Common variations in ALG9 are not associated with bipolar I disorder: a family-based study

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Abstract

Background: A mannosyltransferase gene (ALG9, DIBD1) at chromosome band 11q23 was previously identified to be disrupted by a balanced chromosomal translocation t(9;11)(p24;q23) co-segregating with bipolar affective disorder in a small family. Inborn ALG9 deficiency (congenital disorders of glycosylation type IL) is associated with progressive microcephaly, seizures, developmental delay, and hepatomegaly. It is unknown whether common variations of ALG9 predispose to bipolar affective disorder.

Methods: We tested five polymorphic markers spanning ALG9 (three intragenic and one upstream microsatellite repeats and one common missense variation, V289I (rs10502151) for their association with bipolar I disorder in two pedigree series. The NIMH (National Institute of Mental Health) pedigrees had a total of 166 families showing transmissions to 250 affected offspring, whereas The PITT (The University of Pittsburgh) pedigrees had a total of 129 families showing transmissions to 135 cases. We used transmission disequilibrium test for the association analyses.

Results: We identified three common and distinct haplotypes spanning the ALG9 gene. We found no statistically-significant evidence of transmission disequilibrium of marker alleles or multi-marker haplotypes to the affected offspring with bipolar I disorder.

Conclusion: These results suggest that common variations in ALG9 do not play a major role in predisposition to bipolar affective disorder.
ilies where the disease co-segregates with a chromosomal abnormality among multiple affected relatives [2]. Previously, we described a family with five individuals affected with Bipolar I disorder in three generations and one individual with recurrent unipolar depression [3]. All six affected individuals were carriers of a balanced chromosomal translocation t(9;11)(p24;q23). Although five other individuals carrying the translocation were clinically normal at the time of the diagnostic interview, the absence of any affected individuals without the translocation suggested reduced penetrance of the chromosomal aberration in the causation of BPAD in this family. Subsequently, we captured and characterized the breakpoint junctions of both derivative chromosomes 9 and 11 using somatic cell hybridization methods [4]. We found that a mannosyltransferase gene, ALG9 (Asparagine-Linked Glycosylation mutant 9; DIBD1, Disrupted In Bipolar Disorder 1), at chromosome 11q23 was physically disrupted by the translocation breakpoint [5]. The translocation disrupted ALG9, which is composed of 15 exons spread to approximately 82 kb genomic distance, between exons 9 and 10 (Figure 1). No gene was disrupted at the chromosome band 9p24 breakpoint.

ALG9 encodes a mannosyltransferase protein which is involved in the synthesis of lipid-linked oligosaccharides (LLOs) in the protein N-glycosylation pathway [6]. ALG9 is ubiquitously expressed in different anatomical regions of the central nervous system. N-linked glycosylation is essential for the modification of proteins in eukaryotes.

| Marker         | D11S5028 (Ca)n | V289I   | D11S5027 (TAA)n | D11S5026 (Ca)n | D11S5025 (Ca)n |
|----------------|----------------|---------|----------------|----------------|----------------|
| Alleles (bp or codon) | 1 (106)         | Val (GTA) | 1 (366)         | 1 (164)         | 1 (357)         |
|                | 2 (110)         |         | 2 (372)         | 2 (162)         | 2 (361)         |
|                | 3 (114)         |         | 3 (375)         | 3 (168)         | 3 (others)      |
|                | 4 (116)         |         | 4 (others)      | 4 (others)      |                |
|                | 5 (others)      |         |                |                |                |
| Frequencies of common alleles | 0.31           | 0.69    | 0.62           | 0.34           | 0.67           |
|                | 0.29           | 0.31    | 0.26           | 0.30           | 0.31           |
|                | 0.27           |         | 0.07           | 0.31           | 0.02           |
|                | 0.07           |         | 0.05           | 0.05           |                |
|                | 0.06           |         |                |                |                |

**Figure 1**
A schematic view of the ALG9 gene structure and the relative locations of the five polymorphic markers. Vertical lines represent the exons and lightening bolt represents the location of the translocation breakpoint. Relative positions of the exons and the markers are approximately in scale. The bottom part contains a table that describes common alleles of the markers, which were used in the transmission disequilibrium test, and their frequencies in the PITT sample.
and is one of the most complex metabolic pathways known, involving more than 40 biochemical steps. Congenital disorders of glycosylation type I (CDG-I), an increasingly recognized and expanding group of genetic conditions, are caused by abnormalities in the synthesis of the LLOs or in their attachment to proteins [7]. Most affected children have multi-system involvement indicating the importance of normal protein glycosylation in cellular function. CDG are clinically associated with a broad range of symptoms including the failure to thrive, hypotonia, cerebellar dysfunction, seizures, mental retardation, and coagulopathy.

The deletion of the ALG9 gene in yeast results in accumulation of LLOs and in hypoglycosylation of secreted proteins, suggesting that the ALG9 protein product functions in the addition of terminal α-1, 2- or α-1, 6-mannoses in the endoplasmic reticulum during the stepwise assembly of LLO [8]. Recently, the first human cases of ALG9 deficiency (congenital disorders of glycosylation type II; CDG-II), caused by E523K [9] and Y286C [10] homozygous missense mutations, were described in two infants. The mutations caused an accumulation of lipid-linked-GlcNAc(2)Man(6) and -GlcNAc(2)Man(8) structures and led to transfer of incomplete oligosaccharides precursors to protein. Both infants exhibited progressive microcephaly, seizures, developmental delay, and hepatomegaly. Also, there were additional clinical features common to other forms of CDG, including cystic renal disease, pericardial effusion and tamponade, partial villous atrophy, hypoproteinemia, esotropia, inverted nipples, coagulopathy, and brain imaging findings that suggested cerebral and cerebellar atrophy. Systemic features of the affected cases were consistent with the ubiquitous gene expression pattern and the central nervous system defects suggested a critical role for ALG9 in neurogenesis or in neuronal survival. To test whether common variations in the ALG9 gene are associated with BPAD, we performed a family-based association analysis using two pedigree series.

Methods

We previously evaluated linkage and linkage disequilibrium in the NIMH (National Institute of Mental Health) bipolar pedigrees [5]. The NIMH pedigrees include series 1 which consisted of 22 multiplex families that were previously used in chromosomal and genomic scans and series 2, which is a collection of 97 pedigrees from the NIMH Genetics Initiative for bipolar disorder [11]. In our earlier analyses, two hierarchical phenotype models, Affection Status Model (ASM) I and ASM II were used. ASM I included bipolar I, bipolar II with major depression, and schizoaffective illness, and ASM II included phenotypes in ASM I plus recurrent unipolar disorder. These analyses did not show evidence of statistically significant linkage or linkage disequilibrium to the ALG9 gene using ASM I and ASM II as diagnostic categories. Here, we extend our analyses of the ALG9 gene with a family-based study of genetic association in a new pedigree series that is composed of 133 small nuclear families that have been collected at the University of Pittsburgh (PITT). We confine our analyses to cases with a diagnosis of bipolar I disorder, because this was the diagnosis assigned to the five translocation carriers in the original translocation pedigree. The PITT series contain exclusively bipolar I cases. We also include the NIMH series after recoding of the diagnoses: bipolar I cases are recoded as “affected” and all other psychiatric diagnoses as “unknown” (Table 1). The collection and genetic study of the PITT families were approved by the University of Pittsburgh IRB committee.

Genotyping was performed using four simple tandem repeat polymorphisms (STRPs) and one missense single nucleotide polymorphisms [5]. Our earlier sequence analysis of 60 subjects with BPAD provided us with a polymorphism profile of the gene. A non-synonymous single nucleotide polymorphism (SNP), V289I (rs10502151), had the highest minor allele frequency (0.35) among the four SNPs discovered. The SNP, V289I (rs10502151), in exon 8 of ALG9 was genotyped by PCR amplification and restriction enzyme digestion as described earlier [5]. The STRPs pD115901-GT2 (D11S5025; GenBank: AF113397), -GT1 (D11S5026; GenBank: AF113398), -TAT (D11S5027; GenBank: AF113399), and -CA (D11S5028; GenBank: AF113400) were originally developed during fine mapping of the hereditary paraganglioma type 1 critical region at chromosome band 11q23 [12] and later mapped in and around the ALG9 gene. The information on the allelic distributions of the STRPs is available in Genome Database. These four markers are

| Pedigree Series | Structure of pedigrees | Total No. of nuclear families | Total No. of subjects in the series | No. of families with transmissions to affected offspring | Total No. of bipolar I cases |
|-----------------|------------------------|-----------------------------|-----------------------------------|-------------------------------------------------------|-----------------------------|
| PITT            | trios, duos, sib pairs with two, one or no parents | 133                          | 424                               | 129                                                   | 135                         |
| NIMH            | Multi-generational with multiple affected subjects | 306                          | 1903                              | 166                                                   | 250                         |
using the asymptotic Bootstrap p-values were very close to the ones computed. Bal tests were computed via 1000 bootstrap samples. For robustness, the p-values associated with the glo-rium. For robustness, the p-values associated with the glo-

disequilibrium for each marker allele and for global
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tests. We tested D11S5028-V289I (rs10502151)-

gene for bipolar I using haplotype transmission disequi-

We wished to evaluate Transmission disequilibrium test of haplotypes for association with the five polymorphic markers in the ALG9 gene. The TRANSMIT program provided the observed and the expected number of transmissions for each of the common alleles of the five markers from a parent to an affected offspring. The transmission patterns of the marker alleles to affected offspring did not show statistical significance. Specifically, in the PITIT series, the SNP alleles encoding for Val289 and Ile289 were transmitted 185 and 85 times, similar to the expected transmission numbers of 187.5 and 82.5, respectively. Similarly, transmissions of the SNP alleles occurred 343 and 157 times in the NIMH series, which is very close to the expected numbers of 344 and 156, respectively. TRANSMIT provided \( \chi^2 \)-based significance tests for transmission disequilibrium for each marker allele and for global (including all marker alleles) transmission disequilibrium. For robustness, the p-values associated with the global tests were computed via 1000 bootstrap samples. Bootstrap p-values were very close to the ones computed using the asymptotic \( \chi^2 \) distribution of global tests. None of the common alleles of the five markers yielded statistically significant evidence for transmission disequilibrium. The results of the global \( \chi^2 \) tests are presented in Table 2.

**Results and discussion**

**Transmission disequilibrium test of markers**

We evaluated the PITIT and the NIMH series of pedigrees for association with the five polymorphic markers in the ALG9 gene. The TRANSMIT program provided the observed and the expected number of transmissions for each of the common alleles of the five markers from a parent to an affected offspring. These results are in agreement with our earlier results in the NIMH or the PITIT pedigree series, which collectively contain 295 families showing transmissions to 385 affected offspring. These results are in agreement with our earlier results in the NIMH pedigree series obtained using broader diagnostic criteria and a different analytical approach [5].

Table 2: Global TRANSMIT statistics for transmission disequilibrium tests of association between ALG9 variations and bipolar 1

| Pedigree Series | D11S5025 \( \chi^2 \) (p-value) | V289I \( \chi^2 \) (p-value) | D11S5026 \( \chi^2 \) (p-value) | D11S5027 \( \chi^2 \) (p-value) | D11S5028 \( \chi^2 \) (p-value) |
|-----------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| PITIT           | 0.707 (0.93)                  | 0.299 (0.58)                  | 2.061 (0.57)                  | 0.142 (0.98)                  | 1.541 (0.46)                  |
| NIMH            | 0.385 (0.98)                  | 0.012 (0.91)                  | 2.17 (0.47)                   | 0.349 (0.95)                  | 0.396 (0.76)                  |

To test whether the variations revealed by our multi-marker haplotypes represented the true haplotypic diversity in the ALG9 gene, we assessed its haplotype block structure in Caucasians in the HapMap database [15], because virtually all of our samples were of Caucasian origin. The common haplotypes of ALG9 in the HapMap database were defined by 18 intronic SNPs and the V289I (rs10502151) in 60 Caucasian samples (see Additional File 1). The non-ancestral allele encoding Ile289 (allele T of rs10502151) is associated with a common haplotype (HMB1) that has a frequency of 23%, whereas the remaining four haplotypes, HMA1, HMA1a, HMA2 and HMA2a, are associated with the ancestral allele encoding Val289 (allele C of rs10502151). Notably, only a single intronic SNP distinguishes HMA1 from HMA1a (SNP#19), and HMA2 from HMA2a (SNP#16), suggesting a recent common origin for these haplotype-duos. Thus, HMA1/
HMA1a, HMA2/HMA2a and HMB1 define three common and distinct haplotype groups with frequencies of 45%, 28% and 23%, respectively. These three haplotype groups differ from each other in at least 7 SNP positions.

The ALG9 haplotype structure in the HapMap database is similar to the structure defined by D11S5028-V289I (rs10502151) or D11S5028-V289I (rs10502151)-D11S5027 in our dataset in that both approaches define three distinct and common haplotype groups (Table 3 and Additional File 1). Whereas the non-ancestral allele encoding Ile289 is associated with the common haplotypes 2.2.2 and 2.2, the ancestral allele encoding Val289 is associated with two common distinct haplotype groups 1.1.1 and 3.1.1 (three-marker haplotype) or 1.1 and 3.1 (two-marker haplotype) in our dataset (Table 3). The two common ancestral haplotype groups are tagged by the 106 bp and 114 bp-alleles of D11S5028, whereas the non-ancestral haplotypes are tagged by the 110 bp-allele (Table 3), indicating that the repeat alleles of the three haplotypes differ by at least 4 bp (2 units). Because (CA)n repeat mutations cause an increase or decrease in repeat number by one unit [16], we predict that the two ancestral and one non-ancestral haplotype groups are phylogenetically distant and correspond to the same three haplotype groups defined in the HapMap database.

Whereas the two ancestral haplotypes in the HapMap database, HMA1/HMA1a and HMA2/HMA2a, have frequencies of 45% and 28% respectively (Additional File 1), those in our study have frequencies of 27% and 29% (three-marker haplotypes) or 29% and 28% (two-marker haplotypes) (Table 3). The non-ancestral haplotypes in the HapMap database (HMB1) and in our study (2.2.2 or 2.2) have comparable frequencies of 23% and 22 or 30%, respectively. Thus, the frequencies of the common haplotypes defined by D11S5028-V289I (rs10502151)-D11S5027 or D11S5028-V289I (rs10502151) and HapMap are comparable. Nevertheless, it is conceivable that if there is a susceptibility-variant in the most common ancestral haplotype in HapMap database (HMA1/HMA1a), our method would have reduced power to detect it because the frequency of the most common ancestral haplotypes (1.1.1 or 1.1) in our dataset is lower than that in the HapMap database (27% or 29% vs. 45%), thus reducing the number of informative chromosomes tested for association with bipolar I disorder. If, however, there is a rare susceptibility variant on haplotype 1.1.1 or 1.1, our approach would have more power to detect it because of the stronger allelic association of the susceptibility variant with haplotype 1.1.1 or 1.1 than with HMA1/HMA1a.

If the ALG9 disruption is causally linked to the development of BPAD in the translocation family, several other reasons could be invoked to explain our present failure to detect an association between ALG9 and BPAD. It is conceivable that rare loss-of-function mutations and gross gene aberrations of ALG9 that have dramatic functional consequences, rather than the common allelic and haplotypic variations, could increase predisposition to BPAD. Here, we tested two series of pedigrees. The NIMH pedigrees are enriched for families with multiple affected individuals, whereas 129 of 135 (95.6%) of the PITT pedigrees contain a single affected individual. If rare susceptibility variants in ALG9 contribute to the familial enrichment of BPAD in the NIMH pedigrees, association testing by common alleles and haplotypes would have low power to detect it. Although the PITT series is primarily consisted of small nuclear families with a single affected individual,
and therefore might be more suited to detect common, low-penetrance susceptibility variants, its sample size may not be large enough if the relative risk of susceptibility conferred by the common variant is low. It is also conceivable that certain environmental factors or sex-specific influences may modify the risk conferred by an ALG9 variant. Similar mechanisms may have played a role in failure to support an association between DISC1 variations and schizophrenia in some studies [17-20], although an increasing amount of evidence supports a role for DISC1, a gene disrupted by a translocation breakpoint in an extended family, in the etiology of schizophrenia.

Conclusion
In conclusion, our further analysis of ALG9 gene variations failed to support a major role in predisposition to BPAD. Nevertheless, we cannot rule out a role for ALG9 in BPAD susceptibility because of the limitations of the markers and the pedigrees analyzed here. Interestingly, another protein N-glycosylation pathway gene, MGAT5 (Mannoside acetylgalcosaminyltransferase 5) at chromosome band 22q21, was also found to be disrupted by an unbalanced translocation breakpoint in a schizophrenia case [2]. Thus, the structural aberrations of sugar transferase genes in BPAD and schizophrenia and the recent findings that the germ line homozygous mutations of ALG9 leads to progressive microcephaly, cerebral and cerebellar atrophy underscores the significance of N-glycosylation pathway in central nervous system homeostasis and may warrant further analyses of ALG9 and other mannosyltransferase genes in the etiopathogenesis of psychosis.

Abbreviations
BPAD (Bipolar Affective Disorder), CDG (Congenital Disorders of Glycosylation), DIBD1 (Disrupted in Bipolar Disorder), Ile/I (Isoleucine), IRB (Institutional Review Board), LLO (lipid-linked oligosaccharides), NIMH (National Institute of Mental Health), PIIT (University of Pittsburgh), SNP (Single Nucleotide Polymorphism), STRP (simple tandem repeat polymorphism), TDT (Transmission Disequilibrium Test), Val/V (Valine).

Competing interests
The author(s) declare that they have no competing interests.

Contributors
BEB designed the study, obtained funding and drafted the manuscript, JEBW performed genotyping experiments and data entry, SAB performed statistical analyses, SDW and VLN provided pedigree information and the DNAs. All authors contributed to the manuscript and approved its final version.

Additional material

Acknowledgements
This work was supported by a NARSAD Young Investigator Award to BEB.

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