

Rare variants at 16p11.2 are associated with common variable immunodeficiency

S. Melkorka Maggadottir, MD,^{a,b,*} Jin Li, PhD,^{b,*} Joseph T. Glessner, PhD,^{b,*} Yun Rose Li, PhD,^{b,k} Zhi Wei, PhD,^c Xiao Chang, PhD,^b Frank D. Mentch, PhD,^b Kelly A. Thomas, BA,^b Cecilia E. Kim, BS,^b Yan Zhao, BS,^b Cuiping Hou, BS,^b Fengxiang Wang, PhD,^b Silje F. Jørgensen, MD,^d Elena E. Perez, MD,^e Kathleen E. Sullivan, MD,^a Jordan S. Orange, MD, PhD,^f Tom H. Karlsen, MD, PhD,^d Helen Chapel, MD,^g Charlotte Cunningham-Rundles, MD,^h and Hakon Hakonarson, MD, PhD^{b,i,j}
Philadelphia, Pa, Newark, NJ, Oslo, Norway, St Petersburg, Fla, Houston, Tex, Oxford, United Kingdom, and New York, NY

Background: Common variable immunodeficiency (CVID) is characterized clinically by inadequate quantity and quality of serum immunoglobulins with increased susceptibility to infections, resulting in significant morbidity and mortality. Only a few genes have been uncovered, and the genetic background of CVID remains elusive to date for the majority of patients.

Objective: We sought to seek novel associations of genes and genetic variants with CVID.

Methods: We performed association analyses in a discovery cohort of 164 patients with CVID and 19,542 healthy control subjects genotyped on the Immuno BeadChip from Illumina platform; replication of findings was examined in an independent cohort of 135 patients with CVID and 2,066 healthy control subjects, followed by meta-analysis.

Results: We identified 11 single nucleotide polymorphisms (SNPs) at the 16p11.2 locus associated with CVID at a genome-

wide significant level in the discovery cohort. The most significant SNP, rs929867 ($P = 6.21 \times 10^{-9}$), is in the gene fused-in-sarcoma (*FUS*), with 4 other SNPs mapping to integrin CD11b (*ITGAM*). Results were confirmed in our replication cohort. Conditional association analysis suggests a single association signal at the 16p11.2 locus. A strong trend of association was also seen for 38 SNPs ($P < 5 \times 10^{-5}$) in the MHC region, supporting that this is a genuine CVID locus. Interestingly, we found that 80% of patients with the rare *ITGAM* variants have reduced switched memory B-cell counts. **Conclusion:** We report a novel association of CVID with rare variants at the *FUS/ITGAM* (CD11b) locus on 16p11.2. The association signal is enriched for promoter/enhancer markers in the *ITGAM* gene. *ITGAM* encodes the integrin CD11b, a part of complement receptor 3, a novel candidate gene implicated here for the first time in the pathogenesis of CVID. (J Allergy Clin Immunol 2015;■■■■-■■■■-■■■■.)

Key words: Immunodeficiency, immunogenetics, genome-wide association study, *ITGAM*, rare variants

From ^athe Division of Allergy and Immunology, ^bthe Center for Applied Genomics, Abramson Research Center, and ^cthe Division of Human Genetics, Children's Hospital of Philadelphia; ^dthe Department of Computer Science, New Jersey Institute of Technology, Newark; ^ethe K.G. Jebsen Inflammation Research Centre, Research Institute of Internal Medicine, Division of Cancer Medicine, Surgery and Transplantation, Oslo University Hospital, Rikshospitalet; ^fthe Division of Allergy, Immunology and Rheumatology, Department of Pediatrics, University of South Florida, St Petersburg; ^gthe Section of Immunology, Allergy and Rheumatology, Texas Children's Hospital, Houston; ^hthe Nuffield Department of Medicine, University of Oxford and Oxford Radcliffe Hospital; ⁱInstitute of Immunology and Department of Medicine, Mount Sinai School of Medicine, New York; and ^jthe Department of Pediatrics, ^kMedical Scientist Training Program, Perelman School of Medicine Philadelphia, University of Pennsylvania.

*These authors contributed equally to this work.

Supported by an Institute Development Fund from CHOP, U01HG006830, and a donation from the Kubert Estate Foundation.

Disclosure of potential conflict of interest: Y. R. Li is supported by the Paul and Daisy Soros Fellowship for New Americans and the NIH F30 Individual NRSA Training Grant. E. E. Perez has received consultancy fees from Baxter and CSL Behring; research support from CSL Behring; lecture fees from the Eastern Allergy Conference and the American College of Allergy, Asthma & Immunology (ACAAI); and royalties from UpToDate. K. E. Sullivan has received consultancy fees from the Immune Deficiency Foundation and UpToDate, research support from Baxter, and royalties from UpToDate. J. S. Orange has received consultancy fees from CSL Behring, Baxter, Atlantic Research, Viracor, Octapharma, BPL, Cangene, and Amerisource Bergen; research support from CSL Behring; lecture fees from Baxter; and royalties from UpToDate and Unimed Publishing. H. Chapel is a board member for Baxter Healthcare and has received consultancy fees from Biotest, LFB. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication March 4, 2014; revised December 24, 2014; accepted for publication December 31, 2014.

Corresponding author: Hakon Hakonarson, MD, PhD, Department of Pediatrics, Perelman School of Medicine, Philadelphia, PA 19104. E-mail: Hakonarson@email.chop.edu.

0091-6749/\$36.00

© 2015 American Academy of Allergy, Asthma & Immunology

<http://dx.doi.org/10.1016/j.jaci.2014.12.1939>

Common variable immunodeficiency (CVID) is characterized by susceptibility to bacterial infections caused by inadequate quantity and quality of immunoglobulins.¹ CVID is a primary immunodeficiency disease that is highly heterogeneous in nature, although it is believed to result from intrinsic immunologic deficits. Significant phenotypic heterogeneity in patients with CVID is demonstrated by variability in age at presentation, as well as related comorbidities, including autoimmune disease, malignancy, chronic lung disease, and gastrointestinal disease.^{2,3}

CVID, although relatively rare (prevalence of 1:25,000-1:50,000), is clinically an important cause of immunodeficiency because of the significant morbidity and mortality conferred by its comorbidities. Given its clinical heterogeneity, it is not surprising that the cause of CVID remains poorly understood and complex. A number of different genes have been implicated as candidates for CVID susceptibility in specific patient subsets. These include genes that act in B-cell receptor signaling (*CD19*, *CD20*, *CD21*, *CD81*, *PLCγ2*, *TWEAK*, and *PKCδ*) or pathways of isotype switching and somatic recombination (*BAFFR*, *ICOS*, *MSH5*, *TACI*, and *NFKB2*).⁴⁻¹⁸ Unbiased approaches, including genome-wide association studies (GWASs) and whole-genome copy number variation studies of CVID, have had limited success to date, but among the associations identified is the MHC region and *ORC4L*.^{8,19,20} However, the above associations only account for a minority of CVID cases. Gaining a better understanding of the genetic variability underlying CVID might be a first and

Abbreviations used

CR3:	Complement receptor 3
CVID:	Common variable immunodeficiency
DC:	Dendritic cell
FUS:	Fused-in-sarcoma
GWAS:	Genome-wide association study
IIBDGC:	International Inflammatory Bowel Disease Genetics Consortium
ITGAM:	Integrin CD11b
LD:	Linkage disequilibrium
Mac-1:	Macrophage receptor 1
MAF:	Minor allele frequency
NF-κB:	Nuclear factor κB
OR:	Odds ratio
QC:	Quality control
SLE:	Systemic lupus erythematosus
SNP:	Single nucleotide polymorphism

critical step to developing personalized approaches to treatment, comorbidity monitoring, and overall care of patients with CVID. Therefore the search for novel genetic susceptibility loci in patients with CVID is a major subject of interest in the world of immunology.

METHODS**Patients**

Because CVID is a rare disease, many of the previous studies were underpowered and had limited coverage of immunology-specific genes. Our study included 360 patients with CVID, the diagnosis of which was confirmed by standard diagnostic criteria. The study was approved by institutional review boards at each participating site, and informed consent for DNA collection and genotyping was obtained from all participants. The patients with CVID were from 4 participating sites and reported in a previous study.¹⁹ The 21,610 healthy control subjects were from the International Inflammatory Bowel Disease Genetics Consortium (IIBDGC) and were previously described in a study by Jostins et al²¹ in 2012.

Single nucleotide polymorphism genotyping and quality control

All patients with CVID and all control subjects were genotyped on the Immuno BeadChip from Illumina (iCHIP) platform (San Diego, Calif),^{22,23} with 196,524 single nucleotide polymorphisms (SNPs) and small indels targeting genes implicated in patients with autoimmune and inflammatory diseases based on previous GWASs.²¹ The design of this chip has been reported previously.²¹ This novel array is designed to provide dense coverage of rare polymorphisms and strong candidate genes for major autoimmune and inflammatory diseases from prior GWASs. We sought to associate these rare immune gene SNPs with CVID, as well as with clinical phenotypes, to address the heterogeneity of CVID in our ongoing study aimed at identifying susceptibility loci for CVID.

We obtained the cluster egi file from the IIBDGC and applied it to our data. In the quality control (QC) filtering step, 18,401 SNPs were excluded because of the low genotyping rate (<95%), and 30 samples (29 cases and 1 control subject) were removed from further analysis because of the low sample call rate (<95%). We further filtered out SNPs that deviated from Hardy-Weinberg equilibrium ($P < 10^{-6}$). To adjust for population stratification, we evaluated ancestry-informative markers for all samples by using principal component analysis implemented in EIGENSTRAT²⁴ software. On the basis of these results, we removed an additional 4 outliers (3 cases and 1 control subjects). We further performed genome-wide identity-by-descent analysis with linkage disequilibrium (LD)-pruned SNPs. By using a measure of relatedness P_i -hat cutoff of 0.25, 29 cryptic related pairs of cases were detected, and 1 sample

from each pair was removed from the analysis. An additional 6,813 SNPs fall into the IIBDGC's SNP exclusion list or failed our manual review of Genome Studio Illumina cluster plots and were also excluded from further analysis.

According to principal components plotting (see Fig E1 in this article's Online Repository at www.jacionline.org), we divided our data set into a discovery cohort and a replication cohort. The discovery cohort was composed of 164 cases and 19,542 control subjects. SNP QC was conducted again within the discovery cohort, which left 170,212 SNPs, including both common and rare variants. The replication cohort consists of 135 cases and 2,066 control subjects. Similar SNP QC resulted in a final collection of 169,914 SNPs in the replication cohort. We also further conducted principal component analysis within the discovery and replication cohorts separately.

Association analysis

After sample and SNP-based QC, association testing was carried out in the discovery cohort. Logistic regression under the additive model was used for association analysis, with the first 3 principal components as covariates. The quantile-quantile plot of null SNP P values (see Fig E2 in this article's Online Repository at www.jacionline.org) and an estimated genomic inflation factor λ value of 1.03 do not suggest any substantial population stratification. Conditional regression analysis, LD estimation, and pairwise epistasis tests were conducted with PLINK.²⁵ Association analysis was similarly conducted in the replication cohort. Meta-analysis was performed with PLINK²⁵ and METAL.²⁶

Imputation

Haplotype phasing was first performed with SHAPEIT v2,²⁷ and local imputation at the 16p11.2 locus was carried out with reference to the 1000 Genomes database (http://mathgen.stats.ox.ac.uk/impute/data_download_1000G_phase1_integrated.html) by using IMPUTE2.^{28,29} Afterward, the missing data likelihood score test implemented in SNPTEST V2 software²⁹ was conducted for investigating SNP association with CVID phenotype, with the first 3 principal components as covariates.

RESULTS**Novel locus associated with CVID**

We identified a locus on chromosome 16 (Fig 1), with 11 SNPs surpassing genome-wide significance according to logistic regression (Table I), to be associated with CVID. The most significant SNP, rs929867 ($P = 6.21 \times 10^{-9}$; odds ratio [OR], 89.8), is in the gene fused-in-sarcoma (*FUS*). This maps to an LD block on 16p11.2, which is a gene-rich region (Fig 2) containing 10 other SNPs also associated with CVID in our analysis (P value range = 1.39×10^{-8} to 6.79×10^{-9} ; OR range, 58.55-87.84; Table I). Four of the identified SNPs reaching genome-wide significance are mapped in the gene encoding integrin CD11b (*ITGAM*), with 2 additional SNPs being close to this gene as well. Three of the variants in *ITGAM* are intronic, whereas a fourth (rs8056264) is a synonymous variant in the coding region of *ITGAM* (see Table E1 in this article's Online Repository at www.jacionline.org for details). Detailed inspection of the Genome Studio cluster plots of each SNP confirmed distinct clusters for each SNP (Fig E3 in this article's Online Repository at www.jacionline.org). Consistent with the minor allele frequency (MAF) of control subjects in our data set, in the European population of the 1000 Genomes Project, each of these SNPs had an MAF of less than 0.0001 (see Table E2 in this article's Online Repository at www.jacionline.org). By applying conditional logistic regression on the most significant SNP, rs929867, all other associations were ablated (see Table E3 in this article's Online Repository at www.jacionline.org).

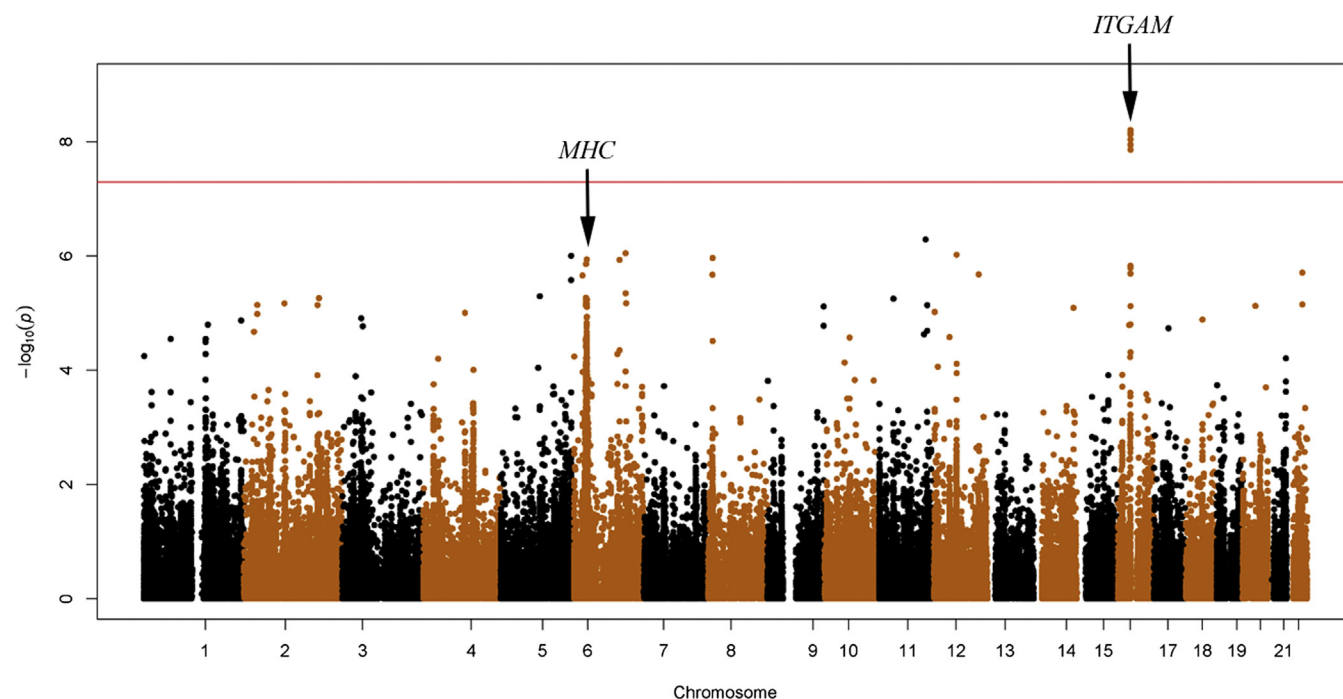


FIG 1. Manhattan plot of genome-wide association results for patients with CVID in the white discovery cohort. The red line indicates the genome-wide significance threshold.

[jacionline.org](http://www.jacionline.org)), suggesting a single association signal at the 16p11.2 locus. In concordance with this, no epistatic interaction was noted among the genome-wide significant SNPs at this locus ($P > .05$ for all epistasis tests).

Next, we sought to replicate these findings in the independent replication cohort. All 11 SNPs identified in the discovery cohort replicated with nominal P values of less than .05 (Table I). When the 2 cohorts were combined by means of meta-analysis, all 11 SNPs were found to be genome-wide significant, and 2 additional genome-wide significant SNPs were uncovered ($P = 2.96 \times 10^{-8}$ and 4.25×10^{-8} ; OR, 9.22 and 24.91, respectively; Table I). The genotype status of each patient with CVID carrying the rare variant allele was further validated by means of Sanger sequencing together with randomly selected patients with CVID without the rare variant allele, who were confirmed to have negative results (see Fig E4 in this article's Online Repository at www.jacionline.org).

Using the 1000 Genomes database as a reference, we conducted imputation around the 16p11.2 locus and uncovered 23 additional SNPs of genome-wide significance in our meta-analysis (see Table E4 in this article's Online Repository at www.jacionline.org), further supporting the importance of this locus.

Association of the MHC region with CVID

The MHC region has previously been reported to be significantly associated with CVID.¹⁹ We found 2 SNPs in the MHC region that reached genome-wide significant P values in the discovery cohort, and a third SNP reached a marginally significant P value of less than 5×10^{-7} . However, these SNPs (rs2517625, rs6933596, and rs7773051) were excluded from the final analysis because of abnormal Genome Studio cluster plots. Despite this, we found a strong trend of association by 38 SNPs

($P < 5 \times 10^{-5}$) in this region (chromosome 6: 28-34 Mb) in the discovery cohort (Fig 1), with the strongest association at rs879882 ($P = 1.156 \times 10^{-6}$). Meta-analysis yielded 20 SNPs in this region with P values of less than 5×10^{-5} (Table II). These results support the MHC region as a genuine CVID locus, as previously reported.¹⁹ Additionally, SNPs in the genes *CR2*, *ICOS*, *MSH5*, and *TACI* were found to be of nominal significance ($P < .05$) in our meta-analysis (Table III). Although known *TACI* carriers were included in our patient population, these SNPs are not covered by the iCHIP and thus did not reach higher significance in our analysis. We detected the nominally significant SNP rs61061086 in the gene *FLJ16124* (Table III), which was reported to be one of the top genes associated with CVID in a previous GWAS.¹⁹ Furthermore, the aforementioned conditional analysis on rs929867 did not ablate the association seen with SNPs across the MHC region, and there were no epistatic interactions observed between SNPs at the 16p11.2 and MHC loci. This suggests that the 2 loci independently affect the risk of CVID.

Functional annotation of the significant SNPs at the *FUS/ITGAM* locus

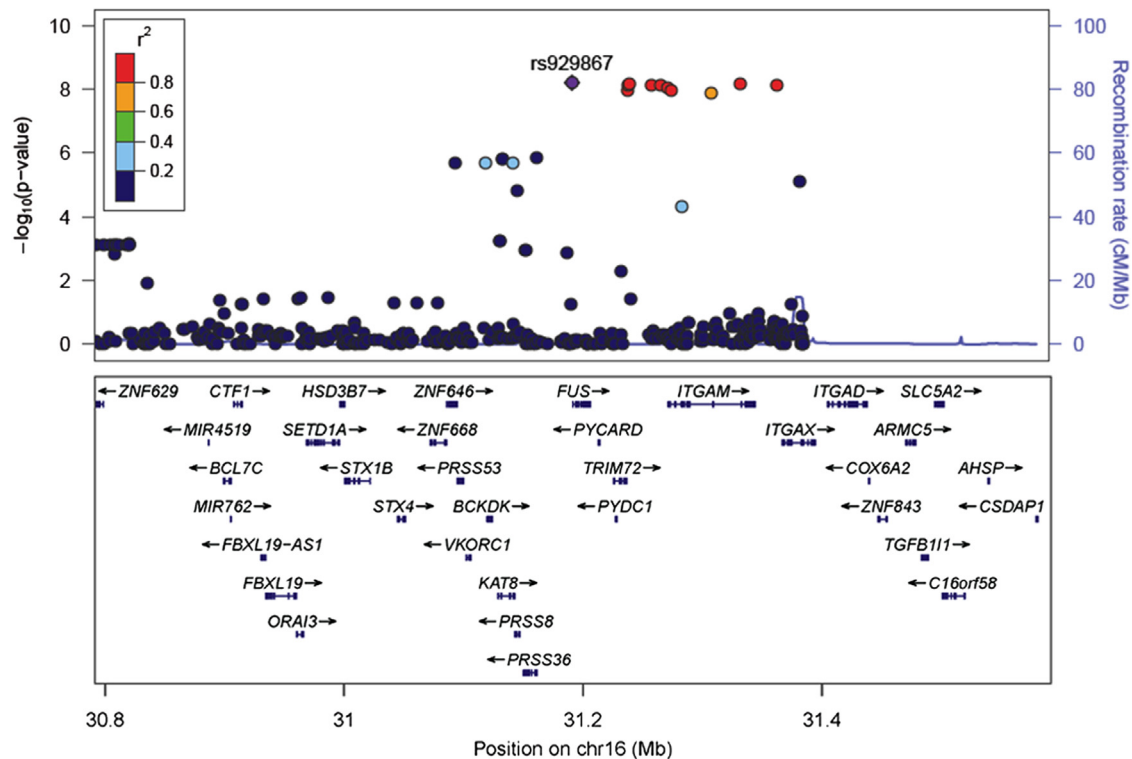
A number of SNP polymorphisms identified to be associated with complex diseases have been shown to affect the disease through regulation of gene expression through the DNA macromolecular complex. To determine whether any of the identified SNPs from this study might have a regulatory role, we searched for prior identified or predicted functional annotations^{30,31} among these SNPs by using the software HaploReg.³² Our results show that the identified genome-wide significant SNPs associated with CVID susceptibility are enriched for enhancer markers (see Table E5 in this article's Online Repository at www.jacionline.org) and DNase sites

TABLE I. Genome-wide significant SNPs at 16p11.2

SNP	Base pair (hg19)	Closest gene	Minor/major allele	Dis MAF cases	Dis MAF control subjects	Dis OR	Dis <i>P</i> value	Rep <i>P</i> value	Meta-analysis OR	Meta-analysis <i>P</i> value
rs750953	31094089	<i>ZNF646</i>	C/T	0.0061	0.000102	66.7	2.05×10^{-6}	1.84×10^{-3}	24.91	4.25×10^{-8}
rs929867	31191482	<i>FUS</i>	A/G	0.00926	0.000102	89.8	6.21×10^{-9}	5.50×10^{-3}	37.33	5.49×10^{-10}
rs7192718	31237920	<i>TRIM72*</i>	T/C	0.00915	0.000128	67.94	1.15×10^{-8}	2.91×10^{-4}	33.37	3.64×10^{-11}
rs10871455	31238690	<i>TRIM72*</i>	T/G	0.00915	0.000102	87.84	7.39×10^{-9}	2.89×10^{-4}	36.61	3.19×10^{-11}
rs12934814	31239855	<i>TRIM72*</i>	T/C	0.00943	0.000128	72.13	7.11×10^{-9}	2.42×10^{-4}	37.9	1.65×10^{-11}
rs4889637	31258238	<i>ITGAM*</i>	C/T	0.00915	0.000128	72.23	7.54×10^{-9}	2.77×10^{-4}	35.19	2.35×10^{-11}
rs4889641	31265541	<i>ITGAM*</i>	A/G	0.00915	0.000103	87.6	7.55×10^{-9}	2.89×10^{-4}	37.29	3.11×10^{-11}
rs8063978	31271700	<i>ITGAM</i>	C/T	0.00938	0.000128	70.39	9.16×10^{-9}	5.64×10^{-3}	32.76	6.67×10^{-10}
rs4889541	31274551	<i>ITGAM</i>	T/C	0.00915	0.000128	68.29	1.12×10^{-8}	7.98×10^{-3}	30.51	1.29×10^{-9}
rs9929801	31283472	<i>ITGAM</i>	C/T	0.00915	0.000744	12.12	4.88×10^{-5}	1.34×10^{-4}	9.22	2.96×10^{-8}
rs8045402	31308281	<i>ITGAM</i>	T/C	0.00915	0.000154	58.55	1.39×10^{-8}	3.38×10^{-5}	30.34	4.73×10^{-12}
rs8056264	31332655	<i>ITGAM</i>	T/C	0.00915	0.000128	73.24	6.79×10^{-9}	2.96×10^{-4}	35.38	2.31×10^{-11}
rs11574629	31362892	<i>ITGAX*</i>	G/A	0.00915	0.000103	87.75	7.44×10^{-9}	3.28×10^{-4}	37.26	3.50×10^{-11}

Dis, Discovery cohort; Rep, replication cohort.

*Not directly on gene.

**FIG 2.** Regional association plot at the 16p11.2 (*ITGAM*) locus. SNPs are plotted by chromosomal position in a 400-kb window against their $-\log_{10}$ transformed *P* values for association with CVID phenotype in the discovery cohort. The color of each SNP reflects their LD with the most significant SNP, rs929867. The r^2 values were calculated from the discovery cohort in PLINK.

(see Table E6 in this article's Online Repository at www.jacionline.org) in certain immune cell types, such as B lymphocytes. Furthermore, genomic annotation (see Table E7 in this article's Online Repository at www.jacionline.org) with HaploReg³² shows each of the genome-wide significant SNPs to overlap with promoter/enhancer histone markers or transcription regulatory motifs, especially in CVID-relevant immune cell types, suggesting these SNPs might affect transcriptional regulation in immune cells.

Network and pathway analysis of *ITGAM*

Because multiple significant SNPs were located in the *ITGAM* gene, we conducted a network and pathway analysis around this gene to further understand its biological function. Using a FunCoup^{33,34} search, we found multiple known and predicted *ITGAM* functional association partners (*ALOX5AP*, *SELL*, *C3*, *FCER1G*, *ICAM1*, *ITGAX*, *ITGB2*, *LYN*, *NCF1*, *NCF2*, *NCF4*, *PLAUR*, *TYROBP*, *VAV1*, *SPI1*, and *CYBA*). Five of these contain SNPs with nominally significant associations with CVID in our

TABLE II. SNPs in the MHC region with a meta-analysis
P value of less than 5×10^{-5}

SNP	Chromosome	Base pair (hg19)	Minor allele	Meta-analysis <i>P</i> value	Meta-analysis OR
rs3130724	6	29118530	A	6.39×10^{-6}	1.55
rs3129151	6	29138315	T	7.31×10^{-6}	1.55
rs3129152	6	29138777	C	1.39×10^{-5}	1.52
rs760804	6	29171039	T	3.86×10^{-6}	1.57
rs3117426	6	29272012	T	3.15×10^{-6}	1.58
rs3131036	6	30728290	A	1.05×10^{-5}	1.49
rs3094117	6	30737486	C	1.56×10^{-5}	1.47
rs3129981	6	30758857	T	3.05×10^{-6}	1.67
rs3095089	6	30933794	T	2.66×10^{-5}	1.54
rs3131932	6	30940328	A	1.88×10^{-5}	1.44
rs3132579	6	30940989	C	3.03×10^{-5}	1.57
rs2073721	6	31129616	A	3.73×10^{-5}	0.61
rs2073723	6	31130078	T	3.38×10^{-5}	0.61
rs1065461	6	31130502	T	1.00×10^{-5}	0.58
rs3132528	6	31131569	C	4.81×10^{-5}	0.61
rs9263805	6	31135735	A	3.15×10^{-5}	0.61
rs3130501	6	31136453	A	3.48×10^{-5}	0.61
rs3130502	6	31136666	A	4.87×10^{-5}	0.62
rs6923313	6	31241370	C	3.63×10^{-5}	0.68
rs2507997	6	31314781	G	3.69×10^{-5}	1.47

study (see Table E8 in this article's Online Repository at www.jacionline.org). To further explore the biological implication of *ITGAM* association with CVID, we input *ITGAM*, the nominally significant CVID susceptibility genes (*CR2*, *ICOS*, *MSH5*, *TNFRSF13B*, and *FLJ16124*), and the nominally significant *ITGAM* functional association partners (*ITGAX*, *C3*, *LYN*, *VAV1*, and *NCF2*) into FunCoup^{33,34} for association network analysis, the results of which are shown in Fig E5 in this article's Online Repository at www.jacionline.org. We also input these genes into STRING,^{35,36} another protein-protein interaction network analysis tool, with similar results. The pathway analysis tool implemented in FunCoup^{33,34} demonstrated enrichment of multiple B cell- and T-cell-signaling pathways, which are highly relevant to CVID pathophysiology (see Table E9 in this article's Online Repository at www.jacionline.org).

Clinical phenotypes of the patients with CVID carrying rare *ITGAM* variants

To determine whether these susceptibility SNPs are associated with certain clinical characteristics or a subgroup of patients with CVID, we examined the available phenotype information for patients with the rare *ITGAM* variant. Results showed that 50% were female and 80% ($n = 4/5$, data not available for $n = 1$) had low switched memory B-cell counts ($<2\%$ of B cells³), which is a higher percentage than seen among the rest of the patients with CVID (60%, $n = 105/175$ with data available) or in the EUROclass study (58%³; Table IV). Reviewing the clinical phenotypes of these subjects, we found only 1 subject to have autoimmune cytopenia (idiopathic thrombocytopenic purpura), and this patient also had nodular regenerative hyperplasia of the liver. Two patients had lung disease, 1 had bronchiectasis, and another was given a diagnosis of lung cancer. A second patient had cancer (Burkitt lymphoma), and this patient also had systemic lupus erythematosus (SLE) with lupus nephritis requiring renal transplantation.

Sanger sequencing of *FUS*, *ITGAM*, and *ITGAX*

To elucidate the genetic association of 16p11.2 with CVID, we performed Sanger sequencing of the plausible candidate genes at this locus. We evaluated 2 patients with CVID carrying the rare variant at all the genome-wide significant SNPs at the 16p11.2 locus and 1 patient with CVID with none of these rare variants. All exons of the 3 plausible candidate genes (*FUS*, *ITGAM*, and *ITGAX*) in strong LD with the most significant SNPs were interrogated by using Sanger sequencing. Again, we confirmed the C>T synonymous variant of rs8056264 in exon 15 of gene *ITGAM*. However, no exonic nucleotide bases were uncovered that demonstrated corresponding rare variant nonsynonymous changes that would lead to an amino acid change in the translated protein product and directly affect gene function in patients with CVID, suggesting that the association signal at the locus is capturing some other regulatory effects on gene function yet to be established.

DISCUSSION

We describe a novel association of CVID with a rare variant using the iCHIP platform, which densely covers immune disease-related genes. The genome-wide significant locus discovered at 16p11.2 presents the first SNP association with CVID in a genome-wide study in a non-MHC region. This locus is downstream of and in separate LD blocks from the 16p11.2 locus (29.5-30.1 Mb), which was previously associated with autism, schizophrenia, and obesity (see Fig E6 in this article's Online Repository at www.jacionline.org).³⁷⁻³⁹ The single association signal at this locus and genomic annotation suggests these rare SNPs can affect transcriptional regulation in lymphocytes. The most significant SNP, rs929867, maps to the gene *FUS*, encoding a multifunctional protein component of the heterogeneous-nuclear-ribonucleoprotein complex. *FUS*, belonging to the FET family of RNA-binding proteins, has an important role in pre-mRNA splicing and nuclear export of processed mRNA and might consequently help maintain genomic integrity.^{40,41} Mutations in *FUS* have been reported in amyotrophic lateral sclerosis type 6.^{42,43}

Two SNPs that are significantly associated with CVID in our study are of particular interest. The SNP rs929867, which has the best *P* value in our discovery cohort, is located in the 5' untranslated region of the *FUS* gene. This region is crucial for translational regulation in many species⁴⁴ by regulating translation efficiency and mRNA stability through secondary structures and RNA-binding proteins.^{44,45} It could function as the binding site for several transcription factors (see Table E7), such as CCCTC-binding factor (CTCF) and nuclear factor κ B (NF- κ B), which both play essential roles in normal B-cell function.^{46,47} NF- κ B has also been associated with development of CVID.¹⁸ Another SNP, rs8056264, causes a synonymous substitution in the *ITGAM* gene. Although synonymous mutations do not alter the amino acid sequence, they can affect mRNA transport, splicing, and even translation because of codon use bias.^{48,49} Using the HaploReg software,³² we found that both SNPs are conserved sites by using the SiPhy-omega (Site-specific PHYlogenetic analysis) algorithm.⁵⁰ Whether these rare variants truly affect mRNA or protein levels of *FUS* or *ITGAM* needs to be further explored.

Among the genes in the 16p11.2 locus, *ITGAM* is the most attractive candidate, with 4 genome-wide significant SNPs

TABLE III. Association results for candidate genes

SNP	Chromosome	Base pair (hg19)	Gene	Distance	Minor/major allele	Meta-analysis <i>P</i> value	Meta-analysis OR
rs4618970	1	207661640	<i>CR2</i> (<i>CD21</i>)	0	C/T	.0429	1.27
rs10932030	2	204803483	<i>ICOS</i>	0	C/T	.0134	1.94
rs9968356	4	151471789	<i>LRBA</i>	0	A/G	.0944	1.42
rs1144708	6	31710020	<i>MSH5</i>	0	T/C	.0236	1.22
rs7897947	10	104157711	<i>NFKB2</i>	0	G/T	.192	1.14
rs35979293	16	28944700	<i>CD19</i>	0	T/G	.235	1.11
rs4889393	16	81844013	<i>PLCG2</i>	0	A/G	.128	0.738
rs7214221	17	16859829	<i>TNFRSF13B</i> (<i>TACI</i>)	0	A/G	.0107	2.39
rs61061086	2	65710170	<i>FLJ16124</i>	0	G/A	2.85×10^{-3}	1.33

Statistically significant values are shown in boldface.

Distance, Distance between the SNP and the gene.

TABLE IV. Phenotypic information for patients with CVID patients with the *ITGAM* rare variants

Patient no.	Age at onset of symptoms (y)	Sex	FHx of PID	IgG (mg/dL)	IgM (mg/dL)	IgA (mg/dL)	CD19 B cells (%)	Switched memory B cells (CD19 ⁺ CD27 ⁺ IgM ⁺ IgD ⁺ [%])
1	3	M	No	80	0	14	14.0	0.00
2	NA	M	No	150	16	<7	2.1	7.32
3	NA	F	No	197	6	<4	17.8	0.35
4	NA	F	No	186	16	6	<1.0	0.00
5	NA	M	Yes	NA	NA	NA	NA	NA
6	11	F	No	120	NA	NA	NA	1.90

Laboratory values were obtained the time of diagnosis.

F, Female; FHx of PID, family history of primary immunodeficiency disease; M, male; NA, not applicable.

mapping to this gene and 2 additional SNPs close to it. *ITGAM* encodes integrin α_M (CD11b). Integrins are heterodimeric integral membrane proteins with key roles in adhesion and cell contact. CD11b, together with β_2 integrin (ITGB2/CD18), forms the leukocyte-specific heterodimer macrophage receptor 1/complement receptor 3 (Mac-1/CR3). CR3 is expressed on mononuclear phagocytes, neutrophils, natural killer cells, a subset of B cells, and some T cells.⁵¹⁻⁵⁴ Its main ligands are intercellular adhesion molecules 1 and 2, inactivated C3b, and fibrinogen. CR3 ligation results in proinflammatory responses, such as leukocyte adhesion, migration, recruitment, and superoxide release.⁵⁵ Importantly, CD11b is involved in phagocyte adhesion and migration, as well as in phagocytosis of opsonized pathogens/particles. Thus the *ITGAM* variant might impair these critical functions of the immune system, and altered trafficking and cell-cell interactions might predispose to defective immune responses in patients with CVID. The immunophenotype of *ITGAM* knockout mice supports the importance of its function for normal immune responses. These mice have reduced leukocyte and neutrophil adhesion, impaired neutrophil degranulation, phagocytosis, and oxidative burst. Also, the numbers of CD4⁺ T cells are reduced, as is T-cell proliferation. Importantly, susceptibility and mortality from pneumococcal infection are significantly increased in these mice (see Table E10 in this article's Online Repository at www.jacionline.org; <http://www.informatics.jax.org/diseasePortal/>).

Interestingly, the nominally significant *ITGAM* association partners, either direct or indirect, identified by our network analyses include a tyrosine kinase involved in B-cell receptor signaling (*LYN*) and a guanine nucleotide exchange factor important for actin cytoskeletal rearrangements involved in both B- and T-cell development and activation (*VAV1*). Thereby our network analyses demonstrate enrichment of B and T cell–signaling pathways, including regulation of activation of

both cell types. Finally, Fc γ R signaling, which has roles in phagocytosis of opsonized pathogens, also is implicated.

Previous studies have demonstrated reduced numbers of dendritic cells (DCs) and defective Toll-like receptor (TLR) signaling in patients with CVID, especially through TLR7 and TLR9.^{56,57} TLR7 and TLR9 are expressed on both naive and memory B cells and can mediate powerful stimulation and potentiate B-cell activation, possibly allowing for autoreactivity to self-RNA/DNA. Interestingly, CD11b can both positively and negatively regulate TLR signaling, depending on the experimental conditions⁵⁸⁻⁶⁴; furthermore, CR3 can interact with Fc receptors on neutrophils/macrophages and modulate the effects of immune complex binding.⁶⁵ CR3 ligation can also suppress TLR4 and TLR7/8 signaling through degradation of downstream components (myeloid differentiation primary response gene 88 [MyD88] and TIR-domain-containing adapter-inducing interferon- β [TRIF]),^{61,66} suggesting negative cross-talk between the TLR and CR3 pathways. This cross-talk and effects of TLR stimulation on B cells might influence responses to self-antigens and thus autoimmunity. Finally, these interactions might affect B-cell activation through other pathways.

Previous GWASs have revealed associations between different SNPs in the *ITGAM* locus and SLE susceptibility,^{65,67-71} where studies found phagocytosis by monocytes/macrophages and firm adhesion of neutrophils to be impaired.^{65-67,72-74} In patients with SLE, *ITGAM* variants influence the risk of renal disease, autoantibody production, and immunologic manifestations,⁵⁹ possibly by affecting clearance of apoptotic cells and leukocyte trafficking. Mac-1 can also induce tolerance through its effects on DCs, including maturation restriction, antigen-presenting cell function, and T-cell activation.^{75,76} Hence decreased phagocytosis could, through decreased binding of Mac-1 to inactivated C3b, contribute to both immune dysfunction and

autoimmunity through reduced clearance of apoptotic cells, activation of DCs, production of inflammatory cytokines, and uptake and presentation of self-antigens to T cells. Also, dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin on DCs can bind CR3 on neutrophils during cross-talk between these cells.^{77,78} Another role for CD11b in tolerance is through restriction of T_H17 cell differentiation, a cell type implicated in autoimmunity.⁷⁹⁻⁸¹

Our limited phenotypic information does not definitively indicate a specific immunophenotype for patients carrying the *ITGAM* variant; however, our sample size might be too small to identify such an association. Importantly, 4 of 6 patients had CVID-related comorbidities, including cancer and lung disease. Also of note, the majority (80%) had low switched memory B-cell counts compared with 60% of patients with CVID without the *ITGAM* variant. This is interesting in the context of the possible association of *ITGAM* dysfunction and autoimmunity because patients with CVID with low switched memory B-cell counts are at higher risk of autoimmune disease.

To determine whether rare nonsynonymous exonic variants might be tagged by the SNP microarray association signal at the 16p11.2 locus, we conducted Sanger sequencing of the 3 plausible candidate genes in this region. However, no corresponding rare variant nonsynonymous mutation was found. Therefore we hypothesize that gene regulation at the expression level of these proteins is the more likely genetic mechanism of perturbation in patients with CVID rather than direct amino acid change in a protein product. In addition to the 2 aforementioned SNPs, rs929867 and rs8056264, the genotyped SNP rs4889637 and the imputed SNPs rs114798394 and rs7202897 are predicted to alter binding motifs for signal transducer and activator of transcription (STAT),³⁰ a component of the Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway, which is critical for many key functions of the immune system.⁸² Furthermore, among the imputed SNPs, binding factor YY1 at rs889552³⁰ is required for early B-cell development in mice,⁸³ and rs2098730 is located at the binding motif for NF-κB.³⁰ Therefore the rare variants we found could be functional variants themselves contributing to the pathogenesis of CVID. A parallel example in this regard would be the SNP rs7903146 in intron 3 of *TCF7L2*, which is considered a plausible causal variant for type 2 diabetes.^{84,85} Furthermore, intronic variants in *FUS*, *ITGAM*, and *ITGAX* could affect alternative splicing, which would not be captured by our Sanger sequencing analysis. In view of the above, coupled with the finding that patients with CVID with the associated variant have a lower percentage of switched memory B cells, our leading hypothesis is that the associated variants affect gene regulation and subsequent protein dosage. Alternatively, it is possible that more distal genes in LD with the gene-rich region of 16p11.2 or even more distal genes based on chromosome conformation in 3-dimensional space are acting as enhancers through chromosome looping.

As in our previous GWAS,¹⁹ the MHC locus stands out at or close to the genome-wide significance level, and SNPs in the gene *FLJI16124* were nominally significant in both studies. The unique SNP coverage in the iCHIP platform allowed identification of the *ITGAM* locus in this study, whereas the previously applied platform (HumanHap 610) had few SNPs in this region (53 in the region of chr16:31,100,000-31,500,000). Previously, the focus has been exclusively on common variants (MAF > 5%). Similarly, the nominally significant signals of other

loci in our previous study only have sparse SNP coverage on the iCHIP (1-2 common variants at each locus), and therefore associations at those loci were not detected in this study.

In summary, our results suggest that the variants uncovered at the 16p11.2 locus involve CD11b and thus could affect many important pathways, such as TLR signaling, with modulating effects on immune responses, B- and T-cell activation, and autoimmunity in patients with CVID. This is supported by our pathway and network analyses, phenotype data, and known knockout mouse phenotypes, making *ITGAM* an exciting candidate gene for functional evaluation. Further understanding of the autoimmune phenomena in patients with CVID would greatly affect the care of these patients because prediction models and treatments for these are scarce to date. Hopefully, further understanding of this pathway and its implications in patients with CVID can improve the future care of these patients.

We thank all the participants enrolled in this study. We also thank the IIBDGC for access to the genotype information of the control subjects used in this study.

Key messages

- We report the results of an association analysis of CVID through dense genotyping of immune-related loci, the first of its kind in patients with CVID.
- We identified an association of CVID with rare variants at the *FUS/ITGAM* (CD11b) locus on 16p11.2 in a cohort of 360 patients with CVID and 21,610 healthy control subjects.
- The locus is enriched for promoter/enhancer histone markers, supporting the hypothesis that it might contribute to the pathogenesis of CVID.
- *ITGAM* encodes the integrin CD11b, an important molecule for cellular contact and adhesion. Knockout mouse models in the literature support the importance of *ITGAM* function for normal immune responses.

REFERENCES

1. Chapel H, Cunningham-Rundles C. Update in understanding common variable immunodeficiency disorders (CVIDs) and the management of patients with these conditions. *Br J Haematol* 2009;145:709-27.
2. Cunningham-Rundles C, Bodian C. Common variable immunodeficiency: clinical and immunological features of 248 patients. *Clin Immunol* 1999;92:34-48.
3. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. *Blood* 2008;111:77-85.
4. Bacchelli C, Buckridge S, Thrasher AJ, Gaspar HB. Translational mini-review series on immunodeficiency: molecular defects in common variable immunodeficiency. *Clin Exp Immunol* 2007;149:401-9.
5. Salzer U, Chapel HM, Webster AD, Pan-Hammarstrom Q, Schmitt-Graeff A, Schlesier M, et al. Mutations in TNFRSF13B encoding TACI are associated with common variable immunodeficiency in humans. *Nat Genet* 2005;37:820-8.
6. Castigli E, Wilson SA, Garibyan L, Rachid R, Bonilla F, Schneider L, et al. TACI is mutant in common variable immunodeficiency and IgA deficiency. *Nat Genet* 2005;37:829-34.
7. Pan-Hammarstrom Q, Salzer U, Du L, Bjorkander J, Cunningham-Rundles C, Nelson DL, et al. Reexamining the role of TACI coding variants in common variable immunodeficiency and selective IgA deficiency. *Nat Genet* 2007;39:429-30.
8. Olerup O, Smith CI, Bjorkander J, Hammarstrom L. Shared HLA class II-associated genetic susceptibility and resistance, related to the HLA-DQB1 gene, in IgA deficiency and common variable immunodeficiency. *Proc Natl Acad Sci U S A* 1992;89:10653-7.

9. Grimbacher B, Huttoff A, Schlesier M, Glocker E, Warnatz K, Drager R, et al. Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. *Nat Immunol* 2003;4:261-8.
10. Kanegane H, Agematsu K, Futatani T, Sira MM, Suga K, Sekiguchi T, et al. Novel mutations in a Japanese patient with CD19 deficiency. *Genes Immun* 2007;8:663-70.
11. Kuijpers TW, Bende RJ, Baars PA, Grummels A, Derks IA, Dolman KM, et al. CD20 deficiency in humans results in impaired T cell-independent antibody responses. *J Clin Invest* 2010;120:214-22.
12. Salzer U, Maul-Pavicic A, Cunningham-Rundles C, Urschel S, Belohradsky BH, Litzman J, et al. ICOS deficiency in patients with common variable immunodeficiency. *Clin Immunol* 2004;113:234-40.
13. van Zelm MC, Reisli I, van der Burg M, Castano D, van Noesel CJ, van Tol MJ, et al. An antibody-deficiency syndrome due to mutations in the CD19 gene. *N Engl J Med* 2006;354:1901-12.
14. van Zelm MC, Smet J, Adams B, Mascart F, Schandene L, Janssen F, et al. CD81 gene defect in humans disrupts CD19 complex formation and leads to antibody deficiency. *J Clin Invest* 2010;120:1265-74.
15. Ombrello MJ, Remmers EF, Sun G, Freeman AF, Datta S, Torabi-Parizi P, et al. Cold urticaria, immunodeficiency, and autoimmunity related to PLCG2 deletions. *N Engl J Med* 2012;366:330-8.
16. Sekine H, Ferreira RC, Pan-Hammarstrom Q, Graham RR, Ziemba B, de Vries SS, et al. Role for Msh5 in the regulation of Ig class switch recombination. *Proc Natl Acad Sci U S A* 2007;104:7193-8.
17. Warnatz K, Salzer U, Rizzi M, Fischer B, Gutenberger S, Bohm J, et al. B-cell activating factor receptor deficiency is associated with an adult-onset antibody deficiency syndrome in humans. *Proc Natl Acad Sci U S A* 2009;106:13945-50.
18. Chen K, Coonrod EM, Kumanovics A, Franks ZF, Durtschi JD, Margraf RL, et al. Germine mutations in NFKB2 implicate the noncanonical NF-kappaB pathway in the pathogenesis of common variable immunodeficiency. *Am J Hum Genet* 2013;93:812-24.
19. Orange JS, Glessner JT, Resnick E, Sullivan KE, Lucas M, Ferry B, et al. Genome-wide association identifies diverse causes of common variable immunodeficiency. *J Allergy Clin Immunol* 2011;127:1360-7.e6.
20. Volanakis JE, Zhu ZB, Schaffer FM, Macon KJ, Palermos J, Barger BO, et al. Major histocompatibility complex class III genes and susceptibility to immunoglobulin A deficiency and common variable immunodeficiency. *J Clin Invest* 1992;89:1914-22.
21. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012;491:119-24.
22. Cortes A, Brown MA. Promise and pitfalls of the Immunochip. *Arthritis Res Ther* 2011;13:101.
23. Trynka G, Hunt KA, Bockett NA, Romanos J, Mistry V, Szperl A, et al. Dense genotyping identifies and localizes multiple common and rare variant association signals in celiac disease. *Nat Genet* 2011;43:1193-201.
24. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006;38:904-9.
25. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559-75.
26. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 2010;26:2190-1.
27. Delaneau O, Zagury JF, Marchini J. Improved whole-chromosome phasing for disease and population genetic studies. *Nat Methods* 2013;10:5-6.
28. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet* 2009;5:e1000529.
29. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet* 2007;39:906-13.
30. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature* 2012;489:57-74.
31. Bernstein BE, Stamatoyannopoulos JA, Costello JF, Ren B, Milosavljevic A, Meissner A, et al. The NIH Roadmap Epigenomics Mapping Consortium. *Nat Biotechnol* 2010;28:1045-8.
32. Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res* 2012;40:D930-4.
33. Alexeyenko A, Sonnhammer EL. Global networks of functional coupling in eukaryotes from comprehensive data integration. *Genome Res* 2009;19:1107-16.
34. Schmitt T, Ogris C, Sonnhammer EL. FunCoup 3.0: database of genome-wide functional coupling networks. *Nucleic Acids Res* 2014;42:D380-8.
35. Snel B, Lehmann G, Bork P, Huynen MA. STRING: a web-server to retrieve and display the repeatedly occurring neighbourhood of a gene. *Nucleic Acids Res* 2000;28:3442-4.
36. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, et al. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res* 2013;41:D808-15.
37. Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, et al. Association between microdeletion and microduplication at 16p11.2 and autism. *N Engl J Med* 2008;358:667-75.
38. McCarthy SE, Makarov V, Kirov G, Addington AM, McClellan J, Yoon S, et al. Microduplications of 16p11.2 are associated with schizophrenia. *Nat Genet* 2009;41:1223-7.
39. Walters RG, Jacquemont S, Valsesia A, de Smith AJ, Martinet D, Andersson J, et al. A new highly penetrant form of obesity due to deletions on chromosome 16p11.2. *Nature* 2010;463:671-5.
40. Gao K, Zheng W, Deng X, Xiong W, Song Z, Yang Y, et al. Genetic analysis of the fused in sarcoma gene in Chinese Han patients with Parkinson's disease. *Parkinsonism Relat Disord* 2014;20:119-21.
41. Shelkovnikova TA, Robinson HK, Connor-Robson N, Buchman VL. Recruitment into stress granules prevents irreversible aggregation of FUS protein mislocalized to the cytoplasm. *Cell Cycle* 2013;12:3194-202.
42. Iguchi Y, Katsuno M, Ikenaka K, Ishigaki S, Sobue G. Amyotrophic lateral sclerosis: an update on recent genetic insights. *J Neurol* 2013;260:2917-27.
43. Baron DM, Kaushansky LJ, Ward CL, Sama RR, Chian RJ, Boggio KJ, et al. Amyotrophic lateral sclerosis-linked FUS/TLS alters stress granule assembly and dynamics. *Mol Neurodegener* 2013;8:30.
44. Mignone F, Gissi C, Liuni S, Pesole G. Untranslated regions of mRNAs. *Genome Biol* 2002;3:REVIEWS0004.
45. Araujo PR, Yoon K, Ko D, Smith AD, Qiao M, Suresh U, et al. Before it gets started: regulating translation at the 5' UTR. *Comp Funct Genomics* 2012;2012:475731.
46. Qi CF, Martensson A, Mattioli M, Dalla-Favera R, Lobanov VV, Morse HC 3rd. CTCF functions as a critical regulator of cell-cycle arrest and death after ligation of the B cell receptor on immature B cells. *Proc Natl Acad Sci U S A* 2003;100:633-8.
47. Baeuerle PA, Henkel T. Function and activation of NF-kappa B in the immune system. *Annu Rev Immunol* 1994;12:141-79.
48. Chamary JV, Parmley JL, Hurst LD. Hearing silence: non-neutral evolution at synonymous sites in mammals. *Nat Rev Genet* 2006;7:98-108.
49. Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, et al. A "silent" polymorphism in the MDR1 gene changes substrate specificity. *Science* 2007;315:525-8.
50. Garber M, Guttman M, Clamp M, Zody MC, Friedman N, Xie X. Identifying novel constrained elements by exploiting biased substitution patterns. *Bioinformatics* 2009;25:i54-62.
51. Dai Z, Turtle CJ, Booth GC, Riddell SR, Gooley TA, Stevens AM, et al. Normally occurring NKG2D+CD4+ T cells are immunosuppressive and inversely correlated with disease activity in juvenile-onset lupus. *J Exp Med* 2009;206:793-805.
52. Green MR, Kennell AS, Larche MJ, Seifert MH, Isenberg DA, Salaman MR. Natural killer T cells in families of patients with systemic lupus erythematosus: their possible role in regulation of IGG production. *Arthritis Rheum* 2007;56:303-10.
53. Griffin DO, Rothstein TL. A small CD11b(+) human B1 cell subpopulation stimulates T cells and is expanded in lupus. *J Exp Med* 2011;208:2591-8.
54. Wagner C, Hansch GM, Stegmaier S, Denefleh B, Hug F, Schoels M. The complement receptor 3, CR3 (CD11b/CD18), on T lymphocytes: activation-dependent up-regulation and regulatory function. *Eur J Immunol* 2001;31:1173-80.
55. Reed JH, Jain M, Lee K, Kandimalla ER, Faridi MH, Buyon JP, et al. Complement receptor 3 influences toll-like receptor 7/8-dependent inflammation: implications for autoimmune diseases characterized by antibody reactivity to ribonucleoproteins. *J Biol Chem* 2013;288:9077-83.
56. Escobar D, Pons J, Clemente A, Iglesias J, Regueiro V, Bengoechea JA, et al. Defective B cell response to TLR9 ligand (CpG-ODN). *Streptococcus pneumoniae* and *Haemophilus influenzae* extracts in common variable immunodeficiency patients. *Cell Immunol* 2010;262:105-11.
57. Yu JE, Knight AK, Radigan L, Marron TU, Zhang L, Sanchez-Ramon S, et al. Toll-like receptor 7 and 9 defects in common variable immunodeficiency. *J Allergy Clin Immunol* 2009;124:349-56, e1-3.
58. Cao C, Gao Y, Li Y, Antalis TM, Castellino FJ, Zhang L. The efficacy of activated protein C in murine endotoxemia is dependent on integrin CD11b. *J Clin Invest* 2010;120:1971-80.

59. Fossati-Jimack L, Ling GS, Cortini A, Szajna M, Malik TH, McDonald JU, et al. Phagocytosis is the main CR3-mediated function affected by the lupus-associated variant of CD11b in human myeloid cells. *PLoS One* 2013;8:e57082.
60. Hajishengallis G, Harokopakis E. *Porphyromonas gingivalis* interactions with complement receptor 3 (CR3): innate immunity or immune evasion? *Front Biosci* 2007;12:4547-57.
61. Han C, Jin J, Xu S, Liu H, Li N, Cao X. Integrin CD11b negatively regulates TLR-triggered inflammatory responses by activating Syk and promoting degradation of MyD88 and TRIF via Cbl-b. *Nat Immunol* 2010;11:734-42.
62. Huynh L, Wang L, Shi C, Park-Min KH, Ivashkiv LB. ITAM-coupled receptors inhibit IFNAR signaling and alter macrophage responses to TLR4 and *Listeria monocytogenes*. *J Immunol* 2012;188:3447-57.
63. Marth T, Kelsall BL. Regulation of interleukin-12 by complement receptor 3 signaling. *J Exp Med* 1997;185:1987-95.
64. Yoshida Y, Kang K, Berger M, Chen G, Gilliam AC, Moser A, et al. Monocyte induction of IL-10 and down-regulation of IL-12 by iC3b deposited in ultraviolet-exposed human skin. *J Immunol* 1998;161:5873-9.
65. Zhou Y, Wu J, Kucik DF, White NB, Redden DT, Szalai AJ, et al. Multiple lupus-associated ITGAM variants alter Mac-1 functions on neutrophils. *Arthritis Rheum* 2013;65:2907-16.
66. Rhodes B, Furnrohr BG, Roberts AL, Tzircotis G, Schett G, Spector TD, et al. The rs1143679 (R77H) lupus associated variant of ITGAM (CD11b) impairs complement receptor 3 mediated functions in human monocytes. *Ann Rheum Dis* 2012;71:2028-34.
67. Han S, Kim-Howard X, Deshmukh H, Kamatani Y, Viswanathan P, Guthridge JM, et al. Evaluation of imputation-based association in and around the integrin- α -M (ITGAM) gene and replication of robust association between a non-synonymous functional variant within ITGAM and systemic lupus erythematosus (SLE). *Hum Mol Genet* 2009;18:1171-80.
68. Hom G, Graham RR, Modrek B, Taylor KE, Ortmann W, Garnier S, et al. Association of systemic lupus erythematosus with C8orf13-BLK and ITGAM-ITGAX. *N Engl J Med* 2008;358:900-9.
69. International Consortium for Systemic Lupus Erythematosus Genetics (SLEGEN), Harley JB, Alarcon-Riquelme ME, Criswell LA, Jacob CO, Kimberly RP, et al. Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXX, KIAA1542 and other loci. *Nat Genet* 2008;40:204-10.
70. Nath SK, Han S, Kim-Howard X, Kelly JA, Viswanathan P, Gilkeson GS, et al. A nonsynonymous functional variant in integrin- α (M) (encoded by ITGAM) is associated with systemic lupus erythematosus. *Nat Genet* 2008;40:152-4.
71. Yang W, Zhao M, Hirankarn N, Lau CS, Mok CC, Chan TM, et al. ITGAM is associated with disease susceptibility and renal nephritis of systemic lupus erythematosus in Hong Kong Chinese and Thai. *Hum Mol Genet* 2009;18:2063-70.
72. Rosetti F, Tsuboi N, Chen K, Nishi H, Hernandez T, Sethi S, et al. Human lupus serum induces neutrophil-mediated organ damage in mice that is enabled by Mac-1 deficiency. *J Immunol* 2012;189:3714-23.
73. Anaya JM, Kim-Howard X, Prahalad S, Chernavsky A, Canas C, Rojas-Villarraga A, et al. Evaluation of genetic association between an ITGAM non-synonymous SNP (rs1143679) and multiple autoimmune diseases. *Autoimmun Rev* 2012;11:276-80.
74. MacPherson M, Lek HS, Prescott A, Fagerholm SC. A systemic lupus erythematosus-associated R77H substitution in the CD11b chain of the Mac-1 integrin compromises leukocyte adhesion and phagocytosis. *J Biol Chem* 2011;286:17303-10.
75. Bai Y, Qian C, Qian L, Ma F, Hou J, Chen Y, et al. Integrin CD11b negatively regulates TLR9-triggered dendritic cell cross-priming by upregulating microRNA-146a. *J Immunol* 2012;188:5293-302.
76. Skoberne M, Somersan S, Almodovar W, Truong T, Petrova K, Henson PM, et al. The apoptotic-cell receptor CR3, but not α phv β 5, is a regulator of human dendritic-cell immunostimulatory function. *Blood* 2006;108:947-55.
77. van Gisbergen KP, Ludwig IS, Geijtenbeek TB, van Kooyk Y. Interactions of DC-SIGN with Mac-1 and CEACAM1 regulate contact between dendritic cells and neutrophils. *FEBS Lett* 2005;579:6159-68.
78. van Gisbergen KP, Sanchez-Hernandez M, Geijtenbeek TB, van Kooyk Y. Neutrophils mediate immune modulation of dendritic cells through glycosylation-dependent interactions between Mac-1 and DC-SIGN. *J Exp Med* 2005;201:1281-92.
79. Coustet B, Agarwal SK, Gourh P, Guedj M, Mayes MD, Dieude P, et al. Association study of ITGAM, ITGAX, and CD58 autoimmune risk loci in systemic sclerosis: results from 2 large European Caucasian cohorts. *J Rheumatol* 2011;38:1033-8.
80. Diamond MS, Garcia-Aguilar J, Bickford JK, Corbi AL, Springer TA. The I domain is a major recognition site on the leukocyte integrin Mac-1 (CD11b/CD18) for four distinct adhesion ligands. *J Cell Biol* 1993;120:1031-43.
81. Sachs UJ, Chavakis T, Fung L, Lohrenz A, Bux J, Reil A, et al. Human alloantibody anti-Mart interferes with Mac-1-dependent leukocyte adhesion. *Blood* 2004;104:727-34.
82. Shuai K, Liu B. Regulation of JAK-STAT signalling in the immune system. *Nat Rev Immunol* 2003;3:900-11.
83. Liu H, Schmidt-Suppran M, Shi Y, Hobeika E, Barteneva N, Jumaa H, et al. Yin Yang 1 is a critical regulator of B-cell development. *Genes Dev* 2007;21:1179-89.
84. Palmer ND, Hester JM, An SS, Adeyemo A, Rotimi C, Langefeld CD, et al. Resequencing and analysis of variation in the TCF7L2 gene in African Americans suggests that SNP rs7903146 is the causal diabetes susceptibility variant. *Diabetes* 2011;60:662-8.
85. Wellcome Trust Case Control Consortium, Maller JB, McVean G, Byrnes J, Vukcevic D, Palin K, et al. Bayesian refinement of association signals for 14 loci in 3 common diseases. *Nat Genet* 2012;44:1294-301.

Rare variants at 16p11.2 are associated with common variable immunodeficiency

S. Melkorka Maggadottir, Jin Li, Joseph T. Glessner, Yun Rose Li, Zhi Wei, Xiao Chang, Frank D Mentch, Kelly A Thomas, Cecilia E. Kim, Yan Zhao, Cuiping Hou, Fengxiang Wang, Silje F. Jørgensen, Elena E. Perez, Kathleen E. Sullivan, Jordan Scott Orange, Tom H. Karlsen, Helen Chapel, Charlotte Cunningham-Rundles, and Hakon Hakonarson

Supplementary Information

Table E1. Genotype counts and Fisher exact test results for 16p11.2 SNPs

SNP (gene)	Genotype	Discovery cohort			Replication cohort			Meta analysis	
		Cases (N=164)	Controls (N=19542)	Fisher Exact P-value	Cases (N=135)	Controls (N=2066)	Fisher Exact P-value	Meta P-value	OR
rs750953	CT	2	4	1.01x10 ⁻³	4	5	1.37x10 ⁻³	5.10x10 ⁻⁹	22.43
	TT	162	19533		131	2059			
rs929867	AG	3	4	1.88x10 ⁻⁵	2	5	6.42x10 ⁻²	6.10x10 ⁻⁹	26.80
	GG	159	19535		133	2060			
rs7192718	TC	3	5	3.12x10 ⁻⁵	3	6	1.46x10 ⁻²	8.76x10 ⁻¹⁰	22.76
	CC	161	19502		132	2056			
rs10871455	TG	3	4	1.95x10 ⁻⁵	3	6	1.46x10 ⁻²	1.01x10 ⁻⁹	24.05
	GG	161	19535		132	2059			
rs12934814	TC	3	5	2.84x10 ⁻⁵	3	5	1.03x10 ⁻²	2.87x10 ⁻¹⁰	26.23
	CC	156	19525		132	2053			
rs4889637	CT	3	5	3.11x10 ⁻⁵	3	6	1.47x10 ⁻²	8.79x10 ⁻¹⁰	22.75
	TT	161	19524		132	2053			
rs4889641	AG	3	4	1.96x10 ⁻⁵	3	6	1.49x10 ⁻²	1.08x10 ⁻⁹	23.91
	GG	161	19490		132	2042			
rs8063978	CT	3	5	2.90x10 ⁻⁵	2	6	8.15x10 ⁻²	1.11x10 ⁻⁸	22.62
	TT	157	19493		132	2055			
rs4889541	TC	3	5	3.11x10 ⁻⁵	2	5	6.43x10 ⁻²	5.76x10 ⁻⁹	24.86
	CC	161	19520		133	2057			
rs9929801	CT	3	29	2.39x10 ⁻³	6	16	1.65x10 ⁻³	5.64x10 ⁻⁸	7.80
	TT	161	19465		129	2045			
rs8045402	TC	3	6	4.65x10 ⁻⁵	4	7	3.27x10 ⁻³	1.37x10 ⁻¹⁰	20.56
	CC	161	19492		131	2053			
rs8056264	TC	3	5	3.10x10 ⁻⁵	3	6	1.45x10 ⁻²	8.56x10 ⁻¹⁰	22.80
	CC	161	19536		132	2060			
rs11574629	GA	3	4	1.96x10 ⁻⁵	3	6	1.46x10 ⁻²	1.02x10 ⁻⁹	24.03
	AA	161	19506		132	2059			

OR=odds ratio.

Table E2. Comparison of minor allele frequency for significant SNPs in cases, controls, and European population of 1000 genome dataset showing absence.

SNPs	Minor /Major Allele	MAF cases	MAF controls	eur.MAF (1000G)
rs750953	C/T	0.0061	0.000102	0.0000
rs929867	A/G	0.00926	0.000102	0.0000
rs7192718	T/C	0.00915	0.000128	0.0000
rs10871455	T/G	0.00915	0.000102	0.0000
rs12934814	T/C	0.00943	0.000128	0.0000
rs4889637	C/T	0.00915	0.000128	0.0000
rs4889641	A/G	0.00915	0.000103	0.0000
rs8063978	C/T	0.00938	0.000128	0.0000
rs4889541	T/C	0.00915	0.000128	0.0000
rs9929801	C/T	0.00915	0.000744	0.0000
rs8045402	T/C	0.00915	0.000154	0.0000
rs8056264	T/C	0.00915	0.000128	0.0000
rs11574629	G/A	0.00915	0.000103	0.0000

MAF=minor allele frequency; eur.MAF(1000G)=minor allele frequency in the European population of 1000 Genome project.

Table E3. Conditional association analysis results.

SNP	P-value	r ²
rs7192718	0.9995	0.875
rs10871455	NA	1
rs12934814	0.9995	0.875
rs4889637	0.9995	0.875
rs4889641	NA	1
rs8063978	0.9995	0.875
rs4889541	0.9995	0.875
rs8045402	0.9992	0.778
rs8056264	0.9995	0.875
rs11574629	NA	1

P-value=P-value in association analysis conditional on rs929867; r²=r² with rs929867

Table E4. Additional genome-wide significant SNPs based on imputed genotype in meta-analysis.

Marker	BP (hg19)	Allele1	Allele2	Effect	StdErr	P-value	Direction ¹
rs7202897	31220869	T	C	4.23	0.59	6.69x10 ⁻¹³	++
rs6565224	31247924	T	C	22.11	2.08	2.49x10 ⁻²⁶	++
rs146286805	31256485	T	C	17.33	1.86	9.61x10 ⁻²¹	++
rs2098730	31257101	T	C	-17.24	1.85	1.22x10 ⁻²⁰	--
rs2081224	31257478	A	G	17.18	1.85	1.41x10 ⁻²⁰	++
rs889552	31260113	T	C	16.69	1.82	5.30x10 ⁻²⁰	++
rs78652532	31262373	A	G	-16.68	1.82	5.45x10 ⁻²⁰	--
rs75887269	31271991	T	C	16.14	1.94	9.29x10 ⁻¹⁷	++
rs3948508	31272117	C	G	-16.16	1.94	8.98x10 ⁻¹⁷	--
rs41374952	31282980	T	C	15.24	1.89	7.34x10 ⁻¹⁶	++
rs78685891	31298689	A	G	15.65	1.92	3.12x10 ⁻¹⁶	++
chr16:31299154:D	31299154	G	GA	15.65	1.92	3.12x10 ⁻¹⁶	++
rs80178085	31299626	T	C	16.08	1.94	1.16x10 ⁻¹⁶	++
rs7198540	31302834	T	C	16.02	1.93	1.22x10 ⁻¹⁶	++
chr16:31303692:I	31303692	A	AT	-16.01	1.93	1.25x10 ⁻¹⁶	--
chr16:31304673:D	31304673	G	GTCT	13.83	1.67	1.17x10 ⁻¹⁶	++
chr16:31304674:D	31304674	T	TCTTC	14.31	1.7	3.27x10 ⁻¹⁷	++
rs59668899	31311339	A	C	13.86	1.67	1.09x10 ⁻¹⁶	++
rs149312445	31312157	T	C	16.25	1.95	7.23x10 ⁻¹⁷	++
rs77769416	31338940	A	T	17.98	2.05	1.53x10 ⁻¹⁸	++
rs114798394	31355894	A	G	16.83	1.98	1.91x10 ⁻¹⁷	++
rs75466689	31360963	T	C	-18.28	2.06	6.81x10 ⁻¹⁹	--
rs74015510	31366848	A	G	17.84	2.09	1.40x10 ⁻¹⁷	++

BP=base pair; Allele1=effect allele; Allele2=reference allele; StdErr=standard error; P-value=meta-analysis P-value.

¹the direction of effects are represented by either the + or the – sign compared to the reference allele in the discovery cohort and replication cohort respectively.

131 Table E5. Significant SNPs at 16p11.2 locus are enriched for enhancers in immune cell
132 types.

Cell type		All enhancers				Strongest enhancers			
ID	Description	Obs	Exp	Fold	P	Obs	Exp	Fold	P
CD34.MBP1508	Mobilized CD34 Primary Cells.Donor RO 01508	3	0.3	10.2	2.77x10 ⁻³	1	0.1	8.5	1.11x10 ⁻¹
CD19.P	CD19 Primary Cells	3	0.4	7.1	7.76x10 ⁻³	2	0.2	9	2.01x10 ⁻²
CD34.P	CD34 Primary Cells	2	0.5	4.2	7.91x10 ⁻²	2	0.2	11.5	1.27x10 ⁻²
SPL	Spleen	5	0.6	7.9	2.59x10 ⁻⁴	4	0.4	10.5	4.21x10 ⁻⁴
BN.HM150	Brain Hippocampus Middle.Donor 150	3	0.6	5	2.02x10 ⁻²	3	0.3	9.8	3.11x10 ⁻³
CD34.MBP1562	Mobilized CD34 Primary Cells.Donor RO 01562	3	0.6	4.7	2.35x10 ⁻²	3	0.3	9.7	3.27x10 ⁻³
CD34.MBP1536	Mobilized CD34 Primary Cells.Donor RO 01536	3	0.5	5.8	1.35x10 ⁻²	1	0.2	6	1.55x10 ⁻¹
CD34.C	CD34 Cultured Cells	3	0.6	4.8	2.23x10 ⁻²	3	0.3	10	3.0x10 ⁻³
CD15.P	CD15 Primary Cells	2	0.5	4.3	7.55x10 ⁻²	2	0.3	7.9	2.57x10 ⁻²
CD34.MBP1549	Mobilized CD34 Primary Cells.Donor RO 01549	2	0.4	5.6	4.74x10 ⁻²	0	0.1	0	1
K562	Leukemia	6	0.6	10	1.3x10 ⁻⁵	5	0.2	26	1.0x10 ⁻⁶
GM12878	B-lymphocyte, lymphoblastoid	4	0.6	6.6	2.45x10 ⁻³	2	0.2	9.2	1.94x10 ⁻²

133 Obs=observed; Exp=expected; Fold=fold of enrichment; P=P-value.

134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165

166 Table E6. Significant SNPs at 16p11.2 locus are enriched for DNase sites in immune cell
 167 types.
 168

Cell type				DNase			
ID	Description	Treatment	Production center	Obs	Exp	Fold	P
Chorion	chorion cells (outermost of two fetal membranes)	None	Duke	2	0.1	14.8	7.81×10^{-3}
H1-hESC	embryonic stem cells	None	AWG	2	0.2	10.1	1.63×10^{-2}
HUVEC	umbilical vein endothelial cells	None	AWG	2	0.1	15.1	7.52×10^{-3}
GM19240	B-lymphocyte, lymphoblastoid	None	Duke	2	0.1	14.8	7.84×10^{-3}
GM19239	B-lymphocyte, lymphoblastoid	None	Duke	2	0.1	21.7	3.71×10^{-3}
GM18507	B-lymphocyte, lymphoblastoid	None	Duke	2	0.1	23.9	3.08×10^{-3}

169 Obs=observed; Exp=expected; Fold=fold of enrichment; P=P-value.

170
 171
 172
 173
 174
 175
 176
 177
 178
 179
 180
 181
 182
 183
 184
 185
 186
 187
 188
 189
 190
 191
 192
 193
 194
 195
 196
 197
 198
 199
 200
 201
 202
 203
 204
 205
 206
 207

208 Table E7. Regulatory elements overlapping with significant SNPs at the locus of
209 16p11.2.
210

rsID	Promoter histone markers	Promoter histone markers in immune cell types*	Enhancer histone markers	Enhancer histone markers in immune cell types*	DNase	DNase in immune cell types*	Proteins bound	Motifs changed	Functional annotation
rs750953			23 cell types	CC.TPC (CD4+ CD25- Th Primary Cells)	5 cell types			Hdx;T3R	intronic
rs929867	88 cell types	GM12878 (B-lymphocyte, lymphoblastoid); CD4.NP (CD4 Naive Primary Cells); CCCRA.NP (CD4+ CD25- CD45RA+ Naive Primary Cells); CCC.TMP (CD4+ CD25int CD127+ Tmem Primary Cells); CD4.MP (CD4 Memory Primary Cells); CCCRO.MP (CD4+ CD25- CD45RO+ Memory Primary Cells); CD8.MP (CD8 Memory Primary Cells); CCC.TREGP (CD4+ CD25+ CD127- Treg Primary Cells); CD8.NP (CD8 Naive Primary Cells); CD3.P (CD3 Primary Cells); CCIP.LSMPTP (CD4+ CD25- IL17- PMA-Ionomycin stimulated MACS purified Th Primary Cells); CD19.P (CD19 Primary Cells); CC.TPC (CD4+ CD25- Th Primary Cells);	2 cell types		111 cell types	GM12878 (B-lymphocyte, lymphoblastoid); Th1 (primary Th1 T cells); GM12891 (B-lymphocyte, lymphoblastoid); GM12892 (B-lymphocyte, lymphoblastoid); GM18507 (B-lymphocyte, lymphoblastoid); GM19239 (B-lymphocyte, lymphoblastoid); GM19240 (B-lymphocyte, lymphoblastoid); Adult_CD4_Th0 (CD4+ cells isolated from human blood and enriched for Th0 populations); CD20+ (B cells); GM06990 (B-lymphocyte, lymphoblastoid); GM12864 (B-lymphocyte, lymphoblastoid); GM12865 (B-lymphocyte, lymphoblastoid); Jurkat (T lymphoblastoid derived from an acute T cell leukemia); Th2 (primary Th2 T cells)	POL2,CTCF,NF KB,BCL11A,BC L3,BCLAF1,EG R1,ETS1,IRF4,M EF2A,NRSF,OC T2,P300,PAX5C 20,PAX5N19,PB X3,POU2F2,SIN 3AK20,SP1,SRF, TAF1,TBP,TCF1 2,YY1,ZBTB33, ZEB1,POL24H8, NANOG,NRF1,T AF7,TCF4,POL2 B,AP2ALPHA,A P2GAMMA,CM YC,GTf2F1,INI 1,MAX,MXI1,P OL2S2,ELF1,FO SL2,HEY1,JUN D,MAFF,CCNT2 ,CHD2	CTCF	5'-UTR
rs7192718			1 cell type		6 cell types	GM19239 (B-lymphocyte, lymphoblastoid)		ERalpha;Esr2;GR; HNF4;NRS F;RAR	
rs10871455			3 cell types					Foxp1, GR, HMG-IY, Hoxb13, RREB-1, THAP1, YY1, Zfp105	
rs12934814			8 cell types		2 cell types			Pax-5	
rs4889637			2 cell types					CEBPG;ST AT	
rs4889641	2 cell types		14 cell types	GM12878 (B-lymphocyte, lymphoblastoid); CD4.MP (CD4 Memory Primary Cells); CD8.MP (CD8 Memory Primary Cells); CCIP.LSTP (CD4+ CD25- IL17+ PMA-Ionomycin stimulated Th17 Primary Cells); CD19.P (CD19 Primary Cells)	1 cell type			Nkx2;Sox	
rs8063978	21 cell	CD19.P (CD19	10 cell types	GM12878 (B-				BDP1;BHL	intronic

	types	Primary Cells); CD3.P (CD3 Primary Cells); CCIP.LSTP (CD4+ CD25- IL17+ PMA- Ionomycin stimulated Th17 Primary Cells)		lymphocyte, lymphoblastoid); CD4.MP (CD4 Memory Primary Cells); CD8.MP (CD8 Memory Primary Cells); CD8.NP (CD8 Naive Primary Cells); CC.TPC (CD4+ CD25- Th Primary Cells)				HE40;NRS F	
rs4889541			9 cell types	GM12878 (B- lymphocyte, lymphoblastoid); CD19.P (CD19 Primary Cells); CD4.NP (CD4 Naive Primary Cells)					intronic
rs9929801			2 cell types.	CCIP.LSMPTP (CD4+ CD25- IL17- PMA- Ionomycin stimulated MACS purified Th Primary Cells)	2 cell types			AP-1;Pax- 6;TBX5	intronic
rs8045402								Hoxa5;RX RA	intronic
rs8056264								BDP1;GAT A	synonymou s
rs11574629	2 cell types		8 cell types	GM12878 (B- lymphocyte, lymphoblastoid); CD19.P (CD19 Primary Cells)	3 cell types	GM18507 (B- lymphocyte, lymphoblastoid); GM19240 (B- lymphocyte, lymphoblastoid)	BCL11A,EBF1,E LF1,IRF4,NFKB, PU1		

* only CVID relevant immune cell types are shown.

211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238

Table E8. Nominally significant known or predicted functional association partners of *ITGAM*. FunCoup^{1, 2} search identified genes known or predicted to be functionally associated with *ITGAM*. Among them, 5 genes contain SNPs of nominally significant association with CVID in our study.

Gene	SNP	CHR	BP (hg19)	Distance	Minor Allele	Meta P	Meta OR
NCF2	rs699242	1	183546473	0	G	1.17x10 ⁻²	0.756
LYN	rs117197109	8	56818550	0	G	7.09x10 ⁻⁴	2.20
ITGAX	rs2230426	16	31382464	0	C	5.55x10 ⁻⁷	57.05
C3	rs2241391	19	6686504	0	T	3.93x10 ⁻²	5.75
VAV1	rs4807901	19	6796734	0	A	1.62x10 ⁻²	1.32

CHR= Chromosome; BP=base pair; Distance=Distance between the SNP and the gene; Meta P=Meta-analysis P-value; Meta OR=Meta-analysis odds ratio.

Table E9. Enriched pathways. *ITGAM*, nominally significant CVID candidate genes and nominally significant *ITGAM* association partners were used as input for pathway enrichment analysis implemented in FunCoup^{1, 2}. Significantly enriched pathways are shown below (q-value < 0.05).

KEGG signaling	Number of Genes	q-value
B cell receptor signaling pathway	3	0.0102
Leukocyte transendothelial migration	3	0.0164
GO biological process	Number of Genes	q-value
negative regulation of B cell proliferation	2	0.00952
regulation of myeloid leukocyte mediated immunity	2	0.021
negative regulation of B cell activation	2	0.0236
regulation of B cell proliferation	2	0.0325
positive regulation of T cell activation	3	0.0325
negative regulation of mononuclear cell proliferation	2	0.0338
negative regulation of lymphocyte proliferation	2	0.0338
negative regulation of leukocyte proliferation	2	0.0338
positive regulation of lymphocyte activation	3	0.0368
regulation of B cell activation	2	0.039
regulation of T cell activation	3	0.039
Fc-gamma receptor signaling pathway	2	0.0451

Table E10. Immunophenotypes of *ITGAM* knockout mice.

	Itgam ^{tm1Blf} /Itgam ^{tm1Blf}	Itgam ^{tm1Myd} /Itgam ^{tm1Myd}	Itgam ^{tm1Rws} /Itgam ^{tm1Rws}
Leukocyte numbers	Increased		
Leukocyte adhesion	Reduced		
Neutrophil numbers	Increased	Increased	
Neutrophil adhesion	Reduced	Reduced	
Neutrophil degranulation	Reduced	Reduced	
Neutrophil phagocytosis	Reduced	Reduced	
Neutrophil oxidative burst		Reduced	
CD4+ T-cell numbers	Reduced		
CD8+ T-cell numbers	Increased		
T-cell proliferation	Reduced to PHA, ConA and Staphylococcal enterotoxins		
Susceptibility to and mortality from pneumococcal infection	Increased		
Macrophage adhesion			Reduced
Macrophage respiratory burst			Reduced

Figure legends

Figure E1. Results of principal component analysis. Principal component 1 (PC1, X-axis) was plotted against principal component 2 (PC2, Y-axis). After removing outliers, samples were divided into a discovery cohort ($PC1 > -0.005$) and a replication cohort ($PC1 \leq -0.005$).

Figure E2. Quantile-quantile plot showing the expected and observed P-values of a list of null SNPs. The list of null common SNPs was of association with schizophrenia, psychosis and reading/mathematics/ability in literature, which are unrelated to CVID.

Figure E3. Genome studio cluster plots for genome-wide significant SNPs at 16p11.2 locus. For each SNP, both the dbSNP rs number and the iCHIP original SNP id are shown. The number of samples in AA, AB and BB clusters of each SNP are indicated.

Figure E4. Sanger sequencing chromatograms of representative rare variants. (A) Five cases carry the rare minor allele A at rs929867. (B) Six cases carry the rare minor allele T at rs8056264. (C) Seven cases carry the rare minor allele T at rs8045402. Rare minor alleles are indicated by red rectangle (carriers) or green rectangle (non-carriers). Based on array genotyping, samples S1-S5 carry the rare minor alleles at all the three SNPs tested; S6 carry the rare minor alleles at rs8056264 and rs8045402; and S7 only at rs8045402. Samples SN1-SN8 are randomly selected cases whose array genotyping does not have any of these rare minor alleles.

Figure E5. Network analysis results. ITGAM, nominally significant CVID candidate genes and nominally significant ITGAM association partners were used as input for network analysis in FunCoup^{1,2} (left panel). Similar functional associations were also found in network work analysis in STRING^{3,4} (right panel). The types of evidence for the associations in STRING are shown at the right hand corner.

Figure E6. LD block structure of the large 16p11.2 region. The image was downloaded from UCSC genome browser track of phased Hapmap CEU LOD. The *ITGAM* locus (31.1Mb-31.4Mb) is in different LD block from the well known 16p11.2 locus (29.5-30.1Mb) associated with autism, schizophrenia, and obesity.

References:

1. Alexeyenko A, Sonnhammer EL. Global networks of functional coupling in eukaryotes from comprehensive data integration. *Genome Res* 2009; 19:1107-16.
2. Schmitt T, Ogris C, Sonnhammer EL. FunCoupe 3.0: database of genome-wide functional coupling networks. *Nucleic Acids Res* 2014; 42:D380-8.
3. Snel B, Lehmann G, Bork P, Huynen MA. STRING: a web-server to retrieve and display the repeatedly occurring neighbourhood of a gene. *Nucleic Acids Res* 2000; 28:3442-4.
4. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, et al. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res* 2013; 41:D808-15.

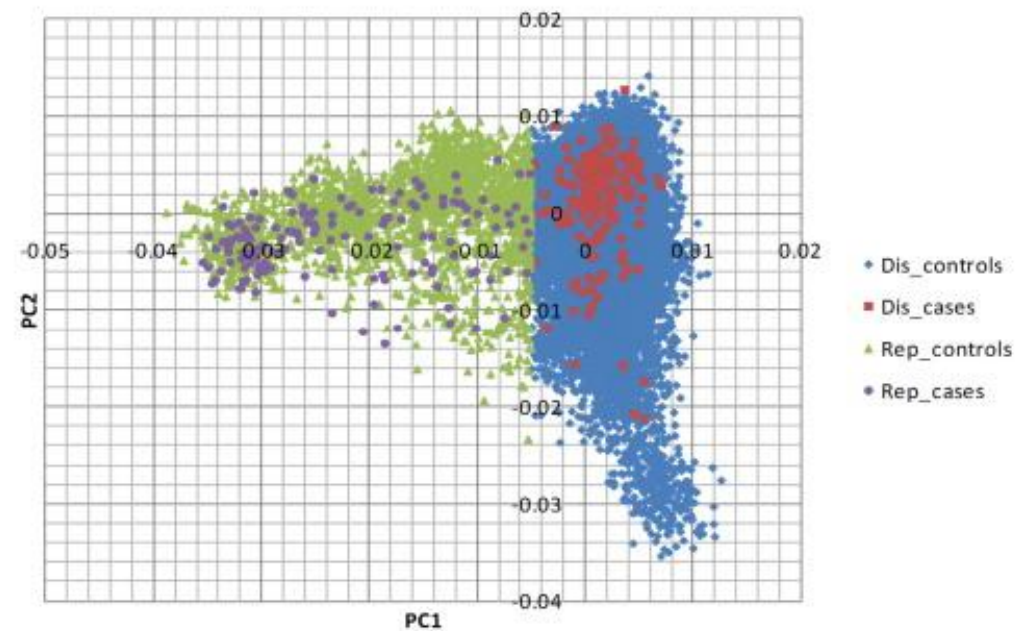


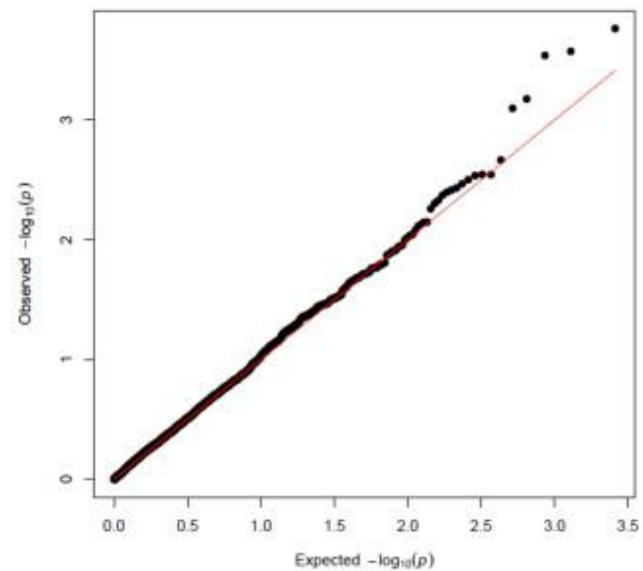
Fig E1

S. Melkorka Maggadottir , Jin Li , Joseph T. Glessner , Yun Rose Li , Zhi Wei , Xiao Chang , Frank D. Mentch , Ke...

Rare variants at 16p11.2 are associated with common variable immunodeficiency

Journal of Allergy and Clinical Immunology, 2015

<http://dx.doi.org/10.1016/j.jaci.2014.12.1939>



Genomic Inflation Factor= 1.03; N(pval)=2579

Fig E2

S. Melkorka Maggadottir , Jin Li , Joseph T. Glessner , Yun Rose Li , Zhi Wei , Xiao Chang , Frank D. Mentch , Ke...

Rare variants at 16p11.2 are associated with common variable immunodeficiency

Journal of Allergy and Clinical Immunology, 2015

<http://dx.doi.org/10.1016/j.jaci.2014.12.1939>

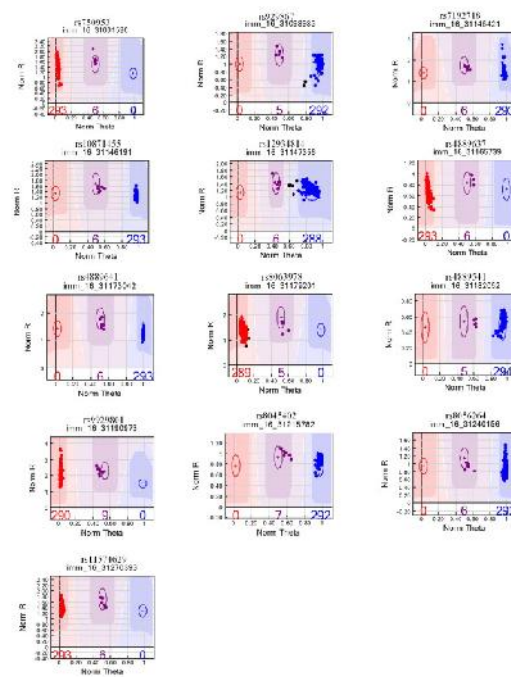


Fig E3

S. Melkorka Maggadottir , Jin Li , Joseph T. Glessner , Yun Rose Li , Zhi Wei , Xiao Chang , Frank D. Mentch , Ke...

Rare variants at 16p11.2 are associated with common variable immunodeficiency

Journal of Allergy and Clinical Immunology, 2015

<http://dx.doi.org/10.1016/j.jaci.2014.12.1939>

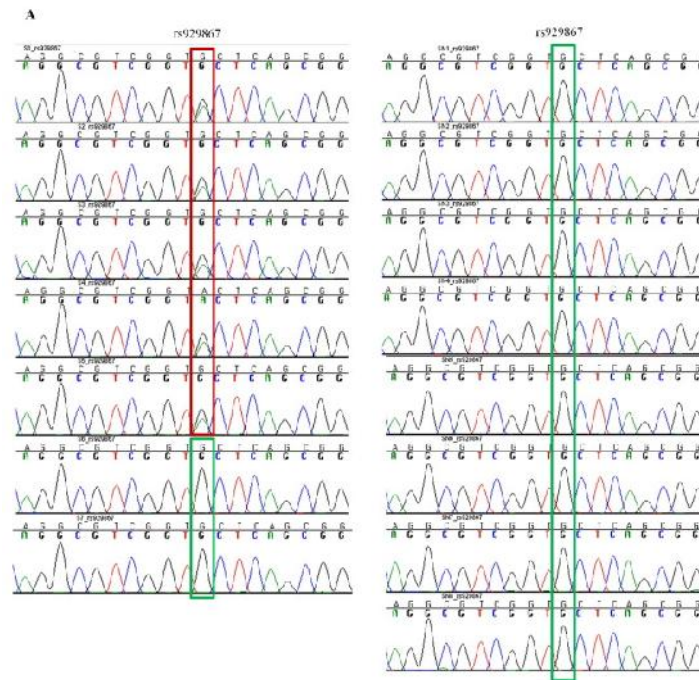


Fig E4

S. Melkorka Maggadottir , Jin Li , Joseph T. Glessner , Yun Rose Li , Zhi Wei , Xiao Chang , Frank D. Mentch , Ke...

Rare variants at 16p11.2 are associated with common variable immunodeficiency

Journal of Allergy and Clinical Immunology, 2015

<http://dx.doi.org/10.1016/j.jaci.2014.12.1939>

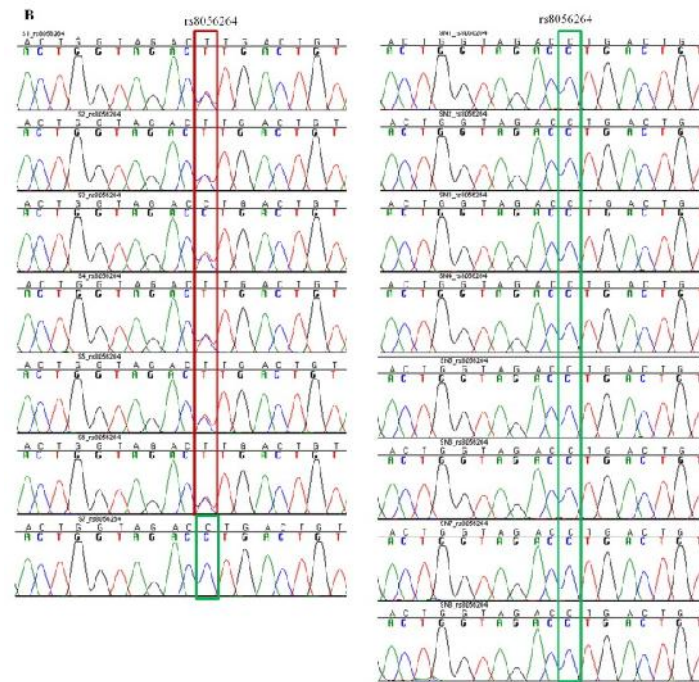


Fig E4

S. Melkorka Maggadottir , Jin Li , Joseph T. Glessner , Yun Rose Li , Zhi Wei , Xiao Chang , Frank D. Mentch , Ke...

Rare variants at 16p11.2 are associated with common variable immunodeficiency

Journal of Allergy and Clinical Immunology, 2015

<http://dx.doi.org/10.1016/j.jaci.2014.12.1939>

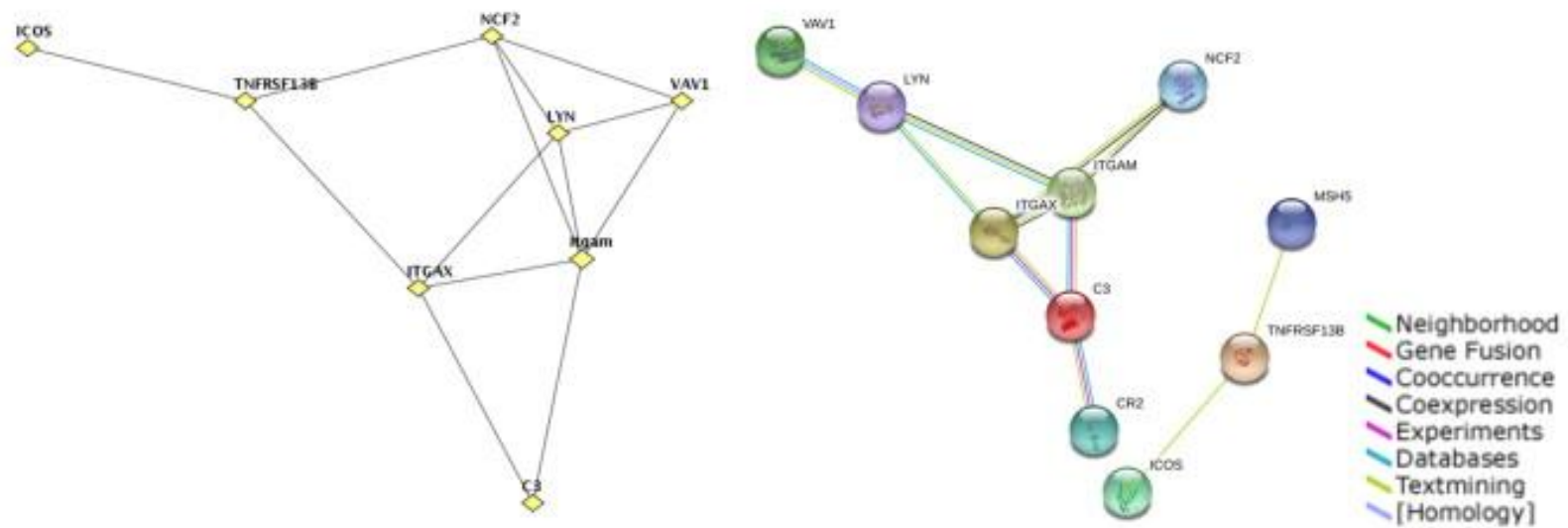


Fig E5

S. Melkorka Maggadottir , Jin Li , Joseph T. Glessner , Yun Rose Li , Zhi Wei , Xiao Chang , Frank D. Mentch , Ke...

Rare variants at 16p11.2 are associated with common variable immunodeficiency

Journal of Allergy and Clinical Immunology, 2015

<http://dx.doi.org/10.1016/j.jaci.2014.12.1939>

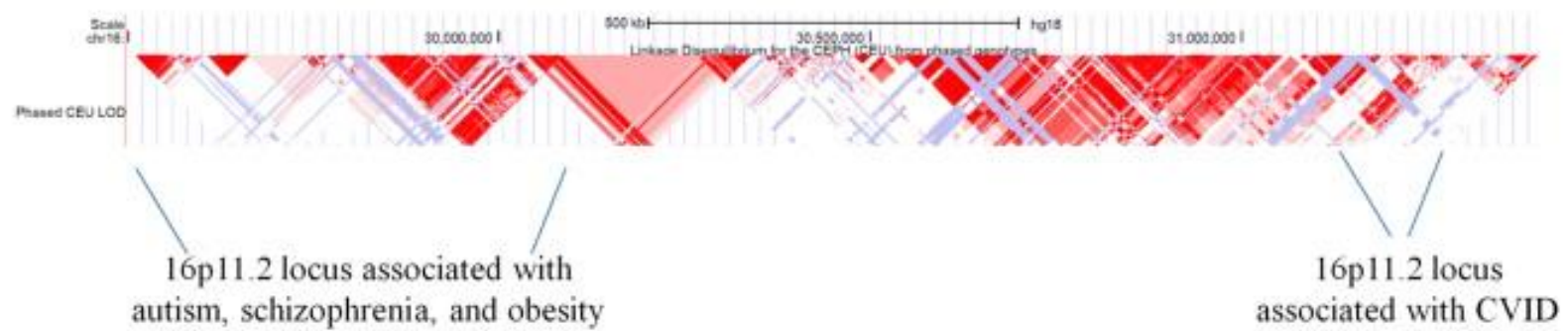


Fig E6

S. Melkorka Maggadottir , Jin Li , Joseph T. Glessner , Yun Rose Li , Zhi Wei , Xiao Chang , Frank D. Mentch , Ke...

Rare variants at 16p11.2 are associated with common variable immunodeficiency

Journal of Allergy and Clinical Immunology, 2015

<http://dx.doi.org/10.1016/j.jaci.2014.12.1939>