A family with hypothyroidism caused by fatty acid synthase and apolipoprotein B receptor mutations

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Abstract. Hypothyroidism is a disease with a genetic component. The present study aimed to identify the potential causative gene mutation in a family with hypothyroidism and to investigate its potential pathology. DNA was extracted from the affected individual and his parents, maternal aunt and maternal grandmother. Whole exome sequencing was used to examine their exomes. The potential causative genes that may have an autosomal dominant mode of inheritance were selected after variant calling and filtering. Bioinformatics analysis was utilized to predict the deleteriousness of the identified variants, and multiple sequence alignment and conserved protein domain analyses were performed using online software. Finally, Sanger sequencing was used to validate the identified variants. In the present study, a total of 50 variants were screened based on the autosomal dominant mode of inheritance. Two variants, the fatty acid synthase (FASN) and apolipoprotein B receptor (APOBR) genes, were further analyzed, as they were highly associated with hypothyroidism. Genotyping results revealed that two mutations, c.G7192T (p.A2398S) in the FASN gene and c.C1883G (p.T628R) in the APOBR gene, were fully co-segregated with established hypothyroidism phenotypes in the family. These mutations were located in the conserved α/β-hydrolase fold and Na^+/Ca^{2+} exchanger superfamily domain of FASN and APOBR, respectively. In conclusion, the present study demonstrated that the FASN c.G7192T and APOBR c.C1883G mutations may be the potential causative variants in this Chinese hypothyroidism pedigree.

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Abbreviations: APOBR, apolipoprotein B receptor; FASN, fatty acid synthase; SNVs, single nucleotide variants; WES, whole exome sequencing

Key words: WES, hypothyroidism, FASN, APOBR

Introduction

Thyroid hormones (triiodothyronine and thyroxine) are major regulators of diverse metabolic pathways via interactions with thyroid hormone nuclear receptors in various tissues (1-3). Maintaining thyroid hormone levels is essential for development and metabolism. Hypothyroidism, one of several thyroid diseases, is a pathological state where thyroid hormone levels are decreased systemically or locally in one or more tissues. The prevalence of hypothyroidism is sizeable and ranges between 3-18% in the adult population, with women, elderly persons and populations with iodine deficiency or excess being more often affected (4,5). Generally, hypothyroidism is diagnosed either in the subclinical or clinical form. Congenital hypothyroidism, if not treated, may lead to severe and irreversible mental retardation (6).

Hypothyroidism is associated with various symptoms, including cold sensitivity, fatigue and lethargy, cognitive dysfunction and delayed growth; these symptoms can be accompanied by distinct signs of tachycardia, weight loss and attention deficit-hyperactivity disorder (7,8). Two of the main characteristics of hypothyroidism are the marked impairment of lipid metabolism and dyslipidemia (9,10). Furthermore, hypercholesterolemia induces increased concentrations of total and low-density lipoprotein cholesterol (11), which will affect normal metabolism. At present, the genetic mechanism implicated in the pathogenesis of hypothyroidism remains poorly understood. It has been reported that mutations in the NK2 homeobox 1 and forkhead box E1 (FOXE1) genes cause hypothyroidism (12,13). In addition, a mutation in immunoglobulin superfamily member 1 has been revealed to be associated with central hypothyroidism (X-linked syndrome) (14). Loss-of-function mutations in thyroglobulin, paired box 8, thyroid-stimulating hormone receptor, FOXE1, NK2 homeobox (NKX2)-1 and NKX2-5 genes are also associated with inherited congenital hypothyroidism (15,16). Therefore, identifying candidate gene mutations may be helpful in understanding the pathology of hypothyroidism.

Whole exome sequencing (WES) is a useful tool for exploring the genetic mechanism of different diseases (17-19). The present study investigated a Chinese hypothyroidism pedigree, which included an affected

proband, mother and maternal grandmother; other relatives were unaffected. The results indicated that the c.G7192T (p.A2398S) mutation in fatty acid synthase (FASN) and the c.C1883G (p.T628R) mutation in apolipoprotein B receptor (APOBR) may be the most likely causes of the disease. These results on the inheritance of mutant genes may provide novel information regarding the pathological mechanism underlying hypothyroidism.

Materials and methods

Subjects and family members. The present study examined a three-generation hypothyroidism pedigree residing in China. The proband (IV:1) was a 3.9-year-old boy who was diagnosed with hypothyroidism. Furthermore, his mother (III:2) and maternal grandmother (II:3) also had hypothyroidism, and all of them shared common characteristics (Fig. 1). In addition, family members I:1 and II:1 were diagnosed with subclinical hypothyroidism, and II:5 was a probable case. The father, maternal aunt and paternal grandparents of the proband were unaffected. Therefore, it was hypothesized that the etiology of hypothyroidism in this family had an autosomal dominant mode of inheritance. Therefore, the proband, along with his parents, maternal aunt and maternal grandmother were enrolled for whole exome sequencing (Fig. 1). Written informed consent was obtained from all individuals enrolled in this study. In addition, the present study was approved by the ethical approval committee of Jinan Central Hospital Affiliated to Shandong University (2016-053-01; Jinan, China).

Exome sequencing and variant calling. Venous blood was obtained from the affected individuals (IV:1, III:2 and II:3) and two unaffected individuals (III:1 and III3) in the hypothyroidism pedigree. The collected blood was stored in EDTA, followed by DNA extraction using a kit (Tiangen Biotech Co., Ltd., Beijing, China). The Agilent SureSelect Human All Exon 50Mb Exon kit (Agilent Technologies, Inc., Santa Clara, CA, USA) was used to perform exome target enrichment of quantified genomic DNA. The amplification process was performed under the following conditions: 15 min at 95˚C followed by 40 cycles of 10 sec at 95˚C, 30 sec at 55˚C, 32 sec at 72˚C, and 15 sec at 95˚C, 60 sec at 60˚C, 15 sec extension at 95˚C. The Illumina HiSeq 4000 Sequencer (Illumina, San Diego, CA, USA) was utilized for WES. Sequencing reads that had paired-ends, were 200-bp long and had a mean coverage of 100x were generated for each sample. After filtering out adapter sequences, and contaminated and low quality reads, the clean paired reads were then mapped to the reference human genome sequence, hg19 (20), using the Burrows-Wheeler alignment tool, generating the sequence alignment/map file. Picard software program (version 1.07) was used to mark and remove polymerase chain reaction (PCR) duplicate reads. MuTect (version 1.1.4) and Genome Analysis Toolkit software (version 3.1) (21,22) were used to identify single nucleotide variants (SNVs), deletions and insertions throughout the genome.

Variant filtering. To identify potential candidate genes, the variants were annotated in a systematic manner. Variant information was annotated using various genetic variation databases by the program ANNOVAR (23). From the reported variant frequencies, variants with a minor allele frequency >0.01 in the 1000 Genomes Project (https://www.nature.com/articles/nature15393) were excluded. Based on the variant location within genes, higher priority was given to the variants in the coding region and variants that altered the coding sequence (nonsynonymous variants) were identified. The deleteriousness of identified variants was then predicted by bioinformatics analysis [e.g. Oncotator v1.5.3.0, sift (http://sift.jcvi.org/) and polyphen (http://genetics.bwh.harvard.edu/pph2/)], and the harmful nonsynonymous variants were obtained. The eligible nonsynonymous variants were identified using the following filtering parameter criteria in the Genome Analysis Toolkit software (v3.1) (https://software.broadinstitute.org/gatk/): QualByDepth>2.0, FisherStrand<60.0, StrandOddsRatio<4.0, RMSMappingQuality (MQ)>40.0, MQRankSumTest>-12.5, ReadPosRankSumTest>-8.0. In addition, minor allele frequency <0.05 was also included.

In silico analysis. The multiple sequence alignment of FASN and APOBR in different species was performed using an online tool (https://www.ncbi.nlm.nih.gov/tools/cobalt/cobalt.cgi?LINK_LOC=BlastHomeLink). In addition, the Conserved Domain Search Service (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) was used to identify the conserved protein domains.

Sanger sequencing. After the systematic filtering, two candidate variants (FASN c.G7192T, p.A2398S and APOBR c.C1883G, p.T628R) were confirmed. Genomic DNA was prepared from the proband, ten family members and twenty additional, unrelated, affected individuals. Primer-Premier 5.0 (Premier Biosoft International, Palo Alto, CA, USA) was used to design oligonucleotide primer sets for the variants. PCR analyses and Sanger sequencing were then performed. The amplification process was: 15 min at 95˚C followed by 40 cycles of 10 sec at 95˚C, 30 sec at 55˚C, 32 sec at 72˚C, and 15 sec at 95˚C, 60 sec at 60˚C, 15 sec extension at 95˚C. The PCR products were used for Sanger sequencing.

Results

Genetic and in silico analysis. Genomic DNA samples from IV:1, III:1, III:2, III:3 and II:3 were analyzed. A total of 50 nonsynonymous variants were identified (Table I). Based on the hypothyroidism in the pedigree and subsequent Sanger sequencing results (Table II), two SNVs, the c.G7192T mutation (a substitution from C to A) in FASN (Chr17: 80037439) and the c.C1883G mutation (a substitution from C to G) in APOBR (Chr16: 28508245), were likely to be associated with the disease. In pedigree individuals, the results of II:3, II:5, III:2 and IV:1 showed the c.G7192T mutation in FASN (Chr17: 80037439) and the c.C1883G mutation in APOBR.

In silico analysis. The c.G7192T mutation in FASN resulted in a protein alteration of p.A2398S. In addition, the c.C1883G mutation in APOBR resulted in a protein alteration of p.T628R. Furthermore, multiple sequence alignment and conserved

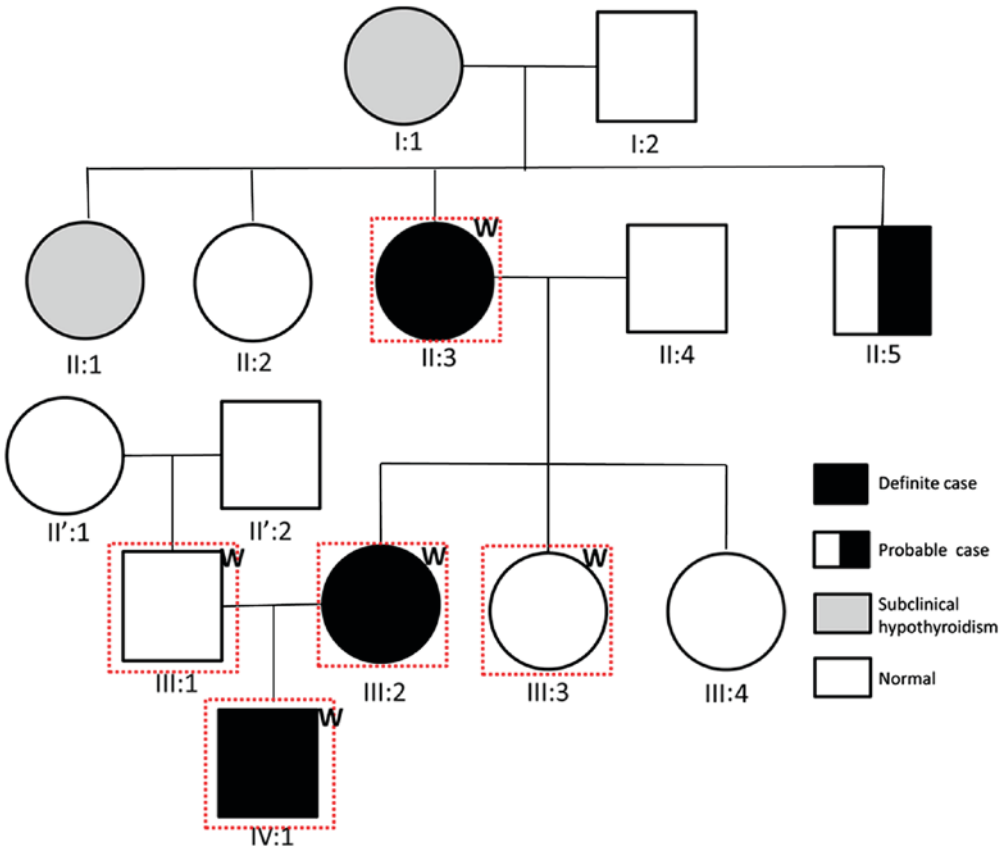

Figure 1. Hypothyroidism pedigree. Exome sequencing was performed on individuals IV:1, III:1, III:2, III:3 and II:3. IV:1 was identified as the proband; I:1 and II:1 were individuals with subclinical hypothyroidism; II:5 was a probable case of hypothyroidism. Circles represent female individuals; squares represent male individuals. W, whole exome sequencing.

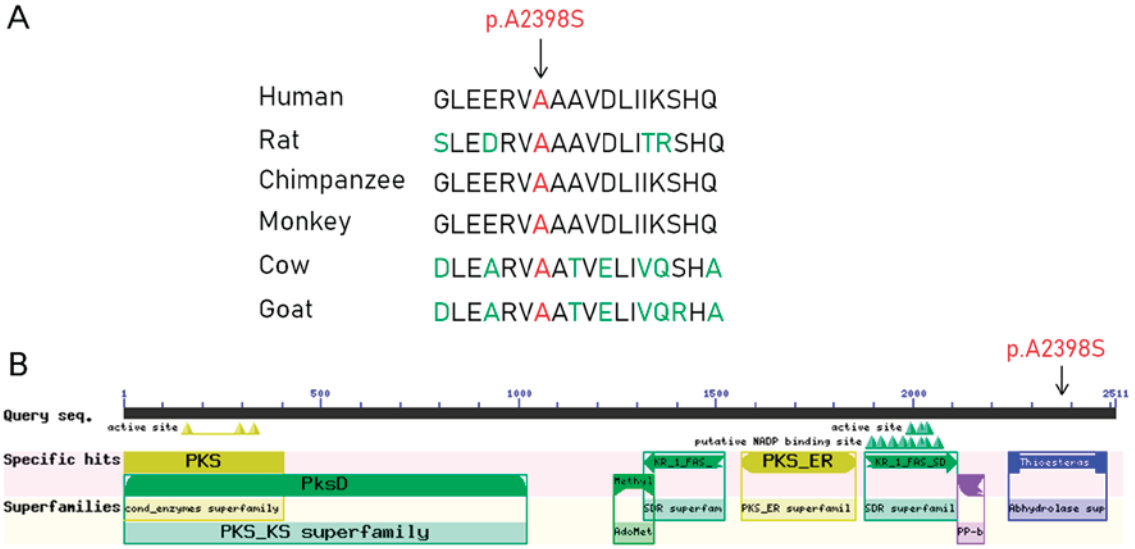

Figure 2. (A) Affected amino acid residue was highly conserved between different species. (B) Conserved domains in fatty acid synthase.

protein domain analyses indicated that the amino acid at position 2,398 in the FASN protein sequence was located in the α/β-hydrolase fold, and it was highly conserved across several species, including humans, rats, chimpanzees, monkeys, cows and goats (Fig. 2). Furthermore, the amino acid at position 628 in the APOBR protein sequence was located in the Na^+/Ca^{2+} exchanger superfamily, and it was highly conserved across several species, including humans, monkeys, camels, chimpanzees, pigs and rats (Fig. 3).

Sanger sequencing of the candidate causative variants. In order to further confirm the c.G7192T and c.C1883G mutations in hypothyroidism, Sanger sequencing was performed on the 11 family members and on 20 additional, unrelated individuals

Table I. A total of 50 nonsynonymous variants in the pedigree.

Chr	Start	End	Ref	Alt	Gene.refGene	DbSNP138
1	16057042	16057042	G	A	PLEKHM2	.
1	23238971	23238971	G	A	EPHB2	.
2	167760021	167760021	A	G	XIRP2	.
2	169801131	169801131	G	A	ABCB11	rs118109635
2	172409920	172409920	T	C	CYBRD1	rs16859487
2	175618404	175618404	C	T	CHRNA1	.
2	179300979	179300979	A	T	PRKRA	rs77419724
2	179597657	179597657	T	G	TTN	.
2	179632619	179632619	C	T	TTN	rs141258018
2	204304489	204304489	G	T	RAPH1	rs191393494
2	234869499	234869499	G	C	TRPM8	.
2	238732983	238732983	G	T	RBM44	.
3	13670739	13670739	G	A	FBLN2	rs201340643
3	38087142	38087142	G	A	DLEC1	rs117463277
3	49701035	49701035	G	A	BSN	rs141950704
3	56597830	56597830	G	A	CCDC66	rs146224729
4	74363387	74363387	A	T	AFM	rs2276444
6	90578678	90578678	A	C	CASP8AP2	.
6	168294583	168294583	G	A	MLLT4	rs150936076
6	170886768	170886768	C	G	PDCD2	rs140493653
9	140323749	140323749	C	T	NOXA1	rs201388549
10	86132217	86132217	G	T	CCSER2	.
11	3249828	3249828	C	T	MRGPRE	rs200334859
11	3681054	3681054	G	A	ART1	rs2280133
11	3744621	3744621	C	T	NUP98	rs148384795
11	4411560	4411560	A	C	TRIM21	.
11	44069816	44069816	G	A	ACCSL	rs182257970
11	58605757	58605757	C	T	GLYATL2	.
11	62381857	62381857	G	A	ROM1	.
11	68748268	68748268	A	G	MRGPRD	rs74390416
11	73067275	73067275	C	T	ARHGEF17	.
11	77838424	77838424	T	C	ALG8	rs138293432
11	93754643	93754643	T	A	HEPHL1	rs192979315
13	96242562	96242562	T	G	DZIP1	.
15	65490592	65490592	C	T	CILP	rs148582730
16	28508245	28508245	C	G	APOBR	rs13306186
16	28948654	28948654	T	C	CD19	.
17	4076694	4076694	A	G	ANKFY1	.
17	4856390	4856390	G	C	ENO3	rs143945974
17	10312678	10312678	A	T	MYH8	rs151091483
17	19246867	19246867	T	C	B9D1	rs7221577
17	79167744	79167744	C	T	CEP131	rs138784674
17	80037439	80037439	C	A	FASN	rs200842352
18	3094191	3094191	C	T	MYOM1	rs149588924
20	76700	76700	G	A	DEFB125	rs116934569
20	60575227	60575227	C	A	TAF4	.
20	60892518	60892518	C	T	LAMA5	rs200632605
20	61167658	61167658	C	G	MIR1-1HG	rs145416632
21	34951831	34951831	C	A	DONSON	rs190773441
21	40568847	40568847	T	C	BRWD1	rs73357824

Alt, alteration allele; Chr, chromosome; Ref, reference allele; rs, accession number in single nucleotide polymorphism database Build 138 (https://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgi?view+summary=view+summary&build_id=138).

Table II. Sanger sequencing validation results.

Individuals	CHRNA1	ABCB11	APOBR	FASN	TRPM8
Affected sporadic individuals					
1	GG	CC	CC	CC	GG
2	GG	CC	CC	CC	GG
3	GG	CC	CC	CC	GG
4	GG	CC	CC	CC	GG
5	GG	CC	CC	CC	GG
6	GG	CC	CC	CC	GG
7	GG	CC	CC	CC	GG
8	GG	CC	CC	CC	GG
9	GG	CC	CC	CC	GG
10	GG	CC	CC	CC	GG
11	GG	CC	CC	CC	GG
12	GG	CC	CC	CC	GG
13	GG	CC	CC	CC	GG
14	GG	CC	CC	CC	GG
15	GG	CC	CC	CC	GG
16	GG	CC	CC	CC	GG
17	GG	CC	CC	CC	GG
18	GG	CC	CC	CC	GG
19	GG	CC	CC	CC	GG
20	GG	CC	CC	CC	GG
Pedigree individuals					
III-2	GG	CC	CG	AC	GG
II-1	GA	CT	CC	CC	GC
II-2	GG	CC	CC	CC	GG
II-1	GG	CC	CC	CC	GG
III-3	GG	CC	CC	CC	GG
III-1	GA	CT	CC	CC	GC
II-5	GA	CT	CG	AC	GC
II-4	GG	CC	CC	CC	GG
II-3	GA	CT	CG	AC	GC
I-1	GG	CC	CC	CC	GG
IV-1	GA	CT	CG	AC	GC

ABCB11, ATP binding cassette subfamily B member 11; APOBR, apolipoprotein B receptor; CHRNA1, cholinergic receptor nicotinic α1 subunit; FASN, fatty acid synthase; TRPM8, transient receptor potential cation channel subfamily M member 8.

with primary hypothyroidism. The clinical information of the 20 additional, unrelated individuals with primary hypothyroidism is presented in Table III. The results demonstrated that the c.G7192T mutation in FASN and the c.C1883G mutation in APOBR were fully co-segregated with established hypothyroidism phenotypes in the family recruited for WES (Figs. 4 and 5). Furthermore, the FASN c.G7192T and APOBR c.C1883G mutations were detected in the patient with probable hypothyroidism (II:5). It is worth mentioning that the FASN c.G7192T and APOBR c.C1883G mutations were not observed in individuals with subclinical hypothyroidism (I:1, II:1). In addition, the mutations were not detected in the unrelated, affected individuals with hypothyroidism (date not shown), which supported the hypothesis of genetic heterogeneity of hypothyroidism in this family.

Discussion

Hypothyroidism is the most common endocrine disorder caused by thyroid hormone deficiency, which can induce metabolic dysfunction (24). At present, the genetic mechanisms underlying hypothyroidism pathogenesis remain poorly understood. It has been reported that the c.1184_1187dup4 mutation in the thyroid peroxidase gene, c.40A>G and c.94G>A mutations in the thyroid-stimulating hormone β gene, p.G488R, p.A649E, p.R885Q, p.I1080T and p.A1206T mutations in the dual oxidase 2 gene, and the p.Y138X mutation in the dual oxidase maturation factor 2 gene are associated with congenital hypothyroidism (25-27). WES technology is an effective method for identifying potential causative genes in disease phenotypes. In the present study,

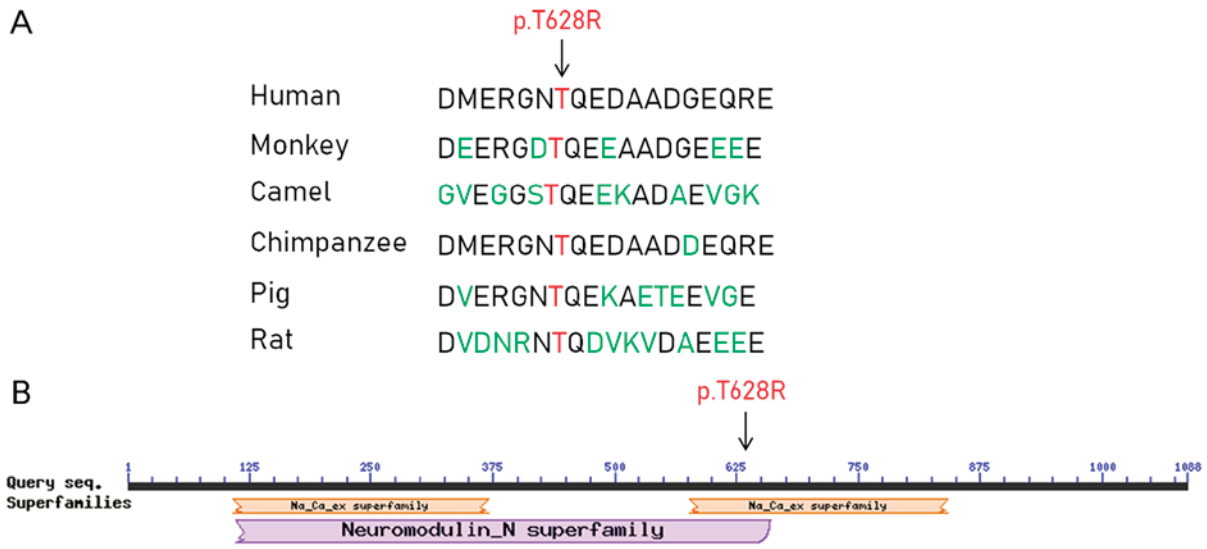

Figure 3. (A) Affected amino acid residue was highly conserved between different species. (B) Conserved domains in apolipoprotein B receptor.

WES was performed to identify potential causative genes in the affected individual, his parents, maternal aunt and maternal grandmother in a hypothyroidism pedigree. Two mutations (c.G7192TT and c.C1883G) in the FASN and APOBR genes, respectively, were revealed to be associated with hypothyroidism. In addition, Sanger sequencing validated these FASN and APOBR mutations in the proband and his mother. Furthermore, the mutations were fully co-segregated with established hypothyroidism phenotypes in the family.

Fatty acid synthesis is a process that entails producing *de novo* fatty acids from carbohydrate- and amino acid-derived carbon sources. The liver serves a key role in fatty acid modulation. The key enzymes in the lipogenic and lipolytic pathways are regulated by thyroid hormones in the liver and adipose tissues (28,29). Disrupted thyroid hormone levels alter hepatic fatty acid composition (30). In addition, it has been reported that subclinical and clinical hypothyroidism are associated with hepatic steatosis (31). Numerous studies have indicated that hypothyroidism is a risk factor for nonalcoholic fatty liver disease and results in metabolic syndrome (32-34). In addition, the effects of hypothyroidism to enhance lipogenesis is amplified in the presence of physiological concentrations of fatty acids (35). FASN is an enzyme involved in fatty acid biosynthesis (36). In mammals, cells manufacture *de novo* fatty acids using different pathways, which synthesize fatty acids from acetyl and malonyl esters of CoA that are catalyzed by dimerized FASN (37). Furthermore, fatty acid synthesis is controlled by FASN (38), and the downregulation of FASN protein levels causes markedly decreased regulation of *de novo* lipogenesis (30). Notably, FASN is downregulated in the livers of patients with hypothyroidism (39). In addition, a microarray analysis revealed that FASN, one of the hepatic target genes, is preferentially activated by triiodothyronine during transition from the hypothyroid state to the euthyroid state (40). The c.G7192T mutation is located in the conserved α/β-hydrolase fold of FASN and was highly conversed among several diverse species, including humans, rats, chimpanzees,

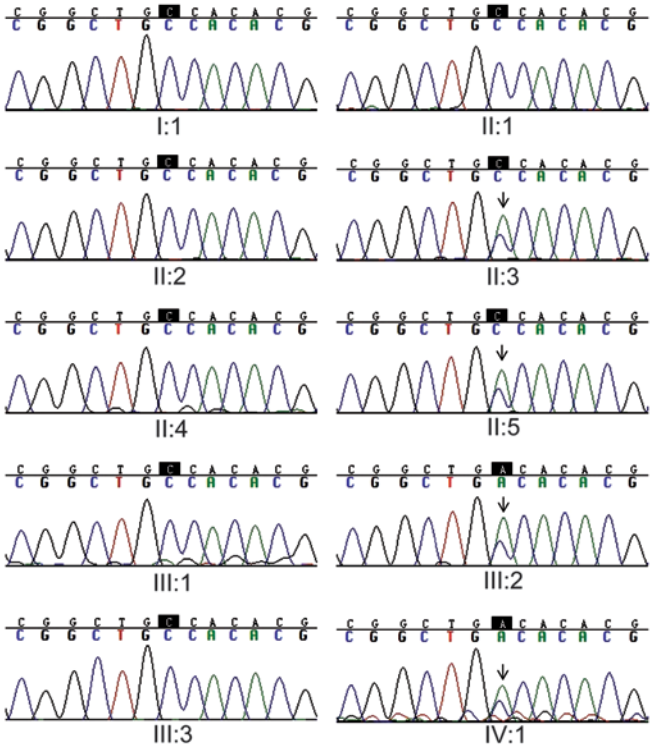

Figure 4. Sanger validation results of fatty acid synthase variants in ten family members of the hypothyroidism pedigree. Arrow represents the mutation site.

monkeys, cows and goats. Therefore, this FASN mutation suggested that FASN may have roles in hypothyroidism.

APOBR is an apolipoprotein E-independent receptor that binds APOB48, allowing cells to uptake postprandial triglyceride-rich lipoproteins, for which APOBR has a high affinity; APOBR functions as a nutritional receptor that provides dietary fatty acids and lipid-soluble vitamins to cells (41). The APOBR protein is distributed in the moieties of plasma triglyceride-rich lipoproteins. It has been reported that APOBR is an important molecule in lipid metabolism,

Table III. Clinical information of 20 additional, unrelated individuals with primary hypothyroidism.

Case	Sex	Age (years)	TSH (mU/l)	FT4 (pmol/l)
1	Male	75	27.7	7.31
2	Male	49	4.85	16.2
3	Male	47	72	0.88
4	Male	67	9.15	10.7
5	Male	47	1.71	15.3
6	Female	64	3.07	15.7
7	Female	31	6.21	12.3
8	Female	21	5.56	11.5
9	Female	29	1.59	10.3
10	Female	25	5.52	13.2
11	Female	27	4.56	12.7
12	Female	31	3.97	16
13	Female	32	2.57	10
14	Female	68	37	7.08
15	Female	54	5.08	11.5
16	Female	30	57.5	7.16
17	Female	36	4.3	12.2
18	Female	29	0.969	11.4
19	Female	35	9.74	12.4
20	Female	27	4.26	11.5

FT4, free thyroxine; TSH, thyroid-stimulating hormone.

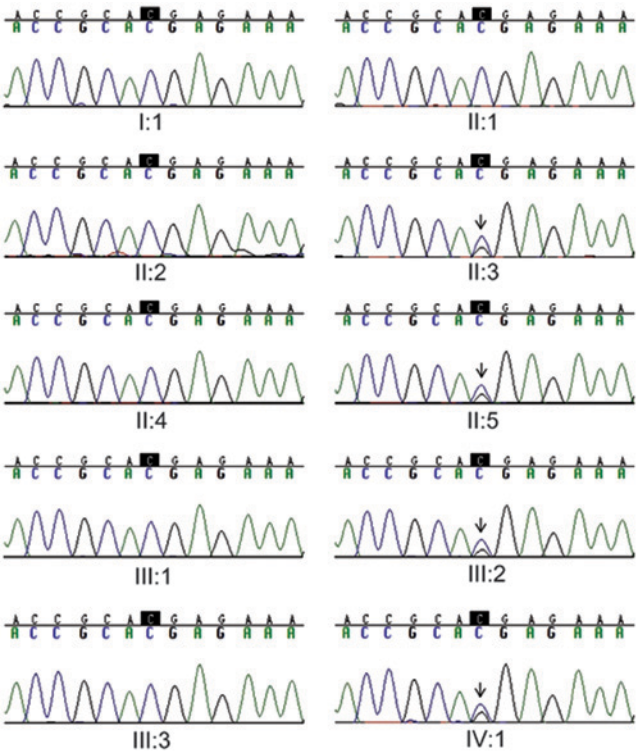

Figure 5. Sanger validation results of apolipoprotein B receptor variants in ten family members of the hypothyroidism pedigree. Arrow represents the mutation site.

and that it primarily serves roles in the sterol transport and metabolism pathways (42). Mutations in the APOBR gene (c.934-960/del and A419P) have been identified as novel hyperlipidemia-associated variants, which may be involved in regulating plasma total cholesterol levels in patients with hypercholesterolemia (43). APOBR is also involved in atherogenesis, which is a lipid metabolism disorder (44). Therefore, it may be hypothesized that the APOBR pathway is a novel therapeutic target, particularly in hypertriglyceridemic patients, such as those with hypothyroidism. In the present study, a mutation (c.C1883G, p.T628R) in the APOBR gene was detected in patients with hypothyroidism. The mutation is located in the conserved Na^+/Ca^{2+} exchanger domain of APOBR and was highly conversed among different species, including humans, monkeys, camels, chimpanzees, pigs and rats. Therefore, this APOBR mutation may be considered another candidate molecule in the pathogenesis of hypothyroidism.

In conclusion, the findings reported here demonstrated that patients with mutations in FASN (c.G7192T, p.A2398S) and APOBR (c.C1883G, p.T628R) may be predisposed to hypothyroidism. These mutations may disrupt the regulation of fatty acid biosynthesis and lipid metabolism. These findings may reveal the high degree of genetic heterogeneity in hypothyroidism phenotypes. Future work will be performed to improve understanding of this disorder. In addition, there is a limitation of the present study; the identified SNVs, FASN (c.G7192T, p.A2398S) and APOBR (c.C1883G, p.T628R), require validation in animal models of hypothyroidism.

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Availability of data and materials

All data generate or analyzed during the present study are included in this published article.

Authors' contributions

JS, LS, WC, XY and YL analyzed and interpreted the data. JS was a major contributor in writing the manuscript. QJ designed the project. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from all individuals enrolled in this study. In addition, the present study was approved by the ethical approval committee of Jinan Central Hospital Affiliated to Shandong University (2016-053-01).

Patient consent for publication

Informed written consent was obtained from all subjects.

Competing interests

The authors declare that they have no competing interests.

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