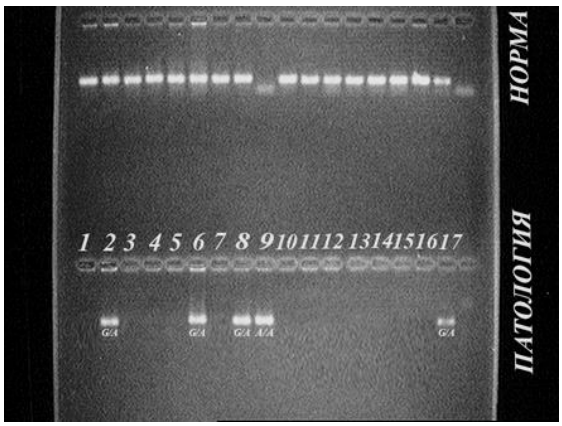

Figure 1: Specificity and number of amplified fragments were tested by electrophoresis in 4% agarose gel.

In this electrophorepgram, G/A 2,6,8 and 17 heterozygous genotypes were noted; A/A 9 homozygous genotype; the remaining G/G 1,3,4,5,7,10-16 normal genotypes.

RESULTS & DISCUSSION

Analysis of the study of the distribution of allele frequencies and genotypes of polymorphism of the P2RY12 gene rs2046934 in the general group of patients revealed closer to a significant increase in the proportion of carriers of adverse allele A in comparison with the control group (16.9% versus 8,3%; $\chi2$=3.61; P=0.057; OR=2.24; 95%CI 0.96-5.22). Quite, the exact pattern determined in the study of the frequency of this allele in the subgroup of patients with hereditary disaggregation thrombocytopathy, that its frequency is significantly exceeded by 2.6 times in relation to control ($\chi2$=4.46; P=0.035; OR=2.62; 95%CI 1.05-6.55) [Table 1].

The findings suggest that the adverse allele A association with risk of development of hemorrhagic syndrome in disaggregation thrombocytopathy. In the total group of patients with disaggregation thrombocytopathy a false increase in the proportion of carriers of the heterozygous genotype G/A in comparison to a control group (22.5% vs. 16.7%, respectively, at .$\chi2$=0.88; P=0.35; RR=1.19; 95% CI 0.85-1.67; OR=1.57; 95% CI 0.61-4.03). In further study of this genotype, a subgroup of patients with HTP (23.1% vs 16.7% at $\chi2$=0.88; P=0.35; RR=1.31; 95% CI 0.77-2.24; OR=1.67; 95% CI 0.57-4.86) and a subgroup of patients with ATP in relation to the control group (21.9% vs. 16.7% at $\chi2$=0.43; P=0.51; RR=1.24; 95% CI 0.66-2.33; OR=1.46; 95% CI 0.47-4.53), respectively.

The carrier frequency of homozygous genotype A/A in the main and control groups was 5.6% and 0.0%, respectively. In this case, the risk of disaggregation thrombocytopathy (the main group of patients) with the carrier of this genotype is 1.8 at $\chi2$=3.04; P=0.08; 95% CI 1.49-2.14, this indicates a trend towards association. In the subgroup of patients with HTP, the frequency of this allele was 2.48% ($\chi2$=4.18; P=0.041) and in the subgroup of patients with ATP 2.67% ($\chi2$=1.63; P=0.20), respectively. It is obvious that our results indicate an association between adverse hetero/homozygous genotypes with the development of disaggregation thrombocytopathy.

The distribution frequencies of the rs2046934 polymorphism genotypes of the P2RY12 gene in both the study and control groups corresponded to the expected distribution according to the Hardy-Weinberg equilibrium (P>0.05).

Table 1: Frequency distribution of alleles and genotypes of polymorphism of the P2RY12 gene rs2046934 in patient and control groups

№	Group	n	Allele frequency				The frequency distribution of genotypes					
			G		A		G/G		G/A		A/A	
			n	%	n	%	n	%	n	%	n	%
1	General group	71	118	83,1	24	16,9	51	71,8	16	22,5	4	5,6
A	HTP	39	63	80,8	15	19,2	27	69,2	9	23,1	3	7,7
B	ATP	32	55	85,9	9	14,1	24	75,0	7	21,9	1	3,1
2	Control group	48	88	91.7	8	8.3	40	83,3	8	16,7	0	0

CONCLUSION

One of the promising approaches in determining the risk of development of disaggregation thrombocytopathy is the study of genetic markers determining the risk of development and course of the disease. With this in mind, it is undoubtedly of great scientific and practical interest to study the significance of polymorphisms of genes responsible for the functional activity of platelets, in particular of polymorphism of the P2RY12 gene rs2046934.[12,13]

In conclusion, it should be noted that the frequency of A/A and G/A genotypes of the P2RY12 gene in our sample was somewhat comparable with the

world data and is consistent with the assumption that they affect the development of hemorrhagic states in disaggregation thrombocytopathy. Results inconsistent with data of other researchers do not contradict conclusions, and can have regional feature.

REFERENCES

1. Andrew D. JohnsonThe Genetics of Common Variation affecting Platelet Development, Function and Pharmaceutical Targeting J ThrombHaemost. Author manuscript; available in PMC 2012 Jul 1. doi:10.3324/haematol.13611
2. Annabel Maclachlan, Steve P. Watson, and Neil V. Morgan Inherited platelet disorders: Insight from platelet genomics using next-generation sequencing Platelets. 2017 Jan 2; 28(1): 14–19. doi:10.1080/09537104.2016.1195492
3. Assaf Arie Barg,Hagit Hauschner et al. From thrombasthenia to next generation thrombocytopenia: Neonatal alloimmune thrombocytopenia induced by maternal Glanzmann thrombasthenia 14 September 2018, https://doi.org/10.1002/pbc.27376
4. Gessica Fontana, Jerry Ware and Marco Cattaneo Haploin sufficiency of the platelet P2Y12 gene in a family with congenital bleeding diathesis Haematologica. 2009 Apr; 94(4): 581–584. doi:10.3324/haematol.13611
5. Isabel Sánchez-Guiu, Ana I Antón et al. Functional and molecular characterization of inherited platelet disorders in the Iberian Peninsula: results from a collaborative study Published online 2014 Dec 24. doi:10.1186/s13023-014-0213-6
6. Juan Fang, Kairbaan Hodivala-Dilke Therapeutic expression of the platelet-specific integrin, αIIbβ3, in a murine model for Glanzmann thrombasthenia Blood. 2005 Oct 15; 106(8): 2671–2679. doi:10.1182/blood-2004-12-4619
7. Katalin Koltai, Gabor Kesmarky Platelet Aggregometry Testing: Molecular Mechanisms, Techniques and Clinical Implications Int J Mol Sci. 2017 Aug; 18(8): 1803. doi:10.3390/ijms18081803
8. Kathleen Freson, Veerle Labarque et al.What's new in using platelet research? To unravel thrombopathies and other human disorders Eur J Pediatr. 2007 Dec; 166(12): 1203–1210. Published online 2007 Jul 10. doi:10.1007/s00431-007-0543-7
9. Konety A. RW Special review: genetics of platelet-derived bleeding disorders. Thrombocytes. 2017 Jan; 28 (1) doi:10.1080/09537104.2016.1262013
10. Leinøe, Eva; Zetterberg, Eva; Kinalis, Savvas et al. Application of whole-exome sequencing to direct the specific functional testing and
11. diagnosis of rare inherited bleeding disorders in patients from the Öresund Region, Scandinavia Published in: British Journal of Haematology DOI:10.1111/bjh.14863
12. Nurden AT, Breillat C, Jacquelin B, Combrie R, Freedman J, Blanchette VS, Schmugge M, Rand ML. Triple heterozygosity in the integrin alpha IIb subunit in a patient with Glanzmann's thrombasthenia. J Thromb Haemost. 2004; 2:813–819. doi: 10.1046/j.1538-7836.2004.00711.x. [PubMed] [Cross Ref]
13. Paolo Gresele, Emanuela Falcinelli, Loredana Bury Inherited platelet function disorders: Diagnostic approach and Management Article in Hamostaseologie August 2016. DOI: 10.5482/HAMO-16-02-0002
14. Piotr K. Janicki 1, Ceren Eyileten 2 et al. Population-Specific Associations of Deleterious Rare Variants in Coding Region of P2RY1–P2RY12 Purinergic Receptor Genes in Large-Vessel Ischemic Stroke Patients International Journal of
15. Molecular Sciences Received: Int. J. Mol. Sci. 2017, 18, 2678; doi:10.3390/ijms18122678
16. Sara Orsini, Patrizia Noris, Loredana Bury et al. Bleeding risk of surgery and its prevention in patients with inherited platelet disorders, Haematologica, (2017)1192–1203. doi:10.3324/haematol.2016.160754
17. Sarah K. Westbury,Matthias Canault, Daniel Greene, et al. Expanded repertoire of RASGRP2 variants responsible for platelet dysfunction and severe bleeding, Blood, 2017, 1026-1030 doi:10.1182/blood-2017-03-776773
18. Swathi J, Gowrishankar A et al. A rare case of bleeding disorder: Glanzmann's thrombasthenia Ann Afr Med. 2017 Oct-Dec; 16(4):196-198.
19. T J Kunicki, D Pidard, J P Cazenave et al. Inheritance of the human platelet alloantigen, PlA1, in type I Glanzmann's thrombasthenia J Clin Invest. 1981 Mar; 67(3): 717–724. doi:10.1172/JCI110088
20. Thomas A. Blair, Alan D. Michelson, and Andrew L. Frelinger, III Mass Cytometry Reveals Distinct Platelet Subtypes in Healthy Subjects and Novel Alterations in Surface Glycoproteins in Glanzmann Thrombasthenia Published online 2018 Jul 9 doi:10.1038/s41598-018-28211-5

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