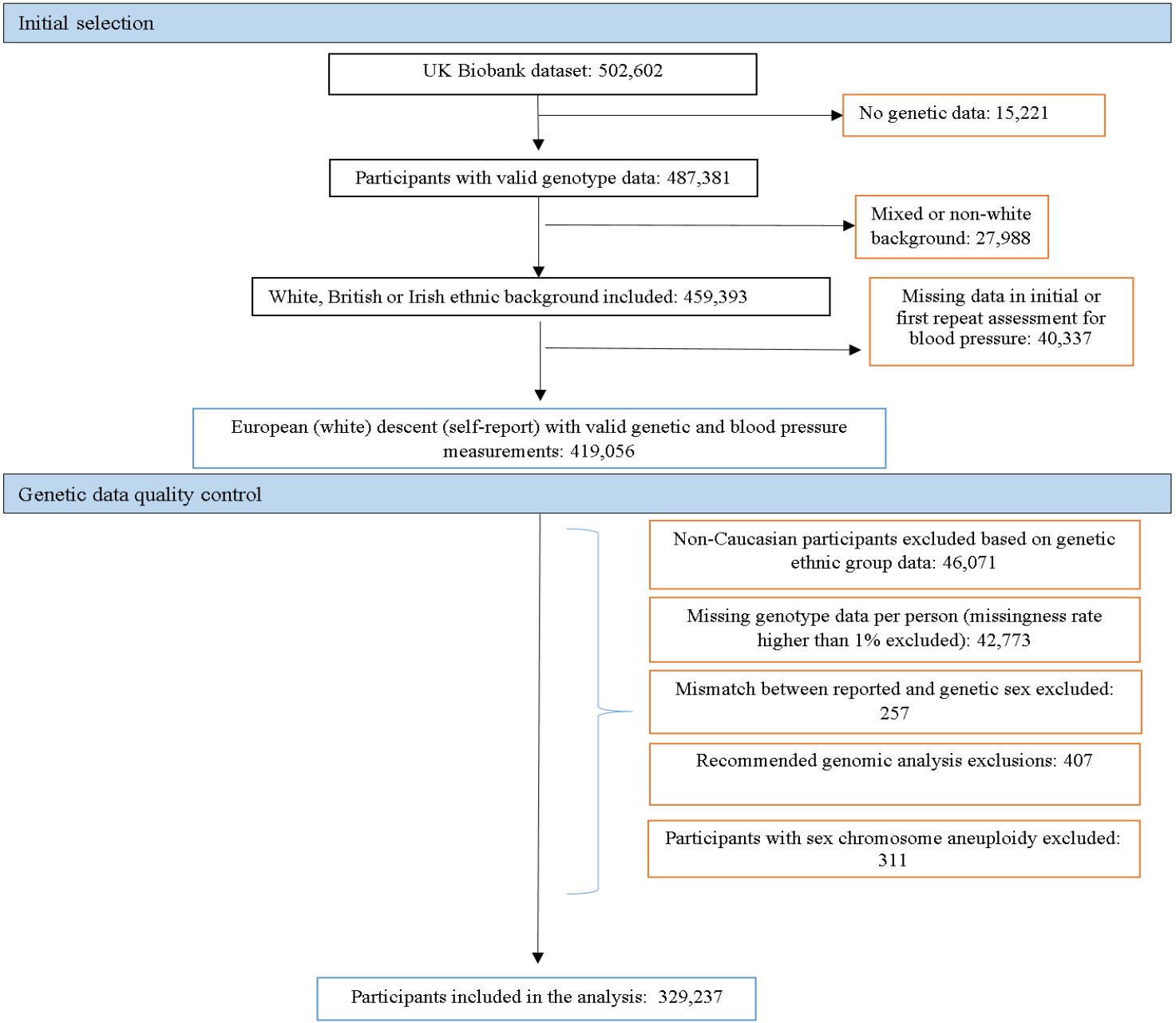
Supplementary Online Content

Nazarzadeh M, Pinho-Gomes A-C, Smith Byrne K, et al. Systolic blood pressure and risk of valvular heart disease: a mendelian randomization study. *JAMA Cardiol*. Published online July 10, 2019. doi:10.1001/jamacardio.2019.2202

This supplementary has been provided by the authors to give readers additional information about their work.



eTable 1. Systolic blood pressure related variants used for generation of weighted genetic risk score (Evangelou et al)[1].

Locus name	rs ID	Chromosome/Position	Effect allele	Combined beta	*P* value	Genome wide association with risk factors of valvular or coronary heart disease
RXFP2	rs9532243	13:32191408	A	0.2709	6.43E-29	No
L2HGDH	rs72683923	14:50735947	T	0.9769	1.40E-25	No
PBX3	rs7023828	9:128498594	T	-0.2436	1.22E-22	No
KDM4B	rs2613765	19:5066330	A	-0.2336	9.01E-22	No
YES1	rs34413141	18:777282	A	-0.3084	2.10E-21	No
LRP4	rs1585453	11:46884713	A	-0.3892	3.53E-21	No
TERT	rs10069690	5:1279790	T	0.2735	3.78E-21	No
MARK3	rs8014182	14:103859962	T	-0.3397	4.99E-21	No
RP11-714L20.1	rs7439567	4:138464842	T	0.2355	7.35E-21	No
WHSC1L1	rs1906672	8:38130025	A	0.2715	1.03E-20	No
TOP3A	rs4925159	17:18185510	A	0.2281	2.27E-20	No
FGF9	rs606950	13:22298923	A	0.2324	3.77E-20	No
LCORL	rs2610990	4:18008232	A	-0.2573	3.82E-20	No
ATP2B1*	rs10858966	12:90567026	C	0.254	5.56E-20	No
APOLD1	rs2024385	12:12888438	A	-0.2326	1.05E-19	No
SZT2	rs839755	1:43856410	A	-0.2274	1.36E-19	No
KLF14	rs34072724	7:130432469	A	-0.2181	4.09E-19	No
MSRA	rs1986971	8:10268736	A	0.2377	3.93E-18	No
FAM193A	rs231708	4:2694773	C	-0.2281	5.24E-18	No
INPP5A	rs1133400	10:134459388	A	-0.2607	7.04E-18	Triglyceride measurement
PDGFC	rs17035181	4:157678511	T	0.2949	2.17E-17	No
WDR7	rs10048404	18:54578482	T	-0.2277	3.10E-17	No
CDKAL1	rs9368222	6:20686996	A	0.2301	3.85E-17	Cardiovascular disease , BMI, Insulin response rate
NFATC2	rs6021247	20:50108980	A	0.2041	4.29E-17	Cardiovascular disease
RP11-158M2.4	rs11632436	15:86295286	C	0.2056	5.11E-17	No
AP000721.4	rs4980515	11:63744609	T	0.2086	9.07E-17	No

TRIM48	rs4385883	11:51539339	T	-0.2331	1.17E-16	No
CTD-2260A17.2	rs709668	5:96174186	A	-0.2534	1.24E-16	No
MEX3C	rs11876341	18:48799991	A	-0.2225	1.93E-16	No
ARHGEF25	rs10437954	12:58003922	A	-0.3471	2.64E-16	No
NCOA7	rs10782230	6:126228512	A	0.1995	5.27E-16	No
SLC30A5	rs246973	5:68007803	T	0.2244	7.25E-16	No
TGFBR2	rs12638085	3:30405936	A	0.2132	8.36E-16	No
KLF5	rs78474310	13:73826901	A	-0.4828	8.38E-16	No
PDE8A	rs3743157	15:85680532	A	0.2602	1.27E-15	No
IGF1	rs5742643	12:102837863	A	-0.2236	2.15E-15	No
SYT1	rs7963801	12:79685226	T	-0.2027	2.29E-15	No
AL672294.1	rs4926499	1:249155909	C	0.2954	2.91E-15	No
PPM1E	rs34430710	17:56876627	A	-0.2069	4.26E-15	No
BCL2	rs12454712	18:60845884	T	0.2038	4.41E-15	BMI, waist-hip ratio, insulin sensitivity
THADA	rs35590893	2:43716933	A	-0.2152	4.65E-15	No
XPR1	rs1043069	1:180859368	T	0.1995	5.19E-15	No
C1orf21	rs4651224	1:184585182	T	0.1944	7.29E-15	No
MEIS1	rs2300481	2:66782467	T	0.1949	8.01E-15	BMI
ZMAT2	rs702395	5:140086677	T	0.191	8.25E-15	No
MEF2A	rs4965529	15:100145224	T	-0.2497	1.42E-14	No
PTPRD	rs1332813	9:9350706	T	0.1965	3.58E-14	No
FOXC1	rs2745599	6:1613686	A	0.1908	4.17E-14	Waist-hip ratio
PLXNB2	rs28578714	22:50727921	T	0.1951	4.17E-14	No
EPB41L2	rs9885632	6:131311909	T	0.2102	5.07E-14	No
LRCH1	rs912434	13:47189928	T	0.2151	6.27E-14	No
PPP2R2D	rs7912283	10:133773019	A	0.1943	6.76E-14	No
C12orf75	rs11112548	12:105871914	A	0.4608	1.44E-13	No
AQP1	rs10233127	7:30933453	A	0.3025	1.48E-13	No
Y_RNA	rs72688070	8:81393697	T	-0.241	1.56E-13	No
C1orf172	rs79598313	1:27284913	T	0.5926	1.82E-13	HDl, LDL, triglyceride

RNASEH2B	rs9526707	13:51489186	A	-0.1939	1.90E-13	No
RGS6	rs11623535	14:72462381	A	0.2022	3.02E-13	No
LTBP2	rs11159091	14:75074316	A	0.1793	3.26E-13	No
CTNNB1	rs6788984	3:41107173	A	0.2558	3.96E-13	No
AC022431.2	rs13179413	5:55868097	T	0.2045	5.57E-13	Cardiovascular disease
ARL14EP	rs11031051	11:30355707	A	-0.1899	5.80E-13	No
PHTF2	rs848445	7:77572461	T	-0.2004	6.57E-13	No
TNKS	rs62491354	8:9730663	A	0.2565	7.82E-13	No
CENPP	rs7045409	9:95201540	A	-0.1823	9.15E-13	No
NRG4	rs11634028	15:76276150	A	0.2258	9.46E-13	No
FARP2	rs139354822	2:242344695	T	0.5473	1.06E-12	No
TSNARE1	rs4129585	8:143312933	A	0.1749	1.06E-12	No
BMPR1B	rs1347345	4:95938386	A	-0.1792	1.45E-12	No
RP11-89M16.1	rs4598218	8:129483956	T	0.1797	1.50E-12	No
PLA2G12B	rs12572586	10:74751579	T	-0.3646	1.83E-12	No
NT5C1B	rs67720684	2:18975439	A	0.2039	2.26E-12	No
FAM129B	rs1891730	9:130309028	T	-0.1785	2.95E-12	No
MERTK	rs28377357	2:112769721	A	-0.1862	3.37E-12	No
RAD52	rs11571376	12:1059556	C	-0.1884	3.49E-12	No
WNT4	rs2807337	1:22577371	T	0.1753	3.60E-12	No
CTC-228N24.3	rs62373688	5:127352807	A	0.2607	5.49E-12	No
GDF2	rs34130368	10:48411796	T	-0.2715	5.64E-12	No
RP11-444A22.1	rs72816333	2:60096560	A	0.2225	5.88E-12	No
CITED2	rs7763294	6:140383733	T	-0.1804	6.85E-12	No
KAT2B	rs189267552	3:20073193	A	-0.783	1.14E-11	No
POM121C	rs6963105	7:75097488	A	-0.1779	1.39E-11	No
ZSWIM2	rs28558491	2:187816321	T	-0.1874	1.47E-11	No
TMEM108	rs9875380	3:132780356	T	-0.1651	1.70E-11	No
BCAR3	rs7514579	1:94051350	A	0.1969	1.73E-11	No
HSPA12A	rs11197813	10:118523933	A	-0.1801	1.84E-11	No

BCAS3	rs1036902	17:58950791	T	-0.2273	1.85E-11	No
RP11-805L22.1	rs1551355	17:30032420	T	0.1932	1.96E-11	No
C11orf24	rs67976715	11:68023742	C	0.1971	2.30E-11	No
TFCP2L1	rs6723509	2:122000745	T	0.2348	2.32E-11	No
GABRA2	rs12511987	4:46595623	T	-0.2153	2.71E-11	No
PCCB	rs863930	3:135949737	A	0.1629	2.89E-11	No
RBMS1	rs79523138	2:161368213	A	-0.2629	3.82E-11	No
ARHGAP29	rs17396055	1:94730954	A	-0.1727	5.73E-11	No
TRIP12	rs1044822	2:230629138	T	-0.2275	6.15E-11	BMI
CRB1	rs12042924	1:197297417	T	-0.1615	6.26E-11	No
ERBB4	rs12694277	2:213188795	T	-0.1765	7.61E-11	No
JAZF1	rs10274928	7:28142088	A	0.1592	8.68E-11	No
LINC00311	rs7187540	16:85318302	A	-0.1766	1.08E-10	No
IRF6	rs7555285	1:209970355	C	0.1961	1.09E-10	No
RP11-1055B8.6	rs112280096	17:79367409	A	-0.1706	1.13E-10	No
GLIS3	rs28558845	9:4334791	C	-0.218	1.22E-10	No
DGKH	rs73187288	13:42738672	A	-0.2558	1.57E-10	No
MLF1	rs78151625	3:158316726	T	-0.2108	1.60E-10	No
ADORA1	rs33996239	1:203109801	T	-0.3538	1.71E-10	No
SEMA4A	rs76719272	1:156129796	T	-0.2394	1.98E-10	No
SPIB	rs138877676	19:50935809	T	-0.6556	2.27E-10	No
RARRES2	rs11771693	7:150050111	A	0.1673	2.72E-10	No
ARIH2	rs6774721	3:49381898	C	0.2457	3.00E-10	No
PRKD1	rs17115145	14:30122409	T	0.1569	3.74E-10	No
ZNF804A	rs6739913	2:185033065	A	0.1675	6.40E-10	No
SOX5	rs7976167	12:24210599	T	0.1638	7.21E-10	No
CNTN3	rs9857362	3:74710462	A	0.151	9.71E-10	No
IER5L	rs184457	9:131940019	A	-0.1659	1.09E-09	No
RTN4	rs2920899	2:55279681	T	0.1851	1.17E-09	No
DENND2A	rs12703989	7:140238048	A	0.1521	1.26E-09	No

ST5	rs10743086	11:8774923	A	-0.1832	1.57E-09	No
SREK1	rs3121685	5:65662133	T	-0.1475	1.92E-09	No
TARS	rs74774746	5:33411769	C	-0.1705	2.08E-09	No
RBFOX1	rs35450617	16:6889675	T	-0.1609	2.33E-09	No
RBM26	rs7988232	13:79808655	A	0.148	2.99E-09	No
CAPRIN1	rs190194639	11:34068037	T	0.2746	4.55E-09	No
THSD7B	rs72844590	2:138421227	T	0.2083	4.77E-09	No
SKI	rs260508	1:2187085	T	0.1485	5.17E-09	No
CCT6A	rs6593297	7:56122058	A	0.1581	8.38E-09	No
MCM9	rs9401090	6:119113317	T	0.1637	9.25E-09	No
NRXN1	rs6545155	2:50429861	T	0.1691	9.84E-09	No
ZBTB20	rs1882289	3:114461208	A	-0.2159	1.26E-08	No
FAM208B	rs56352451	10:5804865	T	0.1992	2.54E-08	No
TOX	rs6996733	8:60535824	T	0.1882	3.34E-08	No

eMethods. Statistical Power Analysis for the Mendelian Randomisation With Binary Outcomes

The statistical power for the Mendelian randomisation with binary outcomes[2,3] was calculated for outcomes using the following parameters: proportion of outcomes in the cohort, estimated odds ratio and proportion of variance explained for the association between the genetic risk score and the measured systolic blood pressure (BP) [2]. **eTable 2** shows the results of power analysis based on the actual number of cases and under different assumptions with respect to effect size. If the observational effect size reported by previous cohort studies (OR < 2) was assumed, power would be low for all outcomes. However, given that Mendelian randomisation studies typically show stronger effects than observational studies (partly because of assessment of life-time exposure), we also estimated power for a OR of up to 4.5. Results showed that we had good statistical power for aortic stenosis, mitral regurgitation and the composite outcome, but weaker power for aortic regurgitation at a plausible range of OR. The PASS software (version 15) was used to calculate statistical power.

eTable 2. Statistical power analysis for Mendelian randomisation with binary outcome.

Outcome (n, %)	OR=1.5	OR=2	OR=3	OR=3.5	OR=4	OR=4.5
Aortic stenosis (1491, 0.45 %)	0.18	0.45	0.83	0.91	0.95	0.97
Aortic regurgitation (634, 0.19 %)	0.10	0.22	0.47	0.58	0.67	0.74
Mitral regurgitation (1736, 0.53 %)	0.21	0.51	0.88	0.95	0.97	0.99
All cases (3570, 1.08 %)	0.38	0.81	0.99	0.99	0.99	1.00

The following parameters were used for power calculation: proportion of variance explained for the association between the genetic risk score and the exposure variable = 0.48%; sample size: 329,237

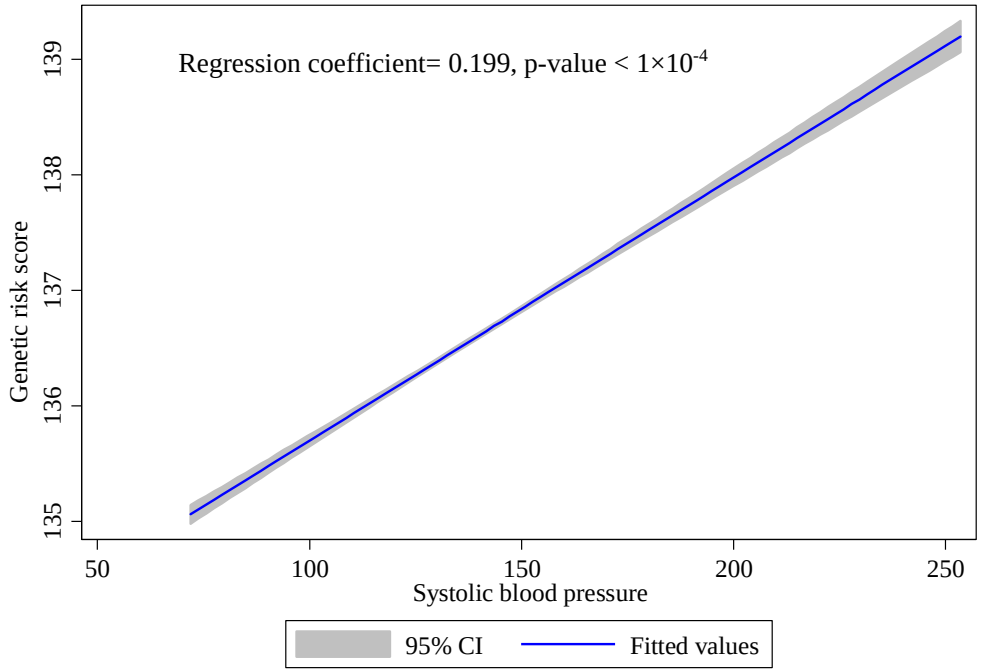
eTable 3. Correlation coefficient (r) between weighted genetic risk score, measured systolic blood pressure, and potential confounders.

	Genetic risk score (r, *P* - value)	Measured systolic BP (r, *P* - value)
Genetic risk score	1	0.0673, $< 1\times10^{-4}$
Age	-0.0031, 0.08	0.3232, $< 1\times10^{-4}$
Sex	-0.0023, 0.18	0.1529, $< 1\times10^{-4}$
BMI	0.0034, 0.06	0.1847, $< 1\times10^{-4}$
Smoking	-0.0008, 0.68	0.0009, 0.61
Alcohol	0.0010, 0.63	-0.0631, $< 1\times10^{-4}$
Townsend index	0.0027, 0.13	-0.0319, $< 1\times10^{-4}$

eTable 4. Associations between systolic blood pressure (per 20 mmHg) and three major outcomes of valve disease using unweighted genetic risk score as instrumental variable.

Outcomes	No. cases	OR (95% CI)	*P*-value
Aortic valve stenosis	1491	3.41 (1.49; 7.77)	0.003
Aortic valve regurgitation	634	2.22 (0.63; 7.80)	0.21
Mitral valve regurgitation	1736	2.02 (0.94; 4.34)	0.06

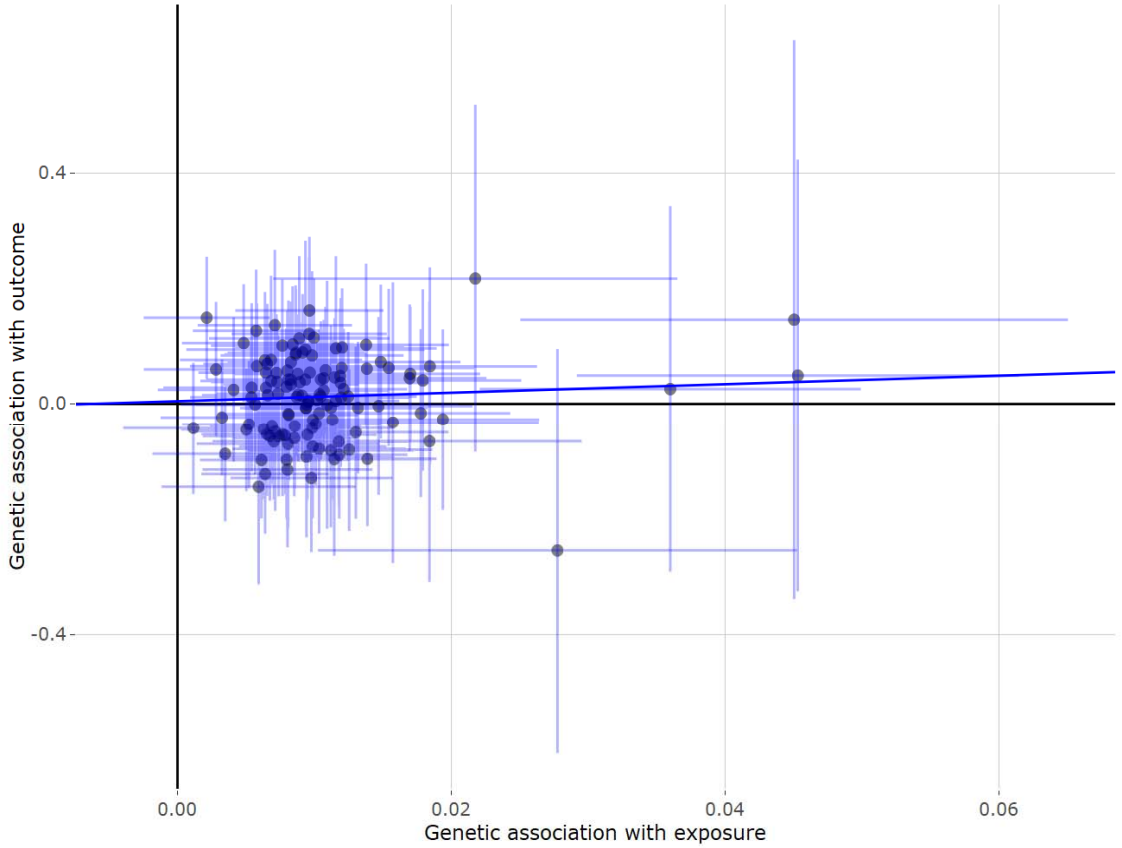
Adjusted for age, sex, body mass index, UK Biobank assessment center, genotype measurement batch, alcohol intake frequency, smoking status, genetic kinship to other participants and 10 genetic principal component.



eFigure 2. Regression line represent intercorrelations between weighted genetic risk score and systolic blood pressure originated from 130 SNPs in the last published GWAS study.

eFigure 3. MR-Egger intercept test to assess validity of the instrumental variable.

Possible pleiotropy assessed visually by a scatterplot similar to Egger's regression plot for assessing small study effect in conventional meta-analysis. Solid points represent each SNPs effect and should be approximately compatible with the linear relationship between SNPs (genetic) associations with the outcome (estimated as ln odds ratio) versus SNPs associations with the systolic BP (estimated as regression coefficients). The plot and Egger test shows that there is no observable pleiotropic effect (in average) (intercept = 0.001, standard error = 0.017, P= 0.94).



Further sensitivity analysis results

eResults 1. Case definition sensitivity analyses.

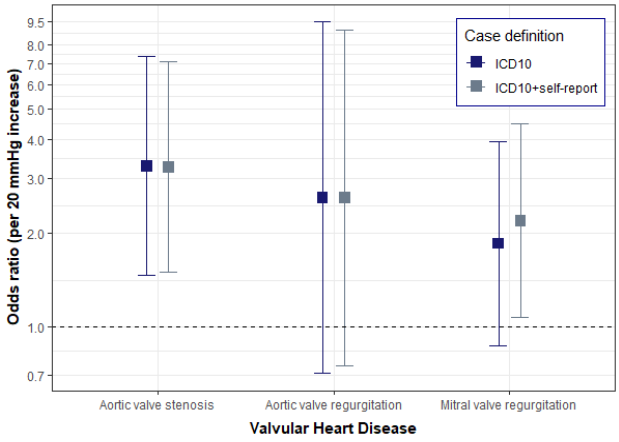
The ICD-10 and UK Biobank self-report codes were used for outcome definition and the number (%) of cases were as following: aortic stenosis (**ICD 10: 1458 [0.44%]; UK Biobank self-report: 84 [0.03%])**; aortic regurgitation **(ICD 10: 564 [0.17%]; UK Biobank self-report: 92 [0.03%])**; mitral regurgitation (**ICD 10: 1664 [0.51%]; UK Biobank self-report: 132 [0.04%])**. Also, the below tables shows concordance between ICD-10 codes and self-report outcomes.

		Aortic stenosis diagnosed using ICD-10 code	
Aortic stenosis diagnosed using self-report		**0**	**1**
	0	327746	1407
	1	33	51

		Aortic regurgitation diagnosed using ICD-10 code	
Aortic regurgitation diagnosed using self-report		**0**	**1**
	0	328603	542
	1	0	92

		Mitral regurgitation diagnosed using ICD-10 code	
Mitral regurgitation diagnosed using self-report		**0**	**1**
	0	327501	1604
	1	0	132

These numbers confirm that most cases were based on routinely collected electronic health records linked to UK Biobank rather than on self-reported data and thus the accuracy and reliability of the former are expected to be satisfactory. In addition, the following figure compare the estimations.



eResults 2. Sensitivity analysis for assessing the effect of severe cases including aortic valve replacement and mitral valve replacement surgery.

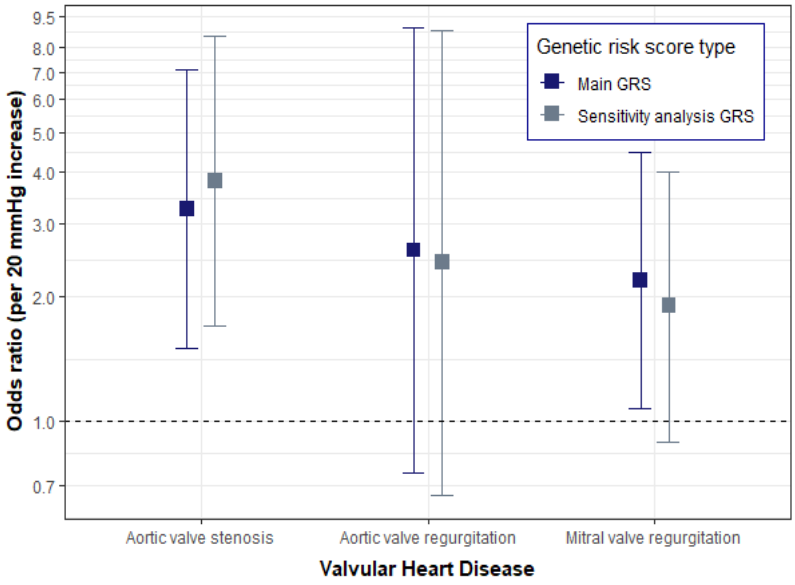
The data for aortic valve replacement and mitral valve replacement surgery was available, but detailed data about the cause of surgery (stenosis or regurgitation) was not available. Although we understand the rationale for using valve replacement surgery as a proxy for more severe valve disease, this is not necessarily true. Patients may undergo valve replacement for reasons other than the severity of the disease, for instance, if they are undergoing coronary artery bypass grafting. We therefore decided not to consider valve replacement surgery as equivalent to severe valve disease as this could be an erroneous assumption. However, we performed sensitivity analysis with inclusion of aortic valve replacement and mitral valve replacement surgery as outcome and this was in keeping with the main results.

	Number of cases (%)	**OR* (95% CI)**
Aortic stenosis AND aortic valve replacement surgery	707 (0.21%)	4.31 (1.35 to 13.75)
Aortic stenosis	1491 (0.41%)	3.26 (1.50 to 7.10)
Aortic regurgitation AND aortic valve replacement surgery	216 (0.07%)	2.15 (0.26 to 17.33)
Aortic regurgitation	634 (0.19%)	2.59 (0.75 to 8.92)
Mitral regurgitation AND mitral valve replacement surgery	339 (0.1%)	1.74 (0.32 to 9.24)
Mitral regurgitation	1736 (0.53%)	2.19 (1.07 to 4.47)
(Aortic stenosis OR Aortic regurgitation OR Mitral regurgitation) **AND** (aortic valve replacement surgery OR mitral valve replacement surgery)	1176 (0.36%)	2.71 (1.10 to 6.66)
Aortic stenosis OR Aortic regurgitation OR Mitral regurgitation	3570 (1.08%)	2.85 (1.69 to 4.78)

*Adjusted for age, sex, body mass index, UK Biobank assessment center, genotype measurement batch, alcohol intake frequency, smoking status, genetic kinship to other participants and 10 genetic principal component.
OR per 20 mmHg increase in systolic blood pressure

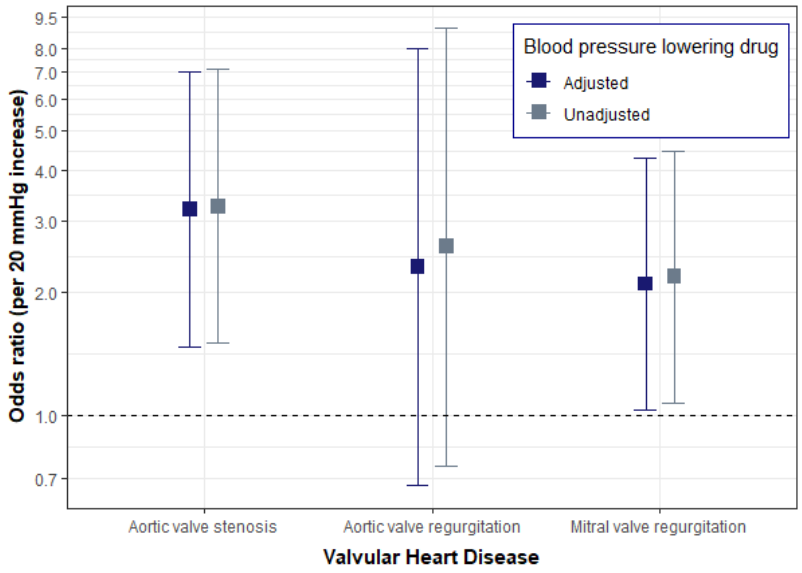
eResults 3. Sensitivity analysis for assessing the pleiotropic effect of variants included in the genetic risk score.

The individual association of each SNP with other risk factors or disease has been checked using the NHGRI-EBI Catalog of published genome-wide association studies (https://www.ebi.ac.uk/gwas/home). We conducted a sensitivity analysis and re-constructed a genetic risk score excluding SNPs associated with any type of well-known cardiovascular risk factor or disease. We excluded 11 SNPs genome widely associated with cardiovascular risk factors or disease (please see **eTable 1** for details). The below figure compare the estimations. We did not find any significant change in the estimations.



eResults 4. Sensitivity analysis for assessing the effect of adjustment for blood pressure lowering treatment on the estimations.

Adjustment for use of BP-lowering treatment did not have a substantial impact on the risk estimations as shown in the figure below. However, this does not mean that BP-lowering treatment cannot reduce the risk of valvular heart disease because Mendelian randomisation assesses the influence of genetically-associated, lifelong exposure to elevated BP, which explains only a small fraction of actual BP variability in the population. In other words, our exposure is not actual measured BP but genetically-associated likelihood of higher BP. In addition, BP-lowering treatment is prescribed only above a certain threshold of actual BP and cardiovascular disease risk. It is also possible that the effect of BP-lowering treatment takes a certain time to reduce risk or it may only reduce risk at an early stage in the pathologic process that eventually leads to clinically evident valvular heart disease.



eReferences

1. Evangelou E, Warren HR, Mosen-Ansorena D, et al. Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. *Nat Genet*. 2018. doi:10.1038/s41588-018-0205-x

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