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Cardiac expression of the microsomal triglyceride transport protein protects the heart function during ischemia

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ABSTRACT

Aims: The microsomal triglyceride transport protein (MTTP) is critical for assembly and secretion of apolipoprotein B (apoB)-containing lipoproteins and is most abundant in the liver and intestine. Surprisingly, MTTP is also expressed in the heart. Here we tested the functional relevance of cardiac MTTP expression.
Materials and methods: We combined clinical studies, advanced expression analysis of human heart biopsies and analyses in genetically modified mice lacking cardiac expression of the MTTP-A isoform of MTTP.
Results: Our results indicate that lower cardiac MTTP expression in humans is associated with structural and perfusion abnormalities in patients with ischemic heart disease. MTTP-A deficiency in mice heart does not affect total MTTP expression, activity or lipid concentration in the heart. Despite this, MTTP-A deficient mice displayed impaired cardiac function after a myocardial infarction. Expression analysis of MTTP indicates that MTTP expression is linked to cardiac function and responses in the heart.
Conclusions: Our results indicate that MTTP may play an important role for the heart function in conjunction to ischemic events.

1. Introduction

The microsomal triglyceride transport protein (MTTP) is a heterodimer consisting of a unique MTTP subunit and a protein disulfide isomerase (PDI) subunit [1]. The MTTP subunit belongs to a lipid transfer protein family which also includes apoB, insect apolipophorin II/I, and the egg yolk precursor vitellogenin [1]. The MTTP subunit preferentially transfers neutral lipids compared with charged lipids and is most abundant in the liver and intestine [2,3]. However, it is capable of binding and transferring a wide variety of lipid molecules [1]. PDI is a ubiquitously enzyme that catalyzes the formation and breakage of disulfide bonds within proteins as they fold. The function of PDI in MTTPMTTP is still uncertain but it has been proposed to maintain MTTP in a soluble form [1].

Three splice variants of MTTP (MTTP-A, −B and −C) have been identified to date [4–7]. All three isoforms display lipid transfer activity but differ in their tissue distribution. MTTP-A is mostly found in the liver and intestine where it localizes mainly in the endoplasmic reticulum, MTTP-B in adipose tissue and natural killer T cells where it is localized mainly in the Golgi apparatus, and MTTP-C in tissues such as

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liver and brain [4–7]. Intriguingly, all three isoforms are also expressed in the heart [4,5,8]. Importantly, in humans only MTTP-A and MTTP-C are translated into protein but the MTTP-C translation is strongly inhibited by regulatory elements within its 5′-UTR [4]. Thus, the MTTP-C protein levels are approximately 10% of MTTP-A [9]. However, there is no major difference in lipid transfer activity between MTTP-A and MTTP-C [4].

In the liver and small intestine, MTTP is critical for the formation of apoB-containing triglyceride-rich lipoproteins. Its role in other tissues is still unclear, but some information has emerged. For example, MTTP is important for the immune response [10]. In antigen presenting cells, MTTP is critical fpr biosynthesis of the lipid antigen presenting protein CD1 [11], and *Mttp*-deficient mice are unable to activate natural killer T (NKT) cells [10,11]. In adipocytes, MTTP has been linked to lipid droplet formation [10]. In the heart, expression of MTTP is critical for cardiac synthesis and secretion of apoB-containing lipoproteins [12–14]. The physiological relevance of cardiac lipoprotein secretion is still unclear but it has been shown that cardiac lipid accumulation is linked to impaired cardiac contractility and conductivity [15] and heart failure [16,17], and increased cardiac secretion of apoB-containing lipoproteins has been proposed to play an important role in unloading potentially toxic lipids that accumulate in cardiomyocytes under ischemic conditions [18,19]. In support of this hypothesis, overexpression of human apoB in transgenic mice has been shown to reduce cardiac remodeling and hypertrophy after a myocardial infarction and to protect against lipotoxic cardiomyopathy [20,21]. In Drosophila cardiomyocyte MTTP has been shown to be the main regulator of the whole-body lipid metabolism since cardiomyocyte-derived apoB-lipoproteins are the main source of circulating lipids [22].

In humans, three common polymorphisms of MTTP have been described that are in complete allelic association ($D' = 1$, $r^2 = 1$); −493G > T (rs1800591), −164 T > C (rs1800804), and Ile128Thr (rs3816873) [1]. The minor C allele at position −164 has been associated with decreased MTTP transcription compared with the major T allele and the net effect of minor alleles of these two promoter polymorphisms −493G > T and −164 T > C is decreased expression mediated by allele-specific binding of transcription factors to the −164 T > C polymorphism [24].

Others and we have earlier reported that the −493G > T polymorphisms associated with increased risk of ischemic heart disease independent of plasma lipids [24,25]. However, different results have been reported [26–30]. The underlying mechanism is unclear but a study in mice has shown that failure to upregulate *Mttp* expression in response to increased fatty acid availability increases cardiac lipid accumulation and reduces heart function [31].

To further our understanding of the biological relevance of cardiac MTTP expression, we combined clinical studies, advanced expression analysis of human heart biopsies and analyses in genetically modified mice. We show that *MTTP* polymorphisms in humans associate with cardiac function in conjuction to ischemic events and are linked to reduced MTTP expression. Our expression analyses reveal that *MTTP* in the human heart is highly co-expressed with genes linked to cardiac arrhythmias, mitochondrial function and lipid metabolism. Finally, we show that knockout of *Mttp-A* in mice hearts reduces heart function after a myocardial infarction despite compensatory upregulation of *Mttp-B*. Together, these studies are consistent with the hypothesis that MTTP plays an important role for heart function during ischemic events.

2. Methods

2.1. The coronary flow reserve and cardiovascular events (CEVENT) study

In total, 441 patients with clinically suspected coronary artery disease (referred for investigation of chest pain) were recruited to participate in the CEVENT study at the Department of Clinical Physiology, Sahlgrenska University Hospital, Gothenburg, Sweden, from 2006 to 2008. Blood samples were taken after an overnight fast. All patients underwent standard myocardial perfusion scintigraphy (MPS) for detection of clinically meaningful myocardial ischemia and a complete ultrasound examination of the carotid arteries within one month of the MPS [32]. Ultrasound-based velocity vector imaging measurements were processed later at a workstation. The presence of perfusion abnormalities was assessed using: (1) the summed stress score (the sum of the segmental scores from the stress images), which reflects the extent and severity of myocardial perfusion abnormalities related to both myocardial ischemia and infarction; (2) the summed rest score (the sum of the segmental scores assessed at rest), which represents the extent of myocardial infarction; and (3) the difference between the first two (the summed difference score), which represents stress-induced ischemia. The absence and presence of left ventricle perfusion defects were scored as 0 and > 0, respectively.

For the present study, we excluded patients with overt ischemic heart disease or who had undergone percutaneous transluminal coronary angioplasty or coronary artery bypass grafting. Therefore, a total of 310 patients with suspected coronary artery disease were included. Of these MPS variables were analyzed in 309 subjects, and genotyping of rs1800804(−164 T > C) polymorphism in 307 individuals.

The study was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent, and the study was approved by the local ethics committee in Gothenburg.

2.2. Genotyping of the CEVENT study population

Genomic DNA was isolated from whole blood. The rs1800804 (−164 T > C) polymorphism in the proximal promoter of MTTP was genotyped by TaqMan technology using a premade assay (C_11944682_20) according to the manufacturer's instructions (Applied Biosystems). The call rate was > 99%. Deviation from the Hardy-Weinberg equilibrium was measured using the $\chi 2$ test.

2.3. The advanced study of aortic pathology (ASAP) study

The ASAP study was designed to investigate the development and underlying causes, including genetic determinants, of valve disease and ascending aortic dilation [33]. The study included patients undergoing aortic valve surgery and/or surgery for aortic aneurysm at the Karolinska University Hospital, Stockholm, Sweden Patients with coronary artery disease were excluded. Fine needle biopsies from left ventricle of the heart were taken during surgery and global gene expression measured using Affymetrix ST 1.0. RNA sequencing was performed in biopsies from the left ventricle of the heart and liver biopsies in a subset of eight patients to identify the presence of different MTTP isoforms. Genotypes of the rs3816873 (Ile128Thr) polymorphism of *MTTP* were determined from results obtained using Illumina Human 610 K chips [33]. The study was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent, and the study was approved by the local ethics committee of the Karolinska Institute.

2.4. Mice

Genetically modified mice with heart-specific MTTP-A deficiency were generated by breeding MTTP LoxP mice (B6;129S-Mttp < tm2Sgy > /J, stock #3902, Jackson Laboratories) with alpha myosin heavy chain (αMHC)-Cre mice (B6.FVB-Tg(Myh6-cre) 2182Mds/J, Jackson Laboratories) to obtain heart-specific *Mttp-A* knockout (*hMttp-A*$^{-/-}$) ($n = 30$) and nontransgenic littermate littermate controls ($n = 30$). Identification of mice genotype was confirmed by PCR of tissue extracts of the tail. The mice were kept under temperature-controlled conditions with free access to food and water. The experiments were approved by the ethics committee on animal experiments in Gothenburg.

2.5. Protein analysis in mouse hearts

Immunoblotting was done as described [34]. Briefly, proteins were extracted from homogenized frozen mouse heart tissue with the Qproteosome Mammalian Protein Prep Kit (Qiagen). Specific antibodies for MTTP (kind gift from Prof L. Swift, Vanderbilt University, USA) and Na^+/K^+ ATPase (Abcam) were used.

2.6. Analysis of gene expression in mouse hearts

Total RNA was isolated from homogenized heart tissue using RNeasy Fibrous Tissue Mini Kit (Qiagen). cDNA was synthesized with the high-capacity cDNA Reverse Transcription Kit (Applied Biosystems) and random primers. mRNA expression of genes of interest was analyzed with TaqMan real-time PCR in an ViiA™ 7 system (Applied Biosystems). The following specific TaqMan assays were used: *Mttp-A* (5´CAGAGGGAGCCAGCATGATC, 3´GCAGAGTAGGAGGAGAAGAA GCA, probe TCTTG GCAGTGCTTTT), *Mttp-B* (5´GGGTTTTGCGGGAAT GGT, 3´CAACGCTTGACCGGGAAA, probe AGTGGCTACCGCGAGG), Gfm2 (Mm00623824_m1), Hadh (Mm00492535_m1), Hprt (Mm01545399_m1), Tmlhe (Mm00454748_m1) (Applied Biosystems).

2.7. MTTP activity in mouse hearts

Hearts from 10-week-old mice were homogenized in 1.5 ml buffer [50 mM Tris-HCl, 50 mM KCl, 5 mM EDTA and protease inhibitor (Roche) pH 7.4]. The heart homogenates were subjected to ultracentrifugation for 60 min at 100000 *g* in a Beckman ultracentrifuge (Beckman Coulter). The supernatant containing the microsomal fraction was added to 1/10 volume of 0.54% sodium deoxycholate (pH 7.5) and incubated on ice for 30 min, followed by overnight dialysis at 4 °C against a buffer (15 mM Tris-HCl, 40 mM NaCl, 10 mM EDTA and 0.02% NaN_3, pH 7.4). The protein concentration was measured using a Pierce BCA protein assay kit (Thermo Scientific). The MTTP activity was determined using MTTP Activity Assay Kit (Sigma Aldrich) according to the manufacturer's protocol.

2.8. Lipid analyses of mouse hearts

Heart lipids from homogenized mouse hearts were extracted using the BUME method [35], and analyzed using a QTRAP 5500 mass spectrometry (Sciex). Cholesteryl esters, triglycerides and phospholipids were analyzed using direct infusion (shotgun approach) with the help of a robotic nanoflow ion source, TriVersa NanoMate (Advion BioSciences) as described previously [36–39]. Ceramides were separated using UPLC (Agilent Technologies) prior to mass spectrometric detection according to previous work [40].

2.9. Induction of myocardial infarction and echocardiography in mice

A myocardial infarction was induced by ligating the left anterior descending coronary artery immediately after the bifurcation of the left coronary artery as described [34]. Echocardiography was performed at baseline and 24 h after the myocardial infarction using the VisualSonics VEVO 2100 system (VisualSonics) as described [34].

2.10. Statistical analyses

Statistical analyses were performed in SAS 9.3 (SAS Institute) and the SPSS 19.0 software (SPSS). Allele frequency was tested for Hardy-Weinberg equilibrium. Comparisons between continuous variables were conducted using Student's *t*-test. Associations between single nucleotide polymorphisms and continuous variables were tested under an additive genetic model, using generalized linear models that can fit heterocatanomic multivariate data (*i.e.,* data that come from different distributions) by the method of maximum likelihood. For ordinal values, associations were tested by logistic regression analyses. The models were adjusted for adjusted for age, sex, body mass index (BMI) and triglycerides. Pearson's correlations were used to explore the relationship between the expression levels of different genes. *P* values < .05 were considered significant. For the systems biology analyses, the human heart and liver data were retrieved from GTEx project [41]. Genes that were differentially expressed between sample groups were identified by performing a negative binomial test using DESeq2 R package [42]. To identify differences between sample groups with regard to biological processes, we also performed gene set enrichment analysis using PIANO R package [43]. Global co-expression networks were generated by calculating Pearson's correlation coefficient of expressions between all gene pairs [44].

3. Results and discussion

3.1. Human MTTP polymorphisms associate with cardiac function after an ischemic event and reduced MTTP expression

To test if a genotype-specific *MTTP* expression in the myocardium renders the heart particularly vulnerable to ischemic damage, we investigated if genetic variation in the MTTP-A promotor associates with altered cardiac function during ischemia.

Of the 307 patients with suspected coronary artery disease (from the CEVENT study) genotyped for the polymorphism rs1800804 (−164 T > C) in the *MTTP-A* promotor, 173, 114 and 20 subjects had the genotypes TT, TC and CC, respectively (Table 1).

The minor allele frequency was 25.1% and the genotype distribution adhered to Hardy-Weinberg equilibrium ($\chi 2 = 0.044$, $P = .83$). Carriers of the minor −164C allele in *MTTP* had significantly higher infarct score ($\beta = 0.974 \pm 0.465$, $P = .036$) and wall motion ($\beta = 1.186 \pm 0.388$, $P = .002$) scores and a trend towards a higher degree of stress-induced ischemia (indicated by summed difference score) compared with subjects carrying the major allele (Table 2). These results show that genetic variation of the human *MTTP* promotor associated with cardiac dysfunction during stress-induced ischemia detected by abnormal wall motion score, increased stress-induced ischemia and clinical infarct scores.

MTTP is polymorphic, with several genetic variants in linkage disequilibrium [45]. The *MTTP*-164 T > C (rs1800804) polymorphism is in complete linkage disequilibrium with the *MTTP* Ile128Thr (rs3816873) and *MTTP*-493G > T (rs1800591) polymorphisms [24]. Thus, all three polymorphisms act as markers for each other and all have been shown to associate with increased risk of ischemic heart disease [24,25,46]. An earlier study analyzed the relationship between the *MTTP*-493G > T (rs1800591) promoter polymorphism and *MTTP* expression in 18 heart muscle biopsies and found borderline results for reduced cardiac *MTTP* expression [25]. Here we analyzed *MTTP* expression in heart biopsies from 126 patients (from the ASAP study) genotyped for the polymorphism rs3816873 (Ile128Thr) and showed that the minor allele of rs3816873 was associated with significantly lower expression of *MTTP* in cardiomyocytes (Fig. 1).

3.2. Humans primarily express the MTTP-A isoform in the heart and liver

To identify whether different MTTP isoforms are expressed in the heart, RNA sequencing of myocardial biopsies from the left ventricle was performed in eight patients undergoing valve surgery. Liver biopsies from the same patients were also RNA sequenced and used as a reference. As expected the RNA expression of MTTP in the heart was much lower than the MTTP expression levels in the liver (Fig. 2). All myocardial and liver biopsies that were RNA sequenced showed a stronger signal for exon 1A compared with exon 1B. In fact, the expression signal from exon 1B in all samples did not differ from the background signal (Fig. 2). This result shows that it is primarily the MTTP-A isoform that is expressed in human heart and liver.

Table 1
Characteristics of the study population in relation to anthropometric and cardiac parameters by rs1800804 (−164 T > C) genotype. The numbers of individuals carrying each genotype are given in brackets at the top of the Table. TT indicates homozygosity for the major T allele, TC indicates heterozygosity and CC indicates homozygosity for the minor C allele of the -164 T > C polymorphism. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TnT, troponin; EF, ejection fraction; LVDD, left ventricle diastolic diameter; LVSD, left ventricle systolic diameter; PWT, posterior wall thickness; IVS, interventricular septum; LVDV, left ventricle diastolic volume; LVSV, left ventricle systolic volume.

Variable	TT (n = 173)		TC (n = 114)		CC (n = 20)	
	n	Mean ± sd	n	Mean ± sd	n	Mean ± sd
Age, years	173	60.8 ± 9.4	114	61.5 ± 8.4	20	60.1 ± 10.7
Men/women	69/104		41/73		7/13	
BMI, kg/m^2	173	26.4 ± 4.2	114	26.0 ± 4.0	20	27.0 ± 3.6
DBP, mmHg	170	84.6 ± 11.2	111	84.9 ± 11.5	20	87.1 ± 8.8
SBP, mmHg	173	143.6 ± 23.8	114	143.6 ± 21.5	20	144.0 ± 20.5
TnT, ng/ml	170	0.16 ± 0.07	114	0.17 ± 0.12	18	0.20 ± 0.11
Echocardiographic variables						
EF, %	147	56.4 ± 6.4	97	54.4 ± 8.3	17	56.8 ± 6.1
LVDD, cm	164	4.5 ± 0.5	107	4.5 ± 0.5	18	4.7 ± 0.6
LVSD, cm	164	3.6 ± 0.5	107	3.6 ± 0.6	18	3.7 ± 0.6
PWT, cm	162	0.9 ± 0.1	104	0.8 ± 0.1	17	0.9 ± 0.1
IVS, cm	162	0.9 ± 0.1	102	0.9 ± 0.1	17	1.0 ± 0.1
LVDV, ml	147	83.1 ± 21.8	97	82.8 ± 25.3	17	86.4 ± 27.2
LVSV, ml	147	36.8 ± 13.8	97	38.7 ± 17.9	17	38.2 ± 18.0

3.3. Co-expression of genes with MTTP differs between human heart and liver

Genes with coordinated expression across a variety of experimental conditions may indicate the presence of functional linkages between genes [47,48]. Therefore, to gain more understanding of the functional role of MTTP in the human heart, we retrieved data from the Genotype-Tissue Expression (GTEx) project [41]. First, we compared mRNA expression of *MTTP* in human heart (left ventricle, n = 272) and liver samples (n = 156) and observed that *MTTP* expression was 60.6 times lower in the heart than the liver (Supplementary Fig. 1). Genes that collaborate in a shared function often require simultaneous expression. We therefore identified genes whose expression was tightly associated with MTTP in the heart and liver biopsies using the global co-expression network (Supplementary Data 1), and showed that *MTTP* was associated with different genes in the two tissues (Supplementary Tables 1 and 2), indicating that the functional roles of MTTP in the heart and liver are different. In the heart, *MTTP* was highly co-expressed with genes linked to cardiac arrhythmias (*e.g.* ryanodine receptor 2), mitochondrial function and lipid metabolism. In the liver, the degree of co-expression was weaker, and *MTTP* mainly co-expressed with genes linked to different forms of metabolism. To analyze if MTTP is expressed in other cell types in the human heart, we collected cardiac needle biopsies from the left ventricle as well as from epicardial fat tissue from ischemic and non-ischemic patients and analyzed the expression of MTTP. The results confirmed that MTTP is expressed in cardiac left ventricle biopsies, but virtually not detected in epicardial fat biopsies (Supplementary Fig. 2). We also analyzed the expression of MTTP in cultured fibroblasts, but were not able to detect any expression

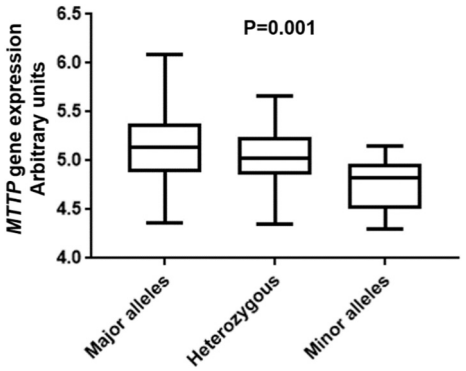

Fig. 1. Cardiac human MTTP expression according to MTTP genotype. Cardiac mRNA expression of *MTTP* as measured by gene expression array, according to *MTTP* genotypes for rs3816873 (Ile128Thr), which is in complete linkage disequilibrium with rs1800804 (−164 T > C). The bottom and the top of the box indicates the interquartile range and the line represents the median. The whiskers under and over the box correspond to the minimum and maximum values. Major alleles denote homozygous for the major alleles (n = 73); heterozygous (n = 42); minor alleles denotes homozygous for the minor alleles (n = 11). The p-value denotes linear regression analysis of variance.

Table 2
Association of *MTTP* rs1800804 (−164 T > C) polymorphism with MPS scores. Generalized linear models were used to explore the relationship between rs1800804 alleles and clinical MPS measurements expressed as ordinal scores. The absence and presence of left ventricle perfusion defects were scored as 0 and > 0, respectively. The β-values refer to the effect of each additional copy of the minor allele with corresponding standard errors (se) and P values adjusted [1]for age and sex, and [2]for age, sex, BMI and plasma triglyceride concentration.

MPS variable	Score = 0	Score > 0	Per C allele effect					
	n (%)	n (%)	β	se	P value[1]	β	se	P value[2]
Infarct score	296 (95.8)	13 (4.2)	0.75	0.43	*0.080*	0.97	0.46	**0.036**
Ischemia score	215 (69.6)	94 (30.4)	0.21	0.20	0.196	0.22	0.21	0.281
Wall motion score	283 (93.09)	21 (6.9)	0.94	0.35	**0.007**	1.19	0.39	**0.002**
Summed rest score	107 (43.3)	140 (56.7)	0.26	0.24	0.286	0.21	0.25	0.392
Summed stress score	79 (31.5)	172 (68.5)	0.11	0.25	0.661	0.11	0.26	0.677
Summed difference score	129 (52.2)	118 (47.8)	0.45	0.22	**0.040**	0.43	0.23	**0.058**

Significant P-values are marked in bold.

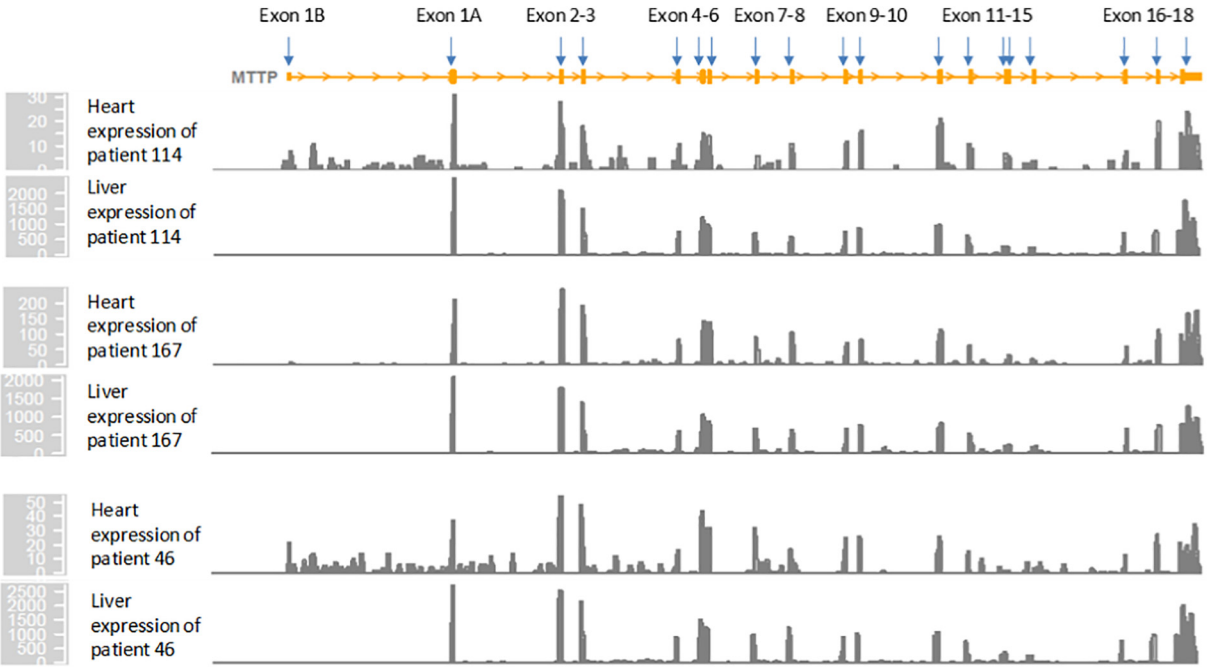

Fig. 2. RNA sequencing of the MTTP gene in human myocardial and liver biopsies. Representative expression signals from RNA sequencing analyses of non-ischemic human biopsies from the left ventricle of the heart and from the liver obtained from three patients undergoing heart valve surgery. At the top the MTTP gene is depicted with the putative exons indicated. The intensities of the signals are given to the left. Subject 114 is homozygous for the minor allele, subject 167 is heterozygous and subject 46 is homozygous for the major allele.

(data not shown).

We next analyzed and compared the global gene expression in heart biopsies from the quartiles with the highest (1.5 ± 0.4 transcripts per million) and lowest (0.28 ± 0.12 transcripts per million) expression of *MTTP* ($n = 68$ for both). In total, 4562 genes showed significant differences in expression (q value < 0.01) between these two groups (Supplementary Data 2). By performing gene set analysis for gene ontology (GO) biological process terms using the PIANO R package [43], we found that heart biopsies with high *versus* low *MTTP* expression showed: (1) significant upregulation of many heart muscle-related gene sets (including regulation of cardiac muscle conduction, regulation of heart rate, and regulation of cardiac muscle contraction); (2) significant association with fatty acid metabolism-related GO biological process terms (*i.e.,* fatty acid, and monocarboxylic acid catabolic processes); and (3) significant downregulation of immune and inflammatory responses (q value < 1E-5 for all, Supplementary Data 3, Supplementary Fig. 3). We also compared global gene expression in liver biopsies from the quartiles with the highest (72.9 ± 17.0 transcripts per million) and lowest (27.8 ± 8.1 transcripts per million) expression of *MTTP* ($n = 39$ for both). In total, 984 genes showed significant differences in expression (q value < 0.01) between these two groups (Supplementary Data 4). Gene set analysis showed, as expected, that lipid metabolism and other central metabolism-related GO biological process terms were significantly (q value < 1E-5) associated with high MTTP expression in the liver biopsies (Supplementary Data 5, Supplementary Fig. 4). Importantly, none of the gene sets associated with cardiac *MTTP* expression were significantly (q value < 1E-5) enriched in the liver biopsies with high *versus* low *MTTP* expression (Supplementary Data 5). Thus, expression of *MTTP* in the heart seems to be linked to biological processes that differ from those in the liver.

3.4. Knockout of Mttp-A in mice hearts does not affect lipid accumulation but reduces heart function after a myocardial infarction

To test if MTTP-A influences cardiac lipid metabolism, gene expression of heart muscle-related genes, and stress responses after a myocardial infarction, we generated heart-specific *Mttp-A* knockout (*hMttp-A*$^{-/-}$) mice using LoxP-Cre technology. We first showed that *hMttp-A*$^{-/-}$ mice had normal hepatic *Mttp-A* mRNA expression (131.4 ± 37.5% of nontransgenic littermates at baseline), but lacked cardiac expression of *Mttp-A* mRNA both at baseline and after a myocardial infarction (Fig. 3A). However, cardiac MTTP protein levels did not differ between the *hMttp-A*$^{-/-}$ mice and their littermate controls either at baseline or after a myocardial infarction (Fig. 3B). Furthermore, *hMttp-A*$^{-/-}$ mice had normal MTTP activity (94.4 ± 10.4% of nontransgenic littermates at baseline). These results indicated

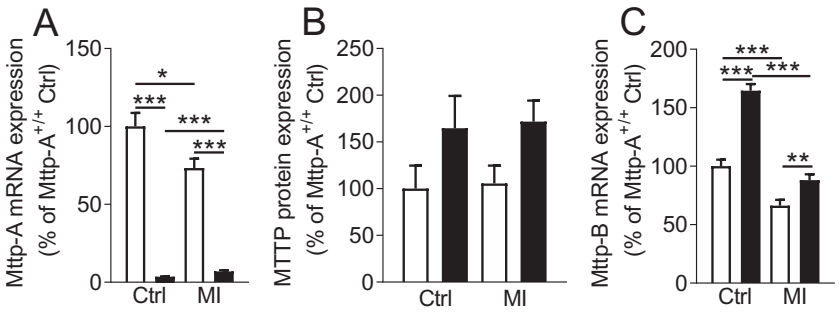

Fig. 3. Mttp heart expression at baseline (Ctrl) and 24 h after MI in heart-specific Mttp-A knockout mice and their littermate controls. mRNA (normalized to Hprt) and MTTP protein (normalized to Na+/K+ ATPase) levels in the heart from Mttp-A heart-specific knockout mice before and after a myocardial infarction. (A) Values are mean ± SEM $n = 8–10$. (B) Values are mean ± SEM $n = 4–5$. Unfilled bars denote nontransgenic littermates and filled bars heart-specific *Mttp-A* knockout mice. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

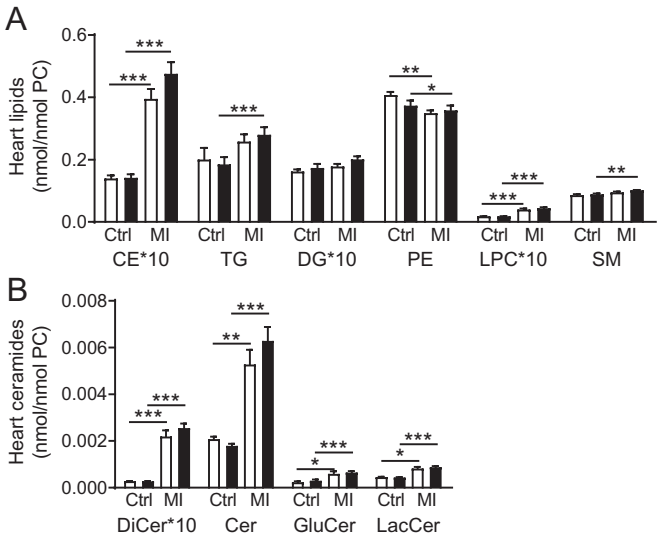

Fig. 4. Lipid content (normalized to phosphatidylcholine (PC)) in the hearts of hMTTP$^{-/-}$ mice and their littermate controls at baseline and 24 h after myocardial infarction (MI). A, Neutral lipids and phospholipids levels in the heart at baseline (Ctrl) and 24 h after MI. B, Ceramide species levels in the heart at baseline (Ctrl) and 24 h after MI. Unfilled bars denote nontransgenic littermates and filled bars heart-specific *Mttp-A* knockout mice. Data are mean ± SEM, n = 7–9, $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

compensatory upregulation of other MTTP isoforms in *hMttp-A*$^{-/-}$ mice. Indeed, expression analysis showed that the expression of *Mttp-B* was upregulated in the heart of *hMttp-A*$^{-/-}$ mice both at baseline and after a myocardial infarction (Fig. 3C). Thus, heart-specific *Mttp-A* deficiency did not result in reduced cardiac total MTTP activity, due to compensatory upregulation of the *Mttp-B*. These results are in agreement with an earlier study showing increased expression of *Mttp-B* in a heart-specific *Mttp-A* knockout mouse model [8].

We next tested if the compensatory upregulation of *Mttp-B* would be sufficient for heart function to be maintained in *hMttp-A*$^{-/-}$ mice after an experimental myocardial infarction. As expected, cardiac accumulation of neutral lipids and ceramide species was increased in mice 24 h after a myocardial infarction (Fig. 4A and B). However, no differences in cardiac lipid content were observed between the heart-specific *Mttp-A*$^{-/-}$ mice and their littermate controls either at baseline or 24 h after the experimental myocardial infarction (Fig. 4A and B). We also performed a more detailed analyses of different ceramide species. Results showed very similar patterns of the heart ceramide species (Supplementary Fig. 5).

Echocardiogram analysis revealed that the *Mttp-A*$^{-/-}$ mice displayed a normal ejection fraction (*i.e.,* a measure of the pumping efficiency of the heart) at baseline but a reduced ejection fraction 24 h after a myocardial infarction (Fig. 5A and B). These results indicate that *Mttp-A*$^{-/-}$ mice display impaired cardiac function after a myocardial infarction despite maintaining normal cardiac lipid equilibrium.

3.5. Reduced cardiac expression of heart muscle-related gene in heart-specific *Mttp-A*$^{-/-}$ mice

In our expression analysis of human heart biopsies, we identified a set of upregulated genes linked to mitochondrial function whose expression was tightly associated with *MTTP* in the human heart (Supplementary Table 1). We therefore investigated expression of three of these mitochondrial genes in hearts from *Mttp-A*$^{-/-}$ mice at baseline and after an experimental myocardial infarction. We showed that these mitochondrial genes were significantly, although modestly, downregulated in *hMttp-A*$^{-/-}$ mice at baseline (Fig. 6). However, only *Tmhle,* that encodes the mitochondrial epsilon-*N*-trimethyllysine

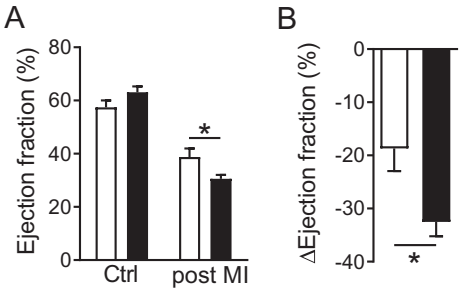

Fig. 5. Heart function as measured by echocardiography at baseline (Ctrl) and 24 h after MI in hMTTP$^{-/-}$ mice and their littermate controls. A, Ejection fraction at baseline (Ctrl) and 24 h after MI. B, Differences in ejection fraction post MI compared with baseline. Unfilled bars denote nontransgenic littermates and filled bars heart-specific *Mttp-A* knockout mice. Data are mean ± SEM, n = 7–9, $^{*}P < .05$

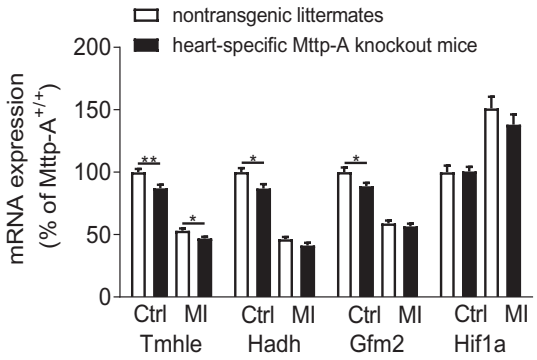

Fig. 6. mRNA expression (normalized to Hprt) in the heart from Mttp-A heart specific knockout mice before and after a myocardial infarction. Tmhle, trimethyllysine hydroxylase, epsilon; Hadh, hydroxyacyl-CoA dehydrogenase; Gfm2, G elongation factor mitochondrial 2; Hif1α, hypoxia-inducible factor 1-alpha. Data are mean ± SEM, n = 7–9, $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

hydroxylase, an important regulator of carnitine biosynthesis and transport of fatty acids across the inner mitochondrial membrane [49], was significantly downregulated after a myocardial infarction (Fig. 6). We also analyzed the expression of additional enzymes and transporters associated with fat uptake into the cardiomyocytes in heart biopsies from nontransgenic littermates and heart-specific Mttp-A knockout mice (Supplementary Fig. 6). No significant differences were detected between MTTP-A deficient mice or littermate control mice, before or after an experimental MI (Supplementary Fig. 6).

4. Conclusion

Epidemiology studies have linked a minor allele of a promoter polymorphisms in MTTP that results in lower gene expression to increased risk of ischemic heart disease independent of plasma lipids. However, the underlying mechanism(s) are still unclear. Here we have tested the hypothesis that MTTP plays an important role for heart function during ischemic events. We show that this functional human MTTP promoter polymorphism is associated with cardiac dysfunction during stress-induced ischemia. Our expression analyses demonstrated that many heart muscle-related gene sets were significantly upregulated in heart biopsies with high *MTTP* expression, and that the expression profile of *MTTP* differs between the heart and liver. Finally, we show that knockout of *Mttp-A* in mice hearts reduced heart function after a myocardial infarction despite compensatory upregulation of *Mttp-B* and no effect on lipid accumulation. Together, these studies indicate that MTTP likely plays an important role for heart function after an ischemic event. However, the underlying mechanism(s) are still unclear. Our

results link MTTP to mitochondrial function, and the physiological/pathophysiological relevance of this needs to be tested in future studies.

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Disclosures

The authors declare no conflicts of interest regarding the present work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yjmcc.2019.09.003.

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