Family studies of type 1 diabetes reveal additive and epistatic effects between \textit{MGAT1} and three other polymorphisms

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In a recent study on multiple sclerosis (MS), we observed additive effects and epistatic interactions between variants of four genes that converge to induce T-cell hyperactivity by altering Asn-(N)-linked protein glycosylation: namely, the Golgi enzyme \textit{MGAT1}, cytotoxic T-lymphocyte antigen 4 (CTLA-4), interleukin-2 receptor-\(\alpha\) (IL2RA) and interleukin-7 receptor-\(\alpha\) (IL7RA). As the CTLA-4, IL2RA and IL7RA variants are associated with type 1 diabetes (T1D), we examined for joint effects in T1D. Employing a novel conditional logistic regression for family-based data sets, epistatic and additive effects were observed using 1423 multiplex families from the Type 1 Diabetes Genetic Consortium data set. The IL2RA and IL7RA variants had univariate association in MS and T1D, whereas the \textit{MGAT1} and CTLA-4 variants associated with only MS or T1D, respectively. However, similar to MS, the \textit{MGAT1} variant haplotype interacted with CTLA4 (\(P = 0.03\)), and a combination of IL2RA and IL7RA (\(P = 0.01\)). The joint effects of \textit{MGAT1}, CTLA4, IL2RA, IL7RA and the two interactions using a multiple conditional logistic regression were statistically highly significant (\(P < 5 \times 10^{-10}\)). The \textit{MGAT1}-CTLA-4 interaction was replicated (\(P = 0.01\)) in 179 trio families from the Genetics of Kidneys in Diabetes study. These data are consistent with defective N-glycosylation of T cells contributing to T1D pathogenesis.

\textit{Genes and Immunity} (2014) \textbf{15}, 218–223; doi:10.1038/gene.2014.7; published online 27 February 2014

Keywords: type 1 diabetes; N-glycosylation; genetic interaction; \textit{MGAT1}

\textbf{INTRODUCTION}

With the advancement of high-throughput genotyping technologies, hundreds of common genetic variants have been identified for complex human traits, such as type 1 diabetes (T1D, MIM 222100). However, it has been reported that these genetic variants explain only a small proportion of heritability.\textsuperscript{1} Gene–gene interactions are likely a major factor in explaining the mystery of missing heritability,\textsuperscript{1} and, thus, characterizing gene–gene interactions is of fundamental importance in unraveling the etiology of complex human diseases. However, successful detection of gene–gene interactions faces many challenges. A major constraint is the issue of multiple hypothesis testing. In a genome-wide search for gene–gene interactions, correcting for the very large number of tests greatly diminishes the power to detect interactions with moderate effects.

Single-gene disorders displaying Mendelian inheritance disrupt molecular pathways at a single step. However, a similar degree of pathway disruption may be obtained through small defects in the multiple genes/environmental inputs that combine to disrupt a single pathway. These interactions may be epistatic or additive and may promote disease only when combined, and are therefore poorly detected by genome-wide association studies. A functional approach that groups candidate variants on the basis of a shared ability to alter a common molecular pathway provides an alternative method to identify interactions. Indeed, we recently reported that multiple environmental factors (vitamin D\textsubscript{3} deficiency and metabolism) and multiple genetic variants (IL7RA, IL2RA, \textit{MGAT1}, \textit{MGAT5} and CTLA-4) converge to dysregulate Golgi \textit{N}-glycosylation and T-cell function in multiple sclerosis (MS).\textsuperscript{2–4} Causality of defective \textit{N}-glycosylation in MS is supported by animal data, where genetic- and metabolic-induced alterations control T-cell growth, T\textsubscript{h}1/T\textsubscript{h}17 differentiation and autoimmunity, including development of a spontaneous MS-like disease in \textit{Mgat5}-deficient PL/J mice.\textsuperscript{5–9} In MS, epistatic interactions and additive effects were observed between the four variants and environmental factors resulting in dysregulated \textit{N}-glycosylation. For example, a haplotype of the Golgi \textit{N}-glycosylation enzyme \textit{MGAT1} promotes MS, alters \textit{N}-glycosylation, T-cell activation thresholds and surface expression of anti-autoimmune cytotoxic T-lymphocyte antigen 4 (CTLA-4) in a manner that is sensitive to metabolic conditions, vitamin D\textsubscript{3} signaling, the number of N-glycans attached to CTLA-4 (CTLA-4, rs2317775) and interleukin-7/interleukin-2 signaling modulation by the IL2RA (rs6897932) and IL7RA (rs2104286) variants. The interaction between the \textit{MGAT1} and CTLA-4 variants was epistatic, as CTLA-4 (rs2317775) lacks univariate association with MS. In contrast, a non-additive interaction was observed between the \textit{MGAT1} risk variant and a combination of the IL7RA and IL2RA risk variants, a result consistent with their opposing effects on mRNA levels of the \textit{MGAT1} enzyme. These data suggest that studies examining only univariate association, such as genome-wide association studies, are unlikely to adequately define heritability.

Studies have shown that genetic risk factors and pathways are frequently shared across different autoimmune diseases, albeit not always in the same direction.\textsuperscript{10–14} For example, the interleukin-2 receptor-\(\alpha\) (IL2RA) gene is significantly associated with both MS and T1D;\textsuperscript{10,11} however, the direction of the effect may be the same or opposite depending upon the specific variant examined.\textsuperscript{12,15} Similarly, \textit{HLA-DR15} is a risk marker for MS but is protective in T1D. These considerations, along with a common molecular target...
(that is, N-glycosylation), motivated us to hypothesize that the four MS variants we detected may also interact in T1D to determine disease susceptibility. By borrowing the interaction information learned from MS, the burden of multiple testing present in a random genome-wide search is significantly reduced. The most common test for genetic association is the case–control design; however, this can be biased by population stratification. A common way to analyze family data is with conditional logistic regression (CLR). Cordell et al. proposed the use of CLR to test genetic interaction between two variants by constructing 15 pseudo controls for each affected child. This approach is difficult to be generalized to examine multiple variants as the number of pseudo controls for each affected child grows exponentially with the number of variants. In addition, analyzing linked variants requires knowledge of recombination rates between variants. One way to avoid these complications is to match each affected child to the pseudo control whose genotype is formed by all of the other non-transmitted alleles by parents. Kotti et al. used this matching strategy to test gene–gene interactions. We have recently shown that Kotti’s matching strategy is suboptimal for testing gene–gene interactions. Therefore, to test both additive and non-additive genetic effects using the multiplex family data collected by the T1DGC, we utilized an easy-to-implement yet efficient method of constructing pseudo controls. Using this method, we identified additive and non-additive effects of MGAT1, CTLA4, IL2RA, and interleukin-7 receptor-α (IL7RA) on T1D risk, with an overall P-value < 5 × 10^-10.

RESULTS

A novel matching strategy

In recent theoretical work, we examined and compared CLR under two matching strategies: 1:1 matching and exhaustive matching. Suppose that we are interested in testing L loci. In 1:1 matching, each affected child is matched to its ‘anti-self’, a pseudo control whose genotype is formed by the non-transmitted alleles. In exhaustive matching, each affected child is matched to 4^L-1 pseudo controls. The two matching strategies at two loci for a case–parent trio are illustrated in Figure 1a. Compared with exhaustive matching, the 1:1 matching strategy is simpler, more straightforward to implement and computationally easier. Furthermore, 1:1 matching does not require knowledge of recombination rates between markers, whereas exhaustive matching does. Intuitively, 1:1 matching utilizes less information from the data. However, we found that 1:1 matching is as efficient as exhaustive matching when the true underlying genetic effects are additive, which requires that there are no intra- or inter-locus interactions. Thus, when the focus is on additive genetic effects, we can safely use 1:1 matching; on the other hand, when the focus is non-additive effects, we should consider exhaustive matching.

On the basis of our prior understanding of MGAT1 and other genetic variants altering N-glycosylation in MS, we expected both additive effects and gene–gene interactions between variants of the following four genes: MGAT1 (rs7726005 and rs2070924), CTLA4 (rs2317775), IL2RA (rs2104286) and IL7RA (rs6897932). At the individual gene level, our studies in MS indicate that the MGAT1 IVaVT-T haplotype (rs7726005 and rs2070924) has a dominant effect, whereas single-nucleotide polymorphisms (SNPs) rs2317775 (CTLA4), rs2104286 (IL2RA) and rs6897932 (IL7RA) demonstrate additive effects. Between genes, we found that the MGAT1 IVaVT-T haplotype interacts with two sets of SNPs: rs2317775 (CTLA4), and a combination of rs2104286 (IL2RA) and rs6897932 (IL7RA). In the following, we show how this prior information can be used to facilitate our construction of pseudo controls for each affected child in the T1DGC study.

The rs2070924 SNP in MGAT1 is almost in complete linkage disequilibrium with rs7726005. The haplotype formed by these two rare SNPs shows a dominant effect, indicating that exhaustive
matching would be more efficient than 1:1 matching; however, the frequency of the haplotype is rare, and in this case a dominant model is close to an additive model. To reduce the complexity of matching, we used 1:1 matching at MGA1. Both rs2104286 (IL2RA) and rs6897932 (IL7RA) show additive individual effects in our previous MS study; therefore, we used 1:1 matching at each of the two genes. As there was no evidence of genetic interaction between rs231775 (CTLA4) and rs2104286 (IL2RA), we assumed that the alleles at rs231775 (CTLA4) and rs2104286 (IL2RA) are co-transmitted from parents to offspring. In addition, there was no evidence of genetic interaction between rs231775 (CTLA4) and rs6897932 (IL7RA). Thus, we could also let rs231775 (CTLA4) co-transmit with rs6897932 (IL7RA) when constructing pseudo controls. Indeed, the two methods presented identical results. Finally, because we wanted to test gene–gene interactions, we considered exhaustive matching among the three groups (MGA1), (IL2RA, CTLA4) and (IL7RA), leading to 1:7 matching. It should be noted that the genes are in linkage equilibrium. Thus, under the null hypothesis, the eight possible offspring genotypes, including that of an affected child and his/her seven matched pseudo controls, are equally likely. We used H(G) to denote the eight genotypes given the parental genotype G*. The final matching strategy to identify both additive and non-additive multi-locus genetic effects of these genes is summarized in Figure 1b.

CLRs

Matched case–control data are often analyzed by CLRs. Let G*0 be the genotype of the ith child among n total affected children and G*i be the genotype of the parents of the ith affected child. Using the matching strategy we described above, the likelihood function of association parameters is

$$L(\beta) = \prod_{i=1}^{n} \exp\left(\beta G_i^0 \sum_{(G_i' \in W(G_i^0))} \exp(\beta G_i')\right)$$

The form of \(\beta\) and G*0 depends upon our model. For example, when testing the association of the dominant MGA1 IVs/VV-T haplotype in T1D, we assigned G*0 to 1 if the offspring has at least one copy of the haplotype, and 0 otherwise. Correspondingly, \(\beta\) is the log of the genotype relative risk (GRR) for carriers of the haplotype to those non-carriers. As another example, when testing the interaction between the MGA1 IVs/VV-T haplotype and rs231775 (CTLA4), G*0 is a vector of numerical values with three elements indicating the presence of the MGA1 IV/V haplotype, the number of copies of the G allele (that is, the risk allele) of rs231775 (CTLA4), and the product of the first two numbers. Correspondingly, \(\beta\) is a vector of coefficients corresponding to the main effect of the MGA1 IV/V haplotype, the main effect of rs231775 (CTLA4) and the interaction of the two variants, respectively.

We characterized the significance of additive and non-additive effects utilizing P-values. P-values of individual terms in a multiple CLR were calculated using the Wald test. When examining the joint effect of multiple terms, we use the likelihood ratio test. CLRs were fitted using the ‘clogit’ function in the survival package in R (http://cran.r-project.org/package=survival).

Gene–gene interactions and joint effects in the T1DGC study

We first examined the individual effects of the four variants. We used the risk alleles (column 3 of Table 1) as the test alleles and the protective alleles as the reference alleles. Many groups have reported association between T1D and CTLA4, IL2RA and IL7RA.21,22 All variants are significantly associated with T1D except the MGA1 IVs/VV-T haplotype (Table 1). This differs from MS, where all variants were associated, except CTLA-4.

Motivated by the interactions between MGA1 and CTLA4, IL2RA and IL7RA for MS susceptibility, we tested their genetic interactions for T1D susceptibility. Although the MGA1 IVs/VV-T haplotype does not show univariate association with T1D, it is a protective, neutral and risk allele for AA, AG and GG CTLA-4 genotypes, respectively (Table 2). For subjects with the AG genotype at rs231775 (CTLA4), the MGA1 IVs/VV-T haplotype shows no association with T1D. For subjects with the AA genotype, that is, the low-risk group based on CTLA-4, the MGA1 IVs/VV-T haplotype leads to an increased risk for T1D with a GRR of 1.53 (P-value = 0.014). For subjects with the GG genotype, that is, the high-risk group based on CTLA-4, the MGA1 IVs/VV-T haplotype has a protective role for T1D with a GRR of 0.58 (P-value = 0.028). The different effects of the MGA1 IVs/VV-T haplotype under the three CTLA-4 genotypes suggest a gene–gene interaction between the two variants. The P-value for interaction is 0.029. Stratified point estimates of GRRs, 95% confidence intervals and P-values can be found in Table 2.

In MS, the MGA1 IVs/VV-T haplotype also interacts with a combination of the IL2RA and IL7RA risk alleles.2 Our CLR indicates that the MGA1 IVs/VV-T haplotype also shows differential effects on T1D susceptibility between subjects with four risk alleles of IL2RA and IL7RA versus other subjects (Table 3). The MGA1 IVs/VV-T haplotype increases T1D risk for subjects at high risk based on IL2RA and IL7RA. It has a protective risk in the rest of the population, as can be seen from the point estimates of the GRRs in Table 3. Testing the interaction between them leads to a P-value of 0.013.

As the variants we considered here are in linkage equilibrium, the interactions we observed cannot be explained by each other.

### Table 1. Individual genetic effects estimated from the T1DGC data

| Alleles | Freq | GRR (95% CI) | P-value |
|---------|------|-------------|---------|
| MGA1 IVs/VV-T (rs7726005, rs2070924) | — | 0.039 | 1.06 (0.87–1.29) | 0.58 |
| rs231775 (CTLA4) | A, G | 0.414 | 1.17 (1.08–1.26) | 5.3 x 10^-5 |
| rs2104286 (IL2RA) | A, G | 0.775 | 1.25 (1.14–1.36) | 6.9 x 10^-7 |
| rs6897932 (IL7RA) | C, T | 0.751 | 1.12 (1.02–1.22) | 1.3 x 10^-2 |

### Table 2. Stratified analysis of MGA1 IVs/VV-T using the T1DGC data based on CTLA4 genotypes

| CTLA4 genotypes | GRR (95% CI) | P-value |
|----------------|-------------|---------|
| AA | 1.53 (1.09–2.15) | 1.4 x 10^-2 |
| AG | 1.00 (0.75–1.33) | 1.0 |
| GG | 0.58 (0.36–0.95) | 2.8 x 10^-2 |

**Abbreviations:** CI, confidence interval; GRR, genotype relative risk; T1DGC, Type 1 Diabetes Genetics Consortium. The P-value for interaction is 2.9 x 10^-2.

Gene–gene interactions and joint effects in the T1DGC study

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As the variants we considered here are in linkage equilibrium, the interactions we observed cannot be explained by each other.
To confirm this and evaluate the overall impact of the variants, we fit a multiple CLR with the four variants and the two interactions. Table 4 indicates that the point estimates of the GRRs and P-values for the four variants in the multiple CLR are similar to those from individual CLRs.

The two interaction terms are significant in the multiple CLR, indicating they are still important after accounting for the main effects, consistent with what we observed in Tables 2 and 3. Therefore, we used a likelihood ratio test of two degrees of freedom to examine the joint effects of the two interaction terms. The P-value based on the likelihood ratio test is 0.002. Finally, we used a likelihood ratio test of seven degrees of freedom to test the joint effect of both main and interaction effects. The P-value is <5 × 10⁻¹⁰.

Replication analysis in the GoKinD study

The MGAT1 IV₉/V₇-T haplotype is not available in public genome-wide association study data sets. Therefore, to attempt replicating the results above, we genotyped all four variants in 379 trios from the Genetics of Kidneys in Diabetes study (GoKinD). The results show that the only significant individual term is IL7RA (P-value 0.016). This is not surprising. First, the sample size is much smaller than that of the T1DGC study. Second, the T1D offspring in the T1DGC study are from variances in metabolism between T1D and MS, coupled with clues and new tools for understanding the pathogenic process of MGAT1.

**DISCUSSION**

In this article, we presented two potential gene–gene interactions involved in regulating N-glycosylation and possibly T1D susceptibility. Our analysis is knowledge-driven and is motivated by the fact that gene–gene interactions were observed in MS. It is known that both MS and T1D are autoimmune diseases and they share many common pathways. Different from a genome-wide search for gene–gene interactions, our analysis avoids multiple testing and thus potentially improves power. We have observed gene–gene interactions between the MGAT1 IV₉/V₇-T haplotype and CTLA-4 in both the T1DGC study and the GoKinD study. This provides encouraging evidence that the observed interaction is likely to be true. The interaction between the MGAT1 IV₉/V₇-T haplotype and IL2RA and IL7RA is observed in the T1DGC families but not in the GoKinD families. As the T1DGC study is larger than the GoKinD and identifying gene–gene interaction requires large sample sizes, it is likely that the interaction is true but the GoKinD study is not powerful enough to detect it. Independent studies are required to further examine how MGAT1, IL2RA and IL7RA jointly affect T1D susceptibility.

Specifically, in both the T1DGC study and the GoKinD trios free from kidney disease, the MGAT1 IV₉/V₇-T haplotype increases the risk of T1D in patients with the AA genotype at CTLA-4 and has a protective role in patients with the AG or GG genotype. The interaction is not significant in the GoKinD trios with kidney disease and it is estimated that the MGAT1 IV₉/V₇-T haplotype has a similar role in the AA and AG/GG groups (Table 5).

We also tested the interaction between the MGAT1 IV₉/V₇-T haplotype and the IL2RA and IL7RA polymorphisms. No interaction was identified for either the separate or combined analysis of the GoKinD families. This is not surprising, given the lower effect size of the interaction and reduced power relative to the T1DGC cohort.
surface expression of the CTLA-4 protein in T cells. The MAGT1 IVα/IV-T haplotype is a gain of function that increases mRNA and protein levels of the Golgi enzyme Mgat1. When metabolism limits substrate availability (that is, UDP-GlcNAc derived from glucose) to the Golgi, the MAGT1 IVα/IV-T gain-of-function haplotype paradoxically lowers N-glycan branching by limiting UDP-GlcNAc availability to downstream Golgi enzymes, resulting in reduced cell surface expression of the anti-autoimmune CTLA-4 protein. The G allele of CTLA-4 (rs2317775) decreases the number of N-glycans attached to CTLA-4 by 50%, thereby also reducing surface expression of the CTLA-4 protein. Thus, when metabolism limits Golgi substrate (UDP-GlcNAc) availability, the MAGT1 IVα/IV-T haplotype and the G allele of CTLA-4 (rs2317775) combine to lower the CTLA-4 cell surface expression,2 consistent with the genetic interaction observed in MS. In contrast, when metabolism increases Golgi UDP-GlcNAc substrate supply, as occurs with high glucose levels,7 the MAGT1 IVα/IV-T haplotype has the opposite effect on N-glycan branching and CTLA-4 surface expression. Thus, under high glucose levels often present in early stages of T1D, the MAGT1 IVα/IV-T haplotype is expected to counteract the G allele of CTLA-4 (rs2317775) to promote CTLA-4 protein expression and inhibit T-cell function, consistent with the protective genetic interaction observed in T1D.

Most existing multi-loci methods for family data only provide an overall significance of multiple loci or specific combinations of alleles. Examples of such methods include haplotype-based methods,4, 30–32 genotype-based methods,30–34 family-based multiple dimension reduction35 and contrasting linkage disequilibrium.36 In comparison, the method we utilized here has two advantages. First, most of the existing multi-focus methods are based upon 1:1 matching, which is not efficient for testing non-additive effects. Second, these methods are mainly for hypothesis testing. In contrast, our method not only provides the significance level of effects but also provides point estimates of GRRs.

PATIENTS AND METHODS

Data sets

We analyzed Caucasian multiplex or trio families in the T1DGC and the GoKinD.23 The SNPs were genotyped using a method previously described.2 We excluded families with missing parents or genotyping errors at any of the four SNPs. For T1DGC, this led to 2858 affected and their parents and (2) 179 T1D patients without kidney disease and their parents and (2) 179 T1D patients without kidney disease and their parents and (2) 179 T1D patients without kidney disease and their parents. Other information, such as genotyping, can be found in a report from the consortium.21 To test genetic effects, we used CLR with a novel matching strategy, as described in the following section.

Statistics

To test genetic effects, CLR with a novel matching strategy was used and is described in detail in the results section. The significance of additive and non-additive effects was characterized by P-values. P-values of individual terms in a multiple CLR are calculated using the Wald test. When examining the joint effect of multiple terms, we used the likelihood ratio test. CLRs were fitted using the 'clogit' function in the survival package in R (http://cran.r-project.org/package=survival).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the National Institute of Diabetes and Digestive and Kidney Diseases for providing access to the T1DGC and GoKinD DNA samples. Research was supported in part by grant R01HG004960 from the National Human Genome Research Institute to ZY and grant R01A082266 from the National Institute of Allergy and Infectious Diseases to MD.

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