The Effect of Common Variants in SLC44A2 on the Contribution to the Risk of Deep Vein Thrombosis after Orthopedic Surgery

Liqiang Zhi¹, Weilou Feng², Jingqi Liang³, Qing Zhong¹, Liaooyuan Ren⁴, Jianbing Ma¹ and Shuxin Yao¹

Liqiang Zhi, Weilou Feng and Jingqi Liang contributed equally to this work.

1 Department of Joint Surgery, Honghui Hospital, Xi’an Jiaotong University, Xi’an, Shaanxi, China
2 Department of Traumatic Orthopedics, Honghui Hospital, Xi’an Jiaotong University, Xi’an, Shaanxi, China
3 Department of Foot and Ankle Surgery, Honghui Hospital, Xi’an Jiaotong University, Xi’an, Shaanxi, China
4 Department of Ultrasonography, Honghui Hospital, Xi’an Jiaotong University, Xi’an, Shaanxi, China

Aim: Deep vein thrombosis (DVT) is a common complication of orthopedic surgery. Multiple lines of evidence indicate that genetic factors play an important role in the development of DVT following orthopedic surgery (DVT-FOS). Recent evidence suggested that the solute carrier family 44 member 2 (SLC44A2) gene may contribute to the risk of DVT. In this study, we aimed to investigate the associations of SLC44A2 and DVT-FOS in Chinese Han individuals.

Methods: In the study, 2,655 subjects, including 689 DVT-FOS patients and 1,966 controls, were recruited. Eighteen SNP were genotyped in the study. Genetic association analyses were performed at both the single marker and haplotype levels. Bioinformatics analyses were conducted to predict the functional consequences of significant SNPs.

Results: SNP rs2288904 of SLC44A2 was identified as being significantly associated with DVT-FOS (P=0.0003, OR [95%CI] =1.28[1.12–1.46]). Allelic analyses showed that the G allele of this SNP significantly elevated the risks of DVT-FOS, which was replicated in the genotypic association analyses. Moreover, a two-SNP haplotype, including rs2288904, was found to be strongly correlated with the risk of DVT-FOS (P=4.15×10⁻¹¹). Wide-spread effects in the expression quantitative trait loci were identified for rs2288904 in multiple tissues.

Conclusion: In summary, our results provide further supportive evidence of the association of SLC44A2 with the risk of DVT-FOS, which also provide clues for understanding the important roles of the SLC44A2 gene in the pathogenesis of DVT-FOS and in the development of preventive strategies.

Key words: Single nucleotide polymorphisms, SLC44A2, Deep vein thrombosis, Case–control study, Genetic association

Introduction

Deep vein thrombosis (DVT) is a major medical disease, with an incidence of 67 per 100,000 cases every year¹, and is caused by venous injury, slow blood flow, or blood hypercoagulability². Severe DVT can result in postphlebitic syndrome, pulmonary embolism, and even death³. The results of some genetic studies have shown that genetic factors might contribute to the development of DVT⁴. In the past decades, family and twin studies have also confirmed that the heritability of DVT is greater than 60%⁵, ⁶. Orthopedics surgery after injury or disease is strongly associated with a risk of developing DVT. In the absence of thromboprophylaxis, venography documented DVT may occur in up to 60% of patients within 2 weeks following lower-extremity orthopedic surgery, which is far greater than the incidence
observed in healthy people\textsuperscript{7}. Postoperative complications of DVT were determined not only by environmental factors but also by genetic factors. Therefore, it is urgent to identify the susceptibility genes for DVT and to elucidate the exact molecular mechanisms of DVT following orthopedic surgery (DVTFOS).

With the development of high-throughput DNA sequencing techniques, genome-wide association (GWA) studies have provided supportive evidence for the polygenic nature of venous thromboembolism (VTE) susceptibility and have identified some SNPs that contribute to the risk of VTE\textsuperscript{8,9}; however, these results can explain only a small portion of the limited heritability due to a lack of biological interpretations. So far, the molecular mechanisms of VTE are still unknown. Recently, a meta-analysis of 12 GWA studies, involving 7,507 VTE cases and 52,632 controls, has identified an association between the exonic SNP rs2288904 within the solute carrier family 44 member 2 (\textit{SLC44A2}) gene and VTE in European populations\textsuperscript{8}). Moreover, the association between \textit{SLC44A2} and thrombosis was subsequently confirmed in another independent study of a European population, which further strengthens the evidence linking \textit{SLC44A2} and VTE\textsuperscript{9}).

As a common form of VTE, DVTFOS might also be associated with the \textit{SLC44A2} gene. Although there is evidence of significant associations with VTE in European-ancestry populations, the contributions of \textit{SLC44A2} to the risk of DVTFOS have not yet been fully investigated. Moreover, due to genetic heterogeneity, large-scale studies in non-European populations are necessary to confirm these results and to understand further the genetic origins of DVTFOS. In addition, current preoperative prevention strategies are not enough to prevent DVTFOS. Investigating the potential genetic markers contributing to the risk of DVTFOS would enable pre-surgery genetic screening and precision prevention for DVTFOS. Thus, we performed a hospital-based, case-controlled study to identify further the association between \textit{SLC44A2} and the risk of DVTFOS in Han Chinese individuals, which could provide clues for understanding the roles of \textit{SLC44A2} in the genetic predisposition to the development of DVTFOS and aid in the development of preventive strategies.

\textbf{Materials and Methods}

\textbf{Study Population}

In our study, 2,655 subjects who underwent orthopedic surgery on the knee or hip were recruited from Honghui Hospital of Xi'an Jiaotong University between April 2011 and May 2017. Among the subjects, 689 cases were diagnosed with DVTFOS (394 females and 295 males), and 1,966 controls experienced none of the typical symptoms or signs of DVT (1,126 females and 840 males) (Fig. 1). For each sub-
SNP Selection and Genotyping

We searched for all SNPs with minor allele frequencies (MAF) ≥ 0.05 within the region of the SLC44A2 gene in the 1,000 Genomes Chinese Han Beijing population. Then, MAF ≥ 0.05, with pairwise tagging, and r² ≥ 0.7 were used as the cut-off criteria during tag SNP selection, which generated 18 tag SNPs for our study. Basic information on the 18 selected SNPs is summarized in Supplemental Table 1. Genomic DNA was isolated from peripheral blood using a Tiangen DNA extraction kit (Tiangen Biotech Co. Ltd, Beijing, China), according to the manufacturer’s protocol. SNP genotyping was performed using a Sequenom MassARRAY platform with iPLEX GOLD chemistry (Sequenom, San Diego, CA, USA), based on the manufacturer’s protocols. The results were processed using Sequenom Typer 4.0 software, and genotype data were generated from the samples.

Genotyping was conducted by laboratory personnel blinded to the case–control status, and the genotyping results, data entry, and statistical analyses were independently reviewed by two authors [10, 11]. We randomly re-performed the analyses on 5% of the sample, with a concordance of 100%.

Statistical Analyses

χ² tests were performed to examine genetic associations at both the single marker and haplotype levels. In addition, logistic models were fitted for each SNP to adjust for the potential confounding effects of age and body mass index (BMI) by including both variables as covariates (because both variables had unbalanced distributions between DVT cases and controls). Plink was utilized for the statistical analyses mentioned above [12]. A Q–Q plot was made to examine the potential inflation of significant hits from single-marker-based analyses to detect the potential effects of population stratifications. Locus zoom was utilized to make a regional association plot [13]. Bonferroni corrections were applied to address multiple comparisons. For single-marker-based association analyses, the threshold for P values was 0.003 (0.05/18).

Bioinformatics Analyses

Functional analyses of SNPs with significant association signals were performed by two bioinformatics tools. For non-synonymous SNPs located within exons, SIFT was utilized to evaluate the functional consequences of SNPs on the protein encoded by its gene [14], and we also examined the expression quantitative trait loci (eQTL) patterns of these significant association hits using the GTEx database [15]. In addition, we investigated the interaction network of SLC44A2 using the STRING database, which is a database of known and predicted protein–protein interactions.

Results

In the study, significant differences in age and BMI were found between DVT FOS patients and controls (Table 1). All SNPs were in Hardy–Weinberg equilibrium in the control group (P>0.05, Supplemental Table 2). No significant clues for the inflation of -log P values can be found from the Q–Q plot (Supplemental Fig. 1). As presented in Table 2 and Fig. 1, only SNP rs2288904 of SLC44A2 was identified to be strongly associated with DVT FOS in our study subjects (P=0.0003) after adjusting for age and BMI (Table 2 and Fig. 2). The association signal was still significant after the Bonferroni correction (Pthreshold =0.05/18). The MAF of the G allele of this SNP was...
296

18 selected SNPs, and the associated SNP rs2288904 was located in block 3 (Fig. 3). Haplotypic association analyses were conducted in all LD blocks, and the results were summarized in Table 3. A two-SNP haplotype in SLC44A2 was identified to be significantly associated with DVTFOS (rs76638997–rs2288904, \(P = 4.15 \times 10^{-11}\)). The associated SNP rs2288904 was also included in this haplotype, which provided supportive evidence of the significant association of rs2288904 with the risk of DVTFOS (Table 3).

For the significant SNP, rs2288904, which results in a non-synonymous change located within the exonic region of SLC44A2, no evidence of significant

| Table 1. Characteristic and clinical information for the 2,655 study subjects |
|---------------------------------------------|-----------------|-------------|-----------------|-----------------|---------------|
|                                              | Cases (N=689)  | Controls (N=1,966) | Statistics | \(P\) |
| Age, mean ± sd                               | 59.6 ± 5.7     | 58.5 ± 6.5           | \(t=4.3\)  | 1.78 \times 10^{-5} |
| BMI, mean ± sd                               | 25.9 ± 1.6     | 25.4 ± 1.7           | \(t=6.8\)  | 1.23 \times 10^{-11} |
| Gender (%)                                   |                |                        |            |                                     |
| Male                                         | 295 (43)       | 840 (43)             | \(\chi^2 = 0\) | 1 |
| Female                                       | 394 (57)       | 1,126 (57)           | \(\chi^2 = 0.11\) | 0.74 |
| Site of Surgery (%)                          |                |                        |            |                                     |
| hip                                          | 396 (57)       | 1,146 (58)           | \(\chi^2 = 0.11\) | 0.74 |
| knee                                         | 293 (43)       | 820 (42)             | \(\chi^2 = 0.11\) | 0.74 |
| Hypertension (%)                             |                |                        |            |                                     |
| Yes                                          | 205 (30)       | 555 (28)             | \(\chi^2 = 0.11\) | 0.48 |
| No                                           | 484 (70)       | 1,411 (72)           | \(\chi^2 = 3.39\) | 0.07 |
| Hyperlipemia (%)                             |                |                        |            |                                     |
| Yes                                          | 197 (29)       | 490 (25)             | \(\chi^2 = 0.11\) | 0.75 |
| No                                           | 492 (71)       | 1,476 (75)           | \(\chi^2 = 0.11\) | 0.75 |
| Diabetes (%)                                 |                |                        |            |                                     |
| Yes                                          | 39 (6)         | 103 (5)              | \(\chi^2 = 0.11\) | 0.75 |
| No                                           | 650 (94)       | 1,863 (95)           | \(\chi^2 = 0.11\) | 0.75 |
| Smoking (%)                                  |                |                        |            |                                     |
| Yes                                          | 117 (17)       | 316 (16)             | \(\chi^2 = 0.11\) | 0.75 |
| No                                           | 572 (83)       | 1,650 (84)           | \(\chi^2 = 0.11\) | 0.75 |
| Location of the thrombosis (%)               |                |                        |            |                                     |
| Proximal                                     | 586 (85)       |                       | \(\chi^2 = 0.11\) | 0.75 |
| Distal                                       | 85 (12)        |                       | \(\chi^2 = 0.11\) | 0.75 |
| Both                                         | 18 (3)         |                       | \(\chi^2 = 0.11\) | 0.75 |

\(\chi^2\), \(P\) values and OR adjusted by age and BMI. Risk allele was highlighted in bold, and OR referred to the risk allele.

| Table 2. Significant SNPs identified in single marker based association analyses |
|------------------------------------|------------------|-----------------|-----------------|-----------------|
| SNPs                              | Status | Genotypes (%) | \(P\) genotype | Genotypes (%) | \(P\) allele | Alleles (%) | \(\chi^2\) | \(P\) allele | OR adj |
|------------------------------------|--------|---------------|----------------|---------------|--------------|-------------|---------|--------------|--------|
| rs2288904                          | Cases (N=689) | 58 (8.4)       | 311 (45.1)    | 320 (46.5)    | 0.00053      | 427 (31.0) | 951 (69.0) | 0.00026      | 1.28   |
|                                    | Controls (N=1,966) | 264 (13.4)   | 904 (46.0)    | 798 (40.6)    | 0.00062      | 1,432 (36.4) | 2,500 (63.6) | 13.00 | 0.00030 (1.12-1.46) |

For the significant SNP, rs2288904, which results in a non-synonymous change located within the exonic region of SLC44A2, no evidence of significant
functional consequences could be found using SIFT, and both alleles were classified as “tolerated”. However, widespread effects of eQTL were identified for rs2288904 in multiple tissues, including the skin, whole blood, mucosa, esophagus, and skeletal muscle. The most significant hit was from the skeletal muscle with a $P$-value of $10^{-27}$ (Table 4), indicating a strong effect on regulating the expression of $SLC44A2$. Furthermore, according to the STRING database, we found that the protein encoded by the $SLC44A2$ gene and the proteins encoded by the other 15 genes constructed a more complex interaction network (Fig. 4).
which also increased the complexity of the effect of the SLC44A2 gene on the risk of DVTFOS to a certain extent.

**Discussion**

Previous large-scale meta-analyses and follow-up studies based on European populations have identified and confirmed the association between rs2288904 in SLC44A2 and VTE. The effect of the SNP rs2288904 was the same compared with the previous meta-analysis. The effect size was also very similar between the two studies. In this sense, we have successfully replicated the finding of this previous meta-analysis for DVTFOS in the Chinese Han population. In addition, compared with the previous genetic association studies focusing on unraveling the genetic etiology of VTE, our study could provide important data to support pre-surgery genetic screening and precision prevention for DVTFOS by identi-
Transfusion-related acute lung injury has been implicated in the pathogenesis of human complex disorders. Previous studies have shown that CTL2 is a binding partner for the von Willebrand factor, which facilitates hemostasis primarily by stabilizing coagulation Factor VIII (FVIII). Increased levels of FVIII have been demonstrated to be a risk factor for first and recurrent episodes of DVT. In addition, one of the primary causes of DVT is damaged blood vessel walls, resulting from oxidative stress and inflammation responses.

Given that it is difficult to draw reliable conclusions only based on SNPs association analyses, bioinformatics analyses of rs2288904 have shown that it is likely to cause very limited functional consequences of the structure of the protein encoded by SLC44A2. Further bioinformatics analyses have confirmed that the significant SNP had a potential functional consequence. These results suggest the important roles of SLC44A2 in the pathogenesis of DVTFOS. Therefore, we believe that population stratification is not a problem for this study. In addition, although we have tried our best to exclude patients with risk factors of VTE in the sample recruitment process, it might still be not enough to control these potential risk factors. Therefore, we need to be careful in interpreting the significant hits identified in the present study, and our results should be considered to be preliminary and confirmed by functional evidence in future research.

In this study, we have obtained evidence for genetic associations between DVTFOS and gene SLC44A2. Further bioinformatics analyses have confirmed that the significant SNP had a potential functional consequence. These results suggest the important roles of SLC44A2 in the pathogenesis of DVTFOS. Further research and wider replications should be conducted to validate in larger, preferably population-based studies to elucidate the exact molecular basis of the relationship between SLC44A2 and DVTFOS risk, which would help to reveal the etiology of DVTFOS and provide intriguing new insight into its biology.

Sources of Funding

This work was financially supported by the China Postdoctoral Science Foundation (No.
Conflict of Interest

All authors declare that they have no conflict of interest.

Author Contributions

L.Q Zhi and W.L. Feng designed the study. W.L. Feng, and J.Q. Liang carried out candidate SNPs selection and statistical analyses. J.B. Ma, Q. Zhong and L.Y. Ren conducted subject screening and contributed to the collection and preparation of control DNA samples. L.Q. Zhi drafted the manuscript, and S.Y. Yao critically revised the manuscript.

References

1) Huang L, Li J and Jiang Y: Association between hypertension and deep vein thrombosis after orthopedic surgery: a meta-analysis. Eur J Med Res, 2016; 21: 13
2) Neglen P, Thrasher TL and Raju S: Venous outflow obstruction: An underestimated contributor to chronic venous disease. J Vasc Surg, 2003; 38: 879-885
3) Qin J, Dai J, Xu Z, Chen D, Qin J, Shi D, Teng H and Jiang Q: Genetic polymorphism of NOS3 with susceptibility to deep vein thrombosis after orthopedic surgery: a case-control study in Chinese Han population. PLoS One, 2013; 8: e70033
4) Souto JC, Almasy L, Borrell M, Blanco-Vaca F, Mateo J, Soria JM, Coll I, Felices R, Stone W, Fontcuberta J and Blangero J: Genetic susceptibility to thrombosis and its relationship to physiological risk factors: the GAIT study. Genetic Analysis of Idiopathic Thrombophilia. Am J Hum Genet, 2000; 67: 1452-1459
5) Larsen TB, Sorensen HT, Skytte A, Johnsen SP, Vaupel JW and Christensen K: Major genetic susceptibility for venous disease. J Vasc Surg, 2003; 38: 381S-453S
6) Neglen P, Thrasher TL and Raju S: Venous outflow obstruction: An underestimated contributor to chronic venous disease. J Vasc Surg, 2003; 38: 879-885
7) Kakkar AK, Rushton-Smith SK: Incidence of Venous Thromboembolism in Orthopedic Surgery. In: Llau J. (eds) Thromboembolism in Orthopedic Surgery, 2013; 2: 11-17
8) Marine G: Meta-analysis of 65,734 individuals identifies TSPAN15 and SLC44A2 as two susceptibility loci for venous thromboembolism. Am J Hum Genet, 2015; 96: 532-542
9) Hinds DA, Buil A, Ziemek D, Martinez-Perez A, Malik R, Folkersen L, Germain M, Malaspina A, Brown A and Soria JM: Genome-wide association analysis of self-reported events in 6,135 individuals and 252,827 controls identifies 8 loci associated with thrombosis. Hum Mol Genet, 2016; 25: 1867-1874
10) Guan F, Zhang C, Wei S, Zhang H, Gong X, Feng J, Gao C, Su R, Yang H, Li S: Association of PDE4B polymorphisms and schizophrenia in Northwestern Han Chinese. Hum Genet, 2012; 131: 1047-1056
11) Guan F, Zhang B, Tan T, Li L, Liu F, Li T, Feng Z, Zhang B, Liu X, Li S: miR137 gene and target gene CACNA1C of miR-137 contribute to schizophrenia susceptibility in Han Chinese. Schizophr Res, 2014; 152: 97-104
12) Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM and Lee JJ: Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience, 2015; 4: 7
13) Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, Boehnke M, Abecasis GR and Willer CJ: LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics, 2010; 26: 2336-2337
14) Ng PC and Henikoff S: SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res, 2003; 31: 3812-3814
15) Consortium GT: The Genotype-Tissue Expression (GTEx) project. Nat Genet, 2013; 45: 580-585
16) Kimmel C, Nair TS, Raphael Y, Telian SA, Kim AH, Arts HA, El-Kashlan HK and Carey TE: Cochlin Isoforms and Their Interaction with CTL2 (SLC44A2) in the Inner Ear. J Assoc Res Otolaryngol, 2007; 8: 435-446
17) Oneil J, O'Regan S and Ruat M: The choline transporter-like family SLC44: properties and roles in human diseases. Mol Aspects Med, 2013; 34: 646-654
18) Le, Liu, Wang, QY, FX, Ding, ZX, Xia and LQ: Isoforms, Expression, Glycosylation, and Tissue Distribution of CTL2/SLC44A2. Protein J, 2010; 29: 417-426
19) Bayat B, Tjahjono Y, Berghofer H, Werth S, Deckmyn H, De Meyer SF, Sachs UF and Santoso S: Choline Transporter-Like Protein-2: New von Willebrand Factor-Binding Partner Involved in Antibody-Mediated Neutrophil Activation and Transfusion-Related Acute Lung Injury. Arterioscler Thromb Vasc Biol, 2015; 35: 1616-1622
20) Koster T, Blann AD, Briet E, Vandenbroucke JP and Rosendaal FR: Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. Lancet, 1995; 345: 152-155
21) Roderik K, Pieterella itA, Marianne K, Pieter R, Martin P, Abraham vdE and Harry B: High Plasma Concentration of Factor VIIIc Is a Major Risk Factor for Venous Thromboembolism. Thromb Haemost, 2000; 83: 5-9
22) Kyrle PA, Minar E, Hirschl M, Bialonczyk C, Stain M, Roderik K, Pieternella itA, Marianne K, Pieter R, Martin P, Abraham vdE, El-Kashlan HK and Carey TE: Cochlin Isoforms and Their Interaction with CTL2 (SLC44A2) in the Inner Ear. J Assoc Res Otolaryngol, 2007; 8: 435-446
23) Akhter MS, Biswas A, Ranjan R, Sharma A, Kumar S and Saxena R: The nitric oxide synthase 3 gene polymorphisms and their association with deep vein thrombosis in Asian Indian patients. Clin Chim Acta, 2010; 411: 649-652
24) Guan F, Zhang T, Han W, Zhu L, Ni T, Lin H, Liu D, Chen G, Xiao J, Li T: Relationship of SNAP25 Variants With Schizophrenia and Antipsychotic-Induced Weight Change in Large-Scale Schizophrenia Patients. Schizophr Res, 2020; 215: 250-255
25) Li J, Zhu L, Guan F, Yan Z, Liu D, Han W, Chen T: Relationship Between Schizophrenia and Changes in the Expression of the Long Non-Coding RNAs Meg3, Miat, Neat1 and Neat2. J Psychiatr Res, 2018; 106: 22-30
26) Sun H, Luo C, Chen X, Tao L: Assessment of Cognitive Dysfunction in Traumatic Brain Injury Patients: A Review. Forensic Sci Res, 2017; 2: 174-179
27) Zhang Z, Gong Q, Feng X, Zhang D, Quan L: Astrocytic Clasmatodendrosis in the Cerebral Cortex of Methamphetamine Abusers. Forensic Sci Res, 2017; 2: 139-144
28) Zhu L, Li J, Dong N, Guan F, Liu Y, Ma D, Goh EL, Chen T: mRNA Changes in Nucleus Accumbens Related to Methamphetamine Addiction in Mice. Sci Rep, 2016; 6: 36993
29) Guan F, Ni T, Han W, Lin H, Zhang B, Chen G, Zhu L, Liu D, Zhang T: Evaluation of the relationships of the WBP1L gene with schizophrenia and the general psychopathology scale based on a case–control study. Am J Med Genet B Neuropsychiatr Genet, 2020; 183: 164-171
30) Han W, Zhang TX, Ni T, Liu L, Sun L, Chen G, Lin H, Chen T, Guan FL: Relationship of common variants in CHRNSA5 with early-onset schizophrenia and executive function. Schizophr Res, 2018; 206: 407-412
31) Zhang TX, Zhu L, Ni T, Liu D, Chen G, Yan Z, Lin H, Guan F, Rice JP: Voltage-gated calcium channel activity and complex related genes and schizophrenia: A systematic investigation based on Han Chinese population. J Psychiatr Res, 2018; 106: 99-105
32) Lotta LA, Tuana G, Yu J, Martinelli I, Wang M, Yu F, Passamonti SM, Pappalardo S, Valsecchi C, Scherer SE, Hale WT, Muzny DM, Randi G, Rosendaal FR, Gibbs RA and Peyvandi F: Next-generation sequencing study finds an excess of rare, coding single-nucleotide variants of ADAMTS13 in patients with deep vein thrombosis. J Thromb Haemost, 2013; 11: 1228-1239
33) RK CY, Merico D, Bookman M, J LH, Thiruvahindrapuram B, Patel RV, Whitney J, Deflaux N, Bingham J, Wang Z, Pellecchia G, Buchanan JA, Walker S, Marshall CR, Uddin M, Zarei M, Deneault E, D'Abate L, Chan AJ, Koyanagi S, Paton T, Pereira SL, Hoang N, Engchuan W, Higginbotham EJ, Ho K, Lamoureux S, Li W, MacDonald JR, Nalpathakkam T, Sung WW, Toor FJ, Wei J, Xu L, Tasse AM, Kirby E, Van Etten W, Twigger S, Roberts W, Drmic I, Jilderda S, Modi BM, Kellam B, Szego M, Cytynbaum C, Weksberg R, Zwaigenbaum L, Woodbury-Smith M, Brian J, Senman L, Iaboni A, Doyle-Thomas K, Thompson A, Chrysler C, Leef J, Savion-Lemieux T, Smith IM, Liu X, Nicolson R, Seifer V, Fedele A, Cook EH, Dager S, Estes A, Gallagher L, Malow BA, Parr JR, Spence SJ, Vorstman J, Frey BJ, Robinson JT, Strug LJ, Fernandez BA, Elsabbagh M, Carter MT, Hallmayer J, Knoppers BM, Anagnostou E, Szatmari P, Ring RH, Glazer D, Pletcher MT and Scherer SW: Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder. Nat Neurosci, 2017; 20: 602-611
34) Zhang T, Hou L, Chen DT, McMahon FJ, Wang JC and Rice JP: Exome sequencing of a large family identifies potential candidate genes contributing risk to bipolar disorder. Science, 2018; 645: 119-123
35) Chen J, Zheng H, Bei JX, Sun L, Jia WH, Li T, Zhang F, Seielstad M, Zeng YX, Zhang X and Liu J: Genetic structure of the Han Chinese population revealed by genome-wide SNP variation. Am J Hum Genet, 2009; 85: 775-785
### Supplemental Table 1. Basic information of the 18 selected SNPs for *SLC44A2*

| CHR | SNP       | POS   | Alleles | GENE | FUNC    |
|-----|-----------|-------|---------|------|---------|
| 19  | rs61127297| 10713387 | C/G     | SLC44A2 | intron   |
| 19  | rs12982370| 10715154 | A/C/T   | SLC44A2 | intron   |
| 19  | rs10408934| 10716256 | A/G     | SLC44A2 | intron   |
| 19  | rs10413422| 10718727 | C/T     | SLC44A2 | intron   |
| 19  | rs76359824| 10721530 | A/G     | SLC44A2 | intron   |
| 19  | rs1865065 | 10724002 | A/G     | SLC44A2 | intron   |
| 19  | rs76434079| 10726601 | G/T     | SLC44A2 | intron   |
| 19  | rs116957244| 10730946 | C/T     | SLC44A2 | intron   |
| 19  | rs8113212 | 10732663 | A/G     | SLC44A2 | intron   |
| 19  | rs116979350| 10733412 | A/G     | SLC44A2 | intron   |
| 19  | rs3745242 | 10736237 | C/G     | SLC44A2 | intron   |
| 19  | rs28860769| 10737581 | A/G     | SLC44A2 | intron   |
| 19  | rs113922991| 10738129 | A/G     | SLC44A2 | intron   |
| 19  | rs76638997 | 10741545 | A/G     | SLC44A2 | intron   |
| 19  | rs2288904  | 10742170 | C/T     | SLC44A2 | missense |
| 19  | rs577950492| 10751769 | A/G     | SLC44A2 | intron   |
| 19  | rs79290735 | 10752252 | A/G     | SLC44A2 | intron   |
| 19  | rs2288903  | 10754735 | A/C/T   | SLC44A2 | intron   |

CHR: chromosome; POS: position; FUNC: function

### Supplemental Table 2. Full results for single marker based association analyses

| CHR | SNP       | POS   | GENE     | MAF | HWE | A1 | OR   | STAT | P     |
|-----|-----------|-------|----------|-----|-----|----|------|------|-------|
| 19  | rs61127297| 10713387 | SLC44A2  | 0.11| 0.72| G  | 0.97 | -0.28| 0.78  |
| 19  | rs12982370| 10715154 | SLC44A2  | 0.09| 0.56| A  | 1.04 | 0.32 | 0.75  |
| 19  | rs10408934| 10716256 | SLC44A2  | 0.27| 0.91| A  | 1.02 | 0.30 | 0.76  |
| 19  | rs10413422| 10718727 | SLC44A2  | 0.28| 0.50| C  | 0.98 | -0.28| 0.78  |
| 19  | rs76359824| 10721530 | SLC44A2  | 0.33| 0.61| A  | 0.97 | -0.43| 0.67  |
| 19  | rs1865065 | 10724002 | SLC44A2  | 0.21| 0.89| C  | 1.03 | 0.35 | 0.73  |
| 19  | rs76434079| 10726601 | SLC44A2  | 0.16| 0.61| T  | 0.98 | -0.29| 0.77  |
| 19  | rs116957244| 10730946 | SLC44A2  | 0.10| 0.53| T  | 1.11 | 1.00 | 0.32  |
| 19  | rs8113212 | 10732663 | SLC44A2  | 0.31| 0.92| A  | 0.97 | -0.42| 0.67  |
| 19  | rs116979350| 10733412 | SLC44A2  | 0.07| 0.58| G  | 1.09 | 0.66 | 0.51  |
| 19  | rs3745242 | 10736237 | SLC44A2  | 0.45| 0.65| G  | 1.04 | 0.54 | 0.59  |
| 19  | rs28860769| 10737581 | SLC44A2  | 0.24| 0.57| A  | 0.99 | -0.17| 0.86  |
| 19  | rs113922991| 10738129 | SLC44A2  | 0.13| 1.00| G  | 0.95 | -0.48| 0.63  |
| 19  | rs76638997 | 10741545 | SLC44A2  | 0.29| 0.96| A  | 1.03 | 0.44 | 0.66  |
| 19  | rs2288904  | 10742170 | SLC44A2  | 0.35| 0.77| A  | 0.78 | -3.63| 2.81 × 10^{-4} |
| 19  | rs577950492| 10751769 | SLC44A2  | 0.13| 1.00| A  | 0.97 | -0.30| 0.76  |
| 19  | rs79290735 | 10752252 | SLC44A2  | 0.06| 0.46| G  | 0.97 | -0.24| 0.81  |
| 19  | rs2288903  | 10754735 | SLC44A2  | 0.15| 0.93| T  | 1.03 | 0.35 | 0.72  |

HWE: P values of Hardy-Weinberg equilibrium tests. Significant results were highlighted in bold
Supplemental Fig. 1. Q-Q plot for results of single marker based association analyses