Genetic risk factors for portopulmonary hypertension in patients with advanced liver disease

Online Data Supplement

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Methods

Study cohort and study sample

The Pulmonary Vascular Complications of Liver Disease (PVCLD) Study enrolled a cohort of 536 patients evaluated for liver transplantation or pulmonary hypertension at seven centers in the United States between 2003 and 2006. The only inclusion criterion was the presence of chronic portal hypertension with or without intrinsic liver disease. We excluded patients with evidence of active infection, recent (< two weeks) gastrointestinal bleeding, or who had undergone liver or lung transplantation. Patients in the cohort were referred for evaluation for liver transplantation or pulmonary hypertension. The institutional review boards at each of the participating centers approved this study, and informed consent was obtained.

We performed a case-control study. The study sample included newly-referred patients who were evaluated with transthoracic echocardiography (routinely performed for pre-transplant evaluation) during the study period. "Prevalent" patients who met the case definition (see below) were also included. We excluded patients with pulmonary function testing showing a significant obstructive ventilatory defect, defined as forced expiratory volume in one second (FEV1)/forced vital capacity (FVC) < 0.70 with FEV1 % predicted < 80% or a significant restrictive ventilatory defect, defined as FVC % predicted and (if performed) total lung capacity % predicted < 70% using standard reference equations (1, 2).

Patients who otherwise fulfilled the case definition (see below) without pulmonary function testing were included if chest radiograph did not show hyperinflation or interstitial lung disease; controls without pulmonary function testing were excluded. We also excluded patients with HIV infection or the presence of more than moderate aortic or mitral valvular disease or significant left ventricular dysfunction by transthoracic echocardiography.

Case and control definitions

Cases with PPHTN met the following criteria at initial evaluation: 1) mean pulmonary artery pressure > 25 mm Hg, pulmonary capillary wedge pressure (or left ventricular end-diastolic pressure) ≤ 15 mm Hg, and pulmonary vascular resistance > 240 dynes•s^{-1}•cm^{-5} measured by right heart catheterization, and 2) no other etiology for pulmonary hypertension. Controls met the following echocardiographic criteria at entry into the cohort: 1) right ventricular (RV) systolic pressure < 40 mm Hg (if estimable) and 2) absence of right atrial or ventricular dilation, hypertrophy, or dysfunction. "Prevalent" cases who had previously undergone evaluation and were subsequently being treated were also included; data from the initial evaluation (before treatment) were used for these cases. During the recruitment period, a screening transthoracic echocardiogram showing RV systolic pressure > 50 mm Hg with abnormal RV morphology was considered an indication for right heart catheterization.

Clinical variables and blood sampling

Data were collected from the patients and from the medical record. The etiology of underlying liver disease (or portal vein thrombosis) and past medical history was recorded. Patients underwent laboratory assessment. The Model for End-stage Liver Disease (MELD) score was calculated (3).

Phlebotomy was performed and blood was collected into EDTA-containing tubes. Samples were centrifuged immediately at 2500 X g for 15 minutes at room temperature. Plasma and buffy coat layers were stored at -80° C.

Candidate genes and single nucleotide polymorphism (SNP) selection

Ninety-three genes affecting vascular function were selected by the investigators (**Table 1**). We selected genes linked to vascular tone and cellular growth regulation, as well as genes coding for key mediators of inflammatory and coagulation cascades. We prioritized those genes previously implicated in human or animal models of pulmonary vascular disease. We also included genes with prior associations with systemic blood pressure regulation and cardiovascular disease. Candidate genes were assigned to functional categories based upon Gene Ontology (GO) Pathways (4). For this study, each candidate gene was defined as a genomic region containing introns, exons, and proximal and distal regulatory regions (coding region ± ~5 kilobases).

Within each candidate region, haplotype block structure was defined using data from the HapMap data release #20/phase 2 Jan 06 (NCBI B35 assembly). SNPs with a minor allele frequency of ≥ 0.05 were selected using the following criteria: 1) minimum of one haplotype tagging SNP per block (Haploview 4.0, $r^2 > 0.8$), 2) nonsynonomous substitution, or 3) a prior published association with cardiovascular phenotype.

For this study, 1079 SNPs in the 93 candidate genes were genotyped. These SNPs were distributed as follows: 79 coding (51 nonsynonymous), 749 intronic, and 251 in untranslated regions (**Table E1**).

Detection of population substructure and stratification

We genotyped an additional set of 60 SNPs (null loci) in order to detect potential population substructure and stratification. Null loci were selected from a validated list of Ancestry Informative Markers (5) using the following criteria: 1) minor allele frequency ≥ 0.10,

2) minimum of 20 Mb between loci, and 3) no linkage ($r^2 < 0.2$) between null and candidate loci. The physical and genetic map positions of the null loci are available from the authors by request.

Genotyping

Genomic DNA was isolated from peripheral leukocytes using standard procedures (Gentra Puregene®, Qiagen, Valencia, CA). SNP genotyping was performed using the GoldenGate® Assay (Illumina, Inc., San Diego, CA).

Quality control data

Three SNPs (0.3%) were unable to be genotyped in ≥ 15% of subjects. These 3 SNPs were not included in the analysis. Seven replicate DNA samples showed 100% reproducibility of genotypes.

Plasma estradiol

To validate the findings from the genetic analyses, we measured plasma estradiol using a double antibody radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). The coefficient of variation of this assay was < 5% and the sensitivity was 1.4 pg/mL.

Statistical analysis

Continuous data were summarized using mean ± standard deviation or median [interquartile range], as appropriate. Categorical variables were summarized using n (%). Unpaired Student's t-tests and Wilcoxon rank sum tests were used to compare continuous

variables between cases and controls, and Chi-square tests and Fisher's exact tests were used for categorical variables.

Hardy-Weinberg equilibrium (HWE) was assessed for genetic alleles using exact tests in controls. The association of genotype with case/control status was assessed using additive models in multivariate logistic regression and expressed with odds ratios (ORs). We adjusted for gender and autoimmune liver disease (previously associated with case status (6)) in the final multivariate logistic regression models. Other liver disease etiologies were assessed as confounders; change in the coefficient for genotype > 20% after inclusion in the regression models was considered to indicate confounding. As the main goal of this study was hypothesis generation, adjustment for multiple comparisons was not performed. Single locus association analyses were performed using SAS/STAT (SAS Institute, Cary, NC).

For genes in which more than one SNP was associated with PPHTN, we determined haplotype structure and pairwise linkage disequilibrium in controls between SNPs using Haploview 4.0 (7). Values of D' and r^2 approach 1 as the likelihood of recombination between two loci decreases to zero, reflecting increased linkage.

The presence or absence of population stratification was assessed by comparing allele frequencies of the 60 null loci between cases and controls using Chi-square tests with a significance threshold of $p < 0.05$ (8). Sensitivity analyses assessed the potential impact of racial differences or cryptic subpopulations. Using estimated allele frequencies at the 60 null marker loci, two genetic ancestry clusters were predicted by *STRUCTURE 2.2* (9). Analyses were then performed including 1) self-identified whites only and 2) subjects with Caucasian genetic ancestry as predicted by *STRUCTURE 2.2*. $P < 0.05$ was considered significant for all analyses.

There was 80% power to detect odds ratios of ≥ 2.4 – 4.0 (or ≤ 0.25 – 0.42), depending upon the minor allele frequency of the SNP (ranged 0.45 – 0.05). Power analysis was performed using *QUANTO 1.2*. (10).

Association testing for loci on the X chromosome

The genes coding for NADPH oxidase 1 (NOX1), cytochrome b-245 (CYBB), and thromboplastin (HEMB) are located on the X chromosome. For the SNPs in these genes, we employed the methods described by Zheng et al. (11) which include the following:

1. Tests for HWE using Fisher's exact tests in female controls.
2. Allele-based Chi-square tests for males
3. Genotype-based tests of trend using additive genetic models for females.

For all tests, $p < 0.05$ was considered significant.

Legend

Figures E1 through E3. Linkage disequilibrium (LD) structure of estrogen receptor 1 (ESR1), aromatase (CYP19A1), and angiopoietin 1 (ANGPT1) in controls (Figures E1 – E3, respectively). The strength of LD is depicted graphically for each pair-wise comparison (squares), such that white and blue represent low levels of LD and red indicates high levels of LD (see color key). The SNPs are identified by their RS numbers and displayed relative to the candidate gene region. The display range of the chromosome (black line) corresponds to the genomic region of the candidate gene (roughly coding sequence ± 5-10 kilobases) targeted by this study. Exon/intron structure of the genes is indicated by thick/thin purple lines according to genome assembly hg17/May 2004. Annotated graphical images were generated using into LocusView 2.0 (12)**.**

Table E1. Genotyped single nucleotide polymorphisms (N = 1079).

SNPs are organized by chromosome (Chr) and candidate gene. NCBI Reference Sequence (RefSeq) is indicated, as is the minor allele frequency (MAF). The Location of the SNP describes the location of the SNP relative to gene exonic structure: coding, intronic, untranslated region (UTR). SNPs located in portions of the UTR immediately proximal to exons are indicated by the additional term 'flanking.'

Chr	Gene	RefSeq	SNP	MAF	Location	Amino Acid
1	**5,10-methylenetetrahydrofolate reductase (MTHFR)**	NM_005957.2	rs2274976	0.06	coding	R593Q
			rs1801131	0.36	coding	E428A
			rs1801133	0.24	coding	A221V
			rs7533315	0.31	intron	
			rs9651118	0.26	intron	
			rs7525338	0.01	intron	
			rs3737964	0.31	intron	
1	**Natriuretic peptide precursor A (NPPA)**	NM_006172.1	rs198414	0.14	3UTR	
	Natriuretic peptide precursor B (NPPB)	NM_002521.1	rs198358	0.27	flanking_3UTR	
			rs198359	0.11	flanking_3UTR	
			rs12024265	na	flanking_3UTR	
			rs198361	0.15	flanking_3UTR	
			rs5065	0.15	coding	*151R
			rs5063	0.06	coding	V31M
			rs198373	0.12	flanking_5UTR	
			rs632793	0.43	flanking_5UTR	
			rs198381	0.12	flanking_3UTR	
			rs198388	0.46	flanking_3UTR	
			rs198389	0.45	flanking_5UTR	
			rs12406089	0.33	flanking_5UTR	
1	**Endothelin converting enzyme 1 (ECE1)**	NM_001397.1	rs3026913	0.17	flanking_3UTR	
			rs2038089	0.43	intron	
			rs9426748	0.43	intron	
			rs212517	0.44	intron	
			rs3026883	0.27	intron	
			rs212539	0.39	intron	
			rs212540	0.39	intron	
			rs3026869	0.03	intron	
			rs4654916	0.05	intron	

			rs213045	0.25	flanking_5UTR	
1	**Small heterodimer partner (NR0B2)**	NM_021969.1	rs17162330	0.13	flanking_3UTR	
			rs17340482	0.16	flanking_3UTR	
			rs7504	0.31	3UTR	
			rs6659176	0.09	coding	G170A
			rs11581460	0.37	flanking_5UTR	
1	**Tyrosine kinase with Ig and EGF Factor homology domains (TIE1)**	NM_005424.2	rs11210831	0.33	flanking_5UTR	
			rs3120047	0.42	flanking_5UTR	
			rs7527092	0.45	intron	
			rs1999595	0.15	intron	
			rs2991990	0.42	intron	
			rs1199039	0.43	coding	L990L
			rs1098182	0.06	intron	
			rs11210834	0.23	intron	
1	**Calcium-binding protein A4 (S100A4)**	NM_002961.2	rs1810765	0.08	flanking_5UTR	
			rs743687	0.16	flanking_3UTR	
			rs730347	0.00	flanking_3UTR	
			rs1051044	0.03	3UTR	
			rs2071631		coding	E48E
			rs1005436	0.16	flanking_5UTR	
1	**C-reactive protein (CRP)**	NM_000567.2	rs2794520	0.33	flanking_3UTR	
			rs2808630	0.28	flanking_3UTR	
			rs1205	0.33	3UTR	
			rs1130864	0.30	3UTR	
			rs1800947	0.07	coding	L183L
			rs1417938	0.33	intron	
			rs3091244	0.05	flanking_5UTR	
			rs3093060	0.00	flanking_5UTR	
2	**Rho-associated protein kinase 2 (ROCK2)**	NM_004850.3	rs2290156	0.31	intron	
			rs12470004	0.38	intron	
			rs2011812	0.42	intron	
			rs3771109	0.01	intron	
			rs9808232	0.42	coding	T430N
			rs1868585	0.31	intron	
			rs6716817	0.50	intron	
			rs3771106	0.38	intron	
			rs1868584	0.19	intron	
			rs4669700	0.20	intron	
			rs4668720	0.24	intron	
			rs10929728	0.36	intron	
			rs11695377	0.20	intron	
			rs7575837	0.24	intron	
			rs7355489	0.24	intron	
2	**Xanthine dehydrogenase (XDH)**	NM_000379.2	rs1042039	0.47	flanking_3UTR	
			rs2268800	0.47	intron	
			rs207444	0.06	intron	

			rs169596	0.48	intron	
			rs4952085	0.23	intron	
			rs1896846	0.27	intron	
			rs7597755	0.02	intron	
			rs992137	0.01	intron	
			rs17038412	0.19	intron	
			rs1366817	0.32	intron	
			rs2281547	0.46	intron	
			rs3769618	0.45	intron	
			rs185925	0.23	intron	
			rs206847	0.22	intron	
			rs206851	0.21	intron	
			rs206855	0.40	intron	
			rs206857	0.18	intron	
			rs206860	0.21	intron	
			rs494852	0.14	intron	
			rs1346644	0.13	intron	
			rs3769616	0.02	intron	
			rs206811	0.20	intron	
			rs206812	0.32	flanking_5UTR	
			rs7575607	0.22	flanking_5UTR	
2	**Bone morphogenetic protein receptor type II (BMPR2)**	NM_001204.5	rs1980153	0.14	intron	
			rs4303700	0.20	intron	
			rs6435149	0.27	intron	
			rs16839149	0.13	intron	
			rs4675278	0.27	intron	
			rs12477602	0.14	intron	
			rs12621870	0.23	intron	
			rs7605442	0.06	intron	
			rs7562876	0.47	intron	
			rs1199496	0.31	intron	
			rs2228545	0.03	coding	S774N
			BMPR2600	na	intron	
2	**Serotonin 2B receptor (HTR2B)**	NM_000867.2	rs10191678	0.00	flanking_3UTR	
			rs6437000	0.23	intron	
			rs10194776	0.33	intron	
			rs1549339	0.26	intron	
			rs17586428	0.03	intron	
			rs3806545	0.06	flanking_5UTR	
			rs765458	0.25	flanking_5UTR	
			rs10498257	0.25	flanking_5UTR	
3	**Caveolin 3 (CAV3)**	NM_033337.1	rs12486403	0.19	flanking_5UTR	
			rs11926335	0.18	flanking_5UTR	
			rs10490801	0.17	flanking_5UTR	
			rs9816472	0.18	flanking_5UTR	
			rs237860	0.42	flanking_5UTR	
			rs237862	0.39	flanking_5UTR	

			rs2268485	0.38	flanking_5UTR	
			rs2072581	0.18	flanking_5UTR	
			rs1008642	0.22	coding	N32N
			rs1558991	0.23	intron	
			rs10490800	0.10	intron	
			rs13061909	0.18	intron	
			rs4686300	0.03	intron	
			rs237870	0.29	intron	
			rs237871	0.25	intron	
			rs237872	0.47	intron	
			rs151462	0.24	intron	
			rs237875	0.46	intron	
			rs13087941	0.28	coding	F40F
3	**Peroxisome proliferator activated receptor, gamma (PPARG)**	NM_005037	rs2972164	0.49	intron	
			rs4684846	0.22	intron	
			rs880663	0.21	intron	
			rs10510418	0.35	intron	
			rs1801282	0.08	coding	
			rs17817276	0.35	intron	
			rs2938395	0.38	intron	
			rs1151996	0.38	intron	
			rs1175540	0.34	intron	
			rs1175542	0.49	intron	
			rs1797912	0.40	intron	
			rs3856806	0.10	coding	H446H
			rs1152003	0.32	flanking_3UTR	
			kr1805192	na	intron	
3	**Retinoic acid receptor, beta (RARB)**	NM_000965.2	rs6550978	0.36	intron	
			rs6550980	0.19	intron	
			rs871963	0.35	intron	
			rs4607073	0.40	intron	
			rs11715516	0.16	intron	
			rs1432603	0.22	intron	
			rs1286656	0.10	intron	
			rs2056777	0.16	intron	
			rs1286641	0.32	intron	
			rs1153584	0.40	intron	
			rs1286646	0.15	intron	
			rs1881706	0.33	intron	
			rs1153589	0.18	intron	
			rs1153597	0.41	intron	
			rs1153598	0.10	intron	
			rs1153604	0.33	intron	
			rs1286665	0.23	intron	
			rs7616062	0.23	intron	
			rs1286773	0.10	intron	
			rs1286772	0.33	intron	

			rs1435706	0.35	intron	
			rs1626875	0.14	intron	
			rs1286765	0.46	intron	
			rs1286761	0.15	intron	
			rs12635379	0.22	intron	
			rs1656465	0.14	intron	
			rs1631354	0.48	intron	
			rs1730226	0.49	intron	
			rs9809535	0.11	intron	
3	**Pregnane X receptor (NR1I2)**	NM_003889.3	rs7643038	0.40	flanking_5UTR	
			rs2472671	0.11	intron	
			rs2472672	0.11	intron	
			rs1403527	0.11	intron	
			rs1403526	0.24	intron	
			rs4440154	0.35	intron	
			rs2461823	0.35	intron	
			rs7643645	0.30	intron	
			rs2472680	0.02	intron	
			rs3732357	0.23	intron	
			rs6784598	0.30	intron	
			rs3732359	0.07	3UTR	
			rs1054190	0.18	3UTR	
4	**Recombination signal-binding protein 1 for J-kappa (RBPSUH)**	NM_005349.2	rs3822223	0.06	intron	
			rs2077777	0.05	intron	
			rs1877207	0.48	intron	
			rs3762930	0.48	intron	
			rs2667065	0.03	intron	
			rs6821126	0.20	intron	
			rs4330343	0.20	intron	
			rs2725303	0.46	intron	
			rs3109836	0.20	intron	
			rs13109703	0.43	intron	
			rs13114911	0.43	intron	
			rs10517097	0.03	intron	
			rs946346	0.10	intron	
4	**Nuclear factor kappa B p105 subunit (NFKB1)**	NM_003998.2	rs980455	0.46	flanking_5UTR	
			rs3774934	0.06	intron	
			rs1599961	0.46	intron	
			rs230533	0.37	intron	
			rs4647992	0.07	intron	
			rs230498	0.38	intron	
			rs4648072	0.02	coding	M506V
			rs11722146	0.34	intron	
			rs4648099	0.00	coding	H711Q
			rs3774968	0.34	intron	
			rs4648110	0.22	intron	
			rs1609798	0.35	intron	

			rs997476	0.10	flanking_3UTR	
4	**Phosphodiesterase 5 (PDE5A)**	NM_001083.3	rs17006190	0.22	3UTR	
			rs3775843	0.22	intron	
			rs1480933	0.44	intron	
			rs10003953	0.00	intron	
			rs1155576	0.22	intron	
			rs10034450	0.19	intron	
			rs11731756	0.22	intron	
			rs2248236	0.23	intron	
			rs3733526	0.18	coding	A92V
4	**Endothelin receptor, type A (EDNRA)**	NM_001957.1	rs6842241	0.16	flanking_5UTR	
			rs6823537	0.29	intron	
			rs7655670	0.33	intron	
			rs4563479	0.10	intron	
			rs1568136	0.23	intron	
			rs6827096	0.16	intron	
			rs1878404	0.18	intron	
			rs10008744	0.22	intron	
			rs2048894	0.24	intron	
			rs5333	0.23	coding	H322H
			rs5343	0.38	3UTR	
5	**Betaine-homocysteine methyltransferase (BHMT)**	NM_001713.1	rs542852	0.38	intron	
			rs492842	0.42	intron	
			rs567754	0.29	intron	
			rs3733890	0.28	coding	R238Q
5	**CD14 molecule (CD14)**	NM_000591.1	rs4914	0.09	coding	L366L
			rs2569190	0.47	flanking_5UTR	
			rs2569193	0.23	flanking_5UTR	
6	**Endothelin 1 (EDN1)**	NM_001955.2	rs3087459	0.18	flanking_5UTR	
			rs1476046	0.25	intron	
			rs2071942		intron	
			rs1630736	0.49	intron	
			rs5370	0.24	coding	K197N
			rs2859338	0.42	flanking_3UTR	
			rs6906760	0.06	flanking_3UTR	
6	**Tumor necrosis factor (TNF)**	NM_000594.2	rs2844482	0.16	flanking_5UTR	
			rs2229094	0.26	coding	C12R
			rs1800630	0.09	flanking_3UTR	
			rs11574936	0.00	coding	I193N
			rs769177	0.03	flanking_3UTR	
6	**Complement component 4A (C4A)**	NM_007293	rs3093559	0.02	flanking_3UTR	
			rs389883	0.36	intron	
			rs389512	0.08	intron	
			rs12525076	0.42	intron	
6	**Vascular endothelial growth factor (VEGF)**	NM_001025366.1	rs833060	0.30	flanking_5UTR	
			rs699947	0.41	flanking_5UTR	

			rs2010963	0.42	5UTR	
			rs833069	0.34	intron	
			rs3025010	0.35	intron	
			rs3025033	0.18	intron	
			rs3025035	0.10	intron	
6	**Estrogen receptor 1 (ESR1)**	NM_000125.2	rs488133	0.32	flanking_5UTR	
			rs2077647	0.48	coding	S9S
			rs532010	0.32	intron	
			rs6902771	0.42	intron	
			rs2234693	0.41	intron	
			rs1709182	0.37	intron	
			rs712221	0.41	intron	
			rs11155819	0.35	intron	
			rs9340835	0.37	intron	
			rs1913474	0.21	intron	
			rs2347867	0.30	intron	
			rs4870062	0.28	intron	
			rs6927072	0.28	intron	
			rs1801132	0.18	coding	P324P
			rs3020314	0.29	intron	
			rs3020317	0.12	intron	
			rs1884051	0.30	intron	
			rs6557177	0.17	intron	
			rs985694	0.12	intron	
			rs3020403	0.23	intron	
			rs932477	0.03	intron	
			rs926777	0.18	intron	
			rs3020328	0.19	intron	
			rs7754762	0.07	intron	
			rs7757956	0.19	intron	
			rs3020411	0.39	intron	
			rs2982712	0.48	intron	
			rs3020368	0.13	intron	
			rs9322354	0.07	intron	
			rs9479190	0.08	intron	
			rs2982896	0.32	intron	
			rs3020381	0.40	intron	
			rs2474148	0.36	intron	
			rs2813544	0.22	flanking_3UTR	
			rs2747649	0.23	flanking_3UTR	
			rs1543403	0.45	flanking_3UTR	
6	**Superoxide dismutase 2, mitochondrial (SOD2)**	NM_00636.2	rs2758329	0.46	flanking_3UTR	
			rs8031	0.43	intron	
			rs5746136	0.28	3UTR	
6	**Plasminogen (PLG)**	NM_000301.1	rs9458005	0.21	flanking_5UTR	
			rs783144	0.27	flanking_5UTR	
			rs2314852	0.19	flanking_5UTR	

			rs1950562	0.39	flanking_5UTR	
			rs1819138	0.02	intron	
			rs4252092	0.42	intron	
			rs1465620	0.25	intron	
			rs783147	0.42	intron	
			rs1321201	0.43	intron	
			rs783146	0.11	intron	
			rs13231	0.32	coding	Q360Q
			rs4252125	0.33	coding	D471N
			rs783145	0.48	intron	
			rs813641	0.11	intron	
			rs3757017	0.25	intron	
			rs4252151	0.47	intron	
			rs4252166	0.22	intron	
			rs783176	0.13	intron	
			rs11060	0.47	coding	G761G
			rs4252200	0.04	flanking_3UTR	
			rs783166	0.13	flanking_3UTR	
7	**Ikaros (IKZF1)**	NM_006060.2	rs6976046	0.03	intron	
			rs7789106	0.02	intron	
			rs10230385	0.21	intron	
			rs6962370	0.19	intron	
			rs6964823	0.47	intron	
			rs6952409	0.28	intron	
			rs6973210	0.32	intron	
7	**Elastin (ELN)**	NM_000501.1	rs3757583	0.13	flanking_5UTR	
			rs868005	0.37	intron	
			rs4717865	0.14	intron	
			rs10949834	0.16	intron	
			rs11770302	0.16	flanking_3UTR	
7	**Plasminogen activator inhibitor 1 (SERPINE1)**	NM_000602.1	rs6950982	0.23	flanking_5UTR	
			rs6465787	0.02	flanking_5UTR	
			rs6956010	0.23	flanking_5UTR	
			rs2227631	0.40	flanking_5UTR	
			rs6090	0.01	coding	V16I
			rs2227708	0.03	intron	
			rs2070682	0.48	intron	
			rs1050813	0.19	3UTR	
			rs2227714	0.06	3UTR	
7	**Caveolin 2 (CAV2)**	NM_001233.3	rs987791	0.10	flanking_5UTR	
	Caveolin 1 (CAV1)	NM_001753.3	rs4730742	0.19	flanking_5UTR	
			rs8940	0.20	coding	A129E
			rs4727833	0.47	3UTR	
			rs1052990	0.38	3UTR	
			rs6466579	0.47	flanking_3UTR	
			rs2024211	0.28	flanking_3UTR	
			rs4236601	0.28	flanking_5UTR	

			rs926198	0.34	intron	
			rs9649394	0.42	intron	
			rs6466583	0.16	intron	
			rs10256914	0.25	intron	
			rs1474510	0.15	intron	
			rs3807986	0.26	intron	
			rs3807989	0.44	intron	
			rs3801993	0.10	intron	
			rs729949	0.28	intron	
			rs3807994	0.28	intron	
			rs6466587	0.18	intron	
			rs1049337	0.25	3UTR	
7	**Nitric oxide synthase 3 (NOS3)**	NM_000603.3	rs10277237	0.27	flanking_5UTR	
			rs2070744	0.00	intron	
			rs3918166	0.00	coding	R111Q
			rs1799983	0.34	coding	D297E
			rs3918227	0.13	intron	
			rs3730006	0.01	intron	
			rs3918232	0.01	coding	V826M
			rs743507	0.18	intron	
			rs3918234	0.02	coding	Q981L
			rs2566518	0.02	intron	
8	**Angiopoietin 1 (ANGPT1)**	NM_001146.3	rs1954727	0.30	3UTR	
			rs2514872	0.13	intron	
			rs2514878	0.36	intron	
			rs10505101	0.23	intron	
			rs10505102	0.11	intron	
			rs4354281	0.11	intron	
			rs4236785	0.21	intron	
			rs4324901	0.38	intron	
			rs6469108	0.47	intron	
			rs7011605	0.24	intron	
			rs4626569	0.08	intron	
			rs1283651	0.24	intron	
			rs1433195	0.15	intron	
			rs2217673	0.13	intron	
			rs2163870	0.43	intron	
			rs2514857	0.47	intron	
			rs1654718	0.20	intron	
			rs1433175	0.32	intron	
			rs1654725	0.40	intron	
			rs1433179	0.40	intron	
			rs1654730	0.40	intron	
			rs4268102	0.25	intron	
			rs1283695	0.15	intron	
			rs1283673	0.43	intron	
			rs1433189	0.23	intron	
			rs1283701	0.40	intron	

			rs1283698	0.11	intron	
			rs4734967	0.31	intron	
			rs2916084	0.36	intron	
			rs1368495	0.31	intron	
			rs4114169	0.37	intron	
			rs1654680	0.15	intron	
			rs1433165	0.43	intron	
			rs1283720	0.17	intron	
			rs1433168	0.43	intron	
			rs10505108	0.06	intron	
			rs17302560	0.34	intron	
9	**Cyclin-dependent kinase inhibitor 2A (CDKN2A)**	NM_000077.3	rs3731257	0.27	flanking_3UTR	
			rs11515	0.13	3UTR	
			rs2518719	0.15	intron	
			rs3731246	0.09	intron	
			rs2811708	0.25	intron	
			rs3731239	0.38	intron	
			rs2811709	0.11	intron	
			rs4074785	0.10	intron	
			rs3731221	0.01	intron	
			rs3731198	0.15	intron	
			rs3218020	0.36	flanking_5UTR	
			rs2811712	0.11	flanking_5UTR	
			rs3218009	0.10	flanking_5UTR	
9	**Tenascin C (TNC)**	NM_002160.1	rs1888221	0.25	flanking_3UTR	
			rs1330362	0.05	flanking_3UTR	
			rs13321	0.29	coding	E2007Q
			rs3789875	0.28	intron	
			rs2274751	0.08	intron	
			rs953288	0.38	intron	
			rs1547691	0.28	intron	
			rs1330368	0.47	intron	
			rs944225	0.25	intron	
			rs1330349	0.43	intron	
			rs1330351	0.48	intron	
			rs944227	0.44	intron	
			rs1617917	0.33	intron	
			rs1250019	0.44	intron	
			rs1330360	0.41	intron	
			rs3748166	0.28	intron	
9	**Endoglin (ENG)**	NM_000118.1	rs4451422	0.34	flanking_3UTR	
			rs1330684	0.33	intron	
			rs5031024	0.03	intron	
			rs10819309	0.50	intron	
			rs12001427	0.04	intron	
			rs1800956	0.01	coding	D365H
			rs3739817	0.08	coding	T342T

			rs10987750	0.33	intron	
			rs11792480	0.36	intron	
			rs10121110	0.44	intron	
			rs10819312	0.13	intron	
			rs4836585	0.14	intron	
			rs4837192	0.14	intron	
			rs10987759	0.09	flanking_5UTR	
			rs7865146	0.27	flanking_5UTR	
10	**Growth differentiation factor 2 (GDF2)**	NM_016204.1	rs9325886	0.08	flanking_3UTR	
			rs9421799	0.37	flanking_3UTR	
			rs3740297	0.02	3UTR	
			rs7923671	0.04	intron	
			rs11204215	0.13	flanking_5UTR	
10	**Bone morphogenetic protein receptor type 1a (BMPR1A)**	NM_004329.2	rs3905377	0.31	flanking_5UTR	
			rs7072166	0.01	intron	
			rs6586034	0.39	intron	
			rs7088641	0.31	intron	
			rs11202169	0.02	intron	
			rs7096781	0.28	intron	
			rs4933411	0.31	intron	
			rs6586039	0.03	intron	
			rs4934268	0.38	intron	
			rs4934272	0.22	intron	
			rs1124482	0.34	intron	
			rs11202221	0.19	intron	
			rs2354354	0.30	intron	
			rs2883420	0.42	intron	
			rs7894198	0.09	intron	
			rs4934275	0.13	intron	
			rs10749542	0.25	intron	
			rs12777504	0.03	intron	
			rs12269120	0.02	intron	
			rs7909264	0.10	flanking_3UTR	
10	**Nuclear factor kappa B p100 subunit (NFKB2)**	NM_01077493.1	rs1572532	0.00	flanking_5UTR	
			rs11574845	0.00	intron	
			rs7897947	0.16	intron	
			rs7077329	na	intron	
			rs1056890	0.36	3UTR	
11	**Tryptophan hydroxylase (TPH1)**	NM_004179.1	rs10741734	0.40	intron	
			rs211102	0.17	intron	
			rs1799913	na	intron	
			rs1800532	0.38	intron	
			rs10488683	0.50	intron	
			rs172423	0.33	intron	
			rs10488682	0.33	flanking_5UTR	
			rs623580	0.28	flanking_5UTR	

11	**Nuclear factor kappa B p65 subunit (RELA)**	NM_021975.2	rs1466462	0.38	flanking_3UTR	
			rs1049728	0.03	flanking_3UTR	
			rs11227247	0.16	intron	
			rs7101916	0.16	flanking_5UTR	
11	**NADPH Oxidase 4 (NOX4)**	NM_016931.2	rs11821838	0.11	intron	
			rs317187	0.40	intron	
			rs1847137	0.31	intron	
			rs7944576	0.08	intron	
			rs317150	0.10	intron	
			rs317155	0.44	intron	
			rs546460	0.43	intron	
			rs2202150	0.38	intron	
			rs317147	0.45	intron	
			rs538102	0.47	intron	
			rs319016	0.45	intron	
			rs957140	0.43	intron	
			rs10830277	0.13	intron	
			rs2164521	0.10	intron	
			rs614128	0.08	intron	
			rs497279	0.27	intron	
			rs3017887	0.09	flanking_5UTR	
			rs585197	0.21	flanking_5UTR	
			rs553635	0.09	flanking_5UTR	
11	**Transient receptor potential cation channel, subfamily C, 6 (TRPC6)**	NM_004621.3	rs11826762	0.09	intron	
			rs11224779	0.33	intron	
			rs11224783	0.25	intron	
			rs11821584	0.36	intron	
			rs7945727	0.12	intron	
			rs4403777	0.48	intron	
			rs7925012	0.47	intron	
			rs7101962	0.11	intron	
			rs7931676	0.32	intron	
			rs10895131	0.12	intron	
			rs4469857	0.37	intron	
			rs4331057	0.36	intron	
			rs10895142	0.08	intron	
			rs10895146	0.48	intron	
			rs10501985	0.48	intron	
			rs7103450	0.16	intron	
			rs7121108	0.15	intron	
			rs10895150	0.09	flanking_5UTR	
11	**Matrix metalloproteinase 3 (MMP3)**	NM_002422.3	rs4754884	0.42	flanking_3UTR	
			rs650108	0.23	intron	
			rs520540	0.41	coding	A361A
			rs566125	0.11	intron	
			rs679620	0.43	coding	K44E

			rs522616	0.13	flanking_5UTR	
12	**Potassium channel, voltage-gated, shaker, member 5 (KCNA5)**	NM_002234.2	rs7973471	0.11	flanking_5UTR	
			rs7298858	0.12	flanking_5UTR	
			rs887353	0.25	flanking_5UTR	
			rs11615552	0.10	flanking_5UTR	
			rs3741930	0.35	5UTR	
			rs1860420	0.48	flanking_3UTR	
			rs12311859	0.25	flanking_3UTR	
			rs10774297	0.09	flanking_3UTR	
			rs7302727	0.20	flanking_3UTR	
12	**Von Willebrand factor (VWF)**	NM_000552.2	rs3809241	0.22	flanking_5UTR	
			rs7976955	0.22	flanking_3UTR	
			rs2270151	0.13	intron	
			rs2286646	0.22	intron	
			rs10774387	0.22	intron	
			rs723189	0.41	intron	
			rs4764478	0.24	intron	
			rs917857	0.44	intron	
			rs917858	0.33	intron	
			rs2239138	0.33	intron	
			rs216856	0.27	intron	
			rs216867	0.08	coding	T2412T
			rs2058473	0.43	intron	
			rs216873	0.13	intron	
			rs216891	0.48	intron	
			rs216893	0.47	intron	
			rs216902	0.26	coding	C1947C
			rs216905	0.24	intron	
			rs216805	0.29	intron	
			rs216812	0.31	intron	
			rs542993	0.46	intron	
			rs216312	0.48	intron	
			rs11609815	0.24	intron	
			rs216330	0.31	intron	
			rs216333	0.25	intron	
			rs11614912	0.22	intron	
			rs10849378	0.26	intron	
			rs11064004	0.29	intron	
			rs216290	0.13	intron	
			rs1063856	0.34	coding	T788A
			rs216303	0.12	intron	
			rs980130	0.31	intron	
			rs980131	0.38	intron	
			rs1800378	0.38	coding	H483R
			rs2238104	0.44	intron	
			rs2109118	0.48	intron	
			rs2239144	0.09	intron	

			rs3782716	0.35	intron	
			rs10849387	0.35	flanking_5UTR	
12	**Elastase 1 (ELA1)**	NM_001971.4	rs4762040	0.37	intron	
			rs4762041	0.28	intron	
			rs3843650	0.38	intron	
			rs7311196	0.18	flanking_5UTR	
			rs7138439	0.37	3UTR	
			rs10876162	0.37	intron	
			rs3847862	0.46	intron	
			rs17860299		coding	R43W
12	**Activin A receptor, type II-like kinase (ACVRL1)**	NM_000020.1	rs3759178	0.35	flanking_5UTR	
			rs11169953	0.23	intron	
			rs706812	0.00	intron	
			rs2071219		intron	
			rs706815	0.17	intron	
			rs706824	0.22	flanking_3UTR	
12	**Retinoic acid receptor, gamma (RARG)**	NM_000966.3	rs1554753	0.20	flanking_3UTR	
			rs3741434	0.12	3UTR	
			rs2229774	0.04	coding	S426L
			rs1465057	0.06	intron	
			rs6580936	0.16	intron	
			rs7398676	0.48	flanking_5UTR	
12	**Tryptophan hydroxylase 2 (TPH2)**	NM_173353.2	rs472197	0.44	intron	
			rs10748185	0.44	intron	
			rs2129575	0.20	intron	
			rs1843809	0.13	intron	
			rs1386494	0.13	intron	
			rs1386493	0.15	intron	
			rs1386492	0.15	intron	
			rs6582078	0.34	intron	
			rs1007023	0.12	intron	
			rs1386497	0.12	intron	
			rs1352250	0.38	intron	
			rs1487278	0.22	intron	
			rs9325202	0.35	intron	
			rs1386486	0.30	intron	
			rs7309440	0.02	flanking_3UTR	
			rs300510	0.46	intron	
12	**Farnesoid X receptor (NR1H4)**	NM_005123.1	rs11110390	0.32	intron	
			rs4764980	0.49	intron	
			rs17030285	0.14	intron	
			rs1030454	0.17	intron	
			rs35738	0.42	intron	
			rs35735	0.40	intron	
			rs35723	0.41	flanking_3UTR	
13	**Endothelin receptor, nonselective type (EDNRB)**	NM_000115.1	rs1924919	0.19	flanking_3UTR	

			rs11149080	0.41	flanking_3UTR	
			rs4885491	0.12	3UTR	
			rs3027095	0.03	3UTR	
			rs3818416	0.21	intron	
			rs5352	0.01	coding	S304N
			rs5351	0.40	coding	L276L
			rs2147555	0.08	intron	
			rs2329046	0.40	intron	
			rs4885493	0.28	intron	
			rs7982910	0.48	intron	
			rs3759475	0.41	intron	
			rs9544638	0.40	intron	
13	**Solute carrier family 10, member 2 (SLC10A2)**	NM_000452.1	rs7992775	0.47	flanking_3UTR	
			rs6491729	0.27	flanking_3UTR	
			rs279941	0.19	3UTR	
			rs190716	0.22	intron	
			rs1854519	0.28	intron	
			rs183963	0.33	intron	
			rs4772525	0.31	intron	
			rs157266	0.18	intron	
			rs1886927	0.16	intron	
			rs3759504	0.24	flanking_5UTR	
			rs466802	0.03	flanking_5UTR	
			rs7319981	0.30	flanking_5UTR	
14	**Hypoxia-inducible factor 1, alpha subunit (HIF1A)**	NM_001530.2	rs1951795	0.15	intron	
			rs4899056	0.06	intron	
			rs1957757	0.06	intron	
			rs11158358	0.13	intron	
			rs2301111	0.16	intron	
			rs2301113	0.18	intron	
			rs11549465	0.09	coding	P581S
			rs1319462	0.17	flanking_3UTR	
14	**Estrogen receptor 2 (ESR2)**	NM_001437.1	rs1152579	0.38	flanking_3UTR	
			rs1256064	0.09	intron	
			rs1256063	0.08	intron	
			rs1256061	0.48	intron	
			rs1256059	0.40	intron	
			rs8017441	0.13	intron	
			rs7157428	0.09	intron	
			rs1256049	0.03	coding	V327V
			rs7154455	0.34	intron	
			rs1256030	0.42	intron	
			rs1952586	0.16	intron	
			rs1887994	0.08	intron	
			rs1271572	0.41	flanking_5UTR	
			rs975232	0.30	flanking_3UTR	

14	**Solute carrier family 10, member 1 (SLC10A1)**	NM_003049.1	rs8013586	0.19	flanking_3UTR	
			rs9323529	0.01	intron	
			rs2296651	0.00	coding	S266F
			rs11624523	0.32	intron	
			rs11622925	0.12	intron	
14	**V-AKT murine Thymoma viral oncogene homolog 1 (AKT1)**	NM_001014431.1	rs2498804	0.33	flanking_3UTR	
			rs2494730	0.33	flanking_3UTR	
			rs2498802	0.34	flanking_3UTR	
			rs3730358		intron	
			rs2494738	0.11	intron	
			rs2494746	0.08	intron	
			rs1130214	0.28	5UTR	
15	**Thrombospondin-1 (THBS1)**	NM_003246.2	rs1478604	0.22	5UTR	
			rs753599	0.10	intron	
			rs2292305	0.10	coding	T522A
			rs2228262	0.08	coding	N699S
			rs1051442	0.12	3UTR	
15	**Dual oxidase 2 (DUOX2)**	NM_014080.3	rs269866	0.36	intron	
	Dual oxidase 1 (DUOX1)	NM_017434.3	rs269863	0.02	intron	
			rs1961660	0.01	intron	
			rs269856	0.03	intron	
			rs1365242	0.46	intron	
			rs1706810	0.27	intron	
			rs1648282	0.38	intron	
			rs2020216	0.11	intron	
			rs3784577	0.10	intron	
			rs1706803	0.16	intron	
			rs1706804	0.24	coding	T1075T
			rs1706808	0.38	intron	
			rs1648312	0.38	3UTR	
			rs2292467	0.26	3UTR	
			rs1706816	0.12	flanking_3UTR	
15	**Aromatase (CYP19A1)**	NM_000103.2	rs1122044	0.50	flanking_3UTR	
			rs2414093	0.17	flanking_3UTR	
			rs4775928	0.38	flanking_3UTR	
			rs3759809	0.25	flanking_3UTR	
			rs934633	0.08	flanking_3UTR	
			rs10046	0.45	3UTR	
			rs28757184	0.06	coding	T200M
			rs2899472	0.27	intron	
			rs700518	0.41	coding	V79V
			rs10519295	0.10	intron	
			rs727479	0.30	intron	
			rs12591359	0.36	intron	
			rs1062033	0.48	intron	
			rs2008691	0.17	intron	

			rs1008805	0.45	intron	
			rs749292	0.46	intron	
			rs1902586	0.05	intron	
			rs936306	0.18	intron	
			rs2470176	0.18	intron	
			rs2470152	0.45	intron	
			rs2470150	0.08	intron	
			rs1902584	0.11	intron	
			rs1004984	0.42	intron	
			rs7175922	0.15	flanking_5UTR	
15	**Homolog of drosophila mothers against dpp 3 (SMAD3)**	NM_005902.3	rs12904944	0.32	intron	
			rs10518707	0.48	intron	
			rs4776881	0.42	intron	
			rs12324036	0.42	intron	
			rs7176870	0.41	intron	
			rs9972423	0.42	intron	
			rs2118612	0.21	intron	
			rs1438386	0.32	intron	
			rs718663	0.07	intron	
			rs4776892	0.21	intron	
			rs920293	0.06	intron	
			rs11071937	0.33	intron	
			rs4776342	0.23	intron	
			rs991157	0.31	intron	
			rs11071938	0.33	intron	
			rs6494633	0.44	intron	
			rs7178117	0.45	intron	
			rs2414937	0.18	intron	
			rs12443188	0.28	intron	
			rs745103	0.38	intron	
			rs893473	0.16	intron	
			rs2289263	0.47	intron	
			rs2033785	0.26	intron	
			rs731874	0.30	intron	
			rs12441344	0.23	intron	
			rs2033784	0.28	intron	
			rs7173698	0.33	intron	
			rs11639295	0.32	intron	
			rs7183244	0.40	intron	
			rs2278545	0.10	intron	
			rs1470003	0.47	intron	
			rs3784681	0.30	intron	
			rs6494636	0.49	intron	
			rs8031440	0.19	3UTR	
16	**Protein kinase C, beta 1 (PRKCB1)**	NM_002738.5	rs3760106	0.18	flanking_5UTR	
			rs2575390	0.20	flanking_5UTR	
			rs2188357		intron	

			rs195999	0.38	intron	
			rs4788192	0.36	intron	
			rs1971276		intron	
			rs3729896	0.01	coding	P201P
			rs7196765		intron	
			rs1013316	0.41	intron	
			rs429342	0.21	intron	
			rs3729904		coding	G589G
			rs3785380	0.02	3UTR	
16	**Matrix metalloproteinase 2 (MMP2)**	NM_004530.2	rs243866	0.24	flanking_5UTR	
			rs243865	0.18	flanking_5UTR	
			rs857403	0.13	intron	
			rs1132896	0.43	coding	G225G
			rs1053605	0.08	coding	T249T
			rs243842	0.36	intron	
			rs243839	0.14	intron	
			rs2287076	0.49	intron	
			rs243836	0.48	intron	
			rs243834	0.46	intron	
			rs17302903	0.50	5UTR	
17	**Sex hormone binding globulin (SHBG)**	NM_001040.2	rs13894	0.10	coding	R125C
			rs858521	0.39	intron	
			rs6258	0.02	coding	P184L
			rs6259	0.14	coding	D355N
			rs2955617	0.38	flanking_3UTR	
			rs1641544	0.07	flanking_3UTR	
17	**Nitric oxide synthase 2 (NOS2A)**	NM_000625.3	rs4795051	0.49	flanking_3UTR	
			rs7406657	0.19	flanking_3UTR	
			rs8068149	0.35	intron	
			rs2314809	0.47	intron	
			rs2297516	0.34	intron	
			rs2297517	0.00	intron	
			rs2248814	0.48	intron	
			rs4796052	0.17	intron	
			rs1137933	0.20	coding	D384D
			rs4795067	0.30	intron	
			rs1113283	0.22	intron	
			rs3729508	0.49	intron	
			rs944725	0.39	intron	
			rs3794764	0.18	intron	
			rs11080358	0.08	flanking_5UTR	
17	**Retinoic acid receptor, alpha (RARA)**	NM_000964.2	rs2715554	0.16	intron	
			rs2715553	0.46	intron	
			rs9303285	0.17	intron	
			rs482284	0.28	intron	
17	**Angiotensin I converting enzyme (ACE)**	NM_152830.1	rs4305	0.40	intron	

			rs4309	0.46	coding	P404P
			rs4311	0.38	intron	
			rs4329	0.48	intron	
			rs4343	0.48	coding	T775T
			rs4344	0.49	intron	
			rs4353	0.48	intron	
			rs4362	0.49	coding	F1128F
			rs4363	0.50	intron	
			rs4461142	0.48	intron	
			rs4267385	0.42	intron	
			rs4459610	0.42	coding	K714N
			rs8066276	0.38	intron	
			rs12451328	0.39	intron	
			rs4968591	0.42	intron	
17	**Protein kinase C, alpha (PRKCA)**	NM_002737.2	rs6504413	0.05	intron	
			rs4328478	0.44	intron	
			rs12450534	0.23	intron	
			rs8078231	0.27	intron	
			rs4435295	0.07	intron	
			rs4561502	0.28	intron	
			rs4417581	0.09	intron	
			rs4790911	0.08	intron	
			rs16959227	0.11	intron	
			rs11079656	0.28	intron	
			rs973753	0.15	intron	
			rs7405806	0.25	intron	
			rs11079657	0.18	intron	
			rs990082	0.32	intron	
			rs228885	0.26	intron	
			rs11654093	0.33	intron	
			rs8074294	0.35	intron	
			rs7211424	0.44	intron	
			rs956952	0.17	intron	
			rs1806448	0.26	intron	
			rs1003598	0.48	intron	
			rs2078153	0.24	intron	
			rs1860984	0.08	intron	
			rs887797	0.40	intron	
			rs10491204	0.14	intron	
			rs1985633	0.39	intron	
			rs1860985	0.11	intron	
			rs8068966	0.38	intron	
			rs11867695	0.48	intron	
			rs8071795	0.10	intron	
			rs17710992	0.28	intron	
			rs3889237	0.36	intron	
			rs8464	0.15	3UTR	
18	**Homolog of drosophila mothers**	NM_005901.4	rs1792666	0.48	flanking_3UTR	

	against dpp 2 (SMAD2)					
			rs8085335	0.08	flanking_3UTR	
			rs1792684	0.48	intron	
			rs9946556	0.45	intron	
			rs1792658	0.22	intron	
			rs1792683	0.43	intron	
			rs1787177	0.08	intron	
			rs1631576	0.47	intron	
			rs11082639	0.48	intron	
			rs4940086	0.34	intron	
18	**Homolog of drosophila mothers against dpp 4 (SMAD4)**	NM_005359.3	rs12958604	0.42	intron	
			rs12968012	0.40	intron	
			rs10502913	0.27	intron	
			rs3764465	0.42	intron	
			rs948588	0.09	intron	
19	**Elastase 2 (ELA2)**	NM_001972.2	rs3761008	0.11	flanking_3UTR	
			rs3826946	0.14	flanking_3UTR	
			rs7260160		flanking_5UTR	
			rs1683564	0.38	flanking_5UTR	
19	**Transforming growth factor, Beta-1 (TGFB1)**	NM_000660.3	rs1800472	0.02	coding	T262I
			rs4803455	0.48	intron	
			rs2241715	0.30	intron	
			rs1800469	0.31	flanking_3UTR	
			rs1982072	0.31	intron	
19	**Apolipoprotein E (APOE)**	NM_000041.2	rs405509	0.50	flanking_5UTR	
			rs429358		coding	C129R
			rs7412	0.28	coding	R175C
			rs439401	0.38	flanking_3UTR	
19	**BCL2-associated X protein (BAX)**	NM_138764.2	rs11667200	0.12	flanking_5UTR	
			rs11667229	0.48	flanking_5UTR	
			rs11667351	0.14	flanking_5UTR	
			rs1009316	0.12	intron	
			rs1805419	0.24	intron	
			rs4645900	0.05	3UTR	
19	**Protein kinase C, gamma (PRKCG)**	NM_002739.3	rs307941	0.09	flanking_5UTR	
			rs454006	0.25	intron	
			rs3745406	0.35	coding	N188N
			rs3745405	0.34	intron	
			rs3844454	0.08	flanking_5UTR	
20	**Thrombomodulin (THBD)**	NM_000361.2	rs6076013	0.40	flanking_3UTR	
			rs3176123	0.18	3UTR	
			rs6048519	0.40	flanking_5UTR	
			rs8123616	0.24	flanking_5UTR	
20	**Lipopolysaccharide binding protein (LBP)**	NM_004139.2	rs1780616	0.35	flanking_5UTR	
			rs2232571	0.11	flanking_5UTR	

			rs12624843	0.40	intron	
			rs1780623	0.37	intron	
			rs1780627	0.47	intron	
			rs1780629	0.26	flanking_3UTR	
			rs737090	0.48	flanking_3UTR	
20	**Proteinase inhibitor 3; elafin (PI3)**	NM_002638.2	rs1983649	0.38	intron	
			rs6032040	0.14	intron	
			rs2664581	0.14	coding	T33P
			rs2267864	0.16	flanking_3UTR	
20	**Matrix metalloproteinase 9 (MMP9)**	NM_004994.2	rs4810482	0.38	flanking_5UTR	
			rs3918241	0.19	flanking_5UTR	
			rs1805088	0.03	coding	A19V
			rs2250889	0.05	coding	R573P
			rs3918261	0.23	intron	
			rs3918270	0.19	flanking_3UTR	
20	**Prostaglandin I2 synthase (PTGIS)**	NM_000961.3	rs491025	0.46	flanking_3UTR	
			rs5602	0.46	3UTR	
			rs729824	0.25	intron	
			rs7271624	0.26	intron	
			rs6090996	0.17	intron	
			rs508757	0.13	intron	
			rs6091000	0.03	intron	
			rs570022	0.13	intron	
			rs491490	0.29	intron	
			rs927068	0.26	intron	
			rs477627	0.13	intron	
			rs693649	0.16	flanking_5UTR	
			rs6019910	0.07	flanking_5UTR	
21	**Superoxide dismutase 1, soluble (SOD1)**	NM_000454.4	rs10432782	0.12	intron	
			rs2070424	0.09	intron	
			rs1041740	0.23	intron	
21	**Runt-related transcription factor 1 (RUNX1)**	NM_001754.3	rs2070369	0.49	flanking_3UTR	
			rs2070370	0.45	flanking_3UTR	
			rs2073354	0.08	intron	
			rs2249650	0.44	intron	
			rs2249884	0.33	intron	
			rs2834642	0.46	intron	
			rs2253319	0.31	intron	
			rs2834646	0.07	intron	
			rs2834647	0.05	intron	
			rs2243988	0.33	intron	
			rs2226303	0.20	intron	
			rs2834650	0.16	intron	
			rs2284612	0.36	intron	
			rs2268281	0.12	intron	
			rs2284613	0.29	intron	

			rs2268284	0.09	intron	
			rs8134380	0.46	intron	
			rs968625	0.22	intron	
			rs2834651	0.36	intron	
			rs2248720	0.48	intron	
			rs2834654	0.22	intron	
			rs2268290	0.14	intron	
			rs2252585	0.30	intron	
			rs2284617	0.20	intron	
			rs2834656	0.20	intron	
			rs2300396	0.22	intron	
			rs2300400	0.18	intron	
			rs13053063	0.14	intron	
			rs2300401	0.32	intron	
			rs2834662	0.25	intron	
			rs2834664	0.13	intron	
			rs1475840	0.48	intron	
			rs2834675	0.09	intron	
			rs2834676	0.46	intron	
			rs4817699	0.09	intron	
			rs2834680	0.05	intron	
			rs2834683	0.45	intron	
			rs2834684	0.31	intron	
			rs2051179	0.49	intron	
			rs2834703	0.36	intron	
			rs2834708	0.39	intron	
			rs2834709	0.41	intron	
			rs1981392	0.37	intron	
			rs2834714	0.36	intron	
			rs762248	0.10	intron	
			rs8130985	0.10	intron	
			rs2834726	0.07	intron	
			rs2834729	0.14	intron	
			rs2834732	0.43	intron	
			rs2834735	0.38	intron	
			rs2834736	0.36	intron	
			rs2242890	0.35	intron	
			rs2294163	0.16	intron	
			rs2834739	0.36	intron	
			rs1883066	0.15	intron	
			rs7280028	0.16	intron	
			rs7277157	0.24	intron	
			rs2834740	0.36	flanking_5UTR	
21	**Cystathionine-beta-synthase (CBS)**	NM_000071.1	rs719037	0.45	flanking_3UTR	
			rs12613	0.09	3UTR	
			rs4920037	0.22	intron	
			rs234705	0.34	intron	
			rs9982015	0.09	intron	

			rs11701048	0.09	intron	
21	**Collagen, type XVIII, alpha-1 (COL18A1)**	NM_130445.2	rs2838665	0.33	flanking_5UTR	
			rs2183589	0.13	flanking_5UTR	
			rs2838906	0.32	flanking_5UTR	
			rs879330	0.06	flanking_3UTR	
			rs2838907	0.36	flanking_3UTR	
			rs4819099	0.21	flanking_3UTR	
			rs4819101	0.29	flanking_3UTR	
			rs2838917	0.25	3UTR	
			rs2838920	0.11	coding	
			rs7281421	0.31	flanking_5UTR	
			rs2838923	0.32	flanking_5UTR	
			rs8126757	0.15	flanking_5UTR	
			rs8129539	0.12	flanking_5UTR	
			rs9985044	0.31	flanking_5UTR	
			rs11089003	0.20	flanking_5UTR	
			rs2015673	0.09	flanking_5UTR	
			rs10854470	0.36	flanking_5UTR	
			rs2838933	0.12	intron	
			rs2236470	0.21	intron	
			rs1556329	0.09	intron	
			rs2236475	0.20	intron	
			rs2236479	0.31	intron	
			rs7409857	0.45	intron	
			rs3753019	0.37	intron	
			rs2236483	0.38	intron	
			rs2838950	0.25	intron	
			rs7278425	0.14	intron	
			rs1050351	0.42	coding	
			rs2838951	0.47	intron	
22	**Heme oxygenase 1 (HMOX1)**	NM_002133.1	rs12168789	0.00	flanking_5UTR	
			rs2071746	0.46	flanking_5UTR	
			rs2071748	0.38	intron	
			rs2071749	0.49	intron	
			rs11912889	0.07	intron	
			rs5755720	0.30	intron	
			rs2285112	0.39	intron	
			rs743811	0.25	flanking_3UTR	
22	**Peroxisome proliferator activated receptor, alpha (PPARA)**	NM_005036.4	rs9627100	0.09	intron	
			rs4253701	0.11	intron	
			rs11703495	0.14	intron	
			rs4253711	0.27	intron	
			rs4823613	0.30	intron	
			rs1800206	0.04	coding	L161V
			rs4253755	0.18	intron	
			rs4253760	0.21	intron	
			rs11090819	0.06	intron	

			kr1800234	na	intron	
X	**Cytochrome b-245, NADPH Oxidase 2, NOX2 (CYBB)**	NM_000397.2	rs6610650	0.13	flanking_5UTR	
			rs6520785	0.13	intron	
			rs4827298	0.22	intron	
			rs5964125	0.11	intron	
			rs12848910	0.12	intron	
			rs5964149	0.11	intron	
X	**NADPH Oxidase 1 (NOX1)**	NM_007052.3	rs4828067	0.37	intron	
			rs4828068	0.39	intron	
			rs5921678	0.40	intron	
			rs2266916	0.40	intron	
			rs4827881	0.24	flanking_5UTR	
			rs6620949	0.42	flanking_5UTR	
			rs1883411	0.38	intron	
X	**Thromboplastin (HEMB)**	NM_000133.2	rs411017	0.27	flanking_5UTR	
			rs371000	0.48	intron	
			rs4149674	0.37	intron	
			rs4149676	0.01	intron	
			rs376165	0.38	intron	
			rs422187	0.33	intron	
			rs6048	0.33	coding	T193A
			rs413957	0.18	intron	
			rs4149762	0.12	intron	
			rs434144	0.20	flanking_3UTR	
			rs3117074	0.18	flanking_3UTR	

Table E2. Pairwise linkage disequilibrium in candidate genes.

Gene	SNP 1	SNP 2	D'	95%CI	LOD	r^2	Base pairs between SNPs
ESR1	rs2234693	rs1913474	0.07	0 – 0.4	0.03	0.001	45387
	rs1913474	rs1801132	0.85	0.75 – 0.91	25.69	0.67	56800
	rs1801132	rs3020317	0.75	0.64 – 0.83	17.63	0.53	13219
	rs3020317	rs985694	0.71	0.57 – 0.81	12.54	0.42	7884
	rs985694	rs932477	0.97	0.85 - 1	20.24	0.61	17971
	rs932477	rs7757956	0.18	0.02 – 0.73	0.04	0.001	12544
	rs7757956	rs3020411	0.96	0.8 – 0.99	12.66	0.31	26623
	rs7757956	rs3020368	0.89	0.76 – 0.96	18.24	0.58	54050
PDE5A	rs3775843	rs1155576	1	0.95 - 1	45.82	0.95	22315
	rs1155576	rs10034450	1	0.95 - 1	45.82	0.95	5490
	rs10034450	rs11731756	1	0.97 - 1	51	1	23047
ANGPT1	rs4324901	rs4268102	0.42	0.08 – 0.69	0.77	0.03	106611
RARB	rs871963	rs1153584	0.37	0.21 - 0.5	3.44	0.11	31350
CYP19A1	rs1902584	rs7175922	1	0.8 - 1	10.84	0.35	35083
SMAD3	rs4776881	rs12324036	1	0.97 - 1	59.57	1	1490
S100A4	rs1810765	rs743687	1	0.88 - 1	21.84	0.71	405

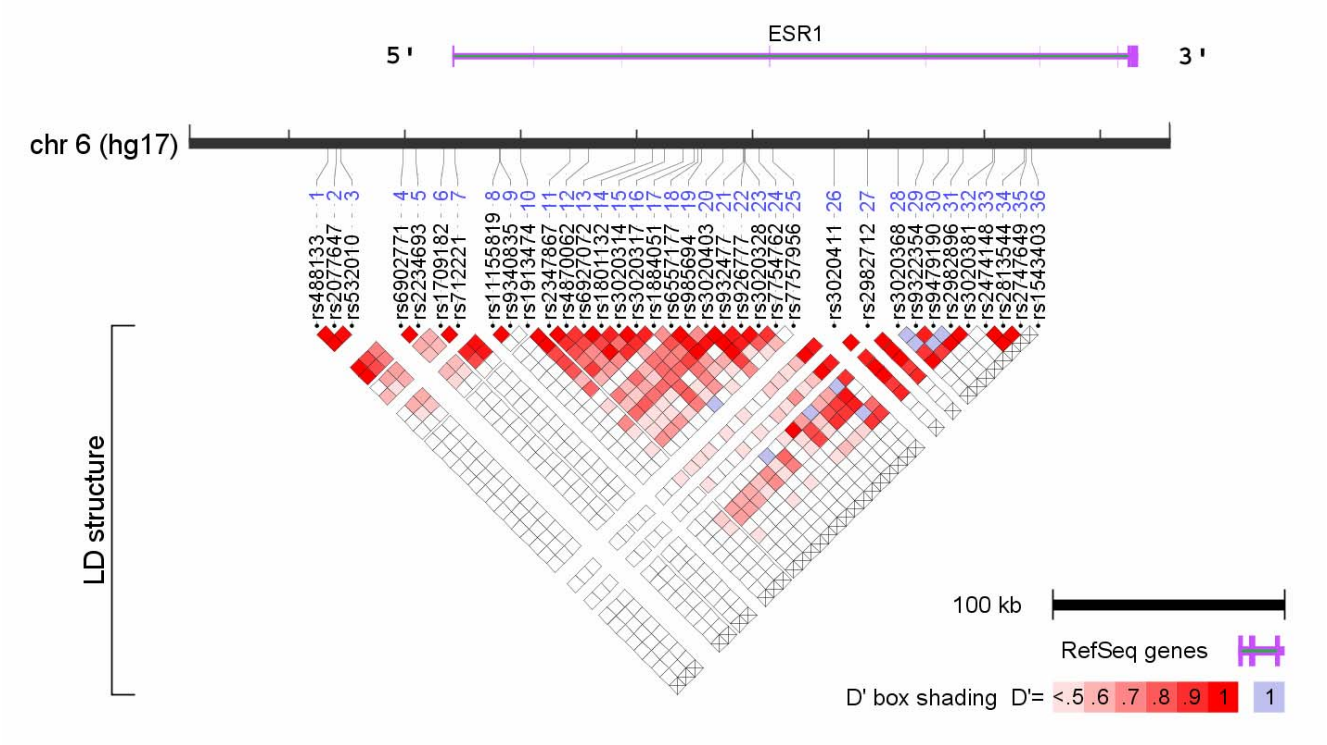
Abbreviations: LOD, Log of the likelihood of the odds ratio; ESR1, estrogen receptor 1; PDE5A, phosphodiesterase 5; ANGPT1, angiopoeitin 1; RARB, retinoic acid receptor beta; CYP19A1, aromatase; S100A4, calcium-binding protein A4.

Table E3. Logistic regression models for SNPs and the risk of PPHTN (unadjusted and adjusted for liver disease etiology)

			Unadjusted				Adjusted for liver disease			
Gene	**SNP**	**Liver Disease**	**OR**	**95%CI**	**P value**	**β**	**OR**	**95%CI**	**P value**	**β**
ESR1	rs1801132	Primary sclerosing cholangitis	2.57	1.14 – 5.83	0.02	0.94	2.81	1.22 – 6.48	0.02	1.03
	rs985694	Hepatitis C infection	2.52	1.03 – 6.15	0.04	0.92	3.26	1.25 – 8.54	0.02	1.18
	rs985694	Autoimmune	2.52	1.03 – 6.15	0.04	0.92	5.34	1.54 – 18.53	0.01	1.68
	rs932477	Hepatitis B infection	3.11	1.02 – 9.49	0.05	1.13	3.08	1 – 9.44	0.05	1.12
	rs7757956	Cryptogenic	0.55	0.26 - 1.13	0.10	-0.60	0.51	0.24 – 1.09	0.08	-0.68
	rs3020368	Primary sclerosing cholangitis	0.53	0.24 – 1.16	0.11	-0.64	0.47	0.21 – 1.06	0.07	-0.75
S100A4	rs1810765	Hepatitis B infection	1.88	0.9 – 3.92	0.09	0.63	2.06	0.96 – 4.41	0.06	0.72
	rs743687	Hepatitis B infection	2.83	1.21 – 6.63	0.02	1.04	3.29	1.34 – 8.09	0.01	1.19
RARB	rs1153584	Hepatitis B infection	1.79	0.97 - 3.32	0.07	0.58	1.79	0.96 - 3.34	0.07	0.58

Abbreviations: ESR1, estrogen receptor 1; S100A4, calcium-binding protein A4; RARB, retinoic acid receptor beta; OR, odds ratio

Figure E1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rs1913474
rs2347867
rs4870062
rs6927072
rs1801132
rs3020314
rs3020317
rs1884051
rs6557177
rs985694
rs3020403
rs932477
rs926777
rs3020328
rs7554762
rs7757956
rs3020411
rs2982712
rs3020368
rs9322354
rs9479190
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rs2474148
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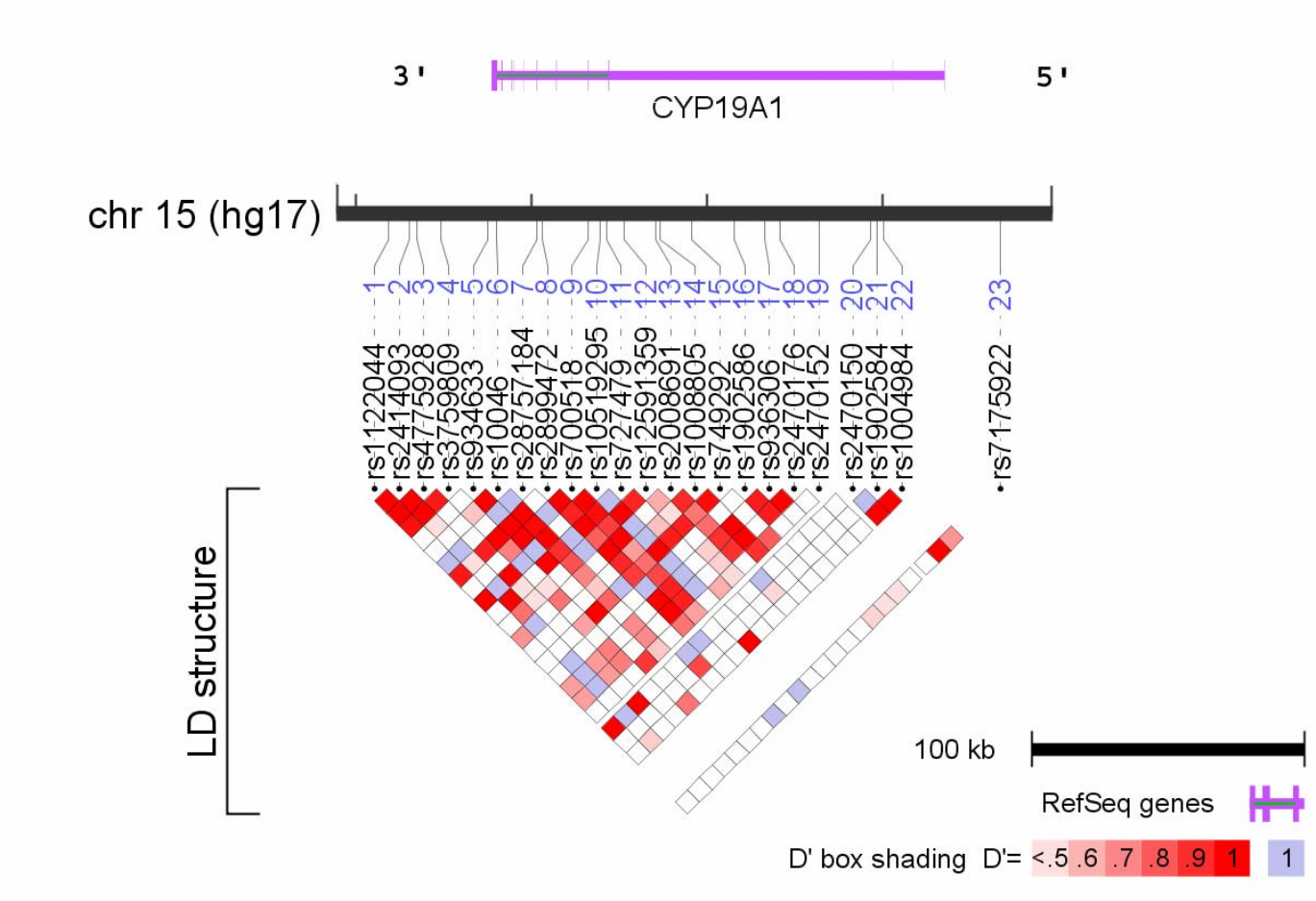
LD structure
100 kb
RefSeq genes
D' box shading D'= <.5 .6 .7 .8 .9 1 1

Figure E2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5'
CYP19A1
chr 15 (hg17)
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rs4775928
rs3759809
rs934633
rs10046
rs2875184
rs2899472
rs700518
rs10519295
rs727479
rs12591359
rs2008691
rs1008805
rs749292
rs1902586
rs936306
rs2470176
rs2470152
rs2470150
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LD structure
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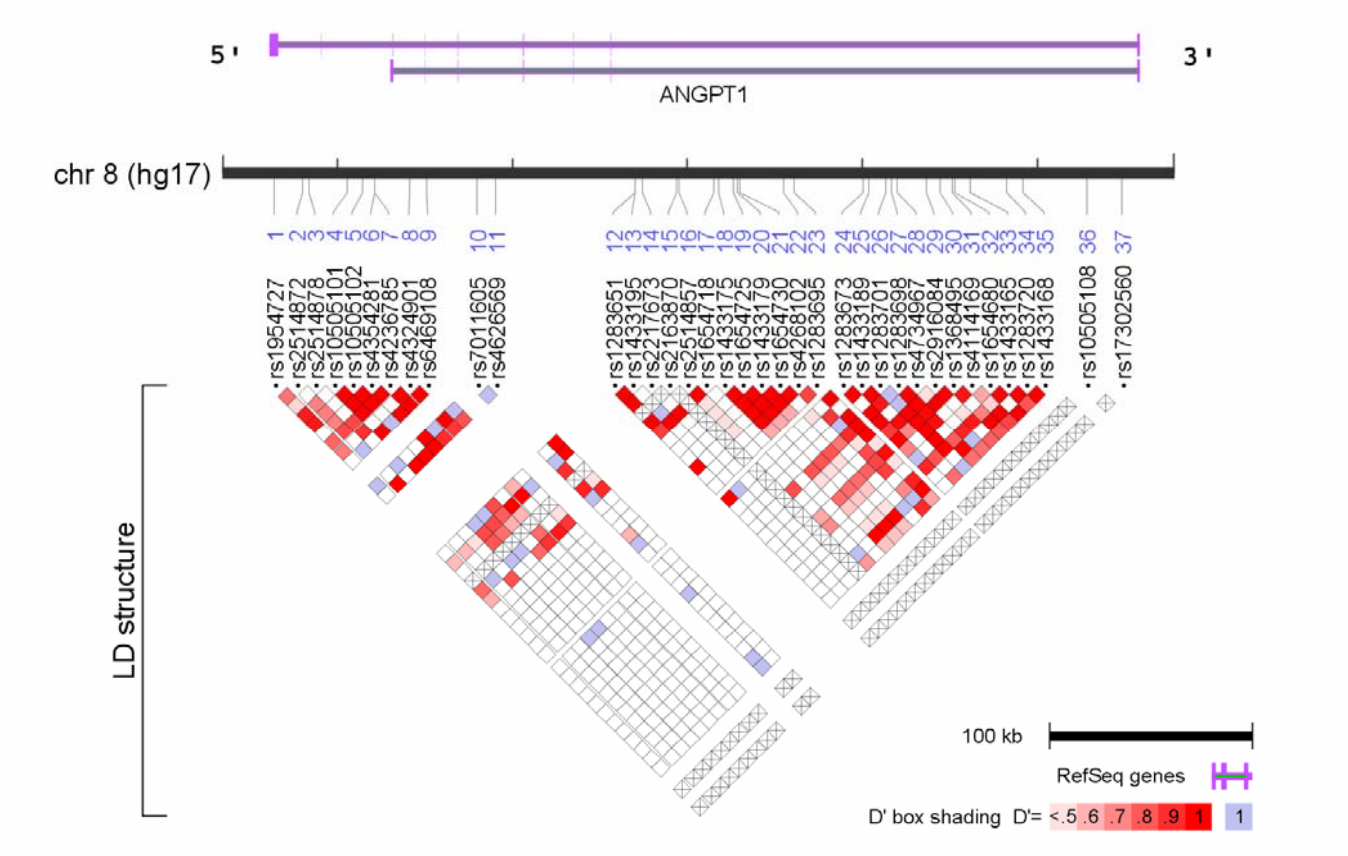
RefSeq genes
D' box shading D'= <.5 .6 .7 .8 .9 1 1

Figure E3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3'
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rs10505102
rs4354281
rs4236785
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rs2217673
rs2163870
rs2514857
rs1654718
rs1433175
rs1654725
rs1433179
rs1654730
rs4268102
rs1283695
rs1283673
rs1433189
rs1433701
rs1283698
rs4734967
rs2916084
rs1368495
rs4114169
rs1654680
rs1433165
rs1283720
rs1433168
rs10505108
rs17302560
LD structure
100 kb
RefSeq genes
D' box shading D'= <.5 .6 .7 .8 .9 1 1

References for Online Data Supplement

E1. Hankinson JL, Odencrantz JR, and Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med* 1999; 159:179-87.

E2. Crapo RO, Morris AH, Clayton PD, and Nixon CR. Lung volumes in healthy nonsmoking adults. *Bull Eur Physiopathol Respir* 1982; 18:419-25.

E3. Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, D'Amico G, Dickson ER, and Kim WR. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; 33:464-70.

E4. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, and Sherlock G. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 2000; 25:25-9.

E5. Seldin MF, Shigeta R, Villoslada P, Selmi C, Tuomilehto J, Silva G, Belmont JW, Klareskog L, and Gregersen PK. European population substructure: clustering of northern and southern populations. *PLoS Genet* 2006; 2:e143.

E6. Kawut SM, Krowka MJ, Trotter JF, Roberts KE, Benza RL, Badesch DB, Taichman DB, Horn EM, Zacks S, Kaplowitz N, Brown RS, Jr., and Fallon MB. Clinical risk factors for portopulmonary hypertension. *Hepatology* 2008; 48:196-203.

E7. Barrett JC, Fry B, Maller J, and Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; 21:263-5.

E8. Pritchard JK, and Rosenberg NA. Use of unlinked genetic markers to detect population stratification in association studies. *Am J Hum Genet* 1999; 65:220-8.

E9. Pritchard JK, Stephens M, Rosenberg NA, and Donnelly P. Association mapping in structured populations. *Am J Hum Genet* 2000; 67:170-81.

E10. Gauderman W, and Morrison J. 2006. QUANTO 1.1: A computer program for power and sample size calculations for genetic-epidemiology studies, http://hydra.usc.edu/gxe.

E11. Zheng G, Joo J, Zhang C, and Geller NL. Testing association for markers on the X chromosome. *Genet Epidemiol* 2007; 31:834-43.

E12. Petryshen T, A. Kirby, M. Ainscow. LocusView 2.0. Broad Institute.