ACOT1 eQTL: a gene involved in lipid metabolism that modulates erectile dysfunction progression via metabolites

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Submitted: 31 October 2024; **Accepted:** 22 January 2025
Online publication: 4 March 2025

Arch Med Sci
DOI: https://doi.org/10.5114/aoms/200393

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Abstract

Introductions: The causal relationship between expression quantitative trait loci (eQTL) and erectile dysfunction (ED) remains underexplored. This study applied Mendelian randomization (MR) analysis to investigate potential causal links between novel susceptibility genes for ED and their underlying mechanisms.
Material and methods: Two-sample MR analysis was employed to examine causal connections between eQTLs, metabolites, and ED progression. Furthermore, summary-data-based MR (SMR) analysis was used to validate the causal association between cis-eQTLs and ED. A castrated rat model was also established to validate gene expression via quantitative real-time polymerase chain reaction (qRT-PCR).
Results: The results provide novel evidence that ACOT1 eQTL promoted ED progression. SMR analysis confirmed a causal relationship between the ACOT1 cis-eQTL and ED progression ($p < 0.05$). Regarding ACOT1's potential role in ED, the study suggested that the ACOT1 eQTL may negatively regulate docosadioate (C22-DC) and octadecanedioylcarnitine (C18-DC), both of which inhibited ED progression. In SD rats, castration led to a decrease in the ratio of intracavernous pressure (ICP) to mean arterial pressure (MAP) and a reduction in smooth muscle to collagen, accompanied by an increase in α-SMA expression in the castration group. These findings confirm the successful establishment of a castrated ED model. Additionally, further analysis of ACOT1 expression revealed significant upregulation in the castrated group ($p < 0.05$).
Conclusions: This study, for the first time, elucidates the mechanisms by which ACOT1, as a novel eQTL-mediated ED susceptibility gene, accelerates ED progression by negatively regulating levels of docosadioate (C22-DC) and octadecanedioylcarnitine (C18-DC) metabolites. These insights offer potential new therapeutic targets for ED.

Key words: Mendelian randomization, eQTL, ACOT1, erectile dysfunction, metabolites, pathology, gene expression, castrated rats.

Introduction

Erectile dysfunction (ED) is characterized by the inability to attain or sustain an erection adequate for satisfactory sexual performance [1].

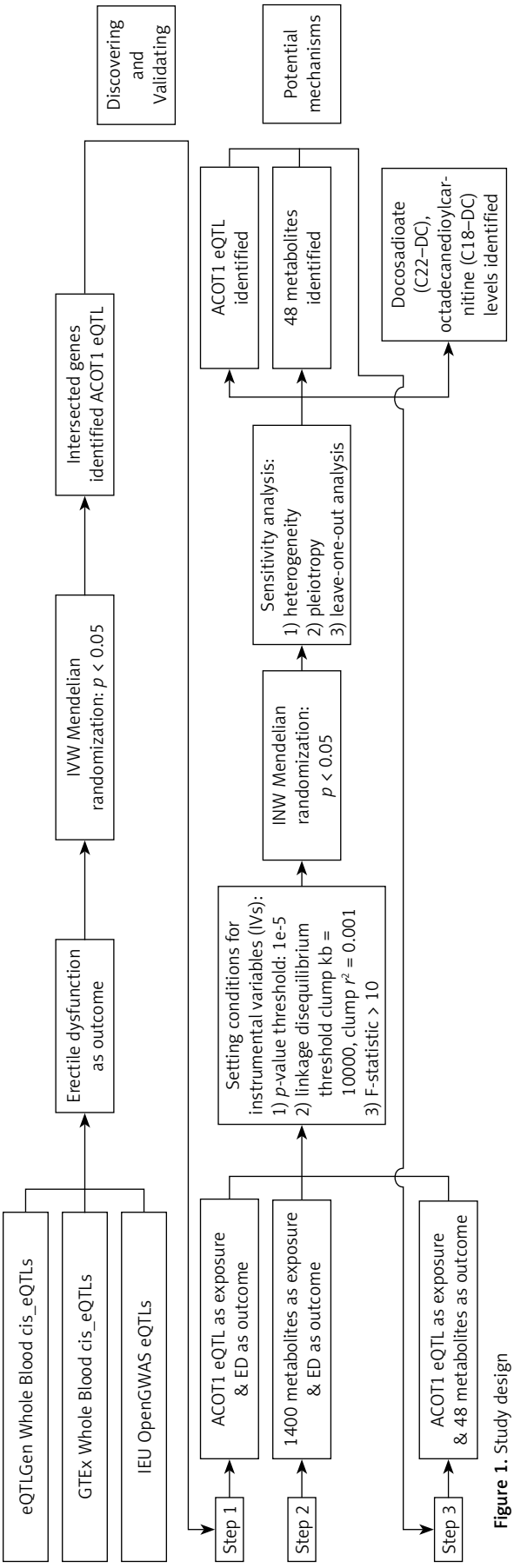

Figure 1. Study design

This condition is not life-threatening but widespread globally and seriously impacts the physical and mental well-being of men and their partners [2]. Epidemiological data confirm its widespread occurrence and increasing incidence rates [3]. A recent study in the United States reported rising prevalence of ED with age: 48.0% of individuals aged 65–74 and 52.2% of those over 75 met the diagnostic criteria for ED [4]. In contrast, the pooled prevalence of ED in men in Mainland China was found to be 49.69% [5]. Pharmacological treatments, particularly phosphodiesterase 5 inhibitors (PDE5Is), are commonly prescribed for ED; however, while effective for many, these medications are associated with adverse effects such as headaches, facial flushing, and visual disturbances [6]. Therefore, identifying novel therapeutic targets for ED remains a critical need.

ED is typically a condition resulting from the interplay of organic and psychogenic factors, with contributing risk factors including aging, metabolic dysfunction, and depression [7]. Among these, the rising prevalence of conditions such as obesity, diabetes, hyperlipidemia, hypertension, cardiovascular disease, and metabolic syndrome has prompted investigations into the role of metabolic factors in erectile function. Recent years have seen an increased focus on metabolites [8]. These small molecules, which act as intermediates or end products in metabolic pathways, are influenced by a variety of factors including genetics, diet, gut microbiota, lifestyle and disease states. As functional intermediates, metabolites offer valuable insights into the underlying mechanisms of disease progression.

While some studies have identified significant associations between certain metabolites, such as high-density lipoprotein, and ED [9], research on the expression of metabolic genes and the role of metabolites in ED remains limited. Mendelian randomization (MR), an analytical approach that employs genetic variation as instrumental variables (IVs), enables the estimation of causal links between exposures and outcomes [9]. However, no MR studies incorporating both genome-wide association studies (GWAS) and expression quantitative trait loci (eQTL) data on ED have been reported to date. This study represents the first application of MR methods to integrate GWAS and eQTL data, aiming to investigate the relationship between metabolites and ED and to identify metabolites as potential biomarkers and therapeutic targets.

Material and methods

Study design

Figure 1 illustrates the study design implemented in this research. Part 1 (Discovery &

Validation) focused on identifying intersected genes across three datasets (GTEx Whole Blood cis_eQTLs, eQTLGen Whole Blood cis_eQTLs and IEU OpenGWAS eQTLs), ultimately pinpointing the ACOT1 gene. Part 2 (Potential Mechanisms) involved three steps. Step 1 examined ACOT1 eQTL as the exposure with ED as the outcome. Step 2 explored 1400 metabolites as the exposure and ED as the outcome, leading to the identification of 48 metabolites. Step 3 accessed ACOT1 eQTL as the exposure, and 48 metabolites as the outcome, ultimately revealing significant associations with docosadioate (C22-DC) and octadecanedioylcarnitine (C18-DC) levels [10].

Data sources

The IEU OpenGWAS eQTLs data, retrieved from the IEU OpenGWAS project (https://gwas.mrcieu.ac.uk/), includes [11]: ED (project ID: ebi-a-GCST006956), a GWAS conducted on 223,805 Europeans (Ncase = 6,175, Ncontrol = 217,630), analyzing 9,310,196 single nucleotide polymorphisms (SNPs); the ACOT1 eQTL dataset (project ID: EQTL-A-ENSG00000184227) based on a GWAS of 28,703 Europeans, examining 18,982 SNPs; metabolite genetic data available through the GWAS Catalog (https://www.ebi.ac.uk/gwas/), covering project IDs GCST90199621 to GCST90201020 [12]; eQTLGen Whole Blood cis_eQTL datasets, accessible via the eQTLGen-cis-eQTLs website (https://www.eqtlgen.org/cis-eqtls.html) [13]; and the GTEx Whole Blood cis_eQTL data (GTEx_V8_cis_eqtl_summary_lite, hg19), obtained from the YangLab website (https://yanglab.westlake.edu.cn/software/smr/#DataResource).

Selection of IVs

To expand the scope of potential SNPs, IVs with a more permissive threshold ($p < 1 \times 10^{-5}$) were utilized. Independent SNPs were then identified through an analysis of linkage disequilibrium (LD) among the selected variants (LD $r^2 < 0.001$ within a 10,000 kb window), and dependent SNPs were excluded using the PLINK clustering method. To minimize bias from weak instrumental variables, the F statistic for each SNP was computed individually, with those having an F statistic < 10 being discarded. The F statistic was defined as $(\text{beta}/\text{SE})^2$ [14].

MR analysis and sensitivity analyses

MR analysis, incorporating five methods – inverse variance weighted (IVW), MR Egger, weighted median, simple mode, weighted mode – was employed to assess potential causality between various exposures and anticipated outcomes [14]. The IVW method constituted the primary analysis, employing a significance threshold set at $p < 0.05$.

To identify outliers and evaluate horizontal pleiotropic effects, the MR-PRESSO global test and MR-Egger intercept were applied [15]. The MR-Egger intercept assesses average pleiotropy (significant at intercept $p < 0.05$), while the slope provides reliable MR estimates in the presence of pleiotropy. It is important to note that factors such as age, race, and obesity influence pleiotropy results. Although methods such as MR-Egger regression can detect and adjust for pleiotropy, their effectiveness remains limited [16].

SMR and sensitivity analyses

The SMR analysis combines aggregated statistics from GWAS and eQTL studies within an MR framework to identify genes whose expression may influence the outcome trait [17]. In this study, SMR analysis was performed using the eQTLGen and GTEx Whole Blood cis-eQTL datasets to investigate potential causal relationships with ED, applying a significance threshold of $p < 0.05$. We conducted sensitivity analysis using the heterogeneity in dependent instruments (HEIDI) test to differentiate pleiotropy from linkage [10].

Construction of castrated rat model

Eight-week-old male SD rats, weighing 200 to 250 g, were procured from the Animal Experiment Center of Nantong University School of Medicine. The rats were randomly assigned to two groups: control, and surgical castration ($n = 8$ per group) [18]. Castration was performed under anesthesia induced by intraperitoneal pentobarbital sodium (40 mg/kg; Sigma-Aldrich, Denmark). A ventral midline incision was made above the scrotum, and the abdominal wall was carefully opened. The spermatic cord was isolated, and the vas deferens, along with the associated blood vessels, was ligated. Bilateral orchiectomy was then performed [19]. The model was successfully established after 4 weeks, with no mortality observed (0% mortality rate). The study was approved by the Institutional Animal Care and Use Committee of Nantong University (No. S20211214-001).

Detection of erectile function

Intracavernous pressure (ICP) and mean arterial pressure (MAP) were measured as described previously [20]. Four weeks after modeling, rats were subjected to anesthesia with an intraperitoneal injection of pentobarbital sodium. A midline abdominal incision was made to expose the prostate and penis by carefully dissociating surrounding tissues and organs. The cavernous nerve and major pelvic ganglia were identified, and one end of a catheter was connected to pin 26 before insertion into the cavernous body. The other end

of the catheter was linked to an ICP measurement device (MP160, Biopac Systems, Goleta, CA). The right carotid artery was then exposed, and a 20-gauge cannula containing heparin saline was inserted to measure MAP, with the cannula connected to a BL-420S via a pressure sensor. We utilized electrodes to stimulate the cavernous nerve to induce penile erection, employing stimulus parameters of 5 V, 20 Hz, a pulse width of 5 ms, and a duration of 50 s [20]. Max ICP/MAP was calculated to evaluate erectile function.

Masson's trichrome staining

Following established experimental protocols [21], rat penis tissue was collected and the midsection immersed in 4% paraformaldehyde. After undergoing a programmed dehydration process, the tissue was embedded in paraffin and sectioned at a thickness of 4 µm. Gradient dehydration with xylene and alcohol was performed before staining with the Masson trichrome stain kit (Servicebio, China) [22]. The smooth muscle and collagen areas were quantified using ImageJ software [23].

Immunofluorescence staining

Rats were euthanized following the assessment of erectile function, and their penises were harvested, fixed, and sectioned into 4 µm slices for subsequent immunofluorescence analysis. Immunofluorescence staining procedures were described previously [24]. Primary antibodies targeting α-SMA (Servicebio, Wuhan, China) were utilized, and 4′,6-diamidino-2-phenylindole (DAPI) was employed for nuclear staining.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Frozen penile tissues from castrated rats were homogenized in a tissue grinder. RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. The RNA was then subsequently reverse-transcribed into complementary DNA using a Reverse Transcriptase Kit (Thermo Fisher, Massachusetts, USA). Gene expression in the penile tissue was quantified via SYBR Green-based PCR [25]. The $2^{-\Delta\Delta CT}$ method was applied to assess gene expression, with GAPDH as the internal control. The primers for the target genes are listed below:

GAPDH-F (from 5′ to 3′): ACGGCAAGTTCAACGGCACAG

GAPDH-R (from 5′ to 3′): CGACATACTCAGCACCAGCATCAC

ACOT1-F (from 5′ to 3′): TGGCCACCCTGAGGTAAAAGG

ACOT1-R (from 5′ to 3′): TGGTTTCTCAGGATAGTCACAGGG

Statistical analysis

The 'TwoSampleMR' R package and R version 4.3.2 (http://www.Rproject.org) were employed to assess evaluate causal relationships between modifiable exposures and disease outcomes. Statistical significance was established as $p < 0.05$ [10]. Groups comparisons were performed using an unpaired *t*-test. Data analysis was carried out with SPSS 26.0 and GraphPad Prism 8.0, with a *p*-value < 0.05 deemed statistically significant [26].

Results

MR analysis showed that ACOT1 promoted progression of ED

ACOT1 was initially identified as the sole gene with a causal link to ED across three independent datasets: eQTLGen, GTEx Whole Blood cis_eQTLs, and IEU OpenGWAS eQTLs. Using the IEU OpenGWAS eQTLs dataset, an MR analysis were performed with ACOT1 eQTL as the exposure and ED as the outcome. Four SNPs were selected as IVs for ACOT1 eQTL, and IVW estimates indicated a positive association with ED progression (β = 0.170, 95% CI: 0.054 to 0.286, $p = 0.004$) (Figure 2). No heterogeneity was detected through global tests and the MR-Egger method ($p > 0.05$, $p = 0.557$), and no evidence of pleiotropy was observed ($p > 0.05$, $p = 0.587$). Scatter plots confirmed a direct relationship between SNP effects on ACOT1 eQTL and ED progression (Supplementary Figure S1), supported by a forest plot and leave-one-out analysis.

SMR analysis confirmed that ACOT1 can promote progression of ED

SMR analysis using SMR 1.3.1 was conducted to confirm the causal relationship between ACOT1

Exposure	Outcome	nsnp	Method	*P*-value	β (95% CI)
ACOT1 eQTL	Erectile dysfunction	4	MR Egger	0.179	0.233 (0.009 to 0.457)
		4	Weighted median	0.003	0.179 (0.060 to 0.297)
		4	Inverse variance weighted	0.004	0.170 (0.054 to 0.286)
		4	Simple mode	0.786	–0.045 (–0.342 to 0.252)
		4	Weighted mode	0.056	0.193 (0.069 to 0.317)

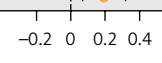

Figure 2. MR analysis demonstrated that genetic susceptibility to ACOT1 eQTL may accelerate ED progression

A

Exposure	Outcome	eQTLs	Datasets	*P*-value	β (95% CI)
ACOT1 eQTL	Erectile dysfunction	cis_eQTLs	GTEx Whole Blood	0.002	0.096 (0.034 to 0.158)
		cis_eQTLs	eQTLGen Whole Blood	0.002	0.269 (0.098 to 0.440)

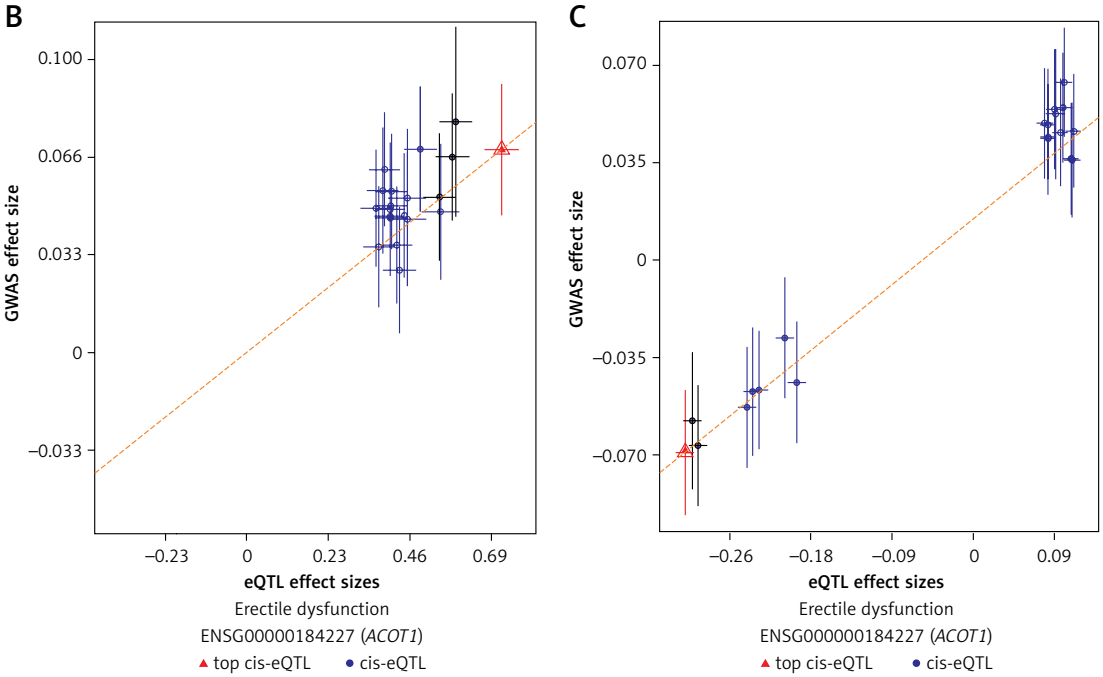

Figure 3. SMR analysis confirmed that genetic susceptibility to ACOT1 cis-eQTL contributed to ED progression. **A** – MR results indicating the causal association between ACOT1 cis-eQTL and ED; **B** – Scatter plots illustrating the causal effects of ACOT1 cis-eQTL on ED in GTEx Whole Blood cis_eQTLs; **C** – Scatter plots showing the causal effects of ACOT1 cis-eQTL on ED in eQTLGen Whole Blood cis_eQTLs

cis-eQTL and ED progression. Default settings and a significance threshold of $p < 0.05$ were applied to two independent datasets: eQTLGen and GTEx Whole Blood cis_eQTLs. A significant causal association between the ACOT1 eQTL and ED progression was observed in both datasets (β = 0.096, 0.269; 95% CI: 0.034 to 0.158,0.098 to 0.440; p = 0.002, 0.002) (Figure 3 A). The SMR correlation plot revealed a positive association between ACOT1 cis-eQTL and ED GWAS data (Figures 3 B, C).

ACOT1 inhibited expression of the metabolites docosadioate (C22–DC) and octadecanedioylcarnitine (C18–DC)

To investigate the potential mechanism of ACOT1 in ED, steps 2 and 3 were followed. The metabolites docosadioate (C22–DC) and octadecanedioylcarnitine (C18–DC) were identified as key candidates. Docosadioate (C22–DC) was classified as an ultra-long chain fatty acid. Using ACOT1 eQTL as the exposure and docosadioate (C22–DC) levels as the outcome, four SNPs were selected as IVs. IVW analysis revealed that an increase in SNP influence on ACOT1 eQTL was associated with a decrease in docosadioate (C22–DC) levels (β = –0.098, 95% CI: –0.189 to –0.007, p = 0.035) (Figure 4). No evidence of heterogeneity or pleiotropy was observed (all $p > 0.05$). Similarly, with ACOT1 eQTL as the exposure and octadecanedioylcarnitine (C18-DC) levels as the outcome, four SNPs were again identified as IVs. IVW analysis demonstrated that increased SNP influence on ACOT1 eQTL was linked to reduced octadecanedioylcarnitine (C18–DC) levels (β = –0.169, 95% CI: –0.278 to –0.060, p = 0.002) (Figure 4). MR-Egger analysis showed no evidence of heterogeneity (p = 0.770) or pleiotropy (p = 0.663). Detailed forest plots, leave-one-out analyses and scatter plots are provided in Supplementary Figure S2.

The metabolites docosadioate (C22-DC) and octadecanedioylcarnitine (C18-DC) inhibited ED progression

Using 26 SNPs as IVs for docosadioate (C22-DC) levels, IVW analysis established a causal relationship between reduced docosadioate (C22-DC) levels and ED (β = –0.113, 95% CI: –0.223 to –0.003, p = 0.044) (Figure 5). Sensitivity analysis

Exposure	Outcome	nsnp	Method	*P*-value	β (95% CI)
ACOT1 eQTL	Docosadioate (C22-DC) levels	4	MR Egger	0.504	–0.073 (–0.250 to 0.104)
		4	Weighted median	0.039	–0.099 (–0.192 to–0.005)
		4	Inverse variance weighted	0.035	–0.098 (–0.189 to –0.007)
		4	Simple mode	0.941	–0.007 (–0.174 to 0.161)
		4	Weighted mode	0.132	–0.101 (–0.198 to –0.005)
	Octadecanedioylcarnitine (C18-DC) levels	4	MR Egger	0.215	–0.223 (–0.467 to 0.021)
		4	Weighted median	< 0.001	–0.179 (–0.272 to –0.086)
		4	Inverse variance weighted	0.002	–0.169 (–0.278 to –0.060)
		4	Simple mode	0.375	–0.126 (–0.363 to 0.111)
		4	Weighted mode	0.027	–0.182 (–0.270 to –0.093)

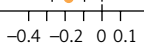

Figure 4. MR analysis showed that genetic susceptibility to ACOT1 eQTL was associated with reduced levels of docosadioate (C22-DC) and octadecanedioylcarnitine (C18-DC) metabolites

affirmed the robustness of the causal estimate and effect size, with no evidence of heterogeneity (p = 0.427) or pleiotropy (p = 0.943). Similarly, 26 SNPs were selected as IVs for octadecanedioylcarnitine (C18-DC) levels, with IVW analysis indicating that elevated octadecanedioylcarnitine (C18-DC) levels were associated with a reduced risk of ED progression (β = –0.072, 95% CI: –0.138 to –0.007, p = 0.030) (Figure 5). MR-Egger analysis further confirmed the absence of heterogeneity (p = 0.825) or pleiotropy (p = 0.463). Supplementary Figure S3 presents scatter plots, forest plots, and leave-one-out analyses.

Animal models and verification of ACOT1 expression in castrated rat models

This study successfully established a castrated model using SD rats, validated through ICP and MAP measurements as well as Masson's trichrome staining. Figure 6 A presents the ICP and MAP results for both the control and castrated groups. The ratio of max ICP to MAP was used as an indicator of erectile function [23], which was significantly reduced in the castrated group (Figure 6 B). Atrophy of the corpus cavernosum smooth muscle was observed in the castrated group (Figure 6 C). The smooth muscle to collagen ratio, indicative of fibrosis in the corpus cavernosum [23], also decreased significantly in the castrated group (Figure 6 D). Furthermore, immunofluorescence staining with an anti-α-SMA antibody revealed a marked reduction in smooth muscle content in the castrated group compared to the control (Figure 6 E). These results confirm the successful establishment of the castrated model. Additionally, ACOT1 expression was assessed using qRT-PCR, which showed a significant increase in ACOT1 levels in the castrated group compared to controls (p < 0.05), as demonstrated in Figure 6 F.

Based on these results, it was concluded that the ACOT1 eQTL promoted ED progression by regulating the levels of docosadioate (C22-DC) and octadecanedioylcarnitine (C18-DC) (Figure 7).

Discussion

The results suggest that genetic susceptibility to ACOT1 eQTL may accelerate ED progression, with SMR analysis confirming a causal link between ACOT1 cis-eQTL and ED development. Further investigation into the underlying mechanisms revealed that ACOT1 eQTL decreases the levels of the metabolites docosadioate (C22-DC) and octadecanedioylcarnitine (C18-DC), while elevated levels of these metabolites are associated with an increased risk of ED. These results highlight a potential metabolic mechanism through which ACOT1 eQTL promotes ED progression by

Exposure	Outcome	nsnp	Method	*P*-value	β (95% CI)
Docosadioate (C22-DC) levels	Erectile dysfunction	26	MR Egger	0.324	–0.121 (–0.355 to 0.114)
		26	Weighted median	0.050	–0.159 (–0.319 to 0.000)
		26	Inverse variance weighted	0.044	–0.113 (–0.223 to –0.003)
		26	Simple mode	0.437	–0.130 (–0.453 to 0.193)
		26	Weighted mode	0.434	–0.130 (–0.451 to 0.191)
Octadecanedioylcarnitine (C18-DC) levels	Erectile dysfunction	26	MR Egger	0.484	–0.039 (–0.148 to 0.069)
		26	Weighted median	0.645	–0.020 (–0.105 to 0.065)
		26	Inverse variance weighted	0.030	–0.072 (–0.138 to –0.007)
		26	Simple mode	0.626	–0.045 (–0.222 to 0.133)
		26	Weighted mode	0.471	–0.032 (–0.119 to 0.054)

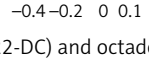

Figure 5. MR analysis revealed that genetic susceptibility to levels of docosadioate (C22-DC) and octadecanedioylcarnitine (C18-DC) metabolites may mitigate the progression of ED

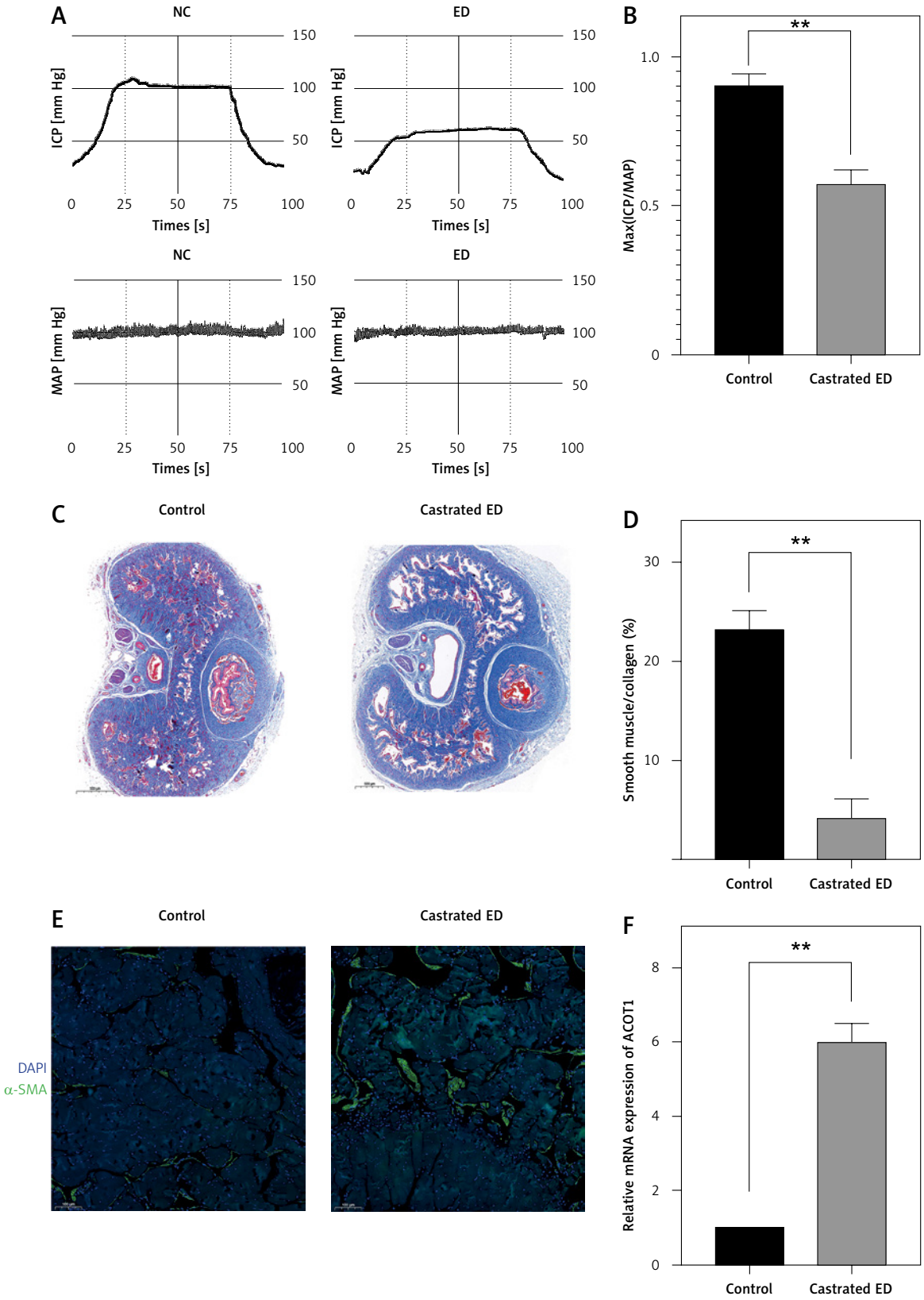

Figure 6. The experiments verified that ACOT1 promoted progression of ED. **A** – Measurement of intracavernous pressure (ICP) and mean arterial pressure (MAP) in the control and castrated ED groups; **B** – Bar graph showing the ICP/MAP ratio; **C** – Masson's trichrome staining of tissues from the control and castrated ED groups; **D** – Bar graph depicting the ratio of smooth muscle to collagen; **E** – Expression levels of α-SMA in the control and castrated ED groups; **F** – Bar graph of ACOT1 transcriptional expression in the control and castrated ED groups; $^{**}p < 0.01$; $^{***}p < 0.001$

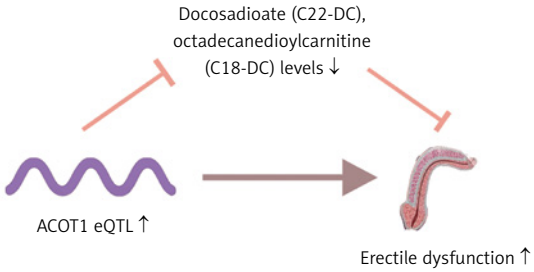

Figure 7. Schematic representation of the potential mechanism

modulating levels of docosadioate (C22-DC) and octadecanedioylcarnitine (C18-DC).

ED, a common male sexual disorder, profoundly affects both the individual's and their partner's quality of life [27]. ED is classified into psychogenic, organic and mixed types [28]. Among the organic causes, vascular factors are considered the predominant contributors, owing to the penile tissue's high vascular density [29]. Consequently, risk factors for ED include obesity, hypertension, metabolic syndrome, and diabetes, all of which promote vascular inflammation and subsequent endothelial dysfunction [30]. Additionally, lipid metabolism disorders play a significant role in ED, contributing to local atherosclerosis via inflammatory pathways [30].

ACOT1 is a cytoplasmic enzyme with substrate specificity for long-chain (C12-C20) saturated and monounsaturated acyl-CoA, playing a key role in lipid metabolism [31]. It catalyzes the hydrolysis of acyl-CoA to free fatty acids and CoA, which are then directed into various metabolic pathways, such as glycerol and sphingoid synthesis, esterification for retinol and cholesterol biosynthesis, or conversion to acylcarnitine via mitochondrial β-oxidation [31]. Previous studies have shown that ACOT1 overexpression reduces reactive oxygen species and improves cardiac function in mouse models of diabetes-induced cardiac dysfunction and septicemia [32, 33]. Additionally, ACOT1 deficiency has been linked to reduced fat mass accumulation induced by high-fat diets, attributed to increased energy expenditure [34]. However, the role of ACOT1 in ED remains underexplored. This study provides the initial evidence that ACOT1 eQTL contributes to ED progression. Further SMR analysis confirms a causal association between ACOT1 cis-eQTL and ED progression, offering a novel therapeutic target for its treatment.

This study suggests that docosadioate (C22-DC) and octadecanedioylcarnitine (C18-DC) may inhibit ED progression. Research has shown that elevated levels of dicarboxylic acids, including dodecanedioic acid and docosadioate (C22-DC), impair fatty acid β-oxidation and peroxisomal activity *in vivo*, thereby reducing the conversion of cholesterol to bile acids and potentially enhancing steroidogenesis [35]. Octadecanedioylcarnitine (C18-DC), a key fatty acylcarnitine involved in long-chain fatty acid metabolism, particularly mitochondrial β-oxidation, serves as a diagnostic marker for complex disorders of fatty acid metabolism. It facilitates early detection of chronic obstructive pulmonary disease with a frequent exacerbator phenotype, serves as a primary diagnostic marker for carnitine-acylcarnitine transferase deficiency, and aids in assessing the link between serum acylcarnitine levels and the severity of coronary artery disease [36–38].

ACOT1 may influence lipid metabolism by reducing steroid synthesis *in vivo*, particularly through the decrease of docosadioate (C22-DC) levels, which could contribute to the progression of ED. Additionally, ACOT1 may impair fatty acid metabolism, specifically by lowering octadecanedioylcarnitine (C18-DC) levels, further promoting ED progression. Disruptions in lipid metabolism lead to vascular changes, including thickening of blood vessel walls, narrowing of the arteries, and arteriosclerosis. These alterations compromise blood flow to the penile arteries, limiting the expansion of the penile spongy tissue and thereby impairing erectile function.

This study has several limitations. First, clinical trials in real-world settings are necessary to validate the role of ACOT1 in ED. Second, the study populations are exclusively of European descent, which may introduce sample selection bias [39]. Future GWAS should include larger, more diverse populations to mitigate this limitation. Finally, the analysis did not stratify the results by etiology, such as cardiogenic or vasogenic factors. These limitations warrant careful consideration in future research. Subsequent studies should build upon these results through more comprehensive experiments to further investigate the role of ACOT1 in ED and explore potential therapeutic strategies.

In conclusion, the study provides initial evidence that ACOT1 eQTL promotes ED progression. Subsequent SMR analysis and qRT-PCR experiments confirm a causal link between ACOT1 cis-eQTL and ED progression. Investigation into the underlying mechanism suggests that ACOT1 eQTL may negatively regulate the levels of docosadioate (C22-DC) and octadecanedioylcarnitine (C18-DC), both of which play a significant role in ED progression. These findings establish a foundation for further exploration of the molecular links between gene expression, metabolic activity, and ED progression, which will require additional clinical and experimental validation.

Data availability

The datasets generated and analyzed throughout our study are available on the website: https://

gwas.mrcieu.ac.uk/;https://www.ebi.ac.uk/gwas/;https://www.eqtlgen.org/cis-eqtls.html; https://yanglab.westlake.edu.cn/software/smr/#DataResource

Zelin Zhang and Yingfei Chen contributed equally to this work.

Funding

National Natural Science Foundation of China, Grant Number: 82370780.

Ethical approval

The IEU open GWAS is a publicly accessible database, with all included patient data having received ethical approval. Researchers can freely download relevant data for analysis and publish associated findings. This study is based solely on publicly available data, ensuring no ethical concerns or conflicts of interest. Approval for the study was obtained from the Institutional Animal Care and Use Committee of Nantong University (No. S20211214-001).

Conflict of interest

The authors declare no conflict of interest.

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