Supplementary Information

Gbadegesin et al: EXOME-WIDE ASSOCIATION STUDY IDENTIFIES *HLA-DQA1* AND *PLCG2* AS CANDIDATE RISK LOCI FOR CHILDHOOD ONSET STEROID SENSITIVE NEPHROTIC

Supplementary Table 7: In silico modeling of the missense variants in *HLA-DQA1*

Supplementary Table 8: Top loci in rare variant analysis

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Supplementary Table 10: Minor allele frequency of rare variants in *PLCG2* in 1000 Genomes Project phase 1 reference populations.

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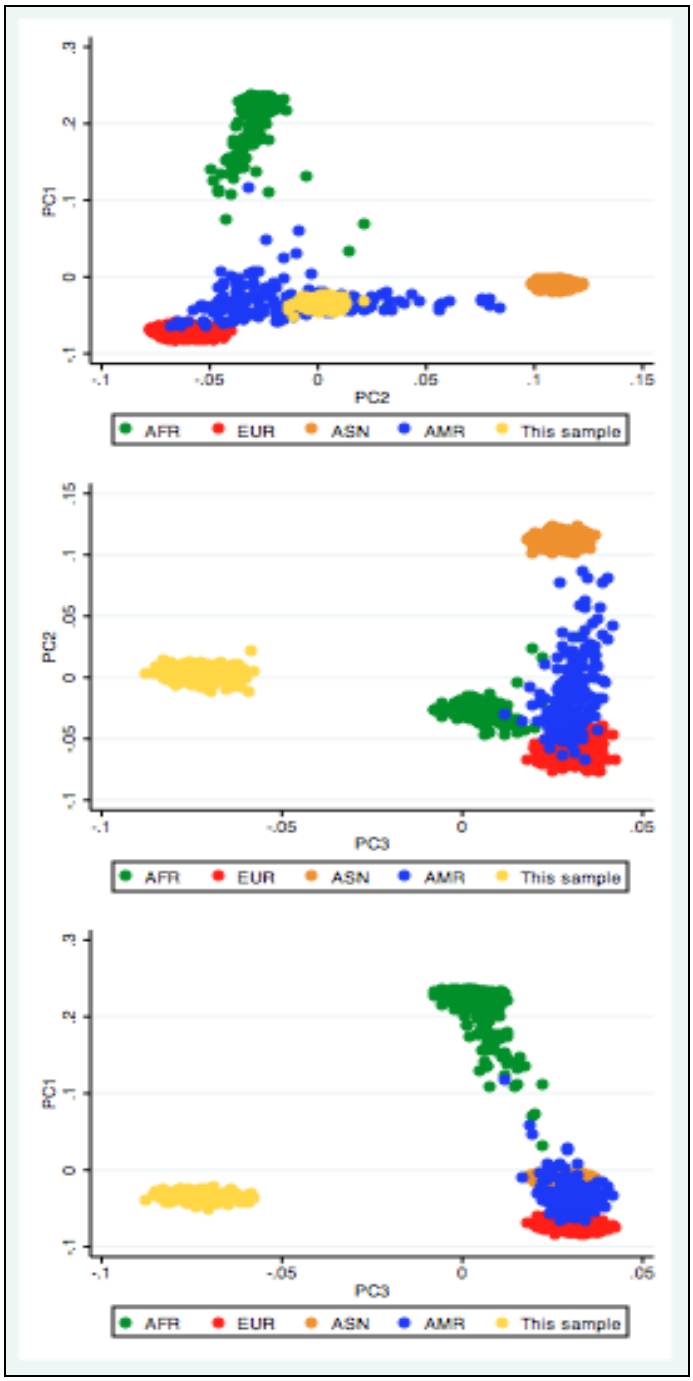
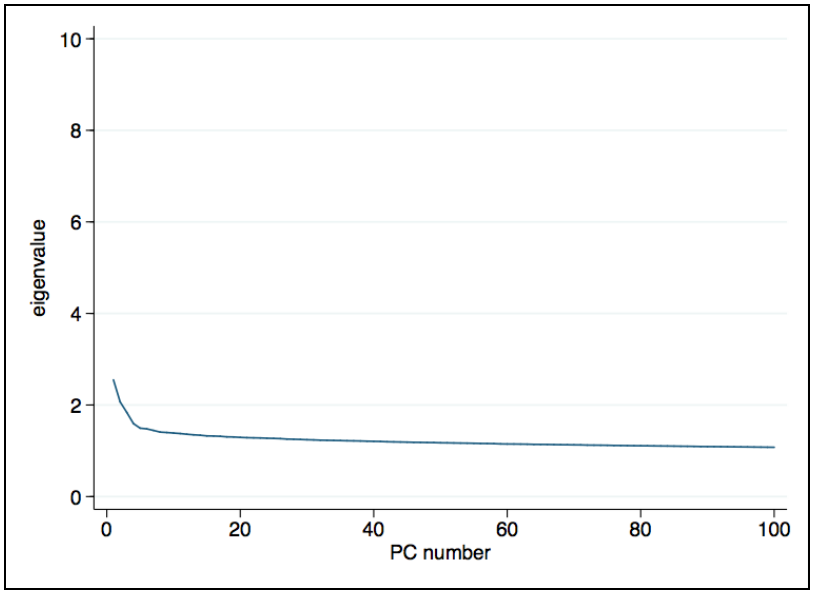
Supplementary Table 13: HWE p-values for control cohorts

Supplementary Figure 1: Plot of principal components (PCs) –PC1 *versus* PC2 - of the genotypes for the discovery sample with case-control status indicated

Supplementary Figure 2: Plots of first three principal components (PCs) of the genotypes projected against 1000 Genomes population reference samples grouped by continental ancestry (AFR= Africa, ASN = Asia (East Asia); AMR = the Americas; EUR = Europe)



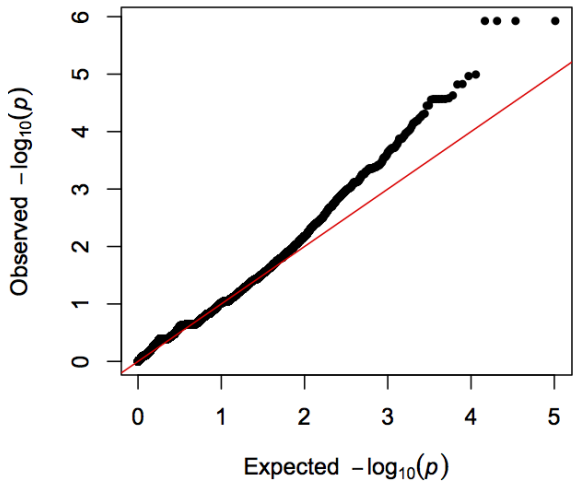
Supplementary Figure 3: Scree plot of the first 100 principal components



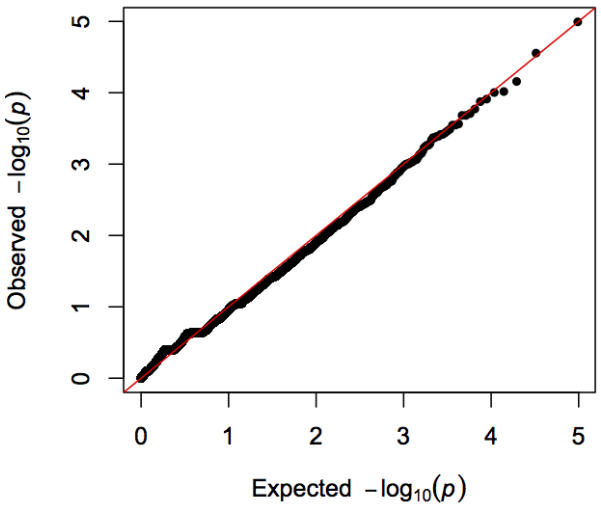
Note: A scree plot of the first 100 PCs shows a nearly flat surface, suggesting that no PC is significant. A formal test of significant PCs using the minimum average partial tests (Shriner D. Improved Eigen analysis of discrete subpopulations and admixture using the minimum average partial test. Hum Hered. 2012; 73(2):73-83) showed that no PC was significant.

Supplementary Figure 4: QQ plots for discovery sample (single variant analysis) (a) All tested markers (b) All tested markers with HLA region removed

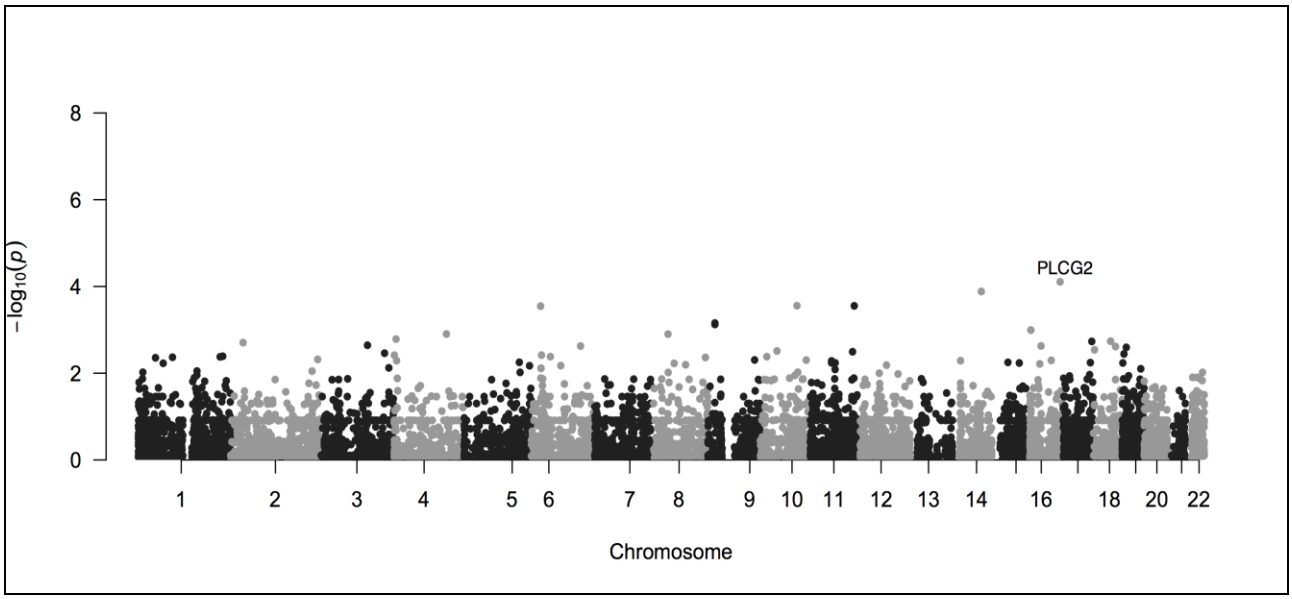
a



b

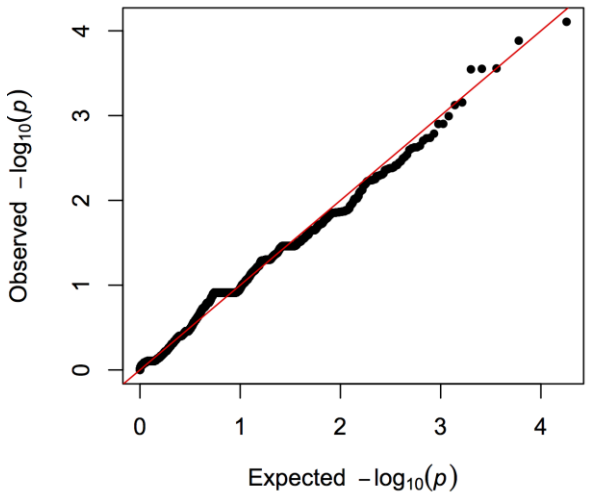


Supplementary Figure 5: Manhattan plot for gene set based analysis

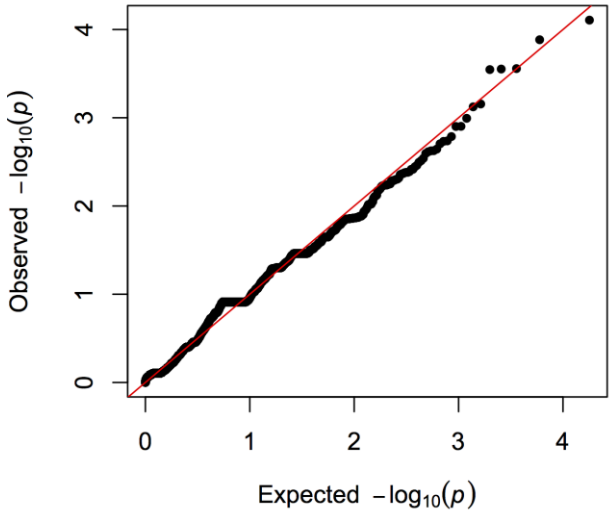


Supplementary Figure 6: QQ plots for gene set based analysis (a) All tested markers (b) All tested markers with HLA region removed

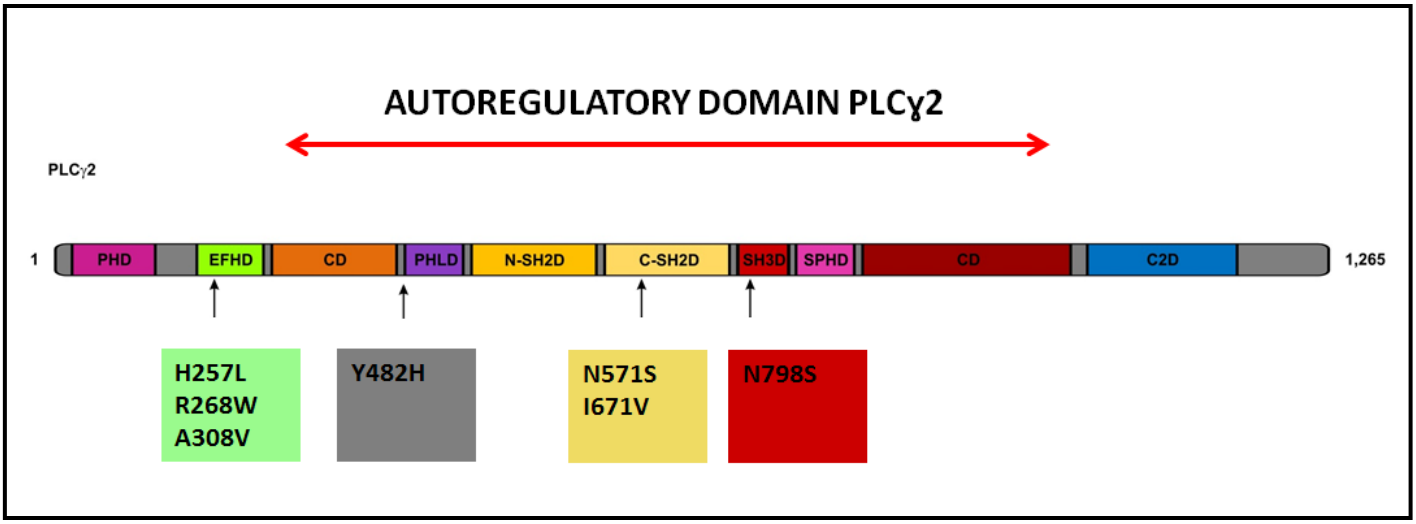
a



b

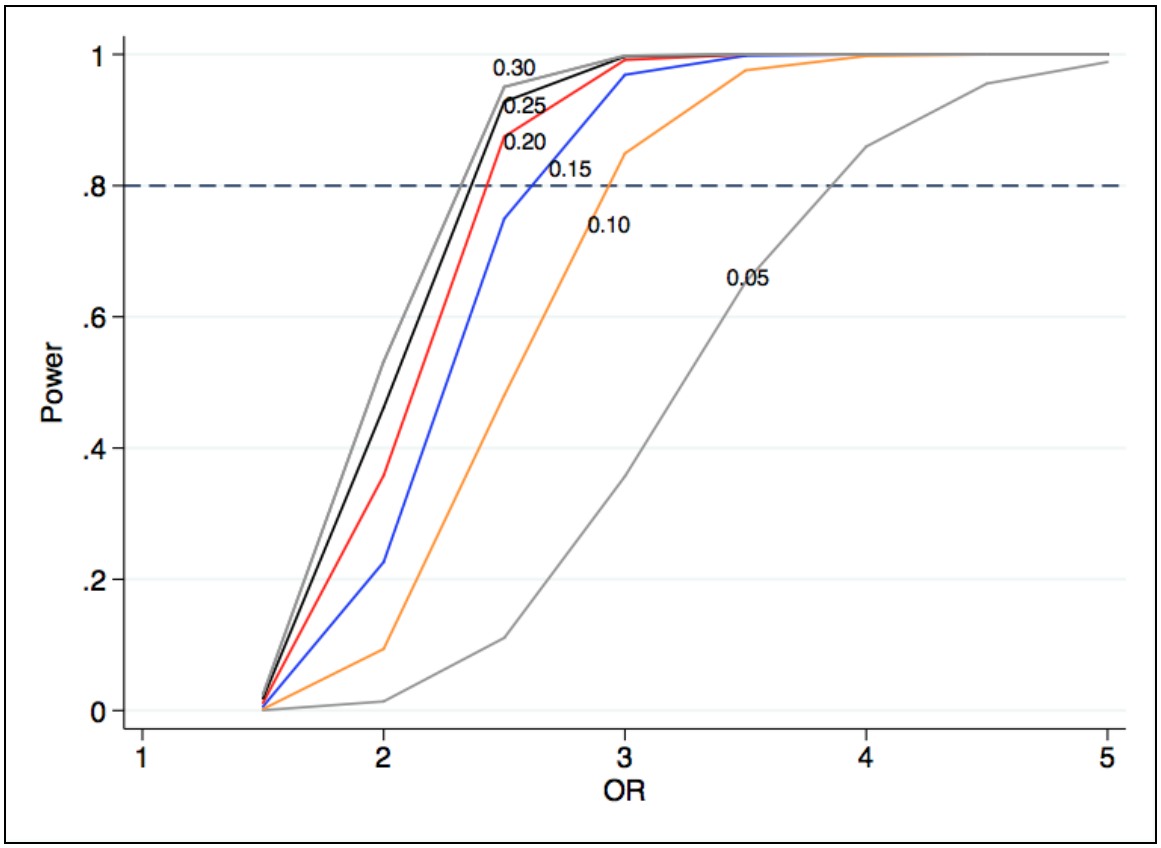


Supplementary Figure 7: Cartoon showing domains of PLCγ2 protein and the location of the variants tested in rare variant analysis



Pleckstrin homology domain (PHD) amino acid (aa) 22-140
EF hand domain (EFHD): aa 243-309
Catalytic domain (CD): aa 311-458
Pleckstrin homology like domain (PHLD): aa 475-510
N-terminal Src Homology 2 domain (N-SH2D): aa 527-627
C-terminal Src Homology domain C-SH2D): aa 641-744
Src Homology 3 domain (SH3D): amino acid 773-827
Split Pleckstrin homology domain (SPHD): aa 840-910
Catalytic domain (CD): aa 926-1031
C2 PLC domain: aa 1067-1187
Rare variants found in *PLCG2* gene in a cohort of children with SSNS are H275V, R268W, A308V, Y482H, N571S, I671V, and N798S

Supplementary Figure 8: Power estimates for the discovery sample



Power estimates for the discovery sample for minor allele frequencies ranging from 0.05 to 0.3 for an α of 1.87 X 10^{-6} (Bonferonni-adjusted p value for the number of testable markers) under an additive genetic model

Supplementary Table 1: Table of top 25 signals on single variant analysis of the discovery dataset

SNP	dbSNP rsID	CHR	BP	A1	F_A	F_U	OR	L95	U95	Fisher test exact P	Allelic model P	Gene(s)_in_EXON
exm536067	rs1129740	6	32609105	A	0.6729	0.4933	2.113	1.559	2.864	1.68E-06	1.19E-06	HLA-DQA1
exm536069	rs1071630	6	32609126	G	0.3271	0.4933	2.113	1.559	2.864	1.68E-06	1.19E-06	HLA-DQA1
exm-rs9273349	rs9273349	6	32625869	G	0.3271	0.4933	2.113	1.559	2.864	1.68E-06	1.19E-06	
exm536255		6	32629137	C	0.3271	0.4933	2.113	1.559	2.864	1.68E-06	1.19E-06	HLA-DQB1
exm-rs6916716	rs6916716	6	30385235	A	0.2313	0.104	2.592	1.678	4.002	8.23E-06	1.08E-05	
exm-rs9261947	rs9261947	6	30394628	A	0.2243	0.1007	2.583	1.663	4.013	1.06E-05	1.51E-05	
exm-rs2844580	rs2844580	6	31333303	G	0.2773	0.1404	2.348	1.585	3.479	1.45E-05	1.49E-05	
exm696263	rs2306899	8	38095662	A	0.05607	0.1544	0.3254	0.1939	0.5463	1.75E-05	1.02E-05	DDHD2
exm-rs9261829	rs9261829	6	30380245	G	0.229	0.1074	2.469	1.605	3.797	2.09E-05	2.63E-05	
exm-rs2844523	rs2844523	6	31368588	A	0.243	0.1174	2.412	1.591	3.657	2.16E-05	2.35E-05	MICA
exm-rs12183946	rs12183946	6	30418533	A	0.2243	0.104	2.49	1.611	3.851	2.62E-05	2.72E-05	
exm-rs9261919	rs9261919	6	30391723	A	0.2243	0.104	2.49	1.611	3.851	2.62E-05	2.72E-05	
exm-rs9261895	rs9261895	6	30389323	A	0.2243	0.104	2.49	1.611	3.851	2.62E-05	2.72E-05	
exm-rs2090192	rs2090192	11	1401890	A	0.3084	0.1711	2.16	1.5	3.11	2.81E-05	2.79E-05	
exm-rs2023372	rs2023372	6	30372018	G	0.215	0.09655	2.563	1.623	4.047	2.89E-05	3.54E-05	
exm-rs9276598	rs9276598	6	32733987	G	0.3318	0.4866	0.5239	0.3868	0.7096	3.06E-05	2.71E-05	
exm-rs1573649	rs1573649	6	32731258	G	0.3318	0.4866	0.5239	0.3868	0.7096	3.06E-05	2.71E-05	HLA-DQB2
exm-rs6906021	rs6906021	6	32626311	A	0.3785	0.5336	0.5324	0.3945	0.7186	4.03E-05	3.50E-05	
exm-rs2281390	rs2281390	6	33059669	A	0.1636	0.06376	2.871	1.689	4.881	4.65E-05	5.51E-05	
exm-rs6902723	rs6902723	6	32731960	G	0.3413	0.4932	0.5325	0.3923	0.7228	5.40E-05	4.88E-05	
exm-rs2844782	rs2844782	6	30150043	A	0.2687	0.1443	2.179	1.479	3.21	5.51E-05	6.45E-05	
exm536445		6	32634306	T	0.1995	0.3333	0.4985	0.355	0.7001	6.03E-05	5.05E-05	HLA-DQB1
exm-rs6909620	rs6909620	6	30448668	C	0.3986	0.2551	1.935	1.396	2.682	6.17E-05	6.57E-05	
exm535981		6	32552095	A	0.3854	0.2415	1.969	1.412	2.747	6.18E-05	5.86E-05	HLA-DRB1
exm-rs9295895	rs9295895	6	30438226	G	0.4042	0.2617	1.914	1.386	2.642	7.18E-05	7.18E-05	

Supplementary Table 2: Table of top signals for association under a dominant model

CHR	SNP	A1	A2	TEST	AFF	UNAFF	P
6	rs9261947	A	G	DOM	89/125	28/121	4.342e-06
6	rs6916716	A	C	DOM	91/123	29/120	4.844e-06
6	exm535981	A	G	DOM	70/135	61/86	7.483e-06
6	rs9261895	A	G	DOM	89/125	29/120	8.24e-06
6	rs9261919	A	G	DOM	89/125	29/120	8.24e-06
6	rs12183946	A	G	DOM	89/125	29/120	8.24e-06
6	rs2844580	G	A	DOM	103/108	37/109	9.244e-06
6	rs6909620	C	A	DOM	72/140	63/84	1.473e-05
6	rs9261829	G	A	DOM	89/125	30/119	1.541e-05
6	rs2844782	A	C	DOM	103/111	38/111	1.769e-05
10	exm2267068	A	G	DOM	58/156	73/76	2.345e-05
6	rs9295895	G	A	DOM	71/143	66/83	2.419e-05
11	rs2090192	A	G	DOM	102/112	45/104	2.721e-05
6	rs2523535	G	A	DOM	101/113	46/102	4.267e-05
6	rs2021720	C	G	DOM	52/162	67/82	4.287e-05
6	exm536067	G	A	DOM	98/116	37/112	4.45e-05
6	exm536069	A	G	DOM	98/116	37/112	4.45e-05
6	exm536255	A	C	DOM	98/116	37/112	4.45e-05
6	rs9273349	A	G	DOM	98/116	37/112	4.45e-05
6	rs2023372	G	A	DOM	76/124	26/119	4.759e-05

Supplementary Table 3: Table of top signals for association under a recessive model

CHR	SNP	A1	A2	TEST	AFF	UNAFF	P
4	exm2269871	G	A	REC	50/164	9/140	5.34e-06
21	exm1567035	A	C	REC	61/153	17/132	8.529e-05
6	exm536453	A	G	REC	36/178	52/97	0.0001041
6	rs6933050	G	A	REC	5/209	19/130	0.0001349
12	rs11047543	A	G	REC	30/184	4/145	0.0001735
6	rs6906021	A	G	REC	32/182	47/102	0.0002611
12	rs17287293	G	A	REC	29/185	4/145	0.0002976
6	exm536067	G	A	REC	24/190	39/110	0.0003672
6	exm536069	A	G	REC	24/190	39/110	0.0003672
6	exm536255	A	C	REC	24/190	39/110	0.0003672
6	rs9273349	A	G	REC	24/190	39/110	0.0003672
9	exm2259123	A	C	REC	24/190	39/110	0.0003672
6	rs2523674	A	G	REC	29/185	43/106	0.0004574
9	exm754321	C	A	REC	9/205	22/127	0.0005088
9	exm754324	A	G	REC	9/205	22/127	0.0005088
10	exm2271266	A	C	REC	35/179	48/101	0.0005611
19	exm1490415	A	G	REC	37/177	8/141	0.0005852
20	rs1877751	G	A	REC	28/186	41/108	0.0006716
3	rs991258	C	G	REC	12/202	25/124	0.0007076
16	exm1267596	A	C	REC	0/214	8/141	0.0007194

Supplementary Table 4: Table of top signals for X chromosome analysis

CHR	SNP	BP	A1	A2	CHISQ	P	OR	SE
23	exm2268548	139942890	A	G	12.98	0.0003143	2.025	0.1971
23	exm2273107	29255861	G	A	11.01	0.0009075	1.931	0.1995
23	exm1635842	45011096	A	G	10.61	0.001126	5.985	0.6185
23	exm2262754	13615118	A	G	10.14	0.001451	2.222	0.2546
23	exm2263050	32102726	C	A	10.01	0.001553	1.854	0.1961
23	exm2262755	136442811	G	A	9.333	0.002251	1.813	0.1956
23	exm2273247	41265035	A	G	9.274e-05	0.9923	1.002	0.2367
23	exm2273302	135743000	A	G	9.109	0.002544	1.952	0.2235
23	exm1639871	50438789	A	G	8.986	0.002721	0.08127	1.065
23	rs7892812	8394253	G	A	7.566	0.005949	1.718	0.1976
23	rs5944185	25853614	G	A	7.413	0.006474	0.5933	0.1924
23	exm1662118	150884620	A	G	6.901e-05	0.9934	1.005	0.6525
23	exm2273227	7575984	G	A	6.247	0.01244	0.5319	0.255
23	rs6610953	44400186	A	G	6.239	0.01249	0.5206	0.2642
23	exm1633338	32380996	G	A	6.151	0.01314	1.673	0.2084
23	exm2273263	63491607	A	C	6.112	0.01342	0.2179	0.6734
23	rs7064929	64367019	A	G	6.052	0.01389	0.1099	1.084
23	exm2268591	64025794	A	G	6.052	0.01389	0.1099	1.084
23	exm1650298	101909785	C	G	5.967	0.01458	0	inf
23	exm2268561	152197812	A	G	5.871	0.01539	1.592	0.1924

Supplementary Table 5: Conditional association analysis (residual association after adjusting for the top exome wide significant hit - rs1129740)

SNP	dbSNP ID	CHR	BP	A1	OR	STAT	P
exm-rs2090192	rs2090192	11	1401890	A	2.17	4.041	5.317e-05
exm-rs416352	rs416352	6	32207393	A	1.909	3.94	8.15e-05
exm-rs6933050	rs6933050	6	31343632	G	0.4788	-3.926	8.63e-05
exm696263		8	38095662	A	0.339	-3.887	0.0001014
exm2271266		10	26636925	A	0.551	-3.861	0.0001129
exm462082		5	75948650	G	1.868	3.859	0.000114
exm2271475		11	23079457	G	0.5324	-3.809	0.0001396
exm2267068		10	134629053	A	1.918	3.795	0.0001476
exm2260679		17	56083934	G	1.937	3.704	0.0002126
exm-rs9295895	rs9295895	6	30438226	G	1.914	3.686	0.0002274
exm-rs6916716	rs6916716	6	30385235	A	2.368	3.644	0.0002688
exm-rs314253	rs314253	17	7091650	G	2.164	3.617	0.0002979
exm-rs6909620	rs6909620	6	30448668	C	1.898	3.616	0.0002987
exm-rs9261947	rs9261947	6	30394628	A	2.365	3.581	0.000342
exm-rs1075496	rs1075496	6	30658239	C	1.77	3.52	0.0004318
exm2270752		7	51527696	A	2.87	3.519	0.0004339
exm-rs7567851	rs7567851	2	178684720	G	0.3476	-3.51	0.0004475
exm2261158		2	208014898	A	0.4694	-3.504	0.0004577
exm-rs9261895	rs9261895	6	30389323	A	2.297	3.493	0.0004776
exm-rs9261919	rs9261919	6	30391723	A	2.297	3.493	0.0004776
exm-rs12183946	rs12183946	6	30418533	A	2.297	3.493	0.0004776
exm-rs9261829	rs9261829	6	30380245	G	2.24	3.488	0.0004859
exm-rs2253907	rs2253907	6	31336870	A	0.5486	-3.479	0.0005038
exm2264392		10	89912106	C	1.862	3.477	0.0005067

Supplementary Table 6: Functional annotation of exome wide significant loci done with HaploReg v2 (http://www.broadinstitute.org/mammals/haploreg/haploreg.php)

Enhancer enrichment analysis
No significant enrichment

DNase enrichment analysis

Cell type				DNase			
ID	Description	Treatment	Production center	Obs	Exp	Fold	p
GM12878	B-lymphocyte, lymphoblastoid	None	AWG	1	0	31.9	0.030981
CD20+	B cells	None	UW	2	0	107.1	0.000116

Query SNP: rs1129740 and variants with r^2 >= 0.8

chr	pos (hg19)	LD (r^2)	LD (D')	variant	Ref	Alt	AFR freq	AMR freq	ASN freq	EUR freq	SiPhy cons	Promoter histone marks	Enhancer histone marks	DNAse	Proteins bound	eQTL tissues	Motifs changed	GENCODE genes	dbSNP func annot
6	32608888	0.86	0.94	rs9272675	G	T	0.53	0.64	0.45	0.54		GM12878			POL24H8,POL2		CTCF,RBP-Jkappa	HLA-DQA1	intronic
6	32609105	1	1	rs1129740	G	A	0.52	0.65	0.45	0.53		GM12878		GM12878,CD20+	POL2,POL24H8		6 altered motifs	HLA-DQA1	missense
6	32609126	0.88	1	rs1071630	T	C	0.56	0.68	0.47	0.58		GM12878		CD20+	POL2,POL24H8		PLZF	HLA-DQA1	missense
6	32609207	0.82	0.96	rs1142324	C	T	0.50	0.59	0.39	0.46		GM12878			POL2,POL24H8		5 altered motifs	HLA-DQA1	missense
6	32610109	0.82	0.98	rs34826728	A	C	0.56	0.69	0.47	0.58		GM12878					Ik-1,Ik-2,Zfp410	HLA-DQA1	intronic

Query SNP: rs1071630 and variants with r^2 >= 0.8

chr	pos (hg19)	LD (r^2)	LD (D')	variant	Ref	Alt	AFR freq	AMR freq	ASN freq	EUR freq	SiPhy cons	Promoter histone marks	Enhancer histone marks	DNAse	Proteins bound	eQTL tissues	Motifs changed	GENCODE genes	dbSNP func annot
6	32607780	0.85	0.96	rs9272611	C	T	0.54	0.66	0.46	0.57		GM12878		GM12878			BAF155,DMRT1,NF-E2	HLA-DQA1	intronic
6	32608014	0.84	0.95	rs9272625	T	C	0.54	0.66	0.43	0.57		GM12878		CD20+	POL2,POL24H8		PU.1	HLA-DQA1	intronic
6	32608093	0.86	0.95	rs9272628	T	C	0.54	0.65	0.46	0.57		GM12878		CD20+,GM12864	POL2,POL24H8		SIX5,Znf143,p53	HLA-DQA1	intronic
6	32608096	0.86	0.95	rs9272629	G	T	0.54	0.65	0.46	0.57		GM12878		CD20+,GM12864	POL2,POL24H8		ZBTB33,p53	HLA-DQA1	intronic
6	32608888	0.87	0.98	rs9272675	G	T	0.53	0.64	0.45	0.54		GM12878			POL24H8,POL2		CTCF,RBP-Jkappa	HLA-DQA1	intronic
6	32609105	0.88	1	rs1129740	G	A	0.52	0.65	0.45	0.53		GM12878		GM12878,CD20+	POL2,POL24H8		6 altered motifs	HLA-DQA1	missens
6	32609126	1	1	rs1071630	T	C	0.56	0.68	0.47	0.58		GM12878		CD20+	POL2,POL24H8		PLZF	HLA-DQA1	missens
6	32609427	0.85	0.96	rs9272723	T	C	0.53	0.66	0.44	0.54		GM12878				Schadt_Liver,Stranger_LCL	Barx1,Cdx2,EBF	HLA-DQA1	intronic
6	32610109	0.91	0.97	rs34826728	A	C	0.56	0.69	0.47	0.58		GM12878					Ik-1,Ik-2,Zfp410	HLA-DQA1	intronic

Query SNP: rs9273349 and variants with r^2 >= 0.8

chr	pos (hg19)	LD (r^2)	LD (D')	variant	Ref	Alt	AFR freq	AMR freq	ASN freq	EUR freq	SiPhy cons	Promoter histone marks	Enhancer histone marks	DNAse	Proteins bound	eQTL tissues	Motifs changed	GENCODE genes	dbSNP func annot
6	32625869	1	1	rs9273349	T	A,C,G	0.00	0.00	0.00	0.00					POL24H8	Montgomery_LCL		1.4kb 3' of HLA-DQB1	

Supplementary Table 7: In silico modeling of the missense variants in *HLA-DQA1*

Variant	**Polyphen score**	**SIFT score**	**Mutation Taster**
rs1129740 (C34Y)	0.1 (Benign)	1 (Tolerated)	Polymorphism
rs1071630 (F41S)	0.01 (Benign)	1 (Tolerated)	Polymorphism

Supplementary Table 8: Top loci in rare variant analysis

Gene Set	P value	Number of rare markers	Number of markers tested
PLCG2	9.478e-05	7	7
GSTZ1	0.000118	4	4
IFT46	0.000248	1	1
LRRC16A	0.000343	5	5
ANKRD2	0.000400	3	3
PLIN2	0.000668	1	1
HAUS6	0.000729	3	3
HLA-DPA1	0.000900	7	7
ENGASE	0.000994	3	3

All p values are genome wide adjusted (Bonferroni correction of α of 0.05 for ~20,000 genes in the human genome)

Supplementary Table 9: Annotation of *PLCG2* SNPs

Location	Transcript	Ensembl protein	Protein pos	Amino Acid	cDNA pos	Cds pos	codons	Sift score	Polyphen2 score	Mass*	Condel score	Condel Label
16:81922781	ENST00000359376	ENSP00000352336	257	H/L	940	770	cAt/cTt	0.68	0	-0.975	0.001	neutral
16:81922813	ENST00000359376	ENSP00000352336	268	R/W	972	802	Cgg/Tgg	0.07	0.999	0.69	0.499	deleterious
16:81925132	ENST00000359376	ENSP00000352336	308	A/V	1093	923	gCg/gTg	0.29	0	0.625	0.006	neutral
16:81939089	ENST00000359376	ENSP00000352336	482	Y/H	1614	1444	Tac/Cac	0.08	0.88	1.59	0.363	neutral
16:81942175	ENST00000359376	ENSP00000352336	571	N/S	1882	1712	aAt/aGt	0.02	0	0.835	0.008	neutral
16:81946278	ENST00000359376	ENSP00000352336	671	I/V	2181	2011	Atc/Gtc	1	0.942	-0.34	0.887	deleterious
16:81957175	ENST00000359376	ENSP00000352336	798	N/S	2563	2393	aAt/aGt	0.01	0.987	3.02	0.842	deleterious

Annotation done with CONDEL (CONsensus DELeteriousness score of missense SNVs - http://bg.upf.edu/condel/home), a tool which assesses the outcome of nonsynonymous SNVs using a consensus deleteriousness score that combines various tools including: SIFT (Sift score above), Polyphen2 (Polyphen2 score above) and MutationAssessor (Mass* above).

Supplementary Table 10: Minor allele frequency of rare variants in *PLCG2* in 1000 Genomes Project reference populations.

	Minor Allele Frequency			
Variants	European	African	Asian*	American#
rs45443101(H257L)	0.02	0.0	0.0	0.04
rs17537869 (R268W)	0.08	0.0	0.0	0.05
rs199636472 (A308V)	0.0	0.0	0.0	0.0
rs187956469 (Y482H)	0.01	0.0	0.0	0.0
rs75472618 (N571S)	0.01	0.01	0.0	0.01
rs150833842 (I671V)	0.01	0.0	0.0	0.01
rs117077093 (N798S)	0.0	0.0	0.0	0.0

* Asian (ASN): Data derived from Southern Han Chinese, Han Chinese in Beijing and Japanese in Tokyo.
# American: Data derived from Medellian in Colombia, Mexicans from Los Angeles and Puerto Ricans from Puerto Rico
All data source: http://browser.1000genomes.org/index.html

Note that the discovery data in this study is from a South Asian population mainly from Sri Lanka and therefore does not reflect the genetic architecture of the ASN population in the 1000 genomes Project phase 1 reference population, that is made up of East Asians (Chinese and Japanese).

Supplementary Table 11: Joint common and rare variant analysis with SKAT (minor allele cut off 0.05)

Gene set	P value	Number of markers in gene set	Number of markers tested	Number of rare variants tested	Number of common variants tested
HLA-DQA1	2.660e-06	7	7	0	7
DDHD2	4.589e-05	1	1	0	1
HLA-DRB1	5.912e-05	3	3	1	2
PLCG2	6.913e-05	7	7	7	0
VMO1	0.000149	2	2	0	2
HLA-DQB1	0.000235	15	15	2	13
PPT2-EGFL8	0.000246	4	4	1	3
KLF7	0.000294	1	1	0	1
SRSF1	0.000299	1	1	0	1
ANKRD2	0.000335	3	3	3	0

Supplementary Table 12: List of centers participating in the replication study

Center	**Number of subjects recruited n (%)**	**Number of subjects successfully genotyped n (%)**
Duke University Medical Center, Durham, NC	32(26)	27(27)
Children's Mercy Hospital, Kansas, Missouri	15(12.2)	13(13)
University of Michigan Hospital, Michigan, MI	13(10.6)	11(11)
Texas Children's Hospital, Houston, Texas	12(9.8)	11(11)
Children's Hospital of Atlanta, Emory University, Atlanta, GA	12(9.8)	10(10)
Akron Children's Hospital, Akron, Ohio	11(9)	6(6)
Monro Carell Children's Hospital, Vanderbilt University, Nashville, TN.	10(8.1)	8(8)
University of Iowa Hospital, Iowa	5(4.1)	4(4)
Brenner Children's Hospital, Wake Forest University Medical Center, Winston Salem, NC	3(2.4)	2(2)
Helen Devos Children's Hospital, Grand Rapids, Michigan	3(2.4)	3(3)
University of Minnesota Amplatz Children's Hospital, Minneapolis, MN	3(2.4)	3(3)
Cardinal Glennon Children's Hospital, Saint Louis University, St Louis, MO	2(1.6)	2(2)
Dayton Children's Hospital, Dayton Ohio	2(1.6)	0(0)
TOTAL	123(100)	100(100)

Supplementary Table 13: HWE p-values for control cohorts

SNP	**Discovery sample**	**CATHGEN**	**1000 Genomes EUR**
rs1129740	0.628	1.06×10^{-5}	0.003
rs1071630	0.628	1.06×10^{-5}	0.172