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Transmission disequilibrium analysis of whole genome data in childhood-onset systemic lupus erythematosus

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Abstract

Childhood-onset systemic lupus erythematosus (cSLE) patients are unique, with hallmarks of Mendelian disorders (early-onset and severe disease) and thus are an ideal population for genetic investigation of SLE. In this study, we use the transmission disequilibrium test (TDT),

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AUTHOR CONTRIBUTIONS
LBL, SH, and MK designed the clinical protocol. CD performed the sequencing protocol. LBL, KV, AM, and JEBW designed the research methodology. ZD, LBL and AM performed the data analysis. LBL, KV, and AM wrote the manuscript. KV, LH, CS, AB, ZD, CD, JEBW, AM, SH, MK and LBL reviewed and revised the full manuscript.

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a family-based genetic association analysis that employs robust methodology, to analyze whole genome sequencing data. We aim to identify novel genetic associations in an ancestrally diverse, international cSLE cohort. Forty-two cSLE patients and 84 unaffected parents from 3 countries underwent whole genome sequencing. First, we performed TDT with single nucleotide variant (SNV)-based (common variants) using PLINK 1.9, and gene-based (rare variants) analyses using Efficient and Parallelizable Association Container Toolbox (EPACTS) and rare variant TDT (rvTDT), which applies multiple gene-based burden tests adapted for TDT, including the burden of rare variants test. Applying the GWAS standard threshold (5.0×10^{-8}) to common variants, our SNV-based analysis did not return any genome-wide significant SNVs. The rare variant gene-based TDT analysis identified many novel genes significantly enriched in cSLE patients, including *HNRNPUL2*, a DNA repair protein, and *DNAH11*, a ciliary movement protein, among others. Our approach identifies several novel SLE susceptibility genes in an ancestrally diverse childhood-onset lupus cohort.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic autoimmune disorder characterized by episodic flares, significant morbidity, and higher mortality than age-matched peers. Patients with childhood-onset SLE (cSLE) have even more severe disease than those with adult-onset, with a higher prevalence of serious manifestations, such as lupus nephritis and neuropsychiatric SLE [1–3]. The prevalence of cSLE ranges from 0.28–2.5/100,000 person-years, varies highly based on sex, race, and cohort location, and is higher in non-White populations [4]. As cSLE patients have aggressive disease with early-onset, genetic investigations targeting this population could lead to important insights into SLE pathogenesis [4–9]. Yet, the majority of genetic studies of SLE to date have focused on adult-onset SLE.

While the cause of SLE has yet to be fully understood, numerous studies of SLE have demonstrated a strong genetic link [10, 11]. The vast majority of genetic studies to date have been genome wide association studies (GWAS) of adult-onset SLE populations, but few GWAS in cSLE have been performed [12]. In the GWAS conducted in adult-onset SLE, variants in the human leukocyte antigen (HLA) region have the most significant association with SLE [13], and have been identified in all ancestral populations studied to date [14].

Both rare and common variants are important in cSLE pathogenesis [15, 16], although the true genetic architecture of cSLE has not been rigorously studied [17]. Monogenic forms of cSLE tend to present at an early age. These include well-established rare variants such as homozygous complement defects, and more recently described monogenic risk genes such as variants in *TREX1*, *DNASEIL3 and TLR7* [18–21]. However, genetic testing for cSLE has largely been limited to targeted testing for a specific gene (i.e. homozygous complement deficiency) or next generation sequencing performed on a small subset of patients with extremely early-onset and uncommon manifestations [22, 23]. Next generation sequencing, including whole exome and whole genome sequencing of a more widely inclusive sampling of cSLE patients may continue to yield important insights.

The transmission disequilibrium test (TDT) is a family-based genetic association test performed on trios (patient and two parents) first established in 1993 [24]. This test determines the allelic transmission from a heterozygous parent to an affected offspring. TDT is often more feasible for childhood-onset diseases because both parents are more likely to be available for genetic testing [25]. Many genetic studies in SLE are confounded by population stratification, where frequencies of many genetic variants that are not associated with disease risk differ between ancestral populations, and those ancestries may differ between cases and controls [26]. These allele frequency differences can lead to false positive results [27]. TDT is performed within families and therefore is more robust to confounding by population heterogeneity substructures or admixture [28–30]. Furthermore, trio-based TDT is powered to detect associations over a broad range of allele frequencies [28]. TDT has been used historically to test single genes or a set of genes of interest and has been successfully used to identify candidate genes in SLE [31, 32]. Although TDT is a powerful and well-established method to identify variants across a wide range of allele frequencies in SLE, cost and accessibility have limited use for whole genome sequence data for cSLE to date.

Ultra-rare (minor allele frequency (MAF) < 0.01), or private rare variants, have been projected to provide a large contribution to the genetic heritability of disease [33]. Rare and private variation may contribute to some of the missing heritability previously described in GWAS and other genetic studies. To address this, we not only assessed individual single nucleotide variants, but also combined different rare variants in the same gene in unrelated cSLE patients using burden testing and rare variant TDT methodology to detect genes associated with cSLE risk. This approach enables us to detect the effect on disease risk of two or more different rare variants in one gene.

We have a diverse international cohort of cSLE patients from the USA, Canada, and South Africa. With the dual approaches of rare and common variant analyses, our aim was to leverage the advantages of the family-based approach of TDT analyzing whole genome data to identify novel genetic links to cSLE.

METHODS

All SLE subjects met at least 4 of 11 revised American College of Rheumatology classification SLE criteria. All participants were cSLE subjects, with disease onset prior to age 18 [34]. Subjects were from Canada, the United Sates, and South Africa. Subject trios were restricted to an affected subject with cSLE and two unaffected parents; only complete trios were included. All participants and/or legal guardians provided informed consent. Clinical and demographic information were entered at the time of enrolment into the genetic study at each site. Clinical and serologic features were entered if positive at diagnosis or any time in the period up to study enrolment during disease course. We captured the following demographic and clinical features in Table 1: age at SLE diagnosis, positive antinuclear antibody test (ANA) (defined as ANA titer > 1:80), anti-double stranded DNA antibody (anti-dsDNA), biopsy proven lupus nephritis (LN), central nervous system SLE (CNS SLE), arthritis, and disease activity score at enrolment (as demonstrated by SLE Disease Activity Index-2K (SLEDAI-2K [35])). Genomic DNA was extracted from whole blood samples (Qiagen Gentra Puregene, USA) and underwent whole genome sequencing via Illumina HiSeq X Ten. The samples had a mean depth coverage of 36x (range 31–40x). After initial quality control with FASTQC, sequences were aligned to human reference sequence library (GRCh37) using Burrows-Wheeler Alignment [36]. Post-alignment quality control was performed with Picard software and analysis ready binary alignment map files were processed jointly using a Genome Analysis Toolkit (GATK) pipeline [37]. We used PLINK 1.9 to generate family pedigree files from resulting variant call files [38].

Patient ancestry was determined using PCAir PCA analysis from GENESis software package in R [39, 40]. Patient ancestry was compared to ancestral groups in publicly available HapMap 3 datasets [41]. ADMIXTURE software (v1.3.0) was used to estimate proportions of ancestral groups within the participants [42]. When an individual displayed an ancestral proportion equal to or greater than 80%, they were classified into that ancestral group, while those who did not were classified as ‘admixed’. The admixed ancestral groups were pooled for analysis of participant ancestry. Participants were thereby stratified into six ancestral groups: African, East Asian, European, South Asian, Amerindian, and Admixed.

TDT was performed to assess for association of each variant allele with cSLE of those alleles from the parents with heterozygous genotypes. TDT compares the rate of transmission of an associated marker allele from the heterozygous parent to an affected offspring compared to its rate of non-transmission $(b - c)^2/(b + c)$, where b is the number of transmissions of the first allele to affected offspring from heterozygous parents, and c is the count of non-transmissions of this same allele to affected offspring from heterozygous parents (i.e. where the second allele was transmitted to the affected offspring from the heterozygous parents). The allelic transmission is compared to the expected transmission rate for each allele at meiosis [24]. TDT was performed via two methods: single nucleotide variant (SNV)-based and gene-based analyses. SNV-based TDT for common variants was performed using PLINK 1.9 and gene-based analysis for rare variants was performed using rvTDT [43]. This study was approved by the Institutional Board Review at the National Institutes of Health, the Ethics committee at the University of Cape Town, and the Institutional Research Ethics Board of the University of Toronto.

SNV-based TDT analysis for common variants

We tested SNVs with threshold MAF > 0.05, allele counts >5 in our cohort, and filtered out intergenic SNVs, which resulted in 3 182 149 SNVs tested. We performed standard SNV-based TDT analysis using PLINK 1.9 [38]. We used the standard GWAS significance threshold of $p \leq 5.0 \times 10^{-8}$ to identify genome-wide significant common variants associated with cSLE in our cohort, correcting for approximate independent common variants [44, 45].

Gene-based analysis for rare variants

In addition to testing each SNV, we also performed gene-based TDT to increase the power to identify genes based on burden of rare variants. Gene-based analyses collapse all rare variants located within a single region (here defined as a gene) into a single gene-based marker. Standard association analysis can then be performed on this new gene-based marker.

We assigned variants to a gene using Efficient and Parallelizable Association Container Toolbox (EPACTS) ; meaning that each variant was assigned to a gene or intergenic region between two genes [46]. Data was phased using PLINK for all rare exonic SNVs (MAF < 0.05, exonic regions). Gene-based TDT was then performed using rvTDT. rvTDT performs multiple tests of gene-based association, including derivations of the popular combined multivariate and collapsing (CMC) method, burden of rare variant (BRV) test and the variable threshold (VT) tests [43, 47–49]. Here, we report the results of the burden of VT-BRV-Haplo test. BRV is a burden style test that counts the number of transmitted alleles from parent to child within a specified region (gene) [43]. Haplotype permutation is used to control for variants in linkage disequilibrium (LD) which are transmitted together. Variable threshold testing (VT) statistical significance is maximized over the various MAF allele frequencies, and allows for variants within a gene to act in both directions (protective and deleterious) [49, 50]. We assess statistical significance using the haplotype permutation as the test statistic. Empirical permutation was conducted to correct for multiple testing, and the significance level used 1×10^{-5}. The p-values reported in Tables 2, 3 and Supplementary Table 1 are adjusted p-values based on the empirical permutation, rounded to 4 decimal places, with the adjusted p-value of 0.05 being genome-wide significant.

RESULTS

Our study included 42 trios of cSLE patients. Seventy-six percent of subjects with cSLE were female, consistent with other cSLE studies which demonstrate strong female predisposition of disease. The median age at SLE diagnosis was 14 years (IQR 12.25–15 years) (Table 1). Nearly 40% of the patients had lupus nephritis (LN), and all of these had proliferative lupus nephritis on biopsy (class III, IV or mixed class III/IV). Thirty-six percent of this cohort had CNS SLE, which is within the wide range of other cohorts which report 30–95% of patients with CNS SLE [51]. The median SLEDAI-2K score at enrolment was 11.5, indicating most patients had highly active disease. The cohort was diverse, with genetic ancestry falling into 6 categories: European, East Asian, South Asian, African, Amerindian, and Admixed. The largest ancestral group of cSLE subjects were those of European ancestry (40%), followed by East Asian (24%) and Admixed (17%) (Table 1).

The SNV based analysis did not return any SNVs below the significance threshold (5×10^{-8}). A SNV of interest (rs11059840, 12–129189369 A-T) closest to this threshold for cSLE association was found ($p = 9.76 \times 10^{-6}$, OR 0.19). This SNV is an intronic variant in the Transmembrane Protein 132 C gene (*TMEM132C*). The SNV of interest was found in 7 of our cSLE trios. These trios were ancestrally diverse: this SNV was transmitted in families of European, South Asian, East Asian, and Admixed ancestries. We cross-referenced our common variant TDT at the SNV level and rare variant results at the gene level. *TMEM132C* was also present in our gene-based analysis, although again it did not reach statistical significance.

In the gene-based TDT analysis, many genes were statistically significantly associated with cSLE risk after permutation-based multiple testing correction. The gene-based rare variant p-values were adjusted based on the permutations and the corrected genome-wide significance level used was 0.05. Overall, our study resulted in 448 genes meeting the significance threshold based on the corrected *p*-value of 0.05 using VT-BRV-Haplo Testing (Supplementary Table 1).

We then filtered these genome-wide significant rvTDT results even more stringently, to those associations with an adjusted *p*-value of ≤0.005 as the most highly significant genes containing rare variants in cSLE patients (Table 2). These include dynein axonemal heavy chain 11 (*DNAH11*) (adjusted $p = 0.0009$), protocadherin beta 15 (*PCDHB15*) (adjusted $p = 0.0009$), *TMEM63A* (adjusted $p = 0.0009$), *FAM160A1* (adjusted $p = 0.0011$) and heterogeneous nuclear ribonucleoprotein U like 2 (*HNRNPUL2*) (adjusted $p = 0.0013$). We also report a list of genes with associations with a slightly less stringent filter for statistical significance (adjusted *p*-value of 0.005–0.01) as genes of interest containing rare variants in cSLE patients (Table 3).

DISCUSSION

Our study aimed to identify common risk alleles across families, and genes containing high-risk rare variants for cSLE in a multi-ancestral population of patients. To the best of our knowledge, this is the first study to apply TDT methodology in SLE agnostically across the entire genome. The rare variant gene-based TDT analysis, identified over 400 genome-wide significant genes after adjusting for the number of genes tested, some of which were both novel and highly significant, with compelling mechanisms for relation to autoimmunity (Tables 2, 3, Supplementary Table 1). We report the results of the VT-BRV-Haplo analysis as this method allows for burden testing while reducing false positives due to SNVs in linkage disequilibrium (LD) and correcting for multiple associations, but we included the results from all six analytic methods for comparison (Supplementary Table 1.) In addition to novel risk associations, there are some genes in our analysis, such as *LAMP1* (Table 3, adjusted *p*-value 0.0055), which have been previously linked to SLE pathogenesis, indicating our methodology is robust [52]. In the SNV-based association testing, none of the SNVs reached the significance threshold. However, a protective SNV rs11059840 (12–129189369 A-T), in an intron of the *TMEM132C* gene was closest to statistical significance ($p = 9.76 \times 10^{-6}$).

SLE is a challenging disease to study due to the heterogeneity of clinical manifestations. Prior studies have provided evidence of a genetic component to SLE from twin and sibling risk studies [10, 11, 53]. These studies have been conducted either at one center or within several centers within one country. Furthermore, the vast majority of genetic studies in SLE have been common variant studies conducted on adult women of White race or European ancestry [54–56]. The focus on common variants in adult populations, and lack of diversity within SLE genetic studies may contribute to the missing heritability that remains in SLE studies. While SLE affects patients of all races and ethnicities, there are differences in the manifestations and severity of disease in these groups- specifically, it is more prevalent and severe in non-White patients. We do not want to confound race and ancestry, as there are unmeasured effects that contribute to health disparities in SLE [57]. Yet, it is important to include a diverse population in genetic studies to understand the full breadth of disease. TDT is a useful analytic tool for analysis of diverse cohorts of childhood onset disease as it addresses admixture and population stratification.

Prior TDT studies of SLE and cSLE have been conducted on a single gene or a predefined group of variants. SLE associations have also been found with different versions of TDT. Single gene TDT established associations between both HLA-DRB1 and HRES-1 locus SNVs and SLE, both using traditional TDT in European populations [55, 58]. A haplotype based test identified the PD1.3 A allele haplotype of the *PDCD1* gene associated with SLE in non-Spanish Europeans, and a Bayesian approach found a novel association with the *PTPRT* gene and SLE in GWAS data, confirming previous associations of *IRF5* gene and SLE [32, 59]. These studies demonstrate the utility of family-based genetic studies in understanding SLE genetic risk.

Our gene-based results identified many novel gene associations not previously described in SLE. The large number of associated genes with multiple rare variants identified in this analysis suggests that rare variants may play a significant role in the genetics of early-onset disease, consistent with other studies of cSLE populations [17]. The most intriguing of the highly significant genes is heterogeneous nuclear ribonucleoprotein U like 2 (*HNRNPUL2*, adjusted $p = 0.0013$). HNRNPUL2 plays a key role in responding to double-stranded DNA breaks [60], and defects in nucleases which lead to accumulation of endogenous nucleic acid have been implicated in SLE pathogenesis [19]. Double-stranded breaks are among the least tolerated forms of DNA damage, and the DNA damage response has evolved in mammals to limit toxicity. HNRNPUL2 is recruited to the site of the double stranded break along with the MRN complex. HNRNPUL2 is required for long range resection by promoting the Bloom syndrome helicase recruitment over Exonuclease 1 to the site of the break [60, 61]. This alteration in response to DNA damage is intriguing and merits further mechanistic study.

The most highly significant (genome-wide adjusted $p = 0.0009$), dynein axonemal heavy chain 11 (*DNAH11*), is involved in ciliary movement. Recently *DNAH11* was identified as a cause of de novo pediatric sarcoidosis in a whole exome analysis, potentially linking the gene to autoimmunity [62]. Ours is the first study to link variants in this gene to SLE. Most of the literature to date describes variants in this protein and an association with primary ciliary dyskinesia and situs inversus [63–65]. Recently, a link between a ciliopathy and autoimmunity was reported in Bardet-Biedl syndrome [66]. The exact mechanism is still under investigation, but there is evidence that T cells utilize ciliary machinery in the immune synapse, and DNAH11 is expressed in T cell subsets in single cell data sets derived from spleen and peripheral blood [67]. Whether this correlation applies to other ciliopathies requires further study.

Many of the genes that we found to be genome-wide significant have been studied in neurological conditions, such as Transmembrane Protein 63 A (*TMEM63A)* and Protocadherin beta 15 (*PCDHB15). PCDHB15*, which we found to be associated with SLE in our study (adjusted $p = 0.0009$), has been associated with deafness and Usher syndrome [68, 69]. Procadherins are cadherin proteins involved in cell-cell adherence, and it is interesting to note that the genes of PCDHB protein family are organized similarly to the B-cell and T-cell receptor gene clusters [70]. *PCDHB15* has not been linked to SLE risk, but other procadherins have been implicated in autoimmunity [71, 72]. Although also described in neurologic conditions, TMEM63A is a protein with a very different mechanism than PCDHB15. TMEM63A is a calcium-permeable mechanosensitive channel [73], channels that are stretch activated at a high threshold. There are no previous studies that link TMEM63A to autoimmunity. Heterozygous missense variants in this gene have been reported with transient hypomyelination in infants [74]. Our study may be the first to link these genes to SLE because we used TDT methodology in an early-onset SLE cohort, which may be enriched in variants contributing to SLE. The approach of gene-based testing to broaden the search for variants across the gene may have also contributed to this discovery. Many previous genetic studies have implicated common genetic drivers in neurologic diseases and immunity [75]. Further investigation to understand more about the link between these phenotypes is needed.

Both rare and common variants may contribute to cSLE pathogenesis, and thus both were assessed in our study. In addition to rvTDT, we also used TDT to determine SLE association with common variants in cSLE. As noted above, TDT has identified a few common SLE risk variants, but the majority of common variants studies in SLE to date are GWAS, which have identified specific risk both in MHC regions and variants outside of MHC [54]. In European populations, the HLA-DRB1:03:01 and HLADRB1:15:01 have been linked to SLE, while in East Asians the highest risk for SLE is associated with HLA-DRB:15:02 [76, 77]. In African Americans, HLA-DRB1:15:03 has been implicated as a risk associated allele [78]. Non-MHC regions associated with SLE risk include tumor necrosis factor (ligand) superfamily member 4, and ubiquitin-conjugating enzyme E2L3, BLK, BANK, PTPN22 among others [79, 80]. Lack of power often limits pediatric GWAS and few common variant studies have targeted the cSLE population [12]. An association between cumulative common SLE risk loci and the risk for lupus nephritis in children was described in a study of adults and children [12]. TDT is a different, family-based methodology to assess common variant association with SLE.

In the SNV-based association testing, none of the SNVs reached statistical significance. The SNV 12–129189369 A-T (rs11059840), in an intron of *TMEM132C* gene was closest to the standard GWAS threshold. *TMEM132C* was not significant in the gene-based analysis. A previous GWAS of SLE identified *TMEM132C* as a candidate for a gene within the same 200 base pair region as an SLE-associated protective locus (rs1059312; 12–129278864-A-G) [13]. While this was a different SNV than the SNV identified in our cohort and was not in LD with our SNV (R^2 0.04), GWAS often identifies SNVs which act as markers for a gene region of interest. The TMEM132 gene family has also been implicated in GWAS studies of schizophrenia and Alzheimer's disease [81, 82]. Future studies of larger cohorts are important to see if our association is more robust with more statistical power. Furthermore, dedicated functional studies are necessary to elucidate the mechanisms by which variants in these genes contribute to autoimmunity.

Our study was limited by a small sample size, a common challenge in cSLE studies. Repeating this analysis on larger cohorts of trios could help to confirm or expand upon our findings. A limitation of TDT methodology is that the parent must be heterozygous at the locus of interest to be detected. Thus, TDT is one method of genetic analysis but may miss important variants for which parents are homozygous at that locus. TDT can be complemented with other genetic analyses (i.e., rare variant burden testing) which help to address this limitation.

Our study is the first that we are aware of to apply TDT methodology to explore genetic associations across the entire genome in SLE. This methodology can be used in future studies of cSLE patients with a larger sample size, as well as many other childhood-onset autoimmune diseases as whole genome sequencing is performed more frequently on these cohorts. Key cSLE-associated genes were identified in our rare variant burden analysis. *HNRNPUL2, DNAH11* and others, may warrant further investigation, as some of these genes have compelling mechanisms such as DNA repair or have been identified in larger GWAS studies. Validation with larger trio analyses and functional studies are needed to fully understand the impact of these variants. Identification of genetic associations in SLE could lead to improvements in the clinical approach to the disease including more specific therapies, targeted genetic testing for SLE risk, and could provide prognostic information to patients and families. TDT is a robust genetic test to use for diverse cohorts, as the method controls for both population stratification and admixture. In this way, our study lays the foundation for future family-based association studies using whole genome data in SLE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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COMPETING INTERESTS

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DATA AVAILABILITY

The data from this paper are available from the corresponding author upon reasonable request.

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Table 1.

Demographic and clinical features of cSLE patients.

	cSLE patients (*n* = 42)
% Female (*n* (%))	32 (76)
Age at diagnosis (years), (median (IQR))	14.0 (12.25–15)
Ancestry (*n* (%))	
European	17 (40)
African	1 (2)
Amerindian	2 (5)
East Asian	10 (24)
South Asian	5 (12)
Admixed	7 (17)
ANA positive (*n* (%))	42 (100)
Anti-dsDNA antibody (*n* (%))	33 (78)
Lupus Nephritis (*n* (%))	16 (39)
Class III (*n* (%of those with LN)	5 (12)
Class IV (*n* (%of those with LN)	7 (44)
Class III/IV (*n* (%of those with LN)	3 (19)
Class IV/V (*n* (%of those with LN)	1 (6)
CNS SLE (*n* (%))	15 (36)
Arthritis (*n* (%))	36 (85)
SLEDAI-2K, median (SD)	11.5 (8.6)

Table 2.

Rare variant genes identified via rvTDT that are highly statistically significant in the VT-BRV-Haplo analysis (adjusted *p*-value ≤ 0.005).

Gene	*p*-value	Description
DNAH11 (dynein axonemal heavy chain 11)	0.0009	Encodes a ciliary outer dynein arm protein; implicated in respiratory cilia movement. Patients with homozygous variants have been reported in primary ciliary dyskinesia, Kartagener Syndrome and male sterility.
PCDHB15 (protocadherin beta 15)	0.0009	Calcium dependent cell adhesion protein, important in neuronal cells, regulated by DNA methylation, methylation changes in PCDHB15 have been reported in cancer and multiple sclerosis
TMEM63A (transmembrane protein 63A)	0.0009	Calcium permeable mechanosensitive channel; May potentiate macrophage recruitment
FAM160A1 (family with sequence similarity 160 member A1)	0.0011	Member of the UPF0518 family of proteins, which contain a conserved retinoic acid induced 16 like domain; part of FHF complex which facilitates vesicle transport
HNRNPUL2 (heterogeneous nuclear ribonucleoprotein U like 2)	0.0013	Involved in DNA repair, specifically in double-stranded breaks.
ADCY10 (adenylate cyclase 10)	0.0018	A soluble adenylyl cyclase that is insensitive to G protein or forskolin regulation, may modulate mitochondrial induced apoptosis, implicated in inflammatory myocardiopathy of Chagas disease
DAB2IP (DAB2 Interacting protein)	0.0018	Ras GTP-ase activating protein; scaffold protein for H-Ras and TRAF-2, involved in malignancy, innate immunity, apoptosis, and atherosclerosis
BCAM (Basal Cell Adhesion Molecule (Lutheran Blood Group)	0.0019	Encodes Lutheran blood group glycoprotein, a member of the immunoglobulin superfamily; receptor for the extracellular matrix protein a5 on laminin. High expression accelerates glomerulonephritis in mice
CEBPZ (CCAAT Enhancer binding protein zeta)	0.0024	DNA-binding transcriptional activator and regulates the heat-shock protein 70 (HSP70) promoter in a CCAAT-dependent manner
SLFN14 (Schlafen family member 14)	0.0026	RNA helicase, involved in platelet formation and function; RNA surveillance recognizes stalled ribosomes and triggering endonucleolytic cleavage of aberrant mRNAs
PEAK3 (PEAK family member 3)	0.0027	Pseudokinase scaffolding protein of important signaling regulators in the EGFR pathway; Enables protein self-association, involved in actin organization, regulates cell motility
PNKD (PNKD Metallo-Beta-Lactamase Domain Containing)	0.0029	Contains G-lactamase domain, Regulation of myofibrillogenesis, associated with paroxysmal non-kinesigenic dyskinesias and tic disorders
GCGR (Glucagon Receptor)	0.0033	Controls blood glucose levels through glucagon, important in fasting
NLGN2 (Neuroligin 2)	0.0038	Family of neuronal cell surface proteins, associated with autism and schizophrenia
ADAM12 (ADAM Metallopeptidase Domain 12)	0.0039	An active metalloproteinase, regulates cell-cell and cell-matrix interactions, costimulatory molecule that determines Th1 cell fate and mediates tissue inflammation
TLE6 (TLE Family Member 6, Subcortical Maternal Complex Member)	0.0040	A component of the mammalian subcortical maternal complex, which is required for preimplantation development; regulates spermatogonia proliferation and cell cycle progression
TTC37 (SKIC3) (Subunit of Superkiller Complex)	0.0041	Part of SKI complex, exosome-mediated RNA decay to degrade aberrant mRNA, associates with transcriptionally active genes in a manner dependent on PAF1 complex
MARCHF10 (Membrane Associated Ring-CH-Type Finger 10)	0.0042	Member of the March family of E3 ubiquitin ligases
PROZ (Protein Z, Vitamin K Dependent Plasma Glycoprotein)	0.0046	A liver vitamin K-dependent glycoprotein synthesized in the liver and secreted into plasma, complexes with protein Z-dependent protease inhibitor to directly inhibit activated factor X at the phospholipid surface
B4GALNT2 (Beta-1,4-N-Acetyl-Galactosaminyltransferase 2)	0.0048	Catalyzes the last step in the biosynthesis of the human Sd(a) blood group antigen

Gene	*p*-value	Description
ART1 (ADP-Ribosyltransferase 1)	0.0050	Catalyzes the ADP-ribosylation of arginine residues in proteins
MROH7 (Maestro Heat Like Repeat Family Member 7)	0.0050	Extracellular protein

Table 3.

rvTDT results for all genes with adjusted *p*-value > 0.005 and ≤0.01 by VT-BRV-Haplo analysis.

Gene name	VT-BRV-Haplo
LAMP1	0.0055
KRTAP10–5	0.0056
RIMS1	0.0056
ZNF862	0.0056
ACTBL2	0.0057
SAGE1	0.0060
MYO15A	0.0063
TMEM53	0.0063
GPRIN1	0.0064
ADGB	0.0065
QSER1	0.0069
CTC1	0.0073
DNAJA3	0.0075
PRRT3	0.0075
PTCH1	0.0078
MAPK8IP2	0.0079
TRPV4	0.0081
ZNF415	0.0081
ARHGEF38	0.0083
PXK	0.0083
SLC38A9	0.0083
AATF	0.0085
LCN10	0.0087
HEATR1	0.0088
SNAI3	0.0088
YY1AP1	0.0093
C8orf74	0.0096
GBP6	0.0096
KIAA1211L	0.0096
DNER	0.0098
NUMA1	0.0099
TKTL1	0.0099