



Article scientifique

Article

2017

Published version

Open Access

This is the published version of the publication, made available in accordance with the publisher's policy.

Molecular pathways associated with blood pressure and hexadecanedioate levels

Menni, Cristina; Metrustry, Sarah J; Ehret, Georg Benedikt; Dominiczak, Anna F; Chowienczyk, Phil;
Spector, Tim D; Padmanabhan, Sandosh; Valdes, Ana M

How to cite

MENNI, Cristina et al. Molecular pathways associated with blood pressure and hexadecanedioate levels.
In: PLOS ONE, 2017, vol. 12, n° 4, p. e0175479. doi: 10.1371/journal.pone.0175479

This publication URL: <https://archive-ouverte.unige.ch/unige:127789>

Publication DOI: [10.1371/journal.pone.0175479](https://doi.org/10.1371/journal.pone.0175479)

© The author(s). This work is licensed under a Creative Commons Attribution (CC BY)

<https://creativecommons.org/licenses/by/4.0>

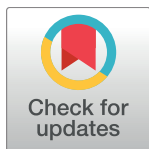
RESEARCH ARTICLE

Molecular pathways associated with blood pressure and hexadecanedioate levels

Cristina Menni^{1*}, Sarah J. Metrustry¹, Georg Ehret², Anna F. Dominiczak³, Phil Chowienczyk⁴, Tim D. Spector¹, Sandosh Padmanabhan³, Ana M. Valdes^{1,5}

1 Department of Twin Research and Genetic Epidemiology, Kings College London, London, United Kingdom, **2** Cardiology, Geneva University Hospital, Geneva, Switzerland, **3** Institute of Cardiovascular and Medical Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom, **4** Department of Clinical Pharmacology, King's College London, London, United Kingdom, **5** Academic Rheumatology Clinical Sciences Building, Nottingham City Hospital, Nottingham, United Kingdom

* cristina.menni@kcl.ac.uk



OPEN ACCESS

Citation: Menni C, Metrustry SJ, Ehret G, Dominiczak AF, Chowienczyk P, Spector TD, et al. (2017) Molecular pathways associated with blood pressure and hexadecanedioate levels. PLoS ONE 12(4): e0175479. <https://doi.org/10.1371/journal.pone.0175479>

Editor: Ines Armando, George Washington University School of Medicine and Health Sciences, UNITED STATES

Received: December 5, 2016

Accepted: March 27, 2017

Published: April 12, 2017

Copyright: © 2017 Menni et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: Data can be shared to third parties through application only via our Data Access Committee (DAC) - the Twins Resource Executive Committee. Applications may be sent to Victoria Vazquez (E-mail: victoria.vazquez@kcl.ac.uk). The committee needs to ensure subject anonymity and that the ethical constraints by which subjects consent to give their data is adhered to. This decision to provide supported access to our data is driven through our

Abstract

The dicarboxylic acid hexadecanedioate is associated with increased blood pressure (BP) and mortality in humans and feeding it to rats raises BP. Here we aim to characterise the molecular pathways that influence levels of hexadecanedioate linked to BP regulation, using genetic and transcriptomic studies. The top associations for hexadecanedioate in a genome-wide association scan (GWAS) conducted on 6447 individuals from the TwinsUK and KORA cohorts were tested for association with BP and hypertension in the International Consortium for BP and in a GWAS of BP extremes. Transcriptomic analyses correlating hexadecanedioate with gene expression levels in adipose tissue in 740 TwinsUK participants were further performed. GWAS showed 242 SNPs mapping to two independent loci achieving genome-wide significance. In rs414056 in the *SCLO1B1* gene (Beta(SE) = -0.088 (0.006) $P = 1.65 \times 10^{-51}$, $P < 1 \times 10^{-51}$), the allele previously associated with increased risk of statin associated myopathy is associated with higher hexadecanedioate levels. However this SNP did not show association with BP or hypertension. The top SNP in the second locus rs6663731 mapped to the intronic region of *CYP4Z2P* on chromosome 1 (0.045 (0.007), $P = 5.49 \times 10^{-11}$). Hexadecanedioate levels also correlate with adipose tissue gene-expression of the 3 out of 4 *CYP4* probes ($P < 0.05$) and of alcohol dehydrogenase probes (Beta(SE) = 0.12(0.02); $P = 6.04 \times 10^{-11}$). High circulating levels of hexadecanedioate determine a significant effect of alcohol intake on BP (SBP: 1.12(0.34), $P = 0.001$; DBP: 0.70 (0.22), $P = 0.002$), while no effect is seen in the lower hexadecanedioate level group. In conclusion, levels in fat of *ADH1A*, *ADH1B* and *CYP4* encoding enzymes in the omega oxidation pathway, are correlated with hexadecanedioate levels. Hexadecanedioate appears to regulate the effect of alcohol on BP.

ethics and Terms of Reference of our governance body.

Funding: This work was supported by: the MRC AimHY (MR/M016560/1) grant; Metabolomic analysis was funded by Pfizer; TwinsUK was funded by the Wellcome Trust; European Community's Seventh Framework Programme (FP7/2007-2013). The study also receives support from the National Institute for Health Research (NIHR) Clinical Research Facility at Guy's & St Thomas' NHS Foundation Trust and NIHR Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. Tim Spector is an NIHR senior Investigator and is holder of an ERC Advanced Principal Investigator award. The GWAS study of the BP extremes and the NORDIL cohort was supported by the following grants: British Heart Foundation (BHF) Chair CH/98001 BHF Program RG/07/005/23633; BHF Special Project Grant SP/08/005/25115 to S. Padmanabhan; European Union Ingenious HyperCare Consortium (LSHM-C7-2006-037093).

Competing interests: The metabolomic analysis was funded by Pfizer. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

Introduction

Hypertension represents a major global disease burden, but discovering pathways for blood pressure (BP) regulation has been challenging. A number of recent studies by our group and others have found several metabolites to be correlated with BP [1–4]. Circulating levels of the dicarboxylic fatty acid hexadecanedioate are associated with increased BP in three independent cohorts and are linked to increased risk of mortality. Evidence for a causal role was obtained by feeding this compound to rats resulting in significant increases in BP, indicating that it is not a by-product, but a cause of high BP [2]. In addition, a recent study has shown a significant effect of hexadecanedioate on incident heart failure which appeared to be causal [5]. Hexadecanedioate is a by-product of omega oxidation of fatty acids, a minor pathway for fatty acid oxidation used when beta oxidation is deficient. The second step is carried out by the enzyme alcohol dehydrogenase. However, the underlying determinants of its variation are still unknown.

We hypothesized that identifying the genetic contribution to circulating levels of hexadecanedioate and genes whose expression is highly correlated to this compound should reveal some of the pathways defining the regulation and pathology of how hexadecanedioate affects BP.

Methods

The study participants were twins enrolled in the TwinsUK Registry, a national register of adult twins recruited via media campaigns without selecting for any particular disease and phenotype [6].

Genome-wide association

Here we dissected the hexadecanedioate genome-wide association scan (GWAS) data that was previously generated and published as part of our GWAS-metabolomics study [7]. Briefly, non targeted mass spec metabolomic profiling and quantification was conducted on fasting serum and plasma samples by the metabolomics provider Metabolon, Inc. (Durham, NC, USA) [8]. GWAS (in the HapMap 2-based imputed genotype data set) was conducted on 6056 individuals from TwinsUK and 1768 from KORA as previously described [7]. Association results were combined in Metal [9] using inverse variance meta-analysis based on effect size estimates and standard errors, adjusting for genomic control.

We tested the multiple single nucleotide polymorphisms (SNPs) associated with hexadecanedioate for association with BP in the International Consortium for Blood Pressure (ICBP) [10] and with hypertension in the BP-extreme GWAS [11] study. Briefly, the ICBP consortium is an international effort to investigate BP genetics. The consortium was formed by two parent consortia, the CHARGE-BP consortium (Cohorts for Heart and Aging Research in Genomic Epidemiology—blood pressure) and the GBPGEN consortium (Global Blood Pressure Genetics Consortium). The BP-extreme GWAS consists of 1621 hypertensive cases and 1699 controls from respectively the top 2% and the lower 9.2% of the BP distribution of the Swedish population [11].

Gene expression

The association of hexadecanedioate with gene-expression levels in fat and lymphoblastoid cell line (LCL) was tested in 740 females from the TwinsUK cohort using random intercept linear regression after adjusting for age, BMI, metabolite batch, expression batch and family relatedness. Gene expression was analysed with the Illumina Human HT-12 V310 as previously reported [12].

Post-genomic functional analysis

Post-genomic functional analysis was undertaken using the Database for Annotation, Visualization and Integrated Discovery (DAVID) [13]. This is an online tool to which a list of genes can be submitted and subsequently results are generated regarding the genes' involvement in biological processes [13]. The gene list was comprised of genes corresponding to all SNPs with a P value of $p < 0.0001$ in the GWAS analysis. The BioCarta and Kegg pathways maps were used for functional annotation.

Pathway analysis

Pathway analysis was carried out on the microarray results using a list of genes corresponding to probes with p values less than $P < 0.00002$ ($n = 52$).

Association with alcohol intake

We assessed the association between alcohol intake (measured via Food Frequency Questionnaire) and BP stratifying by hexadecanedioate levels by using random linear regression adjusting for age, age², BMI and family relatedness.

The study was approved by St. Thomas' Hospital Research Ethics Committee. All participants provided informed written consent.

TwinsUK metabolomics, and phenotypic data are publicly available upon request on the department website (<http://www.twinsuk.ac.uk/data-access/accessmanagement/>).

Results

The flowchart of the study design is depicted in Fig 1.

Genetics

The results of the GWAS study from our previously published metabolomics GWAS [7] are presented in Fig 2. 242 SNPs are associated with circulating levels of hexadecanedioate achieving genome-wide significance ($P < 5 \times 10^{-8}$) [7]. All these SNPs map to two genes: the Solute Carrier Organic Anion Transporter Family, Member 1B1 (*SLCO1B1*) on chromosome 12 and a cytochrome 4 cluster on chromosome 1 which contains the genes *CYP4A11*, *CYP4B1* and *CYP4Z2P*. The locus plots of *SLCO1B1* and the CYP4 cluster are presented in Fig 3(i) and 3(ii) respectively.

Among the top SNPs, rs4149056 is a non-synonymous polymorphism on the *SLCO1B1* gene on chromosome 12 (Beta(SE) = -0.088(0.006) $P = 1.65 \times 10^{-51}$), encoding a Val174Ala amino acid change. This SNP did not show association with BP [10] or hypertension (HTN) [11]. The hexadecanedioate increasing allele of this SNP has previously been associated with statin related myopathy [14], so we further tested whether concomitant use of statins was associated with different levels of hexadecanedioate. We find that use of statins correlates with significantly lower levels of hexadecanedioate (-0.157(0.07), $P = 0.0347$) which remains essentially unchanged if adjusted for SBP (-0.156(0.07), $P = 0.0319$).

SNP rs11045656 on *SLCO1B1* showed nominal association with HTN (Beta(SE) = 0.06(0.03), $P = 0.02$) in the GWAS of BP extremes (Table 1) [11].

A SNP in *SLCO1B1*, monomorphic in Caucasians, has also been implicated in both levels of hexadecanedioate and risk heart failure in African Americans [5].

The top SNP in the second locus rs6663731 mapped to the intronic region of *CYP4Z2P* on chromosome 1 (0.045(0.007), $P = 5.49 \times 10^{-11}$). No association with HTN was detected for the SNPs mapping to the cytochrome 4 cluster. However, circulating levels of hexadecanedioate

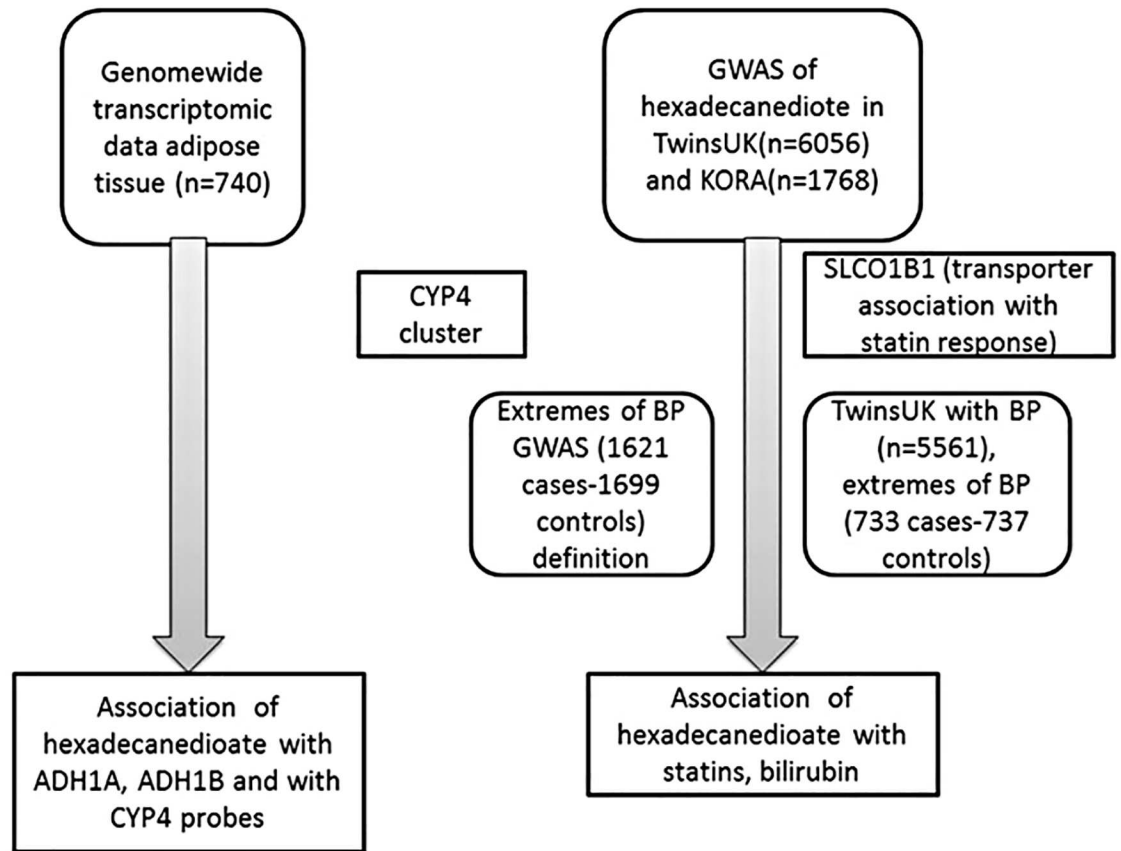


Fig 1. Flowchart of the study design.

<https://doi.org/10.1371/journal.pone.0175479.g001>

correlate with adipose tissue gene-expression levels of probes mapping to *CYP4B1* and *CYP4Z2P* (S1 Table). No probes mapping to *CYP4A11* passed quality control in our gene expression data.

Gene expression

Gene expression in 740 abdominal fat and LCL samples from the TwinsUK cohort were tested for association with hexadecanedioate levels. 23 gene transcripts showed significant association with hexadecanedioate levels in fat after adjusting for multiple testing (Bonferroni $P = 0.05 / [23644 \text{ probes} \times 2 \text{ tissues}] = 1 \times 10^{-6}$). The top association is with the Alcohol Dehydrogenase 1B (Class I), Beta Polypeptide (*ADH1B*) gene on chromosome 4. SNPs on the gene are also associated with circulating hexadecanedioate levels in our metabolomics GWAS [7]. No significant associations were observed in LCL tissue. The significant expression results from the analysis of fat samples are presented in Table 2.

Association with alcohol intake

Because genetic variants at the *ADH1B* gene have been implicated in alcohol induced HTN [15], we explored the association of alcohol and hexadecanedioate on BP in the TwinsUK dataset. We first assessed whether alcohol intake influenced the effect of hexadecanedioate on BP. After adjusting for alcohol consumption (in a log scale) age, age², BMI, family relationship, we

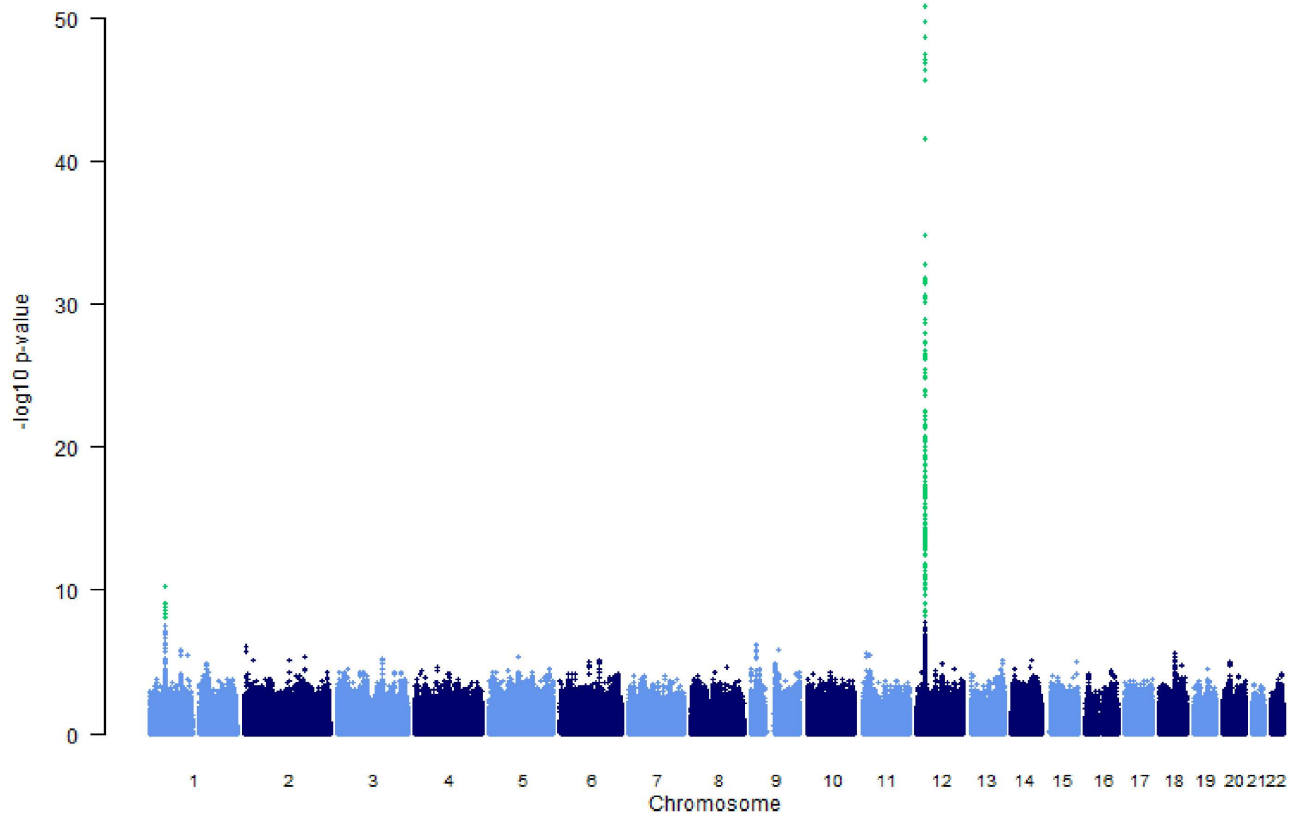


Fig 2. Manhattan plot showing genome-wide P values from association analysis of hexadecanedioate in the TwinsUK-KORA meta-analysis. The y axis shows the $-\log_{10}$ P values of SNPs, and the x axis shows their chromosomal positions.

<https://doi.org/10.1371/journal.pone.0175479.g002>

find that circulating levels of hexadecanedioate are significantly associated with both SBP (Beta (SE) = 1.30(0.29), $P = 1.1 \times 10^{-5}$) and DBP (0.74(0.19), $P = 7.5 \times 10^{-5}$) supporting our previous findings. Overall alcohol consumption is significantly associated with SBP and DBP after adjusting for covariates (SBP: 0.54(0.19), $P = 6.2 \times 10^{-3}$) and DBP (0.49(0.13), $P = 2.6 \times 10^{-4}$). However when stratifying for hexadecanedioate levels, the association between alcohol and BP is present in the individuals in the top tertiles of hexadecanedioate circulating levels (SBP: 1.12(0.34), $P = 0.001$; DBP: 0.70(0.22), $P = 0.002$). On the other hand the level of alcohol intake has no influence on the association between hexadecanedioate levels and BP in those with low hexadecanedioate levels (Table 3). When we stratified the cohort into participants who drink 1 standard alcohol drink per day or less (14 g per day) and those who drink more we find that the effect of hexadecanedioate on blood pressure is the same regardless of alcohol intake (low alcohol: 1.30(0.32), $P = 4.5 \times 10^{-5}$; high alcohol: 1.30(0.36), $P = 3.2 \times 10^{-4}$).

This result suggests that high levels of hexadecanedioate may be indicating a dysfunction or a saturation of enzymatic pathways within the liver related to alcohol metabolism.

Pathway analysis

The results of this analysis in the TwinsUK dataset (see S2 Table) shows some functional clustering of genes, particularly relating to cytoskeleton organisation, cell motility, migration and projection as well as regulation of apoptosis all of which have been involved in endothelial

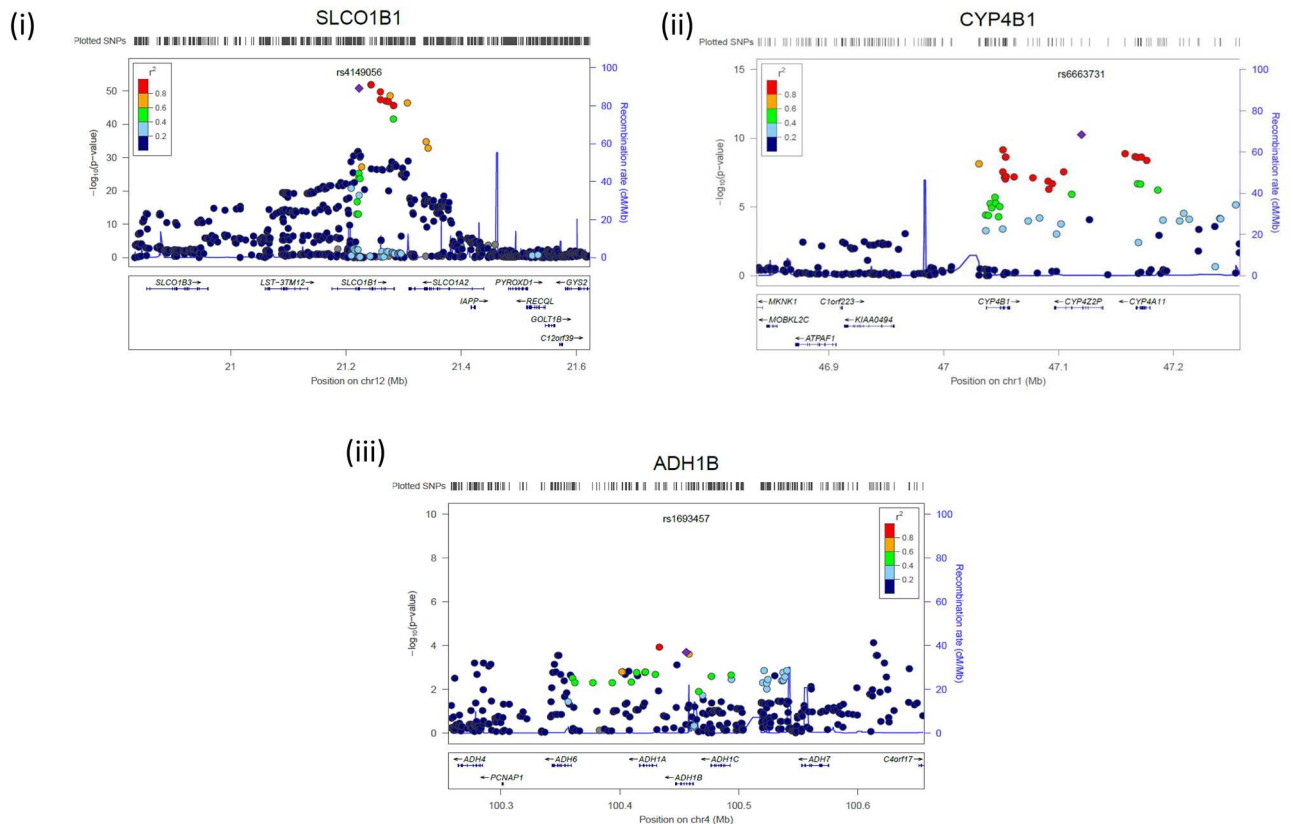


Fig 3. Association plot of the genomic region around (i) *SLCO1B1*, (ii) *CYP4B1* and (iii) *ADH1B* showing both type and imputed SNPs with location of genes and recombination rate. *P* values of SNPs are plotted (as $-\log_{10} P$) against their physical position on the chromosomes (NCBI Build 36). Estimated recombination rates from the HapMap CEU population show the local LD structure. The color of each SNP indicates linkage disequilibrium with the index SNP (rs4149056 or rs6663731 or rs1693457 respectively) based on pairwise r^2 values from HapMap CEU data.

<https://doi.org/10.1371/journal.pone.0175479.g003>

dysfunction. However after applying a Benjamini correction, for multiple tests none of these *P* values remained significant (see [S2 Table](#)).

Discussion

In this study we investigated some of the molecular pathways underlying the fatty acid hexadecanedioate. Exploring our previously published metabolomics GWAS[7], we identified two loci, mapping to *SLCO1B1* and to the cluster encoding *CYP4* genes strongly associated to circulating levels of hexadecanedioate with 242 SNPs passing Bonferroni correction for multiple testing.

Our gene-expression analyses show that circulating levels of hexadecanedioate are nominally correlated with adipose tissue levels of probes mapping to *CYP4B1* and *CYP4Z2P*. The fact that CYP cluster genes correlate with hexadecanedioate but do not reach significance for over-expression may suggest that such over-expression may be occurring in other (not investigated) tissues in which their functions regarding blood pressure are more important (e.g., vessels or kidney). The alcohol dehydrogenase 1B (class I), beta polypeptide (*ADH1B*) is however the strongest gene whose expression is associated with hexadecanedioate after adjusting for multiple testing. Although the effect of hexadecanedioate on BP remains unvaried after

Table 1. Association between SNPs in the *SLCO1B1* gene and *CYP4* cluster and hexadecanedioate levels in the meta analysis carried out by Shin et al (2014)[7], in the extremes of BP carried out by Padmanabhan et al (2010)[11], in the ICBP consortium GWAS(2011)[10] and with risk of statin associated myopathy as reported (SEARCH collaborative group 2008)[14].

Gene	SNP	EA	hexadecanedioate-TUK KORA meta-analysis Nat Gen			HTN extreme GWAS			ICBP			Risk of statin associated myopathy		
			BETA	SE	P	BETA	SE	P	BETA	SE	P	BETA	SE	P
SLCO1B1	rs4149056	T	-0.088	0.006	1.65x10⁻⁵¹	-0.006	0.031	0.86	-0.12	0.13	0.34*	-1.51	0.258	4.0x10⁻⁹
	rs2900478	A	0.087	0.006	2.02x10 ⁻⁵⁰	0.022	0.029	0.50						
	rs7969341	A	-0.086	0.006	2.67x10 ⁻⁴⁹	-0.022	0.029	0.50						
	rs4363657	T	-0.085	0.006	3.92x10⁻⁴⁸	-0.022	0.029	0.49				-1.51	0.258	4.0x10⁻⁹
	rs4149081	A	0.084	0.006	9.67x10 ⁻⁴⁸	0.021	0.029	0.50						
	rs11045879	T	-0.084	0.006	1.56x10 ⁻⁴⁷	-0.022	0.029	0.49						
	rs2199680	A	-0.084	0.006	4.52x10 ⁻⁴⁷	-0.018	0.029	0.57						
	rs12366582	A	-0.083	0.006	2.28x10 ⁻⁴⁶	-0.019	0.029	0.55						
	rs12369881	A	0.083	0.006	2.28x10 ⁻⁴⁶	0.019	0.029	0.54						
	rs12371604	T	-0.069	0.005	2.85x10 ⁻⁴²	-0.002	0.026	0.96						
	rs4149054	A	0.054	0.005	6.26x10 ⁻²⁶	0.027	0.027	0.37						
	rs4149050	T	-0.052	0.005	1.23x10 ⁻²⁴	-0.027	0.027	0.37						
	rs4149058	A	-0.052	0.005	2.06x10 ⁻²⁴	-0.026	0.027	0.37						
	rs4149057	T	0.039	0.004	2.00x10 ⁻¹⁹	0.023	0.023	0.36						
	rs11045656	A	0.026	0.004	5.39x10⁻⁹	0.064	0.025	0.02						
CYP4	rs6663731	A	0.045	0.007	5.49x10 ⁻¹¹	0.067	0.035	0.08	-0.041	0.147	0.92			
	rs9332998	T	0.04	0.007	4.19x10 ⁻⁹	0.068	0.036	0.08	-0.002	0.148	0.99			

* Beta(SE), P Value for SNP rs12317268 that is in complete LD (R2 = 1) with rs4149056

<https://doi.org/10.1371/journal.pone.0175479.t001>

adjustment for alcohol intake [2] these data suggest that there may be an important interaction between alcohol intake and hexadecanedioate levels with regards to their effect on BP.

SLCO1B1

The strongest genetic association seen with hexadecanedioate maps to *SLCO1B1*, an association previously reported [16, 17] in a metabolome-wide genetic study in Caucasians[17] and also in African Americans [5].

The *SLCO1B1* gene encodes OATP1B1, (also named OATP2, OATP-C and LST-1) which is mainly expressed on the sinusoidal membrane of human hepatocytes. Substrates of OATP1B1 include endogenous organic anions such bilirubin, estradiol, prostaglandin 2, leukotrienes C4 and thyroxine, and structurally diverse drugs, such as statins, antibiotics (Rifampicin) antivirals (Saquinavir) and some anti-hypertensive drugs (valsartan)[18].

The functional variants that causes reduced function of OATP1B1 and identified as increasing the risk of statin myopathy (rs414056 and rs4363657 and variants in linkage disequilibrium with them) are the most strongly associated with higher levels of hexadecanedioate.

We find no convincing evidence of an association between variants in *SLCO1B1* with HTN or BP in the two GWAS that we tested [10, 11]. This lack of association is not due to lack of power: the ICBP GWAS is sufficiently powered to detect effects as those expected (0.4 mm Hg per allele) yet we failed to see a significant association with the hexadecanedioate associated SNPs in Caucasians. The effect of the variant allele at rs4149056 is -0.087, the effect of each SD of hexadecanedioate is 1.3 mm Hg per SD of hexadecanedioate. Therefore we expect an effect of 0.44 mmHg per allele difference in rs4149056 if hexadecanedioate is causative of blood

Table 2. Significant expression results for hexadecanedioate in adipocytes.

Gene	Probe	Beta	SE	P
ADH1B	ilmn_1811598	0.12	0.02	6.04x10 ⁻¹¹
GSDMB	ilmn_1666206	0.12	0.02	5.55x10 ⁻¹⁰
CIDEA	ilmn_1788184	0.14	0.02	8.01x10 ⁻¹⁰
CIDEA	ilmn_2390318	0.13	0.02	1.03x10 ⁻⁹
MOCS1	ilmn_1798624	0.06	0.01	4.25x10 ⁻⁹
GSDMB	ilmn_2347193	0.10	0.02	5.02x10 ⁻⁹
CEACAM1	ilmn_1716815	0.05	0.01	8.71x10 ⁻⁹
SFRP2	ilmn_1722898	-0.14	0.02	1.03x10 ⁻⁸
FAM184A	ilmn_1696699	0.05	0.01	1.57x10 ⁻⁸
RGS17	ilmn_1725485	0.07	0.01	1.65x10 ⁻⁸
SLC19A3	ilmn_1716359	0.09	0.02	1.91x10 ⁻⁸
MAGED1	ilmn_1775522	-0.05	0.01	3.12x10 ⁻⁸
HDDC3	ilmn_1781638	0.05	0.01	3.27x10 ⁻⁸
KHDRBS3	ilmn_1691747	0.05	0.01	3.79x10 ⁻⁸
GLYCTK	ilmn_1791222	0.09	0.02	7.24x10 ⁻⁸
PQLC1	ilmn_1798620	0.04	0.01	8.88x10 ⁻⁸
EHBP1	ilmn_1803348	-0.06	0.01	1.07x10 ⁻⁷
ANG	ilmn_1760727	0.06	0.01	2.91x10 ⁻⁷
MT1M	ilmn_1657435	0.06	0.01	5.23x10 ⁻⁷
SPSB1	ilmn_1714170	-0.07	0.01	5.66x10 ⁻⁷
NXNL1	ilmn_1742917	0.06	0.01	5.71x10 ⁻⁷
ECHDC2	ilmn_1671568	0.05	0.01	7.56x10 ⁻⁷
GART	ilmn_1679476	0.03	0.01	8.80x10 ⁻⁷

<https://doi.org/10.1371/journal.pone.0175479.t002>

pressure increase. A sample size of 42,672 individuals is needed to find this as statistically significant with $p < 0.05$ with a MAF of 19% with 80% power, and 67161 for 94% power. The ICBP GWAS used a samples size of 69395 individuals and hence had over 94% power to detect the expected effect under the hypothesis that hexadecanedioate levels are causing an increase in blood pressure. One possible explanation for this finding is that it is intracellular hexadecanedioate levels that influence BP and not necessarily circulating levels. The association with *SLCOB1* reflects circulating levels in large part determined by hepatic uptake of the compound but this association may not be related to intracellular levels of hexadecanedioate.

Hexadecanedioate is a product of omega-oxidation, a secondary fatty acid oxidation pathway. In the first step, an hydroxyl group is introduced onto the omega carbon. This reaction is carried out by certain members of the *CYP4* subfamilies or by two other *CYP450* enzymes, and the electron donor *NADPH*. The next step is the oxidation of the hydroxyl group to an aldehyde by *NAD+* and is catalysed by alcohol dehydrogenase, whose subunits are encoded by

Table 3. Alcohol intake and BP stratified by hexadecanedioate levels adjusting for age, age², BMI and family relatedness.

Hexadecanedioate	SBP		DBP	
	Beta(SE)	P	Beta(SE)	P
Overall	0.54(0.19)	6.2x10 ⁻³	0.49(0.13)	2.6x10 ⁻⁴
Low tertile	0.06(0.35)	0.86	0.21(0.21)	0.84
Middle tertile	0.26(0.33)	0.44	0.45(0.23)	0.05
High tertile	1.12(0.34)	0.001	0.70(0.22)	0.002

<https://doi.org/10.1371/journal.pone.0175479.t003>

genes *ADH1A*, *ADH1B* nad *ADH1C*[19]. The third step is the oxidation of the aldehyde group to a carboxylic acid by NAD⁺. The product of this step is a fatty acid with a carboxyl group at each end, i.e. a dicarboxylic fatty acid, such as hexadecanedioate[20].

CYP4A11 / CYP4B1/ CYP4Z2P

The second strongest genes to be statistically associated with hexadecanedioate are the Cytochromes *CYP4A11*, *CYP4B1* and *CYP4Z2P* on chromosome 1 as shown in Fig 2(ii).

CYP4B1 has been shown to be involved in prostaglandin metabolism[21] through the production of 12-hydroxyeicosatrienoic acid (12-HETE), a potent inflammatory and angiogenic eicosanoid.

In rodent models, decreased expression of *CYP4A* results in increased epithelial sodium channel (ENaC) activity and salt-sensitive hypertension.

Increased *CYP4Z2P*- along with the functional *CYP4Z1*-3'UTR expression has been shown to promote tumor angiogenesis in breast cancer partly via miRNA-dependent activation of PI3K/Akt and ERK1/2[22]. This is relevant to BP regulation as apoptosis of endothelial cells is involved in endothelial dysfunction and the resulting vascular disease. In addition, the mRNA of the *CYP4Z2P* pseudogene has been shown to be expressed in tissues that play a role in cardiovascular regulation such as brain, heart arteries, kidney and adrenals[23]. It is possible that genetic variation at the *CYPZ2P* gene may be influencing apoptosis of endothelial cells or other cells via MAP kinases and PI3K. Hence, there may be a link between endothelial dysfunction and hexadecanedioate levels and the regulation of the two may be linked via the *CYP4* encoded molecules or actions.

ADH1B

The gene whose expression is most strongly associated with regards to hexadecanedioate is *ADH1B*(0.12(0.02), $P = 6.04 \times 10^{-11}$). In addition also gene expression levels of *ADH1A* are associated with hexadecanedioate levels (0.09(0.02), $P = 1.19 \times 10^{-6}$). These genes encode the second enzyme in the omega oxidation making this association logical. It further suggests that study of the relationships between alcohol, hypertension and hexadecanedioate is needed. We also find significant associations between circulating levels of hexadecanedioate and 3 SNPs on the *ADH1B* gene ($P < 0.0005$) as shown in Fig 3(iii). This suggests that *ADH1B* is implicated in hexadecanedioate regulation. Though we find no association between SNPs on *ADH1B* in hypertension and no association has been reported in Caucasians, a role for *ADH1B* in hypertension has been found in Japanese men[15]. Here we report that hexadecanedioate appears to influence the role of alcohol on BP, but the opposite is not true and the association of hexadecanedioate is independent of alcohol intake.

Both cross-sectional and prospective epidemiological studies have established a relationship between hypertension and alcohol consumption [24, 25]. Excessive alcohol use can increase BP and cause antihypertensive drug resistance in a dose-dependent manner [26]. The mechanism(s) by which ethanol consumption leads to elevations in blood pressure is uncertain. However, the available data in humans are not sufficient to allow substantive conclusions [27]. Limitation of daily ethanol intake to no more than 1 ounce (30 mL) of 40% ethanol for most men and 0.5 ounces for women and smaller men results in little blood pressure effect [27]. In some cases, BP control is extremely difficult without total abstinence.

Our finding that alcohol intake has a much stronger effect in individuals with high hexadecanedioate may have therapeutic implications for the treatment of alcohol-induced hypertension suggesting that the strategies for reducing blood pressure may be different depending on the subject's hexadecanedioate levels.

In conclusion, all three genes identified in this study as strongly associated with levels of hexadecanedioate have been previously linked to hypertension, but this association is not strong, or not present in Caucasians. Some of the effects previously reported (in other ethnic groups or in small studies) between *ADH1B*, *SLCOB1* and *CYPB4A11* and BP may be due to their link to hexadecanedioate. *SLCOB1* appears not to be associated with BP in spite of its very strong association with hexadecanedioate levels. Our data lend support to the use of intermediate phenotypes, in this case, a metabolite that contributes to BP regulation, to understand some of the pathways involved in BP regulation and cardiovascular risk.

Supporting information

S1 Table. Association of circulating levels of hexadecanedioate and CYP4 in adipocytes.
(DOCX)

S2 Table. Over-represented pathways for gene expression in adipose tissue correlated with circulating hexadecanedioate levels.
(DOCX)

Acknowledgments

We wish to express our appreciation to all study participants of the TwinsUK, ICBP, MDC and NORDIL studies. We thank the ICBP consortium for allowing us to access their data.

Author Contributions

Conceptualization: CM PC SP AMV.

Formal analysis: CM AMV.

Funding acquisition: AFD PC TDS SP AMV.

Resources: SM.

Validation: GE SP.

Writing – original draft: CM AMV.

Writing – review & editing: CM SM GE AFD PC TDS SP AMV.

References

1. Zheng Y, Yu B, Alexander D, Mosley TH, Heiss G, Nettleton JA, et al. (2013) Metabolomics and incident hypertension among blacks: the atherosclerosis risk in communities study. *Hypertension* 62: 398–403. <https://doi.org/10.1161/HYPERTENSIONAHA.113.01166> PMID: 23774226
2. Menni C, Graham D, Kastenmuller G, Alharbi NH, Alsanosi SM, McBride M, et al. (2015) Metabolomic identification of a novel pathway of blood pressure regulation involving hexadecanedioate. *Hypertension* 66: 422–429. <https://doi.org/10.1161/HYPERTENSIONAHA.115.05544> PMID: 26034203
3. van Deventer CA, Lindeque JZ, van Rensburg PJ, Malan L, van der Westhuizen FH, Louw R (2015) Use of metabolomics to elucidate the metabolic perturbation associated with hypertension in a black South African male cohort: the SABPA study. *J Am Soc Hypertens* 9: 104–114. <https://doi.org/10.1016/j.jash.2014.11.007> PMID: 25577962
4. Holmes E, Loo RL, Stamlor J, Bictash M, Yap IK, Chan Q, et al. (2008) Human metabolic phenotype diversity and its association with diet and blood pressure. *Nature* 453: 396–400. <https://doi.org/10.1038/nature06882> PMID: 18425110
5. Yu B, Li AH, Metcalf GA, Muzny DM, Morrison AC, White S, et al. (2016) Loss-of-function variants influence the human serum metabolome. *Sci Adv* 2: e1600800. <https://doi.org/10.1126/sciadv.1600800> PMID: 27602404

6. Moayyeri A, Hammond CJ, Valdes AM, Spector TD (2013) Cohort Profile: TwinsUK and healthy ageing twin study. *Int J Epidemiol* 42: 76–85. <https://doi.org/10.1093/ije/dyr207> PMID: 22253318
7. Shin SY, Fauman EB, Petersen AK, Krumsiek J, Santos R, Huang J, et al. (2014) An atlas of genetic influences on human blood metabolites. *Nat Genet* 46: 543–550. <https://doi.org/10.1038/ng.2982> PMID: 24816252
8. Menni C, Kastenmuller G, Petersen AK, Bell JT, Psatha M, Tsai PC, et al. (2013) Metabolomic markers reveal novel pathways of ageing and early development in human populations. *Int J Epidemiol* 42: 1111–1119. <https://doi.org/10.1093/ije/dyt094> PMID: 23838602
9. Willer CJ, Li Y, Abecasis GR (2010) METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 26: 2190–2191. <https://doi.org/10.1093/bioinformatics/btq340> PMID: 20616382
10. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, et al. (2011) Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature* 478: 103–109. <https://doi.org/10.1038/nature10405> PMID: 21909115
11. Padmanabhan S, Melander O, Johnson T, Di Blasio AM, Lee WK, Gentilini D, et al. (2010) Genome-wide association study of blood pressure extremes identifies variant near UMOD associated with hypertension. *PLoS Genet* 6: e1001177. <https://doi.org/10.1371/journal.pgen.1001177> PMID: 21082022
12. Grundberg E, Small KS, Hedman AK, Nica AC, Buil A, Keildson S, et al. (2012) Mapping cis- and trans-regulatory effects across multiple tissues in twins. *Nat Genet* 44: 1084–1089. <https://doi.org/10.1038/ng.2394> PMID: 22941192
13. Huang da W, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4: 44–57. <https://doi.org/10.1038/nprot.2008.211> PMID: 19131956
14. Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, et al. (2008) SLC01B1 variants and statin-induced myopathy—a genomewide study. *N Engl J Med* 359: 789–799. <https://doi.org/10.1056/NEJMoa0801936> PMID: 18650507
15. Yokoyama A, Mizukami T, Matsui T, Yokoyama T, Kimura M, Matsushita S, et al. (2013) Genetic polymorphisms of alcohol dehydrogenase-1B and aldehyde dehydrogenase-2 and liver cirrhosis, chronic calcific pancreatitis, diabetes mellitus, and hypertension among Japanese alcoholic men. *Alcohol Clin Exp Res* 37: 1391–1401. <https://doi.org/10.1111/acer.12108> PMID: 23550892
16. Suhre K, Gieger C (2012) Genetic variation in metabolic phenotypes: study designs and applications. *Nat Rev Genet* 13: 759–769. <https://doi.org/10.1038/nrg3314> PMID: 23032255
17. Suhre K, Shin SY, Petersen AK, Mohney RP, Meredith D, Wagele B, et al. (2011) Human metabolic individuality in biomedical and pharmaceutical research. *Nature* 477: 54–60. <https://doi.org/10.1038/nature10354> PMID: 21886157
18. Roth M, Obaidat A, Hagenbuch B (2012) OATPs, OATs and OCTs: the organic anion and cation transporters of the SLC0 and SLC22A gene superfamilies. *Br J Pharmacol* 165: 1260–1287. <https://doi.org/10.1111/j.1476-5381.2011.01724.x> PMID: 22013971
19. Yasunami M, Kikuchi I, Sarapata D, Yoshida A (1990) The human class I alcohol dehydrogenase gene cluster: three genes are tandemly organized in an 80-kb-long segment of the genome. *Genomics* 7: 152–158. PMID: 2347582
20. Sanders RJ, Ofman R, Dacremont G, Wanders RJ, Kemp S (2008) Characterization of the human omega-oxidation pathway for omega-hydroxy-very-long-chain fatty acids. *FASEB J* 22: 2064–2071. <https://doi.org/10.1096/fj.07-099150> PMID: 18182499
21. Seta F, Patil K, Bellner L, Mezentsev A, Kemp R, Dunn MW, et al. (2007) Inhibition of VEGF expression and corneal neovascularization by siRNA targeting cytochrome P450 4B1. *Prostaglandins Other Lipid Mediat* 84: 116–127. <https://doi.org/10.1016/j.prostaglandins.2007.05.001> PMID: 17991614
22. Zheng L, Li X, Gu Y, Lv X, Xi T (2015) The 3'UTR of the pseudogene CYP4Z2P promotes tumor angiogenesis in breast cancer by acting as a ceRNA for CYP4Z1. *Breast Cancer Res Treat* 150: 105–118. <https://doi.org/10.1007/s10549-015-3298-2> PMID: 25701119
23. Fagerberg L, Hallstrom BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, et al. (2014) Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Mol Cell Proteomics* 13: 397–406. <https://doi.org/10.1074/mcp.M113.035600> PMID: 24309898
24. Klatsky AL (1995) Blood pressure and alcohol intake. In: Laragh JH, Brenner BM, editors. *Pathophysiology, Diagnosis, and Management*. Second ed. New York: Raven Press.
25. Marmot MG, Elliott P, Shipley MJ, Dyer AR, Ueshima H, Beevers DG, et al. (1994) Alcohol and blood pressure: the INTERSALT study. *BMJ* 308: 1263–1267. PMID: 7802765

26. (1993) Nonpharmacological interventions as an adjunct to the pharmacological treatment of hypertension: a statement by WHL. The World Hypertension League. *J Hum Hypertens* 7: 159–164. PMID: [8510089](#)
27. Marchi KC, Muniz JJ, Tirapelli CR (2014) Hypertension and chronic ethanol consumption: What do we know after a century of study? *World J Cardiol* 6: 283–294. <https://doi.org/10.4330/wjc.v6.i5.283> PMID: [24944758](#)