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A novel variant of the AGPAT2 mutation in generalized congenital lipodystrophy, detected by next generation sequencing

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CASE STUDY

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ABSTRACT

Inherited lipodystrophies are rare causes of young onset diabetes characterised by abnormal fat distribution with unique set of clinical features. We present a case of 24 year old lady with young onset diabetes mellitus, acromegaloid features, virilisation, hepatomegaly, hypertriglyceridemia with almost complete absence of subcutaneous and visceral adipose tissue as assessed by DXA scan body composition and MRI abdomen. Based on the clinical presentation, a diagnosis of Berardinelli–Seip generalized lipodystrophy was considered. Genetic analysis using next generation sequencing identified a novel homozygous insertion mutation in 1-acylglycerol-3-phosphate O-acyltransferase 2(AGPAT2) gene which was further confirmed with Sanger sequencing.

Key Words
Lipodystrophy, diabetes mellitus, AGPAT 2, next generation sequencing

Implications for Practice:

1. What is known about this subject?
Congenital generalised lipodystrophy (CGL) is a rare autosomal recessive lipodystrophy with a unique clinical phenotype and metabolic complications with only 500 individuals with this disorder being reported.

2. What is the key finding in this case report?
The Characteristic clinical presentation of CGL also known as Berardinelli–Seip generalized lipodystrophy and novel homozygous insertion mutation in 1-acylglycerol-3-phosphate-O-acyltransferase2 (AGPAT2) gene identified using next generation sequencing and further confirmed by Sanger sequencing.

3. What are the implications for future practice?
Genetic analysis using Next Generation Sequencing (NGS) may help in identifying the mutated genes in patients presenting with characteristic clinical phenotype of inherited lipodystrophies.

Background

Lipodystrophies represent a diverse group of congenital and acquired disorders which are characterized by a loss of adipose tissue which has a defined pattern. Congenital lipodystrophies may have an autosomal dominant or a recessive inheritance pattern. The lipodystrophies are further classified based on the pattern of fat loss as generalised (involving all fat depots), partial (involving mainly the limbs) or localized (involving discrete regions on abdomen or thigh).

There are four distinct subtypes of congenital generalised lipodystrophy (CGL): CGL1, CGL2, CGL3 and CGL4 associated with mutations in AGPAT2, BSCL2, CAV1 and PTRF respectively. The products of these genes have crucial roles in phospholipid and triglyceride synthesis and lipid droplets and caveolae formation within the adipocytes. Impairment of these functions results in ectopic triglyceride accumulation in liver and skeletal muscle due to inability to

store triglyceride in the adipose tissue leading to insulin resistance, dyslipidaemia and fatty liver. Congenital generalised lipodystrophy is a rare autosomal recessive inherited form of lipodystrophy with only 500 individuals with this disorder reported worldwide till date.[1]

Mutation in genes 1-acylglycerol-3-phosphate O-acyltransferase 2 (AGPAT2) and Seipen causes Type 1 and Type 2 congenital generalised lipodystrophies which are the most prevalent CGLs with their unique clinical phenotype and adipose tissue distribution.[2]

These patients present with features of insulin resistance since childhood in the form of acanthosis nigricans. Insulin resistance and hyperinsulinemia result in excessive growth of soft tissue which gives an acromegaloid appearance. They also develop abnormal fat distribution in the form of lipoatrophy along with the absence of visceral adipose tissue and muscular appearance during adolescence. Non Alcoholic Steatohepatitis due to fat deposition in liver followed by consequent cirrhosis of liver are also described. Females present with features of polycystic ovary syndrome (PCOS) in the form of hirsutism and in some cases virilisation. A very high degree of Insulin resistance in turn may present as Diabetes mellitus and Hypertriglyceridemia.[3]

We report the clinical profile of a young lady with congenital generalised lipodystrophy whose genetic analysis revealed a novel variant in the AGPAT2 gene.

Case details

A 24-year-old lady presented with oligomenorrhea and hirsutism of 9 years duration. She was detected to have diabetes mellitus, 2 years prior to presentation with an initial HbA1c of 7.4 per cent and had been on metformin. There was no history of acne, voice change, easy bruisability, proximal myopathy or ketoacidosis. She was the third child, born to a non-consanguineous parenthood, full term normal delivery with no perinatal complications and her developmental milestones were normal. There was no significant family history.

On examination, her height was 162cm with a BMI of 21.8kg/m^2. Acanthosis nigricans involving the neck, axilla and groin was present. She had an acromegaloid appearance with coarse facial features, macroglossia (Figure 1a), prominent supraciliary arches, prognathism and enlarged hands and feet. She was also noted to have hirsutism with a modified Ferriman Galleway score of 16/36, masculine body habitus and clitoromegaly (clitoral index-45mm^2). Her Tanner's staging of the breasts and pubic hair was 3 and 5 respectively. She had a prominent umbilicus (Figure 1b) and hepatomegaly.

Her biochemical profile was as follows: HbA1c-9 per cent (N<5.7), Total cholesterol-192mg/dL (N<160), Triglyceride serum-289mg/dL (N<150), HDL cholesterol-37mg/dL (N>50), LDL cholesterol-106mg/dL (N<100), Testosterone-213ng/dL (N=50-120), Sex hormone binding globulin(SHBG)-26nmol/L (N=26-110), Free Testosterone Index-28.2 per cent (N=0.5-6.5), DHEAS-69.9µg/dL (N=35-430), 17 hydroxy-progesterone-1.6ng/mL (N=1-3.8), LH-5.5mIU/ml (N=1.1-11), FSH-1.8mIU/ml (N=2.8-11.3), Insulin like growth factor (IGF-1)- 155ng/mL (N=75-275), HGH(1 hour post 75g glucose)-0.9ng/dL, Prolactin-20.6ng/mL (N=1.9-25). Her ultrasound abdomen revealed features suggestive of a PCOS. Body composition by Dual Energy X ray Absorptiometry (Hologic-DXA-Discovery-QDR-4500) displayed almost a complete absence of adipose tissue with well-preserved muscle mass (Figure 2). The MRI of the abdomen done for the assessment of visceral adipose tissue (VAT) showed hepatomegaly associated with markedly reduced VAT (Figure 3).

Based on the clinical presentation of acromegaloid features, virilisation, diabetes mellitus, hypertriglyceridemia, PCOS with almost complete absence of subcutaneous and visceral adipose tissue and generalized muscular appearance, a diagnosis of congenital generalized lipodystrophy (Berardinelli–Seip generalized lipodystrophy) was considered.

Blood was collected for genetic sampling after obtaining a written informed consent. The Next Generation sequencing (NGS) was performed for a panel of genes implicated in lipodystrophy (lamin A and C(LMNA), 1-acylglycerol-3-phosphate O-acyltransferase(AGPAT2), seipin(BSCL2), peroxisomal proliferator activated receptor γ(PPARG), Insulin receptor (INSR) and zinc metalloproteinase(ZMPSTE24)). Following Polymerase Chain Reaction (PCR) based target enrichment, library preparation using KAPA DNA library preparation kit and NGS based amplicon was performed on the Ion torrent personal genome machine (PGM) using 314 chips and an Ion PGM™ 200 Sequencing Kit (Ion Torrent, Life Technologies). Data analysis was performed on an Ion torrent suit software and DNA star software. We identified a novel homozygous insertion mutation c.258_259insGGCTG, p.Q87Gfs*20 along with the change in amino acid at codon 87, with a frame shift resulting in a stop codon at position 106 instead of 279 in AGPAT2 gene. This was confirmed by Sanger sequencing (Figure 4).

With a confirmed genetic diagnosis of congenital generalised lipodystrophy Type 1, she was prescribed pioglitazone, an insulin sensitizer, in addition to metformin along with cyclical combined oestrogen and progesterone therapy and is scheduled for a follow up after 6 months.

Discussion

Inherited generalised lipodystrophy was first described by Berardinelli in 1954, which was further characterised by Seip after a decade and is thus called as Berardinelli-Seip congenital generalised lipodystrophy syndrome.[4,5] This is an uncommon autosomal recessive inherited form of lipodystrophy presenting with a unique clinical phenotype and metabolic complications.

The clinical phenotype along with body composition by DXA and MRI abdomen of our patient were suggestive of Berardinelli-Seip congenital generalised lipodystrophy, hence the genes associated with the prevalent subtypes of CGL,CGL1 and CGL2 (AGPAT2 and BSCL2) were studied along with for a panel of common genes implicated in other lipodystrophies and insulin resistance (LMNA, PPARG, INSR and ZMPSTE24). Among the 4 types of CGL, type 1 and type 2 are the most common subtypes with Magre et al reporting 92 out of 94 patients with Berardinelli-Seip showing mutation in either AGPAT2 or BSCL2.[6] CAV1 and PTRF causing Type 3 and Type 4 CGL are very rare with one and 30 cases reported worldwide so far respectively.[7,8]

CGL Type 1 is caused due to a mutation in gene AGPAT2. Various premature stop variants resulting in nullifying the functions of AGPAT2 gene have been reported in the literature.[9] The enzyme AGPAT2 which is abundant in adipose tissue acrylates lysophosphatidic acid to phosphatidic acid, a key intermediate molecule in the biosynthesis of triacylglycerol and glycerophospholipids.[10] The affected patients demonstrate a lack of metabolically active adipose tissue in most subcutaneous areas, intra-abdominal, intra-thoracic regions and bone marrow, whereas mechanical adipose tissue in the joints, orbits, palms and soles, scalp, perineum, vulva, and pericalyceal regions of the kidneys, which provide a protective and cushioning function appears to be well preserved.[3]

CGL type 2 appears to be secondary to a mutation in the "BSCL2" gene which encodes Seipin, postulated to have a role in the fusion of small lipid droplets and adipocyte differentiation. Patients with the type 2 form of the disorder lack both metabolically active and mechanical adipose tissue and have a higher prevalence of mild mentally challenged state and hypertrophic cardiomyopathy.[11]

These patients present with features of insulin resistance like acanthosis nigricans since childhood. Hyperinsulinemia result in the excessive growth of soft tissue which may give an acromegaloid appearance. They also develop abnormal fat distribution in the form of lipoatrophy along with the absence of visceral adipose tissue and a muscular appearance during adolescence. Many may develop fatty liver and non-alcoholic steato-hepatitis due to hepatic fat deposition. Females may present with features of the PCOS with hirsutism and occasionally, virilisation. A very high degree of insulin resistance often manifests with young onset diabetes mellitus and hypertriglyceridemia. A prominent umbilicus or umbilical hernia is also described.[12]

We screened the genes associated with the common subtypes of CGL, CGL1 and CGL2 (AGPAT2 and BSCL2) along with for a panel of common genes implicated in other lipodystrophies and insulin resistance (LMNA, PPARG, INSR and ZMPSTE24) using Next generation sequencing based methodology. Further, this NGS methodology has been shown to be sensitive and specific to identify single nucleotide variants[13] and in the present study this methodology has also shown to be useful in identifying insertion mutation. NGS based parallel multi-gene testing against serial Sanger sequencing would make genetic testing cost effective and relatively faster and with a higher chance of definitive diagnosis. Further, PCR coupled NGS based methodology is robust and scalable to screen the increasing number of genes. With the improvements in the sequencing chemistry (Hi-Q sequencing reagents) small insertion and deletions can also be identified.

Therapeutically novel options are emerging to manage this disorder, though at present largely restricted to management of individual components like diabetes, dyslipidaemia and PCOS. Insulin sensitizers such as troglitazone and pioglitazone which increase the levels of adipokines like leptin and adiponectin have been tried. Thiazolidinediones have been shown to improve metabolic control and increase subcutaneous fat in lipodystrophies. Caution is advised with respect to hepatic side effects as an increase in Alanine transaminase with troglitazone[14] and exacerbations of NAFLD in mice exposed to rosiglitazone[15] have been described. However similar side effects have not been described with respect to pioglitazone and may even improve the hepatic steatosis.[16,17]

The role of leptin and its human analogue Metreleptin as a therapeutic modality has been shown to be beneficial in recent studies.[18-20] Fat transplantation as a treatment option has also been explored in the animal models.[21,22]

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PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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PATIENT CONSENT

The authors, Shetty S, Chapla A, Kapoor N, Thomas N, Paul TV, declare that:

1. They have obtained written, informed consent for the publication of the details relating to the patient(s) in this report.

2. All possible steps have been taken to safeguard the identity of the patient(s).
3. This submission is compliant with the requirements of local research ethics committees.

Figure 1: Clinical images showing a: Macroglossia; b: prominent umbilicus

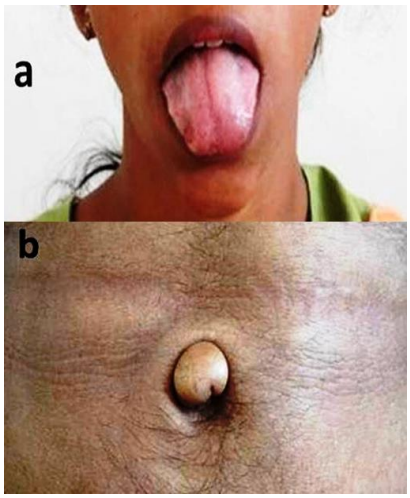
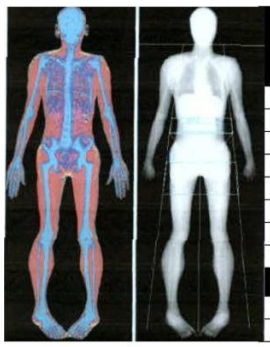


Figure 2: Body composition assessment by DXA displaying almost complete absence of adipose tissue with well-preserved muscle mass



Adipose Indices			
Measure	Result	Percentile YN	Percentile AM
Total Body % Fat	10.1	1	1
Fat Mass/Height² (kg/m²)	1.94	1	1
Android/Gynoid ratio	0.87		
%Fat Trunk % Fat Legs	0.69	37	34
Trunk/Limb Fat Mass Ratio	0.6	21	19
Est VAT Mass (g)	53.7		
ESt VAT Volume(cm³)	58		
ESt VAT Area (cm²)	11.1		
Lean Indices			
Lean/Height² (kg/m²)	16.3	56	54
Appen. Lean/Height² (kg/m²)	8.11	84	83

Figure 3: MRI abdomen showing Hepatomegaly splenomegaly and absence of visceral fat

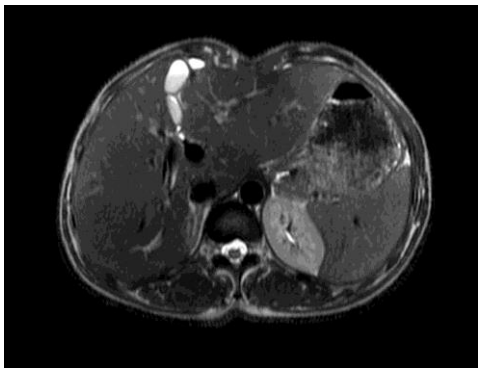
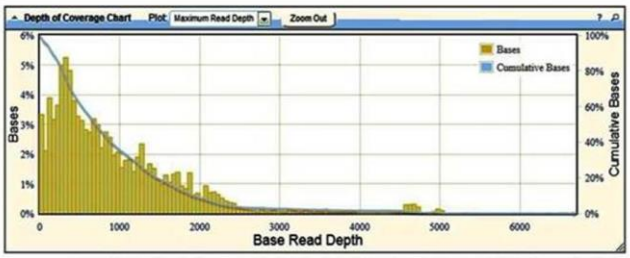


Figure 4: Genetic analysis: a: The coverage details, b: Next Generation sequencing reads with the insertion mutation along with Sanger confirmation

a

Amplicon Read Coverage		Target Base Coverage	
Number of amplicons	42	Bases in target regions	27,213
Percent assigned amplicon reads	93.76%	Percent base reads on target	94.82%
Average reads per amplicon	3,686	Average base coverage depth	929.6
Uniformity of amplicon coverage	97.62%	Uniformity of base coverage	88.46%
Amplicons with at least 1 read	100.00%	Target base coverage at 1x	98.94%
Amplicons with at least 20 reads	100.00%	Target base coverage at 20x	97.71%
Amplicons with at least 100 reads	100.00%	Target base coverage at 100x	94.59%
Amplicons with at least 500 reads	97.62%	Target base coverage at 500x	61.86%
Amplicons with no strand bias	100.00%	Target bases with no strand bias	88.01%
Amplicons reading end-to-end	0.00%	Percent end-to-end reads	0.00%



b