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Case Report

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Whole-exome Sequencing Analysis of a Japanese Patient With Hyperinsulinemia and Liver Dysfunction

Shingo Fujita,[1] Emi Horitani,[1] Yohei Miyashita,[2] Yukari Fujita,.[1,3] Kenji Fukui,[1] Yoshihiro Kamada,[4] Ikuo Mineo,[5] Yoshihiro Asano,[6] Hiromi Iwahashi,[1,5,7] Junji Kozawa,[1,7] and Iichiro Shimomura[1]

[1]Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, Suita, Osaka, 565-0871, Japan
[2]Department of Legal Medicine, Graduate School of Medicine, Osaka University, Suita, Osaka, 565-0871, Japan
[3]Department of Community Medicine, Graduate School of Medicine, Osaka University, Suita, Osaka, 565-0871, Japan
[4]Department of Advanced Metabolic Hepatology, Graduate School of Medicine, Osaka University, Suita, Osaka, 565-0871, Japan
[5]Diabetes Center, Toyonaka Municipal Hospital, Toyonaka, Osaka, 560-8565, Japan
[6]Department of Cardiovascular Medicine, Graduate School of Medicine, Osaka University, Suita, Osaka, 565-0871, Japan
[7]Department of Diabetes Care Medicine, Graduate School of Medicine, Osaka University, Suita, Osaka, 565-0871, Japan

Correspondence: Junji Kozawa, MD, PhD, Department of Diabetes Care Medicine, Graduate School of Medicine, Osaka University, 2-2-B5 Yamada-oka, Suita, Osaka, 565-0871, Japan. Email: kjunji@endmet.med.osaka-u.ac.jp.

Abstract

Hyperinsulinemia is often observed in obese subjects because of insulin resistance, but it may occur in nonobese subjects with unknown etiology. A 72-year-old man was admitted to our hospital for the examination of hyperinsulinemia, reactive hypoglycemia, and liver dysfunction. The patient's body mass index was 23.7 kg/m^2, but he had an elevated visceral fat area (125 cm^2). His laboratory data showed mildly elevated liver enzymes, whereas plasma fasting glucose and serum insulin levels were 91 mg/dL and 52.3 µU/mL, respectively. In a 75-g oral glucose tolerance test, the serum insulin level reached the highest value of 1124 µU/mL at 180 minutes. There was no obvious etiology except for mild liver steatosis shown by liver biopsy. We suspected genetic abnormalities related to hyperinsulinemia. We performed whole-exome sequencing (WES) analyses and identified a heterozygous nonsense variant p.R924X in the insulin receptor (*INSR*) gene, a novel heterozygous missense variant p.V416M in the *AKT1* gene, and a novel hemizygous missense variant p.R310Q in the *PHKA2* gene, which is the causative gene of hepatic injury as glycogen storage disease type IX. It was speculated that the *INSR* gene variant, in addition to visceral fat accumulation, was the main cause of hyperinsulinemia and reactive hypoglycemia, and the remaining 2 variants were also partly responsible for hyperinsulinemia. WES analysis revealed candidate gene variants of hyperinsulinemia and hepatic-type glycogenosis. Thus, WES analysis may be a useful tool for clarifying the etiology when unexplained genetic pathophysiological conditions are suspected.

Key Words: whole exome sequencing, hyperinsulinemia, insulin receptor gene, glycogen storage disease

Abbreviations: CADD, combined annotation-dependent depletion; GSD, glycogen storage disease; *INSR*, insulin receptor; NMD, nonsense-mediated mRNA decay; OGTT, oral glucose tolerance test; WES, whole-exome sequencing; WGS, whole-genome sequencing

Insulin concentration is determined by insulin secretion and insulin resistance [1]. Hyperinsulinemia is often observed because of the deterioration of insulin sensitivity in patients with visceral fat accumulation, whereas it is rarely present in nonobese patients [2]. Several specific conditions (eg, insulinomas, insulin autoimmune syndrome, type B insulin resistance syndrome) can also cause hyperinsulinemia [3, 4]. In addition to these acquired diseases, genetic abnormalities, such as insulin receptor (*INSR*) gene variants, can also cause hyperinsulinemia (eg, Donohue syndrome, Rabson-Mendenhall syndrome, type A insulin resistance syndrome) [5-7]. In our previous report, 0.4% of nonobese healthy Japanese subjects (body mass index, < 25 kg/m^2) had hyperinsulinemia with serum fasting immunoreactive insulin ≥ 15 µU/mL [8]. In addition, 2 of 11 nonobese subjects with hyperinsulinemia, whose *INSR* genes have been analyzed, had novel heterozygous nonsense variants [8], suggesting that *INSR* gene variants may be one of the causes of hyperinsulinemia. However, other genetic factors related to hyperinsulinemia have not been well examined.

In this article, we report a case of significant hyperinsulinemia with reactive hypoglycemia and liver dysfunction, whose genetic assessment was performed by using whole-exome sequencing (WES).

Case Report

A 72-year-old man was admitted to our hospital for examination of hyperinsulinemia, reactive hypoglycemia, and liver dysfunction, which is a mild elevation in transaminases. He was diagnosed with mild liver dysfunction in his 30s, and abdominal ultrasonography revealed mild fatty liver at 52 years of age. He became aware of hypoglycemic symptoms (cold sweat and hand tremor) 4 to 5 times a year at approximately

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60 years of age. At the age of 66 years, blood examination showed hyperinsulinemia with 7% hemoglobin A1c, 89 mg/dL fasting blood glucose, and 34.8 µU/mL serum fasting immunoreactive insulin. After an overnight fast, a 75-g oral glucose tolerance test (OGTT) was performed for 5 hours, and as a result, he was found to have reactive hypoglycemia (53 mg/dL) (Ademolus Classification of Hypoglycemia grade 2 hypoglycemia) [9] at 5 hours. An abdominal dynamic enhancement computed tomography scan detected no space-occupying lesion in the pancreas, suggesting that insulinoma was unlikely in this case. There was no history of gastrectomy.

The patient was 162.8 cm tall and weighed 63 kg (body mass index, 23.7 kg/m^2) on admission. He had mild obesity with visceral fat accumulation, which was defined based on Japanese criteria (visceral fat area, 125 cm^2 [normal range < 100 cm^2]; abdominal circumference, 87.8 cm [normal range < 85cm in men]) [10]. His blood pressure was 113/55 mm Hg, and his pulse rate was 52/min, which was sinus bradycardia. Radiography of the thorax and electrocardiograph revealed normal findings. No signs of acanthosis nigricans or hypertrichosis were noted. The results of the laboratory tests are summarized in Table 1. He had mildly elevated liver enzymes (37 IU/mL aspartate aminotransferase, 50 IU/mL alanine aminotransferase, and 70 IU/mL γ-glutamyltransferase). His hemoglobin A1c level was 5.9%, and his plasma fasting glucose and serum insulin levels were 91 mg/dL and 52.3 µU/mL, respectively. He showed no abnormalities in fasting glucagon, ACTH, cortisol, TSH, free T3, or free T4 levels. His anti-insulin antibody and anti-insulin receptor antibody were both negative. There was no evidence of viral hepatitis or autoimmune liver diseases. A 75-g OGTT was performed again for 5 hours, and the insulin level reached the highest value of 1124 µU/mL at 180 minutes; however, hypoglycemia was not observed in this examination (Table 2). Abdominal ultrasonography revealed no findings of liver cirrhosis. His family history was unremarkable, including for diabetes. He drank 1 to 2 cups of alcohol per day and had a history of smoking 30 cigarettes a day for 35 years. Carotid artery echocardiography examination showed the findings of atherosclerosis (maximum intima-media thickness: 3.0 mm). The patient had persistent liver dysfunction since his 30s and a high NAFIC score of 4 (394 ng/mL ferritin [≥300] [male], 52.3 µIU/mL immunoreactive insulin [≥10], and 5.3 ng/mL type IV collagen 7S [≥5]) [11]. He was suspected of having nonalcoholic steatohepatitis and underwent liver biopsy at 72 years old, which revealed mild steatosis.

There was no obvious etiology for hyperinsulinemia, and we suspected genetic abnormalities related to insulin or insulin signaling as the cause of hyperinsulinemia. We performed WES analyses after obtaining written and signed informed consent from the patient. The Osaka University Research Ethics Committee approved this study procedure on June 1, 2016 (approval number: 702). Blood samples were collected from the patient. Genomic DNA was extracted from whole venous blood using a QIAamp DNA Blood Minikit (Qiagen, Hilden, Germany). WES analysis was performed using the Agilent SureSelect Human All Exon V6 (Agilent Technologies, Santa Clara, CA), and 100-bp paired-end reads were performed using the Illumina HiSeq 3000 platform (Illumina, Inc., San Diego, CA). Image analysis, base calling, and demultiplexing were performed using Illumina bcl2fastq2 Conversion Software v2.20. FASTQ files were quality checked using FASTQC, and low-quality reads were removed using Trimmomatic-0.36. Read alignment was performed using standard parameterized BWA v0.7.17 for human genome assembly hg19 (GRCh37). Single nucleotide variants or short indels were called according to GATK best practice (GATK4.0.3). Called variants were filtered using GATK Variant Filtration, and variants that met the following conditions were analyzed: QD < 2.0, FS > 60.0, MQ < 40.0, MQRankSum ≤ 12.5, ReadPosRankSum ≤ 8.0, and SQR > 4.0. The annotation information was then added to the obtained variant list using Annovar.

We examined 148 candidate genes potentially involved in hyperinsulinemia and diabetes mellitus [12]. The 148 genes were extracted based on the following report: category A, genes associated with the insulin signaling pathway in the Kyoto Encyclopedia of Genes and Genomes database (https://www.kegg.jp/), and genes in the insulin signaling pathways reported by Kadowaki et al [13]; category B, genes involved in transcriptional regulation of pancreatic beta cells reported by Flannick et al [14]; category C, genes involved in serum insulin levels or diabetes susceptibility by genome-wide association studies reported by Flannick et al [14]; and category D, genes responsible for congenital hyperinsulinemia by clinical practice guidelines for congenital hyperinsulinism [15]. We then identified 162 variants in exons of these genes, and among these variants, 70 were found to be of high pathological significance, including the stop codon and missense variants (stop codon, N = 1; nonsynonymous, N = 69) (Table 3), whereas the remaining 92 variants are shown elsewhere [12]. These nonsynonymous variants contained 15 variants with a combined annotation-dependent depletion (CADD) score of 20 or more, which suggested pathogenicity in the highest 1% [16] and has also been used as the pathogenic cutoff value in previous reports [17, 18] (https://cadd.gs.washington.edu/). Two of these 15 variants, in addition to a stop codon variant described previously, are variants with low frequency in the Human Genetic Variation Database (https://www.hgvd.genome.med.kyoto-u.ac.jp/) and Tohoku Medical Megabank Organization (https://jmorp.megabank.tohoku.ac.jp/202102/) databases with minor allele frequency < 0.01, which has been often used as a cutoff value for filtering against common variants in previous reports [19, 20]. Finally, we demonstrated that the patient had the following 3 heterozygous variants: c.2770C > T (p.R924X) in the *INSR* gene; c.1246G > A (p.V416M) in the *AKT1* gene; and c.929G > A (p.R310Q) in the *PHKA2* gene (Fig. 1A). Furthermore, confirmatory Sanger sequencing was performed on PCR (Table 4, Fig. 1B).

A heterozygous nonsense variant (c.2770 C > T [p.R924X], rs387906538) at exon 14 in the *INSR* gene is rare with minor allele frequency 0 in the generic and Tohoku Medical Megabank Organization databases and had a high CADD score of 43, suggesting that it is highly pathogenic. A novel heterozygous missense variant (c.1246 G > A [p.V416M]) at exon 13 in the *AKT1* gene with no single nucleotide polymorphism registration had a high CADD score of 25.6, suggesting that it is also pathogenic. A novel heterozygous missense variant (c.929 G > A [p.R310Q]) at exon 10 in the *PHKA2* gene with no single nucleotide polymorphism registration also had a high CADD score of 32. The *PHKA2* gene is a major causative gene in approximately 75% of hepatic glycogen storage disease (GSD) IX alpha [21]. The *PHKA2* gene is located on the X gene, which is the sex chromosome, and when a variant is found in males, this variant is considered a hemi variant, suggesting that it is highly pathogenic.

Table 1. Results of laboratory tests

CBC			Blood chemistry			Urinalysis		
WBC	4680	/μL (3300-9400)	Alb	4.1	g/dL (3.6-4.7)	Specific gravity	1.015	
RBC	443×10^4	/μL ($443\text{-}560 \times 10^4$)	Na	141	mEq/L (138-145)	Urobilinogen	(±)	
Hb	14.1	g/dL (13.8-17.0)	K	4.4	mEq/L (3.6-4.8)	Bilirubin	(-)	
Plt	29.4×10^4	/μL ($13\text{-}32 \times 10^4$)	Cl	108	mEq/L (100-108)	Protein	(-)	
			Ca	9.0	mg/dL (8.4-10)	Occult blood	(-)	
Virus markers			P	3.3	mg/dL (2.9-4.8)	Ketone body	(-)	
HBs antigen		(-)	UN	17	mg/dL (7-22)	Glucose	(-)	
HCV antibody		(-)	Cr	0.99	mg/dL (0.6-1.2)	WBC	(-)	
HCV-RNA		(-)	UA	7.7	mg/dL (3.6-7.2)	RBC	0-1/HPF	
			T.Bil	0.9	mg/dL (0.2-1.2)	Granular casts	(-)	
Antibody			AST	37	U/L (<40)	Epithelial casts	(-)	
ANA		(-)	ALT	50	U/L (<40)			
AMA		20<	γGTP	70	U/L (12-69)	**Endocrine**		
AMA-M2 titer		1.5 < IU/mL	ALP	188	U/L (134-359)	GH	0.35	ng/mL (0-2.47)
Insulin antibody		0.4<	CK	84	U/L (54-286)	ACTH	23	pg/mL (7-63)
			Glucose	91	mg/dL (70-110)	Cortisol	8.3	μg/dL (4-18.3)
Tumor markers			HbA1c	5.9	% (4.6-6.2)	Glucagon	141	pg/mL (70-174)
CEA	1	ng/mL (0-35.4)	CRP	0.08	mg/dL (0-0.2)	Adrenalin	0.04	ng/mL (<0.17)
CA19-9	9	U/mL (0-5.0)	TG	140	mg/dL (30-150)	Noradrenalin	0.43	ng/mL (0.15-0.57)
PIVKA-II	16	mAU/mL (<6)	HDL-Cho	49	mg/dL(40-80)	TSH	1.47	μU/L (0.45-3.72)
AFP	5	ng/mL (0-7)	LDL-Cho	110	mg/dL (<140)	FT4	1.0	ng/dL (0.8-1.7)
			Fe	159	μg/L (55-157)	FT3	2.2	pg/mL (2.1-3.1)
Coagulation			Ferritin	394	ng/mL (46-332)	IRI	52.3	μIU/mL (1.1-9.0)
PT	93	% (70-125)	Type IV collagen7S	5.3	ng/mL (<6)	C peptide	3.0	ng/mL (0.7-2.5)
APTT	31	Second (24-39)						

Abbreviations: Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMA, anti-mitochondrial antibody; AMA-M2, anti-mitochondrial antibody M2; ANA, anti-nuclear antibody; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Ca, calcium; CBC, complete blood count; CK, creatine kinase; Cl, chlorine; Cr, creatinine; CRP, C-reactive protein; FDP, fibrinogen degradation product; Fe, iron; FT3, free triiodothyronine; FT4, free thyroxine; Hb, hemoglobin; HbA1c, hemoglobin A1c; HDL-Cho: high-density lipoprotein cholesterol; IRI, immunoreactive insulin; K, potassium; LDL-Cho, low-density lipoprotein cholesterol; Na, sodium; P, phosphorus; Plt, platelet; PT, prothrombin time; RBC, red blood cells; T.Bil: total bilirubin; TG, triglyceride; UA, uric acid; WBC, white blood cell; γGTP, γ-glutamyltransferase.

Table 2. The results of 75-g oral glucose tolerance tests

66 years old								
Time (min)	**0**	**30**	**60**	**90**	**120**	**180**	**240**	**300**
Glucose (mg/dL)	82	152	170	194	145	111	85	53
IRI (μU/mL)	19.9	161	197	439	350	373	141	39
C-peptide (ng/mL)	1.6	5.7	7.8	13.4	11.8	12	7	3.4
On admission								
Time (min)	0	30	60	120	180	240	300	
Glucose (mg/dL)	80	157	186	210	159	148	91	
IRI (μU/mL)	34.2	483	415	1071	1124	907	440	
C-peptide (ng/mL)	2.2	11.9	12.3	21.5	23.9	17.5	11.4	
1 y after treatment								
Time (min)	0	30	60	120	180	240		
Glucose (mg/dL)	94	172	158	108	130	144		
IRI (μU/mL)	32.7	591.5	536.4	310.6	387.6	438.5		
C-peptide (ng/mL)	2.7	15.6	14.5	10.7	13.2	13.7		

Abbreviation: IRI, immunoreactive insulin.

Table 3. Variants of high pathological significance, including stop codon and missense variants

Chromosome	Position	Abbreviated gene	Reference base	Variant base	Nature of variant	CADD Phred	HGVD	ToMMo
19	7132241	INSR	G	A	Stop gain	43	N/A	N/A
X	18956857	PHKA2	C	T	Nonsynonymous	32	N/A	N/A
2	56420296	CCDC85A	C	T	Nonsynonymous	31	0.194376	0.2034
19	33878977	PEPD	C	T	Nonsynonymous	29.8	0.082634	0.1057
20	25259006	PYGB	G	T	Nonsynonymous	26.5	0.32254	0.3133
2	56420006	CCDC85A	A	T	Nonsynonymous	26.2	0.195598	0.2041
12	121416650	HNF1A	A	C	Nonsynonymous	25.6	0.495386	0.4879
14	105238716	AKT1	C	T	Nonsynonymous	25.6	N/A	N/A
7	113518434	PPP1R3A	C	A	Nonsynonymous	24.7	0.677686	0.6878
17	2268311	SGSM2	G	A	Nonsynonymous	24.7	0.828534	0.8288
4	6302889	WFS1	G	A	Nonsynonymous	23.8	0.11249	0.106
2	135744007	MAP3K19	T	C	Nonsynonymous	23.4	0.320889	0.3408
8	118184783	SLC30A8	C	T	Nonsynonymous	23.2	0.404229	0.4296
9	3856011	GLIS3	T	C	Nonsynonymous	23.1	0.018182	0.0193
20	25262769	PYGB	G	A	Nonsynonymous	21.7	0.315789	0.3126
2	165476253	GRB14	A	T	Nonsynonymous	19.79	0.413679	0.4347
17	2266799	SGSM2	G	A	Nonsynonymous	19.13	0.838695	0.8293
2	135744416	MAP3K19	C	G	Nonsynonymous	16.6	0.323554	0.3405
17	64783081	PRKCA	G	A	Nonsynonymous	16.45	1	1
2	56411817	CCDC85A	C	T	Nonsynonymous	16.3	0.876162	0.8981
19	49481243	GYS1	T	C	Nonsynonymous	16.06	0.1	0.099
9	97369149	FBP1	C	T	Nonsynonymous	15.69	0.99944	0.9999
12	121435427	HNF1A	G	A	Nonsynonymous	15.32	0.550977	0.5436
1	219383905	LYPLAL1	A	G	Nonsynonymous	14.86	0.999587	1
9	97349666	FBP2	C	G	Nonsynonymous	13.87	0.88401	0.8706
19	18272190	PIK3R2	A	C	Nonsynonymous	12.64	0.945336	0.9348
15	62221745	VPS13C	T	A	Nonsynonymous	12.43	0.003719	0.0055
4	6303354	WFS1	G	A	Nonsynonymous	11.69	0.85	0.8633
14	23771734	PPP1R3E	T	C	Nonsynonymous	11.15	N/A	0.0503
2	135740863	MAP3K19	C	A	Nonsynonymous	10.95	N/A	0.3408
4	108931039	HADH	T	C	Nonsynonymous	10.8	0.966306	0.9637
11	47306585	MADD	G	A	Nonsynonymous	10.54	0.593388	0.6037

Table 3. Continued

Chromosome	Position	Abbreviated gene	Reference base	Variant base	Nature of variant	CADD Phred	HGVD	ToMMo
6	34824107	UHRF1BP1	A	C	Nonsynonymous	10.45	0.226971	0.2276
13	75884216	TBC1D4	C	T	Nonsynonymous	10.45	0.757903	0.7455
11	73689104	UCP2	G	A	Nonsynonymous	10.13	0.472727	0.4851
6	34824636	UHRF1BP1	A	G	Nonsynonymous	8.907	0.335275	0.3195
15	62456358	C2CD4B	A	C	Nonsynonymous	7.72	0.527372	0.5277
15	62259637	VPS13C	C	T	Nonsynonymous	7.541	0.890668	0.8795
11	47346145	MADD	G	A	Nonsynonymous	6.931	0.594982	0.6039
6	5086558	PPP1R3G	C	A	Nonsynonymous	6.717	0.905394	0.9024
12	121437382	HNF1A	A	G	Nonsynonymous	5.414	0.995439	0.9994
2	182543455	NEUROD1	T	C	Nonsynonymous	5.076	0.921009	0.9195
4	6302519	WFS1	G	A	Nonsynonymous	5.055	0.997521	0.9987
11	72408055	ARAP1	G	C	Nonsynonymous	4.928	0.14329	0.1589
13	23898664	SGCG	A	G	Nonsynonymous	4.804	1	1
1	99156634	SNX7	T	A	Nonsynonymous	4.611	0.325142	0.3635
20	56137895	PCK1	G	C	Nonsynonymous	3.717	0.744215	0.7171
2	135781035	MAP3K19	C	T	Nonsynonymous	3.46	N/A	0.2363
7	113519796	PPP1R3A	C	T	Nonsynonymous	3.389	0.99876	1
12	21713402	GYS2	T	C	Nonsynonymous	2.948	0.80976	0.8277
2	169764176	G6PC2	G	C	Nonsynonymous	2.779	0.442562	0.4519
10	71060610	HK1	A	G	Nonsynonymous	1.781	0.994628	0.9987
19	18266699	PIK3R2	C	T	Nonsynonymous	1.285	0.034971	0.0356
9	4118111	GLIS3	G	T	Nonsynonymous	0.622	0.902663	0.8697
15	62226423	VPS13C	T	G	Nonsynonymous	0.502	0.044538	0.0628
19	7293898	INSR	G	C	Nonsynonymous	0.499	0.999396	1
12	121437221	HNF1A	T	C	Nonsynonymous	0.398	N/A	0.9058
1	229772693	URB2	T	G	Nonsynonymous	0.095	0.82438	0.8435
12	121435475	HNF1A	G	A	Nonsynonymous	0.078	0.553853	0.5442
X	107976940	IRS4	G	C	Nonsynonymous	0.028	0.664463	0.7348
1	113456546	SLC16A1	A	T	Nonsynonymous	0.02	0.657438	0.6696
19	18273047	PIK3R2	T	C	Nonsynonymous	0.006	0.96099	0.9565
9	4118208	GLIS3	A	G	Nonsynonymous	0.004	0.999108	1
5	55407542	ANKRD55	C	T	Nonsynonymous	0.002	0.328784	0.3426
7	113518502	PPP1R3A	A	T	Nonsynonymous	0.002	0.999174	1
7	113519719	PPP1R3A	A	T	Nonsynonymous	0.002	0.997107	1
2	135744356	MAP3K19	G	A	Nonsynonymous	0.001	0.02686	0.0365
9	139222174	GPSM1	T	C	Nonsynonymous	N/A	0.997839	1
10	71052083	HK1	C	T	Nonsynonymous	N/A	.	0.7
14	24567498	PCK2	A	C	Nonsynonymous	N/A	0.999586	1

HGVD, Human Genetic Variation Database; N/A, not available; ToMMo, Tohoku Medical Megabank Organization.

We stained the liver biopsy tissue sample, which was obtained at the age of 72, and retrospectively confirmed abundant glycogen accumulation based on positive periodic acid–Schiff staining and negative diastase digestion periodic acid–Schiff staining, consistent with hepatic glycogenosis (Fig. 2).

For treatment, the patient was instructed to avoid excessive carbohydrate intake and consume divided meals. He started treatment with metformin, and the dose was increased to 1000 mg/day. In the 75-g OGTT after 1 year of treatment, the peak immunoreactive insulin level reached 30 minutes earlier than before the treatment, and the level decreased from 1124 to 591.5 µU/mL. In addition, the peak glucose level decreased, suggesting that his insulin sensitivity was improved. The results of the 75-g OGTTs are shown in Table 2. Since discharge, he has experienced no episode of reactive hypoglycemia.

Discussion

We performed WES analysis in a patient with hyperinsulinemia and liver dysfunction, and we identified a heterozygous nonsense variant in the *INSR* gene, a novel heterozygous missense variant in the *AKT1* gene, and a novel hemizygous missense variant in the *PHKA2* gene. The *INSR* gene variant, in addition to visceral fat accumulation, is considered the main cause of hyperinsulinemia, and the remaining 2 variants may also be partly responsible for hyperinsulinemia in this case. WES analysis may be a valuable

A

Gene	Variant			Allele frequency		CADD score	SNP No.
	Nucleotide	Amino acid		HGVD	ToMMo		
INSR	c.2770C>T	p.R924X	Hetero	N/A	N/A	43	rs387906538
AKT1	c.1246G>A	p.V416M	Hetero	N/A	N/A	25.6	No registration
PHKA2	c.929G>A	p.R310Q	Hemi	N/A	N/A	32	No registration

HGVD, Human Genetic Variation Database; ToMMo, Tohoku Medical Megabank Organization; CADD, Combined Annotation-Dependent Depletion.

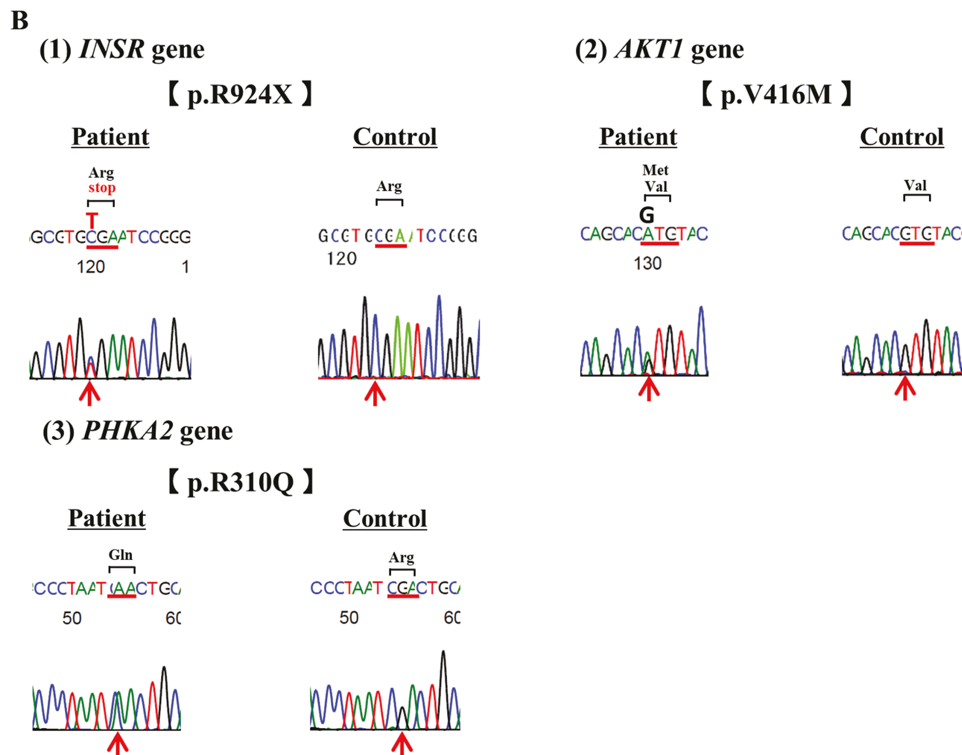

Figure 1. Variants in the patient. (A) Genetic characteristics of the variants detected by WES analysis. (B) Sanger sequencing confirmed detected variants in the patient. The red arrow points to the variant sites.

tool for clarifying the etiology when an unexplained genetic pathophysiological condition is suspected.

In this case, there was a heterozygous nonsense variant in the *INSR* gene. The mechanism of hyperinsulinemia in the *INSR* gene variant is as follows: a variant in the insulin receptor decreases the expression or function of the insulin receptor and induces insulin resistance, resulting in compensative excessive insulin secretion from pancreatic beta cells [5]. The variant found in the patient was a heterozygous nonsense variant, which is rare and pathogenic based on the CADD values. Previously, Kadowaki et al [22] also reported a patient with Donohue syndrome who had a compound heterozygote for 2 cis-acting dominant variants in the *INSR* gene. The paternal allele had a variant in c.2770C > T (p.R924X), which was the same as the one found in our case, resulting in decreased levels of insulin receptor mRNA [22]. Our previous report found that 2 of 11 nonobese healthy subjects with hyperinsulinemia had a heterostop codon in the *INSR* gene [8]. These stop codons should result in nonsense-mediated mRNA decay (NMD). NMD is a widely known mRNA quality control mechanism by which the mRNA with a premature stop codon is degraded, preventing the production of truncated abnormal proteins [23]. Thus, we considered that the 2 stop codons lead to the decreased function of insulin receptor by NMD, which might be related to insulin resistance. These results suggested that the variant in the *INSR* gene may be one of the main causes of hyperinsulinemia in this case.

We also found a novel heterozygous nonsynonymous variant in the *AKT1* gene, which is located downstream of insulin signaling [24]. There are 3 subtypes of the *AKT* gene (*AKT1*, *AKT2*, and *AKT3*). *AKT2* is mainly involved in glucose metabolism [24, 25], whereas *AKT1* is mainly involved in cell proliferation [26, 27]. In humans, there are no

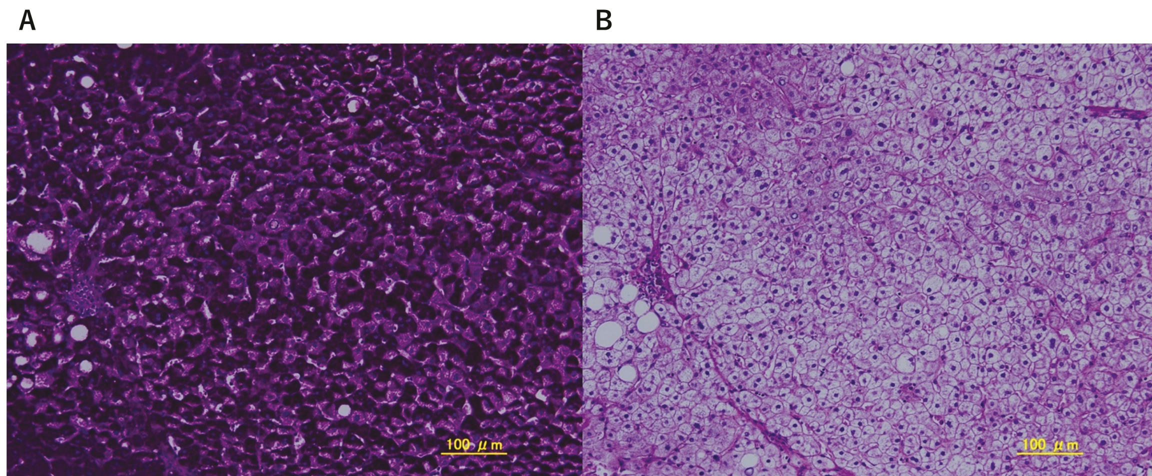

Figure 2. Histopathological examination of liver biopsy. (A) PAS staining (×400). (B) D-PAS staining (×400). The hepatocytes showed marked accumulation of glycogen with positive PAS staining and negative PAS staining after diastase digestion.

Table 4. Primer sequences

Gene	Primers	Sequence (5′->3′)
INSR	Forward	TGGACACTCCCAGATGTGCA
	Reverse	ACCATGCTCAGTGCTAAGCA
AKT1	Forward	GCCCTACATCACAGGAGGAA
	Reverse	GCGTGAGTGTGGATATGTGG
PHKA2	Forward	CCCATGAGGCACAATGGTAT
	Reverse	ACACCCAGCATTTTCTCACC

Confirmatory Sanger sequencing was performed on PCR. Primer designs were designed using Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/). After initial denaturation for 5 min at 95°C, PCR was performed (37 cycles: 30 s at 95°C, 30 s at 5 °C, and 60 s at 72°C) using Ex-Taq DNA polymerase (Takara Shuzo Co., Biomedical Group, Shiga, Japan). PCR products were sequenced after purification with a QIA quick PCR Purification kit (Qiagen, Hilden, Germany). The sequencing reaction was performed using an ABI Prism dye terminator cycle sequencing kit (Applied Biosystems), and the products were analyzed on an ABI gene analyzer 1100 system according to the manufacturer's protocol (Applied Biosystems).

reports that suggest a relationship between hyperinsulinemia and the *AKT1* gene variants. However, 1 report has suggested the relationship between the development of type 2 diabetes mellitus and the *AKT1* gene variants [28]. In addition, a previous report has found that islets isolated from homozygous *AKT1*-deficient mice show significantly higher glucose-responsive insulin secretion than islets isolated from wild-type mice [29], suggesting that the novel variant in the *AKT1* gene may be partially responsible for postprandial hyperinsulinemia.

A novel hemizygous variant p.R310Q in the *PHKA2* gene was detected in this case. *PHKA2* gene is a causative gene of hepatic GSD type IX. Because this disease is an X-linked condition, symptoms often occur in males. Patients with GSD type IX may present with hepatomegaly, ketonic hypoglycemia, and elevated liver transaminases [30], whereas these clinical and biochemical abnormalities gradually disappear, and most adult patients are asymptomatic [31]. Thus, GSD type IX has been regarded as a benign condition with minimal complications; however, patients with liver cirrhosis have recently been reported [30, 31]. Therefore, it is recommended to follow patients even if they are asymptomatic [32].

The *PHKA2* gene is not only a causative gene of hepatic GSD but also one of the target genes for insulin [21]. In addition to the high value of the CADD score, the variant in this male case may be highly pathogenic as a hemi variant. Although no association between *PHKA2* variants and hyperinsulinemia has been reported, chronic hepatic injury derived from hepatic glycogenosis may be partly associated with insulin resistance, resulting in hyperinsulinemia in this case.

In this report, we used WES, whereas whole-genome sequencing (WGS) analysis is believed to be a better method for genetic analysis. WGS is the process of determining the entirety, or nearly the entirety, of the DNA sequence of an organism's genome at a single time. According to a previous report [33], the sequence coverage of WES target regions is not uniform. In addition, WES showed more batch effects/artifacts because of laboratory processing, so there was more significant variability between runs, between laboratories, and possibly between researchers preparing the libraries. As a result, WES was thought to be less reproducible than WGS. However, the overall concordance and correlation of results from WES and WGS were good, and the advantages of WES over WGS are that it is less expensive and has a better coverage/cost ratio. WES might be also superior to WGS because WGS has a huge amount of data, which is difficult to manage. These are the reasons why we applied WES in this case.

In conclusion, we performed WES analysis of a patient with hyperinsulinemia and hepatic dysfunction. We found a heterozygous nonsense variant in the *INSR* gene, a novel heterozygous missense variant in the *AKT1* gene, and a novel hemizygous missense variant in the *PHKA2* gene, which may contribute to hyperinsulinemia and reactive hypoglycemia. We also unexpectedly identified hepatic glycogen storage disease. Thus, WES analysis may a valuable tool for clarifying the etiology when an unexplained genetic pathophysiological condition is suspected.

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Disclosures

The authors have nothing to disclose.

Ethics Approval

The authors have read, understood, and—where appropriate—complied with the policies and guidelines on Ethical Issues.

Informed Consent

We performed whole-exome sequencing analyses after obtaining written and signed informed consent from the patient.

Data Availability

Some or all data generated or analyzed during this study are included in this published article or in the data repositories listed in Reference.

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