Polymorphisms in *FCGR2A* (131H/R) and *FCGR2B* (232I/T) are associated with the development of inhibitors in Chinese hemophilia A patients

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Abstract

Background: Present study was to explore the association between gene polymorphisms in Fc gamma receptor IIa (*FCGR2A*) and IIb (*FCGR2B*) and factor VIII (FVIII) inhibitor development in patients with hemophilia A (HA) in a Chinese Han population.

Methods: *FCGR2A* (131H/R) and *FCGR2B* (232I/T) polymorphisms were genotyped using PCR and direct sequencing method in 108 HA patients, including 23 cases with inhibitors and 85 without inhibitors. Chi-square (χ^2) test was applied to compare the genotype and allele frequencies between two groups. Odds ratio (OR) and 95% confidence interval (95%CI) were calculated to indicate the relative susceptibility of HA.

Results: *FCGR2A* 131RH genotype frequency had a significantly increased trend in inhibitor group compared with the non-inhibitor group, suggesting a momentous role of 131H/R polymorphism for inhibitor development in HA patients. Individuals carrying 131RH genotype showed higher risk to be attacked by the inhibitor development in HA patients (OR=4.929; 95%CI=1.029-23.605). However, there were no significant differences of *FCGR2B* (232I/T) polymorphism genotype and allele frequencies between inhibitor and non-inhibitor groups.

Conclusion: *FCGR2A* (131H/R) was in relation to the susceptibility of FVIII inhibitors development in HA patients.

Background

Hemophilia A (HA) is an X-linked recessive bleeding disorder resulting from a quantitative or qualitative deficiency in the factor VIII (FVIII) protein [1-5]. Manifestation of neutralizing antibodies (inhibitors) against infused FVIII protein is the most burdensome complication of HA patients [6]. In China, overall prevalence of FVIII inhibitors in severe HA patients is showed to reach 4.3% [7], which is much lower than the value reported in other ethnic groups. As a typical multifactorial trait, there are several risk factors identified for inhibitor formation in HA patients, which divided into two groups as follows: environmental factors and genetic factors [8-10]. Main genetic predisposition for inhibitor development in HA patients is the causative FVIII genotype, but it also includes a group of auxiliary risk factors that are weaker than the FVIII genotype, including a family history of inhibitors, ethnicity, human leucocyte antigen haplotype, and polymorphisms of immune system-related genes, such as interleukin (*IL*)-1, *IL*-2, *IL*-10, tumor necrosis factor (*TNF*)- α and cytotoxic T lymphocyte-associated protein (*CTLA*)-4 [11-14].

Receptors of the Fc region of IgG (Fc γ R) are glycoproteins that are usually detected at hematopoietic system. These receptors have a pivotal relevance of cellular with humoral immunity via binding to the Fc domain of the IgG sub-types (*IgG*1-4). Low-affinity Fc γ Rs (Fc γ RIIa, Fc γ RIIb, Fc γ RIIc, Fc γ RIIIa and Fc γ IIb) modulate both pro- and anti-inflammatory response, and vary in their affinity with the antibody Fc-fragment and in the signaling pathways induced. They are encoded by the FCGR genes (*FCGR2A*, *FCGR2B*, *FCGR2C*, *FCGR3A* and *FCGR3B*) clustered on chromosome 1q23-24 [15]. Recently, *FCGR* gene

polymorphisms are demonstrated to be associated with autoimmune diseases. But few is known about the *FCGR2A* and *FCGR2B* gene polymorphisms and the inhibitor development of HA.

The current study was aimed at evaluating whether *FCGR2A* 131H/R (rs1801274) and *FCGR2B* 232I/T (rs1050501) polymorphisms influence the risk of inhibitors development in 108 patients with HA in Chinese Han population.

Methods

Patients

Among 108 unrelated HA patients, 23 patients had been diagnosed as having FVIII inhibitors, and 85 patients had no inhibitors. All patients received on-demand treatment with plasma-derived FVIII. All non-inhibitors patients had more than 5 exposure months. Nijmegen-modified Bethesda assay was used for quantification of the FVIII inhibitor titre. Inhibitor titres ≥ 0.6 BU/mL at any time were considered to be inhibitor positive [16]. Antibody titers <5 BU/mL was defined as low-response inhibitors, while inhibitor titres persistently ≥ 5 BU/mL were defined as high-response inhibitors [17]. Severity of haemophilia was classified according to the criteria of White et al. [18]. The study was permitted by the medical ethics committee of PanYu Central Hospital Hospital. Subjects were signed the written informed consent.

Detection of polymorphisms

Genomic DNA was extracted from the peripheral blood of every individual using vacuum vessel collection tube. TIANGEN biochemical blood genome DNA extraction Kit (TIANGEN, BEIJING) was used to extract the genomic DNA based on the manufacturer's specification. Primers of *FCGR2A* 131H/R and *FCGR2B* 232I/T single nucleotide polymorphisms (SNPs) were designed and synthesized by Sangon Biotech (Shanghai) Co., Ltd. Genotyping of the *FCGR2A* and *FCGR2B* was conducted by PCR amplification and direct sequencing method on an ABI PRISM Sequence Detection System 7500 (Applied Biosystems Foster City, CA, USA) by DNA sequencing.

Statistical analysis

All the data were analyzed by SPSS software 18.0 (SPSS Inc., Chicago, IL, USA). Allele and genotype frequencies were calculated using direct counting. Data in with or without inhibitors groups were compared by χ^2 test. Hardy-Weinberg equilibrium (HWE) was detected in these two groups. Odd ratio (OR) and 95% confidence interval (95%CI) were calculated using Chi-square test. *P* value less than 0.05 was regarded to be statistical significant.

Results

Basic information of patients

A total of 108 HA patients were enrolled in this study, including 82 cases with severe disease, 21 cases with moderate disease and 5 cases with mild disease. 23 patients had inhibitors, 85 patients did not have inhibitors.

HWE test

Genotypes of *FCGR2A* 131H/R and *FCGR2B* 232I/T polymorphisms in HA patients with and without inhibitors did not deviated from HWE test, revealing the reliability of the study samples.

Relationship of FCGR2A (131H/R) and FCGR2B (232I/T) gene polymorphisms with HA susceptibility

FCGR2A (131H/R) exhibited significant differences between the inhibitor and non-inhibitor groups. Frequency of 131RH genotype was higher in inhibitor group than in non-inhibitor group ($P=0.032$). When the 131RR genotype was taken as a reference, the 131RH genotype was associated with an increased risk of FVIII inhibitor development (OR=4.929, 95%CI=1.029-23.605). Although 131HH genotype had higher frequency in non-inhibitor group than in inhibitor group, the difference was not significant. 131H allele was also had no significant association with the development of FVIII inhibitor.

232II, 232IT, and 232TT genotype frequencies of *FCGR2B* 232I/T SNP were 75.29%, 22.35%, and 2.35% in non-inhibitor group and 69.57%, 21.74%, 8.70% in inhibitor group, respectively (**Table 1**). Besides, the allele 232I and 232T frequencies were 86.47% and 13.53% in non-inhibitor and 80.43%, 19.57% in inhibitor group. But all differences of genotype and allele distributions did not reach a significant level ($P>0.05$). It suggested that 232I/T might not be in relation to the susceptibility of the FVIII inhibitor development in HA patients.

Discussion

As is known to all, cytokines are more or less directly in relation to the antibody-modulated immune responses [19, 20]. In autoimmune diseases, polymorphisms in the immune response genes were reported to be related to the formation of antibody. In addition, SNPs in the regulatory regions of cytokine genes are crucial components in the pathogenesis of various diseases, including HA [21, 22]. Individual immune response traits might influence a patient's reaction to exogenous factor VIII. The development of FVIII inhibitor is the main complication of replacement therapy in HA patients. Therefore, it is necessary to recognize indicators which could induce the inhibitors formation for applying appropriate treatments and avoiding the inhibitor response.

FcγR give a link between antigen-specific antibody and non-specific cellular responses of the native immune system. These reports are mainly detected at haemopoietic cells, and have important roles for activating and modulating immune responses. In human autoimmune diseases, published studies have demonstrated that genetic variants in FcγRs, particularly *FCGR2A* 131H/R, *FCGR2B* 232I/T, were showed to be in relation to rheumatoid arthritis, systemic lupus erythematosus [23, 24]. In the autoimmune disorders above mentioned, *FCGR2A* 131H/R polymorphism influences FcγRIIa affinity to IgG2,

phagocytosis, and immune complexes clearance. Nevertheless, to date, no examination was performed about the association of FcγR polymorphisms with the inhibitor development in HA patients.

In present study, association of *FCGR2A* and *FCGR2B* SNPs with inhibitors development was detected in HA patients in a Chinese Han Population. When the allelic and genotypic frequencies of *FCGR2A* 131R/H were compared, 131RH genotype frequency showed remarkable increase trend in inhibitor group, and 131RH genotype carriers had higher risk to be attacked by inhibitors development. It was suggested that *FCGR2A* 131R/H displayed significant association with positive inhibitor development in HA patients. In recent years, increasing studies have reported that there are significant differences between SNPs of the *FCGR* genes and their association with the inhibitors development of HA patients in different ethnic groups. In a Caucasian population, the polymorphism 131R>H of *FCGR2A* gene was in relation to an increased risk of inhibitor development [25]. Our study results were in accordance with the previous evidences. Meanwhile, *FCGR2A* has also been largely researched in other human diseases. For instance, Schneider et al. reported that *FCGR2A* 131R/H polymorphism is correlated with impaired endothelium-dependent vasodilatation [26]. However, genotype and allele distributions of *FCGR2B* 232I/T polymorphism had no significant difference between inhibitor and non-inhibitor groups. It suggested that *FCGR2B* 232I/T polymorphism had no significant correlation with FVIII inhibitor development in HA patients.

Conclusion

Taken together, *FCGR2A* 131R/H polymorphism was closely correlated with FVIII inhibitor development of HA patients in the Chinese Han Population, while no association was found between *FCGR2B* 232I/T polymorphism and FVIII inhibitor development.

Even so, some shortages remains to be stated in the present study. Firstly, the sample size might be not large enough for the correlation analysis. Secondly, the OR value was not corrected by clinical data, which may reduce the statistical power for the determination of differences between two groups. In additional, functional study would also be necessary to identify the molecular mechanisms underlying the association. Further researches should be performed to explore whether the *FCGR2A* and *FCGR2B* gene polymorphisms were in relation to the inhibitor development of HA patients in other ethnic groups.

Abbreviations

Fc gamma receptor IIa (FCGR2A)

Fc gamma receptor lib (FCGR2B)

Factor VIII (FVIII)

Hemophili A (HA)

Odds ratio (OR)

95% confidence interval (95%CI)

Tumor necrosis factor (TNF)

Cytotoxic T lymphocyte-associated protein (CTLA)

Fc region of IgG (FcγR)

IgG sub-types (IgG1-4)

single nucleotide polymorphisms (SNPs)

Hardy-Weinberg equilibrium (HWE)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of PanYu Central Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

Consent for publication

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Data availability

b)All data generated or analysed during this study are included in this published article

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Competing interest

The authors declare that they have no competing interests.

Authors' contributions

H.Q. design of the work; Y.C. the acquisition, analysis, W.Z. interpretation of data; X.H. the creation of new software used in the work; S.C. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Tables

Table 1. Genotype and allele distributions of *FCGR2A* 131H/R and *FCGR2B* 232I/T polymorphisms in non-inhibitor and inhibitor patients.

Genotype/allele	Non-inhibitor n=85 (%)	Inhibitor n=23 (%)	P value	OR (95%CI)
131H/R				
131RR	23 (27.06)	2 (8.70)	-	-
131RH	35 (41.18)	15 (65.22)	0.032	4.929 (1.029-23.605)
131HH	27 (31.76)	6 (26.09)	0.265	2.556 (0.470-13.908)
131R	81 (47.65)	19 (41.30)	-	-
131H	89 (52.35)	27 (58.70)	0.184	0.640 (0.331-1.239)
P_{HWE}	0.107	0.182		
232I/T				
232II	64 (75.29)	16 (69.57)	-	-
232IT	19 (22.35)	5 (21.74)	0.929	1.053 (0.341-3.249)
232TT	2 (2.35)	2 (8.70)	0.154	4.000 (0.523-30.612)
232I	147 (86.47)	37 (80.43)	-	-
232T	23 (13.53)	9 (19.57)	0.307	1.555 (0.664-3.640)
P_{HWE}	0.681	0.138		