SUPPLEMENTAL MATERIAL

Four genetic loci influencing electrocardiographic indices of left ventricular hypertrophy. Sonia Shah et al.

The Supplementary Materials consists of the following information:

1. Additional details on the Discovery and Replication Cohorts

Discovery Cohorts

British Women's Heart and Health Study (BWHHS): The BWHHS is a prospective cohort study of heart disease in British women, randomly selected from 23 British Towns who were aged 60 to 79 years at their initial assessment (1999-2001).[1] At baseline each woman completed a medical questionnaire, was interviewed, examined, provided a blood sample and a 12-lead ECG.[1] Illumina HumanCVD genotype data are available on 3443 Caucasian women from the cohort.

Genetic Regulation of Arterial Pressure of Humans in the Community (GRAPHIC) Study: The GRAPHIC Study comprises 2024 individuals from 520 nuclear families recruited from the general population in Leicestershire, UK between 2003-2005 for the purpose of investigating the genetic determinants of blood pressure and related cardiovascular traits.[2] Families were included if both parents aged 40-60 years and two offspring ≥18 years wished to participate. A detailed medical history was obtained from study subjects by standardized questionnaires and clinical examination was performed by research nurses following standard procedures. Measurements obtained included height, weight, waist-hip ratio, clinic and ambulatory blood pressure and a 12-lead ECG.

Whitehall II Study: The Whitehall II study recruited 10,308 participants (70% men) between 1985 and 1989 from 20 London-based Civil service departments.[3] Clinical measurements are taken every 5 years. Blood samples for DNA were collected in 2002–2004 from more than 6000 participants. 12-lead ECG measurements and other phenotype information from phase 7 (2002-2004) examinations were used in the calculation of the ECG-LVH indices investigated in this analysis.

Replication cohorts

British Regional Heart Study (BRHS): The British Regional Heart Study recruited 7735 men aged 40-59. Full details are reported elsewhere.[4] Men were recruited from 24 medium sized British towns between 1978 and 1980. Twenty years later, when aged 60-79, participants were re-measured, including the application of 12-lead ECG, and provided a whole blood sample for DNA analysis.

British Genetics of Hypertension (BRIGHT) Study: The MRC BRIGHT study comprises hypertensive probands ascertained from families with multiplex affected sibships or as

parent-offspring trios, recruited between 1996 and 2002. Case ascertainment and phenotyping has been described previously.[5] Briefly, cases had BP readings ≥150/100 mmHg based on one reading or ≥145/95 mmHg based on the mean of three readings. ECGs were obtained at the time of recruitment and analysed in the same Glasgow Centre as all the discovery cohorts. Only single individuals from each family were genotyped and included in the analysis.

Prevention of Renal and Vascular End stage Disease (PREVEND) Study: The PREVEND Study is a prospective investigation of the natural course of albuminuria, and its relationship to renal and cardiovascular disease, in a large cohort drawn from the general population in the Netherlands. Details of the study protocol are described elsewhere.[6] In total, 8592 subjects were included in the PREVEND baseline cohort (n=7768 with urinary albumin concentration of at least 10mg/L). After exclusion of non-Caucasians, non-available DNA, QRS duration more than 120ms or less than 50 ms, history of myocardial infarction, extreme QRS axis and missing phenotype or covariates, 7060 individuals were included in the present analysis.

2. eQTL analysis in monocyte transcriptomes

Analyses of eQTLs related to the 4 novel ECG-LVH loci were carried out in monocyte transcriptomes from 395 healthy blood donors (recruited from one Centre) and 363 patients with premature myocardial infarction (recruited from 4 centres) assembled by the Cardiogenics consortium (http://www.cardiogenics.eu). All subjects were of white European descent. RNA was extracted from monocytes isolated from whole blood with CD14 micro beads (AutoMacs Pro, Miltenyi). Genomic DNA was extracted from peripheral blood by standard procedures. Gene expression profiling was performed using Illumina Human Ref-8 arrays (Illumina Inc., San Diego, CA) containing 24,516 probes. mRNA was amplified and labelled using the Illumina Total Prep RNA Amplification Kit (Ambion, Inc., Austin, TX). After hybridization, array images were scanned using the 7 Illumina BeadArray Reader and probe intensities were extracted using the Gene expression module of the Illumina Bead Studio software. Variance Stabilization Transformation (VST) was applied to the raw intensities and quantile normalization was performed in the R statistical environment using the Lumi and Beadarray packages. Whole-genome genotyping was carried out using either the Human Custom 1.2M or the Human Quad Custom 670 arrays from Illumina. The expression level of

all genes within 1 Mb of each lead SNP was first assessed. For those genes showing expression, expression was assigned to indicate whether expression was within the top 20%, the bottom 20% or between 20-80% of genes expressed to give an indication of the relative level of expression (high, low or medium, respectively) for that gene in monocytes. The associations of lead SNPs with transcript levels of expressed genes at individual loci was assessed using additive regression models adjusted for age, gender and centre status using Stata 11 software (http://www.stata.com/) . Proxy SNPs (r^2>0.9) were used (if available) where the lead SNP was not present on the genotyping platform. Not all genes were represented on the expression platform. Where a lead SNP showed a significant association with expression, the region was assessed for additional SNPs also showing an eQTL effect on the relevant gene. If another SNP was found showing a stronger association with expression while in weak linkage with the lead SNP (r^2<0.5), a conditional analysis was carried out to determine if the association of the lead SNP with expression was independent of the other variant. The full findings from this analysis are shown in **Supplementary Table 4**. The principle findings are reported in the main paper.

3. eQTL association in publicly-available datasets

We used the Genevar 3.5.0 software (http://www.sanger.ac.uk/resources/ software/ genevar/) to query two publicly available eQTL datasets.[7,8] The first dataset consists of gene expression assessed using the Illumina HumanHT-12 version 3 array in 156 Lymphoblastoid cell line (LCL) samples, 160 skin and 166 fat samples derived simultaneously from a subset of healthy female twins of the MuTHER resource.[7] The same individuals were genotyped using the Illumina 1M-Duo and 1.2M-Duo arrays. The study design (Matched Co-Twin Analysis) permits immediate replication of eQTLs using co-twins.[7] The second dataset consists of 75 samples genotyped on Illumina 550K SNP array with gene expression measured for the same individuals in primary fibroblasts, LCLs and primary T cells samples using the Illumina WG-6 v3 expression array.[8]

We looked for associations of the 4 replicated SNPs and/or their proxies (R2>0.9) with expression of genes up to 1Mb upstream or downstream of the SNP. Significant associations were identified using the default settings (permutation p-value < $1x10^{-03}$). The minor allele of rs2290893 was significantly association with higher *RBMS2* expression in both fibroblast twin sets.[7] Although this SNP was not present in the Dimas et al dataset,[8] several of its

proxies were significantly associated with *RBMS2* expression in fibroblasts. No significant associations were found for these SNPs with other genes and no significant association were found for the other 3 replicated SNPs and/or their proxies.

4. Additional details in the ECHOGEN Study.

Association statistics for echo-LV mass were obtained from the meta-analysis described previously by Vasan et al.[9] The discovery analysis for this study combined data from 5 cohorts (Cardiovascular Health Study, Framingham Heart Study, Rotterdam Study, Multinational Monitoring of Trends and Determinants in Cardiovascular Disease study (MONICA-KORA), and Gutenberg Heart Study) with total sample size N=12,612. Subjects underwent routine trans-thoracic echocardiography, and methodology for measurement of LV dimensions, and calculation of LV mass, are given in detail in Supplementary Ref 9. Within cohort association analyses regressed LV mass on to additively coded (expected) genotype dose, with age, sex, height and weight as covariates, using linear regression (with random effects to account for relatedness where necessary). Results were combined across cohorts using an inverse variance weighted meta-analysis.

5. Estimated power for replication

Power of the replication sample was calculated using the Russ Lenth online power calculator for linear regression (http://www.stat.uiowa.edu/~rlenth/Power) with the standard deviation of the model error taken from GRAPHIC using founders only, a significance threshold of 4.17x10-3 and a replication sample size of 11,777. Initially power was calculated at over 95% to detect the betas observed in the discovery cohort analysis. These estimates did not account for the winner's curse.

In order to formally assess the potential bias introduced by any Winner's Curse, further power calculations were estimated based on an adjusted discovery beta, corrected as described by Zhong and Prentice[10]. This method calculates the expected effect estimate given the error variance, effect size, and p-value threshold used for SNP selection for replication. The detectable beta used in the power calculation is taken as a weighted average of the corrected estimator and the uncorrected estimator. Power was then calculated using Russ Lenth as before.

6. Supplementary References

1. Ebrahim S, Lawlor DA, Shlomo YB, Timpson N, Harbord R, Christensen M, Baban J, Kiessling M, Day I, Gaunt T, Davey-Smith G. Alcohol dehydrogenase type 1C (ADH1C) variants, alcohol consumption traits, HDL-cholesterol and risk of coronary heart disease in women and men: British Women's Heart and Health Study and Caerphilly cohorts. *Artherosclerosis.* 2008; 196:871-878.

2. Tobin MD, Tomaszewski M, Braund PS, Hajat C, Raleigh SM, Palmer TM, Caulfield M, Burton PR, Samani NJ. Common variants in genes underlying monogenic hypertension and hypotension and blood pressure in the general population. *Hypertension*. 2008; 51:1658-1664.

3. Marmot M, and Brunner E. Cohort Profile: the Whitehall II study. *Int J Epidemiol.* 2005; 34: 251-256

4. Walker M, Whincup PH, Shaper AG. The British Regional Heart Study 1975-2004. *Int J Epidemiol.* 2004; 33:1185-1192.

5. Caulfield M, Munroe P, Pembroke J, Samani N, Dominiczak A, Brown M, Benjamin N, Webster J, Ratcliffe P, O'Shea S, Papp J, Taylor E, Dobson R, Knight J, Newhouse S, Hooper J, Lee W, Brain N, Clayton D, Lathrop GM, Farrall M, Connell J. Genome-wide mapping of human loci for essential hypertension. *Lancet*. 2003; 361:2118-2123.

6. Smilde TDJ, Asselbergs FW, Hillege HL, Voors AA, Kors JA, Gansevoort RT, Gilst WH, Jong PE, Van Veldhuisen DJ. Mild renal dysfunction is associated with electrocardiographic left ventricular hypertrophy. *Am J Hypertens. 2005;* 18:342-347.

7. Nica AC, Parts L, Glass D, Nisbet J, Barrett A, Sekowska M, Travers M, Potter S, Grundberg E, Small K, Hedman AK, Bataille V, Tzenova Bell J, Surdulescu G, Dimas AS, Ingle C, Nestle FO, di Meglio P, Min JL, Wilk A, Hammond CJ, Hassanali N, Yang TP, Montgomery SB, O'Rahilly S, Lindgren CM, Zondervan KT, Soranzo N, Barroso I, Durbin R, Ahmadi K, Deloukas P, McCarthy MI, Dermitzakis ET, Spector TD; MuTHER Consortium. The architecture of gene regulatory variation across multiple human tissues: the MuTHER study. *PLoS Genet.* 2011; 7:e1002003

8. Dimas AS, Deutsch S, Stranger BE, Montgomery SB, Borel C, Attar-Cohen H, Ingle C, Beazley C, Gutierrez Arcelus M, Sekowska M, Gagnebin M, Nisbett J, Deloukas P, Dermitzakis

ET, Antonarakis SE. Common regulatory variation impacts gene expression in a cell type-dependent manner *Science*. 2009; 325(5945):1246-1250

9. Vasan RS, Glazer NL, Felix JF, Lieb W, Wild PS, Felix SB, Watzinger N, Larson MG, Smith NL, Dehghan A, Grosshennig A, Schillert A, Teumer A, Schmidt R, Kathiresan S, Lumley T, Aulchenko YS, König IR, Zeller T, Homuth G, Struchalin M, Aragam J, Bis JC, Rivadeneira F, Erdmann J, Schnabel RB, Dörr M, Zweiker R, Lind L, Rodeheffer RJ, Greiser KH, Levy D, Haritunians T, Deckers JW, Stritzke J, Lackner KJ, Völker U, Ingelsson E, Kullo I, Haerting J, O'Donnell CJ, Heckbert SR, Stricker BH, Ziegler A, Reffelmann T, Redfield MM, Werdan K, Mitchell GF, Rice K, Arnett DK, Hofman A, Gottdiener JS, Uitterlinden AG, Meitinger T, Blettner M, Friedrich N, Wang TJ, Psaty BM, van Duijn CM, Wichmann HE, Munzel TF, Kroemer HK, Benjamin EJ, Rotter JI, Witteman JC, Schunkert H, Schmidt H, Völzke H, Blankenberg S. Genetic variants associated with cardiac structure and function: a meta-analysis and replication of genome-wide association data. *JAMA*. 2009; 302:168-78.

10. Zhong H, Prentice RL. Bias-reduced estimators and confidence intervals for odds ratios in genome-wide association studies. *Biostatistics*. 2008; 9:621-634.

7. Funding sources

The BWHHS is supported by funding from the British Heart Foundation (BHF) and the Department of Health Policy Research Programme (England). HumanCVD genotyping of the BWHHHS was funded by the BHF (PG/07/131/24254). Recruitment and genotyping of the GRAPHIC Study was funded by the BHF. The GRAPHIC Study is part of the research portfolio supported by the Leicester NIHR Biomedical Research Unit in Cardiovascular Disease. The WHII study has been supported by grants from the Medical Research Council; Economic and Social Research Council; BHF; Health and Safety Executive; Department of Health; National Heart Lung and Blood Institute (HL36310), US, NIH: National Institute on Aging (AG13196), US, NIH; Agency for Health Care Policy Research (HS06516); and the John D and Catherine T MacArthur Foundation Research Networks on Successful Midlife Development and Socio-economic Status and Health. Genotyping in WHII was supported by BHF grant PG/07/133/24260. J.F.F. is member of the Netherlands Consortium on Healthy Ageing. N.J.S., S.H and A.F.D hold Chairs funded by the BHF. Support for the replication cohorts, Cardiogenics and ECHOGEN are described in Supplementary Materials.

8. List of members of the ECHOGEN, Cardiogenics and BRIGHT Studies

ECHOGEN Study: Ramachandran S. Vasan, MD,[1] Wolfgang Lieb,[4] MD, Philipp S. Wild, MD,[5] Stephan B. Felix, MD,[6] Norbert Watzinger, MD,[7] Martin G. Larson ScD, [1] Nicholas L. Smith, PhD,[2] Abbas Dehghan, MD, DSc,[3] Anika Großhennig, PhD,[4] Arne Schillert, PhD,[5] Alexander Teumer,[6] Reinhold Schmidt, MD,[7] Sekar Kathiresan[1], MD, Thomas Lumley, PhD,[2] Yurii S. Aulchenko, PhD,[3] Inke R. König, PhD,[4] Tanja Zeller, PhD,[5] Georg Homuth, PhD,[6] Maksim Struchalin,[3] Jayashri Aragam[1], MD, Joshua C. Bis, PhD,[2] Fernando Rivadeneira, MD, PhD,[3] Jeanette Erdmann, PhD,[4] Renate B. Schnabel, MD,[5] Marcus Dörr, MD,[6] Robert Zweiker, MD,[7] Lars Lind, MD, PhD,[8] Richard J. Rodeheffer, MD,[9] Karin Halina Greiser, MD,[10] Daniel Levy [1,11], MD, Talin Haritunians, PhD,[2] Jaap W. Deckers, MD, PhD,[3] Jan Stritzke, MD,[4] Karl J Lackner, PhD,[5] Uwe Völker, PhD,[6] Erik Ingelsson, MD, PhD,[8] Iftikhar Kullo, MD,[9] Johannes Haerting, PhD,[10] Christopher J. O'Donnell,[1] MD, Susan R. Heckbert, MD, PhD,[2] Bruno H. Stricker, MB, PhD,[3] Andreas Ziegler, PhD,[5] Thorsten Reffelmann, MD,[6] Margaret M. Redfield, MD,[9] Karl Werdan, MD,[10] Gary F. Mitchell, MD,[1] Kenneth Rice, PhD,[2] Donna Arnett, PhD,[2] Albert Hofman, MD, PhD,[3] John S. Gottdiener, MD,[2] Andre G. Uitterlinden, PhD,[3] Thomas Meitinger, MD,[4] Maria Blettner, PhD,[5] Nele Friedrich, PhD,[6] Thomas J Wang[1], MD, Bruce M. Psaty,[2] MD, Cornelia M van Duijn, PhD,[3] H.-Erich Wichmann, MD,[4] Thomas F. Munzel, MD,[5] Heyo K. Kroemer, PhD,[6] Emelia J. Benjamin[1], MD, ScM, Jerome I. Rotter, MD,[2] Jacqueline C. Witteman, PhD,[3] Heribert Schunkert, MD,[4] Helena Schmidt, MD, PhD,[7] Henry Völzke, MD,[6] Stefan Blankenberg, MD.[5]

[1]National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, MA; [2]The Cardiovascular Health Study; [3]Rotterdam Study, Erasmus MC, The Netherlands; [4]MONICA/KORA Augsburg Echocardiographic Substudy, Germany ; [5]Gutenberg Heart Study, Germany; [6]Study of Health in Pomerania, Germany; [7]Austrian Stroke Prevention Study, Austria; [8]PIVUS Study, Sweden; [9]Mayo Clinic, Rochester, MN; [10]CARLA Study, Germany; [11]Center for Population Studies, National Heart, Lung, and Blood Institute, Bethesda, MD

Acknowledgments and funding sources for the ECHOGEN Study are given in Reference 17.

Cardiogenics: Tony Attwood[1], Stephanie Belz[2], Jessy Brocheton[3], Jason Cooper[4], Abi Crisp-Hihn[1], Nicola Foad[1], Panos Deloukas[5], Jeanette Eardman[2], Tiphaine Godfroy[3], Jay Gracey[6], Rhian Gwilliams[5], Susanne Heimerl[7], Jennifer Jolley[1], Unni Krishnan[6], Heather Lloyd-Jones[1], Per Lundmark[8], Seraya Maouche[3], Gilles Montalescot[3], Jasbir S Moore[6], David Muir[1], Elizabeth Murray[1], Chris P Nelson[6], David Niblett[5], Karen O'Leary[1], Helen Pollard[6], Carole Proust[3], Angela Rankin[1], Augusto Rendon[9], Catherine M Rice[5], Hendrick Sager[2], Jennifer Sambrook[1], Gerd Schmitz[10], Michael Scholz[11], Laura Schroeder, Heribert Schunkert[2], Ann-Christine Syvannen[8], Chris Wallace[4]

[1]Department of Haematology, University of Cambridge, Long Road, Cambridge, CB2 2PT, UK and National Health Service Blood and Transplant, Cambridge Centre, Long Road, Cambridge, CB2 2PT, UK; [2]Medizinische Klinik 2, Universität zu Lübeck, Lübeck Germany; [3]INSERM UMRS 937, Pierre and Marie Curie University (UPMC, Paris 6) and Medical School, 91 Bd de l'Hôpital 75013, Paris, France; [4]Juvenile Diabetes Research Foundation/Wellcome Trust Diabetes and Inflammation Laboratory, Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, Wellcome Trust/MRC Building, Cambridge, CB2 0XY, UK; [5]The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK; [6]Department of Cardiovascular Sciences, University of Leicester, Glenfield Hospital, Groby Road, Leicester, LE3 9QP, UK; [7]Klinik und

Poliklinik für Innere Medizin II, Universität Regensburg, Germany, [8] Molecular Medicine, Department of Medical Sciences, Uppsala University, Uppsala, Sweden; [9]European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SD, UK; [10]Institut für Klinische Chemie und Laboratoriumsmedizin, Universität, Regensburg, D-93053 Regensburg, German;, [11] Trium, Analysis Online GmbH, Hohenlindenerstr. 1, 81677, München, Germany

The Cardiogenics Project is funded by the European Union (LSHM-CT 2006-037593). Healthy subjects for the Cardiogenics eQTL study were recruited from the NIHR funded Cambridge BioResource.

BRIGHT Study: Toby Johnson,[1] Stephen J. Newhouse,[1] Mark Caulfield,[1]Patricia B. Munroe,[1] Philip J. Howard,[1] Abiodun Onipinla,[1] Anna Dominiczak,[2] Nilesh J. Samani,[3] Martin Farrall,[4] Morris Brown,[5] Mark Lathrop,[6] John Connell.[7]

[1]Clinical Pharmacology and Barts and the London Genome Centre, William Harvey Research Institute, Barts and the London School of Medicine, Queen Mary University of London, London, UK; [2]BHF Glasgow Cardiovascular Research Centre, Division of Cardiovascular and Medical Sciences, University of Glasgow, Western Infirmary, Glasgow, UK; [3]Department of Cardiovascular Sciences, University of Leicester, Glenfield Hospital, Leicester, UK; [4]Department of Cardiovascular Medicine, University of Oxford, Wellcome Trust Centre for Human Genetics, Oxford, UK; [5]Clinical Pharmacology and the Cambridge Institute of Medical Research, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK; [6]Centre National de Genotypage, Evry, France; 7 University of Dundee, Medical School, Ninewells Hospital and Medical School Dundee, UK.

Recruitment and phenotyping of the BRIGHT Study subjects was funded by the Medical Research Council and the British Heart Foundation. Human CVD50 genotyping of the BRIGHT study was funded by the BHF (PG/07/129/24243). The work of T.J., M.C and P.B.M is part of the research themes contributing to the translational research portfolio of Barts and the London Cardiovascular Biomedical Research Unit which is supported and funded by the National Institute of Health Research.

9. Supplementary Tables and Figures

Supplementary Table 1: Pearson correlation coefficients for traits and covariates.

		Cornell Product	Sokolow-Lyon	QRS Voltage Sum	QRS Voltage Product
Cornell Product	BWHHS GRAPHIC WHII	*1.000*			
Sokolow-Lyon	BWHHS GRAPHIC WHII	-0.0139 -0.0588 -0.0265	*1.000*		
QRS Voltage Sum	BWHHS GRAPHIC WHII	0.3917 0.1199 0.330	0.614 0.7499 0.684	*1.000*	
QRS Voltage Product	BWHHS GRAPHIC WHII	0.6138 0.2121 0.444	0.429 0.6425 0.500	0.8434 0.9269 0.880	*1.000*
Sex	BWHHS GRAPHIC WHII	- 0.0626 0.121	- -0.2232 -0.149	- -0.4614 -0.321	- -0.5212 -0.3423
Age	BWHHS GRAPHIC WHII	0.1276 0.0539 0.149	0.0077 -0.2964 -0.0154	0.1196 -0.3974 0.0117	0.1561 -0.3249 0.0385
BMI	BWHHS GRAPHIC WHII	0.1994 0.0703 0.203	-0.167 -0.2265 -0.289	-0.0832 -0.2299 -0.154	-0.0035 -0.1602 -0.0919
Systolic BP	BWHHS GRAPHIC WHII	0.2483 0.1433 0.298	0.1797 0.1590 0.0767	0.2781 -0.2178 0.179	0.2894 0.2444 0.187

Supplementary Table 2: Heritability of ECG indices of Left Ventricular Hypertrophy in the GRAPHIC Study

Trait	Adjusting for Age, Age^2, Sex,		Adjusting for further covariates†	
	Heritability (SE)	**P-Value**	**Heritability (SE)**	**P-Value**
Sokolow-Lyon Index	0.3980 (0.0462)	1.28E-18	0.3880 (0.0482)	8.75E-17
QRS Voltage Product	0.3236 (0.0459)	8.93E-14	0.3179 (0.0485)	3.38E-12
QRS Voltage Sum	0.4356 (0.0446)	1.18E-23	0.4455 (0.0470)	2.06E-22
Cornell Product	0.4013 (0.0452)	9.73E-20	0.3866 (0.0476)	5.73E-17

Heritability calculated using SOLAR in 1,799-1,881 subjects dependent upon trait. †Further adjustments made for SBP, DBP, BMI, HDL, LDL, total cholesterol, triglycerides, history of hypertension, history of diabetes, waist-girth, waist-hip ratio, ever-smoked, plasma sodium, plasma blood potassium, CRP and eGFR.

Supplementary Table 3: Quality filters and SNP exclusion criteria

Number of SNPs excluded in the three discovery cohorts prior to meta-analysis based on specific quality filters.

Exclusion criteria	BWHHS	GRAPHIC	WHII
No. of SNPs with missingness >5%	4	0	821
No. of SNPs with MAF<1%	11,737	12,583	12,494
No. of SNPs with HWE p<0.0001	124	130	171
Other	2908	484	636
No. of SNPs entering analysis	34,321	35,897	34,972

MAF = minor allele frequency; HWE = Hardy-Weinberg equilibrium (p-value estimated for controls/founders only).; Other = SNPs that had model convergence errors during the analysis process.

Supplementary Table 4: Expression QTL (eQTL) analysis in monocytes for the 4 ECG-LVH associated loci.

Region (lead SNP)	Gene Name	Gene expressed?	Lead SNP monocyte association P-value	Strength +	SNP used for conditional analysis (r^2 with lead SNP)	Conditional SNP monocyte association p-value	P-value of lead SNP conditional on secondary SNP
PTGES3 (rs2958155)	SMARCC2		0.01679	2			
	RNF41		0.67552	2			
	OBEC2B	NO					
	SLC39A5	NO					
	ANKRD52	N/A					
	COQ10A		0.26685	2			
	CS		0.00839	3			
	CNPY2	N/A					
	PAN2		0.54765	2			
	IL23A	NO					
	STAT2		0.01746	3			
	APOF	NO					
	MIP	NO					
	TIMELESS		0.38097	2			
	SPYRD4	NO					
	GLS2	NO					
	RBMS2		$1.85x10^{-37}$	2	rs2958139 (0.498)	$5.80x10^{-59}$	0.40209
	BAZ2A	NO					
	ATP5B		0.08482	3			
	PTGES3		0.20874	3			
	NACA		0.02300	3			
	PRIM1		2.59E-08	2	rs2277339 (0.048)	$6.98x10^{-10}$	$3.06x10^{-5}$
	HSD17B6	NO					
	SDR-O	NO					
	RDH16	NO					
	GPR182	NO					
	ZBTB39		0.31714	2			
	TAC3		0.27768	2			
	MYO1A		0.16332	1			
	TMEM194A	N/A					
	NAB2		0.41266	1			
	STAT6		0.04233	3			
	LRP1		0.26583	2			
NMB (rs2292462)	ZSCAN2		0.01280	1			
	WDR73		9.15E-09	2	rs3817193 (0.209)	$1.43x10^{-6}$	$6.17x10^{-5}$
	NMB		9.27E-11	1	rs2271431 (0.476)	$3.67x10^{-20}$	0.9588
	SEC11A		0.11803	3			
	ZNF592	NO					
	ALPK3	NO					
	SLC28A1		0.76695	1			
	PDE5A	NO					
SCN5A (rs6797133)	DLEC1	NO					
	ACAA1		0.14294	3			
	MYD88		0.46627	3			
	OXSR1		0.82544	3			
	SLC22A13	NO					
	SLC22A14	NO					
	XYLB	N/A					
	ACVR2B	NO					
	ENDOGL1		0.50560	2			
	SCN5A	NO					
	SCN10A	NO					
	SCN11A	NO					
	WDR48		0.40001	2			
	GORASP1		0.97697	2			
	TTC21A		0.53859	2			
IGF1R (rs4616271)	FAM169B	NO					
	IGF1R	NO					
	LOC145814	N/A					
	DMN	N/A					
	TTC23		0.99562	1			

Supplementary Table 5: Percentage of total variance explained by each of the four novel ECG-LVH loci in each study individually and combined*

SNP	Gene Locus	Trait	BWHHS	GRAPHIC	WHII	Combined
rs6797133	*SCN5A*	Cornell Product	0.12	0.08	0.25	0.12 (0.02-0.30)
rs2290893	*PTGES3*	QRS Voltage Sum	0.26	0.46	0.06	0.28 (0.09 - 0.06)
rs2292462	*NMB*	QRS Voltage Sum	0.11	0.17	0.34	0.17 (0.05 - 0.38)
rs4966014	*IGF1R*	QRS Voltage Sum	0.28	0.01	0.11	0.09 (0.001 - 0.33)

*Combined variance calculated from a meta-analysis of the regression correlation coefficients and shown with 95% confidence intervals.

Supplementary Table 6: Association analysis of the four ECG-LVH associated loci with echo-determined LV mass

The echo LV mass–SNP associations were analysed *in silico* in the EchoGen Consortium GWAS meta-analysis of echocardiographically determined LV mass by Vasan et al.[9]

SNP	Gene	Chr	Position	Coded Allele	Non-Coded Allele	Meta Beta	Meta SE	Meta p-value	Mean MAF
rs2290893	PTGES3	12	55364887	A	G	-0.0774	0.4574	0.8656	0.3609
rs2292462	NMB	15	83001758	G	T	-0.3252	0.4482	0.4681	0.4570
rs4966014	IGF1R	15	97065541	C	T	0.1816	0.5375	0.7355	0.3193
rs6797133	SCN5A	3	38631037	A	G	0.1697	0.4526	0.7077	0.3924

Supplementary Table 7: Association analysis of candidate renin-angiotensinogen system polymorphisms with ECG-LVH traits

SNP	A1	A2	Gene (Polymorphism)	Index of Left Ventricular Hypertrophy	Discovery Beta (SE)	Discovery P-value
rs5186	C	A	AGTR1 (A1166C)	Cornell Product	-1.64 (0.86)	0.058
				QRS Voltage Sum	-13.6 (46.2)	0.77
				QRS volatge Product	-3.58 (5.73)	0.53
				Sokolow-Lyon	-4.81 (9.56)	0.61
rs699	C	G	AGT (M235T)	Cornell Product	0.25 (0.79)	0.75
				QRS Voltage Sum	46.2 (43.5)	0.29
				QRS volatge Product	13.0 (5.36)	0.016
				Sokolow-Lyon	7.89 (8.99)	0.38
rs1799998	C	T	CYP11B2 (-344 C/T)	Cornell Product	-0.0052 (0.81)	0.99
				QRS Voltage Sum	48.1 (82.6)	0.56
				QRS volatge Product	4.16 (10.8)	0.70
				Sokolow-Lyon	-5.92 (12.4)	0.63
rs4343	A	G	ACE (G2350A, I/D)	Cornell Product	-0.27 (0.80)	0.74
				QRS Voltage Sum	34.7 (43.1)	0.42
				QRS volatge Product	1.83 (5.30)	0.36
				Sokolow-Lyon	6.68 (8.86)	0.45

Supplementary Table 8: Association of variants in LVH candidate genes with ECG-LVH traits.

We identified genes that are either annotated with the gene ontology term cardiac muscle hypertrophy (GO:0003300) or with the OMIM term hereditary ventricular hypertrophy (MIM: 192600). Additional genes implicated in LVH were also identified through literature search. Relevant references are included below the Table. The table shows the discovery meta-analysis summary statistics for SNPs in candidate genes. Only the most significant association with any one of the indices of left ventricular hypertrophy are shown for each gene.

Biological Process	SNP	CHR	BP	A1	A2	Gene	Ref	SNPs on 50K array	Index of Left Ventricular Hypertrophy	Discovery Beta (95% CI)	Discovery P-value
Calcium Homeostasis	rs2746073	1	191045850	A	T	RGS2	GO:0003300	2	Cornell Product	-2.03 (-3.78 , -0.28)	0.0229
	rs7554607	1	235333226	G	A	RYR2	Yamaguchi et al	247	QRS Voltage Sum	137.3 (57.03 , 217.5)	0.000799
	rs3752581	6	118976423	G	A	PLN	Shanmugam et al	3	Sokolow-Lyon	-15.19 (-32.3 , 1.92)	0.0818
	rs10849860	12	120152637	G	A	P2RX4	GO:0003300	5	Cornell Product	-1.93 (-3.97 , 0.11)	0.0632
Hemodynamic load	rs2493129	1	228907565	A	G	AGT	Kaufman et al	88	Cornell Product	-5.62 (-10.37 , -0.86)	0.0206
	rs12721272	3	149929654	A	G	AGTR1	Kaufman et al	47	QRS Voltage Sum	293.5 (31.68 , 555.3)	0.028
	rs4714384	6	12405839	G	A	EDN1	GO:0003300	27	QRS Voltage Product	-11.93 (-22.6 , -1.25)	0.0286
	rs2853796	7	150334848	C	A	NOS3	Xin et al	24	QRS Voltage Product	-13.97 (-24.05 , -3.9)	0.00656
	rs4917675	10	115789467	G	A	ADRB1	Fu et al	12	QRS Voltage Product	12.13 (0.65 , 23.61)	0.0384
	rs4354	17	58925184	A	G	ACE	Kaufman et al	43	QRS Voltage Product	-39.16 (-72.32 , -6.01)	0.0206
	rs4646124	23	15526717	A	G	ACE2	Lieb et al	18	Cornell Product	-2.27 (-4.25 , -0.29)	0.0244
Structural Proteins	rs868407	1	199607964	G	A	TNNT2	OMIM: 192600	12	Sokolow-Lyon	17.25 (-1.48 , 35.99)	0.071
	rs936175	3	46879712	A	C	MYL3	OMIM: 192600	8	QRS Voltage Product	11.54 (-3.89 , 26.98)	0.143
	rs10865971	3	52456446	G	A	TNNC1	OMIM: 192600	3	QRS Voltage Product	13.91 (-0.66 , 28.47)	0.0613
	rs3729989	11	47326617	G	A	MYBPC3	OMIM: 192600	9	Cornell Product	3.6 (1.32 , 5.88)	0.00197
	rs7931539	11	107091031	G	A	SLN	Shanmugam et al	5	QRS Voltage Product	-11.6 (-23.91 , 0.7)	0.0646
	rs3729823	14	22956249	G	C	MYH7	OMIM: 192600	1	QRS Voltage Product	-7.88 (-69.15 , 53.38)	0.801
	rs893132	15	32877352	G	A	ACTC1	OMIM: 192600	13	QRS Voltage Product	-16.11 (-26.19 , -6.03)	0.00173
	rs17752921	15	61130684	G	A	TPM1	OMIM: 192600	10	Cornell Product	-2.99 (-5.51 , -0.47)	0.0202
	rs3729709	19	60359618	G	A	TNNI3	OMIM: 192600	6	Cornell Product	-4.27 (-7.16 , -1.38)	0.00374

Other	rs12032720	1	153541584	G	C	FDPS	GO:0003300	3	Cornell Product	1.45 (-0.32 , 3.22)	0.108
	rs3817368	2	68951366	C	A	BMP10	GO:0003300	3	Cornell Product	-1.02 (-2.6 , 0.55)	0.202
	rs4973377	2	231690236	A	G	HTR2B	GO:0003300	10	Cornell Product	2.26 (0.17 , 4.35)	0.0344
	rs3791596	2	239796293	G	C	HDAC4	GO:0003300	77	QRS Voltage Product	19.63 (4.52 , 34.74)	0.0109
	rs7647790	3	8747089	A	G	CAV3	GO:0003300	22	QRS Voltage Sum	330.4 (30.56 , 630.3)	0.0308
	rs34417936	5	55283892	A	G	IL6ST	GO:0003300	20	QRS Voltage Sum	285.4 (28.7 , 542)	0.0293
	rs11771414	7	151073786	A	G	PRKAG2	OMIM: 192600	118	QRS Voltage Product	-16.25 (-26.3 , -6.2)	0.00153
	rs2061821	15	83923658	A	G	AKAP13	GO:0003300	12	Cornell Product	1.85 (0.25 , 3.45)	0.0233

References:

Kaufman BD, Auerbach S, Reddy S, Manlhiot C, Deng L, Prakash A, Printz BF, Gruber D, Papavassiliou DP, Hsu DT, Sehnert AJ, Chung WK, Mital S. RAAS gene polymorphisms influence progression of pediatric hypertrophic cardiomyopathy. *Hum Genet.* 2007; 122(5): 515-523.

Shanmugam M, Gao S, Hong C, Fefelova N, Nowycky MC, Xie LH, Periasamy M, Babu GJ. Ablation of phospholamban and sarcolipin results in cardiac hypertrophy and decreased cardiac contractility. *Cardiovasc Res.* 2011; 89(2):353-361.

Yamaguchi N, Takahashi N, Xu L, Smithies O, Meissner G. Early cardiac hypertrophy in mice with impaired calmodulin regulation of cardiac muscle Ca release channel. *J Clin Invest*. 2007; 117(5):1344-1353.

Takimoto E, Champion HC, Li M, Belardi D, Ren S, Rodriguez ER, Bedja D, Gabrielson KL, Wang Y, Kass DA. Chronic inhibition of cyclic GMP phosphodiesterase 5A prevents and reverses cardiac hypertrophy. *Nat Med*. 2005; 11(2):214-222.

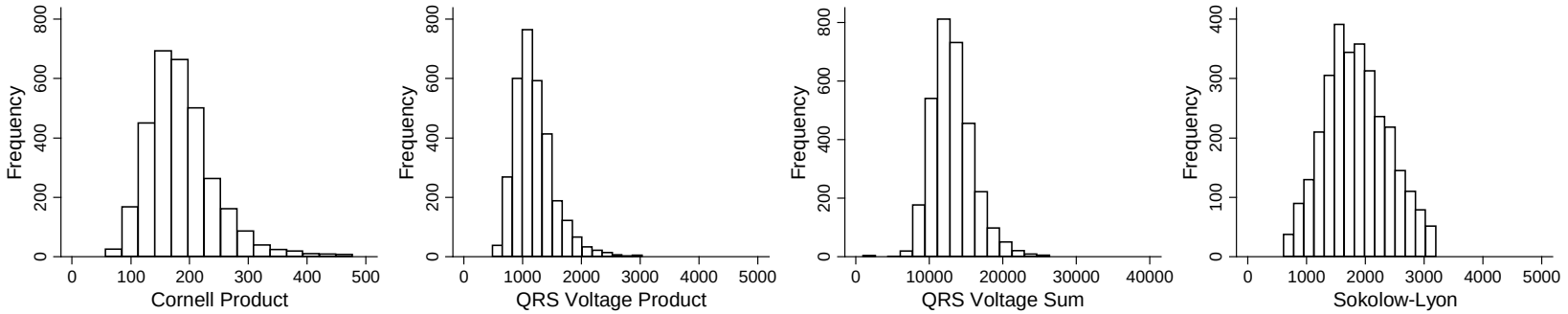
Xin Y, Song X, Xue H, Liu Z, Wang X, Wang H, Sun K, Bai Y, Liu J, Hui R. A common variant of the eNOS gene (E298D) is an independent risk factor for left ventricular hypertrophy in human essential hypertension. *Clin Sci (Lond)*. 2009; 117(2):67-73.

Fu C, Wang H, Wang S, Shi Y, Zhou X, Sun K, Chen J, Song X, Xue H, Hui R. Association of beta 1-adrenergic receptor gene polymorphisms with left ventricular hypertrophy in human essential hypertension. *Clin Biochem*. 2008;41(10-11):773-778.

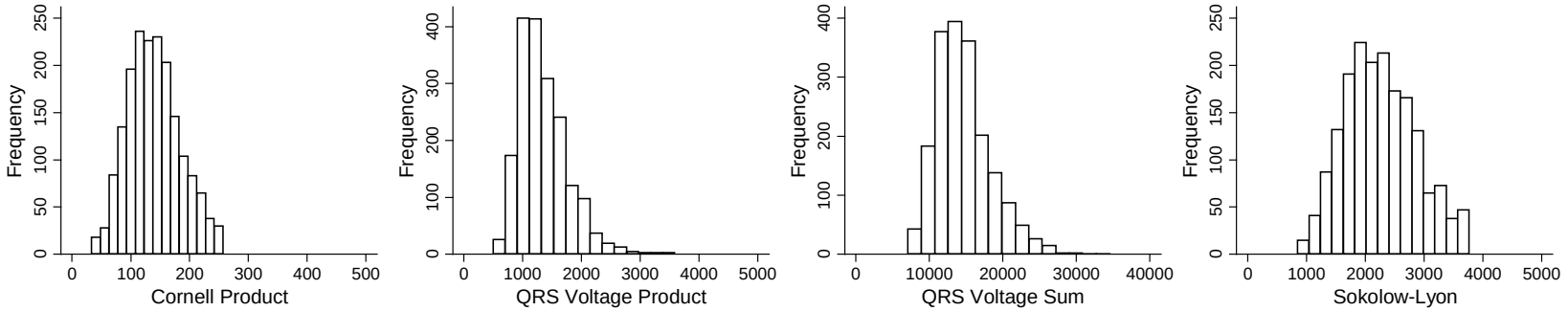
Lieb W, Graf J, Gotz A, Konig IR, Mayer B, Fischer M, Stritzke J, Hengstenberg C, Holmer SR, Doring A, Lowel H, Schunkert H, Erdmann J. Association of angiotensin-converting enzyme 2 (ACE2) gene polymorphisms with parameters of left ventricular hypertrophy in men. Results of the MONICA Augsburg echocardiographic substudy. *J Mol Med*. 2006; 84:88–96.

Supplementary Figure 1: ECG-LVH Trait Distributions in the Discovery Cohorts.

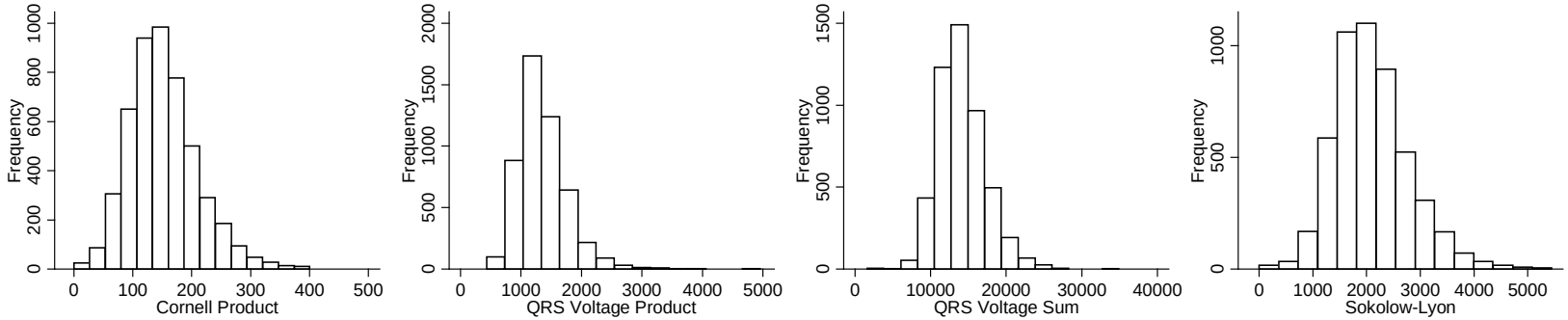
A) BWHHS Trait Distributions



B) GRAPHIC Trait Distributions

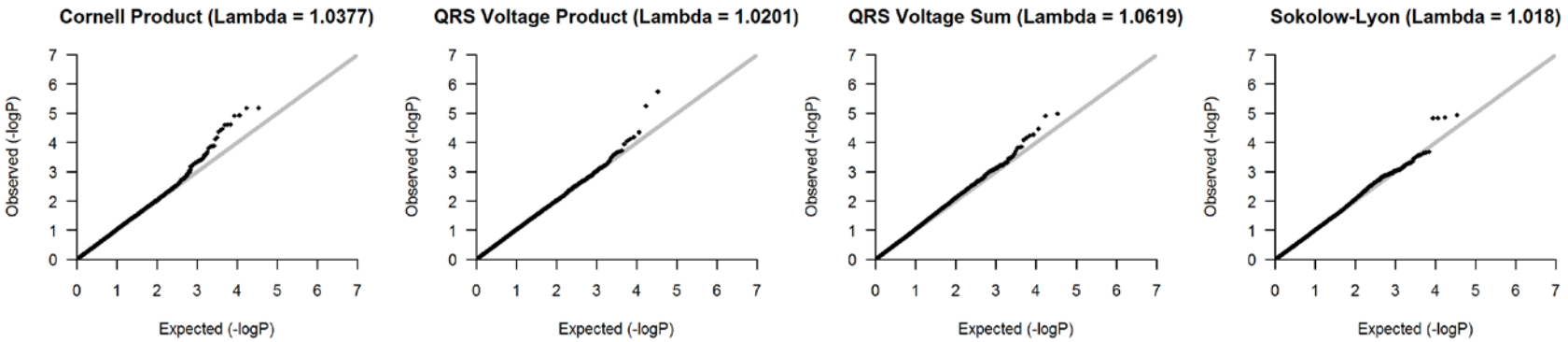


C) Whitehall II Trait Distributions

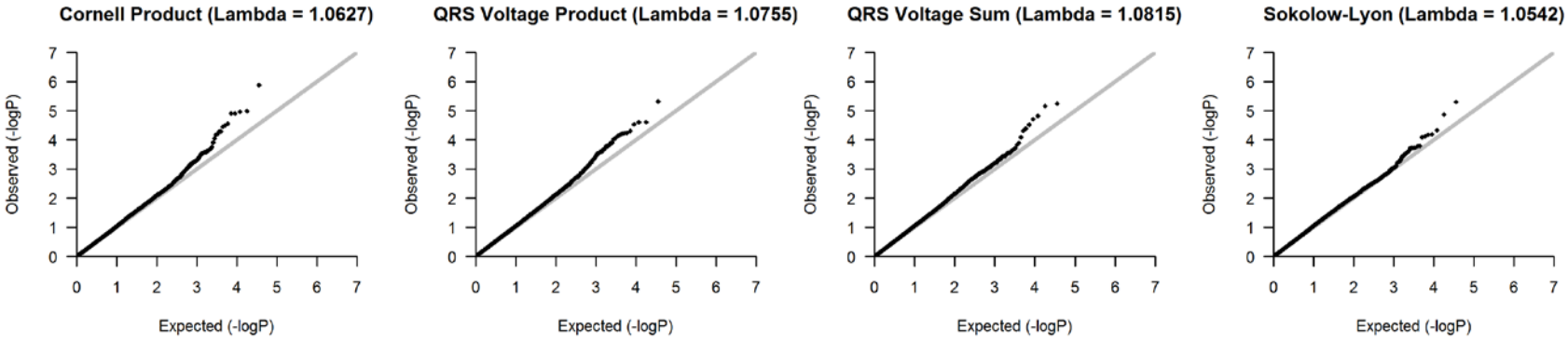


Supplementary Figure 2: QQ plots showing genomic control by study per trait

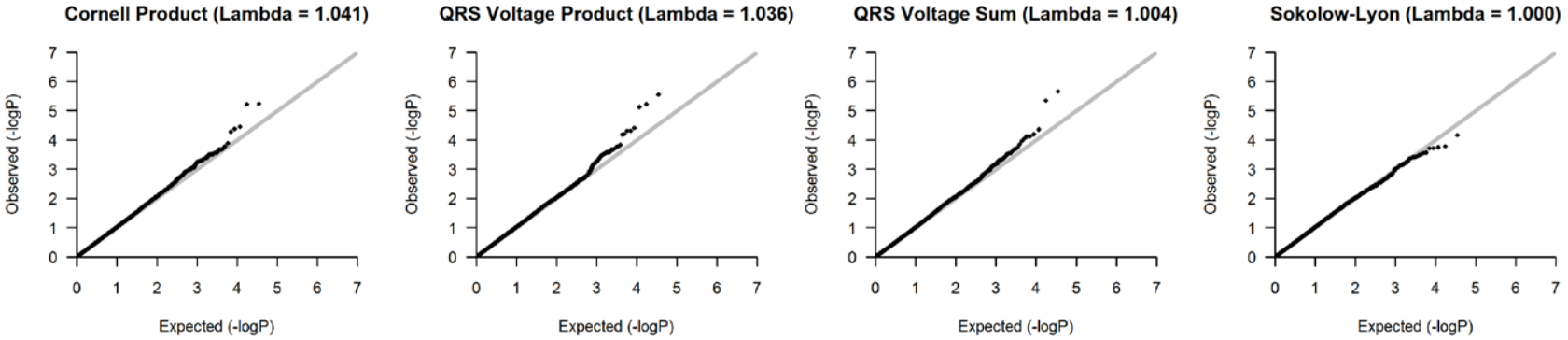
A) BWHHS



B) GRAPHIC

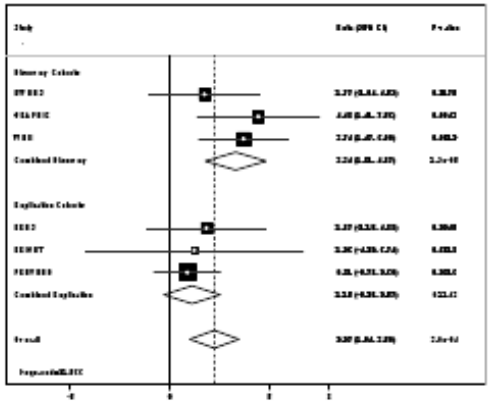


C) Whitehall II

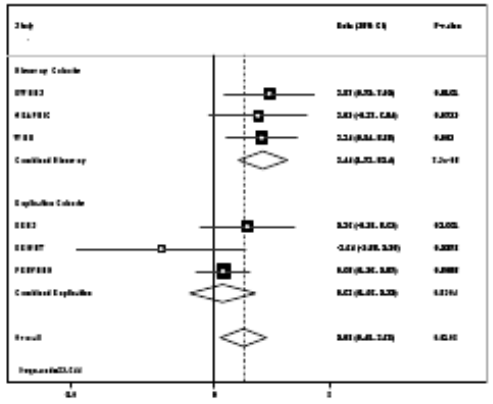


Supplementary Figure 3: Forest plots for SNPs that did not show association, after Bonferroni correction, in the replication samples. Plots show beta coefficients with 95% confidence intervals and p-values in each individual cohort as well as the meta-analysis of the discovery and replication cohorts.

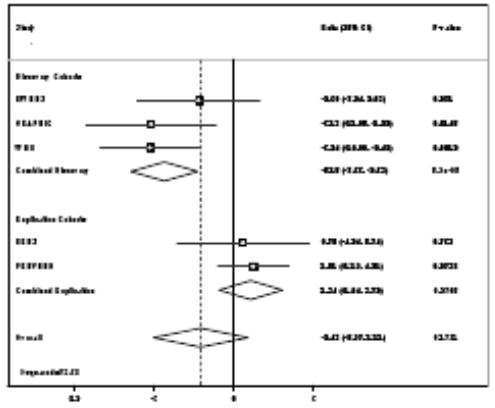
Cornell Product *MYOZ2*
rs999310



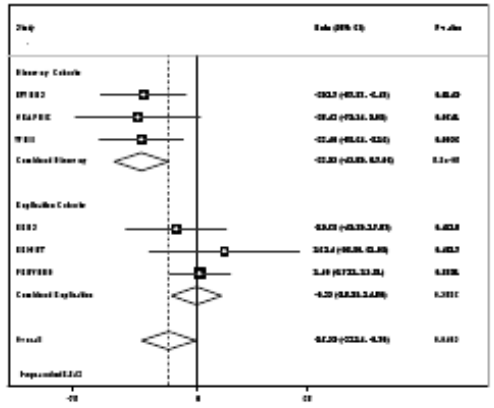
Cornell Product *SERPING1*
rs11603020



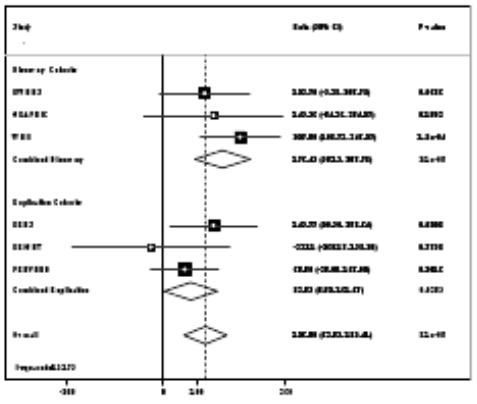
Cornell Product *TMEM117*
rs860867



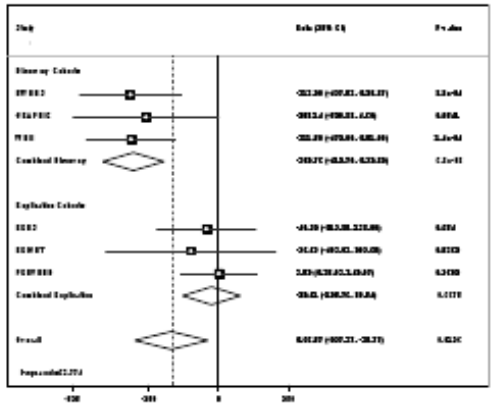
QRS Voltage Product *PPARD*
rs4713845



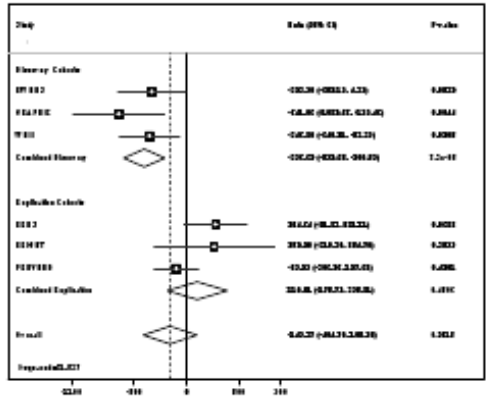
QRS voltage Sum *FABP2*
rs11724758



QRS Voltage Sum *MTRR*
rs3815743



QRS Voltage Sum *TCN1*
rs17597065



Sokolow-Lyon *TTN*
rs10497520