Genome-Wide Association Study of Peripheral Artery Disease

GoLEAD Consortium

2021-10

GoLEAD Consortium , SUMMIT Consortium , van Zuydam , N R , Stiby , A , Abdalla , M , Dahlström , E H , Vlachopoulou , E , Sandholm , N , Forsblom , C , Sinisalo , J , Perola , M , Kallio , M , Groop , P-H , Groop , L & Kullo , I J 2021 , ' Genome-Wide Association Study of Peripheral Artery Disease ' , Circulation-Genomic and precision medicine , vol. 14 , no. 5 , 002862 . https://doi.org/10.1161/CIRCGEN.119.002862

http://hdl.handle.net/10138/336969
https://doi.org/10.1161/CIRCGEN.119.002862

other
publishedVersion

Circulation: Genomic and Precision Medicine

ORIGINAL ARTICLE

Genome-Wide Association Meta-Analysis Supports Genes Involved in Valve and Cardiac Development to Associate With Mitral Valve Prolapse

Mengyao Yu, PhD; Sergiy Kyryachenko, PhD; Stephanie Debette, MD, PhD; Philippe Amouyel, MD, PhD; Jean-Jacques Schott, PhD; Thierry Le Tourneau, MD, PhD; Christian Dina, PhD; Russell A. Norris, PhD; Albert A. Hagège, MD, PhD; Xavier Jeunemaitre, MD, PhD; Nabila Bouatia-Naji, PhD

BACKGROUND: Mitral valve prolapse (MVP) is a common cardiac valve disease, which affects 1 in 40 in the general population. Previous genome-wide association study has identified 6 risk loci for MVP. But these loci explained only partially the genetic risk for MVP. We aim to identify additional risk loci for MVP by adding data set from the UK Biobank.

METHODS: We reanalized 1007 cases and 1469 controls and the MVP-France study and 479 cases and 862 controls from the MVP-Nantes study for genotype reimputation using haplotype reference consortium and Trans-Omics for Precision Medicine panels. We also incorporated 434 MVP cases and 4527 controls from the UK Biobank for discovery analyses. Genetic association was conducted using SNPTEST and meta-analyses using METAL. We used Functional Mapping and Annotation of Genome-Wide Association Studies for post-genome-wide association study annotations and Multi-marker Analysis of GenoMic Annotation for gene-based and gene-set analyses.

RESULTS: We found Trans-Omics for Precision Medicine imputation to perform better in terms of accuracy in the lower ranges of minor allele frequency below 0.1. Our updated meta-analysis included UK Biobank study for ≈8 million common single-nucleotide polymorphisms (minor allele frequency >0.01) and replicated the association on Chr2 as the top association signal near *TNS1*. We identified an additional risk locus on Chr1 (*SYT2*) and 2 suggestive risk loci on chr8 (*MSRA*) and chr19 (*FBXO46*), all driven by common variants. Gene-based association using Multi-marker Analysis of GenoMic Annotation revealed 6 risk genes for MVP with pronounced expression levels in cardiovascular tissues, especially the heart and globally part of enriched GO terms related to cardiac development.

CONCLUSIONS: We report an updated meta-analysis genome-wide association study for MVP using dense imputation coverage and an improved case-control sample. We describe several loci and genes with MVP spanning biological mechanisms highly relevant to MVP, especially during valve and heart development.

Key Words: association ■ computational biology ■ heart valve diseases ■ meta-analysis ■ mitral valve prolapse

Heart valves are key functional elements of the heart that display specific biological mechanisms in health and disease. During the heart morphogenesis, the mitral valve develops soon after cardiac looping. The complex shape of the mitral valve, with 2 leaves, allows a very precise balance of force to maintain unidirectional blood flow through the mitral orifice. The importance of valve development in the origin of mitral valve disease has

Correspondence to: Nabila Bouatia-Naji, PhD, Paris Cardiovascular Research Center, INSERM UMR970, 56 Rue Leblanc, F-75015, Paris, France. Email nabila.bouatia-naji@inserm.fr

The Data Supplement is available at https://www.ahajournals.org/doi/suppl/10.1161/CIRCGEN.120.003148.

For Sources of Funding and Disclosures, see page 597.

Nonstandard Abbreviations and Acronyms

GWAS	genome-wide association study
MAF	minor allele frequency
MSRA	methionine sulfoxide reductase A gene
MVP	mitral valve prolapse
MVP-F	MVP-France study
MVP-N	MVP-Nantes study
OR	odds ratio
SNP	single-nucleotide polymorphism
TGF	transforming growth factor

been established through the discovery of the genetic causes of rare syndromes, such as Marfan, Loeys-Dietz, and Ehlers-Danlos and familial nonsyndromic cases.[1,2] The causal genes play key roles in extracellular matrix deposition and organization, being influenced by TGF (transforming growth factor)-β and ciliogenic signaling nodes.

The adult valve can lose its flexibility under the action of permanent mechanical stress and a degenerative process gradually takes place leading to prolapse (mitral valve prolapse [MVP]), and in many patients, the incapacity of the valve to close. The resulting mitral regurgitation requires surgery repair or replacement, as it greatly increases the risk of heart failure, arrhythmia, and even sudden death.[3] MVP is common, affecting ≈1 in 40 individuals in the general population.[3] As for many heart diseases, the establishment of MVP occurs as a result of mild dysfunctions of the many complex biological mechanisms required during development and valve function. We have shown through family studies the requirement of dachsous cadherin-related 1 gene (*DCHS1*), a member of the cadherin superfamily, for cell alignment during valve development.[1] More recently, we have shown that loss of primary cilia during development leads to progressive myxomatous degeneration of the mitral valve in mice and humans.[2] Using genome-wide association study (GWAS), we have identified predisposition loci,[1,4] particularly those near tensin 1 gene (*TNS1*), are involved in cell adhesion. This finding further supported the importance of cytoskeleton organization revealed by the study of the polyvalvulopathy syndrome caused by filamin A gene (*FLNA1*) mutations.[5] However, taken together, these loci explain only a small fraction of the genetic contribution to MVP. For instance, the relevance of fine regulation of valve and cardiac development mechanisms is established to be at play for early-onset syndromic and nonsyndromic valve disease. However, this is unknown for late-onset and aging-related valve disease. Identification of additional risk loci will likely provide a better and more complete understanding of the genetic and biological basis of MVP.

One of the limitations of the genetic study of MVP is the lack of large cohorts with genome-wide genotyping that would allow increasing the power to discover new predisposition genes of the current GWAS involving only 1500 patients.[1] In addition, since our initial study, it has become possible to achieve much denser and more accurate GWAS through the high-density genetic imputation panels provided by haplotype reference consortium (HRC),[6] and more recently Trans-Omics for Precision Medicine (TOPMed)[7] consortia. These panels offer a theoretical increase in power, genomic coverage, and fine mapping, notably through the study of low-frequency variants.[7,8]

In this work, we first compare the imputation performance of the newly generated HRC and TOPMed panels in the context of our cohorts. We thus describe comparable results using these 2 panels, in favor of a better coverage for low-frequency variants for the TOPMed panel. Then, we performed a GWAS meta-analysis including a new case-control study defined using the UK Biobank resource.[9] We replicate the *TNS1* locus and described 3 new suggestive loci. The gene-based association analysis identifies several new loci that inform established and original mechanisms for the biology underlying the genetic risk for MVP.

MATERIALS AND METHODS

Details on the populations and methods are available in the Data Supplement. The experimental data that support the findings of this study are available from the corresponding author upon request. Summary statistics of the meta-analysis will be publicly available soon after article acceptance. MVP-France (MVP-F) approvals were obtained from CPP Ile-de-France VI (approval no. 60-08, June 25, 2008), the Commission Informatique et Libertés (approval no. 908359, October 14, 2008) and the French Ministry of Health (ID-RCB: 2008-A00568-47) and was registered on the clinicaltrial.gov website (protocol ID: 2008–01). MVP-Nantes (MVP-N) was approved by CPP Ouest IV-Nantes (No. 215/2013, on March 6, 2013, by 913630 of October 16, 2014) and was registered on the https://www.clinicaltrials.gov website (Unique identifier: NCT03884426).

RESULTS

Imputation Accuracy Between HRC and TOPMed in MVP GWAS Data Sets

We first aimed to assess the usefulness of using larger imputation reference panels in the 2 French MVP case controls studies. MVP-F included 1007 cases and 1469 controls with 492 438 genotyped variants. MVP-N included 479 cases and 862 controls with 370 697 genotyped variants. We generated 229 973 672 and 230 053 813 imputed variants in MVP-F and MVP-N, respectively, from the TOPMed reference panel, which represent 5.8-fold more variants compared with HRC (39 117 105 in MVP-F and MVP-N; Figure 1A). However, most of these variants had an imputation score Rsq smaller than 0.3: 84% (MVP-F-TOPMed), 88% (MVP-N-TOPMed), 51% (MVP-F-HRC), and 60% (MVP-N-HRC; Figure 1A). Compared with MVP-N, the relatively larger sample size of MVP-F study generates a slightly larger number of well-imputed variants (Figure 1A).

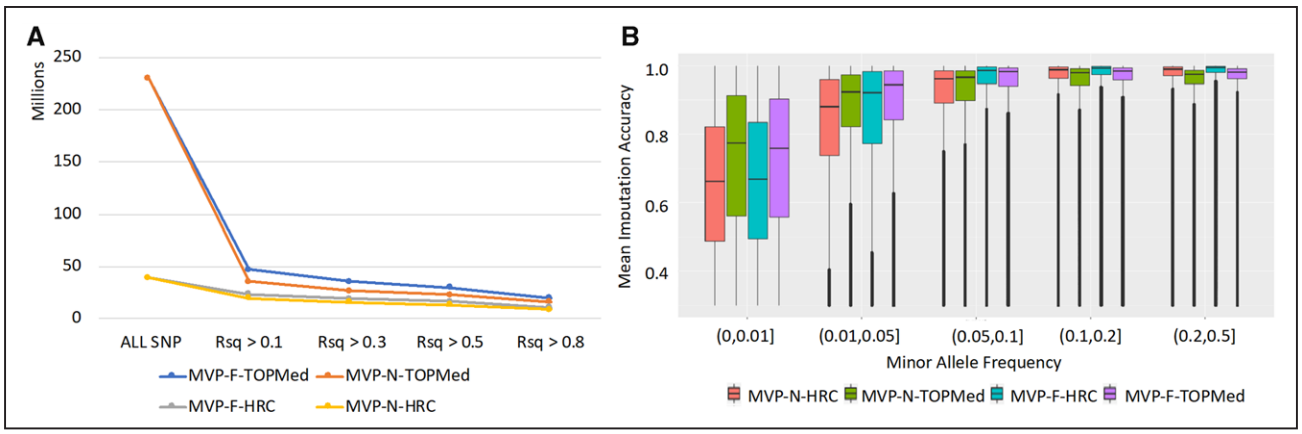

Figure 1. Comparison of the imputation quality of the results for MVP-France (MVP-F) and MVP-Nantes (MVP-N) using HRC and TOPMed as reference panel, respectively.

A, The numbers of variants generated using different imputation accuracy thresholds. **B**, The imputation quality at different minor allele frequency (MAF) region using all the cleaned single-nucleotide polymorphisms (SNPs; Rsq>0.3) in cohorts MVP-F and MVP-N cohorts. HRC indicates haplotype reference consortium; Rsq, imputation accuracy; MVP, mitral valve prolapse; TOPMed, Trans-Omics for Precision Medicine.

We then classified minor allele frequency (MAF) into 5 regions ([0,0.01], [0.01,0.05], [0.05,0.1], [0.1,0.2], and [0.2,0.5]). Most of the variants with Rsq <0.3 are rare variants which MAF is smaller than 0.01, as illustrated for all single-nucleotide polymorphisms (SNPs) generated on chromosome 22 (Figure IA and IB in the Data Supplement). We also found that TOPMed panel imputed variants present, on average, a better accuracy for low-frequency ranges (0,0.01), (0.01,0.05), and (0.05,0.1), while relatively stable accuracy at MAF ranges (0.1,0.2) and (0.2,0.5) compared with HRC panel in both studies (Figure 1B). Consistent results were also found at the variant level (Figure IC and ID in the Data Supplement). We randomly selected 173 804 variants (≈8000 for each chromosome) to compare the imputation accuracy between the 2 panels. The low-frequency ranges (0,0.01) represented 75% of SNPs, compared with other MAF categories (Figure IC in the Data Supplement). The ratios of the number of variant where TOPMed Rsq was higher than HRC Rsq divided by the number of variants where HRC Rsq was higher than TOPMed Rsq were nearly 1.5 for all MAF categories (Figure ID in the Data Supplement). Taken together, we use TOPMed as the imputation panel to allow analyzing more well-imputed variants, especially in the low-frequency (0.01<MAF<0.1) category.

Genetic Association Analyses in French and UK Biobank Case-Control Studies

We meta-analyzed 3 GWAS involving a total of 1920 MVP cases and 6858 controls and ≈8 million (8 021 974) genotyped or imputed common SNPs (MAF>0.01). We confirmed a deviation from the expected levels of significance in this updated meta-analysis (Figure 2A).

We first looked-up the association with MVP in the UK Biobank data set of the previously reported 6 loci in Dina et al.[1] We found nominal association for 2 loci and consistent direction of effects of 5 out of 6 loci in the UK Biobank case-control study (Table I in the Data Supplement).

The strongest association signal was observed on chromosome 2 at the *TNS1* locus, where we report 46 variants with a genome-wide significant $P<5\times10^{-8}$ (Figure 2B). In addition to successfully replicating the previously reported top associated SNP rs12465515 ($P=8.61\times10^{-10}$; odds ratio [OR] =1.30 [1.19–1.42]), we found a new top associated variant on chromosome 2 (lead SNP: rs7595393; $P=4.68\times10^{-10}$; OR=1.31 [1.20–1.42], Table, Figure II in the Data Supplement). Both SNPs are highly correlated ($r^2=0.92$, 1000G Phase 3: CEU). As expected, conditional analyses both on rs12465515 and rs7595393 at the *TNS1* locus resulted in disappearance of the association signal (Figure 3). Functional annotation of 107 SNPs in linkage disequilibrium ($r^2>0.6$) with 4 independent significant SNPs at this locus indicated the existence of 4 SNPs predicted to be deleterious alleles (Combined Annotation Dependent Depletion score >12) and 7 SNPs likely to lie within regulatory elements according to the regulome database score (regulome database score >3a; Table II in the Data Supplement).

We also report a new genome-wide significant locus and 2 suggestive and original association signals (Figure 2, Table). On chromosome 1, the lead SNP (rs199723025, effect allele frequent =0.05, $P=4.55\times10^{-8}$, OR=1.69 [1.40–2.04]) is an intergenic deletion variant of Synaptotagmin 2 gene (*SYT2*; Table, Figure III in the Data Supplement). We found that the lead variant is a significant expression quantitative trait locus (eQTL) in atrial appendage and artery aorta ($P=2.4\times10^{-5}$ and 1×10^{-8}) for the lysine demethylase 5B gene (*KDM5B*) involved in DNA stability and repair. The second locus on chromosome 8 (rs56028519, effect allele frequent =0.74, $P=1.04\times10^{-8}$, OR=1.29 [1.17–1.41]) is an intronic variant of the methionine sulfoxide reductase A gene (*MSRA*; Table, Figure IV in the Data Supplement). The lead SNP rs56028519 is an eQTL of a long non

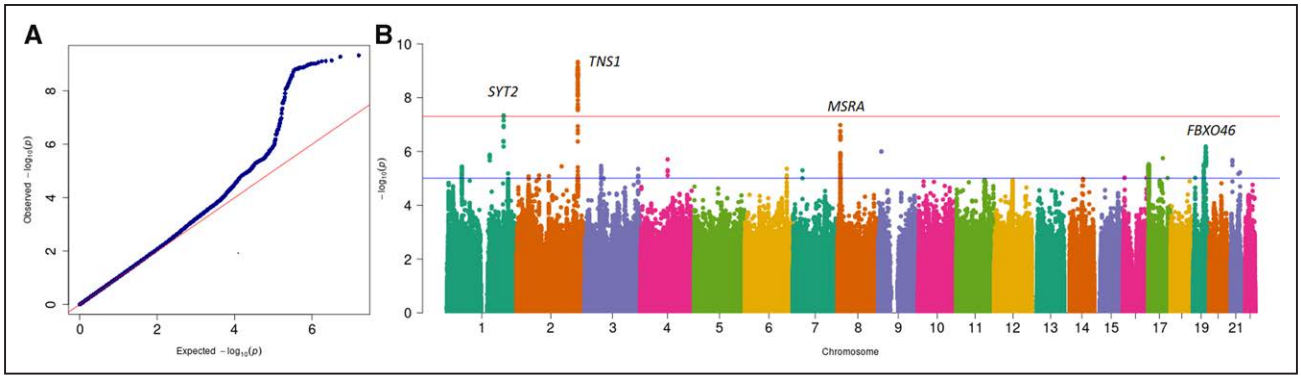

Figure 2. Single-nucleotide polymorphism (SNP)-based association results with mitral valve prolapse (MVP) in a genome-wide association study (GWAS) meta-analysis involving MVP-France (MVP-F), MVP-Nantes, and MVP-UK Biobank case-control studies (lambda GC=1.08).

A, The Q-Q plot represents the expected (x axis) vs the observed (y axis) *P* values. **B**, Manhattan plot summarizes the $-\log_{10}$ (*P*) of each SNP by chromosome obtained from the GWAS meta-analysis. The blue line indicates the threshold for suggestive association ($P<1\times10^{-6}$) and the red line indicates the genome-wide significance threshold ($P<5\times10^{-8}$). Both plots represent the association of ≈8 million SNPs genotyped and imputed using TOPMed panel. TOPMed indicates Trans-Omics for Precision Medicine.

coding RNA gene (AF131215.2) in heart atrial appendage ($P=4.5\times10^{-7}$) and a long non coding RNA gene (RP11-981G7.6) in left ventricle ($P=1.3\times10^{-7}$). We found that several SNPs in linkage disequilibrium ($r^2>0.6$) with the 2 independent significant SNPs are located near the regulatory elements, especially rs11249991 and rs11781529 that showed high potential to be regulatory (regulome database score >3a). Four SNPs in total are likely to be deleterious, including rs11783281 (Combined Annotation Dependent Depletion score =19.34, Table II in the Data Supplement). The third suggestive association signal was located in a particularly gene-rich region on chromosome 19 (rs4802272, effect allele frequent =0.55, $P=6.54\times10^{-7}$, OR=1.24 [1.14–1.34]; Table, Figure V in the Data Supplement). Although the lead SNPs mapped to *FBXO46* (F-box protein 46 gene), the association signal spans 5 genes in total, including *SIX5* (SIX homeobox 5), *FOXA3* (forkhead box A3), *RSPH6A* (radial spoke head 6 homolog A), *DMPK* (DM1 protein kinase), and *DMWD* (DM1 locus, WD repeat containing; Figure V in the Data Supplement). The lead SNP is an eQTLs of *DMPK* and *DMWD* in artery aorta ($P=2.1\times10^{-9}$, 3.4×10^{-11}). Two SNPs in high linkage disequilibrium with the lead SNP showed high deleteriousness scores (rs62111759, Combined Annotation Dependent Depletion score =13.28 and rs672348, Combined Annotation Dependent Depletion score =15.42; Table II in the Data Supplement).

Genome-Wide Gene-Based Association and Pathway Analyses

We performed a genome-wide gene-based association analysis using Multi-marker Analysis of GenoMic Annotation (v1.08)[10] to estimate the gene-level association on the basis of all SNPs in a gene. This method is a complementary approach to single SNP GWAS analyses and usually reveals genes with locally consistent associated SNPs that individually may do not reach genome-wide significance.

In total, we highlight 18 suggestively associated genes ($P<10^{-4}$), including 6 at the genome-wide level ($P<2.61\times10^{-6}$; Table III and Figure VI in the Data Supplement). We highlight *GLIS1* ($P=1.86\times10^{-5}$) that we have previously identified to associate with MVP using single SNP and pathway analyses,[4] *TGFB2* ($P=1.00\times10^{-5}$), a high-profile candidate gene for myxomatous valve disease, *MSRA* ($P=6.31\times10^{-6}$), one of the suggestive loci

Table. Associations of Top SNPs With MVP Obtained in the GWAS Meta-Analysis

					MVP-France	MVP-Nantes	MVP-UKB	All	
RSID	CHR	PosB38	RA	Frequency	*P* value, OR [95% CI]	*P* value, OR [95% CI]	*P* value, OR [95% CI]	*P* value, OR [95% CI]	Heterogeneity *P* value
rs199723025	1	202712600	A	0.05	1.40×10^{-4}, 1.61 [1.25–2.08]	0.31, 1.26 [0.88–1.83]	1.92×10^{-5}, 1.72 [1.34–2.22]	4.55×10^{-8}, 1.69 [1.40–2.04]	0.26
rs7595393	2	217006531	G	0.38	1.40×10^{-6}, 1.34 [1.19–1.52]	9.69×10^{-3}, 1.44 [1.22–1.70]	7.40×10^{-4}, 1.27 [1.10–1.48]	4.68×10^{-10}, 1.31 [1.20–1.42]	0.73
rs56028519	8	10341263	A	0.74	2.94×10^{-3}, 1.19 [1.04–1.35]	1.21×10^{-2}, 1.31 [1.10–1.57]	1.11×10^{-4}, 1.34 [1.16–1.56]	1.04×10^{-7}, 1.29 [1.17–1.41]	0.67
rs4802272	19	45732692	A	0.55	2.10×10^{-3}, 1.18 [1.06–1.33]	3.39×10^{-4}, 1.25 [1.07–1.47]	1.97×10^{-2}, 1.18 [1.03–1.36]	6.54×10^{-7}, 1.24 [1.14–1.34]	0.39

GWAS indicates genome-wide association study; MVP, Mitral valve prolapse; OR, odds ratio; RA, risk allele; and SNP, single-nucleotide polymorphism.

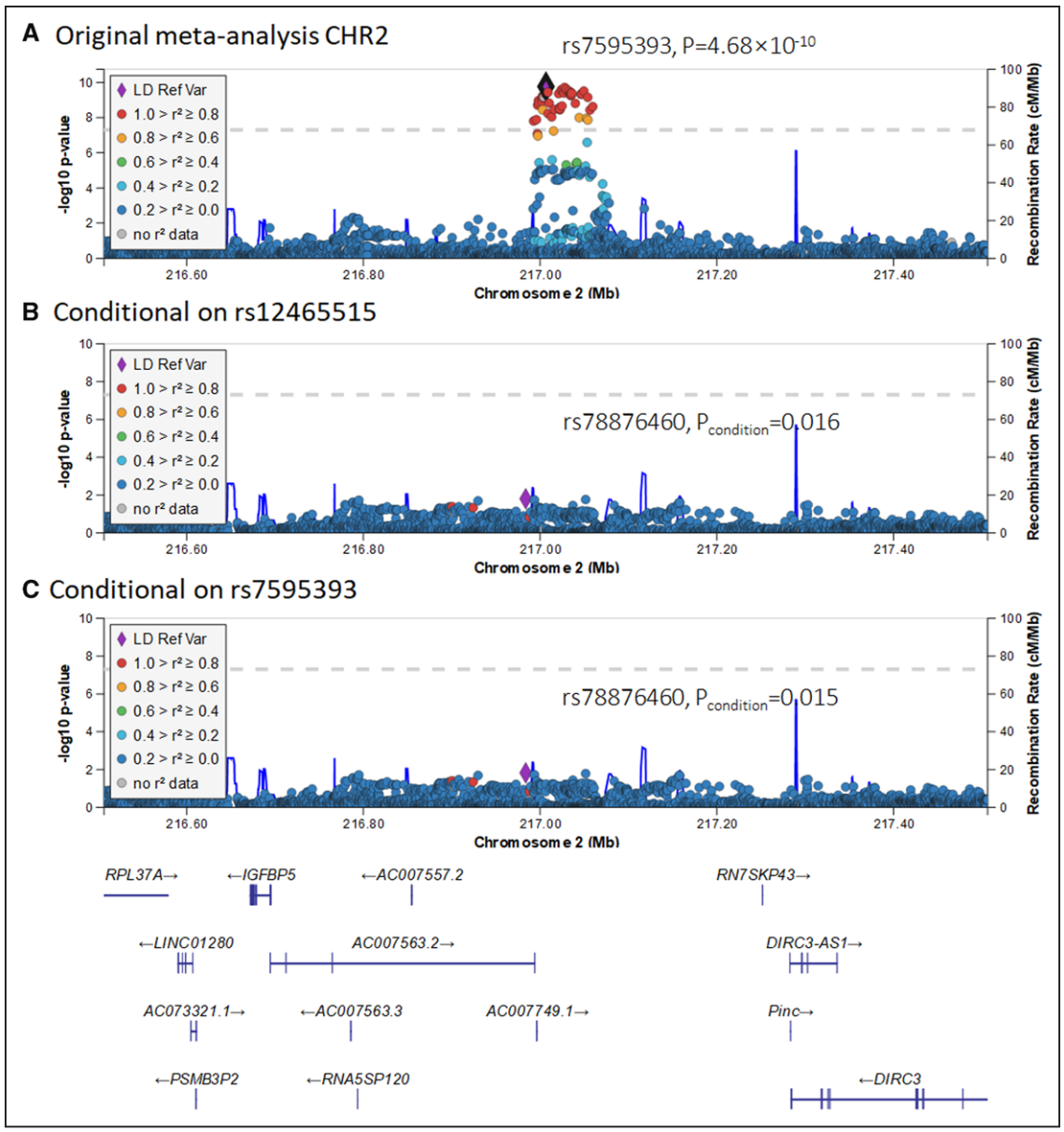

Figure 3. Conditional analysis of the TNS1 locus.

Locus zoom plots of the TNS1 locus demonstrate conditional analysis of Dina et al reported lead single-nucleotide polymorphism (SNP) rs12465515, the top SNP rs7595393 using genome-wode complex trait analysis condition and joint analysis. Linkage disequilibrium of SNPs with the conditioned SNPs is based on data from mitral valve prolapse (MVP)-F cohort and is shown by the color of the points. The best signals in each plot are marked as a purple rhombus. **A**, Association of SNP with MVP; (**B**) MVP association conditioned on Dina et al reported lead SNP rs12465515 (rs12465515 added as an additional covariate); and (**C**) MVP association conditioned on the top SNP rs7595393.

in the SNP GWAS, and *TBX5* (T-box transcription factor 5; $P=1.62\times10^{-5}$), a key regulator of heart development (Table III in the Data Supplement). The 10 remaining genes mapped into 2 gene-rich loci on chromosome 17 and 19. On chromosome 17, 3 genes reached gene-wide significance including *SRR* (serine rasemase gene) ($P=1.07\times10^{-7}$), in addition to *TSR1* (ribosome maturation factor; $P=3.22\times10^{-7}$), and *SGSM2* (small G protein signaling modulator 2 gene; $P=2.05\times10^{-6}$; Table III in the Data Supplement). On chromosome 19, we report significant association *SIX5* ($P=4.30\times10^{-7}$), *DMPK* ($P=6.23\times10^{-7}$), and *DMWD* ($P=2.43\times10^{-6}$; Table III in the Data Supplement). Tissue expression analyses using the genotype tissue expression data resource showed that *DMPK* and *DMWD* are ubiquitously expressed, including in the heart atrial appendage and heart left ventricle (Figure VI in the Data Supplement). We note that low-frequency variant contributed little to the association of the genes above mentioned, where the associations were driven by common variants (Figure VII in the Data Supplement). Section in situ hybridization lookups validated developmental expression of many of these genes within the mitral valves, including *Syt2*, candidate gene at the new locus we describe in the SNP GWAS (Figure VIII in the Data Supplement).

Multi-marker Analysis of GenoMic Annotation pathway analysis using 9996 gene sets (GO terms obtained from the Molecular Signatures Database) indicated that several enriched gene sets for MVP-associated genes are related to cardiac biology. Among top 10 enriched GO terms we found cardiac ventricle formation (P=1.42×10^{-5}), cardiac chamber formation (P=3.01×10^{-5}), cardiac right ventricle morphogenesis (P=2.12×10^{-4}), cardiac atrium development (P=3.00×10^{-4}), and cardioblast differentiation (P=6.78×10^{-4}; Table IV in the Data Supplement).

DISCUSSION

Here, we describe a high genetic coverage meta-analysis of GWAS based on TOPMed imputation involving ≈8 million common variants in ≈2000 MVP patients and ≈6800 controls. In addition to replicate the association at the *TNS1* locus, we identified several associated variants and genes, involving established and original mechanisms for the biology underlying the genetic risk for MVP.

Our results using the TOPMed imputation panel provided a higher resolution association map for the risk of MVP in a reasonably well powered data set. However, from our previous GWAS, only *TNS1*, *LMCD1*, and *SMG6*, showed association at the genome-wide level of significance when adding the UK Biobank data set. The differences in MVP definition (electronic health records in UK Biobank versus predominantly surgery reports) may have influenced the estimates of effects and their significance at the nonreplicating loci. Larger data sets with more homogeneous definition of phenotype are needed to definitely conclude about their role in genetic susceptibility to MVP.

We provide confirmatory results and fine mapping at the *TNS1* locus where we now report rs7595393 as the new lead associated SNP. The association signal on Chr2 is located in a gene-desert genomic region. We have previously provided solid biological evidence, which included a myxomatous valve phenotype in the heterozygote knockout mouse supporting the gene encoding Tensin 1, a focal adhesion protein, to be causal.[1] Functional annotations at this locus describe several SNPs belonging to the association block of rs7595393 as plausible causal variants. In the absence of eQTL, functional annotation for open chromatin and enhancer marks specifically in the mitral valve, we acknowledge that in silico annotation has limited ability to point at the causal variants. In parallel to this work, we have recently explored the functional properties of potential causal SNPs in this locus.[11] Using open chromatin maps that we generated in mitral valve tissue we found that one SNP (rs6723013) from the same association block than rs7595393 is located in open chromatin region specific to mitral valve tissues. Interestingly, the deletion using CRISPR-Cas9 of the sequence surrounding rs6723013 caused a significant change of the expression of *TNS1* specifically, confirming this sequence to harbor a long-range regulator and the most likely causal variant in this locus.[11]

One important addition in the current study is the improved coverage of low-frequency variants (0.01<MAF<0.10). Our current data do not allow us to firmly conclude about the putative role of low-frequency variants in MVP genetic risk, which needs to be explored further using larger data sets, given the limited power of our study for this category of variants (eg, at MAF=0.05, for a significance level of 5×10^{-8}, and a genetic relative risk of 1.50, the power of our current sample is 0.39). Nonetheless, one unprecedented genome-wide significant (*SYT2,* Chr1) association signal that we report here is driven by low-frequency (0.02≤MAF≤0.05) variants, although none the genes in the vicinity of this association signal present biological link to valve development or biology. Follow-up validation in larger studies is needed to confirm this association signal.

We also report 2 suggestive association signals in *MSRA* on Chr8 and *FBXO46* on Chr19. Interestingly, we also report *MSRA* suggestive association at the gene level with MVP. *MSRA* encodes methionine sulfoxide reductase A, a ubiquitously expressed and conserved enzyme, including in the heart, with the highest levels in the kidney and the nervous system. Several GWAS association signals near *MSRA* were reported for blood pressure,[12] neuroticism,[13] and glomerular filtration.[14] The biological implication of MSRA in the degenerative process of the valve is unclear and could be through this enzyme protective role against oxidative stress during aging.[15]

As for *FBXO46* locus on Chr 19, we found evidence for association of several genes with MVP including suggestive association with *FBXO46*, and significant association for *SIX5*, *DMPK*, and *DMWD*. This locus overlaps a complex genomic region where CTG repeats in the 3′untranslated region of *DMPK* are involved in myotonic dystrophy, an autosomal dominant disorder characterized by myotonia, muscular dystrophy, cataracts hypogonadism, and cardiac disorders including arrhythmia and mitral valve prolapse (MVP).[16,17] We found that SNPs in this locus are eQTLs of *DMPK* and *DMWD* cardiovascular tissues in genotype tissue expression, although genotype correlation with expression in mitral valve is not known.

We report several genes involved in cardiac development among the top associated genes and enriched GO terms. This applies to *GLIS1* that we have previously implicated in MVP risk,[4] the TGF-β family member *TGFB2*, an essential growth factor for myocardial cells endothelial to mesenchymal transition and valve elongation during valve development,[18] the inhibitor of DNA binding 2 gene (*ID2*) regulated by TGF-β1 and bone morphogenetic protein-7[19] and whose expression is lost in valve forming regions of Smad4-deficient endocardium mice,[20] and *TBX5*, a key regulator of cardiac development.[21] *TBX5*

expression is not detected in human heart valves,[22] which may suggest the valve prolapse phenotype to result from defaults in the interplay between myocardium, conduction tissue, and mitral valve apparatus during heart development or later as a consequence of valve aging. We also report evidence of valve expression in mouse developing hearts for less obvious associated genes, including *SMG6*, *SRR*, and *ABCC3*, whose function in mitral valve myxomatous process may deserve future investigation.

Our study presents several limitations. The inclusion of a new data set from the UK Biobank and the generation of a dense association map did not compensate the limited power of our study, especially for rare and low-frequency variants, and the original suggestive loci described will need to be replicated in future studies. The gene-based results are not able to detect association signals involving gene-desert genomic regions with long-range enhancers, as the one we observe on Chr2 upstream *TNS1.* Another limitation of this method is that gene-rich genomic regions provide redundant association signals involving the same sets of variants and do not allow to point specifically at a potential causal gene. Functional annotation is based on existing eQTLs and gene expression pattern in cardiovascular tissues, especially heart atrial appendage and left ventricle, where cell composition and gene expression may differ from gene expression in the mitral valve.

CONCLUSIONS

To summarize, we report an updated meta-analysis GWAS for MVP using dense imputation coverage and an increased case-control sample. We describe several established and original associated loci and genes with MVP spanning biological mechanisms highly relevant to heart valve disease. Follow-up biological studies in cell and animal models are needed to better understand their direct effect on the valve degenerative process.

ARTICLE INFORMATION

Received July 16, 2020; accepted July 21, 2021.

Affiliations

PARCC, Inserm, Université de Paris, F-75015, Paris, France (M.Y., S.K., X.J., N.B.-N.). Bordeaux Population Health Research Center, Inserm Center U1219, University of Bordeaux, France (S.D.). Department of Neurology, Bordeaux University Hospital, Inserm U1219, France (S.D.). University of Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1167 - RID-AGE - Labex DISTALZ - Risk Factors and Molecular Determinants of Aging-Related Disease, Lille, France (P.A.). Université de Nantes, INSERM, CNRS, CHU Nantes, l'institut du thorax, F-44000, France (J.-J.S., T.L.T., C.D.). Department of Regenerative Medicine and Cell Biology (R.A.N.) and Department of Medicine (R.A.N.), Medical University of South Carolina, Charleston, SC. Assistance Publique – Hôpitaux de Paris, Department of Cardiology, Hôpital Européen Georges Pompidou, France (A.A.H.).

Acknowledgments

We acknowledge the contribution of the Leducq Foundation, Paris, for supporting the genetic study in the French case-control studies. This research has been conducted using the UK Biobank Resource under Application Number 32360.

Sources of Funding

This study was supported by a PhD scholarship from the China Scholarship Council to Dr Yu and French Agency of Research (ANR-16-CE17-0015-02). Drs Kyryachenko and Bouatia-Naji are supported by a European Research Council grant (ERC-Stg-ROSALIND-716628). The recruitment of the mitral valve prolapse (MVP) France cohort was supported by the French Society of Cardiology (SFC). The recruitment of the MVP-Nantes cohort was supported by Fédération Française de Cardiologie, Fondation Coeur et Recherche, French Ministry of Health PHRC-I 2012, and INSERM Translational Research Grant. The genotyping of the controls from the Three-City Study (3C) was supported by the nonprofit organization Fondation Alzheimer (Paris, France). This work was supported in part by grants from the National Institutes of Health (GM103444 to Dr Norris; R01HL131546, RO1HL149696, P20GM103444, and R01HL127692 to Dr Norris; and American Heart Association (19TPA34850095 to Dr Norris, 17CSA33590067 to Dr Norris).

Disclosures

None.

Supplemental Materials

Supplemental Methods
Supplemental Tables I–IV
Supplemental Figures I–VIII
References [23–40]

REFERENCES

1. Dina C, Bouatia-Naji N, Tucker N, Delling FN, Toomer K, Durst R, Perrocheau M, Fernandez-Friera L, Solis J, Le Tourneau T, et al; PROMESA investigators; MVP-France; Leducq Transatlantic MITRAL Network. Genetic association analyses highlight biological pathways underlying mitral valve prolapse. *Nat Genet.* 2015;47:1206–1211. doi: 10.1038/ng.3383
2. Toomer KA, Yu M, Fulmer D, Guo L, Moore KS, Moore R, Drayton KD, Glover J, Peterson N, Ramos-Ortiz S, et al. Primary cilia defects causing mitral valve prolapse. *Sci Transl Med.* 2019;11:eaax0290. doi: 10.1126/scitranslmed.aax0290
3. Freed LA, Levy D, Levine RA, Larson MG, Evans JC, Fuller DL, Lehman B, Benjamin EJ. Prevalence and clinical outcome of mitral-valve prolapse. *N Engl J Med.* 1999;341:1–7. doi: 10.1056/NEJM199907013410101
4. Yu M, Georges A, Tucker NR, Kyryachenko S, Toomer K, Schott JJ, Delling FN, Fernandez-Friera L, Solis J, Ellinor PT, et al. Genome-wide association study-driven gene-set analyses, genetic, and functional follow-up suggest GLIS1 as a susceptibility gene for mitral valve prolapse. *Circ Genom Precis Med.* 2019;12:e002497. doi: 10.1161/CIRCGEN.119.002497
5. Le Tourneau T, Le Scouarnec S, Cueff C, Bernstein D, Aalberts JJJ, Lecointe S, Mérot J, Bernstein JA, Oomen T, Dina C, et al. New insights into mitral valve dystrophy: a Filamin-A genotype-phenotype and outcome study. *Eur Heart J.* 2018;39:1269–1277. doi: 10.1093/eurheartj/ehx505
6. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, Kang HM, Fuchsberger C, Danecek P, Sharp K, et al; Haplotype Reference Consortium. A reference panel of 64,976 haplotypes for genotype imputation. *Nat Genet.* 2016;48:1279–1283. doi: 10.1038/ng.3643
7. Taliun D, Harris DN, Kessler MD, Carlson J, Szpiech ZA, Torres R, et al. Sequencing of 53,831 diverse genomes from the NHLBI TOPMed program. *Nature.* 2021;590:290–299. doi: 10.1038/s41586-021-03205-y
8. Das S, Abecasis GR, Browning BL. Genotype imputation from large reference panels. *Annu Rev Genomics Hum Genet.* 2018;19:73–96. doi: 10.1146/annurev-genom-083117-021602
9. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, Downey P, Elliott P, Green J, Landray M, et al. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med.* 2015;12:e1001779. doi: 10.1371/journal.pmed.1001779
10. de Leeuw CA, Mooij JM, Heskes T, Posthuma D. MAGMA: generalized gene-set analysis of GWAS data. *PLoS Comput Biol.* 2015;11:e1004219. doi: 10.1371/journal.pcbi.1004219
11. Kyryachenko S, Georges A, Yu M, Barrandou T, Guo L, Bruneval P, Rubio T, Gronwald J, Baraki H, Kutschka I, et al. Chromatin accessibility of human mitral valves and functional assessment of MVP risk loci. *Circ Res.* 2021;128:e84–e101. doi: 10.1161/CIRCRESAHA.120.317581
12. Giri A, Hellwege JN, Keaton JM, Park J, Qiu C, Warren HR, Torstenson ES, Kovesdy CP, Sun YV, Wilson OD, et al; Understanding Society Scientific Group; International Consortium for Blood Pressure; Blood Pressure-International Consortium of Exome Chip Studies; Million Veteran Program. Trans-ethnic

association study of blood pressure determinants in over 750,000 individuals. *Nat Genet.* 2019;51:51–62. doi: 10.1038/s41588-018-0303-9
13. Luciano M, Hagenaars SP, Davies G, Hill WD, Clarke TK, Shirali M, Harris SE, Marioni RE, Liewald DC, Fawns-Ritchie C, et al. Association analysis in over 329,000 individuals identifies 116 independent variants influencing neuroticism. *Nat Genet.* 2018;50:6–11. doi: 10.1038/s41588-017-0013-8
14. Wuttke M, Li Y, Li M, Sieber KB, Feitosa MF, Gorski M, Tin A, Wang L, Chu AY, Hoppmann A, et al; Lifelines Cohort Study; V. A. Million Veteran Program. A catalog of genetic loci associated with kidney function from analyses of a million individuals. *Nat Genet.* 2019;51:957–972. doi: 10.1038/s41588-019-0407-x
15. Cabreiro F, Picot CR, Friguet B, Petropoulos I. Methionine sulfoxide reductases: relevance to aging and protection against oxidative stress. *Ann N Y Acad Sci.* 2006;1067:37–44. doi: 10.1196/annals.1354.006
16. Tokgozoglu LS, Ashizawa T, Pacifico A, Armstrong RM, Epstein HF, Zoghbi WA. Cardiac involvement in a large kindred with myotonic dystrophy. Quantitative assessment and relation to size of CTG repeat expansion. *JAMA.* 1995;274:813–819.
17. Bhakta D, Lowe MR, Groh WJ. Prevalence of structural cardiac abnormalities in patients with myotonic dystrophy type I. *Am Heart J.* 2004;147:224–227. doi: 10.1016/j.ahj.2003.08.008
18. Levine RA, Hagége AA, Judge DP, Padala M, Dal-Bianco JP, Aikawa E, Beaudoin J, Bischoff J, Bouatia-Naji N, Bruneval P, et al; Leducq Mitral Transatlantic Network. Mitral valve disease–morphology and mechanisms. *Nat Rev Cardiol.* 2015;12:689–710. doi: 10.1038/nrcardio.2015.161
19. Kowanetz M, Valcourt U, Bergström R, Heldin CH, Moustakas A. Id2 and Id3 define the potency of cell proliferation and differentiation responses to transforming growth factor beta and bone morphogenetic protein. *Mol Cell Biol.* 2004;24:4241–4254. doi: 10.1128/MCB.24.10.4241-4254.2004
20. Moskowitz IP, Wang J, Peterson MA, Pu WT, Mackinnon AC, Oxburgh L, Chu GC, Sarkar M, Berul C, Smoot L, et al. Transcription factor genes Smad4 and Gata4 cooperatively regulate cardiac valve development. [corrected]. *Proc Natl Acad Sci U S A.* 2011;108:4006–4011. doi: 10.1073/pnas.1019025108
21. Steimle JD, Moskowitz IP. TBX5: a key regulator of heart development. *Curr Top Dev Biol.* 2017;122:195–221. doi: 10.1016/bs.ctdb.2016.08.008
22. Hatcher CJ, Goldstein MM, Mah CS, Delia CS, Basson CT. Identification and localization of TBX5 transcription factor during human cardiac morphogenesis. *Dev Dyn.* 2000;219:90–95. doi: 10.1002/1097-0177(200009)219:1<90::AID-DVDY1033>3.0.CO;2-L
23. 3C Study Group. Vascular factors and risk of dementia: design of the Three-City Study and baseline characteristics of the study population. *Neuroepidemiology.* 2003;22:316–325. doi: 10.1159/000072920
24. Anderson CA, Pettersson FH, Clarke GM, Cardon LR, Morris AP, Zondervan KT. Data quality control in genetic case-control association studies. *Nat Protoc.* 2010;5:1564–1573. doi: 10.1038/nprot.2010.116
25. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet.* 2006;38:904–909. doi: 10.1038/ng1847
26. Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, Vrieze SI, Chew EY, Levy S, McGue M, et al. Next-generation genotype imputation service and methods. *Nat Genet.* 2016;48:1284–1287. doi: 10.1038/ng.3656
27. Fuchsberger C, Abecasis GR, Hinds DA. minimac2: faster genotype imputation. *Bioinformatics.* 2015;31:782–784. doi: 10.1093/bioinformatics/btu704
28. Delaneau O, Marchini J, Zagury JF. A linear complexity phasing method for thousands of genomes. *Nat Methods.* 2011;9:179–181. doi: 10.1038/nmeth.1785
29. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, et al; 1000 Genomes Project Analysis Group. The variant call format and VCFtools. *Bioinformatics.* 2011;27:2156–2158. doi: 10.1093/bioinformatics/btr330
30. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet.* 2007;39:906–913. doi: 10.1038/ng2088
31. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics.* 2010;26:2190–2191. doi: 10.1093/bioinformatics/btq340
32. Watanabe K, Taskesen E, van Bochoven A, Posthuma D. Functional mapping and annotation of genetic associations with FUMA. *Nat Commun.* 2017;8:1826. doi: 10.1038/s41467-017-01261-5
33. GTEx Consortium; Laboratory, Data Analysis &Coordinating Center (LDACC)—Analysis Working Group; Statistical Methods groups—Analysis Working Group; Enhancing GTEx (eGTEx) groups; NIH Common Fund; NIH/NCI; NIH/NHGRI; NIH/NIMH; NIH/NIDA; Biospecimen Collection Source Site—NDRI; Biospecimen Collection Source Site—RPCI; Biospecimen Core Resource—VARI; Brain Bank Repository—University of Miami Brain Endowment Bank; Leidos Biomedical—Project Management; ELSI Study; Genome Browser Data Integration &Visualization—EBI; Genome Browser Data Integration &Visualization—UCSC Genomics Institute, University of California Santa Cruz; Lead analysts; Laboratory, Data Analysis &Coordinating Center (LDACC); NIH program management; Biospecimen collection; Pathology; eQTL manuscript working group; Battle A, Brown CD, Engelhardt BE, Montgomery SB. Genetic effects on gene expression across human tissues. *Nature.* 2017;550:204–213. doi: 10.1038/nature24277
34. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res.* 2019;47(D1):D886–D894. doi: 10.1093/nar/gky1016
35. Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, Kasowski M, Karczewski KJ, Park J, Hitz BC, Weng S, et al. Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res.* 2012;22:1790–1797. doi: 10.1101/gr.137323.112
36. Visel A, Thaller C, Eichele G. GenePaint.org: an atlas of gene expression patterns in the mouse embryo. *Nucleic Acids Res.* 2004;32(Database issue):D552–D556. doi: 10.1093/nar/gkh029
37. Diez-Roux G, Banfi S, Sultan M, Geffers L, Anand S, Rozado D, Magen A, Canidio E, Pagani M, Peluso I, et al. A high-resolution anatomical atlas of the transcriptome in the mouse embryo. *PLoS Biol.* 2011;9:e1000582. doi: 10.1371/journal.pbio.1000582
38. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, Boehnke M, Abecasis GR, Willer CJ. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics.* 2010;26:2336–2337. doi: 10.1093/bioinformatics/btq419
39. Glusman G, Caballero J, Mauldin DE, Hood L, Roach JC. Kaviar: an accessible system for testing SNV novelty. *Bioinformatics.* 2011;27:3216–3217. doi: 10.1093/bioinformatics/btr540
40. Liberzon A, Subramanian A, Pinchback R, Thorvaldsdóttir H, Tamayo P, Mesirov JP. Molecular signatures database (MSigDB) 3.0. *Bioinformatics.* 2011;27:1739–1740. doi: 10.1093/bioinformatics/btr260