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Genetic Risk Factors for Hepatopulmonary Syndrome in Patients With Advanced Liver Disease

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Abstract

Background & Aims—Hepatopulmonary syndrome (HPS) affects 10%–30% of patients with cirrhosis and portal hypertension and significantly increases mortality. Studies in experimental models indicate that pulmonary angiogenesis contributes to the development of HPS, but pathogenesis in humans is poorly understood. We investigated genetic risk factors for HPS in patients with advanced liver disease.

Methods—We performed a multicenter case-control study of patients with cirrhosis being evaluated for liver transplantation. Cases had an alveolar-arterial oxygen gradient ≥15 mm Hg (or ≥20 mm Hg if age > 64 years) and contrast echocardiography with late appearance of microbubbles after venous

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injection of agitated saline (intrapulmonary vasodilatation); controls did not meet both criteria for case status. The study sample included 59 cases and 126 controls. We genotyped 1086 common single nucleotide polymorphisms (SNPs) in 94 candidate genes.

Results—Forty-two SNPs in 21 genes were significantly associated with HPS after adjustments for race and smoking. Eight genes had at least 2 SNPs associated with disease: *CAV3*, *ENG*, *NOX4*, *ESR2*, *VWF*, *RUNX1*, *COL18A1*, and *TIE1*. For example, rs237872 in *CAV3* showed an odds ratio of 2.75 (95% confidence interval: 1.65–4.60, P =.0001) and rs4837192 in *ENG* showed an odds ratio of 0.35 (95% confidence interval: 0.14–0.89, P =.027). Furthermore, variation in *CAV3* and *RUNX1* was associated with HPS in gene-based analyses.

Conclusions—Polymorphisms in genes involved in the regulation of angiogenesis are associated with the risk of HPS. Further investigation of these biologic pathways might elucidate the mechanisms that mediate the development of HPS in certain patients with severe liver disease.

Keywords

Genetic Polymorphism; Portal Hypertension

The hepatopulmonary syndrome (HPS) occurs when vascular alterations in the pulmonary microvasculature lead to abnormalities in systemic oxygenation in the setting of liver disease or portal hypertension.[1]1 This syndrome is found in 10%–30% of patients with cirrhosis being evaluated for liver transplantation, and we and others have shown that HPS is associated with worse quality of life and increased mortality.[2–4] Currently, the only established treatment for HPS is liver transplantation, although postoperative survival may be lower in patients with HPS relative to those without HPS.[5] Together, these observations support the need to define the pathogenesis of HPS to develop effective medical therapies. Identification of genetic risk factors for this prevalent and morbid complication of liver disease could suggest novel therapeutic approaches.

The mechanisms for HPS in patients with cirrhosis are unclear. Early experimental and human studies implicated pulmonary microvascular dilation, in part related to excess nitric oxide production and altered estrogen signaling in disease pathogenesis.[6–8] Although impaired vasomotor tone contributes to the pathophysiology of HPS, incomplete response to pharmacologic blockade of these pathways implies additional mechanisms.[9,10] Observations of increased angiogenesis in the splanchnic and hepatic microvascular beds[11] and increased pulmonary capillary density[12] in advanced liver disease suggest that vascular remodeling and pulmonary angiogenesis may also play a role. This hypothesis is supported by the recent demonstration of increased in pulmonary microvessels as well as up-regulation of vascular endothelial growth factor-mediated angiogenic pathways in the common bile duct-ligated rat, an animal model of human HPS.[13]

We therefore hypothesized that variation in genes responsible for vascular phenotype and homeostasis contributes to the risk of developing HPS. We performed a high-throughput candidate gene study in an attempt to identify common genetic variation associated with the risk of HPS in a group of patients undergoing liver transplantation evaluation. This work has been previously published in abstract form.[14]

Patients and Methods

For other information regarding patients and methods, please see Supplementary Patients and Methods.

Study Cohort and Study Sample

The Pulmonary Vascular Complications of Liver Disease Study enrolled a cohort of 536 patients evaluated for liver transplantation at 7 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 (<2 weeks) gastrointestinal bleeding, or those who had undergone liver or lung transplantation. 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 new patients from the Pulmonary Vascular Complications of Liver Disease Study cohort evaluated with contrast transthoracic echocardiography, spirometry, and arterial blood gas sampling (routinely performed for pretransplant evaluation) during the study period with available genetic data. We excluded patients with pulmonary function testing showing a significant obstructive or restrictive ventilatory defect (see Supplementary Patients and Methods). We also excluded patients with intracardiac shunting (or with uninterpretable shunt timing) by transthoracic echocardiography as described below.

Contrast transthoracic echocardiography was performed and interpreted at each center. Agitated saline was injected via a peripheral vein during imaging. Appearance of microbubbles in the left heart ≥ 3 cardiac cycles after venous injection of agitated saline was considered "late," consistent with intrapulmonary shunting. Appearance of microbubbles in the left heart < 3 cardiac cycles after venous injection was considered "early," consistent with intracardiac shunting.

Case and Control Definitions

Cases and controls were identified from those patients fulfilling the inclusion and exclusion criteria defined above. HPS was defined by (1) contrast echocardiography with late appearance of microbubbles after venous injection of agitated saline and (2) an alveolar-arterial (A-a) oxygen gradient ≥15 mm Hg (or ≥20 mm Hg if age > 64 years), as recommended by the European Respiratory Society Task Force Pulmonary-Hepatic Vascular Disorders Scientific Committee.[1] Patients who did not meet both criteria were considered in the "non-HPS" group (controls). Patients with either "early" or indeterminate timing of the appearance of microbubbles in the left heart after agitated saline injection were excluded from the study.

Clinical Variables and Blood Sampling

Data were collected from subjects and the medical record. The Model for End-stage Liver Disease score was calculated, without inclusion of exception points for either hepatocellular carcinoma or HPS.[15] Phlebotomy was performed, and blood was collected into EDTA-containing tubes. Plasma and buffy coat layers were stored at −80°C.

Candidate Genes and Single Nucleotide Polymorphism Selection

Ninety-four genes affecting vascular function were selected by the investigators (Table 1). For this study, 1086 single nucleotide polymorphisms (SNP) in the 94 candidate genes were genotyped (Supplementary Table 1). We genotyped an additional set of 61 SNPs (null loci) from a validated list of Ancestry Informative Markers (AIM)[16] to detect potential population stratification. (See Supplementary Patients and Methods for details of gene and SNP selection.)

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). SNP assays that failed to generate results in >10% of subjects were considered to have failed and not used for analyses.

Statistical Analysis

Continuous data were summarized using mean ± standard deviation or median (interquartile range), as appropriate. Categorical variables were summarized using number and percentage. To test for differences in covariates between cases and controls, Student *t* tests, Wilcoxon rank-sum tests, χ^2 tests, and Fisher exact tests were used, as appropriate.

Genotype distributions were tested for consistency with expected Hardy–Weinberg equilibrium (HWE) proportions in controls. Single locus association analyses were assessed assuming an additive genetic model using multivariable logistic regression, with adjustment for race and smoking (previously associated with case status[4]). The association of genotype with case/control status was expressed with odds ratios (ORs). Potential population stratification within our sample was tested using multidimensional scaling using AIM.[17] These analyses were performed in PLINK v1.02 (http://pngu.mgh.harvard.edu/purcell/plink/).[18]

For genes in which more than 1 SNP was associated with HPS, we identified linkage disequilibrium blocks containing 3 or more SNPs using Haploview 4.0.[19] We used an expectation-maximization algorithm to estimate haplotypes. Association between disease status and haplotypes was assessed using a generalized linear model approach via the R package Haplo.stats.[20] Both global tests of haplotype association and haplotype-specific analysis (providing ORs with respect to a referent haplotype) were conducted.

Principal component (PC) regression analysis was used to synthesize information across several SNPs within a gene in a gene-based approach.[21,22] Each SNP was assigned a score based on the per-allele model, and PCs were constructed to be linear combinations of these scores. We used the PCs in a logistic regression model to investigate the association between each gene and case status. For each gene, we calculated PCs using the pcreg procedure in R.[23]

In a second gene-based approach, we used classification and regression trees (CART) to help select a small initial subset of interesting markers with high probability for further investigation.[24] In the CART analysis, we specified a minimum group size of 7 and minimum splitting size of 20 in R. Furthermore, we conducted a Random Forests analysis, which creates an ensemble of CART trees using random two-thirds samples of the data then tests the tree with the remaining one third of the data.[25] Missing data were replaced using the multiple imputation algorithm and the Random Forests algorithm.

There was 80% power to detect ORs of ≥ 1.91–3.92 (or ≤0.26–0.52), depending on the minor allele frequency of the SNP (0.05–0.45). Power analysis was performed using QUANTO 1.2.[26] Because the main goal of this study was hypothesis generation, adjustment for multiple comparisons was not performed. $P < .05$ was considered significant for all analyses.

Results

There were 59 cases and 126 controls included in this analysis (Table 2). The mean age of the subjects was 53 ± 10 years, 39% were female, and the majority (83%) was non-Hispanic. The majority of subjects in both groups had liver disease because of hepatitis C infection (44%) or alcohol (41%). Subjects with HPS had a mean PaO_2 of 75 ± 13 mm Hg and a median alveolar-arterial oxygen gradient of 25 mm Hg (interquartile range, 19–35 mm Hg).

Of the 1086 SNPs genotyped in candidate genes, 3 assays failed, 13 SNPs were monomorphic, and 65 SNPs did not conform to HWE (P <.05), leaving 1005 SNPs in the analysis. Of the 61 AIM SNPs, 3 were out of HWE (P <.05) and were thus not used for assessment of population stratification. There was no evidence of population stratification in our study population based on these AIMs.

Single SNP Analysis

Forty-two SNPs in 21 genes were significantly associated with HPS after adjustment for race and smoking (Table 3). Thirty-two of these SNPs were clustered in 8 genes: Caveolin 3 (*CAV3*); Endoglin (*ENG*); NADPH Oxidase 4 (*NOX4*); Estrogen receptor 2 (*ESR2*); von Willebrand Factor (*VWF*); Runt-related transcription factor 1 (*RUNX1*); Collagen, type XVIII, α-1 (*COL18A1*); and Tyrosine kinase with immunoglobulin g and EGF Factor homology domains (*TIE1*). Polymorphisms associated with an increased risk of HPS included 2 *CAV3* SNPs, rs237872 (OR, 2.75; 1.65–4.60, P =.0001) and rs237875 (OR, 2.11; 1.29–3.45, P =.003). In addition, a missense variant (R126C) in spermidine/spermine N1-acetyltrans-ferase family member 2 (*SAT2*), a regulator of Hypoxia-inducible factor 1, α subunit (*HIF1A*) activity, was associated with HPS (OR, 3.65; 1.43–9.31, P =.007).

Other variants that were associated with the risk of HPS included 2 tightly linked ($D' = 0.969$, $r^2 = 0.912$) intron 1 SNPs in *ENG*, rs4836585 (OR, 0.38; 0.15-1.00, P =.49) and rs4837192 (OR 0.35, 0.14–0.89, P =.027). In addition, 2 *NOX4* SNPs (rs585197 and rs2164521) were associated with case status in our subjects. Among genes in steroid hormone signaling pathways, 4 of 14 tested SNPs in *ESR2* affected the risk for HPS, as did a steroid hormone binding globulin (*SHBG*) missense variant in exon 4 (P184L).

Finally, 10 of 11 associated SNPs in *VWF* conferred an increased risk for HPS (OR, 1.66–2.18). One of these SNPs, rs1063856, encodes a missense variant (T789A) in exon 18 previously demonstrated to associate with higher circulating levels of VWF.[27] In our cohort, possession of the alanine allele was significantly associated with case status (OR, 2.18; 95% confidence interval [CI]: 1.35–3.52, P =.002).

Haplotype Analyses

The haplotype block 3 of *CAV3*, (Table 4, Supplementary Figure 1) was significantly associated with the risk for HPS (Global, P =.003). Haplotype-specific analyses demonstrated that possession of haplotype AGAAA confers the greatest increase in HPS risk (OR, 5.28; 95% CI: 2.02-13.82, P =.0009) in comparison with the most common haplotype. In *VWF*, haplotype block 6 (Table 4, Supplementary Figure 2) was significantly associated with HPS (Global, P =.008). In the individual haplotype analysis, the possession of the rare haplotype CGAGG was associated with a significantly lower risk of HPS (OR, 0.21; 95% CI: 0.06–0.76, P =.02).

Gene-Based Analyses

Elastase 1 (*ELA1*) (P <.005), *CAV3* (P <.04), *BMPR2* (P <.03), and *NFKB1* (P <.03) were all significantly associated with HPS in the PC analysis. In the CART analysis, the following SNPs were identified as being most predictive of HPS phenotype: rs2834650 (*RUNX1*), rs1800472 (*TGFB1*), rs11224779 (*TRPC6*), rs429342 (*PRKCB1*), and rs237870 (*CAV3*) (Figure 1). The Random Forests algorithm was run 1000 times, and the 3 most important SNPs identified in each iteration were recorded. The following SNPs were most frequently identified as being influential: rs2834650 (*RUNX1*), rs2274751 (*TNC*), rs3729904 (*PRKCB1*), rs1800472 (*TGFB1*), rs237872 (*CAV3*).

Discussion

Using a hypothesis-generating approach, we have identified that the possession of common genetic variation in genes associated with vascular growth and development and estrogen action and signaling was associated with HPS in this case-control study. In contrast, we did not find any association between HPS and vasoregulatory genes such as nitric oxide, heme oxygenase, and the endothelin-B receptor, which have been specifically implicated in HPS.[28–31] Our findings are in line with recent experimental results that demonstrate an important role for pulmonary angiogenesis in HPS.[13]

We have identified a number of genetic risk factors for HPS that modulate angiogenesis or vascular development. For example, endostatin, the proteolytic fragment of the C-terminus *COL18A1*, inhibits angiogenesis.[32,33] In addition to the genetic association reported here, we have recently demonstrated that overexpression of endostatin in an animal model of HPS blocks the expansion of pulmonary microvessels as well as the oxygen diffusion impairment characteristic of that model.[13] Endoglin is a transmembrane auxillary receptor for transforming growth factor (TGF)-β that is predominantly expressed on proliferating endothelial cells. Mutations in endoglin and activin receptor-like kinase 1 (*ALK1*), an endothelial specific TGF-β type I receptor, have been linked to hereditary hemorrhagic telangiectasia, an autosomal dominant vascular dysplasia characterized by telangiectasias and arteriovenous malformations.[34,35] Interestingly, among patients with hereditary hemorrhagic telangiectasia, pulmonary arteriovenous malformations are significantly more likely in subjects with endoglin mutations.[36] Last, *TIE1*, an endothelial specific receptor tyrosine kinase, is essential for the activation of *TIE2* by vascular endothelial growth factor (*VEGF*), thus modulating vascular remodeling and blood vessel development.[37]

Low oxygen tension (hypoxia) is a potent stimulator of vascular growth and remodeling, and, in the pulmonary vasculature, oxygen sensing is critical for maintenance of normal gas exchange via adjustments in vascular tone. Four of the genes implicated here–*HIFA1*, *SAT2*, *RUNX1* and *NOX4*–play central roles in oxygen-dependant vascular phenotypes. *HIF1A* stimulates endothelial cell angiogenesis under hypoxic conditions by activating the transcription of numerous transcription and growth factors[38] and is regulated by *SAT2*.[39] Variation in both genes was associated with HPS case status. *RUNX1* is a hematopoetic transcription factor that contributes to the angio- and vasculogenic phenotype via its interaction with other transcription factors such as *HIF1A* and insulin growth factor binding protein 3.[40–42] Last, *NOX4* is one of the enzymes responsible for generation of reactive oxygen species in endothelial cells that modulate angiogenesis and has been implicated in hypoxia-induced proliferation.[43] These results identify variation in specific genes that may contribute to susceptibility in HPS and be candidates for future studies.

Three specific signaling pathways–carbon monoxide, nitric oxide, and endothelin–have been implicated in pulmonary vasodilatation in experimental and human HPS. Increased production of the gaseous vasodilators nitric oxide and carbon monoxide has also been associated with vascular dilatation in HPS,[30,44,45] and, thus, we tested variants in the inducible and endothelial forms of nitric oxide synthase (NOS) as well as heme oxygenase 1 (*HMOX1*), the rate-limiting enzyme in the production of carbon monoxide. A recent report found that the Glu298Asp (rs1799983) variant in *NOS3* was associated with risk of HPS in 20 subjects with pediatric (predominately anatomic or metabolic) liver disease. We did not replicate this observation in our cohort (OR, 0.75; 95% CI: 0.43–1.31, P =.31). Altered endothelin signaling has been implicated in experimental HPS, with the liver producing increased circulating ET-1, which signals through up-regulated ET-B receptors on pulmonary endothelial cells.[46] We analyzed SNPs in endothelin converting enzyme as well as both endothelin A and B receptors. Germ-line variation in none of these genes was associated with risk of HPS in our study population.

In addition to our single SNP analyses, we undertook gene- and pathway-based approaches to provide additional insight into the relationship between genotype and disease phenotype. Two genes with single SNP associations–*CAV3* and *RUNX1*–were also identified in these analyses. *CAV3* gene had an overall association with HPS using PC analysis, and SNPs from *CAV3* were found in the CART and Random Forests approaches. A SNP from *RUNX1* was identified as the most discriminating polymorphism (first split) in the CART tree, and this was confirmed by the Random Forests algorithm. Because these 2 genes were shown to be important using multiple methodologies, this provides stronger evidence that *CAV3* and *RUNX1* are associated with HPS. In addition to supporting these associations, these analyses also indicated 3 genes not found in the single SNP analysis—*TGFB1*, *TNC*, and *TRPC6*–may actually be associated with the disease.

There are several limitations to this study. First, the sample size was small, limiting our ability to find genetic alleles associated with HPS that were rare, had small effect sizes, or whose effect depended on gene-gene or gene-environment interaction. However, this is the largest reported epidemiologic study of HPS with strict case and control phenotypes and the first in HPS to employ high-throughput genotyping.

A fundamental challenge in high-throughput genetic analyses is the control of type I error. Given that we analyzed multiple SNPs for each of more than 90 genes, we can reasonably expect a certain number of statistically significant associations because of chance alone. We attempted to minimize the chance of “false-positives” by using a curated candidate gene list, thusly increasing the prior probability that one or more of these genes has mechanistic importance in HPS. There are commonly utilized frequentist methods to adjust for multiple comparisons in high-throughput studies, such as the Bonferroni correction and false discovery rate.[47] Both methodologies assume that the association of each individual SNP with case status is entirely independent of those of the other SNPs. We have documented patterns of linkage disequilibrium between genotyped SNPs (data not shown). Because most accepted methods to account for multiple comparisons do not consider such relatedness, they are overly conservative for this purpose. We have therefore presented the results without adjustment and consider these results to be hypothesis generating. Whereas replication would be important, the biologic plausibility of our findings, the multiple gene “hits” in certain pathways, and the demonstration of association via both single loci and gene-based approaches is reassuring that type I error does not explain the findings.

In conclusion, our results implicate common genetic variation in the pathogenesis of HPS. Future studies should focus on replication in other populations and the mechanisms that explain the associations between the SNPs of interest and HPS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A listing of additional members of the Pulmonary Vascular Complications of Liver Disease Study Group can be found in Appendix 1.

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Appendix: 1

Additional members of the Pulmonary Vascular Complications of Liver Disease Study Group are as follows: Columbia University College of Physicians and Surgeons: Evelyn M. Horn, MD; Jeffrey Okun, BA; Sonja Olsen, MD; Daniel Rabinowitz, PhD; Jenna Reinen, BS; Lori Rosenthal, NP; Debbie Rybak, BS. Mayo Clinic: Russell Wiesner, MD; Linda Stadheim, RN. University of Alabama: Raymond Benza, MD; J. Stevenson Bynon, MD; Devin Eckhoff, MD; Dorothy Faulk; Harpreet Singh; Rajasekhar Tanikella; Keith Wille, MD. University of Colorado: David Badesch, MD; Lisa Forman, MD; Ted Perry. The University of North Carolina at Chapel Hill: Roshan Shrestha, MD; Carrie Nielsen, RN. University of Pennsylvania School of Medicine: Vivek Ahya, MD; Michael Harhay, BS; Sandra Kaplan, RN; Harold Palevsky, MD; Rajender Reddy, MD; Darren Taichman, MD, PhD. University of Southern California: Neil Kaplowitz, MD.

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Abbreviations used in this paper

95% Cl	95% confidence interval
AIM	Ancestry Informative Marker
CART	classification and regression trees
CAV3	Caveolin 3
COL18A1	collagen, type XVIII, α-1
ENG	endoglin
ESR2	Estrogen receptor 2
HIF1A	Hypoxia-inducible factor 1, α subunit
HPS	hepatopulmonary syndrome
HWE	Hardy-Weinberg equilibrium
MELD	Model for End-stage Liver Disease
NOX4	NADPH Oxidase 4
OR	odds ratio
PC	principal component regression analysis
RUNX1	Runt-related transcription factor 1
SAT2	Spermidine/spermine N1-acetyltransferase family member
SHBG	Steroid hormone binding globulin
SNP	single nucleotide polymorphism
TIE1	Tyrosine kinase with Ig and EGF factor homology domains 1
VWF	von Willebrand factor

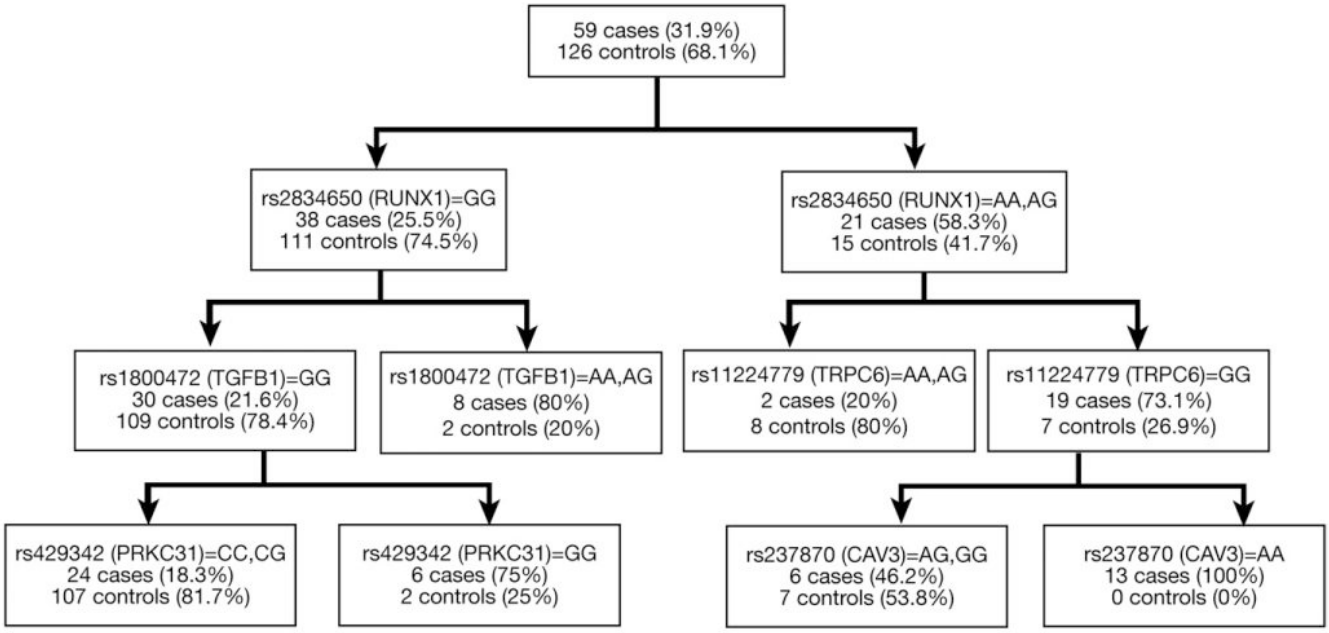

Figure 1.
Classification and regression tree (CART). Each split in the tree maximizes the separation of cases and controls based on SNP genotypes.

Table 1

Candidate Genes: Gene Ontology Annotation

Pathway	Gene	Reference sequence	Chr	SNPs
Control of blood circulation GO: 0008015	Angiotensin I converting enzyme (ACE)	NM_152831	17q23	15
	Elastin (ELN)	NM_000501	7q11	5
	Endothelin 1 (EDN1)	NM_001955	6p24	7
	Endothelin converting enzyme 1 (ECE1)	NM_001397	1p36	10
	Endothelin receptor, nonselective type (EDNRB)	NM_000115	13q22	13
	Endothelin receptor, type A (EDNRA)	NM_001957	4q31	11
	Heme oxygenase 1 (HMOX1)	NM_002133	22q13	8
	Natriuretic peptide precursor A (NPPA)	NM_006172	1p36	13[a]
	Natriuretic peptide precursor B (NPPB)	NM_002521	1p36	13[a]
	Nitric oxide synthase 2 (NOS2A)	NM_000625	17q11	15
	Phosphodiesterase 5 (PDE5A)	NM_001083	4q26	9
	Potassium channel, voltage-gated, shaker, member 5 (KCNA5)	NM_002234	12p13	9
	Rho-associated protein kinase 2 (ROCK2)	NM_004850	2p24	15
	Transient receptor potential cation channel, subfamily C, 6 (TRPC6)	NM_004621	11q21	18
Cell growth	Activin A receptor, type II-like kinase (ACVRL1)	NM_000020	12q11	6
Apoptosis GO:0008283 GO:0006915	Apolipoprotein E (APOE)	NM_000041	19q13	4
	BCL2-associated X protein (BAX)	NM_138764	19q13	6
	Bone morphogenetic protein receptor type 1a (BMPR1A)	NM_004329	10q22	20
	Bone morphogenetic protein receptor type 2 (BMPR2)	NM_001204	2q33	12
	Caveolin 1 (CAV1)	NM_001753	7q31	20[a]
	Caveolin 2 (CAV2)	NM_001233	7q31	20[a]
	Caveolin 3 (CAV3)	NM_033337	3p25	19
	CD14 molecule (CD14)	NM_000591	5q22	3
	Cyclin-dependent kinase inhibitor 2A (CDKN2A)	NM_000077	9p21	13
	Growth differentiation factor 2 (GDF2)	NM_016204	10q11	5
	Homolog of drosphila mothers against dpp 3 (SMAD3)	NM_005902	15q21	34
	Homolog of drosphila mothers against dpp 4 (SMAD4)	NM_005359	18q21	5
	Nitric oxide synthase 3 (NOS3)	NM_000603	7q36	10
	Nuclear factor κB p100 subunit (NFKB2)	NM_001077493	10q24	5
	Nuclear factor κB p105 subunit (NFKB1)	NM_003998	4q23	13
	Nuclear factor κB p65 subunit (RELA)	NM_021975	11q13	4
	Prostaglandin I2 synthase (PTGIS)	NM_000961	20q13	13
	Protein kinase C, α (PRKCA)	NM_002737	17q22	33
	Protein kinase C, β 1(PRKCB1)	NM_002738	16p11	13
	Protein kinase C, γ (PRKCG)	NM_002739	19q13	5
	Transforming growth factor, β-1 (TGFβ1)	NM_000660	19q13	5
	V-AKT murine Thymoma viral oncogene homolog 1 (AKT1)	NM_005163	14q32	7

Pathway	Gene	Reference sequence	Chr	SNPs
Blood vessel growth and development GO: 0001568	Angiopoietin 1 (ANGPT1)	NM_001146	8q22	37
	Calcium-binding protein A4 (S100A4)	NM_019554	1q21	6
	Endoglin (ENG)	NM_000118	9q34	15
	Hypoxia-inducible factor 1, α subunit (HIF1A)	NM_001530	14q21	8
	Plasminogen (PLG)	NM_000301	6q26	21
	Runt-related transcription factor 1 (RUNX1)	NM_001754	21q22	58
	Thrombospondin-1 (THBS1)	NM_003246	15q15	5
	Tyrosine kinase with Ig and EGF Factor homology domains (TIE1)	NM_005424	1p34	8
	Vascular endothelial growth factor (VEGF)	NM_00125366	6p12	7
Inflammation GO:0006954	Complement component 4A (C4A)	NM_007293	6p21	4
	C-reactive protein (CRP)	NM_000567	1q21	8
	Cytochrome b-245, NADPH Oxidase 2, NOX2 (CYBB)	NM_000397	Xp21	6
	Lipopolysaccharide binding protein (LBP)	NM_004139	20q11	7
	Tumor necrosis factor (TNF)	NM_000594	6p21	5
Oxidation reduction GO: 0006979	Dual oxidase 1 (DUOX1)	NM_017434	15q15	15[a]
	Dual oxidase 2 (DUOX2)	NM_014080	15q15	15[a]
	NADPH Oxidase 1 (NOX1)	NM_007052	Xq22	7
	NADPH Oxidase 4 (NOX4)	NM_016931	11q14	19
	Superoxide dismutase 1, soluble (SOD1)	NM_000454	21q22	3
	Superoxide dismutase 2, mitochondrial (SOD2)	NM_00636	6q25	3
	Xanthine dehydrogenase (XDH)	NM_00379	2p23	24
Tissue development GO:0009888	Homolog of drosphila mothers against dpp 2 (SMAD2)	NM_005901	18q21	10
	Ikaros (IKZF1)	NM_006060	7p12	7
	Peroxisome proliferator activated receptor, γ (PPARG)	NM_005037	3p25	13
	Recombination signal-binding protein 1 for J-$_\kappa$ (RBPSUH)	NM_005349	4p15	13
Steroid hormone GO:0008202 GO:0030518	Aromatase (CYP19A1)	NM_000103	15q21	24
	Estrogen receptor 1 (ESR1)	NM_000125	6q25	36
	Estrogen receptor 2 (ESR2)	NM_001437	14q24	14
	Farnesoid × receptor (NR1H4)	NM_005123	12q	7
	Pregnane × receptor (NR1I2)	NM_003889	3q13	13
	Sex hormone binding globulin (SHBG)	NM_001040	17p13	6
	Small heterodimer partner (NR0B2)	NM_021969	1p36	5
Extracellular matrix structure and regulation GO:0043062 GO:0006508	Collagen, type XVIII, α-1 (COL18A1)	NM_130445	21q22	29
	Elastase 1 (ELA1)	NM_001971	12q13	8
	Elastase 2 (ELA2)	NM_001972	19p13	4
	Matrix metalloproteinase 2 (MMP2)	NM_004530	16q13	11
	Matrix metalloproteinase 3 (MMP3)	NM_002422	11q23	6
	Matrix metalloproteinase 9 (MMP9)	NM_004994	20q11	6
	Proteinase inhibitor 3; elafin (PI3)	NM_002638	20q12	4
	Tenascin C (TNC)	NM_002160	9q33	16
Coagulation GO:0050817	Plasminogen activator inhibitor 1 (SERPINE1)	NM_000602	7q21	9

Pathway	Gene	Reference sequence	Chr	SNPs
	Thrombomodulin (THBD)	NM_000361	20p11	4
	Thromboplastin (HEMB)	NM_000133	Xq27	11
	Von Willebrand factor (VWF)	NM_000552	12p13	39
Serotonin GO:0006587 GO:0007210	Serotonin 2B receptor (HTR2B)	NM_000867	2q36	8
	Serotonin transporter (SLC6A4)	NM_001045	17q11	7
	Tryptophan hydroxylase (TPH1)	NM_004179	11p15	8
	Tryptophan hydroxylase 2 (TPH2)	NM_173353	12q21	16
Na/bile acid transporter GO:0008508	Solute carrier family 10, member 1 (SLC10A1)	NM_003049.1	14q24	5
	Solute carrier family 10, member 2 (SLC10A2)	NM_000452.1	13q33	12
Metabolism GO:0008152	5,10-Methylenetetrahydrofolate reductase (MTHFR)	NM_005957	1p36	7
	Betaine-homocysteine methyltransferase (BHMT)	NM_001713	5q13	4
	Cystathionine-β-synthase (CBS)	NM_000071	21q22	6
	Peroxisome proliferator activated receptor, α (PPARA)	NM_005036	22q12	9
Retinoic acid signaling GO:0048384	Retinoic acid receptor, α (RARA)	NM_000964	17q21	4
	Retinoic acid receptor, β (RARB)	NM_016152	3p24	29
	Retinoic acid receptor, γ (RARG)	NM_000966	12q13	6

Chr, chromosome; SNP, single nucleotide polymorphism.

[a]Indicates adjacent genes which were defined by a single genomic region and tagging SNPs. Thus the number of SNPs indicated refers to the total number of SNPs assayed in the region containing both genes.

Table 2

Demographic and Clinical Data

Variable	HPS (n = 59)	No HPS (n = 126)	*P* value
Age (*y*), mean ± SD	53 ± 9	53 ± 10	.71
Female, n (%)	28 (48)	46 (37)	.23
Race/ethnicity, n (%)			
Non-Hispanic white	53 (90)	101 (80)	.02
Hispanic white	2 (3)	16 (13)	
Non-Hispanic black	1 (2)	8 (6)	
Other	3 (5)	1 (1)	
Etiology of liver disease, n*(%)*			
Alcohol	23 (39)	54 (43)	.62
Hepatitis C infection	26 (44)	55 (44)	.96
Nonalcoholic steatohepatitis	8 (14)	16 (13)	.87
Cryptogenic cirrhosis	7 (12)	9 (7)	.29
Autoimmune hepatitis	2 (3)	8 (6)	.72
Primary scelrosing cholangitis	2 (3)	8 (6)	.51
Hepatitis B infection	0 (0)	9 (7)	.06
Primary biliary cirrhosis	2 (3)	4 (3)	1
Smoking, n *(%)*	28 (48)	81 (64)	.03
MELD score, mean ± SD	14 ± 4	13 ±5	.7
Intrapulmonary shunt, n *(%)*	59 (100)	56 (44)	<.0001
Arterial blood gas			
pH, mean ± SD	7.44 ± 0.03	7.43 ± 0.04	.05
pCO_2 (*mm Hg*), mean ± SD	34 ± 4	35 ± 5	.32
pO_2 (*mm Hg*), mean ± SD	75 ± 13	90 ± 15	<.0001
Alveolar-arterial O_2 gradient, mm Hg, median (IQR)	25 (19–35)	10 (4–16)	.0001

HPS, hepatopulmonary syndrome; MELD, Model for End-Stage Liver Disease; $DLCO_{corr}$, diffusing capacity of the lung for carbon monoxide corrected for hemoglobin (% predicted).

Table 3

Multivariable Logistic Regression Models for SNPs and the Risk of HPS, Adjusted for Race and Smoking

		SNP			Risk allele frequency				
Chr	Gene	Identification	Location	Risk allele	Cases	Controls	Per-allele OR	95% CI	*P* value
1	NPP	rs198388	3UTR flank	A	0.37	0.49	0.58	0.35–0.94	.027
1	TIE1	rs7527092	Intron 1	A	0.51	0.39	1.72	1.05–2.82	.030
1	TIE1	rs2991990	Intron 14	A	0.36	0.46	0.58	0.35–0.97	.039
1	TIE1	rs1199039	Exon 18	G	0.36	0.47	0.60	0.36–0.98	.041
1	TIE1	rs11210834	Intron 22	G	0.34	0.23	1.83	1.06–3.15	.029
3	CAV3	rs237872	Intron 1	A	0.61	0.41	2.75	1.65–4.60	.0001
3	CAV3	rs237875	Intron 1	G	0.57	0.40	2.11	1.29–3.45	.003
6	ESR1	rs1543403	3UTR flank	G	0.32	0.44	0.59	0.37–0.94	.027
8	ANGPT1	rs1283695	Intron 1	G	0.13	0.22	0.52	0.28–0.99	.046
9	ENG	rs4836585	Intron 1	C	0.06	0.16	0.38	0.15–1.00	.049
9	ENG	rs4837192	Intron 1	G	0.05	0.15	0.35	0.14–0.89	.027
11	RELA	rs1466462	3UTR flank	G	0.45	0.36	1.69	1.05–2.70	.029
11	NOX4	rs2164521	Intron 2	A	0.05	0.15	0.30	0.12–0.77	.012
11	NOX4	rs585197	5UTR flank	G	0.15	0.25	0.53	0.29–0.98	.043
11	TRPC6	rs7931676	Intron 1	G	0.36	0.25	1.66	1.02–2.72	.043
12	VWF	rs4764478	Intron 45	A	0.25	0.17	1.86	1.06–3.27	.030
12	VWF	rs216902	Exon 35	A	0.32	0.46	0.54	0.34–0.87	.011
12	VWF	rs216312	Intron 27	A	0.56	0.41	1.68	1.06–2.66	.028
12	VWF	rs11609815	Intron 24	G	0.35	0.24	1.75	1.03–2.97	.039
12	VWF	rs216330	Intron 18	C	0.47	0.35	1.67	1.04–2.67	.032
12	VWF	rs11614912	Intron 18	G	0.34	0.21	1.77	1.04–2.99	.034
12	VWF	rs10849378	Intron 18	A	0.38	0.25	1.71	1.03–2.82	.037
12	VWF	rs11064004	Intron 18	C	0.40	0.25	1.79	1.10–2.94	.020
12	VWF	rs1063856	Exon 18	G	0.50	0.31	2.18	1.35–3.52	.002
12	VWF	rs980130	Intron 13	A	0.46	0.31	1.85	1.15–2.97	.011
12	VWF	rs980131	Intron 13	A	0.55	0.38	2.04	1.25–3.32	.004
12	ELA1	rs4762041	3UTR flank	C	0.37	0.26	1.66	1.02–2.70	.043

		SNP			Risk allele frequency				
Chr	**Gene**	**Identification**	**Location**	**Risk allele**	**Cases**	**Controls**	**Per-allele OR**	**95% CI**	***P* value**
14	HIF1A	rs2301113	Intron 9	C	0.15	0.27	0.53	0.29–0.97	.039
14	ESR2	rs1256061	Intron 7	A	0.55	0.41	1.74	1.09–2.78	.020
14	ESR2	rs1256059	Intron 7	A	0.35	0.45	0.60	0.38–0.96	.032
14	ESR2	rs1256049	Exon 6	A	0.08	0.03	3.20	1.08–9.46	.036
14	ESR2	rs1256030	Intron 2	A	0.37	0.49	0.58	0.36–0.93	.024
15	SMAD3	rs6494636	Intron 6	C	0.38	0.52	0.51	0.31–0.84	.008
17	SAT2	rs13894	Exon 6	A	0.12	0.04	3.65	1.43–9.31	.007
17	SHBG	rs6258	Exon 4	A	0.04	0.00	10.35	1.15–93.02	.037
17	ACE	rs4311	Intron 9	G	0.57	0.46	1.64	1.06–2.55	.028
20	PTGIS	rs6091000	Intron 5	G	0.07	0.03	3.93	1.21–12.78	.023
21	RUNX1	rs2248720	Intron 4	C	0.56	0.43	1.83	1.13–2.96	.015
21	RUNX1	rs2834726	Intron 1	G	0.02	0.06	0.19	0.04–0.87	.032
21	COL18A1	rs2838920	Intron 2	A	0.07	0.16	0.42	0.18–0.98	.044
21	COL18A1	rs7278425	Intron 37	A	0.21	0.14	2.05	1.05–4.03	.036
22	PPARA	rs11090819	Intron 6	A	0.14	0.10	2.23	1.02–4.86	.044

Chr, chromosome; UTR, untranslated region; SNP, single nucleotide polymorphism; OR, odds ratio.

Table 4

Distribution of Haplotypes in CAV3 and VWF Associated With HPS

					95% Confidence interval		
	Frequency	Case frequency	Control frequency	Odds ratio	Lower	Upper	*P* value
Caveolin 3							
Block 3 (Global, *P* =.003)							
A-G-A-A-G	0.47	0.36	0.52	Referent group	—	—	—
A-A-A-A-G	0.05	0.03	0.06	0.81	0.20	3.17	.76
A-G-A-A-A	0.07	0.12	0.04	5.28	2.02	13.82	.0009
A-G-G-C-A	0.28	0.30	0.27	2.03	1.12	3.68	.02
T-G-A-A-A	0.11	0.16	0.09	2.89	1.33	6.28	.01
Rare	0.03	0.03	0.02	4.17	0.79	22.09	.09
VWF							
Block 6 (Global, *P* =.008							
G-A-G-G-A	0.43	0.46	0.41	Referent group	—	—	—
C-G-A-A-G	0.25	0.27	0.25	0.90	0.52	1.54	.69
C-G-A-G-A	0.07	0.03	0.08	0.29	0.08	1.01	.05
C-G-A-G-G	0.08	0.03	0.11	0.21	0.06	0.76	.02
C-G-G-G-A	0.15	0.21	0.12	1.78	0.90	3.52	.10
Rare	0.02	0.01	0.03	0.38	0.04	3.30	.38

NOTE. Caveolin 3 haplotype block 3 is composed of the following 5 SNPs: rs13061909, rs4686300, rs237870, rs237871, rs237872. VWF haplotype block 6 is composed of the following 5 SNPs: rs216891, rs216893, rs216902, rs216905, rs216805.

VWF, von Willebrand Factor.