POLYGENIC SCORE ANALYSIS TO FIND GENETIC FACTORS OF INHIBITOR DEVELOPMENT IN KOREAN SEVERE HEMOPHILIA A PATIENTS USING WES

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Introduction

The development of inhibitor against factor VIII is serious complication in the treatment of hemophilia A. *F8* mutation type play an important role in inhibitor development, however, patients who share the same *F8* mutation with discordant inhibitor status suggest the presence of other genetic risk factors. Studies on genetic determinants other than *F8* mutation yielded inconsistent results, therefore the genetic background of the risk remain largely unknown.

Aims

This study was designed to investigate the genetic risk factors underling the development of inhibitor in Korean severe hemophilia A patients by whole exome sequencing.

Methods

A total of 250 severe hemophilia A patients (142 with inhibitors and 108 without inhibitors) underwent sequencing of the exome on NextSeq500 (Illumina) following capture by SureSelect Human All Exon V5 UTR kit (Agilent). Mapping of reads was performed on reference genome (hg19) by BWA and variant calling by GATK. Initially, we screened *F8* mutations and the candidate mutations were confirmed by Sanger sequencing, MLPA and inversion 22 test. Then we performed case (inhibitor) control (non-inhibitor) association studies using SNP-set Kernel Association Test (SKAT-O test, gene-based test) and Fisher's exact test and applied polygenic risk scores.

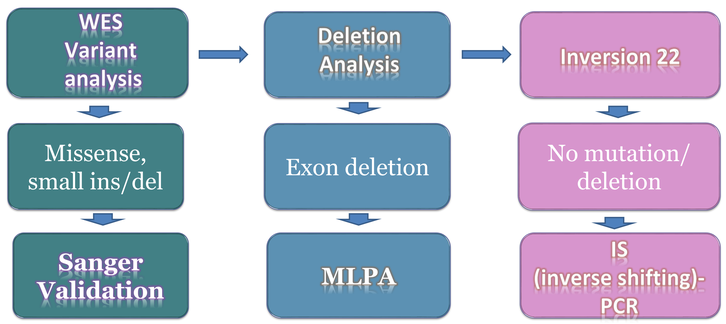

Figure 1. *F8* mutation Work up.

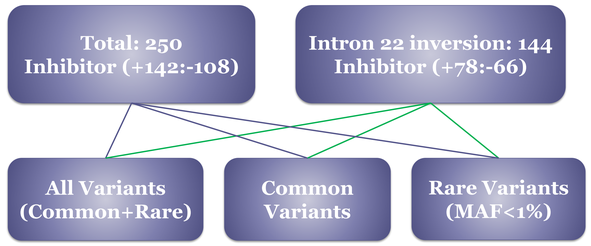

Figure 2. Scheme of WES Analysis

Results

A mean depth of 92X was achieved and the mean 72,125 variants were identified per patient. Large deletion, nonsense, small insertion/deletion, missense mutation and inversion 22 of *F8* were found in 11, 27, 22, 46 and 144 patients, respectively

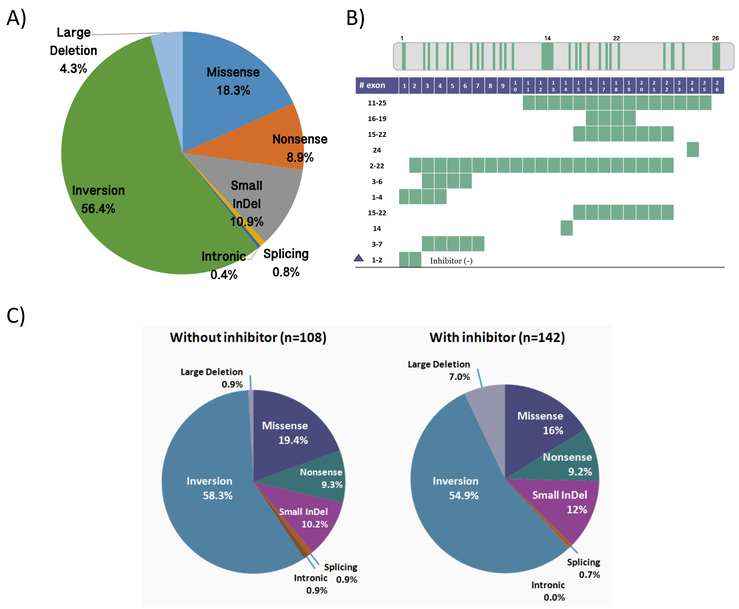

Figure 3. Summary of F8 variants . A) *F8* variant type of 250 samples. B) *F8* exon deletion pattern. C) *F8* mutation patterns by the presence of inhibitor

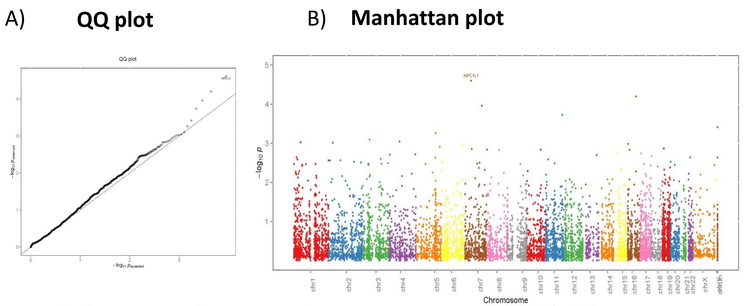

Figure 4. Results of Exome-wide association study A) QQ plot. B) Manhattan plot (SNP-Set Kernel Association Test)

All except one case with large deletions developed inhibitors. We found the 12 immune genes at *P<0.05* and odds ratio>1 by SKAT-O test and 24 immune related variants at *P<0.05* and odds ratio>1 by Fisher's exact test. When we narrowed the patients with inversion 22, 7 immune related genes were identified which had *P<0.05* and odds ratio>1 and among them, CD226 (PTA1 (platelet and T cell activation antigen 1)) gene had the highest odds ratio (6.8). We applied the polygenic scores and achieved AUC 90.8% using 68 variants which had *P<0.000365*.

Table 1. Result of gene-level analysis: Immune-related genes (OR>3) in inversion patients

Gene	*P* value	Variant No.	Case No.	Control No.	Case No. Var alleles	Control No. Var alleles	OR
CD226	0.031	1	78	63	8	1	6.75

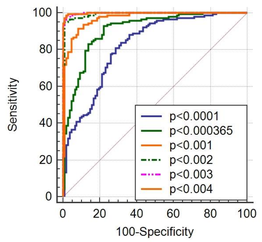

Figure 5. Polygenic Risk Score to predict the risk of development inhibitor

Conclusion

This is fist study which applied polygenic scores to predict the risk of development inhibitor in Korean hemophilia A patients.

References

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Ionita-Laza, I.*, Lee, S.*, *et al.* Sequence kernel association tests for the combined effect of rare and common variants. *American Journal of Human Genetics,* (2013) *92, 841–853*
Euesden, Jack, Cathryn M. Lewis, and Paul F. O'Reilly. "PRSice: Polygenic Risk Score software." Bioinformatics (2014): btu848.