Genome-Wide Significance and Replication of the Chromosome 12p11.22 Locus Near the \textit{PTHLH} Gene for Peripartum Cardiomyopathy

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\textbf{Background}—Peripartum (PP) cardiomyopathy (CM) is a rare condition of unknown etiology that occurs in late pregnancy or early postpartum. Initial evidence suggests that genetic factors may influence PPCM. This study evaluated and replicated genome-wide association of single nucleotide polymorphisms with PPCM.

\textbf{Methods and Results}—Genome-wide single nucleotide polymorphisms in women with verified PPCM diagnosis (n=41) were compared separately with local control subjects (n=49 postmenopausal age-discordant women with parity ≥1 and no heart failure) and iControls (n=654 women ages 30 to 84 years with unknown phenotypes). A replication study of independent population samples used new cases (PPCM2, n=30) compared with new age-discordant control subjects (local2, n=124) and with younger control subjects (n=89) and obstetric control subjects (n=90). A third case set of pregnancy-associated CM cases not meeting strict PPCM definitions (n=29) was also studied. In the genome-wide association study, 1 single nucleotide polymorphism (rs258415) met genome-wide significance for PPCM versus local control subjects \((P=2.06\times10^{-8});\) odds ratio [OR], 5.96. This was verified versus iControls \((P=7.92\times10^{-10});\) OR, 8.52.

In the replication study for PPCM2 cases, rs258415 (ORs are per C allele) replicated at \(P=0.009\) versus local2 control subjects (OR, 2.26). This replication was verified for PPCM2 versus younger control subjects \((P=0.029);\) OR, 2.15) and versus obstetric control subjects \((P=0.013);\) OR, 2.44). In pregnancy-associated cardiomyopathy cases, rs258415 had a similar effect versus local2 control subjects \((P=0.06);\) OR, 1.79), younger control subjects \((P=0.14);\) OR, 1.65), and obstetric control subjects \((P=0.038);\) OR, 1.99).

\textbf{Conclusions}—Genome-wide association with PPCM was discovered and replicated for rs258415 at chromosome 12p11.22 near \textit{PTHLH}. This study indicates a role of genetic factors in PPCM and provides a new locus for further pathophysiological and clinical investigation. \textit{(Circ Cardiovasc Genet. 2011;4:359-366.)}

\textbf{Key Words:} pregnancy \textbullet{} cardiomyopathy \textbullet{} genetic association \textbullet{} genome-wide association study \textbullet{} epidemiology

Peripartum cardiomyopathy (PPCM) is a rare condition afflicting women with no cardiac history. It is characterized by left ventricular dysfunction developed within the last month of pregnancy through the fifth postpartum month.\(^1\) PPCM incidence in the United States is 1 in 3000 to 4000 live births\(^2,3\) but is higher elsewhere (Haiti: 1 in 300).\(^4\) PPCM is a leading cause of pregnancy-related death, with mortality rates of 3\% to 50\%.\(^1,5,7\)

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Current understanding of PPCM is limited. Presumed risk factors include maternal age >30 years, multiparity, multiple gestation, history of preeclampsia, and use of tocolytics.\(^1,5\) African ancestry may be a risk factor\(^2,3\) but is not confirmed.\(^5\)
PPCM pathophysiology may be distinct from idiopathic dilated cardiomyopathy, but this is uncertain. PPCM may arise from an immunologic response to autoantibodies\(^8\)–\(^10\) or virus-associated inflammation, based on the finding of viral genomes in endomyocardial biopsy.\(^11\) It may also occur as a maladaptive response to the stress of pregnancy, perhaps from catecholamine myocyte toxicity.\(^1\) PPCM may require long-term pharmacological therapy, but this is unclear, as are its maternal and fetal risks in subsequent pregnancies.

Genetic factors may play a role in PPCM. Various other cardiomyopathies have a genetic basis,\(^12\) and PPCM has disparate incidences across ethnic groups.\(^2\)–\(^5\) Familial influences on PPCM are reported,\(^13\)–\(^15\) but evidence of a genetic component has not been proven. The genome-wide association study (GWAS) is a case-control approach for discovering single nucleotide polymorphisms (SNPs) associated with a phenotype (eg, References 16 through 20).\(^16\)–\(^20\) The present study sought to identify genetic loci associated with PPCM, using a 2-stage GWAS design.

**Methods**

**Study Populations**

The Utah Affiliated Hospitals Heart Failure Clinical Research Network PPCM registry was created in 2006. Ascertainment of PPCM cases utilized 4 mechanisms: (1) patients within Intermountain Healthcare were identified by ICD-9 codes, (2) patients treated in the Heart Failure Prevention and Treatment Program at Intermountain Medical Center were identified by records review, (3) health care providers across numerous specialties were invited to refer PPCM patients, and (4) a media campaign across Utah was conducted. The Intermountain Healthcare and University of Utah Institutional Review Boards approved this project and all participants provided written informed consent. A description of the study design is provided in Figure 1. Genotyping methods and variable definitions are provided in the online-only Data Supplement text.

**Genome-Wide Association Study**

A total of 77 patients \(\geq 18\) years of age were identified from 2006 to 2008 as potential PPCM cases. PPCM phenotype was assigned based on clinical parameters gathered locally or through medical records (ie, history and physical examination, echocardiogram or—in its absence—another measure of left ventricular function, discharge summary, and other outpatient records). Patients meeting the strict definition of PPCM, based on the National Institutes of Health (NIH) criteria had symptomatic left systolic dysfunction (left ventricular ejection fraction [LVEF] \(< 55\%\) ), diagnosis within the last month of pregnancy through the fifth postpartum month, unknown heart failure etiology, and no diagnosis of heart disease before the final month of pregnancy.\(^1\) Categorization of cases was performed independently by a cardiologist with heart failure expertise and 2 nurse practitioners, and these assignments were verified or reassigned by another heart failure cardiologist. Of the 77 potential PPCM cases, 41 were found to meet definite criteria for PPCM. Five cases were of non-European ancestry, but no correction was made for population stratification (ie, confounding by ethnicity) in favor of determining the ability to replicate in an independent population.

Two independent sets of control groups were ascertained for GWAS. The first and primary control set was European-ancestry postmenopausal women (n = 49) with parity \(\geq 1\) who were free from cardiovascular diseases. These patients, designated “local control subjects,” were identified from the cardiac catheterization laboratory registry of the Intermountain Heart Collaborative Study. Exclusion of cardiovascular diseases was based on a lack of clinically diagnosed heart failure, angiographic evidence of no coronary disease (no coronary stenosis was \(\geq 10\%\)), and a lack of cardiac valve diseases, cardiac arrhythmias, and cerebrovascular and peripheral vascular conditions.

DNA samples from the 41 PPCM case women and the 49 local control subjects were genotyped using the Illumina HumanHap 550K BeadChip SNP microarray, a sample size similar to another recent GWAS we reported.\(^21\) Genotyping was performed by deCODE Genomic Services (Reykjavik, Iceland). One internal CEPH control was used on each DNA plate, and comparison of all control subjects was made across this and other GWAS projects run by the laboratory to track genotype calling. SNP calling and genotype clustering used automated Illumina software. Call rates (genotypes/total number of SNPs) were computed for each DNA sample. Quality control evaluated the per-SNP and per-participant quality, with a minimum threshold of 95% genotyping success designated for inclusion in analyses of cases and local control subjects. SNPs were also excluded if they were subpolymorphic (minor allele frequency [MAF] <0.01). For SNPs, 99.8% of those with \(P < 0.05\) passed genotyping quality control. All PPCM cases and local control subjects passed genotyping quality control (89 had 99.0% to 99.3% success; 1 had 96.9%). Hardy-Weinberg equilibrium was considered violated at \(P < 0.0001\).

The second GWAS control set was drawn from anonymous participants in Illumina’s iControlDB. Genotyping of iControls was

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**Figure 1.** A 2-stage genome-wide association study design with multiple additional control verifications for independent discovery and replication peripartum cardiomyopathy (PPCM) case populations, and an exploratory evaluation for pregnancy-associated cardiomyopathy (PAMC) cases. SNP indicates single nucleotide polymorphism.
performed previously using the Illumina HumanHap 550K BeadChip. For more information on iControls and iControlDB, navigate to http://www.illumina.com/science/icontroldb.IM. Others have used iControls to study cancer. The ethnicity, age, and sex of iControls were known, but phenotypes were not known. From the thousands of possible iControls to study, those included in the present study (n=654) were women of European ancestry who were ≥30 years of age and had 550K GWAS data available.

Replication Study
For replication, a new set of European-ancestry PPCM cases (designated “PPCM2” to distinguish from GWAS cases) and 3 control groups were ascertained. PPCM2 cases (n=30) were recruited during 2008 to 2010, using the same methods as GWAS cases. Another set of age-discordant European-ancestry female control subjects (designated “local2,” n=124) were recruited based on the same methods and cardiac registry source as the GWAS local control subjects, with the additional caveat that local2 were free from hypertension, diabetes, and renal failure. The comparison of PPCM2 to local2 was designated as the primary hypothesis test to match the primary GWAS comparison.

The second replication control set was younger-age female European-ancestry control subjects (YC, n=89) of reproductive age and unknown parity who underwent coronary angiography and were phenotypically similar to local and local2 control subjects, except in age. The third replication control set included European-ancestry obstetric patients who were free from both preeclampsia and PPCM and who delivered at the University of Utah Hospital (obstetric control subjects [OBC], n=90).

Pregnancy-associated cardiomyopathy (PACM) was defined as women diagnosed with cardiomyopathy who did not meet all of the National Institutes of Health PPCM criteria. Ten PACM cases were enrolled during the GWAS phase and 19 during the replication phase, resulting in a PACM sample of n=29 cases.

As an exploratory analysis, obstetric patients from the University of Utah who had preeclampsia but were free from PPCM/PACM were ascertained to test whether rs258415 predicts preeclampsia.

Statistical Analysis
GWAS associations of SNPs with PPCM were performed under the additive genetic model using the allelic χ² test (Fisher exact test) in DiseaseMinerLite (deCODE Genetics, Reykjavik, Iceland). Odds ratios (ORs) and 95% confidence intervals (CI) for PPCM were calculated by comparing carriage of the minor allele to the major allele. The probability threshold for statistical significance was set at P<9.0×10⁻⁸ to correct for the increased type I error, due to the number of potentially independent SNP tests. Suggestive GWAS significance was taken to be P≤1×10⁻⁷. Correction for underlying population stratification was performed using efficient mixed-model association (EMMA-X), and the genomic inflation factor (GIF) was calculated in PLINK and compared with results from EMMA-X.

Analyses in the replication study were performed under the additive genetic model, using the Armitage test of trend (which is asymptotically equivalent to the allelic χ² test). Because of the small case sample size that was available and the success in detecting one SNP at genome-wide significance, the primary replication hypothesis test was designated as the association of rs258415 with PPCM2 cases versus local2 control subjects. Statistical significance was set at P=0.05 for replication. This test was estimated to have 80% power to detect an OR of 3.5 per allele (α=0.05, 2-sided testing) when evaluating 30 PPCM2 cases and 4 local2 control subjects per case, assuming rs258415 MAF in control subjects was the same as in GWAS for local control subjects (MAF=0.27).

Comparisons of PPCM2 cases to YC or OBC were used to verify the MAF of the local2 control subjects. Analyses of PACM cases compared with the control sets were considered hypothesis-generating. Age adjustment was performed when comparing PPCM2 with YC to evaluate if the age-discordant design (used to maximize phenotypic differences between cases and control subjects in primary GWAS and replication analyses) produced aberrant results as the result of age-dependent differences (eg, exceptional longevity of control subjects). Analysis of rs258415 was also performed for PPCM2 versus PACM cases.

To determine whether rs258415 simply predicts preeclampsia, MAFs were examined between GWAS cases with and without preeclampsia and in PPCM2 cases. The non-PPCM preeclampsia patients were also evaluated for rs258415 compared with OBC to evaluate prediction of preeclampsia.

Finally, HapMap linkage disequilibrium (LD) data for 252 SNPs including rs258415 was queried from http://hapmap.ncbi.nlm.nih.gov/ for a 200 kb region on chromosome 12p11.22 from approximately 10 kilobases beyond KLHDC5 to 10 kilobases beyond PTHLH.

Results

Genome-Wide Association Study
In the 41 PPCM patients, average age at diagnosis was 50 years less than the local control diagnosis age and 18 years less than the iControl enrollment age (Table 1). LVEF range was also discordant: 10% to 44% in PPCM cases and 56% to 89% in local control subjects. Among PPCM cases, only 1 had a first-degree relative with cardiomyopathy, and 10% underwent cardiac transplantation. Other characteristics are shown in Table 1. A plot of observed and expected probability values (online-only Data Supplement Figure SI) demonstrated that more than the expected number of SNPs were found to have P≤1×10⁻⁵.

PPCM Cases Versus Local Control Subjects
One SNP on chromosome 12 (rs258415) achieved genome-wide significance for PPCM cases compared with local control subjects (MAFs: 0.68 versus 0.27; P=2.06×10⁻⁸, OR, 5.96 per C allele; 95% CI, 3.13 to 11.38). Post hoc analysis excluded the 5 minority PPCM cases (12% of cases) and showed a similar result (P=7.91×10⁻⁸, OR, 5.90 per C allele; 95% CI, 3.02 to 11.51).

On the basis of the potential function of PTHLH in preeclampsia, rs258415 MAFs were compared between cases with and without preeclampsia. The frequency of the C allele was not different (0.72 versus 0.68, P=0.78), based on preeclampsia status.

Suggestive significance compared with local control subjects was found for 13 other SNPs representing 7 distinct loci (Table 2). Although a prior animal model for PPCM implicated the STAT3 gene, no SNP in the STAT3 gene had P<0.05. The closest SNP on chromosome 17 with suggestive significance was rs12881957, rs7152674, and rs3784064) had MAFs compared between cases with and without preeclampsia. The frequency of the C allele was not different (0.72 versus 0.68, P=0.78), based on preeclampsia status.

Suggestive significance compared with local control subjects was found for 13 other SNPs representing 7 distinct loci (Table 2). Although a prior animal model for PPCM implicated the STAT3 gene, no SNP in the STAT3 gene had P<0.05. The closest SNP on chromosome 17 with suggestive significance was rs12881957, rs7152674, and rs3784064) had P=0.016.

Rs258415 retained genome-wide significance (P=1.62×10⁻¹¹) when corrected for population stratification using EMMA-X, but only 6 other SNPs (rs7102702, rs10048187, rs4458717, rs12881957, rs7152674, and rs3784064) had P≤1×10⁻⁵. EMMA-X analysis produced GIF=0.993 compared with the uncorrected GIF of 1.106.

PPCM Versus iControls
The rs258415 association was verified for PPCM versus iControls (MAF: 0.68 versus 0.21; P=7.92×10⁻¹⁹, OR, 8.31 per C allele; 95% CI, 5.10 to 13.52), including when the 5 minority cases were excluded (P=1.91×10⁻¹⁸; OR, 7.48 per C allele; 95% CI, 4.34 to 12.90). Furthermore, 10 of those
SNPs had \( P \leq 0.001 \) for PPCM versus iControls (online-only Data Supplement Table SI).

**PPCM SNP Replication Study**

Characteristics of replication cases and control subjects are provided in Table 1. LVEF range was 10% to 45% in PPCM2 cases versus 45% to 89% in local control subjects and 45% to 83% in YC. No transplants have been performed as yet in these cases, but 10% required mechanical circulatory support as a bridge to transplant.

**PPCM2 Cases Versus Local2 Control Subjects**

Rs258415 was different for PPCM2 cases and local2 control subjects (MAFs: 0.32 versus 0.16; \( P = 0.009 \); OR, 2.26 per C allele; 95% CI, 1.20 to 4.27). As in the GWAS, preeclampsia-stratified cases did not differ in allele frequencies (MAFs: 0.36 versus 0.30 for preeclampsia versus none; \( P = 0.71 \)). Furthermore, rs258415 was not different for obstetric preeclampsia cases compared with OBC (MAFs: 0.12 versus 0.16; \( P = 0.32 \); OR, 0.76 per C allele; 95% CI, 0.45 to 1.30).

**PPCM2 Versus YC and Versus OBC**

The replication findings above were further verified (see Figure 2) by comparison of PPCM2 to YC (MAF: 0.32 versus 0.182; \( P = 0.029 \)) and to OBC (MAF: 0.32 versus 0.178; \( P = 0.013 \)). Adjustment for age in comparisons of PPCM2 with YC changed the regression \( \beta \)-coefficient of the SNP only 2.5%, indicating that age did not confound the association. PPCM2 compared with all 3 control sets combined produced \( P = 0.006 \).

**PPCM2 Versus iControls**

Post hoc comparison of PPCM2 cases to iControls also showed nominal significance (\( P = 0.041 \); OR, 1.79 per C allele; 95% CI, 1.02 to 3.14).

**PACM Cases and Replication Control Subjects**

No difference in rs258415 genotype distribution was found between PPCM2 and PACM cases (0.32 versus 0.27; \( P = 0.65 \)). For PACM cases versus local2 control subjects (\( P = 0.06 \), YC (\( P = 0.14 \)), and OBC (\( P = 0.038 \)), rs258415 had an effect similar to its effect in PPCM2 cases (Figure 2), although the statistical significance was borderline. PACM compared with the combined 3 control groups produced \( P = 0.035 \).
LD Structure

Figure 3 shows the LD between rs258415 and 251 SNPs (based on $r^2$) across chromosome 12p11.22 in HapMap data and online-only Data Supplement Figure SII shows the D' pattern in the same region.

Discussion

The primary objective of this study was to discover genetic markers across the genome that are associated with PPCM. Using an agnostic approach for locus candidacy and a genome-wide SNP array for genotyping, 41 women with a clean, narrowly defined PPCM phenotype were compared with 49 elderly women who were free from heart diseases. An independent replication population of similar sample size validated GWAS findings for a SNP that achieved genome-wide significant association with PPCM: rs258415 on chromosome 12p11.22. That SNP had a similar effect on PACM, a less stringently delineated phenotype that some assert is the same as PPCM, although statistical significance for PACM (excluding PPCM cases) was borderline. This study indicates

<table>
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<th>SNP</th>
<th>$P$ Value</th>
<th>Minor Allele Frequencies</th>
<th>Genetic Information</th>
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<tr>
<td></td>
<td></td>
<td>Cases</td>
<td>Local Control Subjects</td>
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<tr>
<td>1. rs258415</td>
<td>$2.06 \times 10^{-8}$</td>
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<td>0.27</td>
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<tr>
<td>2. rs7102702</td>
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<tr>
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<td>0.51†</td>
<td>0.18</td>
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<td>0.09‡</td>
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<td>0.37</td>
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<tr>
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</table>

SNP indicates single nucleotide polymorphism; PPCM, peripartum cardiomyopathy; OR, odds ratio; and CI, confidence interval.

One SNP (rs258415) achieved genome-wide significance versus local control subjects despite the unorthodox sample size ($P$ values for discovery PPCM cases versus iControls are also indicated).

For discovery PPCM cases versus iControls: *$P=7.92 \times 10^{-10}$, †$P<9.0 \times 10^{-8}$, and ‡$P<0.001$.

Figure 2. Replication study. Forest plot for rs258415 associations with peripartum cardiomyopathy (PPCM2) cases compared with local2 control subjects, young control subjects, obstetric control subjects, and the combination of all 3 control groups. Results for the same comparisons using pregnancy-associated cardiomyopathy (PACM) cases are also provided. All odds ratios are per C allele and the x-axis is on the log10 scale.
that genetic factors contribute to PPCM and provides a significant, validated locus near the \textit{PTHLH} and \textit{KLHDC5} genes.

As anticipated in the study design of this small-sample-size GWAS, a SNP with replicable association with PPCM was enriched for its minor allele in the GWAS discovery cases (MAF = 0.68 for rs258415) compared with the replication PPCM2 and PACM cases (MAF = 0.32 and 0.27, respectively). The MAF of that SNP was very similar and stable across all control subjects, with MAF = 0.27 in the GWAS local control subjects and MAF from 0.16 to 0.21 for iControls, local2 control subjects, YC, and OBC. Because of the strength of association of that locus and the extreme-phenotype GWAS/replication design, statistical significance could be detected and replicated despite the unorthodox sample size.

PPCM occurs among “healthy” women during a period of life that would otherwise be “normal” and can be shocking and devastating to individuals and their families. Genetic evidences in the context of enhanced knowledge regarding the natural history of PPCM may assist in alleviating concerns for the at-risk population. This may include development of better screening strategies, enhanced understanding of the necessary duration of medical management, possible preventative strategies, and improved knowledge regarding the likelihood of recurrent PPCM with a subsequent pregnancy. Because of the potential for clinical genetic testing among women who are pregnant, this study compared rs258415 between PPCM cases and a secondary control group of otherwise healthy obstetric patients. Prospective studies are required to evaluate the clinical utility of genetic screening for PPCM.

PPCM is considered a rare condition but may be misrepresented by the narrow time window that strict definitions impose. One population-based estimate of PPCM incidence suggests a rate of 1 in 3189 live births.\textsuperscript{2} PPCM is often missed as a diagnosis because, although greater in severity, symptoms mimic usual concerns during pregnancy and often occur before the last antepartum month. PACM is a more inclusive definition of the condition but may encompass a more heterogeneous phenotype. Various risk factors and mechanisms for PPCM/PACM have been proposed,\textsuperscript{1–3,5} yet answers are scarce. Diagnosis carries a similar burden as other forms of heart failure where structural heart pathology may not return to normal and symptoms persist.

A variety of genes for idiopathic cardiomyopathy have been identified.\textsuperscript{12,26} Those loci include genes for the angiotensin I–converting enzyme and \(\beta\)-adrenergic receptors.\textsuperscript{26,27} The \(\beta\)-adrenergic receptor genes and others (eg, \textit{CYP2D6}) are also implicated in pharmacogenetic interactions.\textsuperscript{26–28}

A genetic basis for PPCM is controversial, with some mostly anecdotal reports noting familiality,\textsuperscript{13–15} but other large case series not reporting family history as a PPCM risk factor.\textsuperscript{5} Indeed, the European Society of Cardiology currently classifies PPCM as a nonfamilial, nongenetic form of dilated cardiomyopathy.\textsuperscript{29} A genetic basis for PPCM has not been systematically studied.

Previously, gene-hunting studies were laborious and expensive.\textsuperscript{30} GWAS using large SNP panels permits evaluation of unrelated individuals using traditional statistics and can be a more powerful means to assess genetic risk associations and isolate them to a small genomic region. Although a flood of recent GWAS have been published, (eg, chromosome 9p21 in coronary heart disease\textsuperscript{17}), most use large sample sizes because of small SNP effect sizes in common, heterogeneous diseases. Identifications of genome-wide loci using fewer than 100 cases has been performed for phenotypes such as macular degeneration,\textsuperscript{18} esophageal cancer,\textsuperscript{19} and bladder cancer.\textsuperscript{20} This study extends the list to PPCM, a rare pheno-
type that was tightly defined herein and studied using a small sample of cases.

On the basis of the population frequency of PPCM, a rare genetic variant would be expected to affect the disease, but rs258415 is common (ie, MAFs of 0.16 to 0.21 in iControls and replication study control subjects). On the basis of $r^2$, high LD with other SNPs is limited to a 30-kb region (Figure 3); thus, rs258415 may tag risk arising from intergenic variation. It may reside at a noncoding RNA locus (similar to the coronary disease 9p21 locus$^{31}$) or a regulatory element related to nearby genes. However, LD measured by D' (online-only Data Supplement Figure SII) may be useful if the MAF of the causal SNP is small; thus, if a rare causal variant in LD with rs258415 has an even larger effect on risk, it may be tagged by rs258415 at the distances of the KLHDC5 or PTHLH genes, but this requires further evaluation.

Little is known about KLHDC5, a gene near rs258415, except that it is overexpressed in Sézary syndrome, an aggressive CD4$^+$ cutaneous T-cell lymphoma.$^{32}$ Because PPCM may be an autoimmune disease with specific environmental triggers related to pregnancy,$^3$ that involvement in immune function may connect KLHDC5 to PPCM, but this is tenuous and speculative, requiring further study.

PTHLH (or PTHrP), the closest gene in the other genomic direction, is a member of the parathyroid hormone family. It has multiple alternatively-spliced transcripts encoding 2 isoforms and may have alternate non-AUG translation start codons.

The product of PTHLH, parathyroid hormone-related hormone (PTHrP), is involved in calcium transfer in the placenta and uterus where it regulates blood flow.$^{33}$ It is expressed due to mechanical uterine stretch during pregnancy and appears to be a key factor in preventing contractions until term.$^{33,34}$ It is also potentially involved in the etiology of preeclampsia, with diminished levels resulting in decreased trophoblast apoptosis and abnormal trophoblast invasion.$^{24}$

PTHrP also plays a cardiovascular role by modulating ventricular contraction and by exerting control of pacing at the sinus node.$^{24}$ It influences contraction by reducing systemic blood pressure and by causing an inotropic effect on cardiac myocytes through calcium channel activation. PTHrP is produced in the myocardium and may be expressed as the result of mechanical stretch. It has higher levels in patients with heart failure,$^{35}$ and those levels correlate positively with left ventricular end-diastolic and end-systolic parameters and negatively with LVEF.$^{35}$ Given these various roles, we hypothesize that an increase in PTHrP during pregnancy to prevent uterine contraction may lead a compromised heart into PPCM/PACM.

If the causal variant at chromosome 12p11.22 is common, like the C allele at rs258415, it may interact with another locus or an environmental factor to alter PPCM risk. Conversely, if rs258415 tags a rare variant, that rare variant may modify PTHLH transcription, structure, or translation. These hypotheses require further investigation. Additional investigation is also required to examine the suggestively-significant loci discovered by GWAS.

Strengths and Limitations

Limitations include the small sample size. Despite statistical significance, findings may not be generalizable to other PPCM cases because of the small set of chromosomes available for analysis. As noted in the online-only Data Supplement Materials, the minor allele in GWAS cases should be overrepresented because of the extreme phenotype strategy for locus identification and is not expected to represent the allele frequencies in the general population. Although unlikely because of the high quality of the genotyping by deCODE, the small case sample size may have allowed uncontrollable GWAS laboratory testing, allele assignment, or genotype clustering/calling errors to drive the results. Validations of the SNP associations among other populations (such as the replication sample herein) are required to understand the true allele frequencies in cases and control subjects, including in other ethnic groups.

Study strengths include the tightly defined case phenotype that maximized the detection of genetic factors and minimized statistical noise from indeterminate diagnoses. The 2-stage GWAS design with independent case sets for discovery and replication was also a strength.

Conclusions

A genome-wide significant association with PPCM was found for a genetic locus on chromosome 12p11.22, and this was replicated in an independent set of cases and control subjects. Although these associations do not prove causality, they indicate that genetic factors play a role in PPCM and set the stage for further clinical and pathophysiological investigation of genetic loci in PPCM, including for the rs258415 SNP. Additionally, these data suggest that PACM may be genetically similar to PPCM, but this requires additional evaluation.

Sources of Funding

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Peripartum cardiomyopathy (PPCM) is an uncommon diagnosis but is often devastating for those it affects: younger women who are in their childbearing years. Although evidence is sparse, risk factors for PPCM and its more heterogeneous relative, pregnancy-associated cardiomyopathy, may include maternal age >30 years, multiparity, multiple gestation, a history of preeclampsia, use of tocolytics, and African ancestry. Heritable factors may also influence PPCM, and this study reports the discovery and validation of a novel PPCM locus on chromosome 12. Although not located within a classic gene, the implicated single nucleotide polymorphism is near the parathyroid hormone–like hormone gene, which is an excellent candidate gene for PPCM. This discovery may signal important directions for pathophysiological research of PPCM etiology, while also providing a fertile bed for further genetic association studies of this and other possible causal sequence variants in the genomic region. Additional studies are warranted to evaluate potential clinical applications of these observations.
Genome-Wide Significance and Replication of the Chromosome 12p11.22 Locus Near the *PTHLH* Gene for Peripartum Cardiomyopathy


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Supplemental Material

Supplemental Text

Methods

Methodology. This extreme-phenotype design in which narrowly-defined, carefully clinically phenotyped cases were compared to age-discordant, clinically normal controls was used to maximize the distinction between phenotypes. This was meant to minimize phenotypic overlap and reduce statistical noise in an attempt to increase power by phenotypic methods, countering the low power typically expected for a small sample size. The expectation in performing a GWAS using a small sample size was that the risk allele at some SNPs would be, by random chance, substantially more (or less) prevalent among the specific cases selected for the GWAS compared to the controls under study. While it is unlikely that all disease-associated variants would be found in the specific cases under study, the hope was that the SNPs that were over-represented in this case set might provide sufficient power to find genome-wide significance. (21) Numerically it meant that, for example, the variant allele in the GWAS phase could be much more frequent among cases than among controls and more frequent than its true frequency in the general population—such was the case for rs258415. While the minor allele frequency among cases in the initial GWAS may have been >0.50 in some situations, deciphering the true allele frequencies among the general PPCM case population and among disease-free individuals is one function of the replication phases performed in independent populations.
Population. Because of the internet and word-of-mouth communication, some respondents were from outside Utah, but the ethnic composition of each PPCM case set reflected generally the ethnic composition of that state. The 5 PPCM cases included in the GWAS analyses were: 1 of Asian, 2 of Hispanic White, and 2 of African ancestry.

While the primary GWAS analysis included PPCM cases of both Caucasian and non-European ancestry, all Local controls, iControls, and replication cases and controls included in the analyses were of self-reported European ancestry. However, during the ascertainment phase of the replication study, 4 PPCM2 cases and 4 PACM cases of non-European-ancestry were also recruited who were excluded from the genetic association testing. In both of those case groups, the ancestries of the four cases were: 2 of Hispanic White, 1 of African, and 1 of Polynesian ancestry.

Genotyping. In the replication phase, one SNP (rs258415) was genotyped for potential replication because it achieved genome-wide significance \(p<9.0 \times 10^{-8}\) in GWAS. Additional SNPs that achieved suggestive significance in the GWAS phase were genotyped in the PPCM2 cases and in the Local2 controls and YC samples for exploratory purposes to determine the value in pursuing them in additional cohorts. Genotyping of PPCM2 cases and Local Controls was performed at Intermountain Healthcare using 5′ exonuclease (Taqman) chemistry on the ABI Prism 7000 (Applied Biosystems, Foster City, CA). Quality control was performed by resequencing a random selection of 5% of samples to confirm genotype results, which demonstrated high reproducibility and accuracy (>99%). Genotyping of OBC and the preeclampsia cases was performed at Taueret Laboratories (Salt Lake City, UT) with Taqman-Taqman allele
discrimination assay (Applied Biosystems) on a 7900 real time PCR instrument. Genotypes were called using automated software SDS V2.3 (Applied Biosystems).

Clinical Variables. Data regarding the Local Controls, Local2 Controls, and Young Controls were based on physician exam, ICD-9 codes from the medical record, and patient interview. These included information about the cardiovascular conditions that were used to exclude cardiac catheterization laboratory patients from these control groups.

Clinical co-variables were determined primarily from electronic medical records based on provider report, including mode of delivery (vaginal or cesarean), hypertension history, and medications prescribed for PPCM treatment or, for controls, at hospital discharge (including ACE inhibitors/ARBs, diuretics, β-blockers, digoxin, and aldosterone antagonists). Ethnicity was self-reported. LVEF was measured by standard transthoracic 2-dimensional echocardiography at the time of PPCM diagnosis or by ventriculography for coronary angiogram patients. ECG measurements at PPCM diagnosis were dichotomized to determine the presence of a QRS>120 ms. Blood pressure was measured at the time of PPCM diagnosis for cases and just before the beginning of angiography for Local Controls, Local2, and YC. Preeclampsia was defined as diastolic blood pressure >90 mmHg on two occasions (4 hours-14 days apart) within 14 days of evident significant proteinuria (>300 mg protein in 24 hours, urinary protein/creatinine ratio >0.35, at least 2+ proteinuria from a single dipstick evaluation or 1+ proteinuria from ≥2 measurements obtained 4 hours-14 days apart) in a previously normotensive nonproteinuric patient. Treatment for refractory PPCM included cardiac transplant or mechanical support (i.e., implantation of a left ventricular assist device).
**Supplemental Table S1.** Comparison of GWAS discovery PPCM cases (n=41) to iControls (n=654) for SNPs with GWAS $p \leq 1 \times 10^{-5}$ for cases vs. Local Controls.

<table>
<thead>
<tr>
<th>SNP</th>
<th>p-value</th>
<th>Cases</th>
<th>iControls</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. rs258415</td>
<td>7.92x$10^{-19}$</td>
<td>0.68</td>
<td>0.21</td>
<td>8.31 (5.10, 13.52)</td>
</tr>
<tr>
<td>2. rs7102702</td>
<td>1.35x$10^{-7}$</td>
<td>0.05</td>
<td>0.28</td>
<td>0.12 (0.05, 0.34)</td>
</tr>
<tr>
<td>3. rs10048187</td>
<td>0.001</td>
<td>0.24</td>
<td>0.11</td>
<td>2.57 (1.51, 4.37)</td>
</tr>
<tr>
<td>4. rs4458717</td>
<td>2.64x$10^{-11}$</td>
<td>0.68</td>
<td>0.30</td>
<td>4.94 (3.04, 8.04)</td>
</tr>
<tr>
<td>5. rs17284876</td>
<td>0.001</td>
<td>0.05</td>
<td>0.18</td>
<td>0.24 (0.09, 0.66)</td>
</tr>
<tr>
<td>6. rs7225404</td>
<td>0.003</td>
<td>0.24</td>
<td>0.12</td>
<td>2.38 (1.40, 4.05)</td>
</tr>
<tr>
<td>7. rs2169876</td>
<td>2.35x$10^{-14}$</td>
<td>0.51</td>
<td>0.13</td>
<td>6.80 (4.25, 10.90)</td>
</tr>
<tr>
<td>8. rs12881957</td>
<td>1.85x$10^{-4}$</td>
<td>0.09</td>
<td>0.26</td>
<td>0.27 (0.12, 0.59)</td>
</tr>
<tr>
<td>9. rs7152674</td>
<td>2.80x$10^{-4}$</td>
<td>0.09</td>
<td>0.25</td>
<td>0.27 (0.13, 0.60)</td>
</tr>
<tr>
<td>10. rs3784064</td>
<td>2.79x$10^{-4}$</td>
<td>0.09</td>
<td>0.25</td>
<td>0.28 (0.13, 0.60)</td>
</tr>
<tr>
<td>11. rs12880291</td>
<td>2.39x$10^{-4}$</td>
<td>0.10</td>
<td>0.27</td>
<td>0.29 (0.14, 0.61)</td>
</tr>
<tr>
<td>12. rs17178387</td>
<td>2.38x$10^{-4}$</td>
<td>0.10</td>
<td>0.27</td>
<td>0.29 (0.14, 0.61)</td>
</tr>
<tr>
<td>13. rs11848785</td>
<td>2.40x$10^{-4}$</td>
<td>0.10</td>
<td>0.27</td>
<td>0.29 (0.14, 0.61)</td>
</tr>
<tr>
<td>14. rs2273509</td>
<td>1.84x$10^{-4}$</td>
<td>0.33</td>
<td>0.16</td>
<td>2.64 (1.63, 4.29)</td>
</tr>
</tbody>
</table>
**Supplemental Table 2. Replication Study.** Comparison among Caucasians only for PPCM2 cases to Local2 controls and PPCM2 to young controls for SNPs with GWAS p-value: $9.0 \times 10^{-8} < p \leq 1 \times 10^{-5}$.

<table>
<thead>
<tr>
<th>SNP</th>
<th>MAFs*</th>
<th>p-value</th>
<th>OR (95% CI)</th>
<th>MAFs*</th>
<th>p-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. rs7102702</td>
<td>0.33, 0.37</td>
<td>0.61</td>
<td>0.86† (0.49, 1.53)</td>
<td>0.33, 0.35</td>
<td>0.85</td>
<td>0.94 (0.51, 1.75)</td>
</tr>
<tr>
<td>3. rs10048187</td>
<td>0.05, 0.13</td>
<td>0.08</td>
<td>0.35 (0.10, 1.19)</td>
<td>0.05, 0.11</td>
<td>0.18</td>
<td>0.41 (0.11, 1.52)</td>
</tr>
<tr>
<td>4. rs4458717</td>
<td>0.22, 0.21</td>
<td>0.84</td>
<td>1.07 (0.55, 2.08)</td>
<td>0.22, 0.18</td>
<td>0.58</td>
<td>1.24† (0.58, 2.65)</td>
</tr>
<tr>
<td>5. rs17284876</td>
<td>0.10, 0.19</td>
<td>0.13</td>
<td>0.50† (0.20, 1.23)</td>
<td>0.10, 0.21</td>
<td>0.07</td>
<td>0.43† (0.17, 1.10)</td>
</tr>
<tr>
<td>6. rs7225404</td>
<td>0.11, 0.14</td>
<td>0.62</td>
<td>0.79 (0.31, 2.02)</td>
<td>0.111, 0.112</td>
<td>0.99</td>
<td>0.99 (0.35, 2.81)</td>
</tr>
<tr>
<td>7. rs2169876</td>
<td>0.09, 0.14</td>
<td>0.32</td>
<td>0.60 (0.21, 1.67)</td>
<td>0.09, 0.12</td>
<td>0.60</td>
<td>0.77 (0.29, 2.04)</td>
</tr>
<tr>
<td>8. rs12881957</td>
<td>0.22, 0.26</td>
<td>0.52</td>
<td>0.78† (0.36, 1.67)</td>
<td>0.22, 0.215</td>
<td>0.91</td>
<td>1.04 (0.50, 2.15)</td>
</tr>
<tr>
<td>9. rs7152674</td>
<td>0.21, 0.27</td>
<td>0.39</td>
<td>0.72† (0.34, 1.53)</td>
<td>0.214, 0.215</td>
<td>0.99</td>
<td>0.99 (0.48, 2.05)</td>
</tr>
<tr>
<td>10. rs3784064</td>
<td>0.20, 0.27</td>
<td>0.23</td>
<td>0.63† (0.30, 1.34)</td>
<td>0.20, 0.21</td>
<td>0.85</td>
<td>0.93 (0.46, 1.91)</td>
</tr>
<tr>
<td>11. rs12880291</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>12. rs17178387</td>
<td>0.23, 0.29</td>
<td>0.40</td>
<td>0.74† (0.36, 1.50)</td>
<td>0.23, 0.25</td>
<td>0.83</td>
<td>0.93 (0.47, 1.85)</td>
</tr>
<tr>
<td>13. rs11848785</td>
<td>0.20, 0.29</td>
<td>0.15</td>
<td>0.56† (0.25, 1.25)</td>
<td>0.20, 0.24</td>
<td>0.52</td>
<td>0.77† (0.35, 1.68)</td>
</tr>
<tr>
<td>14. rs2273509</td>
<td>0.26, 0.14</td>
<td>0.020†</td>
<td>2.54† (1.14, 5.69)</td>
<td>0.26, 0.16</td>
<td>0.11</td>
<td>1.76† (0.87, 3.58)</td>
</tr>
</tbody>
</table>

*MAFs: minor allele frequencies for cases and controls, respectively; †Risk effect for PPCM2 vs. Local2 controls has OR>1.10 or OR<0.90 and is in the same direction as discovered by GWAS; ‡SNP failed quality control; ¶Not significant after correction for multiple comparisons: for these 13 originally (in GWAS) suggestively-significant SNPs, $p<0.00625$ was required to correct for 8 hypothesis tests (including the primary hypothesis test and the 7 independent loci that these 13 SNPs represented [SNPs 3 and 6 {rs10048187 and rs7225404} are both at C17orf52 and SNPs 8-13 are near the SIPA1L1 gene]).
Supplemental Figure S1. The distribution of observed and expected probability values for GWAS SNPs with p<0.05 (based on GWAS discovery PPCM cases compared to Local Controls) shows that an excess of SNPs were observed at lower p-values.
Supplemental Figure S2. HapMap data for chromosome 12p11.22. Linkage disequilibrium (LD) of rs258415 with 251 HapMap SNPs across approximately 200 kilobases on chromosome 12p11.22 based on LD measured by $D'$. The base pair position of rs258415 is indicated at the top of the graph. High $D'$ (>0.7) was measured throughout the region including with SNPs in both $PTHLH$ and $KLHDC5$ (which genes are shown in their relative positions 93 kilobases and 62 kilobases away from rs258415, respectively).