SUPPLEMENTAL MATERIALS

PTPRD GENE ASSOCIATED WITH BLOOD PRESSURE RESPONSE TO ATENOLOL AND RESISTANT HYPERTENSION

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Detailed Methods

The Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) Study

The PEAR study (clinicaltrials.gov identifier NCT00246519) was a randomized controlled clinical trial evaluating genetic determinants of BP and adverse metabolic responses to atenolol and hydrochlorothiazide (HCTZ) monotherapy and in combination.[1] Briefly, PEAR recruited male and female individuals of any race between the ages of 17 and 65, with uncomplicated hypertension, at the University of Florida (Gainesville, FL), Mayo Clinic (Rochester, MN), and Emory University (Atlanta, GA). Participants who were treated with antihypertensives at study entry went through an average 4 weeks washout of their medications. Participants were then randomized to either atenolol 50 mg daily or HCTZ 12.5 mg daily for 3 weeks, followed by dose titration to 100 mg and 25 mg daily, respectively. BP and metabolic responses to monotherapy were assessed after an average of 9 weeks of antihypertensive treatment. BP was measured using three different methods: home, office and ambulatory.[1] For the BP genome-wide association analyses, a composite weighted average of the office, home, ambulatory daytime and nighttime BP responses was calculated based on the row sums of the inverse of the inter-method covariance matrices.[2] This weighted average blood pressure had higher signal-to-noise ratio and therefore provides the best power to detect differences compared to any single measurement of BP phenotypes.[2]

PEAR Genotyping and Quality Control

Genomic DNA from PEAR participants was genotyped using the Illumina Human Omni1MQuad BeadChip (Illumina, San Diego, CA, USA). Genotypes were called using GenomeStudio software version 2011.1 and the Genotyping Module version 1.9 calling algorithm (Illumina, San Diego, CA, USA). Participants were excluded if sample call rates were below 95% and SNPs were excluded if genotype call rates were below 95%. Sample contamination was detected by checking gender mismatches using X chromosome genotype data and cryptic relatedness was estimated by pairwise identity-by-descent (IBD) analysis implemented using PLINK (http://pngu.mgh.harvard.edu/purcell/plink/). Principal component analysis was performed to assess the ancestral background. Participant's self-identified race information was confirmed with principal component analysis for genetic ancestry. Hardy-Weinberg Equilibrium was assessed within each race group with chi-square test of one degree of freedom. Genotype imputation was performed using the MaCH software program[3] (version 1.0.16) with SNPs that passed QC filtering and HapMap III phased haplotypes as the reference panel. SNPs were filtered and excluded from analysis if the minor allele frequency was <3% or the imputation quality was <0.3. This analysis focused on the 461 participants that were genetically identified as whites that were treated either with atenolol (n = 233) or HCTZ monotherapy (n = 228) and the 150 participants who were genetically confirmed as blacks that were treated with atenolol monotherapy.

Genetics of Drug Responsiveness Study (GENRES)

The GENRES study was a randomized, double-blind, cross-over, placebo-controlled trial in 313 moderately hypertensive Finnish men, aged between 35 and 60 years, measuring BP response to antihypertensives [4]. Any previously prescribed antihypertensive medication was discontinued for at least 4 weeks prior to initiating study medication. Each study participant received bisoprolol 5 mg, losartan 50 mg, HCTZ 25 mg, and amlodipine 5 mg daily, each as a monotherapy in randomized order for 4 weeks. The study started with a 4-week run-in placebo period, and all four drug treatment periods were separated by four-week placebo periods. Twenty-four-hour ambulatory BP readings were recorded at the end of each treatment period with a device equipped with a QRS complex detector and a position sensor (Diasys Integra; Novacor, Rueil-Malmaison, France). The ambulatory BP phenotype was used for this analysis since it represented the best single BP phenotype for this study and was available for 208 subjects treated with bisoprolol therapy. A total of 207 participants were successfully genotyped using the Illumina HumanOmniExpress-12 BeadChip (Illumina, San Diego, CA, USA).

INternational VErapamil SR Trandolapril STudy GENEtic Substudy (INVEST GENES)

INVEST-GENES collected DNA samples from 5,979 INVEST study participants (clinicaltrials.gov identifier: NCT00133692).[5] INVEST recruited hypertensive individuals with clinically stable coronary artery disease (CAD) residing in the continental United States and Puerto Rico. Briefly, INVEST participants were

randomly assigned to an atenolol based β-blocker strategy or verapamil-SR based calcium channel blocker strategy, and were followed every 6 weeks for the first 6 months and every 6 months until the last participant was enrolled. Hydrochlorothiazide and trandolapril were added as needed to achieve BP control in a protocol-defined manner. Resistant hypertension (RHTN) was defined as BP ≥ 140/90 mmHg despite use of at least 3 antihypertensive agents, or treated with 4 or more antihypertensives regardless of BP[6]. Participants without RHTN were defined as controlled hypertensive participants who had controlled BP (BP<140/90 mmHg) on 0 - 3 antihypertensive medications. Those who were uncontrolled (BP≥140/90 mmHg), but on <3 antihypertensive medications were excluded from analysis. INVEST GENES samples were genotyped on the Illumina OmniExpressExome chip.

INVEST Genotyping and Quality Control

INVEST GENES genomic DNA was extracted from buccal cells collected in mouthwash samples according to standard protocols.[7] Samples were genotyped on the Illumina OmniExpressExome chip. Samples were excluded if call rates were below 95% and SNPs were excluded if call rates were below 95%. The quality control procedures were similar to those used in PEAR samples. Principal component analysis was performed with a linkage disequilibrium (LD) pruned data set using the EINGENSTRAT method.[8] Principal components 1, 2 and 3 provided the best separation of ancestry clusters in the INVEST data and were used as covariates in the subsequent

analysis. The post-QC dataset included 657 whites, 537 Hispanics and 155 blacks.

Statistical Analysis

INVEST RHTN analyses were adjusted for variables that were associated with risk of RHTN in the overall INVEST analysis [9]: age, sex, body mass index, and history of diabetes, heart failure, myocardial infarction, stroke, left ventricular hypertrophy, peripheral vascular disease, treatment assignment; and three principal components for ancestry. Meta-analysis of INVEST whites, Hispanics and blacks was performed using METAL software.[10] Based on the number of independent SNPs in the regions analyzed, we used an alpha level of $1*10^{-4}$ for the meta-analysis to be considered significant. For SNPs significant in the overall analysis, we also assessed the association in the β-blocker and calcium channel blocker-based treatment strategies separately.

Table S1. SNPs Associated with DBP response after atenolol monotherapy with p < 10-5

SNP	CHR	Coordinate	Gene/region	n	coded allele	non coded allele	coded allele frequency	beta	SE	p
rs4742955	9	107416918	FKTN	233	A	G	0.35	2.60	0.52	4.60E-07
rs4742654	9	15730246	FKTN	233	T	G	0.33	2.61	0.53	1.02E-06
rs4742956	9	107420542	FKTN	233	G	A	0.33	2.64	0.54	1.13E-06
rs7640608	3	163243302	OTOL1	233	G	A	0.05	5.40	1.11	1.15E-06
rs12115847	9	107302564	FSD1L	233	A	G	0.33	2.62	0.54	1.18E-06
rs2812312	9	107291221	FSD1L	233	C	A	0.33	2.62	0.54	1.19E-06
rs2771028	9	107284209	FSD1L	233	G	T	0.33	2.62	0.54	1.19E-06
rs2771045	9	107283314	FSD1L	233	T	C	0.33	2.62	0.54	1.19E-06
rs10491808	9	15635492	FKTN/FSD1L	233	C	T	0.33	2.62	0.54	1.20E-06
rs17309137	9	107387959	FKTN	233	A	G	0.33	2.62	0.54	1.22E-06
rs17309806	9	107420176	FKTN	233	A	C	0.33	2.56	0.53	1.61E-06
rs922484	8	11445810	BLK	233	T	C	0.87	-3.45	0.72	1.79E-06
rs2812306	9	15542574	FSD1L	233	A	T	0.33	2.58	0.54	1.86E-06
rs17251166	9	15730819	FKTN	233	C	T	0.34	2.53	0.53	2.01E-06
rs755320	8	11460848	BLK	233	A	G	0.87	-3.25	0.69	2.72E-06
rs409161	6	167100518	RPS6KA2	233	G	A	0.75	2.85	0.61	2.79E-06
rs12346562	9	11008077	PTPRD	233	A	C	0.27	-2.44	0.52	3.22E-06
rs1104514	9	11018275	PTPRD	233	A	G	0.28	-2.29	0.51	5.92E-06
rs4524290	3	163183890	OTOL1	233	G	T	0.05	4.96	1.10	6.13E-06
rs11776081	8	3906572	BLK	233	G	A	0.88	-3.21	0.72	7.66E-06
rs732947	8	11466556	BLK	233	G	A	0.88	-3.21	0.72	7.82E-06

*Coordinates are base pair location on NCBI build 36.
Abbreviations: CHR: chromosome; SE: standard error

Table S2. SNPs Associated with SBP response after atenolol monotherapy with p < 10-5

SNP	CHR	Coordinate	Gene/region	n	coded allele	non coded allele	coded allele frequency	beta	SE	p
rs4742955	9	107416918	FKTN	233	A	G	0.35	4.08	0.79	2.72E-07
rs4742654	9	15730246	FKTN	233	T	G	0.33	4.10	0.82	6.18E-07
rs7579183	2	35411849	CRIM1	233	G	A	0.73	-4.23	0.85	6.55E-07
rs1607412	18	24746727	CDH2	233	C	T	0.45	3.62	0.73	7.32E-07
rs17309137	9	107387959	FKTN	233	A	G	0.33	4.09	0.83	9.04E-07
rs12115847	9	107302564	FSD1L	233	A	G	0.33	4.08	0.83	9.83E-07
rs2812312	9	107291221	FSD1L	233	C	A	0.33	4.07	0.83	9.83E-07
rs2771028	9	107284209	FSD1L	233	G	T	0.33	4.07	0.83	9.84E-07
rs2771045	9	107283314	FSD1L	233	T	C	0.33	4.07	0.83	9.85E-07
rs10491808	9	15635492	FKTN/FSD1L	233	C	T	0.33	4.07	0.83	1.01E-06
rs17309806	9	107420176	FKTN	233	A	C	0.33	4.01	0.82	1.14E-06
rs17251166	9	107449435	FKTN	233	C	T	0.34	3.99	0.82	1.15E-06
rs8084673	18	24721537	CDH2	233	G	A	0.45	3.52	0.73	1.24E-06
rs1464216	18	24725862	CDH2	233	C	T	0.45	3.52	0.73	1.24E-06
rs4742956	9	107420542	FKTN	233	G	A	0.33	4.06	0.84	1.35E-06
rs2812306	9	15542574	FSD1L	233	A	T	0.33	4.03	0.84	1.38E-06
rs7274162	20	4715346	RASSF2	233	C	T	0.91	-6.40	1.33	1.44E-06
rs8122874	20	4719336	RASSF2	233	G	T	0.91	-6.40	1.33	1.46E-06
rs11083301	18	24607722	CDH2	233	A	G	0.45	3.50	0.73	1.64E-06
rs4371344	2	35416295	CRIM1	233	G	A	0.72	-3.91	0.82	1.99E-06
rs6543931	2	35425657	CRIM1	233	G	T	0.72	-3.91	0.82	2.05E-06
rs12457391	18	24559684	CDH2	233	T	G	0.45	3.47	0.73	2.14E-06
rs1860510	7	30766223	INMT	233	G	T	0.53	-3.52	0.75	2.43E-06

rs755320	8	11460848	BLK	233	A	G	0.87	-4.96	1.07	3.65E-06
rs1941195	18	24679449	CDH2	233	G	A	0.45	3.43	0.75	4.28E-06
rs17034538	2	67953962	C1D	233	C	T	0.94	-7.02	1.54	5.05E-06
rs1518589	18	24658770	CDH2	233	C	T	0.48	3.34	0.73	5.35E-06
rs4533454	2	35426063	CRIM1	233	G	A	0.72	-3.85	0.85	5.50E-06
rs10853692	18	24688225	CDH2	233	G	A	0.48	3.34	0.73	5.57E-06
rs2171324	18	24699038	CDH2	233	C	T	0.48	3.34	0.73	5.57E-06
rs485916	18	24692853	CDH2	233	G	A	0.48	3.34	0.73	5.57E-06
rs7603696	2	35427381	CRIM1	233	C	T	0.72	-3.82	0.85	6.65E-06
rs11776081	8	3906572	BLK	233	G	A	0.88	-4.96	1.11	7.69E-06
rs732947	8	11466556	BLK	233	G	A	0.88	-4.96	1.11	7.78E-06
rs1841660	13	34909882	NBEA	233	A	C	0.78	-4.01	0.90	8.70E-06
rs10050053	4	1533980	FAM53A	233	C	T	0.36	-3.62	0.82	9.47E-06

*Coordinates are base pair location on NCBI build 36.
Abbreviations: CHR: chromosome; SE: standard error

Table S3. Baseline characteristics of GENRES participants.

Characteristics*	**GENRES (n = 207)**
Age (mean ± SD)	50.5 ± 6.4
Female gender (n, %)	0 (0%)
BMI (kg/m^2)	26.7 ± 2.8
Pretreatment systolic BP (mmHg)	151.3 ± 12.7
Pretreatment diastolic BP (mmHg)	99.4 ± 6.7

*Numeric characteristics were presented as mean± standard deviation or median and interquartile range if not normally distributed; categorical variables were presented as number and percentages. Abbreviation: SD: standard deviation; BMI: body mass index. BP: blood pressure.

Table S4. Baseline characteristics of INVEST GENES participants.

Characteristics	Whites (n = 657)		Hispanics (n = 537)		Blacks (n = 155)	
	Controlled HTN (n=431)	RHTN (n=226)	Controlled HTN (n=394)	RHTN (n=143)	Controlled HTN (n=84)	RHTN (n=71)
Age, mean± SD	69.2± 9.9	69.9 ± 9.3	65.8 ± 10.2	65.8 ± 10.2	65.7 ±10.3	66.5 ± 10.3
Female	184 (42.7%)	114 (50.4%)	225 (57.1%)	87(60.8%)	54 (64.3%)	47 (66.2%)
BMI, kg/m², mean ± SD	28.7 ± 5.6	29.3 ± 5.5	28.5 ± 4.7	29.7± 5.1[#]	32.2 ± (6.5)	31.9 ± 5.8
Smoking history	204 (47.3%)	113 (50.0%)	137 (34.8%)	44 (30.8%)	28 (33.3%)	30 (42.3%)
Baseline SBP, mm Hg, mean ± SD	147 ±17.4	154 ±19.0*	147 ± 19	149 ±18.7	150 ± 19.1	153 ± 18.6
Baseline DBP, mm Hg, mean ± SD	83 ± 10.1	82 ± 10.7	87 ± 10.3	88 ± 11.5	88 ± 9.2	90 ± 12.1
Treatment arm β-blocker	229 (53.1%)	113 (50.0%)	193(49.0%)	81 (56.6%)	37 (44.1%)	34 (47.9%)
Medical History						
Diabetes[a]	64 (14.9%)	66 (29.2%)*	51 (12.9%)	25 (17.5%)	14 (16.7%)	28 (39.4%)**
Heart Failure (class I-III)	27 (6.3%)	17 (7.5%)	6 (1.5%)	8 (5.6%)[#]	1 (1.2%)	6 (8.5%)**
Myocardial Infarction	170(39%)	92(41%)	35 (9%)	23 (16%)	17 (20.2%)	19 (26.8%)
Renal insufficiency[b]	14 (3.3%)	7 (3.1%)	2 (0.51%)	1 (0.7%)	0 (0%)	4 (5.6%)**
Stroke/TIA	38 (8.8%)	29 (12.8%)	14 (3.6%)	8 (5.6%)	4 (4.8%)	8 (11.3%)
Left ventricular hypertrophy	57 (13.2%)	37 (16.4%)	51 (12.9%)	29 (20.3%)[#]	17 (20.2%)	14 (19.7%)
Peripheral vascular disease	40(9.3%)	35 (15.5%)*	34 (8.6%)	23 (16.1%)[#]	8 (9.5%)	10 (14.1%)
Percutaneous coronary intervention	113 (26.2%)	52 (23.0%)	7 (1.8%)	6 (4.2%)	9 (10.7%)	9 (12.7%)

Numbers represent n (percentage) unless stated otherwise. HTN, hypertension; RHTN, resistant hypertension; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure. *p ≤0.05 compared to Controlled HTN white

Americans; # p ≤0.05 compared to Controlled HTN Hispanics, **p ≤0.05 compared to Controlled HTN black Americans. a. History of or currently taking antidiabetic agent at baseline. b. History of or currently have elevated serum creatinine level but less than 4mg/dl

Figure S1. Distribution of blood pressure responses in PEAR white participants treated with atenolol monotherapy (top panel), PEAR black participants treated with atenolol monotherapy (middle panel) and PEAR white (bottom panel) participants treated with hydrochlorothiazide monotherapy. Each bin represents 5 mmHg. HCTZ: hydrochlorothiazide.

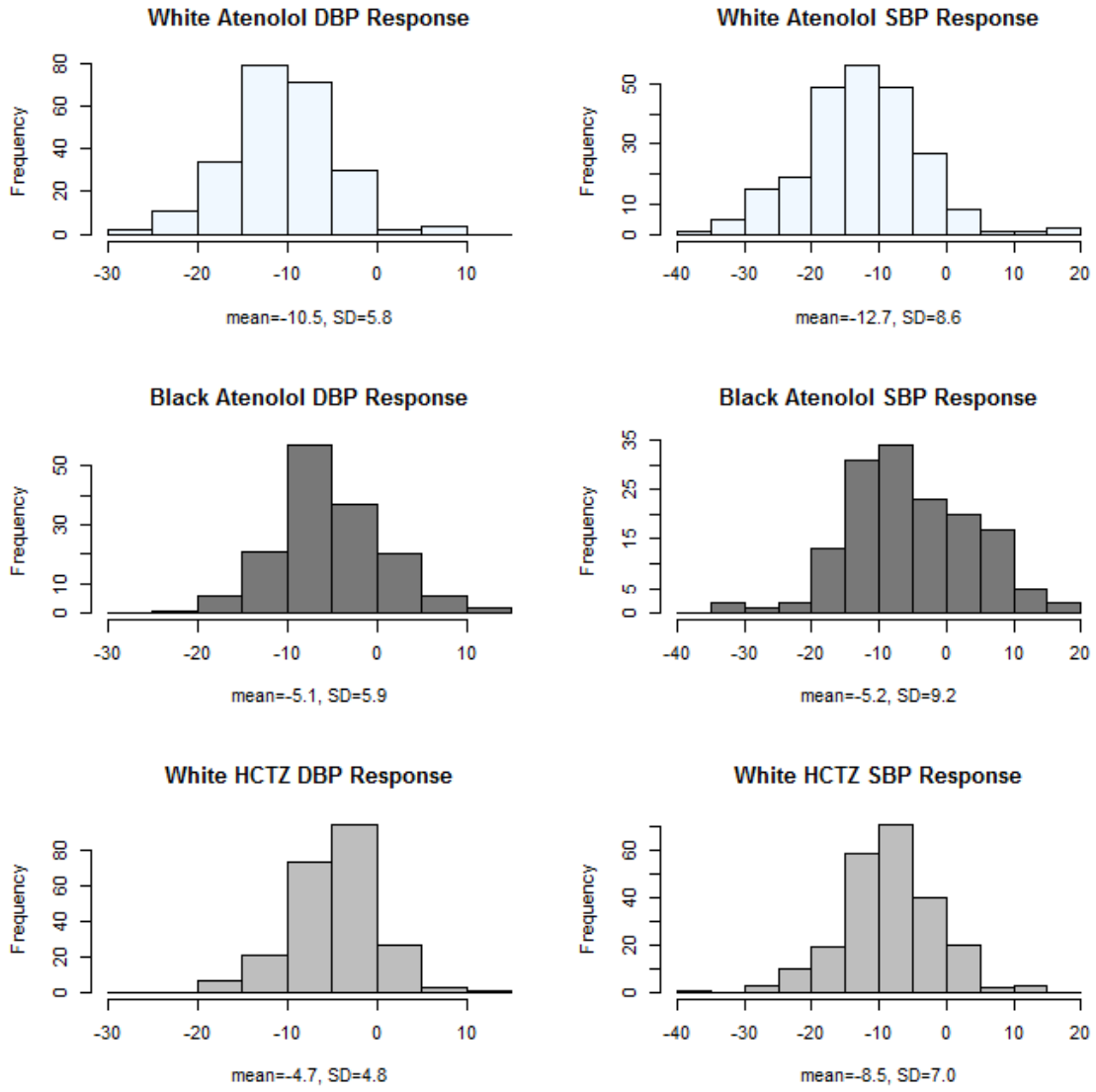
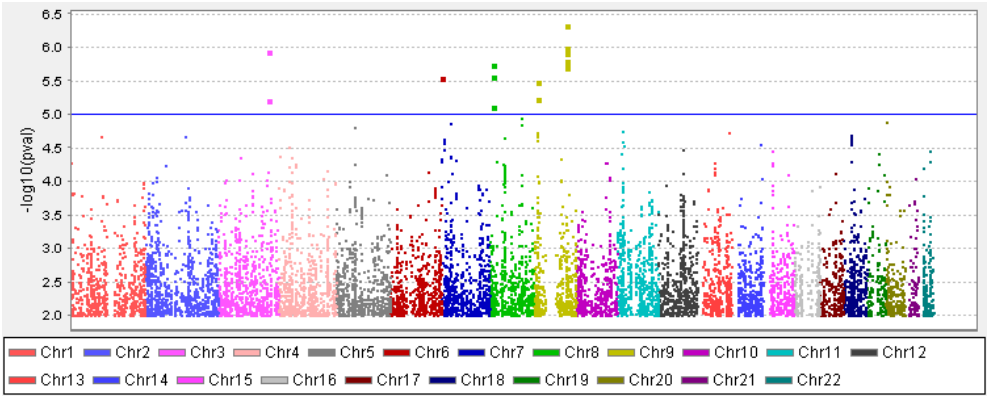
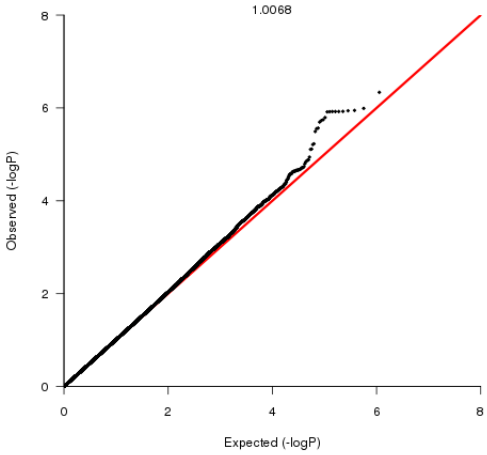


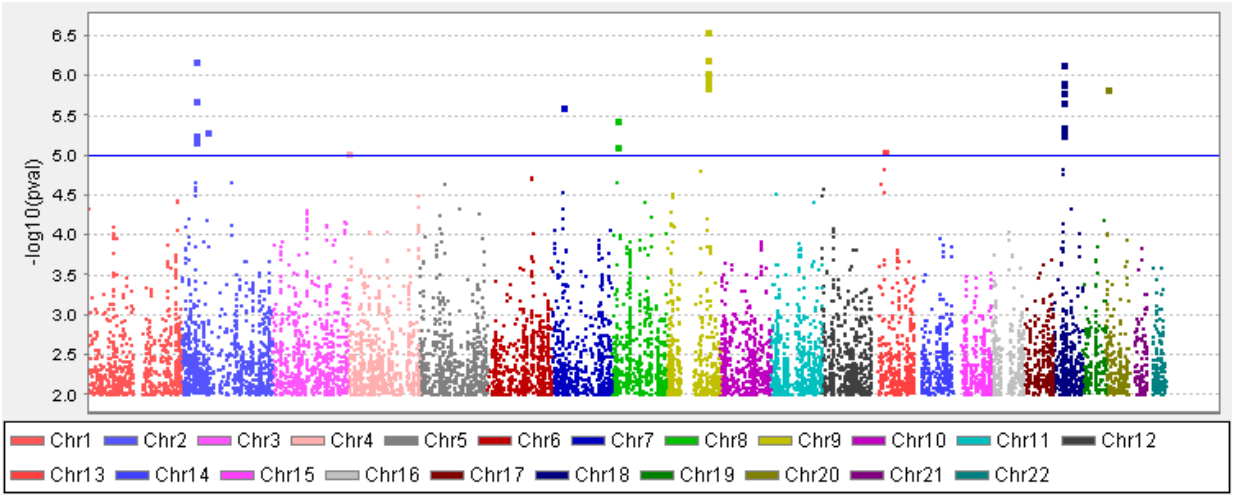
Figure S2. Manhattan plots and Q-Q plots for SNPs associated with blood pressure response to atenolol monotherapy in PEAR white participants. A. diastolic blood pressure response; B. systolic blood pressure response

A.





B.


-log10(pval)
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Chr1 Chr2 Chr3 Chr4 Chr5 Chr6 Chr7 Chr8 Chr9 Chr10 Chr11 Chr12
Chr13 Chr14 Chr15 Chr16 Chr17 Chr18 Chr19 Chr20 Chr21 Chr22

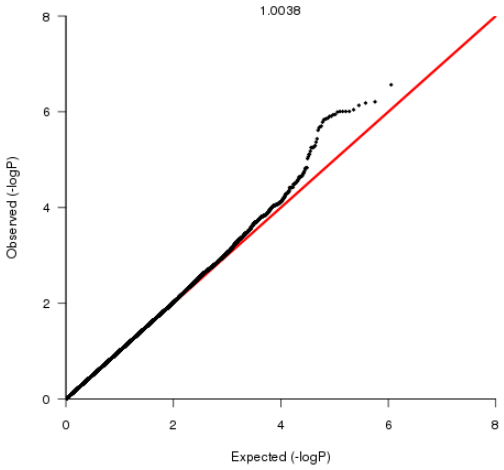
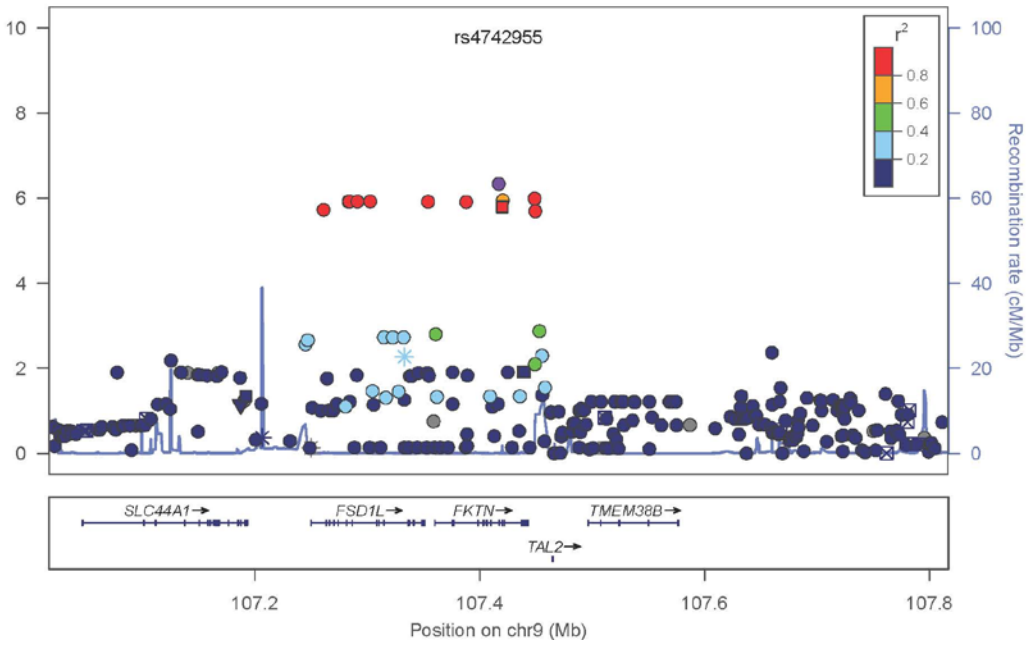

1.0038
Observed (-logP)
Expected (-logP)
0
2
4
6
8

Figure S3. Regional Plot for chromosome 9 rs4742955 associated with diastolic blood pressure response (A) and systolic blood pressure response (B) after atenolol in PEAR white patients.

A.



B.

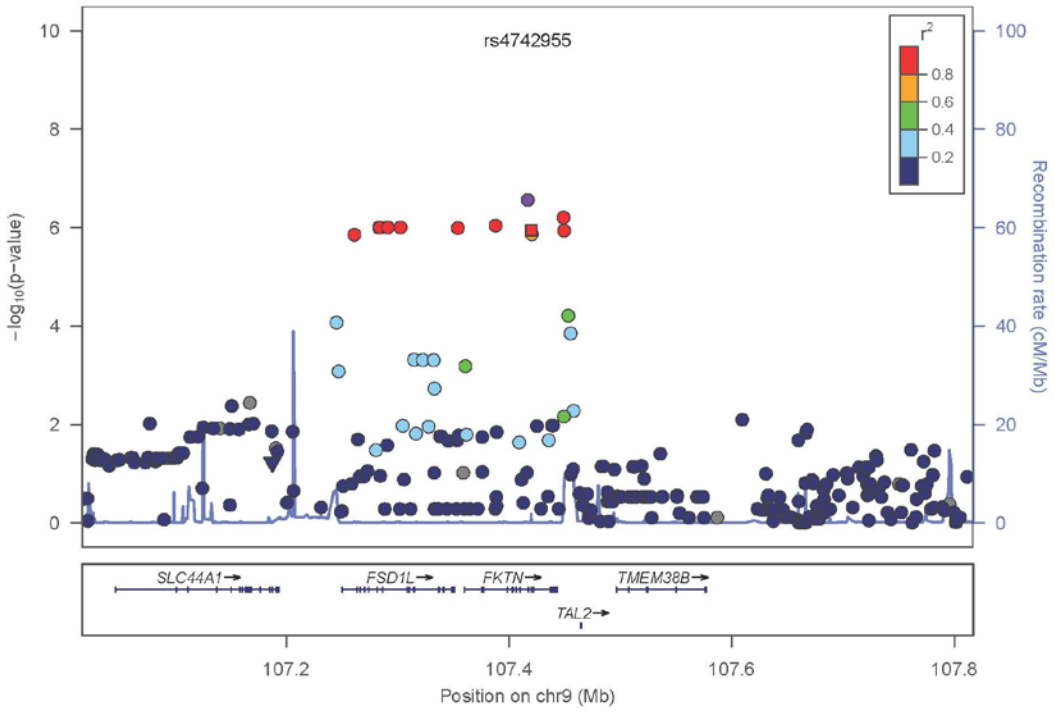
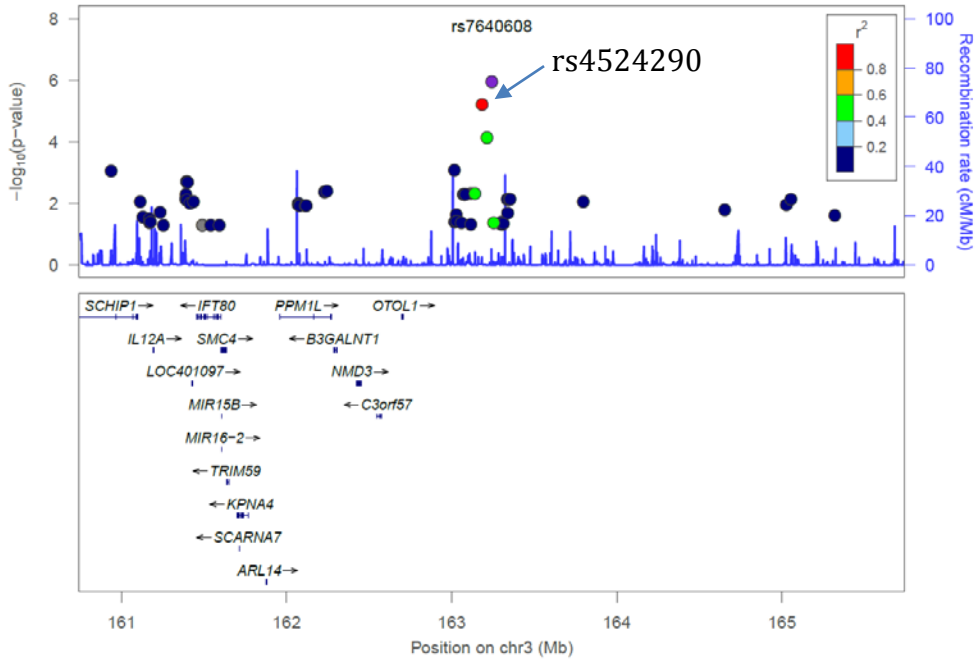


Figure S4. Regional plots for Chromosome 3 SNPs near OTOL1 associated with blood pressure response to atenolol in PEAR white (A, top SNPs: rs7640608 and rs4524290, highlighted with an arrow) and PEAR black participants (B, top SNP: rs12486357).

A.



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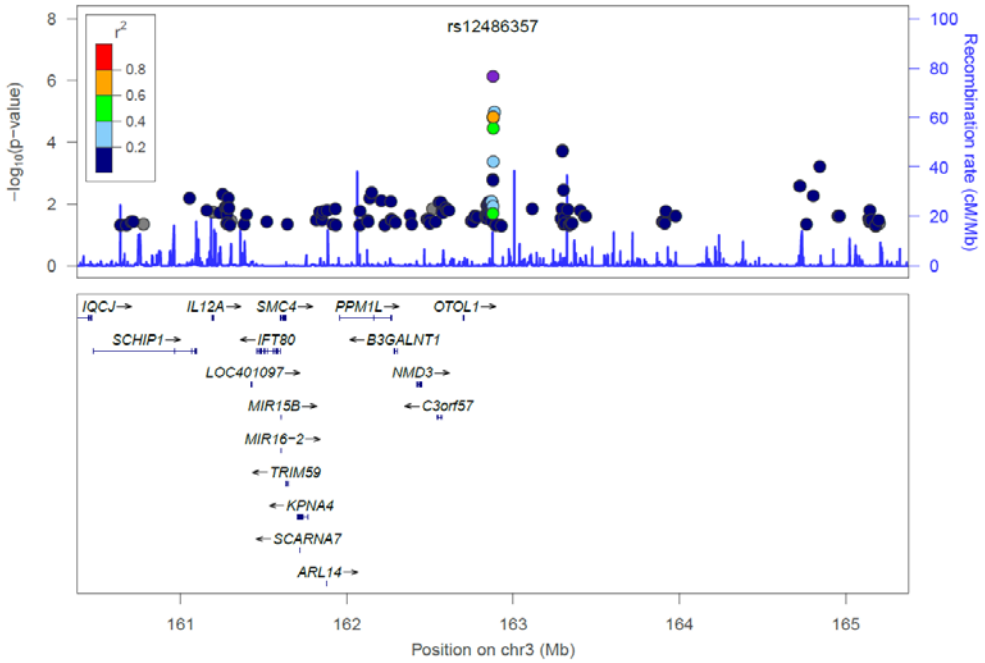

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