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Exome-Wide Rare Variant Analyses in Sudden Infant Death Syndrome

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Abstract

Objective: To determine if a monogenic basis explains sudden infant death syndrome (SIDS) using an exome-wide focus.

Study design: A cohort of 427 unrelated cases of SIDS (257 males; average age = 2.7 ± 1.9 months) underwent whole exome sequencing (WES). Exome-wide rare variant analyses were carried out with 278 European ancestry SIDS cases (173 males; average age = 2.7 ± 1.98 months) and 973 ethnic-matched controls based on six genetic models. Ingenuity Pathway Analysis was also performed. The cohort was collected in collaboration with coroners, medical examiners, and pathologists by St George's University of London, UK and Mayo Clinic, Rochester, Minnesota, USA. WES was performed at the Genomic Laboratory, Kings College London, UK or Mayo Clinic's Medical Genome Facility, Rochester, Minnesota.

Results: Although no exome-wide significant ($P < 2.5 \times 10^{-6}$) difference in burden of ultra-rare variants was detected for any gene, 405 genes had a higher prevalence ($p < 0.05$) of ultra-rare non-synonymous variants among cases with seventeen genes at $p < 0.005$. Some of these potentially overrepresented genes may represent biologically plausible novel candidate genes for a monogenic basis for a portion of patients with SIDS. The top canonical pathway identified was glucocorticoid biosynthesis ($p = 0.01$).

Conclusions: The lack of exome-wide significant genetic associations indicates an extreme heterogeneity of etiologies underlying SIDS. Our approach to understanding the genetic mechanisms of SIDS has far reaching implications for the SIDS research community as a whole and may catalyze new evidence-based SIDS research across multiple disciplines. Perturbations in glucocorticoid biosynthesis may represent a novel SIDS-associated biological pathway for future SIDS investigative research.

Keywords

inherited cardiac conditions; molecular autopsy; sudden infant death syndrome; whole exome sequencing

Sudden infant death syndrome (SIDS) is the sudden unexpected death, which remains unexplained despite comprehensive clinical and pathological investigations.[1] SIDS represents 70-80% of all sudden unexpected infant deaths with an incidence of 0.4/1000 live births in the UK and 0.5/1000 live births in the USA.[2, 3] The peak incidence occurs between 2 – 4 months of age and is more common in males. It is associated commonly with environmental risk factors such as co-sleeping or prone sleeping position.[4] Despite successful targeted risk reduction campaigns, the number of SIDS cases have plateaued. SIDS, also referred to as sudden unexplained infant death (SUID) remains the leading cause of post-neonatal mortality.[4]

A triple-risk model for SIDS suggest the convergence of the vulnerable infant in the setting of exogenous stressors occurring in a critical development period.[5] Although many pathophysiologic theories have been proposed, decisive pathogenic substrates/mechanisms triggering an infant's sudden demise remain unclear.[6-9] Several studies have implicated both common and rare genetic variants involved in autonomic function, neurotransmission, energy metabolism, response to infection, and cardiac repolarization.[10-14] Potentially lethal inherited genetic heart diseases including long QT syndrome (LQTS), Brugada syndrome), catecholaminergic polymorphic ventricular tachycardia (CPVT), and hypertrophic cardiomyopathy have been implicated as monogenic causes for a small proportion (<10%) of SIDS cases.[10, 13, 15-27] [28]

However, less than 100 investigations of genetic variation in population-based SIDS cohorts have been published to date. These studies are largely based on hypothesis-driven, candidate gene/pathway approaches that recognize established environmental risk factors for SIDS, with an average cohort size of just 125 SIDS cases.[13] Although one may postulate that the genetics of SIDS is most likely multigenic and complex, no studies have attempted to elucidate the underlying genetic basis in a single SIDS cohort across multiple biological pathways in an unbiased manner. Here, using whole exome sequencing (WES), we conducted an exome-wide analysis of rare protein-altering variation followed by biological Ingenuity Pathway Analysis (IPA) in a cohort of nearly 300 unrelated Caucasian SIDS cases.

Methods

As previously described, the SIDS cohort (N=427) consisted of 95 coroners' cases from the United Kingdom (UK; London, Sheffield, Edinburgh and Bristol) and 332 coroner/medical examiner/forensic pathologist-referred cases collected from six ethnically and geographically diverse United States (US) populations.[28] Because of the lack of uniformity in procedures and reporting between medical examiner offices in the US, differences in protocols may exist. Nonetheless, both gross and histological examinations of all major organs were performed and all cases satisfied our enrolment criteria that included sudden unexplained death of an infant < 1 year of age, European descent, and a comprehensive negative medico-legal autopsy including a negative toxicology screen and death scene investigation. Infants, clearly demonstrated to have experienced asphyxia or specific disease causing death, were excluded. Ethnicity was self-reported by the referring coroner/medical examiner. This anonymous necropsy study only had limited medical information such as the sex, ethnicity, age at the time of death, and sleep position available. This study complies with the Declaration of Helsinki; locally appointed ethics committees, including Mayo Clinic's Institutional Review Board, have approved the research protocol.

Control Population

Caucasian control exomes (509 females, 464 males) from the ICR1000 UK exome sequencing of the 1958 Birth Cohort study were included for analysis.[29] As previously reported, exome sequencing was performed using the Illumina TruSeq and Illumina instruments.[29]

Whole Exome Sequencing

Genomic DNA isolated from each SIDS case underwent WES at the KCL-GSTT Biomedical Research Centre Genomics Platform, London, UK or Mayo Clinic's Medical Genome Facility, Rochester, Minnesota as described previously.[28] Cases were excluded from further analysis if < 75% of the Gencode defined protein coding exome was covered by < 20 reads. A set of 3847 common variants located outside of regions of the genome, where there is extensive linkage disequilibrium, were used to estimate relatedness within the study cohort. To avoid potential confounding due to population stratification, ancestry estimation was undertaken using the first two dimensions of Multidimensional Scaling (MDS) using Euclidean distance undertaken with the King software package. Quality control metrics excluded 7 cases.[28] The resulting SIDS cases (N = 278) and European controls (N = 973) that formed a homogeneous cluster on the first two principal components were included in the case-control rare variant analysis.[28]

Exome-Wide Rare Variant Analysis

The 278 European Caucasian SIDS cases and 973 ethnic-matched controls underwent a case-control simple collapsing exome-wide rare variant association analysis using the Efficient and Parallelizable Association Container Toolbox (EPACTS).[30] Cases and controls were coded with a binary indicator according to the presence/absence of a “qualifying” variant in each gene based on six genetic models: dominant non-synonymous (amino acid altering), dominant non-synonymous-combined annotation dependent depletion (CADD 20: equivalent to a probability of 0.99 that the variant has a functional impact. [31]), dominant putative loss-of-function (i.e. nonsense, frame-shift, and canonical splice-site errors), recessive non-synonymous, recessive non-synonymous-CADD 20, and recessive putative loss-of-function.

For all models, only “ultra-rare” variants with a minor allele frequency (MAF) < 0.00005 (1:20,000 alleles) derived from the Exome Aggregation Consortium (ExAC)[32] were included. Deviation from the null model of no excess of rare variation in cases was evaluated with a one-tailed Fisher exact test. Despite being too stringent, a Bonferroni-adjusted p-value threshold of $< 2.5 \times 10^{-6}$ was considered as exome-wide significant.

Ingenuity Pathway Analysis

An Ingenuity Pathway Analysis (IPA) was used to analyze a list of 405 genes (p-value<0.05) obtained by performing an exome-wide rare variant comparative analysis between European SIDS cases and ethnic-matched controls. IPA is a web-based tool that is used for discovery and visualization of significant pathways and networks, and close correlations with disease and functional classes. Data were analyzed with QIAGEN's Ingenuity Pathway Analysis (IPA, QIAGEN Redwood City, www.qiagen.com/ingenuity) using default analysis settings. [33]

Statistical analyses

Categorical variables were expressed as absolute numbers and percentage, and compared with Fisher exact or Chi-square tests. Probability values were based on two-sided tests

considered significant at $P < 0.05$. Analysis was conducted with SPSS version 18.0 software (SPSS Chicago III). The significance of the association between the data set (ie, 405 “SIDS genes”) and the IPA canonical pathway was measured as a ratio of the number of genes from the data set that map to the pathway divided by the total number of genes that map to the canonical pathway.) Fisher exact test right-tailed was used to calculate a p-value determining the probability that the association between the genes in the dataset and the canonical pathway is explained by chance alone.[33]

Results

The cohort consisted of 278 cases (173 male infants, 105 female infants; average age = 2.7 ± 1.98 months). The epidemiologically high risk age group of 2–4 months (55.4%) and male sex (62.9%) accounted for the majority of the cases. Sleep characteristics were known in 60% of the cohort (Table I).

Exome-wide rare variant analyses were performed with the 278 European ancestry cases and 973 matched controls. None of the estimated 20,000 genes within the human genome were associated with SIDS when considering an exome-wide significant p-value threshold of $< 2.5 \times 10^{-6}$ with any of the 6 aforementioned inheritance models.

There were 405 genes with potential case:control, ultra-rare ($MAF < 0.00005$), non-synonymous variant over-representation at $p < 0.05$ threshold (Table 2; **available at www.jpeds.com**) with 17 genes reaching $p < 0.005$ (Table 3). Online Mendelian Inheritance in Man (OMIM) disease[34] and PathCards SuperPathway [35] associations for each gene is listed in Table 4 (**available at www.jpeds.com**). The prevalence of ultra-rare, non-synonymous variants with a CADD score > 20 was higher ($p < 0.005$) in cases versus controls in 10 genes (Table 5; **available at www.jpeds.com**). Some of these genes may represent novel, biologically plausible candidate genes for SIDS. For example, 6/278 (2.2%) SIDS cases hosted an *NR3C2* (MIM: 600983) variant compared with only 2/973 (0.21%) controls ($p = 0.000778$). Variants in the *CHRM3* (MIM: 118494)-encoded cholinergic receptor muscarinic-3 were over-represented in SIDS cases (4/278 [1.4%]) compared with controls (0/973, $p = 0.002$), and *LHX9* (MIM: 606066) variants were also over-represented in SIDS cases (5/278 [1.8%]) compared with controls (0/973, $p = 0.000527$).

Ingenuity Pathway Analysis (IPA)

IPA was performed on the aforementioned 405 genes from our dominant non-synonymous ultra-rare variants analysis with a p-value < 0.05 . IPA identified 5 canonical pathways with a p-value < 0.05 (Table 6). The top canonical pathway involved glucocorticoid biosynthesis ($p = 0.0107$, Table 6). Of the 8 genes within the glucocorticoid biosynthesis pathway, 2 genes (25%) were present among the 405 candidate genes. Ultra-rare *CYP17A1* ([MIM: 609300], 4/278 [1.4%] vs 1/973 [0.1%], $p = 0.0099$), and *CYP11B2* ([MIM: 124080], 6/278 [2.2%] vs 3/973 [0.3%], $p = 0.0052$) non-synonymous variants were both over-represented in the cases versus controls ($p = 0.0001$ when considered together).

Discussion

We report genetic insights derived from WES in a large cohort of unrelated SIDS cases. We employed a first ancestry matched case-control burden analysis of ultra-rare variants involving nearly 300 cases and 1000 healthy controls to identify potential novel monogenic causes for SIDS. Although no genes reached the very strict Bonferroni-corrected exome-wide significance threshold of $p < 2.5 \times 10^{-6}$, 405 genes showed a potential over-representation of ultra-rare, non-synonymous variants. However, variants were observed in less than 2% of the SIDS cohort for 90% of these 405 genes. This confirms prior speculation that SIDS is a highly heterogeneous condition and likely results from a complex interplay of genetic and environmental factors rather than from a small number of highly penetrant monogenic disorders. In addition, at the p-value threshold of $p < 0.05$, one might expect 1000 genes to surface as seemingly over-represented in cases just by chance (i.e. 20,000 genes divided by 20). In other words, this study strongly suggests that the vast majority of SIDS is NOT monogenetically-mediated at least by ultra-rare genetic variation localizing to the exome. Although it remains to be confirmed or refuted, much of SIDS may not have a genetic component at all.

Nevertheless, some of these genes identified are appealing pathobiologically and could represent novel, monogenic causes for a small minority of SIDS cases. For example, there was potential over-representation ($p < 0.005$) of *NR3C2*, *CHRM3*, and *LHX9* variants in SIDS cases compared with controls. These three genes may represent biologically plausible candidates for SIDS pathogenicity that could account independently for a small subset of SIDS cases (~2%).

The *NR3C2* gene encodes for a mineralocorticoid receptor that mediates aldosterone activity and is responsible for autosomal dominant pseudohypoaldosteronism Type 1 (adPHA1) which is a rare disorder characterized by renal resistance to aldosterone, with salt-wasting, hyperkalemia, and metabolic acidosis[36]. If undetected and untreated, adPHA1 may present with neonatal lethality owing to severe hyponatremia, metabolic acidosis, or hyperkalemia-mediated arrhythmia and cardiorespiratory arrest.[37, 38] Interestingly, conditional targeted cardiomyocyte over-expression of mineralocorticoid receptor in mice lead to early sudden death without cardiac structural alteration as a result of prolonged ventricular repolarization and spontaneous and triggered ventricular arrhythmias associated with ion channel remodeling.[39]

The *CHRM3* gene encodes for cholinergic receptor muscarinic 3 (M_3 -receptor). Muscarinic acetylcholine receptors are G-protein-coupled receptors that play a key role in parasympathetic acetylcholine neurotransmission for a variety of physiological functions within the brain, heart, and respiratory system.[40] In the respiratory system, muscarinic M_3 -receptors mediate smooth muscle contraction for respiration.[40] In the heart, M_3 -receptors may play a role in the regulation and maintenance of cardiac function.[41, 42] The M_3 -receptors activate the delayed rectifying potassium current I_{KM3} to participate in the regulation of heart rate, cardiac resting membrane potential, and cardiac repolarization.[43] The M_3 -receptor also interacts with gap-junctional channel connexin 43 (Cx43) to maintain cell-cell communication and excitation propagation in the heart.[44] Interestingly, Cx43

mutations leading to gap junction loss were implicated previously in some cases of SIDS. [24] In 2010, cardiac muscarinic receptor over-expression in left ventricular heart samples was shown to be associated with SIDS.[45] In 1995, Kinney et al provided evidence that deficiency of muscarinic receptor binding in the arcuate nucleus of the medulla may be associated with an increase in the probability of death from SIDS.[46]

LHX9-encoded Lhx9 is a member of the apterous group of the LIM-homeodomain family of evolutionary conserved transcription factors crucial for the correct development of many organs including the heart and the nervous system, including the forebrain, thalamus, hypothalamus, and pineal gland.[47-51] Sparks and Hunsaker reported a significant reduction in the size of the pineal gland in SIDS cases compared with controls.[52] In mice, Lhx9 expression is maintained after birth in the hippocampal formation and restricted to the dentate gyrus.[51] Recently, Kinney identified dentate gyrus abnormalities as a potential developmental vulnerability in sudden unexplained death in infants that may lead to autonomic/respiratory instability or autonomic seizures, and sleep-related sudden death of the vulnerable infant when exposed to homeostatic stressors.[53, 54]

Whether *NR3C2*, *CHRM3*, *LHX9* or any of the other genes that may have an over-representation of ultra-rare, nonsynonymous variants in SIDS are indeed responsible for the pathogenic basis for SIDS will require substantial functional analyses and replication studies before being considered as a new pathogenic substrate for some infant deaths.

The Ingenuity Pathway Analysis has suggested a potential association between the glucocorticoid biosynthesis signaling pathways and SIDS. Glucocorticoids and mineralocorticoid, such as aldosterone and cortisol, are hormones that regulate numerous physiological processes, including intermediate metabolism, immune function, and cardiovascular function.[55, 56] They are synthesized and released from the adrenal gland in a circadian manner and in response to stress. Nearly 3.5% of the European Caucasian cases had a heterozygote, ultra-rare, non-synonymous variant within either the *CYP17A1* or *CYP11B2* compared with 0.4% of controls. Although the *CYP17A1*-encoded steroid 17-alpha-hydroxylase is involved in the synthesis of cortisol, the *CYP11B2*-encoded aldosterone synthase is involved in the synthesis of aldosterone.[55] Aldosterone, through increased activation of the mineralocorticoid receptor, increases calcium influx in ventricular myocytes, prolongs ventricular repolarization, and leads to triggered cardiac arrhythmias. [57] Additional investigation and replication will be necessary before invoking genetic perturbations in glucocorticoid biosynthesis signaling as a potentially novel pathogenic substrate for SIDS.

From the perspective of ultra-rare genetic variation in the exome, the lack of significant genetic associations suggests an extreme heterogeneity of etiologies underlying SIDS. Until or unless some of the genes identified herein emerge as true SIDS-susceptibility genes with sufficient monogenic penetrance, then monogenic SIDS, stemming from the cardiac channelopathies and other established sudden death predisposing pathways, comprises less than 10% of all SIDS. We conclude that the vast majority of SIDS is not monogenic, at least within the exome. Whether oligo/polygenic models of genetic variation that account for the “vulnerable infant” will emerge remains to be determined.

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LIST OF ABBREVIATIONS

CADD	Combined annotation dependent depletion
CPVT	Catecholaminergic polymorphic ventricular tachycardia
EPACTS	Efficient and parallelizable association container toolbox
ExAC	Exome aggregation consortium
IPA	Ingenuity pathway analysis
LQTS	Long QT syndrome
MAF	Minor allele frequency
SIDS	Sudden infant death syndrome
WES	Whole exome sequencing

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Table 1.

Summary of the Sudden Infant Death Syndrome Cohort Demographics

Demographics		European Ancestry (n=278)
Sex	Male	173 (62.2%)
	Female	105 (37.8%)
Age	Average (months)	2.7 ± 1.98
	Range (months)	0.1 –12
Age Group	< 2 months	81 (29.1%)
	2 – 4 months	154 (55.4%)
	> 4 months	43 (15.5%)
Gender	Male	173 (62.2%)
	Female	105 (37.8%)
sleep position	Supine	85 (30.6%)
	Prone	52 (18.7%)
	Side	29 (10.4%)
	Seated	2 (0.72%)
	Unknown	110 (39.6%)
Co-sleeping	Yes	66 (23.7%)
	No	106 (38.1%)
	Unknown	106 (38.1%)

Table 2.

Summary of 405 Genes with a Higher Prevalence ($p < 0.05$) of Ultra-Rare, Non-Synonymous Variants in Cases Compared to Controls

Number	Gene ID	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
1	<i>LRP1</i>	0.00026851	3.8948	16 (5.8%)	15 (1.5%)
2	<i>LHX9</i>	0.00052687	INF	5 (1.8%)	0
3	<i>PDS5A</i>	0.00065675	21.389	6 (2.2%)	1 (0.1%)
4	<i>ULK1</i>	0.0021371	10.68	6 (2.2%)	2 (0.2%)
5	<i>NR3C2</i>	0.0021371	10.68	6 (2.2%)	2 (0.2%)
6	<i>OR4C6</i>	0.0023978	INF	4 (1.4%)	0
7	<i>CHRM3</i>	0.0023978	INF	4 (1.4%)	0
8	<i>CYB5D1</i>	0.0023978	INF	4 (1.4%)	0
9	<i>GBP2</i>	0.0023978	INF	4 (1.4%)	0
10	<i>ZNF506</i>	0.0023978	INF	4 (1.4%)	0
11	<i>IFRD2</i>	0.0023978	INF	4 (1.4%)	0
12	<i>BICD2</i>	0.0023978	INF	4 (1.4%)	0
13	<i>KDM4DL</i>	0.002584	17.751	5 (1.8%)	1 (0.1%)
14	<i>TNFRSF10D</i>	0.002584	17.751	5 (1.8%)	1 (0.1%)
15	<i>CTIF</i>	0.0035877	6.2452	7 (2.5%)	4 (0.4%)
16	<i>COL20A1</i>	0.0035877	6.2452	7 (2.5%)	4 (0.4%)
17	<i>ARHGEF16</i>	0.0045947	4.7675	8 (2.9%)	6 (0.6%)
18	<i>KIAA1875</i>	0.0051873	4.03	9 (3.2%)	8 (0.8%)
19	<i>CNKSR1</i>	0.0052208	7.1175	6 (2.2%)	3 (0.3%)
20	<i>ABR</i>	0.0052208	7.1175	6 (2.2%)	3 (0.3%)
21	<i>CYP11B2</i>	0.0052208	7.1175	6 (2.2%)	3 (0.3%)
22	<i>MEGF9</i>	0.0052208	7.1175	6 (2.2%)	3 (0.3%)
23	<i>AKAP13</i>	0.00628	2.5831	15 (5.4%)	21 (2.1%)
24	<i>EIF2C4</i>	0.0069909	4.9923	7 (2.5%)	5 (0.5%)
25	<i>ZMYM2</i>	0.0074023	8.8715	5 (1.8%)	2 (0.2%)
26	<i>APOBR</i>	0.0074023	8.8715	5 (1.8%)	2 (0.2%)
27	<i>SEZ6</i>	0.0074023	8.8715	5 (1.8%)	2 (0.2%)
28	<i>ALOX15</i>	0.0074023	8.8715	5 (1.8%)	2 (0.2%)
29	<i>ZNF131</i>	0.0074023	8.8715	5 (1.8%)	2 (0.2%)
30	<i>LPL</i>	0.0074023	8.8715	5 (1.8%)	2 (0.2%)
31	<i>CYP17A1</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
32	<i>MTHFR</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
33	<i>ISG20L2</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
34	<i>SLC22A25</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)

Number	Gene ID	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
35	<i>SELPLG</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
36	<i>PFKM</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
37	<i>OR6C1</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
38	<i>TMEM53</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
39	<i>EME2</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
40	<i>CCDC55</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
41	<i>SUZ12</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
42	<i>RAB40B</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
43	<i>ZSCAN4</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
44	<i>TCP10L</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
45	<i>TMEM131</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
46	<i>MSH3</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
47	<i>RSPH3</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
48	<i>ME1</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
49	<i>SMURF1</i>	0.0098818	14.151	4 (1.4%)	1 (0.1%)
50	<i>CTCI</i>	0.01064	5.3343	6 (2.2%)	4 (0.4%)
51	<i>POLR1B</i>	0.01064	5.3343	6 (2.2%)	4 (0.4%)
52	<i>RTN4</i>	0.01064	5.3343	6 (2.2%)	4 (0.4%)
53	<i>CPB1</i>	0.01064	5.3343	6 (2.2%)	4 (0.4%)
54	<i>VAR5</i>	0.01064	5.3343	6 (2.2%)	4 (0.4%)
55	<i>NCOA2</i>	0.01064	5.3343	6 (2.2%)	4 (0.4%)
56	<i>SORT1</i>	0.010882	INF	3 (1.1%)	0
57	<i>PITPNM1</i>	0.010882	INF	3 (1.1%)	0
58	<i>RNF121</i>	0.010882	INF	3 (1.1%)	0
59	<i>ZNF678</i>	0.010882	INF	3 (1.1%)	0
60	<i>CACNB3</i>	0.010882	INF	3 (1.1%)	0
61	<i>EIF2C1</i>	0.010882	INF	3 (1.1%)	0
62	<i>GDE1</i>	0.010882	INF	3 (1.1%)	0
63	<i>PSKH1</i>	0.010882	INF	3 (1.1%)	0
64	<i>COTL1</i>	0.010882	INF	3 (1.1%)	0
65	<i>NEGR1</i>	0.010882	INF	3 (1.1%)	0
66	<i>GSG2</i>	0.010882	INF	3 (1.1%)	0
67	<i>SCPEP1</i>	0.010882	INF	3 (1.1%)	0
68	<i>FAM73A</i>	0.010882	INF	3 (1.1%)	0
69	<i>C19orf26</i>	0.010882	INF	3 (1.1%)	0
70	<i>DUSP15</i>	0.010882	INF	3 (1.1%)	0
71	<i>TRMT6</i>	0.010882	INF	3 (1.1%)	0
72	<i>RRP7A</i>	0.010882	INF	3 (1.1%)	0

Number	Gene ID	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
73	<i>TEX264</i>	0.010882	INF	3 (1.1%)	0
74	<i>PCBD2</i>	0.010882	INF	3 (1.1%)	0
75	<i>ERGIC1</i>	0.010882	INF	3 (1.1%)	0
76	<i>MIER3</i>	0.010882	INF	3 (1.1%)	0
77	<i>HBP1</i>	0.010882	INF	3 (1.1%)	0
78	<i>CPA1</i>	0.010882	INF	3 (1.1%)	0
79	<i>ZFP41</i>	0.010882	INF	3 (1.1%)	0
80	<i>NRBP2</i>	0.010882	INF	3 (1.1%)	0
81	<i>TRUB2</i>	0.010882	INF	3 (1.1%)	0
82	<i>KIAA0556</i>	0.011783	2.8191	11 (4.0)	14 (1.4%)
83	<i>CHIT1</i>	0.012308	4.1568	7 (2.5%)	6 (0.6%)
84	<i>SLC4A5</i>	0.012308	4.1568	7 (2.5%)	6 (0.6%)
85	<i>SEC31A</i>	0.012308	4.1568	7 (2.5%)	6 (0.6%)
86	<i>TBC1D1</i>	0.012927	3.5691	8 (2.9%)	8 (0.8%)
87	<i>PXDNL</i>	0.012927	3.5691	8 (2.9%)	8 (0.8%)
88	<i>KLHDC7A</i>	0.016178	5.9107	5 (1.8%)	3 (0.3%)
89	<i>ATG2B</i>	0.016178	5.9107	5 (1.8%)	3 (0.3%)
90	<i>THSD4</i>	0.016178	5.9107	5 (1.8%)	3 (0.3%)
91	<i>TMEM143</i>	0.016178	5.9107	5 (1.8%)	3 (0.3%)
92	<i>RRP1B</i>	0.016178	5.9107	5 (1.8%)	3 (0.3%)
93	<i>VPS8</i>	0.016178	5.9107	5 (1.8%)	3 (0.3%)
94	<i>C4orf41</i>	0.016178	5.9107	5 (1.8%)	3 (0.3%)
95	<i>PCDHA4</i>	0.016178	5.9107	5 (1.8%)	3 (0.3%)
96	<i>SH2D4A</i>	0.016178	5.9107	5 (1.8%)	3 (0.3%)
97	<i>FANCC</i>	0.016178	5.9107	5 (1.8%)	3 (0.3%)
98	<i>PEX5</i>	0.019102	4.2641	6 (2.2%)	5 (0.5%)
99	<i>NYNRIN</i>	0.019102	4.2641	6 (2.2%)	5 (0.5%)
100	<i>PTPRM</i>	0.019102	4.2641	6 (2.2%)	5 (0.5%)
101	<i>PLD1</i>	0.019102	4.2641	6 (2.2%)	5 (0.5%)
102	<i>FAM193A</i>	0.019102	4.2641	6 (2.2%)	5 (0.5%)
103	<i>KIAA1731</i>	0.019807	3.1699	8 (2.9%)	9 (0.9%)
104	<i>KDM6B</i>	0.019807	3.1699	8 (2.9%)	9 (0.9%)
105	<i>PRR14L</i>	0.019807	3.1699	8 (2.9%)	9 (0.9%)
106	<i>CPSF1</i>	0.019807	3.1699	8 (2.9%)	9 (0.9%)
107	<i>RNF111</i>	0.020022	3.5597	7 (2.5%)	7 (0.7%)
108	<i>TGS1</i>	0.020022	3.5597	7 (2.5%)	7 (0.7%)
109	<i>MUC17</i>	0.023956	2.076	15 (5.4)	26 (2.6%)
110	<i>KIAA1279</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)

Number	Gene ID	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
111	<i>WDR47</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
112	<i>ANKK1</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
113	<i>AP4B1</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
114	<i>SMTNL1</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
115	<i>CD247</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
116	<i>RBMXL2</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
117	<i>SETD1B</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
118	<i>PIWIL1</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
119	<i>ATN1</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
120	<i>HECTD3</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
121	<i>RIN3</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
122	<i>DAPK2</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
123	<i>C15orf58</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
124	<i>PLCG1</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
125	<i>HIC2</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
126	<i>MTMR14</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
127	<i>KLF3</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
128	<i>AC104650.1</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
129	<i>NUP155</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
130	<i>ADAMTS6</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
131	<i>ZNF618</i>	0.024477	7.0729	4 (1.4%)	2 (0.2%)
132	<i>SPTBN5</i>	0.026701	2.2634	12 (4.3%)	19 (1.9%)
133	<i>SDK2</i>	0.028944	2.8502	8 (2.9%)	10 (1.0%)
134	<i>SART1</i>	0.029874	4.4299	5 (1.8%)	4 (0.4%)
135	<i>SLC29A2</i>	0.029874	4.4299	5 (1.8%)	4 (0.4%)
136	<i>FAM161B</i>	0.029874	4.4299	5 (1.8%)	4 (0.4%)
137	<i>NUP85</i>	0.029874	4.4299	5 (1.8%)	4 (0.4%)
138	<i>LRRN1</i>	0.029874	4.4299	5 (1.8%)	4 (0.4%)
139	<i>PIGG</i>	0.029874	4.4299	5 (1.8%)	4 (0.4%)
140	<i>HELQ</i>	0.029874	4.4299	5 (1.8%)	4 (0.4%)
141	<i>UGT3A1</i>	0.029874	4.4299	5 (1.8%)	4 (0.4%)
142	<i>ABCA3</i>	0.030564	3.1122	7 (2.5%)	8 (0.8%)
143	<i>FNI</i>	0.030564	3.1122	7 (2.5%)	8 (0.8%)
144	<i>AKAP12</i>	0.030564	3.1122	7 (2.5%)	8 (0.8%)
145	<i>KCNH5</i>	0.031213	3.5503	6 (2.2%)	6 (0.6%)
146	<i>NLRP11</i>	0.031213	3.5503	6 (2.2%)	6 (0.6%)
147	<i>TKTL2</i>	0.031213	3.5503	6 (2.2%)	6 (0.6%)
148	<i>PCDHGB1</i>	0.031213	3.5503	6 (2.2%)	6 (0.6%)

Number	Gene ID	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
149	<i>HPSE2</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
150	<i>MPP7</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
151	<i>C11orf92</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
152	<i>PANX3</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
153	<i>LDHC</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
154	<i>OR52R1</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
155	<i>APLNR</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
156	<i>RNPEP</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
157	<i>PPP1R12A</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
158	<i>NUFIP1</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
159	<i>HSP90AA1</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
160	<i>OR4E2</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
161	<i>LTB4R</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
162	<i>DPH2</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
163	<i>OTUD7A</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
164	<i>AC090651.1</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
165	<i>GCOM1</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
166	<i>PARP16</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
167	<i>HAPLN3</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
168	<i>NOMO2</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
169	<i>GLYR1</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
170	<i>SF3B3</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
171	<i>DHRS7B</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
172	<i>ETV4</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
173	<i>SLC16A5</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
174	<i>FAM69C</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
175	<i>IL12RB1</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
176	<i>S1PR4</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
177	<i>ACPT</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
178	<i>IGLON5</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
179	<i>ZNF561</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
180	<i>CPXM1</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
181	<i>SP140</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
182	<i>SP100</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
183	<i>PHF21B</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
184	<i>PROM2</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
185	<i>CEP63</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
186	<i>AADACL2</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)

Number	Gene ID	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
187	<i>MFSD1</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
188	<i>MYD88</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
189	<i>CYB561D2</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
190	<i>OR5AC2</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
191	<i>NFXL1</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
192	<i>AASDH</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
193	<i>CDKL2</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
194	<i>BTNL9</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
195	<i>LACE1</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
196	<i>SNX9</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
197	<i>SLC17A1</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
198	<i>SLC17A3</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
199	<i>KIAA1009</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
200	<i>SERPINE1</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
201	<i>MLXIPL</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
202	<i>ABHD11</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
203	<i>HTRA4</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
204	<i>PHF19</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
205	<i>STRBP</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
206	<i>NR5A1</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
207	<i>FAM69B</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
208	<i>VPS13A</i>	0.036334	10.575	3 (1.1%)	1 (0.1%)
209	<i>ZNF469</i>	0.038845	2.1842	11 (4.0)	18 (1.8%)
210	<i>ZFHX4</i>	0.04373	2.2301	10 (3.6)	16 (1.6%)
211	<i>MYO1E</i>	0.044281	2.7639	7 (2.5%)	9 (0.9%)
212	<i>DIDO1</i>	0.044281	2.7639	7 (2.5%)	9 (0.9%)
213	<i>ATP10B</i>	0.044281	2.7639	7 (2.5%)	9 (0.9%)
214	<i>CTTNBP2NL</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
215	<i>TSSC4</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
216	<i>ASPM</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
217	<i>NT5DC3</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
218	<i>TRPV4</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
219	<i>PAFAH2</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
220	<i>C1orf94</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
221	<i>IPP</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
222	<i>DOC2A</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
223	<i>CHST5</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
224	<i>ADAT1</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)

Number	Gene ID	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
225	<i>RAPGEFL1</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
226	<i>MAP2K6</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
227	<i>ZNF763</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
228	<i>TNPO2</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
229	<i>CC2D1A</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
230	<i>PNKP</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
231	<i>ZNF544</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
232	<i>TM9SF4</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
233	<i>DNMT3B</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
234	<i>KRTAP10-12</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
235	<i>ZNF804A</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
236	<i>IL17RA</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
237	<i>RASGRP3</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
238	<i>INTU</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
239	<i>SEC24A</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
240	<i>AC008661.1</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
241	<i>TRPC7</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
242	<i>PCDHGA12</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
243	<i>PHIP</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
244	<i>ANKRD6</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
245	<i>RBM33</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
246	<i>CALN1</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
247	<i>ELN</i>	0.047244	4.7125	4 (1.4%)	3 (0.3%)
248	<i>RBP3</i>	0.047417	3.0407	6 (2.2%)	7 (0.7%)
249	<i>FARP1</i>	0.047417	3.0407	6 (2.2%)	7 (0.7%)
250	<i>GREB1L</i>	0.047417	3.0407	6 (2.2%)	7 (0.7%)
251	<i>MOV10L1</i>	0.047417	3.0407	6 (2.2%)	7 (0.7%)
252	<i>FRMD4B</i>	0.047417	3.0407	6 (2.2%)	7 (0.7%)
253	<i>ADAMTS14</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
254	<i>NCAPD3</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
255	<i>CGN</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
256	<i>CNTN1</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
257	<i>LTA4H</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
258	<i>SH3D21</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
259	<i>ZNF785</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
260	<i>ABCA9</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
261	<i>SERPINB8</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
262	<i>MYO1F</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)

Number	Gene ID	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
263	<i>MMP9</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
264	<i>NRIP1</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
265	<i>AOX1</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
266	<i>GIGYF2</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
267	<i>YTHDC2</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
268	<i>HSPA4</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
269	<i>DMGDH</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
270	<i>GPR116</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
271	<i>KIAA0368</i>	0.049108	3.5409	5 (1.8%)	5 (0.5%)
272	<i>AKAP9</i>	0.049138	2.29	9 (3.2%)	14 (1.4%)
273	<i>SMC3</i>	0.049244	INF	2 (0.7%)	0
274	<i>SHOC2</i>	0.049244	INF	2 (0.7%)	0
275	<i>PSTK</i>	0.049244	INF	2 (0.7%)	0
276	<i>FRMD4A</i>	0.049244	INF	2 (0.7%)	0
277	<i>ZNF22</i>	0.049244	INF	2 (0.7%)	0
278	<i>PAPSS2</i>	0.049244	INF	2 (0.7%)	0
279	<i>CASP1</i>	0.049244	INF	2 (0.7%)	0
280	<i>ADAMTS8</i>	0.049244	INF	2 (0.7%)	0
281	<i>FCGR1B</i>	0.049244	INF	2 (0.7%)	0
282	<i>TDRKH</i>	0.049244	INF	2 (0.7%)	0
283	<i>RORC</i>	0.049244	INF	2 (0.7%)	0
284	<i>TRIM46</i>	0.049244	INF	2 (0.7%)	0
285	<i>CCT3</i>	0.049244	INF	2 (0.7%)	0
286	<i>OR5B2</i>	0.049244	INF	2 (0.7%)	0
287	<i>CRP</i>	0.049244	INF	2 (0.7%)	0
288	<i>CCDC86</i>	0.049244	INF	2 (0.7%)	0
289	<i>SCGB2A1</i>	0.049244	INF	2 (0.7%)	0
290	<i>KCNK4</i>	0.049244	INF	2 (0.7%)	0
291	<i>C11orf24</i>	0.049244	INF	2 (0.7%)	0
292	<i>P2RY2</i>	0.049244	INF	2 (0.7%)	0
293	<i>OLFML1</i>	0.049244	INF	2 (0.7%)	0
294	<i>RGS1</i>	0.049244	INF	2 (0.7%)	0
295	<i>KLRC2</i>	0.049244	INF	2 (0.7%)	0
296	<i>RP11-277P12.6</i>	0.049244	INF	2 (0.7%)	0
297	<i>C12orf24</i>	0.049244	INF	2 (0.7%)	0
298	<i>SLC30A1</i>	0.049244	INF	2 (0.7%)	0
299	<i>IFLTD1</i>	0.049244	INF	2 (0.7%)	0
300	<i>PTHLH</i>	0.049244	INF	2 (0.7%)	0

Number	Gene ID	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
301	<i>AMN1</i>	0.049244	INF	2 (0.7%)	0
302	<i>SLC6A13</i>	0.049244	INF	2 (0.7%)	0
303	<i>TRIM58</i>	0.049244	INF	2 (0.7%)	0
304	<i>ARF3</i>	0.049244	INF	2 (0.7%)	0
305	<i>C12orf44</i>	0.049244	INF	2 (0.7%)	0
306	<i>TBK1</i>	0.049244	INF	2 (0.7%)	0
307	<i>TFDP1</i>	0.049244	INF	2 (0.7%)	0
308	<i>SCEL</i>	0.049244	INF	2 (0.7%)	0
309	<i>MFSD2A</i>	0.049244	INF	2 (0.7%)	0
310	<i>EDDM3B</i>	0.049244	INF	2 (0.7%)	0
311	<i>SLC39A2</i>	0.049244	INF	2 (0.7%)	0
312	<i>PPP2R3C</i>	0.049244	INF	2 (0.7%)	0
313	<i>ENTPD5</i>	0.049244	INF	2 (0.7%)	0
314	<i>ISM2</i>	0.049244	INF	2 (0.7%)	0
315	<i>TCL1B</i>	0.049244	INF	2 (0.7%)	0
316	<i>AVEN</i>	0.049244	INF	2 (0.7%)	0
317	<i>JMID7-PLA2G4B</i>	0.049244	INF	2 (0.7%)	0
318	<i>MAP2K5</i>	0.049244	INF	2 (0.7%)	0
319	<i>CLN6</i>	0.049244	INF	2 (0.7%)	0
320	<i>MORF4L1</i>	0.049244	INF	2 (0.7%)	0
321	<i>HES3</i>	0.049244	INF	2 (0.7%)	0
322	<i>ANKS3</i>	0.049244	INF	2 (0.7%)	0
323	<i>TERF2</i>	0.049244	INF	2 (0.7%)	0
324	<i>TP53I13</i>	0.049244	INF	2 (0.7%)	0
325	<i>MSL1</i>	0.049244	INF	2 (0.7%)	0
326	<i>CNP</i>	0.049244	INF	2 (0.7%)	0
327	<i>KCNJ2</i>	0.049244	INF	2 (0.7%)	0
328	<i>NXN</i>	0.049244	INF	2 (0.7%)	0
329	<i>SOX15</i>	0.049244	INF	2 (0.7%)	0
330	<i>PYCR1</i>	0.049244	INF	2 (0.7%)	0
331	<i>SLC25A35</i>	0.049244	INF	2 (0.7%)	0
332	<i>ELTD1</i>	0.049244	INF	2 (0.7%)	0
333	<i>PIAS2</i>	0.049244	INF	2 (0.7%)	0
334	<i>FBXO15</i>	0.049244	INF	2 (0.7%)	0
335	<i>VAPA</i>	0.049244	INF	2 (0.7%)	0
336	<i>ZNF326</i>	0.049244	INF	2 (0.7%)	0
337	<i>CNN1</i>	0.049244	INF	2 (0.7%)	0
338	<i>GAMT</i>	0.049244	INF	2 (0.7%)	0

Number	Gene ID	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
339	<i>TECR</i>	0.049244	INF	2 (0.7%)	0
340	<i>APIM1</i>	0.049244	INF	2 (0.7%)	0
341	<i>LGALS14</i>	0.049244	INF	2 (0.7%)	0
342	<i>NTN5</i>	0.049244	INF	2 (0.7%)	0
343	<i>HSD11B1L</i>	0.049244	INF	2 (0.7%)	0
344	<i>ZIM3</i>	0.049244	INF	2 (0.7%)	0
345	<i>CENPB</i>	0.049244	INF	2 (0.7%)	0
346	<i>SRSF6</i>	0.049244	INF	2 (0.7%)	0
347	<i>TNFRSF6B</i>	0.049244	INF	2 (0.7%)	0
348	<i>C21orf91</i>	0.049244	INF	2 (0.7%)	0
349	<i>MRPL39</i>	0.049244	INF	2 (0.7%)	0
350	<i>TNFAIP6</i>	0.049244	INF	2 (0.7%)	0
351	<i>HOXD1</i>	0.049244	INF	2 (0.7%)	0
352	<i>SGOL2</i>	0.049244	INF	2 (0.7%)	0
353	<i>SCG2</i>	0.049244	INF	2 (0.7%)	0
354	<i>TIMP3</i>	0.049244	INF	2 (0.7%)	0
355	<i>AQP12B</i>	0.049244	INF	2 (0.7%)	0
356	<i>NRBP1</i>	0.049244	INF	2 (0.7%)	0
357	<i>HHLA2</i>	0.049244	INF	2 (0.7%)	0
358	<i>MORC1</i>	0.049244	INF	2 (0.7%)	0
359	<i>ABHD10</i>	0.049244	INF	2 (0.7%)	0
360	<i>CD200</i>	0.049244	INF	2 (0.7%)	0
361	<i>ZNF639</i>	0.049244	INF	2 (0.7%)	0
362	<i>AC022498.1</i>	0.049244	INF	2 (0.7%)	0
363	<i>ZDHHC19</i>	0.049244	INF	2 (0.7%)	0
364	<i>FHIT</i>	0.049244	INF	2 (0.7%)	0
365	<i>SNTN</i>	0.049244	INF	2 (0.7%)	0
366	<i>ARL6IP5</i>	0.049244	INF	2 (0.7%)	0
367	<i>TTC29</i>	0.049244	INF	2 (0.7%)	0
368	<i>STOX2</i>	0.049244	INF	2 (0.7%)	0
369	<i>KCNIP4</i>	0.049244	INF	2 (0.7%)	0
370	<i>TMPRSS11BNL</i>	0.049244	INF	2 (0.7%)	0
371	<i>CXCL6</i>	0.049244	INF	2 (0.7%)	0
372	<i>ROPN1L</i>	0.049244	INF	2 (0.7%)	0
373	<i>DAP</i>	0.049244	INF	2 (0.7%)	0
374	<i>RELL2</i>	0.049244	INF	2 (0.7%)	0
375	<i>NOP16</i>	0.049244	INF	2 (0.7%)	0
376	<i>RADI</i>	0.049244	INF	2 (0.7%)	0

Number	Gene ID	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
377	<i>LMBRD2</i>	0.049244	INF	2 (0.7%)	0
378	<i>GZMA</i>	0.049244	INF	2 (0.7%)	0
379	<i>SREK1</i>	0.049244	INF	2 (0.7%)	0
380	<i>TAS2R1</i>	0.049244	INF	2 (0.7%)	0
381	<i>C6orf52</i>	0.049244	INF	2 (0.7%)	0
382	<i>NRSN1</i>	0.049244	INF	2 (0.7%)	0
383	<i>HIST1H2AE</i>	0.049244	INF	2 (0.7%)	0
384	<i>DAXX</i>	0.049244	INF	2 (0.7%)	0
385	<i>RRP36</i>	0.049244	INF	2 (0.7%)	0
386	<i>CRISP3</i>	0.049244	INF	2 (0.7%)	0
387	<i>LY86</i>	0.049244	INF	2 (0.7%)	0
388	<i>WNT2</i>	0.049244	INF	2 (0.7%)	0
389	<i>OPN1SW</i>	0.049244	INF	2 (0.7%)	0
390	<i>STRA8</i>	0.049244	INF	2 (0.7%)	0
391	<i>GIMAP8</i>	0.049244	INF	2 (0.7%)	0
392	<i>TAX1BP1</i>	0.049244	INF	2 (0.7%)	0
393	<i>STX1A</i>	0.049244	INF	2 (0.7%)	0
394	<i>SGCE</i>	0.049244	INF	2 (0.7%)	0
395	<i>CYP3A5</i>	0.049244	INF	2 (0.7%)	0
396	<i>DEFB135</i>	0.049244	INF	2 (0.7%)	0
397	<i>EFHA2</i>	0.049244	INF	2 (0.7%)	0
398	<i>ADRA1A</i>	0.049244	INF	2 (0.7%)	0
399	<i>CRISPLD1</i>	0.049244	INF	2 (0.7%)	0
400	<i>DPY19L4</i>	0.049244	INF	2 (0.7%)	0
401	<i>MRPL50</i>	0.049244	INF	2 (0.7%)	0
402	<i>C9orf150</i>	0.049244	INF	2 (0.7%)	0
403	<i>C9orf78</i>	0.049244	INF	2 (0.7%)	0
404	<i>GALT</i>	0.049244	INF	2 (0.7%)	0
405	<i>CDC37L1</i>	0.049244	INF	2 (0.7%)	0

INF = infinite

Table 3.
Summary of Genes with an Over-Representation of Ultra-Rare Variants with a $MAF < 0.00005$

Gene ID	Gene/Protein Name	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
<i>LRP1</i>	LDL Receptor Related Protein 1	0.000269	3.8948	16 (5.8%)	15 (1.5%)
<i>LHX9</i>	LIM Homeobox 9	0.000527	INF	5 (1.8%)	0
<i>PDS5A</i>	PDS5 Cohesin Associated Factor A	0.000657	21.389	6 (2.2%)	1 (0.1%)
<i>ULK1</i>	Unc-51 Like Autophagy Activating Kinase 1	0.002137	10.68	6 (2.2%)	2 (0.21%)
<i>NR3C2</i>	Nuclear Receptor Subfamily 3 Group C Member 2	0.002137	10.68	6 (2.2%)	2 (0.21%)
<i>OR4C6</i>	Olfactory Receptor Family 4 Subfamily C Member 6	0.002398	INF	4 (1.4%)	0
<i>CHRM3</i>	Cholinergic Receptor Muscarinic 3	0.002398	INF	4 (1.4%)	0
<i>CYB5D1</i>	Cytochrome B5 Domain Containing 1	0.002398	INF	4 (1.4%)	0
<i>GBP2</i>	Guanylate Binding Protein 2	0.002398	INF	4 (1.4%)	0
<i>ZNF506</i>	Zinc Finger Protein 506	0.002398	INF	4 (1.4%)	0
<i>IFRD2</i>	Interferon-Related Developmental Regulator 2	0.002398	INF	4 (1.4%)	0
<i>BICD2</i>	BICD Cargo Adaptor 2	0.002398	INF	4 (1.4%)	0
<i>KDM4DL</i>	Lysine Demethylase 4E	0.002584	17.751	5 (1.8%)	1 (0.1%)
<i>TNFRSF10D</i>	Tumor Necrosis Factor Receptor Superfamily Member 10d	0.002584	17.751	5 (1.8%)	1 (0.1%)
<i>CTIF</i>	CBP80/20-Dependent Translation Initiation Factor	0.003588	6.2452	7 (2.5%)	4 (0.4%)
<i>COL20A1</i>	Collagen Type XX Alpha 1	0.003588	6.2452	7 (2.5%)	4 (0.4%)
<i>ARHGEF16</i>	Rho Guanine Nucleotide Exchange Factor 16	0.004595	4.7675	8 (2.9%)	6 (0.62%)

Genes are listed in ascending order by their p-value. INF = infinite.

Summary of Genes with an Over-Representation of Ultra-Rare Variants with a $MAF < 0.00005$

Table 4.

Gene ID	Gene/Protein Name	OMIM Disease Associations	PathCards SuperPathways	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
<i>LRP1</i>	LDL Receptor Related Protein 1	1) Association with Alzheimer disease 2) Association with Abdominal Aortic Aneurysm 3) Intellectual Disability	1) Statin Pathway 2) A-beta Pathways: Uptake and Degradation 3) Alzheimer's Disease Pathway 4) Malaria 5) PDGFR-beta signaling pathway 6) amb2 Integrin signaling 7) Binding and Uptake of Ligands by Scavenger Receptors 8) Blood-Brain Barrier and Immune Cell Transmigration: Pathways Overview 9) Metabolism of fat-soluble vitamins 10) Non-Canonical Wnt Pathway 11) Metabolism of water-soluble vitamins and cofactors 12) Wnt signaling pathway (KEGG) 13) Alzheimer's disease 14) Vesicle-mediated transport 15) Signaling by GPCR 16) Metabolism	0.000269	3.8948	16 (5.8%)	15 (1.5%)
<i>LHX9</i>	LIM Homeobox 9	None	None	0.000527	INF	5 (1.8%)	0
<i>PDS5A</i>	PDS5 Cohesin Associated Factor A	None	None	0.000657	21.389	6 (2.2%)	1 (0.1%)
<i>ULK1</i>	Unc-51 Like Autophagy Activating Kinase 1	None	1) Regulation of autophagy 2) Senescence and Autophagy 3) Longevity regulating pathway - multiple species 4) AMPK signaling pathway 5) p53 Pathway (RnD) 6) mTOR signalling 7) Glucose / Energy Metabolism 8) Neuroscience 9) Translation Insulin regulation of translation 10) Cellular Senescence	0.002137	10.68	6 (2.2%)	2 (0.21%)
<i>NR3C2</i>	Nuclear Receptor Subfamily 3 Group C Member 2	1) Pseudohypoaldosteronism Type I, Autosomal Dominant 2) Hypertension, Early-Onset, Autosomal Dominant, with Severe Exacerbation in Pregnancy	1) Aldosterone-regulated sodium reabsorption 2) Agents Acting on the Renin-Angiotensin System Pathway, Pharmacodynamics 3) Nuclear Receptor transcription pathway 4) Gene Expression	0.002137	10.68	6 (2.2%)	2 (0.21%)
<i>OR4C6</i>	Olfactory Receptor Family 4 Subfamily C Member 6	None	1) Olfactory Signaling Pathway 2) Signaling by GPCR	0.002398	INF	4 (1.4%)	0
<i>CHRM3</i>	Cholinergic Receptor Muscarinic 3	1) Pume Belly Syndrome (autosomal recessive)	1) Proton Pump Inhibitor Pathway, Pharmacodynamics 2) Monoamine GPCRs	0.002398	INF	4 (1.4%)	0

Gene ID	Gene/Protein Name	OMIM Disease Associations	PathCards SuperPathways	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
			3) GPCRs, Other 4) Taste transduction 5) Pancreatic secretion 6) Integration of energy metabolism 7) Salivary secretion 8) Insulin secretion 9) Myometrial Relaxation and Contraction Pathways 10) Calcium signaling pathway 11) Regulation of actin cytoskeleton 12) Circadian entrainment 13) Peptide ligand-binding receptors 14) Interleukin-3, 5 and GM-CSF signaling 15) Signaling by GPCR 16) Metabolism				
<i>CYB5D1</i>	Cytochrome B5 Domain Containing 1	None	None	0.002398	INF	4 (1.4%)	0
<i>GBP2</i>	Guanylate Binding Protein 2	None	1) Immune response IFN alpha/beta signaling pathway 2) Interferon gamma signaling 3) Interleukin-3, 5 and GM-CSF signaling 4) Immune System	0.002398	INF	4 (1.4%)	0
<i>ZNF506</i>	Zinc Finger Protein 506	None	1) Gene Expression	0.002398	INF	4 (1.4%)	0
<i>IFRD2</i>	Interferon-Related Developmental Regulator 2	None	None	0.002398	INF	4 (1.4%)	0
<i>BICD2</i>	BICD Cargo Adaptor 2	1) Autosomal dominant lower extremity-predominant spinal muscular atrophy-2	1) COP1-independent Golgi-to-ER retrograde traffic 2) Golgi-to-ER retrograde transport 3) Vesicle-mediated transport	0.002398	INF	4 (1.4%)	0
<i>KDM4DL</i>	Lysine Demethylase 4E	None	None	0.002584	17.751	5 (1.8%)	1 (0.1%)
<i>TNFRSF10D</i>	Tumor Necrosis Factor Receptor Superfamily Member 10d	None	None	0.002584	17.751	5 (1.8%)	1 (0.1%)
<i>CTTF</i>	CBP80/20-Dependent Translation Initiation Factor	None	None	0.003588	6.2452	7 (2.5%)	4 (0.4%)
<i>COL20A1</i>	Collagen Type XX Alpha 1	None	1) Collagen biosynthesis and modifying enzymes 2) Degradation of the extracellular matrix 3) Integrin Pathway 4) Phospholipase-C Pathway 5) ERK Signaling	0.003588	6.2452	7 (2.5%)	4 (0.4%)
<i>ARHGEF16</i>	Rho Guanine Nucleotide Exchange Factor 16	None	1) NgR-p75(NTR)-Mediated Signaling 2) Signaling by Slit 3) Interferon Pathway 4) p75 NTR receptor-mediated signalling	0.004595	4.7675	8 (2.9%)	6 (0.62%)

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Gene ID	Gene/Protein Name	OMIM Disease Associations	PathCards SuperPathways	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
			5) Guidance Cues and Growth Cone Motility 6) G-AlphaQ Signaling 7) RhoGDI Pathway 8) fMLP Pathway 9) Actin Nucleation by ARP-WASP Complex 10) Signaling by Rho GTPases 11) TGF-Beta Pathway 12) Phospholipase-C Pathway 13) GPCR Pathway 14) Apoptotic Pathways in Synovial Fibroblasts 15) Interleukin-3, 5 and GM-CSF signaling 16) ERK Signaling 17) Signaling by GPCR				

INF = infinite

Summary of Genes with an Over-Representation of Ultra-Rare Variants with a $MAF < 0.00005$ and a CADD Score > 20

Table 5.

Gene ID	Gene/Protein Name	OMIM Disease Associations	PathCards SuperPathways	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
<i>PTPRM</i>	Protein Tyrosine Phosphatase, Receptor Type M	None	1) Nectin adhesion pathway 2) Cell adhesion_Cadherin-mediated cell adhesion 3) Adherens junction 4) Adhesion 5) Cell adhesion molecules (CAMs) 6) PAK Pathway	0.000657	21.389	6 (2.2%)	1 (0.1%)
<i>CNKSR1</i>	Connector Enhancer Of Kinase Suppressor Of Ras 1	None	1) MAP2K and MAPK activation 2) Ceramide Pathway 3) Toll-like receptor Signaling Pathways 4) ERK Signaling 5) Developmental Biology 6) Interleukin-3, 5 and GM-CSF signaling 7) Immune System 8) Signaling by GPCR	0.002137	10.68	6 (2.2%)	2 (0.21%)
<i>KDM6B</i>	Lysine Demethylase 6B	None	1) Chromatin Regulation / Acetylation 2) Chromatin organization 3) Cellular Senescence 4) Activated PKN1 stimulates transcription of AR (androgen receptor) regulated genes KLK2 and KLK3	0.002137	10.68	6 (2.2%)	2 (0.21%)
<i>NR3C2</i>	Nuclear Receptor Subfamily 3 Group C Member 2	1) Pseudohypoadosteronism Type I, Autosomal Dominant 2) Hypertension, Early-Onset, Autosomal Dominant, with Severe Exacerbation in Pregnancy	1) Aldosterone-regulated sodium reabsorption 2) Agents Acting on the Renin-Angiotensin System Pathway, Pharmacodynamics 3) Nuclear Receptor transcription pathway 4) Gene Expression	0.002137	10.68	6 (2.2%)	2 (0.21%)
<i>ABCA2</i>	ATP Binding Cassette Subfamily A Member 2	1) Alzheimer Disease	1) ABC-family proteins mediated transport 2) Lysosome 3) Transport of glucose and other sugars, bile salts and organic acids, metal ions and amine compounds	0.002137	10.68	6 (2.2%)	2 (0.21%)
<i>LHX9</i>	LIM Homeobox 9	None	None	0.002398	INF	4 (1.4%)	0
<i>ABR</i>	Active BCR-Related	none	1) G-protein signaling_Rac2 regulation pathway 2) Regulation of RAC1 activity 3) G-protein signaling_Regulation of CDC42 activity 4) G-protein signaling_Regulation of RAC1 activity 5) Regulation of RhoA activity 6) p75 NTR receptor-mediated signalling 7) Signaling by Rho GTPases 8) Interleukin-3, 5 and GM-CSF signaling 9) Signaling by GPCR	0.002398	INF	4 (1.4%)	0

Gene ID	Gene/Protein Name	OMIM Disease Associations	PathCards SuperPathways	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
<i>ERGIC1</i>	Endoplasmic Reticulum-Golgi Intermediate Compartment 1	None	None	0.002398	INF	4 (1.4%)	0
<i>DMGDH</i>	Dimethylglycine Dehydrogenase	1) Dimethylglycine Dehydrogenase Deficiency Inborn Error of Metabolism	1) Glycine, serine and threonine metabolism 2) Glycerophospholipid biosynthesis 3) Metabolism	0.002584	17.751	5 (1.8%)	1 (0.1%)

Table 6.

Ingenuity® Pathway Analysis – Top Canonical Pathway Genes

Pathway	IPA derived pathway level pValue	IPA Overlap (%)	Gene Symbol	Entrez Gene Name	pValue	Odds Ratio (OR)	Number of Variant Positive Cases (n=278)	Number of Variant Positive Controls (n=973)
Glucocorticoid Biosynthesis	0.0107	2/8 (25)	<i>CYP11B2</i>	cytochrome P450 family 11 subfamily B member 2	0.005221	7.1175	6 (2.2%)	3 (0.3%)
			<i>CYP17A1</i>	cytochrome P450 family 17 subfamily A member 1	0.009882	14.151	4 (1.4%)	1 (0.1%)
Melatonin Signaling	0.0133	5/69 (7.2%)	<i>MAP2K5</i>	mitogen-activated protein kinase kinase 5	0.049244	INF	2 (0.7%)	0
			<i>MAP2K6</i>	mitogen-activated protein kinase kinase 6	0.047244	4.7125	4 (1.4%)	3 (0.3%)
			<i>OPN1SW</i>	opsin 1 (cone pigments), short-wave-sensitive	0.049244	INF	2 (0.7%)	0
			<i>PLCG1</i>	phospholipase C gamma 1	0.024477	7.0729	4 (1.4%)	2 (0.2%)
			<i>RORC</i>	RAR related orphan receptor C	0.049244	INF	2 (0.7%)	0
Role of IL-17A in Psoriasis	0.0279	2/13 (15.4%)	<i>CXCL6</i>	C-X-C motif chemokine ligand 6	0.049244	INF	2 (0.7%)	0
			<i>IL17RA</i>	interleukin 17 receptor A	0.047244	4.7125	4 (1.4%)	3 (0.3%)
Sulfate Activation for Sulfonation	0.0404	1/2 (50%)	<i>PAPSS2</i>	3'-phosphoadenosine 5'-phosphosulfate synthase 2	0.049244	INF	2 (0.7%)	0
Glycine Degradation (Creatine Biosynthesis)	0.0404	1/2 (50%)	<i>GAMT</i>	guanidinoacetate N-methyltransferase	0.049244	INF	2 (0.7%)	0

INF=infinite