Missense mutation (Ser654Leu) in the *ITGA8* gene associated with renal hypodysplasia: A case report

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Abstract. Renal hypodysplasia is a congenital kidney anomaly that occurs when part of the kidney does not fully develop in the womb. Numerous genes have been identified that when mutated, result in renal dysplasia. This has encouraged the authors to search for additional genes and variants potentially linked to renal anomalies. Using next-generation sequencing combined with Sanger sequencing, a missense variant in the *ITGA8* gene (NM_003638.2:c.1961C>T; p.Ser654Leu) associated with renal hypodysplasia was identified. Detailed studies on this variant revealed that Ser654 is conserved across species, and the mutation is located in the extracellular domain of the protein, which plays an essential role in ligand binding and protein-protein interactions. This is the first study presenting the clinical correlation of the *ITGA8* variant (Ser654Leu) with renal hypodysplasia.

Introduction

Congenital disorders of kidney are a major disorder that frequently affect children and contribute to prenatal and perinatal deaths (1). The most prevalent kidney disorders include polycystic kidney disease (2), unilateral renal agenesis (3), and bilateral renal agenesis (4). Renal hypodysplasia is one of the most lethal renal disorders resulting in fetal death *in utero*. It is a highly phenotypically heterogeneous autosomal recessive disease (5). Key characteristics of renal hypodysplasia include oligohydramnios *in utero* and Potter facies, which can both be detected using ultrasonography (6). Other less common clinical observations include facial dysmorphism, pulmonary hypoplasia, congenital hip dislocation and clubfoot (7).

Kidney anomalies are estimated to account for 20-50% of all congenital anomalies in developing fetus, with renal hypodysplasia affecting 0.1-0.2% of the global population (4). Renal hypodysplasia can be inherited in both autosomal recessive and autosomal dominant pattern, with a with a male-to-female ratio of 2.7:1 (8).

Studies have identified pathogenic variants in several genes as causes of renal hypodysplasia, some of which are syndromic (9), impacting other organs such as the eyes and limbs; other gene mutation leads to non-syndromic form of renal hypodysplasia (10). While heterozygous mutations in several genes can cause renal hypodysplasia with varying severity, only mutations in the RET and *ITGA8* genes are associated with bilateral renal hypodysplasia (5,11).

In the present clinical study, a 25 year-old woman was reported with a history of multiple pregnancy losses presented within 18-25 weeks of pregnancy with oligohydramnios, facial dysmorphism, and spontaneous abortion at 25 weeks. Clinical observations indicated that the fetus may have been affected by renal hypodysplasia. To investigate further, whole exome sequencing was performed using DNA from both parents and the current pregnancy. Among several variants identified, evidence was provided associating a novel missense variant, Ser654Leu in the *ITGA8* gene, with renal hypodysplasia. This variant was confirmed in the current pregnancy through Sanger sequencing.

Case report

A 25-year-old woman was admitted to Sanjeevani Multi-speciality Hospital and Trauma Centre in December 2021. She was born to second-degree consanguineous parents (Fig. 1) and had a history of two prior pregnancy losses. Her first spontaneous abortion occurred at age 22 after 20 weeks of gestation, and her second at age of 23 after 18 weeks of gestation. Both instances were accompanied by mild abdominal pain and severe vaginal bleeding, that was unresponsive to medication. Genetic counselling revealed that her previous pregnancies had involved facial dysmorphism and oligohydramnios in the fetus.

During her third pregnancy, she reported similar features of abdominal pain and blood spotting which were managed with medication. Initial

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ultrasonography at 14 weeks revealed mild oligohydramnios, with no congenital anomalies detected. However, at 20 weeks, ultrasonography revealed facial abnormalities and severe oligohydramnios (Fig. 2), with normal placental attachment and development. Karyotyping confirmed the absence of chromosomal anomalies in both parents and the current pregnancy.

During early pregnancy, amniotic fluid is produced by the mother's body; however, after 10 weeks, fetal urine becomes the primary source of amniotic fluid (12). Usually, oligohydramnios in the developing fetus is primarily caused by an undeveloped kidney or abnormal placenta (13). In the present case, since placenta development was completely normal, an abnormal kidney development was considered to be the cause. Due to a strong history of oligohydramnios associated with all pregnancies in a span of 3 years, genetic testing was recommended by the clinician, and trio whole exome sequencing was performed, followed by Sanger sequencing.

There was a history of six pregnancy losses in the paternal grandparents and three pregnancy losses in the maternal grandparents. As per information collected in genetic counselling sessions, there were no major other health condition present in the family. All pathological tests were negative.

Genomic DNA was isolated from 5 ml of blood from each parent using QIAamp DNA Blood Mini Kits (cat. no. 51104; Thermo Fisher Scientific, Inc.) as per manufacturer's protocol. Whole exome sequencing was performed with AmpliSeq Exome RDY kit (cat. no. A38264; Thermo Fisher Scientific, Inc.) in an Ion GeneStudio S5 Plus System (Thermo Fisher Scientific, Inc.), covering all exonic regions with a depth of at least 120X. Raw data were quality trimmed to remove low-quality data having Phred score of <30, followed by mapping to the human genome (hg19), and variant calling was performed with Torrent Suite v5.5.5 (Thermo Fisher Scientific, Inc.). Variants were annotated using the gnomAD population database (https://gnomad.broadinstitute.org/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), mutation impact prediction scores, and conservation databases. Finally screening of the variants was performed manually by removing all variants with an allelic frequency of >1% or those predicted to be benign as determined by the mutation impact prediction tools consensually; only variants present in the genes known to cause renal dysplasia were eventually evaluated. Consequently, a single variant in *ITGA8* gene was linked to the clinical condition.

To confirm the presence of same variant in the current pregnancy, amniotic fluid was obtained from current pregnancy and maternal cell contamination (MCC) followed by whole exome sequencing and Sanger sequencing were performed. Using MCC testing it was confirmed that the obtained amniotic fluid doesn't contains any maternal DNA. Sanger sequencing amniotic fluid were performed using primers (forward, 5'-CCAAAC CACAGGCTAACCCA-3' and reverse, 5'-GAGGAAAGC TCTGGTTCCGT-3') to amplify 332 bp region of the *ITGA8* gene.

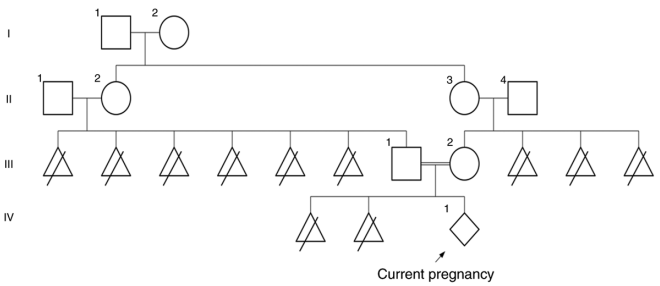

Figure 1. Pedigree demonstrating the family history of the disorder. There is a history of seven pregnancy losses among the father's siblings and three pregnancy losses among the mother's siblings. The mother who has experienced two previous pregnancy losses, has 25 weeks of pregnancy.

Discussion

After following all the steps, a single carrier missense variant (NM_003638.2:c.1961C>T; p.Ser654Leu) in *ITGA8* gene was identified, which was present in both parents. Whole exome sequencing was also performed using the amniotic fluid of the pregnant mother, confirming that no other *de novo* variant was causative in the current pregnancy. This carrier missense variant (NM_003638.2:c.1961C>T; p.Ser654Leu) in the *ITGA8* gene was found to be homozygous in the amniotic fluid of the pregnant mother (Fig. 3A-C).

This variant was classified a variant of uncertain significance (PM2, PP3 and PP4) based on ACMG guidelines (14) following these criteria: i) The homozygous variant c.1961C>T in the *ITGA8* gene is absent in both the gnomAD genome and gnomAD exome databases, while the heterozygous state of the variant has a frequency of 0.001209 in South Asian population and 0.0001835 worldwide in the gnomAD exome database (PM2); ii) As demonstrated in Table SI, various software predicted this variant as damaging, and it is conserved across several species tested, as indicated in Table SII (PP3); and iii) the family history demonstrated a high specificity to multiple pregnancy losses with oligohydramnios (PP4).

After confirming the presence of this variant using whole exome sequencing and Sanger sequencing, its impact on the protein was evaluated. Homology modelling was performed to predict the structures of both the native *ITGA8* protein and the protein with the variant, using Robetta (15) tool. The generated structures were compared using DynaMut (16) and SDM (17) tools to assess changes in stability ($\Delta\Delta G$). Both DynaMut and SDM predicted this change as stabilizing with $\Delta\Delta G$ value of 0.980 and 1.570 kcal/mol, respectively (Table SIII). Vibrational entropy ($\Delta\Delta S_{Vib}$) was estimated using the ENCoM tool (18) between both protein structures (wild-type and mutated) to evaluate the change in molecular flexibility, which revealed that the Ser654Leu variant decreases protein flexibility ($\Delta\Delta S_{Vib}$=-0.0992kcal/mol). Structural analysis using PyMOL (19), revealed that substitution of the polar uncharged amino acid serine with the hydrophobic amino acid leucine altered the intermolecular interaction pattern, resulting in the loss of carbonyl contact and ionic interaction, along with the gain of a new carbonyl contact (Fig. 3D and E). These

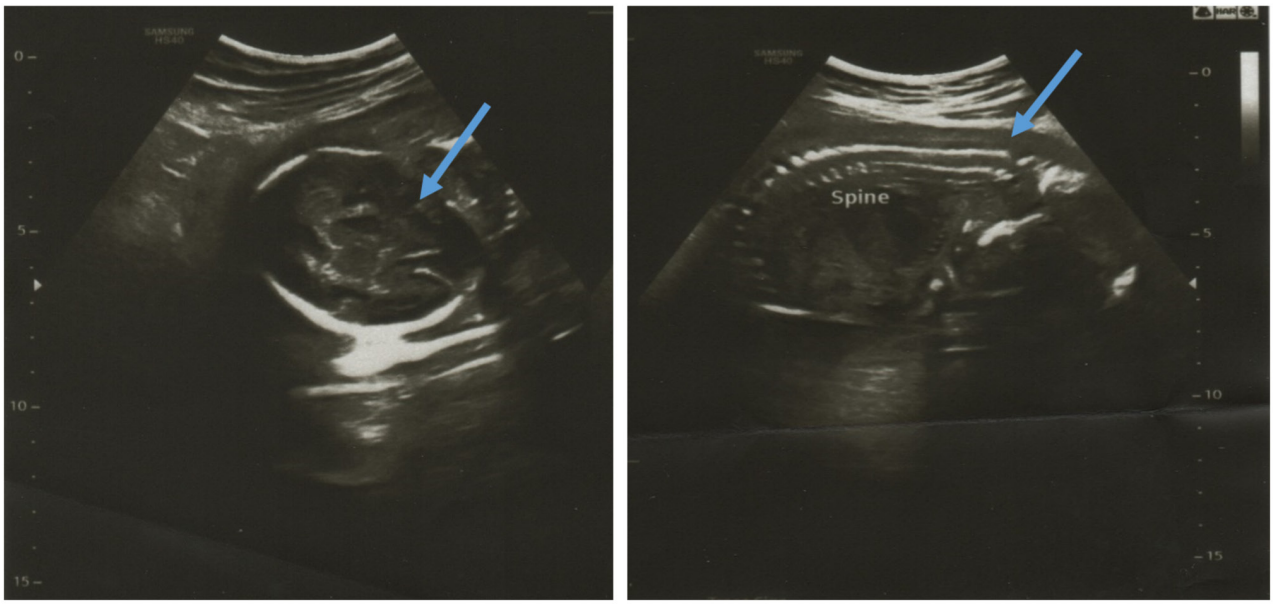

Figure 2. Trans-abdominal ultrasound revealing a normal fetal spine and reduced amniotic fluid at 20 weeks of pregnancy. The blue arrow indicates to the area of decreased amniotic fluid.

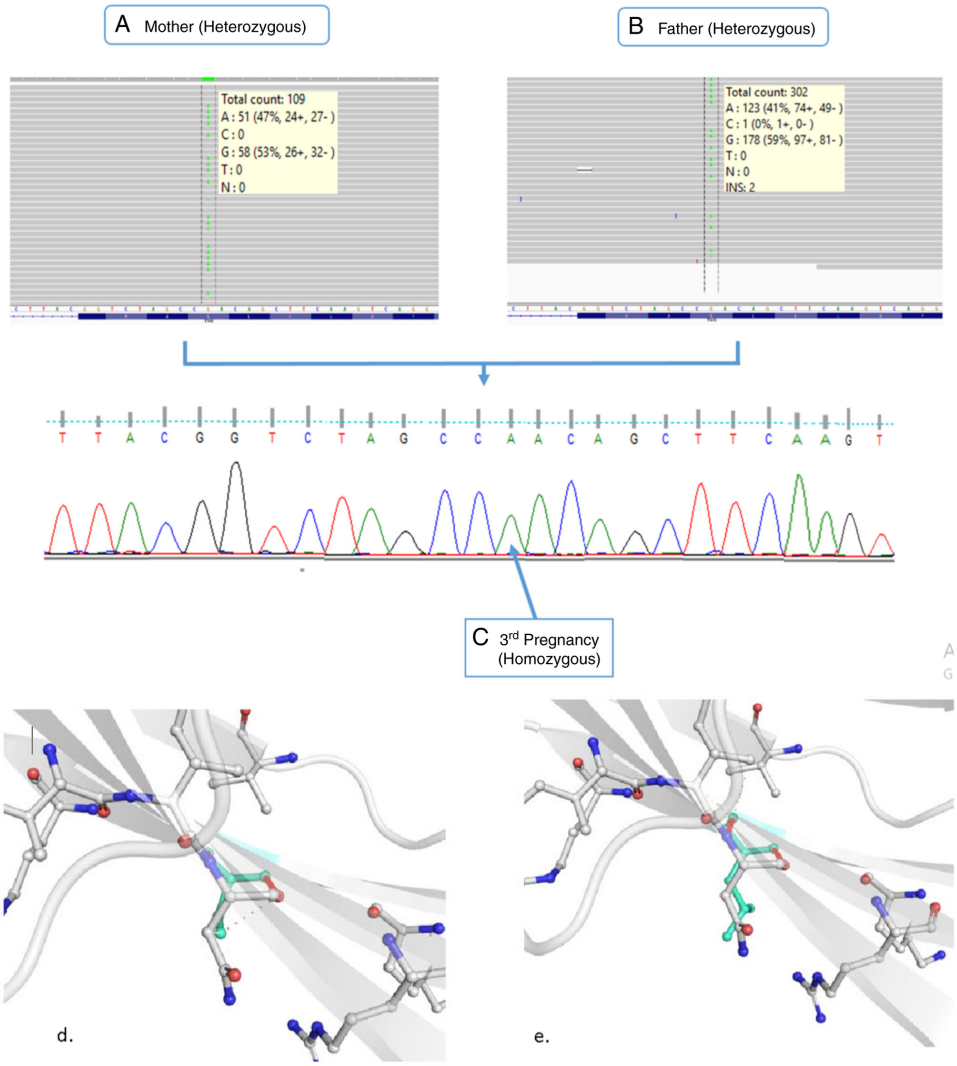

Figure 3. Screenshot from Integrative Genomics Viewer demonstrating the heterozygous variant in (A) the mother, (B) the father and (C) the Sanger sequencing trace for the current pregnancy indicating a homozygous variant for c.1961C>T. The read depth supporting the reference and alternate alleles are displayed in the square boxes. (D) The light green coloured residue represents the wild-type amino acid revealing inter-atomic interactions with surrounding residues and (E) light green coloured residue demonstrates the mutated amino acid with altered inter-atomic interactions with surrounding residues.

comparative observations confirmed the damaging impact of this missense variant, leading to the amino acid change from serine to leucine at position 654.

ITGA8 proteins are heterodimeric transmembrane receptors composed of alpha and beta subunits. They possess a functionally active domain known as 'integrin alpha-2', which contains FG-GAP repeats, proven to be active participants in ligand binding (20). These repeats are also essential for cell-cell interaction (21), host pathogen recognition, and regulation of neurite outgrowth in the sensory and motor neurons and play a vital role in kidney organogenesis. Absence of ITGA8 gene product can potentially affect normal epithelial mesenchymal transition that results in renal hypodysplasia (22). Improved understanding of mechanism underlaying the impact of mutation in this gene will help in increasing the prenatal diagnostic yield (23).

In the present study, both parents were heterozygous for the *ITGA8* (Ser654Leu) missense variant, while the fetus was homozygous for the same variant. This suggests that the identified variant in the *ITGA8* gene has a significant role in proper kidney development. The conservation score and variant impact prediction indicated that the variant is deleterious, and structural analysis further supported its damaging effect on protein structure. The present study reports a novel variant of the *ITGA8* gene and provides clinical evidence for the role of this genetic variant in renal development. *In vitro* or *in vivo* functional study will add more strength to this finding for use in prenatal diagnosis.

Prenatal genetic diagnosis offers wide variety of information about the health of the fetus. The present case study demonstrated the impact of missense mutation (Ser654Leu) in *ITGA8* gene and will help in risk assessment for the renal hypodysplasia in further pregnancies thereby, enabling clinicians to take proactive measures for surveillance and preventions. As both parents were carrier for the same mutation, the women may be suggested to conceive through donor sperm.

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Availability of data and materials

The data generated in the present study may be found in the Sequence Read Archive database under accession numbers SRR31344619, SRR31344620 and SRR31344621 or at the following URL: https://www.ncbi.nlm.nih.gov/sra/?term=SRR31344619, https://www.ncbi.nlm.nih.gov/sra/?term=SRR31344620 and https://www.ncbi.nlm.nih.gov/sra/?term=SRR31344621.

Authors' contributions

AM conceived the idea and designed the project. KGS performed the experiments. Both authors read and approved the final version of the manuscript. KGS and AM confirm the authenticity of all the raw data.

Ethics approval and consent of participation

The study design and protocol were conducted in accordance with the guidelines of the ACMG, and were approved (approval no. IECH/2022/SEP-014) by the ethical review committee of Sanjeevani Multi-Speciality Hospital and Trauma Centre, (Rampur, India).

Patient consent for publication

Written informed consent for the publication of her data and associated images was obtained from the patient.

Competing interests

The authors declare that they have no competing interests.

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