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chip assays were conducted by using the chip kit ab500 (abcam, usa) and nf-b antibody (proteintech) according to the manufacturers instructions. the immunoprecipitate was eluted with 100l dna purifying slurry, and 2l of dna was used in qpcr. input dna and nf-b-enriched dna fragments were amplified by using qpcr in a 7500 fast real-time pcr system (applied biosystems) with primers 5-attctcaccatgggaatgg-3 and 5gaggacagcaaggtaatag-3. the results are shown as percentage input. based on the results of the chip assay, we found that the gg type of nf-b (p50p65) binds to the mlip-it1 region, which is located on human chromosome 11 and contains an nf-b binding site, rs9395890. we found that the transcriptional activity of the nf-b binding site rs9395890 with the tt type was higher than that of the gg type. and we assessed the functionality of the nf-b binding site rs9395890 t/g polymorphism for effects activity by luciferase reporter assay. our experimental fig. 3 showed that the transcriptional activity of the t allele was higher than g allele, but the relative light units (rlu) data was no significant difference between with t and g allele. so far there were no study about the rs9395890 and mlip-it1. however it might be the distance between rs9395890 and mlip-it1 is too far away. chip-seq data from the jaspar database were used to confirm whether the genetic variants had a combination of the following sites [24]: e2f4 (matrix id: ma0470.1) [25], egr1 (matrix id: ma0162.2) [26], jun (matrix id: ma0488.1) [27], sp1 (matrix id: ma0079.3) [28], tcf7l2 (matrix id: ma0523.1) [29], and tp53 (matrix id: ma0106.2) [30]. as chip-seq data were not available for ctnnb1, further examination of this tf did not perform in this study. finally, a total of 14 snps were successfully selected: rs55785541, rs2295624, rs79436692, rs1243673, rs6108246, rs6688233, rs130347, rs3758354, rs3803353, rs77796751, and rs28481460 (figure 1).

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We downloaded the human reference gene sequence of GRCh37/hg19 from the National Center for Biotechnology Information (NCBI) in combination with the human biological database in Taiwan and found that NF-B (p50p65) contained 271,063 potential variants in the human genome based on the sequence of the above binding sites. Of the TFBSs, we compared the remaining 36,041,790 SNPs in the first step and found that 3,121,467 SNPs were near the 271,063 potential TFBSs, of which 40,137 SNPs were even in the TFBS of NF-B. Additionally, mutation of this site will likely result in NF-B (p50p65) being unable to bind. Finally, a total of 5766 SNPs with a minor allele frequency>5% were screened for further follow-up by ChIP-Seq analysis due to the limited number of samples subject to subsequent analysis in this study [36]. We downloaded the human reference gene sequence of GRCh37/hg19 from the National Center for Biotechnology Information (NCBI) in combination with the human biological database in Taiwan and found that NF-B (p50p65) contained 271,063 potential variants in the human genome based on the sequence of the above binding sites. We studied the outcome variable of VTE case status and predictor variable of single-nucleotide variant (SNV) with covariates of age, sex, chip array at runtime, and principal components using R and SNPTEST () statistical software programs. 35 In addition, we performed a secondary analysis where previously associated SNPs were included as covariates (ie, conditional analysis). We used the reSEM package to perform robust linear regression (random coefficients model) with outcome variable of case status, predictor variable of genotype coded in an additive fashion, and covariates of age, sex, chip array at runtime, and principal components using R and SNPTEST ( ) statistical software programs. 36 5ec8ef588b

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