*Experimental Neurobiology*
Vol. 17, pages 7~10, June 2008

# A Polymorphism of Fibrinogen Beta Chain (*FGB*) Gene is Not Associated with Autistic Spectrum Disorder in Korean Population

Shihwan Chang[1], Ah Rang Cho[1,2], Bum Shik Kim[1,3] and Joo-Ho Chung[1]*

[1]Kohwang Medical Research Institute, Department of Pharmacology, [2]Department of Neuropsychiatry, [3]Department of Thoracic and Cardiovascular Surgery, School of Medicine, Kyung Hee University, Seoul 130-701, Korea

## ABSTRACT

Evidences has been accumulated the difference of cardiovascular phenotypes in autistic spectrum disorder (ASD). To determine the genetic association between fibrinogen beta chain (*FGB*) gene and ASD in Korean population, we genotyped single nucleotide polymorphism (SNP) (rs4220, Arg478Lys, exon 8) in the *FGB* gene by using direct sequencing. Among nonsynonymous SNPs in the coding region of *FGB*, only one SNP's heterozygosity (rs4220) is more than 0.05. Therefore, we analyzed the association between rs4220 and ASD. Three hundred six control and 196 ASD subjects were evaluated. For the analysis of genetic data, SNPStats, SNPAnalyzer, and Helixtree programs were used. Multiple logistic regression analysis (codominant, dominant, and recessive models) was also used. The result showed that a SNP (rs4220) in the *FGB* gene was not significantly difference between ASD and controls in three alternative models. This result suggests that the *FGB* gene may have no relation to the development of ASD.

**Key words:** association, autistic spectrum disorder, fibrinogen beta chain, single nucleotide polymorphism

## INTRODUCTION

Autistic spectrum disorder (ASD) is a complex developmental disorder that is classified as one of pervasive developmental disorders (PDD) (Shastry, 2003; Baron-Cohen, 2004; Muhle et al., 2004). ASD shows a variety of symptoms, such as slow language, problems in social relationship, inconsistent sensory reaction, irregularity in intellectual functioning, and limited interests. ASD occurs regardless of racial, ethnic, or social backgrounds. Most patients (80%) are males, and it is generally accepted that ASD occurs in about 4 or 5 out of every 10000 births (Muhle et al., 2004). Data from several epidemiological twin and family studies reveal that ASD is one of the most heritable complex disorders (Shastry, 2003).

Fibrinogen, together with fibrin, serves as the active agent for coagulation. Fibrinogen molecule is an elongated 45 nm structures that is made of two sets of three polypeptide chains, $\alpha$, $\beta$, and $\gamma$ (Mosesson, 2001, 2005; Lord, 2007). These polypeptide chains are encoded by 3 corresponding genes located on chromosome 4 in region q28

*To whom correspondence should be addressed.
TEL: 82-2-961-0281, FAX: 82-2-968-0560
e-mail: jhchung@khu.ac.kr
Received May 15, 2008
Accepted for publication June 15, 2008

(Iacoviello et al., 2001). Fibrinogen beta chain (*FGB*) gene encodes $\beta$ chains of fibrinogen. It has been reported that fibrinogen represents a major cardiovascular risk factor (Ernst and Resch, 1993; Danesh et al., 2005). Sandy et al. (2007) reported the role of *FGB* −455 G/A polymorphism in cardiovascular disease risk in the STANISLAS cohort. Some studies revealed possible relationships between cardiovascular phenomena and autism. Yao et al. (2006) showed biochemical evidence for abnormal platelet reactivity and altered blood flow in children with autism. Another study measured cardiac parasympathetic activity in autistic children, and found that there was significantly lower parasympathetic activity in association with a significant elevation in sympathetic activity (Ming, 2005). Based on these findings, we hypothesized that the *FGB* gene may be related to autistic spectrum disorder.

In this study, we investigated the association between single nucleotide polymorphism (SNP) in the *FGB* gene and autism.

## MATERIALS AND METHODS

### *Subjects and DNA Samples*

Each subject was recruited from Kyung Hee University Medical Center. A total of 306 control and 196 ASD subjects were recruited for this study. Clinical diagnosis was conducted strictly according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 1994). Controls with no clinical evidence of any other disorder were recruited. DNA was isolated using the Core One Blood Genomic DNA Isolation Kit (CoreBioSystem, Seoul, Korea). This study was approved by the ethics Review Committee of the Medical Research Institute, Kyung Hee University Medical Center, Seoul, Korea (IRB number, 20040915; genetic institute, no89). Written informed consent was obtained from each subject.

### *Selection and Genotyping of SNPs*

We selected nonsynonymous SNPs within the coding region of the *FGB* gene using human SNP websites (http://www.ensembl.org; www.ncbi.nlm.nih.gov/SNP). When the SNPs with unknown heterozygosity and minor allele frequency (below 5%) were excluded, the rs4220 is only missense SNP (BUILD 129). SNP genotyping was performed by direct sequencing method. Genomic DNA was amplified using the following primers (sense, 5'-CTTGACCACCGTAGTTCTGTTT-3'; antisense, 5'-CTTGGTGAGCAAGAGAAATGAAG-3'; size, 484 bp). The samples were sequenced using an ABI Prism 377 automatic sequencer (PE Applied Biosystems, Foster City, CA, USA), and data were analyzed using the SeqManII software (DNASTAR Inc., Madison, WI, USA).

### *Statistical Analysis*

Multiple logistic regression models (codominant, dominant, and recessive) were calculated for the odds ratio (OR), 95% confidence interval (CI), and corresponding p values, controlling for age and gender as covariables. We used SNPStats (Sole et al., 2006) and SNPAnalyzer (ISTECH Inc., Goyang, Korea) to analyze the association between SNP and schizophrenia. We set the level of significance at 0.05 of p-value in statistical test.

## RESULTS

The rs4220 SNP in the *FGB* gene was polymorphic. In the control group, genotype frequencies for rs4220 were in Hardy–Weinberg equilibrium ($p > 0.05$, data not shown). As shown in Table 1, genotype distributions of the SNP in ASD and control

**Table 1.** Logistic regression analysis and genotype frequencies of fibrinogen beta chain (*FBG*) gene in Korean autistic spectrum disorder (ASD) and control subjects

| Locus | Genotype | ASD | Controls | Model | OR (95% CI) | p |
|---|---|---|---|---|---|---|
| | | n=196 (%) | n=306 (%) | | | |
| rs4220 (Arg478Lys) (Exon8) | G/G | 125 (63.78) | 223 (72.88) | Codominant | 0.73 (0.17～3.19) | 0.19 |
| | G/A | 67 (34.18) | 77 (25.16) | Dominant | 0.67 (0.43～1.03) | 0.069 |
| | A/A | 4 (2.04) | 6 (1.96) | Recessive | 0.83 (0.19～3.59) | 0.80 |

OR: Odds ratio, CI: confidence interval.

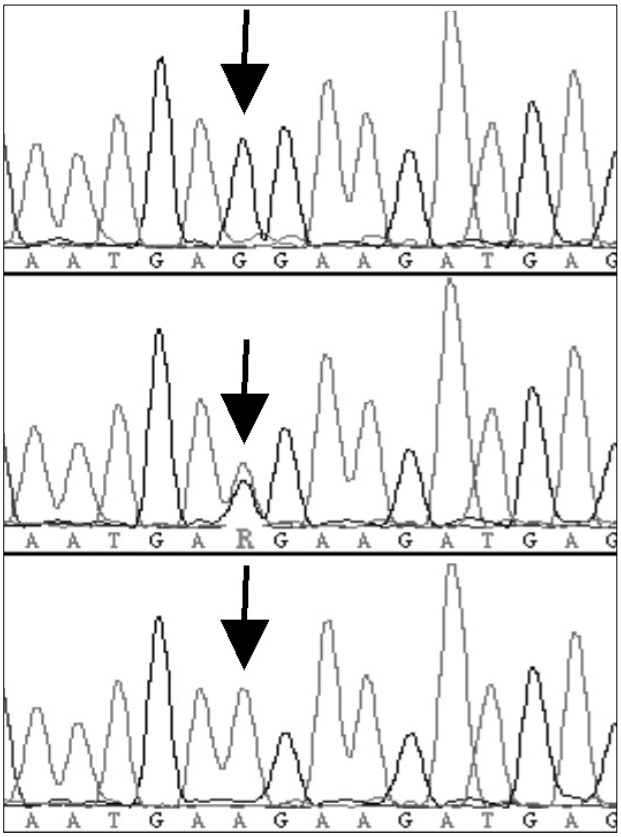


**Fig. 1.** Direct sequencing of PCR-amplified DNA including rs4220 polymorphism of the fibrinogen beta chain (*FGB*) gene. Arrows indicate electropherograms of nucleotide showing the homotype GG (top), heterotype GA (middle), and homotype AA (bottom). R means G and A nucleotides. Green color indicates the A allele, red the T allele, and blue the G allele.

subjects are summarized. Genotype frequencies of rs4220 showed no significant differences between ASD and health controls. The rs4220 (+1458G>A) is located on exon 8, and is a missense SNP (Arg478Lys) with 0.250 heterozygosity (http://www.ncbi.nlm.nih.gov/SNP). GG, GA, and AA genotype frequencies are reported to be 0.617, 0.350, and 0.033 in European, 0.689, 0.267, and 0.044 in Chinese, 0.773, 0.205, and 0.023 in Japanese, 0.850, 0.133, and 0.017 in Sub-Saharan African, respectively (http://www.ncbi.nlm.nih.gov/SNP). In this study, GG, GA, and AA genotype frequencies in Korean normal population were 0.729, 0.252, and 0.020, respectively, which are alsosimilar to those in Japanese. However, in Korean ASD group, the GG, GA, and AA genotypefrequencies were 0.638, 0.342, and 0.020, respectively (Fig. 1, Table 1).

## DISCUSSION

To our knowledge, this study is a first attempt to reveal an association between polymorphisms in the *FGB* gene and autism. Conversion of fibrinogen to fibrin is triggered by thrombin, which cleaves fibrinopeptides A and B from alpha and beta chains. FGB protein (P02675) consists of 491 amino acids, and contains 2 sets of 3 non-identical chains (alpha, beta and gamma). Amino acids from 45 to 491 convert fibrinogen beta chain, 31 to 44 potential fibrinopeptide B, 237 to 487 potential fibrinogen C-terminal domain, 45 to 47 binding distal domain of another fibrin. A long coiled coil structure formed by 3 polypeptide chains connects the central nodule to the C-terminal domains. The long C-terminal ends of the alpha chains fold back, contributing a fourth strand to the coiled coil structure (UniProt, http://beta.uniprot.org; SwissProt, http://www.expasy.org). Recently, Xing et al. (2006) reported that the association between fibrinogen B beta −1420G/A, −993C/T, and −854G/A polymorphisms and coronary heart disease. Sun et al. (2004) also reported that the relationship between fibrinogen B beta gene −455G/A polymorphism and atherosclerotic cerebral infarction. However, we did not find the association between rs4220 and ASD.

In conclusion, this study reveals that rs4220 polymorphism of the *FGB* gene is not associated with the susceptibility of ASD in Korean population.

## ACKNOWLEDGMENTS

This study was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (R11-2005-014), and partially supported by the Governance Program of Kyung Hee University.

## REFERENCES

Baron-Cohen S (2004) The cognitive neuroscience of autism. *J Neurol Neurosurg Psychiatry* 75:945-948.

Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R and Kostis JB, et al. (2005) Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality - an individual participant meta-analysis. *JAMA* 294:1799-1809.

Ernst E and Resch KL (1993) Fibrinogen as a cardiovascular risk factor. *Ann Intern Med* 118:956-963.

Faul F, Erdfelder E, Lang AG and Buchner A (2007) G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 39:175-191.

Iacoviello L, Vischetti M, Zito F and Benedetta Donati M (2001) Genes encoding fibrinogen and cardiovascular risk. *Hypertension* 38:1199-1203.

Lord ST (2007) Fibrinogen and fibrin: scaffold proteins in hemostasis. *Curr Opin Hematol* 14:236-241.

Ming X, Julu PO, Brimacombe M, Connor S and Daniels ML (2005) Reduced cardiac parasympathetic activity in children with autism. *Brain Dev* 27:509-516.

Mosesson MW, Siebenlist KR and Meh DA (2001) The structure and biological features of fibrinogen and fibrin. *Ann N Y Acad Sci* 936:11-30.

Mosesson MW (2005) Fibrinogen and fibrin structure and functions. *J Thromb Haemost* 3:1894-1904.

Muhle R, Trentacoste SV and Rapin I (2004) The genetics of autism. *Pediatrics* 113:472-486.

Sandy M, Bérangère M, Monique V, Gérard S and Sophie V (2007) Analysis of the effect of multiple genetic variants of cardiovascular disease risk on insulin concentration variability in healthy adults of the STANISLAS cohort. *Atherosclerosis* 191:369-376.

Scott EM, Ariëns R and Grant PJ (2004) Genetic and environmental determinants of fibrin structure and function. *Arterioscler Thromb Vasc Biol* 24:1558-1566.

Shastry BS (2003) Molecular genetics of autism spectrum disorders. *J Hum Genet* 48:495-501.

Solé X, Guinó E, Valls J, Iniesta R and Moreno V (2006) SNPStats: a web tool for the analysis of association studies. *Bioinformatics* 22:1928-1929.

Sun H, Lu FH, Tian Q, Wen PE, Wu F and Wang X (2004) Relationship between fibrinogen B beta gene FGB -455G/A polymorphism and atherosclerotic cerebral infarction][Article in Chinese. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 21: 382-385.

Xing HY, Cai WW, Li XM, Fu Q and Liu GX (2006) Association of fibrinogen B beta -1420G/A, -993C/T and -854G/A gene polymorphism with coronary heart disease. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 23:622-626.