**A Bayesian framework for generalized linear mixed modeling identifies new loci for late-onset Alzheimer’s disease**

Xulong Wang1, Vivek M. Philip1, Guruprasad Ananda2, Ankit Malhotra2, Paul J. Michalski2, Krishna R. Murthy Karuturi2, Michael Sasner1, Gareth R. Howell1, Gregory W. Carter1

1The Jackson Laboratory, Bar Harbor, ME

2The Jackson Laboratory for Genomic Medicine, Farmington, CT

**Abstract**

Recent technical and methodological advances have greatly enhanced genome-wide association studies (GWAS). The advent of low-cost whole-genome sequencing facilitates high-resolution variant identification, and the development of linear mixed models (LMM) allows improved identification of putatively causal variants. While essential for correcting false positive associations due to sample relatedness and population stratification, LMMs have commonly been restricted to quantitative variables. However, phenotypic traits in association studies are often categorical, coded as binary case-control or ordered variables describing disease stages. Furthermore, optimally integrating the results of prior studies remains a methodological challenge. To address these issues, we have devised a method for genomic association studies that implements a generalized linear mixed model (GLMM) in a Bayesian framework, called *Bayes-GLMM*. *Bayes-GLMM* has four major features: (1) support of categorical, binary and quantitative variables; (2) cohesive integration of previous GWAS results for related traits; (3) correction for sample relatedness by mixed modeling; and (4) model estimation by both Markov chain Monte Carlo (MCMC) sampling and maximal likelihood estimation. We applied *Bayes-GLMM* to the whole-genome sequencing cohort of the Alzheimer's Disease Sequencing Project (ADSP). This study contains 570 individuals from 111 families, each with Alzheimer's disease diagnosed at one of four confidence levels. The profound population structure in these data required a mixed model approach, and the categorical trait necessitated a generalized model. With *Bayes-GLMM* we identified three new loci significantly associated with Alzheimer’s disease. In summary, this work provides the first implementation of a flexible, generalized mixed model approach in a Bayesian framework for association studies.

**Introduction**

Linking genomic variants to traits is central to discovering the mechanisms of genetic diseases. To date, NHGRI has curated 1,751 publications of genome-wide association studies (GWAS) that considered at least 100,000 single nucleotide polymorphisms (SNP) (Welter et al., 2014, Manolio, 2010). The adoption of high throughput sequencing technology has facilitated the rapid identification of potentially causal variants. The 1000 Genomes Project has characterized roughly 88 million variants by whole genome sequencing of 2504 individuals from 26 populations (1000 Genomes Project Consortium, 2015). Such sequencing approaches to genomic association will soon enable discovery at base pair resolution. Meanwhile, statistical methods for GWAS have evolved from odds ratio tests, to generalized linear regression models, to more sophisticated multivariate linear mixed models (LMMs). LMM approaches have the capacity to correct population structures and sample relatedness (Henderson, 1953), thereby minimizing false positives due to allelic co-segregation. Consequently, the number of LMM-compatible computational tools for genetic studies is rapidly increasing, including ASReml, TASSEL, EMMA, QTLRel, FaST-LMM, DOQTL, GEMMA, and GMMAT (Gilmour et al., 1995; Zhang et al., 2010; Kang et al., 2010; Cheng et al., 2011; Lippert et al., 2011; Gatti et al., 2014; Zhou and Stephens, 2014; Chen et al., 2016).

While LMMs are efficient in correcting sample relatedness, response variables are restricted as numerical. Meanwhile, phenotypic traits in GWAS are often categorical, such as binary variables in case-control studies or multi-level ordered categorical variables corresponding to disease stages. To model discrete response variables in the context of mixed models for population relatedness correction, generalized linear mixed models (GLMMs) are required. Chen et al. published a method that handles binary response variable in the context of mixed model (GMMAT, Chen et al., 2016). However, multiple level categorical variables are not supported. Current approaches commonly transform categorical variables into continuous variables to fit LMMs following the assumption that the trait has constant residual variance. However, the constant residual variance assumption is often violated by categorical trait, which can bias effect estimates.

The proliferation of multiple GWAS for a single disease has also generated a need for methods to systematically combine results from multiple studies. Such efforts, often pursued as meta-analyses, can dramatically boost statistical power through an increase in sample size (Kavvoura and Ioannidis, 2008). However, association strengths of a given variant or a genetic locus typically fluctuate across studies, which may be due to different population compositions, environmental exposures, clinical reporting standards, and experimental platforms. As a result, it is often difficult or impossible to merge raw data of different studies into a single association model. Furthermore, a more general integration of prior information is often desirable, such as co-expression or other correlations between genes. Integration approaches with more flexibility are needed to address these issues.

To address these challenges, we created the *Bayes-GLMM* method that exploits the flexibility of a Bayesian modeling framework and the computing efficiency of the recently developed statistical programming language Stan (<http://mc-stan.org>; Carpenter et al., 2015). As a Bayesian strategy, model parameters are assumed to be stochastic rather than fixed as in the case in frequentist approaches (Gelman et al., 2014). The stochastic nature of Bayesian modeling provides a coherent solution to combine published results of a related GWAS by configuring the prior distributions of the statistics of interest and computing posterior probabilities given new data (Verzilli et al., 2008; Newcombe et al., 2009; Stephens and Balding, 2009). *Bayes-GLMM* priors are determined from reported effect sizes and corresponding *p*-values, thereby allowing integration of published studies based on summary statistics. *Bayes-GLMM* is available as an R package for public use.

**Results**

**Alzheimer’s disease sequencing project**

Development of *Bayes-GLMM* was motivated by the analysis of the whole-genome sequencing association studies, such as the Alzheimer’s disease sequencing project (ADSP). ADSP was initiated to discover novel genomic variants for late-onset Alzheimer’s disease (LOAD). The whole genome sequence (WGS) cohorts of ADSP contained 570 participants from 111 families. This family-based design generated profound sample relatedness that warranted a mixed model approach. Further, phenotypic traits were four levels of Alzheimer’s diagnoses: no (N = 78), possible (N = 81), probable (N = 356), and definite (N = 55), which necessitated a generalized model. Family pedigree, race, ethnicity, age, sex, and APOE e2/e3/e4 genotype were also reported for each participant. The population was 61% female. Interquartile range of sample ages was 67 to 80 years. In APOE genotypes, homozygous APOE/e3 took 56.7% (N = 323) of the population, followed by 35.1% (N = 200) of e3/e4, 6.84% (N = 39) of e2/e3, 1.05% (N = 6) of e2/e4, and 0.351% (N = 2) of e2/e2 (Figure 1).

Additive effects of age, sex (female), and *APOE* allele-types (*ε2*, *ε3*, *ε4*) were tested with *Bayes-GLMM* (Figure 2B). To account for sample relatedness, kinship structure was computed from autosomal variants, and included as the variance-covariance matrix of a random effect that followed a multivariate normal distribution (mvNormal). The covariance matrix of the mvNormal was constrained by the kinship matrix of the samples. All model parameters were estimated by MCMC sampling. As expected, we observed that the *APOEε4* allele significantly increased risk of Alzheimer’s (*p* = 0.00014) while the *APOEε2* allele reduced risk (*p* = 0.0033) relative to the baseline *APOEε3* allele. Sex was also a significant factor, with females at higher risk (*p* = 0.032). Increasing age corresponded to a small but significant risk increase (*p* = 0.00036), with the weakness of the association potentially due to the narrow age range and possible longevity of non-affected individuals. All covariate pairs were tested with fixed-effect interaction terms, but no significant interactions were observed (Supplementary Figure 1).

**GWAS of ADSP WGS cohort by *Bayes-GLMM***

The ADSP consortium identified a total of 27.9 million SNP from the WGS cohort, of which 10.3 million had minor allele frequency larger than 0.01 (Supplementary Figure 2). Associations of the 10.3 million SNP to LOAD diagnosis were tested by *Bayes-GLMM* in two steps (Figure 3). To first screen for potential candidate variants, a generalized linear model (ordered categorical model) was applied to each of the 10.3 million variants without the random term. The model parameters in this pre-scan were estimated by the maximal likelihood estimation (MLE) method for computational efficiency. Variants with *p* < 0.0001 were identified as potential candidate variants (*N* = 9726, Figure 4A). In the second step, these pre-scan candidate variants were tested with the full GLMM, including the random term to correct sample relatedness. Model parameters were estimated by MCMC sampling to avoid the instability in estimating GLMM by MLE. Final *p*-values for every variant were obtained from their empirical posterior distributions (Figure 4b) (Methods).

**Top LOAD-associated variants from ADSP WGS**

We identified four variants in three independent loci that were genome-wide significant (*p* < 5 x 10-8). These four variants (rs10490263, rs74944275, rs149372995, rs140233081) were all intergenic. The SNPs are located as follows: rs10490263 is 233,714 bp upstream of SLC8A1 and 337 bp upstream of lincRNA AC007317.1; rs74944275 is 111,711 downstream of C5orf30 and 18,568 bp downstream of lincRNA CTD-2154H6.1; and rs140233081 and rs149372995 are 8,097 and 8,292 downstream of PRKAR1B, respectively. To assess the functional relevance of these four variants, we queried the Roadmap Epigenomics (Bernstein et al., 2010) and ENCODE (Dunham et al., 2012) resources using HaploReg (Ward and Kellis, 2012) for their chromatin states and protein binding annotations. We found rs10490263 lies in promoter-associated histone marks in circulating T cells and the hippocampus, and rs74944275 lies in both promoter- and enhancer-associated histone marks in multiple brain regions. Furthermore, rs149372995 resides in a candidate-binding site of CTCF, rs74944275 resides in a candidate-binding site of CCNT2, Evi-1, GATA, and HDAC2, rs140233081 and rs149372995 lie in candidate bindings sites of NERF1a, SMC3, and TCF12. We also explored the curated eQTL datasets by GTEx to explore the effects of these SNPs on gene expression, and found rs10490263 had a significant, local association with the expression of AC007317.1 in skin and testis (Ardlie et al., 2015).

Furthermore, we identified 55 variants in 28 loci with *p* < 1 x 10-6 (Table 1, Figure 5). The overwhelming majority of these top 55 variants increased risk of LOAD, 52 of which had positive effects on AD diagnosis. Further, variants with strong effects tended to occur at lower allele frequency, suggesting that these variants might be under negative selection. The top 55 variants led to 146 genetic consequences in the transcript level, in which 73 were intron-related, followed by 31 intergenic, 27 upstream gene variants (within 5K upstream from the 5’ end), 11 downstream gene variants (within 5K downstream from the 3’ end) and 4 regulatory region variants (Supplementary table 1).

The 73 intron-related consequences in the transcript level mapped to 19 variants and 18 unique genes. 12 out of the 18 genes appeared in the NHGRI GWAS category (Welter et al., 2014). Top traits of the 12 genes were obesity-related traits (PTPRD, SORCS2 and SLC24A4), Alzheimer’s disease (SLC24A4, GABRG3), acute lymphoblastic leukemia (ERC2 and ST6GALNAC3), adiponectin levels (CMIP and HIVEP2 in 3 studies), bipolar disorder and schizophrenia (ERC2), and type-2 diabetes (PTPRD). NEED A SUMMARY SENTENCE HERE STATING THE IMPORTANCE OF THESE FINDINGS.

**Integrating prior knowledge**

Prior knowledge integration is a prominent feature of Bayesian modeling. In GWAS, prior information of a variant can be implemented with multiple strategies, each allowing posterior estimations to carry different weights of the priors. In Bayes-GLMM, we implemented a method to configure priors that targeting the unique challenges of GWAS, such as the different meanings of effect sizes from studies with different statistical models, and the particularly small p-values from published large-scale studies. Our method took the reported standardized effect sizes as the prior information and integrated it into the hierarchical model of the variant effect (Methods). To demonstrate the performance of this method, we simulated a binary phenotypic trait (coded as 0 or 1) and genotype of a variant (coded as 0, 1, or 2), and used a logistic regression model (LR) to test their association. We assessed the effect of prior information on the estimated variant effect by testing a range of prior standardized effect sizes. This method of configuring priors effectively modulating the information from the data (Figure 6), regardless of the differences between the prior cohort and the population at hand (*e.g*. differences in sample size).

**Discussion**

We proposed a new GWAS method, *Bayes-GLMM*, and applied it on ADSP’s whole-genome sequencing cohort. This method efficiently addresses three major challenges in GWAS: categorical phenotypes, population structure and sample relatedness, and prior knowledge integration. Furthermore, our generalized approach has the flexibility to operate on binary and quantitative traits in addition to ordered categorical phenotypes. These features enabled identification of three new loci that significantly increased the risk of Alzheimer’s disease. We therefore consider *Bayes-GLMM* to be a powerful addition to existing GWAS methods.

The flexibility of the Bayesian modeling allows the convenient configuration of sophisticated models, such as a GLMM. In *Bayes-GLMM*, logistic and ordered logistic regression likelihoods were used to model binary and ordered categorical variables, respectively. Conditional factors were included as model covariates and, although our study was underpowered for epistasis analysis, interaction terms can be straightforwardly included. Sample relatedness was modeled by a random term that followed a multivariate normal distribution. Model parameters can be estimated by either L-BFGS maximal likelihood estimation (MLE), or Hamilton Markov chain Monte Carlo sampling, as implemented in Stan.

While the MLE method was efficient and reliable in estimating generalized linear models, it was unreliable in estimating generalized linear mixed models. We found that MLE of the random term was skewed toward initial values, suggesting the optimizer was trapped into local optima and limiting reliability in estimating the GLMM. On the other hand, MCMC sampler allows an improved assessment of the robustness and stability of model inferences by reporting the full posterior distributions of model parameters and the convergence of multiple sampling chains. This information allows one to dissect how multiple factors contribute to model estimation, including poorly defined prior distributions, collinearity of predictors, and inappropriate initial sampling values. These properties are especially appealing for estimating complex genetic models in *Bayes-GLMM*.

*Bayes-GLMM* method was optimized in multiple ways to minimize the computational expense: (1) parallel computing; (2) conjugate prior distributions; (3) vectorization of model statements to exploit efficient matrix operations in Stan; and (4) parameterization of multivariate normal distribution for the random effect by Cholesky factoring. Nevertheless, the primary drawback of MCMC sampling was efficiency. Testing at a 2.3G Hz Intel processor, MLE took roughly 0.12 seconds to estimate the GLM model of the ADSP dataset (Methods, Figure 3). In comparison, the MCMC sampler took roughly 30 seconds to generate 1000 samples for each parameter of the same GLM model, and 15 minutes to process 1000 samples for the GLMM model. Our pre-scan with MLE followed by more precise estimation by MCMC proved an effective approach to overcome these processing limitations.

To reduce the computational burden in fitting GLMMs, categorical diagnoses could be collapsed into binary variables. For the ADSP data, the “no” and “possible” diagnoses become “control”, while the “possible” and “definite” diagnoses are “case”. Logistic mixed models, or binary mixed models, were implemented in *Bayes-GLMM* to accommodate binary variables. The MCMC sampler implemented in Stan took approximately 10 minutes to collect 1000 samples for parameters of such a binary mixed model, as opposed to 15 minutes for the four-level categorical mixed model. Alternatively, the recently released GMMAT (generalized linear mixed model association test) method that utilized penalized quasi-likelihood method to fit a binary mixed model was significantly faster than the MCMC sampling approach (Chen et al., 2016). However, this practice reduced precision due to the information loss in collapsing multiple categories. We tested this practice in the ADSP data, and found the association results by binary-GLMM and categorical-GLMM disagreed with each other dramatically (Supplementary Figure 3).

Another strategy to reduce computational requirements is to transform categorical variables into continuous variables to accommodate efficient LMM methods (Kang et al., 2010; Cheng et al, 2011). However, this practice is prone to yield incorrect type I error rate because categorical studies do not satisfy LMM’s constant residual variance assumption; that is, linear models assume residual variances are constant with respect to different values of model predictors. This practice also yields incorrect effect estimates due to the unbalanced sampling in different phenotypic categories, which is prominent in the ADSP study in which the “probable” diagnosis accounted for 62% of the total while the other three categories accounted for only 10-14%. We also found the inferences results of LMM by QTLRel were sensitive to different quantitative coding of categorical variables (Supplementary Figure 4, Methods). Taking rs34827707 as an example, the LOD value for rs34827707 dropped from 29 to 15 by changing the coding from no/possible/probably/definite as 0/0.25/0.5/1 to 0/0.33/0.66/1. In contrast, the GLMM robustly estimated three cut points to separate the four categories.

Bayesian modeling naturally allows the integration of prior information by specifying model parameter’s prior distribution. However, how to best specify a variant’s prior information is an open question when the prior study does not precisely match the design at hand. Association results of each variant in a GWAS are commonly reported by effect size and *p*-value. While critical in describing the association strength, exact values of effect sizes are often specific to the given study because of dependencies on the statistical model, genotype coding strategies, and covariates. Therefore, it can be misleading to use the reported effect sizes to configure the priors. As opposed to effect sizes, *p*-values that quantify deviation from a null hypothesis can be less specific to the given study. Equivalently, standardized effect sizes can describe the confidence of true association as a measure of effect size in units of standard error. However, standardized effect sizes are highly influenced by the sample size, and using standardized effects from a large-scale study as priors will dominate the posterior estimation of a variant’s association, thereby masking the information of the current study. To tackle this problem, we proposed a strategy that models the variant effect by a hierarchical model, in which variant effect was firstly modeled by a normal distribution with expected mean represented as the multiplication of the standardized expected mean and the standard deviation. The standardized expected mean was further modeled by a standard normal with expected mean specified as the prior standardized effect. Simulation results showed our method in configuring the priors effective in allowing priors only modulating information of the data under study (Figure 6).

While powerful, *Bayes-GLMM* has several drawbacks. First, quantitative meanings of parameter values are hard to explain. Second, heritability estimation is elusive due to a difficulty in estimating residual variance. Third, as implemented, only one variance component is supported. Although Bayesian modeling can readily encompass multiple variance components, this becomes impractical for GWAS due to computational limitations for most researchers. Fourth, sampling-based estimations remain computationally intensive and may not be suitable for larger data sets. We expect that advances in model estimation techniques, improved algorithms, and broad application of could-based computational resources will alleviate these problems in the near future.

To summarize, here we have proposed a method for GWAS with three major features: (1) a generalized model to support multiple types of phenotypic data; (2) a Bayesian strategy to effectively integrate previous GWAS results for the same trait; and (3) a mixed-model implementation to correct population structure. With genome-wide association transitioning to whole-genome and whole-exome platforms, statistical methods for large-scale association studies are essential for uncovering the genetic basis of complex disease (this ending is not strong). The ability to integrate existing GWAS as prior information can further power these studies to prioritize specific variants at known loci.

**Deleted**

Variants in *APOE* are the greatest known genetic factors for LOAD. The *APOEε3* allele is considered neutral while *APOEε2* and *APOEε4* are decrease and increase LOAD risk, respectively. General frequencies of the three alleles are 8.4%, 77.9%, and 13.7% (in which population? Source? Also, reported frequencies for genotypes, instead of alleles). As comparison, frequencies of the three APOE alleles in the ADSP dataset are 4.4%, 77.5%, and 18.1%, reflecting a selected enrichment of *APOE* risk alleles in the study population. The risk profile of the three *APOE* alleles is reproduced in our study when *APOE* genotype is explicitly included as an independent variable (Figure 2). However, the specific *APOE* variants, rs429358 and rs7412, were not genome-wide significant. P-values of rs429358 and rs7412 were which reflected the relatively small sample size of the ADSP WGS cohort (570). (Say little more, figure required, state p values to show how close they were). (This paragraph was deleted because the two variants were not reported by the ADSP call).

To demonstrate these issues, we built a LMM for the ADSP dataset by transforming the four categorical AD statuses into numerical probabilities (no to 0, possible to 0.25, probable to 0.5, definite to 1). The LMM realization was estimated with *QTLRel* (Cheng et al., 2011). Results by LMM and GLMM were similar in general. Pearson’s correlation coefficient of P-values by LMM and GLMM was xxx. However, by comparing the top 0.1% variants in either model, the LMM increased LRT by 1.97 on average. Interquartile range of LRT differences between LMM and GLMM for the top 0.1% variants were 0.88 to 2.86 (a figure required). This result showed LMM was incompetent to control type I error.

By inspecting the variants that returned the most different LOD values (top 76 with minimal LOD difference 8) for the two models, we found: (1) these variants were rare, with. MAF ranging from 0.010 to 0.036, (2) MAF of these variants across the four AD diagnoses varied nonlinearly (This is the non-constant residual variance issue as described in the GMMAT paper). Taking rs34827707 for example, while the minor allele appeared frequently in the definite AD population with MAF 0.18, it was rare in all the other AD populations. MAF in no, possible, and probable populations were 0.01, 0, and 0.01, respectively. This suggested that transformation of ordered categories into continuous probabilities is unstable for rare variants.

Leaving the APOE locus, other top AD variants show weak association with LOAD in the ADSP dataset. P-values of the other top 20 AD variants from Alzheimer’s disease were xxx to xxx (table required). This is consistent by using both GLMM and LMM models (Supplementary Figure, say more).

Our method comes with several drawbacks. Firstly, model parameters are hard to explain. Secondly, heritability estimation is elusive because of the hidden residual variances. Thirdly, only one variance component is supported. Although Bayesian modeling has no difficulty handling multiple variance components, this becomes impractical for the GWAS settings given the available computing resources nowadays. Fourthly, the speed is not satisfactory. We expect superior algorithms to be developed for generalized linear mixed model.

Disease phenotypes as defined by ADSP fit an ordered categorical variable. We also collapse the four AD categories into two to simulate a case-control study (control: no, possible; case: probable, definite). Further, the family-wise study design and multiple-races sample pool suggested the necessity of sample relatedness correction. Age and sex was included as conditional covariates for their potential confounding effects to the variants under study.

From the NHGRI GWAS category, obesity-related traits has been studied in 958 publications, Type 2 diabetes in 320 studies, Schizophrenia in 256 studies, and so on.

A total of 244 genomic variants in 73 independent LD blocks passed the LOD threshold of 0.05 FDR (Figure 3a, Table 1). Effect size, MAF, and LOD of the top variants in each LD block matched well. Interestingly, 85% of the 244 variants are risky. Rare variants show bigger effect size (correlation coefficient: -0.785), suggesting extreme protective and risky variants are less heritable. The 244 variants lead to 1101 genetic consequences, in which 51.8% were intron-related (570 out of 1101). This ratio is the same as that of all intron-related consequences relative to consequences by all variants (29.2 million out of 56.5 million). This suggests associations between intron variants and LOAD is not stronger than variants in other genetic regions. The 570 intron-consequences mapped to 153 unique variants and 53 genes. These genes are significantly enriched in metabolic process (14) and calcium ion binding activity (6, Supplementary Table). Further, 23 out of the 53 genes are included in the NHGRI GWAS category (Welter et al., 2014) including PDE7B, a gene that was associated with Alzheimer’s disease (Sherva et al., 2013). Other top traits of the 23 GWAS category genes are rheumatoid arthritis, IgG glycosylation, obesity-related traits, and type-2 diabetes (Supplementary Table). Interestingly, variants of the 23 overlapping GWAS genes are either intron (65) or intergenic (19).

Noncoding transcript variants are the second most abundant (15.3% and 168 incidences). This corresponds to 76 unique variants and 37 genes. Majority of the noncoding transcript consequences are also in the introns (158 out of 168). The other 10 exon-consequences mapped to 8 variants affecting 6 genes: hsa-mir-6723, AC144450, LINC00870, LINC00700, FDPSP3, RP11-560L11. LINC00870 and LINC00700, as lincRNA, were associated with migraine and metabolite levels, respectively (Yu et al., 2013; Anttila et al., 2013). Additionally, we have 79 intergenic, 23 significant regulatory region, and 1 missense variant consequences (Supplementary Table).

The one missense variant is rs191267549 (chr10: 120789413). The *C* to *A* mutation causes a *P* to *T* amino acid change in NANOS1 protein. 18 out of the 19 samples who carry the rs191267549 minor allele (A) are either probable or definite LOAD, whereas all 3 homozygous rs191267549 A/A cases are definite LOAD (Figure 5). The 18 minor allele carriers spread in 10 families. NANOS1 is widely expressed in the neural and immune system and functionally rich. It affects both gene transcription as a transcription factor (TF) and translation as a RNA-binding protein (RBP). As a TF, the 11 targeting genes are enriched in multiple KEGG and GO terms, including neurotrophin signaling, focal adhesion, cell cycle, axon guidance, Jak-STAT signaling, regulation of immune response, regulation of cellular metabolic process, and regulation of apoptotic process (Supplementary Table). As a RBP, NANOS1 contains a zinc-finger motif, which regulates translation of specific mRNAs by forming a complex with PUM2 that associates with the 3’UTR of mRNA targets. Interestingly, both NANOS1 and APOE are associated atherosclerosis, suggesting the two proteins might function together in a shared pathway.