Genetic basis of hypertrophic cardiomyopathy
Bos, J.M.

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Chapter 6

TGFβ-Inducible Early Gene-1 (TIEG1):
A Novel Hypertrophic Cardiomyopathy-
Susceptibility Gene

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Ackerman

Manuscript in preparation
Abstract

Background: Hypertrophic cardiomyopathy (HCM) is the most common heritable cardiovascular disease and the most common cause of sudden cardiac death in the young. Over 24 genes have been implicated in the pathogenesis of HCM. However, for about half of patients with HCM, the genotypic substrate remains elusive. A recent study showed that male TGFβ Inducible Early Gene-1 (TIEG1)-knock-out (TIEG1-/-) mice develop HCM after 16 months. Microarray analysis on the mice hearts showed a 13-fold up-regulation of PTTG1-encoded pituitary tumor-transforming gene 1. We therefore speculated that TIEG1 could be a novel candidate gene in the pathogenesis of genotype negative HCM, possibly through a loss of its repression on PTTG1 expression.

Methods: For this study, we analyzed a cohort of 923 unrelated patients from two independent cohorts of patients with HCM (664 male, age at diagnosis 47.6 ± 18, mean left ventricular wall thickness (MLVWT) 20.0 ± 7 mm). All patients were genotype negative with respect to the 9 genes responsible for myofilament/sarcomeric-HCM. Open reading frame/splice site mutational analysis of TIEG's 4 translated exons was performed using DHPLC and direct DNA-sequencing. Site directed mutagenesis was performed to clone novel variants. The effect of wild type and mutant TIEG1 on the PTTG1 and SMAD7 promoters was studied using transient transfection and luciferase-assays. Cardiac HCM tissue was studied by immunohistochemistry to determine levels of PTTG1 protein expression.

Results: Six novel missense mutations (A12T, M27T, T216A, E137K, A204T and S225N) in TIEG1 were discovered in 6/923 patients (2 males/4 females, mean age at diagnosis 56.2 ± 23 years, MLVWT 20.8 ± 4 mm). Each missense mutation was absent in 800 ethnically-matched reference alleles and involved residues that were conserved across species. Compared to the 50% repression of PTTG1 promoter function by wild type TIEG1, 5 TIEG1 mutants had this repression significantly attenuated resulting in marked accentuation of PTTG1 promoter function similar to the TIEG1-/- KO-mice. One TIEG1 mutant significantly altered TIEG1-function on SMAD7-expression. By immunohistochemistry, PTTG1-protein expression was increased in myectomy specimens from all patients with HCM, irrespective of TIEG1 mutation status, compared to normal hearts.
Conclusions: This is the first paper to associate mutations in *TIEG1* to human disease with the discovery of 6 novel, HCM associated variants. Functional assays suggest a role for PTTG1 and SMAD7 in the pathogenesis of TIEG1-mediated HCM. Up-regulation of PTTG1 could be a final common pathway response in HCM. Future studies are needed to elucidate the precise role of PTTG1 in the pathogenesis of TIEG1-HCM as well as HCM in general.

Keywords

Hypertrophic cardiomyopathy, TIEG1, KLF10, genes, PTTG1
Background

In the last two decades, over 24 disease-susceptibility genes have been elucidated for hypertrophic cardiomyopathy (HCM), a disease characterized by unexplained cardiac hypertrophy that affects approximately 1 in 500 individuals [1, 2]. Currently over 80% of reverse-curve HCM and 10% of sigmoidal-HCM is explained by mutations in genes encoding the myofilaments of the cardiac sarcomere[3], making a large portion of sigmoidal HCM genetically elusive. More recently, rare mutations in genes encoding Z-disc proteins and calcium handling proteins have been linked to the pathogenesis of HCM[1], but the search for novel HCM-causing genes continues.

*Ti*E*G*1-encoded TGFβ-inducible early gene-1 (TIEG1)(also referred to a *KLF*10-encoded Krüppel-Like Factor 10) was discovered originally as an early response gene following TGFβ treatment of human osteoblast and is expressed in many tissues, including cardiac myocardium[4, 5]. It is a member of the Krüppel-like family of transcription factors, which are known to be involved in anti-proliferative and apoptotic inducing functions following TGFβ-induction[6]. Subsequent studies in engineered TIEG1-knock out (TIEG1−/−) mice[7] showed that male mice develop HCM with significant, but relatively late-onset cardiac hypertrophy at 16 months of age[8]. Microscopic examination of TIEG1−/−-male mice hearts showed characteristic hallmarks of HCM: cardiomyocyte hypertrophy, fibroblast hyperplasia and myocyte disarray[8]. Furthermore, microarray analysis revealed a significant up-regulation of PTTG1-encoded pituitary tumor transforming gene-1, demonstrating that TIEG1 plays an important role in the repression of proliferative and hypertrophic pathways, possibly through the actions of PTTG1. Based on these findings, we hypothesized that *Ti*E*G*1 could be a novel HCM-susceptibility gene.
Methods

Study cohort
Our study cohort consisted of 923 unrelated patients with HCM from two large cardiac referral centers – Mayo Clinic (Rochester, MN USA) and the Academic Medical Center (AMC, Amsterdam, The Netherlands). All patients were genotype negative for mutations in the 9 HCM-associated genes, currently included in commercially available genetic tests (MYBPC3, MYH7, TNNT2, TNNI3, TNNC1, TPM1, MYL2, MYL3, and ACTC). Clinical data were collected on all patients, including pertinent personal and family history (especially with regard to HCM or sudden cardiac arrest (SCA), and an echocardiogram to determine maximum left ventricular wall thickness (MLVWT) and resting left ventricular outflow tract gradient (LVOT). Clinical diagnosis of HCM was made when subjects had a MLVWT over 13mm in the absence of hypertrophy inducing conditions such as aortic stenosis or hypertension.

Genetic analysis
DNA of all patients was extracted from peripheral blood lymphocytes (Gentra Inc, Minneapolis, MN). After design of intronic primers, for each patient all 4 translated exons of TIEG1 (Nm_005646) were amplified by polymerase chain reaction (PCR) and subsequently analyzed for genetic variations by denaturing high performance liquid chromatography (DHPLC) (WAVE®, Transgenomic, Omaha, NE). Abnormal DHPLC-elution profiles were subjected to direct DNA sequencing (ABI Prism 377, Applied Biosystems, Foster City, CA) to determine the nature of nucleotide substitution. All translated exons were analyzed for 800 Caucasian reference alleles from ostensibly healthy, ethnically-matched controls to distinguish novel HCM-associated mutations from rare or common polymorphisms.

Site-directed mutagenesis and luciferase assays
After design of sequence specific primers, identified mutations and control variants were created using site-directed mutagenesis, cloned into the pcDNA4.0 expression vector (Invitrogen, Carlsbad, CA) and transformed into XL-10 ultracompetent cells (Quickchange® II, Stratagene, La Jolla, CA). DNA was purified from bacteria (Miniprep®, Qiagen, Valencia, CA) and all constructs were confirmed by direct DNA sequencing.
Effects of the various TIEG1 mutations on PTTG1-promoter activity (PTTG1-promoter including the 5'-flanking region (-1,321 to -3) cloned in front of a luciferase reporter as previously described[8]) were studied using luciferase assays. Blinded to the observer, the PTTG1-promoter construct (1μg) was transfected into AKR2B mouse embryo fibroblasts along with 1μg of empty expression vector, wild-type (WT) TIEG1-expression vector or the various mutant TIEG1 expression vectors. Following 24h of transfection, cell lysates were prepared and analyzed for luciferase activity. Luciferase assays were repeated at least 3 times and values were normalized to total protein concentrations and expressed as fold-change relative to empty-vector promoter activity.

To assess the effect of mutations on the cardiac expressed SMAD7-promoter, 1μg of empty expression vector, and either WT-TIEG1 or TIEG1 mutants were transfected into H9c2 rat cardiocytes along with a SMAD7-promoter construct (1ug). Eight hours after transfection, culture media (DMEM + 10% horse serum (HS)) was replaced with DMEM with 1% HS to induce cardiocyte differentiation. Thirty-six to forty-eight hours after transfection, cells were lysed, and luciferase assays were performed and analyzed as described above.

**Immunohistochemistry**

To determine expression of PTTG1-protein, cardiac tissue obtained following surgical myectomy in patients with HCM and from non-failing left ventricular hearts at autopsy (controls) was stained with monoclonal PTTG1-antibody (Epitomics, Burlingame, CA). Formalin fixed, paraffin embedded tissue was retrieved from 2 TIEG1-genotype positive patients, 2 genotype negative HCM patients, and 2 autopsy-negative, non-cardiac death subjects. Paraffin-blocks were sectioned at 5 μm for immunohistochemical staining. Deparaffinization with xylene and subsequent rehydration with graded ethanol preceded heat induced epitope retrieval with EDTA buffer (pH 8) in a Lab Vision PT Module (Fremont, CA). The staining procedure was carried out by an automated immunohistochemistry-staining machine (DAKO Technmate 500, DAKO, Denmark) using the Envision program. Titration for correct dilution of antibody was performed and after review, a dilution of 1:75 was selected for all assays. We compared expression of PTTG1-protein in tissue of TIEG1-genotype positive patients to genotype negative HCM patients as well as cardiac tissue of two autopsy negative, non-cardiac death subjects.
Statistical analyses

Statistical analyses were performed using JMP 7.0 statistical software (JMP, Cary, NC) using analysis of variance (ANOVA) and Student’s t-test. A p-value <0.05 was considered statistically significant.
Results

Demographics of the study cohort are summarized in Table 1. Overall 923 patients with HCM (664 male) were enrolled in this study – 739 from Mayo Clinic (USA) and 184 from Academic Medical Center (AMC) (NL). Patients had an average age at diagnosis of 47.6 ± 18 years and mean MLVWT of 20.0 ± 7 mm. Twenty-six percent of patients reported a family history of HCM and 15% of patients had a family history of SCA. Thirty percent of patients had undergone surgical myectomy and 14% of patients had received an ICD. The specific demographics of each cohort (Mayo and AMC) are detailed in Table 1. Overall, patients from the AMC cohort had a lower MLVWT (17.5 ± 5.4 vs. 17.5 ± 5.4 (p = 0.03) and were more likely a family history of HCM (35% vs. 25%, p = 0.005) or sudden cardiac arrest (SCA) (47% vs. 15%, p < 0.001). Patients at Mayo were likely to have undergone surgical septal myectomy (38% vs. 6%, p < 0.001), reflecting the referral bias for Mayo Clinic as a surgical center for treatment of obstructive HCM.

Table 1: Demographics of study cohort

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Mayo</th>
<th>AMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>923</td>
<td>739</td>
<td>184</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>664/359</td>
<td>441/298</td>
<td>123/61</td>
</tr>
<tr>
<td>Age at Dx, years</td>
<td>47.6 ± 18</td>
<td>47.4 ± 18</td>
<td>48.3 ± 19</td>
</tr>
<tr>
<td>Septal thickness, mm</td>
<td>20.0 ± 7</td>
<td>20.6 ± 8</td>
<td>17.5 ± 5.4*</td>
</tr>
<tr>
<td>LVOT gradient, mm Hg</td>
<td>45.1 ± 40</td>
<td>45.2 ± 44</td>
<td>44.7 ± 29</td>
</tr>
<tr>
<td>Family history of HCM, n (%)</td>
<td>249 (26)</td>
<td>184 (25)</td>
<td>65 (35)*</td>
</tr>
<tr>
<td>Family history of SCA, n (%)</td>
<td>199 (22)</td>
<td>113 (15)</td>
<td>86 (47)*</td>
</tr>
<tr>
<td>Myectomy, n (%)</td>
<td>289 (31)</td>
<td>281 (38)</td>
<td>8 (6)*</td>
</tr>
<tr>
<td>ICD, n (%)</td>
<td>126 (14)</td>
<td>103 (14)</td>
<td>23 (14)</td>
</tr>
</tbody>
</table>

Dx, diagnosis; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter defibrillator; LVOT, left ventricular outflow tract; SCA, sudden cardiac arrest. *, p<0.05
Genetic results

Genetic analysis of TIEG1’s open reading frame (exome) revealed 6 novel missense mutations (A12T, M27T, E137K, A204T, T216A and S225N) in 6 patients with HCM that were absent in 800 reference alleles (Figure 1). One novel variant (Q10H) was discovered in HCM patients as well as healthy controls at similar frequencies (allelic frequency 0.4%). One previously reported rare polymorphism (S249F, rs4734653) was seen in 3 HCM-patients, but not in our cohort of 400 ethnically matched controls. This variant, however, was previously described in 0.8% of healthy Europeans.

Figure 1: Topology of TIEG1-protein. Schematic representation of 480 amino acid containing TIEG1 protein with HCM-associated (black circles) and control variants/polymorphisms (white circles) identified in two cohorts of HCM patients.
All variants and surrounding residues found in patients with HCM (Figure 2A) as well as control variants (Figure 2B) were conserved across species and the mutant residues were not seen in other species. In certain species, general sequence homology was poor or even absent for the first 90 residues of TIEG1.

![Sequence conservation](image)

**Figure 2: Sequence conservation.** Shown is the conservation across species of (a) novel, HCM-associated TIEG1-mutations (top panel) and (b) novel and previously described control variants (bottom panel).
**Patient characteristics**

Patient characteristics for TIEG1-positive patients are summarized in Table 2. Each mutation was found once in 2 male and 4 female patients. All patients were of Caucasian ethnicity. Overall, there did not seem to be a specific phenotype associated with TIEG1-mediated HCM, although in most cases -- except for case 2 - HCM was late onset (mean age at diagnosis 56.2 ± 23 years). The mean MLVWT of TIEG1-positive patients was 20.8 ± 4 mm and family history of HCM was found in 2/6 (cases 3 and 6) and SCA in 1 case (case 6). Three patients had undergone surgical septal myectomy for relief of symptoms (cases 2-4), a number relatively high compared to the annual average rate of myectomy in HCM (~5-10%). The most severely affected patient (case 2) was a man with M27T-TIEG1. He was diagnosed at 15 years of age, with extreme hypertrophy (MLVWT, 26mm) and obstruction (117 mmHg gradient) for which a surgical myectomy was performed. To date, both parents and most siblings do not meet the diagnostic criteria of HCM following frequent echocardiographic screening suggesting variable penetrance of the disease or a de novo mutation in this patient. His family, as well as the others’, have been contacted but have declined or have not yet enrolled for co-segregation studies.
Table 2: Patient characteristics

<table>
<thead>
<tr>
<th>Case</th>
<th>Cohort</th>
<th>Nucleotide change</th>
<th>Mutation</th>
<th>Sex</th>
<th>Age at Dx (yrs)</th>
<th>MLVWT (mm)</th>
<th>LVOT gradient (mm Hg)</th>
<th>FH HCM</th>
<th>FH SCA</th>
<th>Treatment</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AMC</td>
<td>c.GCG&gt;GAG</td>
<td>p.A12T</td>
<td>M</td>
<td>65</td>
<td>17</td>
<td>13</td>
<td>No</td>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Mayo</td>
<td>c.ATG&gt;ACG</td>
<td>p.M27T</td>
<td>M</td>
<td>15</td>
<td>26</td>
<td>117</td>
<td>No</td>
<td>No</td>
<td>Myectomy</td>
<td>Parents + most siblings echo negative</td>
</tr>
<tr>
<td>3</td>
<td>Mayo</td>
<td>c.GAA&gt;AAA</td>
<td>p.E137K</td>
<td>F</td>
<td>48</td>
<td>20</td>
<td>55</td>
<td>Yes</td>
<td>No</td>
<td>Myectomy, ICD</td>
<td>Two sons echo neg</td>
</tr>
<tr>
<td>4</td>
<td>AMC</td>
<td>c.ACA&gt;GCA</td>
<td>p.T216A</td>
<td>F</td>
<td>58</td>
<td>16</td>
<td>20</td>
<td>No</td>
<td>No</td>
<td>Myectomy</td>
<td>Father died suddenly at age 63 unknown cause</td>
</tr>
<tr>
<td>5</td>
<td>Mayo</td>
<td>c.GCT&gt;ACT</td>
<td>p.A204T</td>
<td>F</td>
<td>80</td>
<td>20</td>
<td>49</td>
<td>No</td>
<td>No</td>
<td>-</td>
<td>Patient deceased at age 76 of HF and COPD</td>
</tr>
<tr>
<td>6</td>
<td>Mayo</td>
<td>c.AGT&gt;AAT</td>
<td>p.S225N</td>
<td>F</td>
<td>71</td>
<td>26</td>
<td>16</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

COPD, chronic obstructive pulmonary disease; Dx, diagnosis; HCM, hypertrophic cardiomyopathy; HF, heart failure; ICD, implantable cardioverter defibrillator; LVOT, left ventricular outflow tract; SCA, sudden cardiac arrest.
**SMAD7 promoter activity in H9c2-cardiocytes**

To study the effect of TIEG1 on SMAD7-promoter expression in a more native environment, we performed a luciferase assay in H9c2 rat cardiocytes. Thirty-six to forty-eight hours after transfection, cardiocytes were lysed and analyzed for luciferase activity as described above. As expected, WT TIEG1 repressed SMAD7-promoter activity by ~70%. Four of the 6 HCM-associated variants as well as the two control variants exhibited normal TIEG1-like function relative to SMAD7 (Figure 3). Two of the putative TIEG1-HCM mutations – A12T and S225N – altered SMAD7-promoter expression with S225N-TIEG1 showing significantly increased expression of SMAD7 promoter activity as compared to wild-type (Figure 3).

**Figure 3: SMAD7-promoter activity in H9c2-cardiocytes.** Bar diagram showing SMAD7-promoter activity in H9c2-cardiocytes. Wild-type TIEG1 (WT) repressed SMAD7-expression. Four of six mutations as well as control variants act like WT, whereas S225N-TIEG1 significantly alters TIEG1-function on SMAD7-expression.
**PTTG1 promoter activity in AKR2B-fibroblasts**

Twenty-four hours after transfection with the PTTG1 promoter and either WT-TIEG1, mutant TIEG1, or control variant expression constructs, AKR2B-cells were lysed and analyzed for luciferase activity. As expected, WT TIEG1 repressed PTTG1-promoter activity by ~55% (Figure 4). Akin to data from TIEG1<sup>-/-</sup>-mice, 5 of the 6 putative TIEG1-HCM mutations resulted in luciferase activity that was significantly higher than that of WT (p < 0.05 compared to WT), and in the case of T216A-TIEG1, up to 2 fold higher than vector control (Figure 4). In contrast, PTTG1-promoter activity seen in Q10H-TIEG1 control variant was identical to WT TIEG1 effect, while on the other hand, S249F-TIEG1 showed increased PTTG1 activity.

![Figure 4: PTTG1-promoter activity in AKR2B-fibroblasts. Bar diagram showing PTTG1-promoter activity in AKR2B-fibroblasts. Wild-type TIEG1 (WT) repressed PTTG1-expression, where 5 of 6 mutations altered PTTG1-expression significantly.](image)

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**PTTG1 protein expression in cardiac tissue**

In order to determine the expression of PTTG1 protein in HCM patients, we performed immunohistochemical analysis on paraffin embedded tissue sections derived from patients with TIEG1-HCM (cases 2 and 3), genotype negative-HCM and autopsy normal hearts (Figure 5). Characteristic hallmarks of HCM, such as myofibrillar disarray as well as fibrosis could be seen in all myocardial specimens from all 4 patients with HCM (Figure 5C-F). Only mild to no PTTG1-protein expression was seen in normal heart tissue (Figure 5A+B) as previously described[9]. In contrast, PTTG1 protein expression was dramatically increased in tissue of both HCM genotype negative patients (Figure 5C + D) as well as the two patients (Cases 2 and 3) with TIEG1-HCM (Figure 5E + F). PTTG1 localized mostly to cytoplasm and myofibers of cardiomyocytes and strongest expression was seen in TIEG1-mediated disease. This data suggests PTTG1 could be a biomarker for HCM in general, although more data is needed to determine whether there is a different expression of PTTG1 between genotype negative and TIEG1-mediated HCM.
Immunohistochemical staining for PTTG1 protein expression demonstrates little PTTG1-protein expression in autopsy normal hearts (A and B), but severely increased levels of PTTG1 protein in myocardial specimens derived from patients with genotype negative-HCM (C and D) and TIEG1-HCM. Characteristic hallmarks of HCM – myofibrillar disarray and fibrosis – can also be seen in HCM tissues (C-F).
Discussion

Over the past two decades, multiple genes encoding proteins involved in various processes in the cell have been implicated in the pathogenesis of HCM. Since the discovery of the first gene, MYH7-encoded β-myosin heavy chain for HCM in 1989, over 24 HCM-susceptibility genes have been reported and commercial genetic testing is now available for a large subset of these genes[1, 10]. However, for many patients, the underlying genetic cause remains elusive and research continues to discover novel HCM-associated genes. Recently, male TIEG1−/− mice exhibited features of late-onset HCM, including asymmetric cardiac hypertrophy, increased ventricular size at 16 months of age, increased heart weight to body weight ratio, increased fibrosis and increased wall thickness compared to WT mice[8]. Furthermore, Masson’s Trichrome-staining demonstrated evidence of myocyte disarray and fibrosis which led us to hypothesize that TIEG1 could be a candidate gene in the pathogenesis of HCM.

Herein, we analyzed 923 unrelated patients with HCM from the USA and the Netherlands. After comprehensive analysis of all translated regions of TIEG1, we discovered 6 novel, HCM-associated missense mutations in patients which were absent in 800 ethnically-matched Caucasian healthy control subjects. Furthermore, we discovered a novel control variant. The Q10H-variant was seen in our patients as well as in our 800 reference alleles (allelic frequency 0.4%). Notably, S249F-TIEG1 (rs4734653) was seen in our patients (Allelic frequency 0.5%), was absent in our reference alleles; but was seen in 0.8% of European controls.

Overall, no TIEG1-specific phenotype seemed to be associated with human TIEG1-HCM, although similar to the TIEG−/− mice, the patient’s cardiac hypertrophy was of late-onset and obstructive in most cases. While HCM-phenotype was seen exclusively in male- TIEG−/− mice, no gender predilection could be demonstrated among the small cohort of patients with putative TIEG1-HCM. Female TIEG1−/− mice are known to have skeletal defects which have been characterized as osteopenia[11]. Careful examination of TIEG1-mutation positive patients’ charts – especially women - revealed no specific skeletal problems in our patients, although it must be acknowledged that most patients were specifically referred to a cardiologist for HCM evaluation.
Transforming growth factor-β (TGFβ) is a key mediator of cardiac adaptations to hemodynamic overload and plays a critical role in induction of cardiac hypertrophy, heart failure and fibrosis[12]. This is caused by TGFβ-induced expression of collagen mRNA and subsequent collagen deposition in fibroblasts, induced expression of factors of the fetal gene program (MYH7 and ACTC) in cardiomyocytes, and through activation of the MAPK signaling pathway and p38-induced transcription factors[13, 14]. TIEG1 plays a critical role in the regulation of TGFβ in multiple cell types. First, it was demonstrated that expression of TIEG1 is increased within 30 minutes following TGFβ treatment in osteoblast cells[4]. Normal TIEG1 function then subsequently attenuates TGFβ-signaling through either activation of SMAD2 or repression of the inhibitory co-factor SMAD7[15, 16, 17].

SMAD7 is a member of the SMAD-family of proteins involved in TGFβ-signaling, and with SMAD6, comprises the subgroup of inhibitory SMADS that antagonize TGFβ-family members [18, 19]. While most mice devoid of the indispensible MH2-domain of SMAD7 die in utero, surviving mice have impaired cardiac functions (such as ejection – and shortening fraction) and cardiac arrhythmias[20]. Because of its known role in TIEG1-TGFβ-signaling, as well as the mutant mouse phenotype, we sought to examine the effects of our novel HCM-associated TIEG1-variants on the activity of the SMAD7-promoter. We found that two of the 6 mutants and none of the control variants alter TIEG1-function on SMAD7, with one variant showing significantly altered function compared to WT TIEG1.

Further studies of TIEG1−/− male mice demonstrated the mice develop characteristic features of HCM during aging with a marked upregulation of PTTG1[8]. PTTG1 is typically overexpressed in a variety of endocrine-related tumors, especially pituitary, thyroid, breast, ovarian, and uterine tumors as well as non-endocrine tumors. PTTG1 functions in cell replication, proliferation, DNA damage/repair, organ development, and metabolism (reviewed in [21]). In vitro luciferase assays studies demonstrated that TIEG1 acts directly on the promoter of PTTG1 causing a 60-70% drop in PTTG1’s promoter activity suggesting that the observed myocyte hypertrophy and fibrosis in male TIEG−/− mice may be mediated by loss of TIEG1’s normal inhibition over PTTG1 and consequential accentuation in PTTG1 gene expression [8]. Akin to these observations, our current study showed that 5 of our 6 TIEG1 mutations resulted in a significant increase in PTTG1 promoter activity relative to WT TIEG1.
In addition, protein levels of PTTG1 in surgically resected, hypertrophic myocardium of patients with TIEG1-HCM, was markedly increased. Further, accentuation in PTTG1 might be a final common pathway hypertrophic response as two patients with genotype negative HCM also displayed this finding. These data suggest that these putative HCM-associated TIEG1 mutations dysregulate TIEG1’s normal repressive control over either SMAD7 or PTTG1 and that PTTG1 protein expression might be a ‘final common pathway’ biomarker in HCM[22, 23]. Further studies are therefore needed to dissect the biological role of PTTG1. Conceivably, gain-of-function PTTG1 mutations in humans or transgenic overexpression of PTTG1 in mice could precipitate HCM.
Conclusions

This is the first report to associate mutations in the TIEG1 gene with human disease. We have identified 6 novel, HCM associated TIEG1 missense mutations and have demonstrated that a number of these variants have abnormal function with regard to mutant TIEG1’s ability to regulate either the PTTG1 or SMAD7 promoters, two genes known to be associated with hypertrophic pathways. Furthermore, tissue expression of PTTG1 seemed to be associated with HCM in general with highest expression seen in TIEG1-mediated HCM suggesting PTTG1 might be a biomarker for HCM. While these studies have implicated TIEG1 in human HCM, additional in vitro and in vivo functional studies are needed to further elucidate the exact pathway(s) leading to HCM in TIEG1-genotype positive patients. Furthermore, studies are needed to examine the potential role of PTTG1 as a biomarker in the pathogenesis of HCM.
References


