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Genome-Wide Screening of Alpha-Tocopherol Sensitive Genes in Heart Tissue from Alpha-Tocopherol Transfer Protein Null Mice (ATTP$^{-/-}$)

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Abstract

Alpha tocopherol transfer protein (ATTP) null mice (ATTP$^{-/-}$) have a systemic deficiency of alpha-tocopherol (AT). The heart AT levels of ATTP$^{-/-}$ are <10% of those in ATTP$^{+/+}$ mice. The genomic responses of heart to AT deficiency were determined in 3 months old male ATTP$^{-/-}$ mice and compared with their ATTP$^{+/+}$ littermate controls using Affymetrix 430A 2.0 high density oligonucleotide arrays. Differential analysis of ~13,000 genes identified repression of genes related to immune system and activation of genes related to lipid metabolism and inflammation with no significant change in the expression of classical antioxidant genes (catalase, superoxide dismutase, glutathione peroxidase) in ATTP$^{-/-}$ as compared to ATTP$^{+/+}$ mice. The present data identifies novel classes of AT sensitive genes in heart tissue.

Keywords

alpha-tocopherol; alpha-tocopherol transfer protein null mice; heart; gene expression profiling

1. Introduction

Vitamin E (VE) was first discovered as a “reproductive factor” important in the maintenance of pregnancy in rats [1]. α-tocopherol (AT), a lipid-soluble antioxidant, is the most abundant biologically active form of VE [2]. VE is absorbed from the intestine, initially transported to systemic tissues in chylomicrons, then following uptake of chylomicrons and their remnants by the liver, with >90% of the absorbed tocopherols still intact, incorporated into the secreted very low density lipoproteins, resulting in recirculation of VE within lipoprotein compartments [3]. AT is preferentially incorporated into the secreted lipoproteins due to its high affinity of AT for transfer protein (ATTP), a protein found predominantly in the liver [4]. The biological activity of AT is thus dependent upon its delivery to tissues, and decreases in the binding capacity or affinity of ATTP for AT will limit the secretion of AT into lipoproteins and the subsequent delivery to peripheral tissues. In the ATTP$^{-/-}$ mice, low AT concentrations are present in plasma (approx. 5% of ATTP$^{+/+}$) and in extra-hepatic tissues, including heart (2–20%), but liver concentrations remain approximately 40% of those of ATTP$^{+/+}$ mice [5].

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Atherosclerotic cardiovascular diseases, a major cause of death in the United States, are believed to be related in part to lipid peroxidative processes occurring in blood vessel walls. As AT represents the major biologic lipophilic antioxidant, many clinical trials have tested the efficacy of AT alone or in combination with other antioxidants to ameliorate cardiovascular diseases, but with results that are generally negative and at best far from clear [6,7]. Little has been reported concerning heart tissue genomic responses to AT. The present study was carried out to identify global gene expression changes in heart tissue as a result of AT deficiency secondary to deletion of the ATTP gene.

2. Materials and Methods

2.1 Animals and Diet

The protocols for the care and use of animals were approved by the Institutional Care and Use Committee at the University of California, Davis. Male C57BL/6 mice with a deletion of ATTP gene (ATTP$^{-/-}$) and littermate wild type mice (ATTP$^{+/+}$) were used from our colonies, which originated from that described by Terasawa et al [8]. The mice were housed in polycarbonate cages in a room maintained at 21–23 °C and 60–70% humidity on a 12 h light/dark schedule and with ad libitum access to water and food. The offsprings were genotyped as previously described [8]. After weaning, the offsprings were fed diets containing 35 IU dl-tocopheryl acetate per kg diet (USB Corporation, Cleveland, OH). At 12–14 weeks the animals were sacrificed by i.p. injection of beuthanasia (120 mg/kg body weight), blood was obtained by cardiac puncture and tissue samples were obtained and stored at −80°C until further processing.

2.2 RNA Extraction, Gene Chip Analysis & Statistics

Total RNA from heart tissue was extracted and processed for GeneChip analysis. RNA from heart tissues were extracted with Trizol reagent and purified and quantified according to the manufacturer's (Invitrogen) protocol. An equal aliquot (5 μg) of total RNA extract in RNAase- and DNAase-free water from each tissue from the group of mice ($n = 4$) was combined. An aliquot (20 μg) of pooled RNA solution was used for preparation of biotinlabeled RNA for hybridization using Affymetrix Mouse 430A 2.0 arrays containing oligonucleotide probes for ~22,690 genes (Santa Clara, CA, USA). The scanned images of hybridization signals were analyzed with the Affymetrix GeneChip Operating Software (GCOS 1.4) and Data Mining Tool software. The absolute mRNA expression (present or absent) and differential (ATTP$^{+/+}$ vs ATTP$^{-/-}$) mRNA expression data were obtained from the pivot data. GeneChips contain 11 pairs of probes to obtain specific and non-specific binding. Net binding intensities for each mRNA were computed by GCOS 1.4, each pair of probes resulting in a total of 11 intensities for each mRNA. Mean, standard deviation and p values are calculated from these data. When the P value for detection signal was <0.049 (range of P value 0.0002–0.049), the expression of the mRNA was classified as present (P). All mRNAs with the p value for detection >0.05 were considered absent (A). Genes whose expression changed by ≥2-fold were considered for further analyses. We also performed Gene Ontology (GO) analysis to assess the content of differentially expressed genes for characterizing the biological properties and generated heat-map by dChip software [9].

3. Results

Though the direct action of AT on heart tissue is not clear, early studies suggested a relationship of nutritional VE deficiency to cardiomyopathy [10,11] and postulated it to be the consequences of oxidative stress [12]. In the present study, we analyzed the global gene expression profile of heart tissue of ATTP$^{-/-}$ mice which have systemic deficiency of AT. The microarray analysis by Affymetrix gene chips detected ~13192 genes out of which 65 genes were affected in heart tissue of ATTP$^{-/-}$ mice as compared to that of ATTP$^{+/+}$ mice. Of these 65 genes, 34 were upregulated (30 with known functions; 4 unknown functions) and 31 were downregulated (28 with known functions; 3 unknown functions). The differentially expressed genes were classified by GO ontology (www.geneontology.org) using dChip software. Genes related to immune responses (19%) were seen to be largely affected by AT deficiency and genes related to the regulation of cellular physiological processes and protein modification (10% and 8% respectively) were also seen to be affected [Fig 1]. The heat-map of the differentially expressed genes with known functions generated by dChip software is reported in Fig 2. Downregulated and Upregulated genes listed according to molecular function are reported in Tables 1 and 2, respectively.

Of note, a cluster of genes related to immune functions (*Igk-V8–16, Igj, Ighg, Igh-6, Ubd, Slpi, Cfd*) were downregulated (Table 1) whereas genes related to lipid metabolism (*Hmgcr, Lip1, Alox12*, *Fads3*, *Ucp1*) and inflammatory response (*Gp9, Tnfrsf11b, Gp1bb, Hamp1, Clec1b, Ccr5, Cxcl7*, *Irak1*) were upregulated (Table 2).

4. Discussion

The present study was carried out to analyze the global gene expression profiling of heart tissue of ATTP$^{-/-}$ mice deficient in AT. We have previously reported low heart AT levels (1.97 ± 0.52 nmol/g wet weight tissue) in ATTP$^{-/-}$ mice as compared to their respective ATTP$^{+/+}$ controls (16.02 ± 4.46 nmol/g wet weight tissue) fed a basal diet [13], heart AT levels in ATTP$^{-/-}$ mice thus being approximately 10% of levels of their ATTP$^{+/+}$ littermates. AT levels from human myocardial biopsies have been reported to be 61 ± 4 nmol/g wet weight tissue [14], which is approximately 4 times higher than the myocardial levels found in the ATTP$^{+/+}$ mice [13]. The plasma AT levels of ATTP$^{+/+}$ mice, ATTP$^{-/-}$ mice and humans are reportedly ~ 4.5 μmol/l, 0.5 μmol/l [13] and 17 μmol/l [15], respectively, and suggest that myocardial AT levels are related to plasma AT levels.

ATTP gene expression was not detected in heart tissues of ATTP$^{+/+}$ mice, confirming our earlier reported data on heart ATTP mRNA by RT-PCR analysis in ATTP$^{+/+}$ and ATTP$^{-/-}$ mice [15] and in human heart tissue by Arita et al [16]. Hence the drop in heart AT levels in ATTP$^{-/-}$ mice is likely attributed to lower plasma AT concentrations [5]. Though AT deficiency is suggested to cause oxidant-antioxidant imbalance leading to oxidative stress [12], in the present study no significant changes were detected in the classical antioxidant genes such as catalase, superoxide dismutase or glutathione peroxidase in heart tissues of ATTP$^{-/-}$ mice as compared to their ATTP$^{+/+}$ littermates. We have recently reported similar observations in lung tissues of ATTP$^{+/+}$ mice fed an AT deficient diet [17] and in lungs of ATTP$^{-/-}$ mice [18].

Several antioxidant clinical trails testing the efficacy of AT on cardiovascular and related diseases were inconclusive [6,7]. This and other considerations have prompted researchers to explore the modulation of biological systems by AT beyond its “non-antioxidant” properties. In 1988, Mahoney and Azzi, for the first time, reported the inhibitory effect of AT on brain protein kinase C (PKC) activity in vitro [19]. AT-induced PKC inhibition was also reported to inhibit smooth muscle cell proliferation and platelet adhesion, aggregation and release reactions, both believed to be independent of AT antioxidant properties [20–23]. AT also inhibited native and oxidized low density lipoprotein (LDL)-induced PKC activity and proliferation of vascular smooth muscle cells, thus affecting processes related to atherogenesis [24]. Of note, AT itself has not been noted to be deficient in human atherosclerotic plaques in spite of the presence of co-existing oxidized lipids [25]. One possible explanation is that the oxidation of lipoproteins by two electron reactions, such as by hypochlorite and peroxynitrite, is not known to be quenched by AT [26,27].

Meydani and co-workers have reported that AT is essential for maintaining optimal immune system functions [28] and that AT deficiency dysregulates immune responses and increase susceptibilities to various infections [29,30]. Supplementation of AT to old mice infected by influenza virus was reported to improve immune response by increasing T helper 1 (Th1) cytokines [31] and by decreasing the age-associated decline in CD(+) T cells signalling [32]. In the present study, a cluster of genes related to immune function was seen to be downregulated in heart tissues of ATTP$^{-/-}$ mice (Table 1). The immunoglobulin related genes, such as immunoglobulin kappa chain variable 8 (V8)–16 (*Igk-V8–16*), immunoglobulin joining chain (*IgJ*), immunoglobulin heavy chain (gamma polypeptide) (*Ighg*) and immunoglobulin heavy chain 6 (heavy chain of IgM) (*Igh-6*) were downregulated by −19.7, −14.9, −13 and −7 fold, respectively (Table 1). We had previously reported similar observations in lung genomic profiles of ATTP$^{-/-}$ mice [18]. AT deficiency was also reported to intensify viral infections and myocardial injury in mice fed AT-deficient diets [33].

Complement factor D (*Cfd*; or adipsin), a key component in the activation of the alternate pathway innate immunity [34], was downregulated by −2.6 fold. Cfd, secreted from cells of nervous tissue and adipocytes, has been implicated to regulate fat metabolism [35]. Secretory leukocyte peptidase inhibitor (*Slpi*) which protects against microbial infection and subsequent inflammation [36], was also downregulated by −2.1 fold. Ubiquitin D (*Ubd*; or FAT10) was downregulated by −9.2 fold. Of note, the lymphocytes of Ubd deficient mice were seen to be more prone to spontaneous apoptotic death and demonstrated high sensitivity to endotoxin challenge [37]. Casein kinase 1 (*Csnk1a1*), a serine/threonine protein kinase which phosphorylates a variety of substrates [38], was seen to be downregulated by −26 fold. This is particularly interesting as casein kinase 1 is known as a positive regulator of Wnt pathway [39], and the interruption of this pathway in heart might lead to impaired tissue remodeling [40].

The upregulation of mRNA encoding 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (*Hmgcr*), the key enzyme for cholesterol biosynthesis, lysosomal acid lipase 1 (*Lip1*), fatty acid desaturase 3 (*Fads3*) [41,42] by 8.0, 2.6 and 2.5 fold, respectively, suggests dysregulated lipid metabolism in hearts of ATTP$^{-/-}$ mice. The upregulation of lipid homeostasis related genes were also observed in lungs of ATTP$^{-/-}$ mice (**18**). Uncoupling protein 1 (*Ucp-1*), a mediator of proton leakage in mitochondria and believed to be involved in thermogenesis and energy expenditure [43], and suggested to play a role in the transport of fatty acids across mitochondrial membrane [44], was upregulated by 3.2 fold (Table 2).

Genes encoding glycoprotein 9 (*Gp9*) and glycoprotein Ib, beta polypeptide (*Gp1bb*), factors relating to platelet activation and aggregation [45], were upregulated by 8.6 and 2.8 fold, respectively (AT supplementation was reported to inhibit platelet aggregation [21]). Tumor necrosis factor receptor superfamily, member 11b (*Tnfrsf11b*; osteoprotegerin), which was upregulated by 4.6 fold, was recently reported to be elevated in patients with atherosclerotic cardiovascular disease [46]. The above mentioned genes, as well as the upregulation of other genes related to inflammatory processes such as those encoding arachidonate 12-lipoxygenase (*Alox12*) [47], chemokine (C-C motif) receptor 5 (*Ccr5*) [48], chemokine (C-X-C motif) ligand 7 (*Ccl7*) [49] and interleukin-1 receptor-associated kinase 1 (*Irak-1*) [50], suggest increased inflammatory responses in ATTP$^{-/-}$ cardiac tissues as a result of AT deficiency, as observed in our earlier reports in ATTP$^{-/-}$ lung and liver tissues [51]. Expression of alpha 2-HS-glycoprotein (*Ahsg*), which shows negative acute-phase reactant properties, was upregulated by 2.1 fold and has been identified in the mineralized matrix of calcified plaques of atherosclerotic human aortas [52]. It is interesting to note that cytochrome P450 -2E1 (*Cyp2e1*), which has a capability of initiating lipid peroxidation by generation of reactiveoxygen species [53], was downregulated by −2.1 fold.

Patients with 744 del A mutation on ATTP gene were reported to show cardiomyopathy as evidenced by echocardiography, but this was not observed in all patients with this disorder [54]. In the present studies we have not investigated for evidences of cardiomyopathy in ATTP$^{-/-}$ mice. Further studies are warranted in these regard.

In summary, we report a preliminary characterization of the genomic profile in heart tissues of AT deficient mice occurring as a result of ATTP gene deletion. The data suggest dysregulation in lipid metabolism and immune-related functions in ATTP$^{-/-}$ mice. The implications of the current observations to human cardiomyopathies associated with activation of inflammatory processes and oxidative stress remain to be characterized. The present microarray data failed to detect modifications of classical antioxidant genes (catalase, superoxide dismutases or glutathione peroxidases), further suggesting that non-antioxidant properties of AT are likely responsible for the presently archived modulations in cardiac gene expression (**55,56**).

Acknowledgments

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Abbreviations

Ahsg	alpha 2-HS-glycoprotein
Alox12	arachidonate 12-lipoxygenase
AT	alpha-tocopherol
ATTP	alpha-tocopherol transfer protein
Ccr5	chemokine (C-C motif) receptor 5
Cfd	complement factor D
Clec1b	C-type lectin domain family 1, member b
Csnk1a1	casein kinase 1
Cxcl7	chemokine (C-X-C motif) ligand 7
Cyp2e1	cytochrome P450, family 2, subfamily e, polypeptide 1
Fads3	fatty acid desaturase 3
GCOS	gene chip operating software

Gp1bb	glycoprotein 1b, beta polypeptide
Gp9	glycoprotein 9
Hamp1	hepcidin antimicrobial peptide 1
Hmgcr	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
Igh-6	immunoglobulin heavy chain 6 (heavy chain of IgM)
Ighg	immunoglobulin heavy chain (gamma polypeptide)
Igk-V8–16	immunoglobulin kappa chain variable 8 (V8)–16
Irak1	interleukin-1 receptor-associated kinase 1
Lip1	lysosomal acid lipase 1
PKC	protein kinase C
Slpi	secretory leukocyte peptidase inhibitor
Tnfrsf11b	tumor necrosis factor receptor superfamily, member 11b
Ubd	ubiquitin D
Ucp1	uncoupling protein 1
VE	vitamin E

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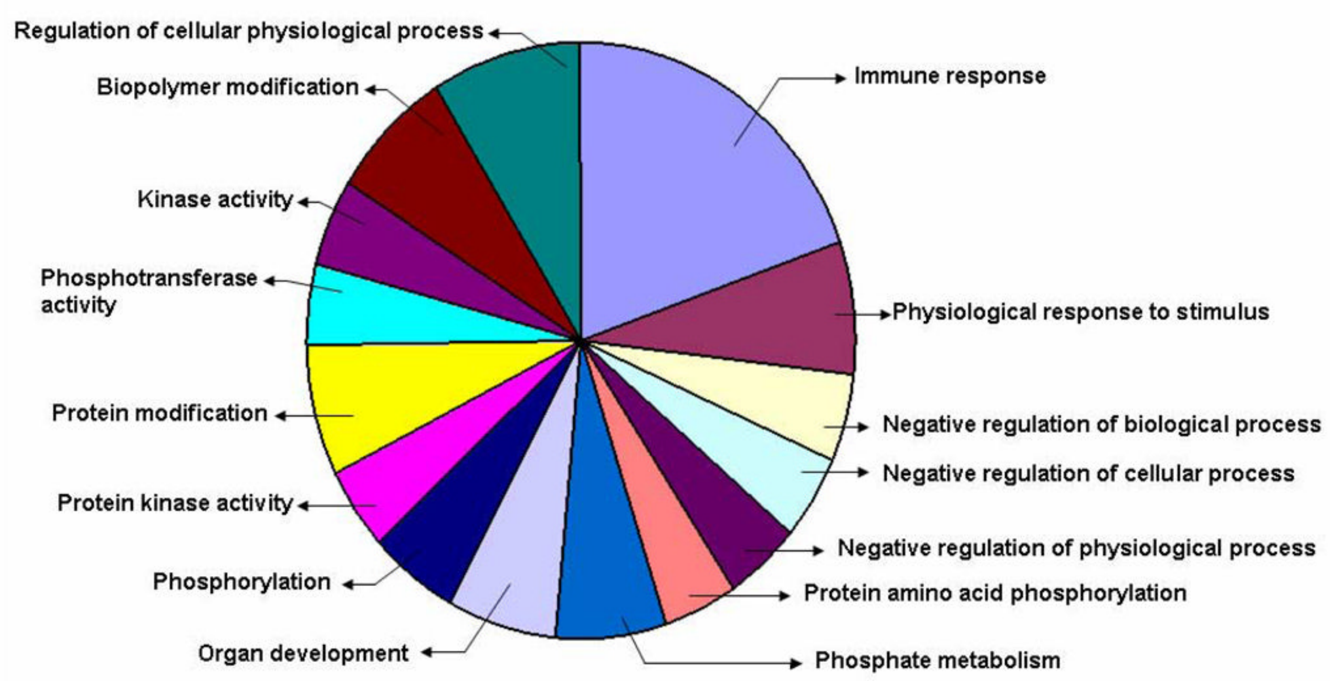

Fig 1.

Functional classification of differentially expressed heart genes in ATTP$^{-/-}$ mice as compared to ATTP$^{+/+}$ mice.

The list of differentially expressed genes was obtained with GCOS 1.4 software. The list was then edited to select genes of known function that change by ≥ 2-fold. The edited list of genes was subjected to GO ontology software and classified according to biological functions. Note that same genes might be included in several functional groups as per its biological function.

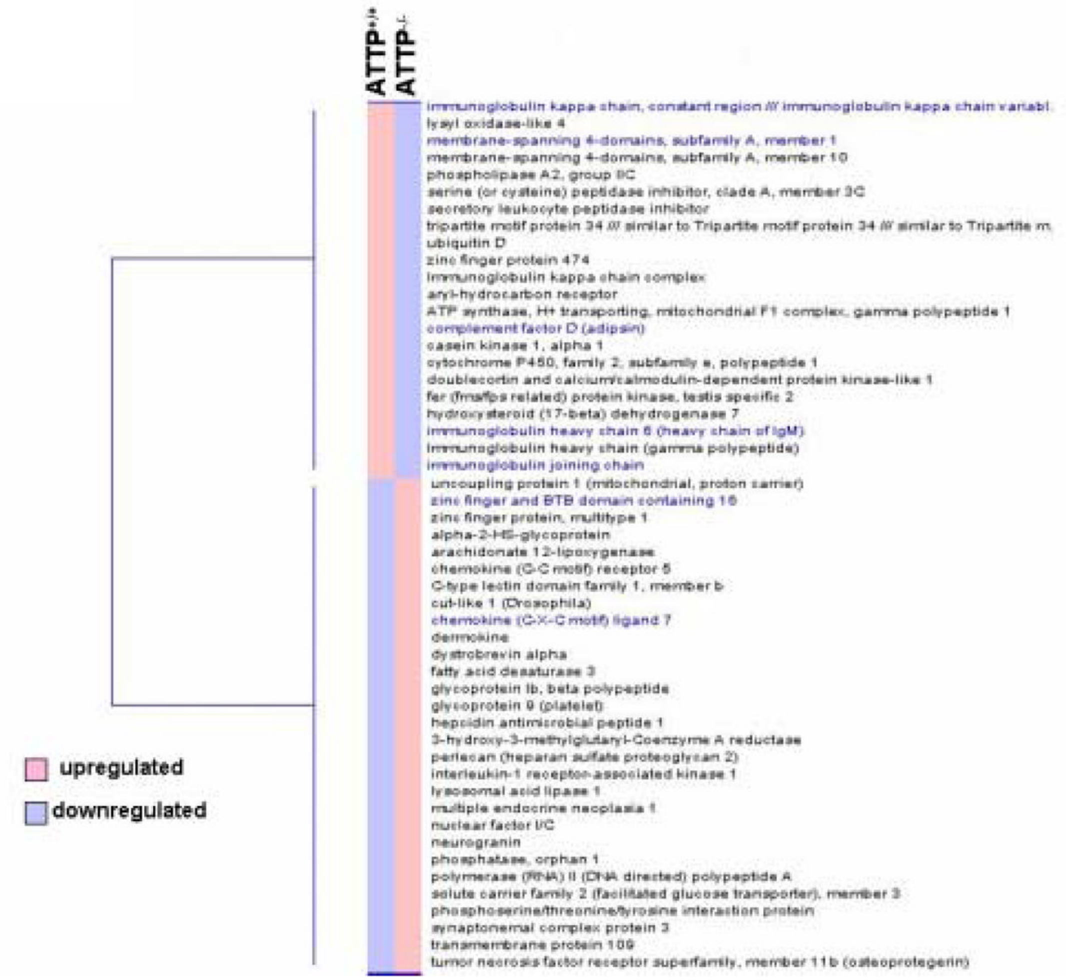

Fig 2.

Heat-map of identified differentially expressed heart genes in ATTP$^{-/-}$ mice as compared to ATTP$^{+/+}$ mice generated by d-Chip software.

The list of differentially expressed, AT-sensitive genes was obtained with GCOS software. The list was further edited to delete genes of unknown function and to focus on genes that change by ≥ 2-fold. The edited list of AT-sensitive genes was subjected to "hierarchial cluster analysis" with d-Chip software. The analysis identified two major clusters. The "two-colour" heat map shows downregulated (blue) and upregulated (pink) genes.

Table 1

Downregulated genes in heart tissue of ATTP$^{-/-}$ mice as compared to ATTP$^{+/+}$ mice

Probe Set ID	Gene Title	Gene Symbol	fold change
Immune Response			
1452417_x_at	immunoglobulin kappa chain variable 8 (V8)–16	Igk-V8–16	−19.7
1424305_at	immunoglobulin joining chain	Igj	−14.9
1424631_a_at	Immunoglobulin heavy chain (gamma polypeptide)	Ighg	−13.0
1452463_x_at	Immunoglobulin kappa chain complex	--	−12.1
1427329_a_at	immunoglobulin heavy chain 6 (heavy chain of IgM)	Igh-6	−7.0
1419762_at	ubiquitin D	Ubd	−9.2
1448377_at	secretory leukocyte peptidase inhibitor	Slpi	−2.1
1417867_at	complement factor D (adipsin)	Cfd	−2.6
1421564_at	serine (or cysteine) peptidase inhibitor, clade A, member 3C	Serpina3c	−2.3
Signal Transduction			
1451497_at	casein kinase 1, alpha 1	Csnk1a1	−26.0
1432453_a_at	membrane-spanning 4-domains, subfamily A, member 10	Ms4a10	−10.6
1450863_a_at	doublecortin and calcium/calmodulin-dependent protein kinase-like 1	Dcamkl1	−4.0
1426090_a_at	fer (fms/fps related) protein kinase, testis specific 2	Fert2	−3.7
1423226_at	membrane-spanning 4-domains, subfamily A, member 1	Ms4a1	−2.6
Extracellular Matrix Protein			
1450134_at	lysyl oxidase-like 4	Loxl4	−5.3
Lipid Metabolism			
1417871_at	hydroxysteroid (17-beta) dehydrogenase 7	Hsd17b7	−4.6
1420584_at	phospholipase A2, group IIC	Pla2g2c	−3.5
Transcription factors			
1450695_at	aryl-hydrocarbon receptor	Ahr	−4.0
1451059_at	zinc finger protein 474	Zfp474	−2.8
1421550_a_at	tripartite motif protein 34	Trim34	−2.1
Xenobiotic Metabolism			
1415994_at	cytochrome P450, family 2, subfamily e, polypeptide 1	Cyp2e1	−2.1
Ion Transport			
1438809_at	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1	Atp5c1	−2.0

Table 2

Upregulated genes in heart tissue of ATTP$^{-/-}$ mice as compared to ATTP$^{+/+}$ mice

Probe Set ID	Gene Name	Gene Symbol	Fold change
Lipid Metabolism			
1427229_at	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	Hmgcr	8.0
1423140_at	Lysosomal acid lipase 1	Lip1	2.6
1422699_at	Arachidonate 12-lipoxygenase	Alox12	2.5
1449219_at	Fatty acid desaturase 3	Fads3	2.5
1418197_at	Uncoupling protein 1 (mitochondrial, proton carrier)	Ucp1	3.2
Inflammatory Response			
1919720_at	Glycoprotein 9	Gp9	8.6
1449033_at	Tumor necrosis factor receptor superfamily, member 11b	Tnfrsf11b	4.6
1422977_at	Glycoprotein 1b, beta polypeptide	Gp1bb	2.8
1419196_at	Hepcidin antimicrobial peptide 1	Hamp1	2.5
1421182_at	C-type lectin domain family 1, member b	Clec1b	2.3
1424727_at	Chemikine (C-C motif) receptor 5	Ccr5	2.1
1418480_at	Chemokine (C-X-C motif) ligand 7	Cxcl7	2.1
1455093_a_at	Alpha-2-HS-glycoprotein	Ahsg	2.1
1460649_at	Interleukin-1 receptor-associated kinase 1	Irak1	2.0
1418669_at	Perlecan (heparin sulfate proteoglycan 2)	Hspg2	2.0
Cytoskeletal function			
1456069_at	Dystrobrevin alpha	Dtna	4.6
1428781_at	Dermokine	Dmkn	2.6
Binding Protein			
1423231_at	neurogranin	Nrgn	3.5
Transporter			
1455898_x_at	Solute carrier family 2 (facilitated glucose transporter), member 3	Slc2a3	3.2
Transcription factors			
1422565_s_at	Nuclear factor I/C	Nfic	2.3
1419874_x_at	Zinc finger and BTB domain containing 16	Zbtb16	2.3
1416348_at	Multiple endocrine neoplasia 1	Men1	2.1
1449534_at	Synaptonemal complex protein 3	Sycp3	2.1
1423603_at	Zinc finger protein, multitype 1	Zfpm1	2.1
1424667_a_at	Cut-like 1 (Drosophila)	Cutl1	2.0
1426242_at	Polymerase (RNA) II (DNA directed) polypeptide A	Polr2a	2.0
Other functions			

Probe Set ID	Gene Name	Gene Symbol	Fold change
1416032_at	Transmembrane protein 109	Tmem109	2.6
1421664_a_at	Phosphoserine/threonine/tyrosine interaction protein	Styx	2.3
1452485_at	Phosphatase, orphan 1	Phospho1	2.0