

The Utility of Whole Exome Sequencing for Prenatal Diagnosis of Bartter Syndrome: A Case Report

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INTRODUCTION

Polyhydramnios is identified in 1-2% of pregnancies [1]. It can result in the second trimester as the fetal kidneys become the predominant source of amniotic fluid. Polyhydramnios may be related to maternal or fetal conditions including gestational diabetes, congenital infection, congenital anomalies and genetic disorders [2]. Evaluation typically includes maternal testing for diabetes, targeted anatomic ultrasound, maternal serology for infectious diseases and occasionally offering genetic amniocentesis. Despite thorough investigation, 50-60% of cases will be idiopathic [3]. In 10% of these cases, an anomaly will only be found after birth [4]. Polyhydramnios has been associated with adverse pregnancy outcomes, including fetal death, preterm delivery and increased neonatal morbidity [5]. There are only a few genetic diseases associated with polyhydramnios, including antenatal Bartter syndrome.

Bartter syndrome is a rare autosomal recessive salt losing tubulopathy first described in 1962 [6]. It is caused by defective salt reabsorption in the thick ascending loop of Henle [7]. It can present in two ways: antenatal and classical Bartter syndrome. The classic form has insidious onset in infancy; children present with severe polyuria, dehydration, hypokalemic metabolic alkalosis and failure to thrive [8]. Typically, the prenatal presentation is very severe with marked polyhydramnios, fetal growth restriction, preterm delivery, postnatal polyuria, dehydration, hypokalemic metabolic alkalosis, marked hypercalciuria leading to nephrocalcinosis and failure to thrive [9,10]. Because symptoms at birth can be life threatening, prenatal diagnosis is important for management both prenatally and in the immediate postnatal period.

An accurate diagnosis is an integral component of patient care for children with rare genetic disorders. However, precise diagnosis of an underlying genetic defect is often challenging as most cases lack a family history and predisposing factors. Currently, prenatal testing for a sonographically identified abnormality includes fluorescence

in situ hybridization for rapid aneuploidy testing, G-banded karyotyping and chromosomal microarray analysis for detecting copy number variants. These cytogenetic tests are limited by their resolution. If these techniques are used in conjunction in fetuses with structural abnormalities, they will identify an underlying etiology in up to 40% of dysmorphic fetuses [11]. This leaves a majority of cases without a diagnosis, particularly in the absence of a structural abnormality. Significant advances in next generation sequencing technology over the recent years, particularly whole exome sequencing (WES), have revolutionized the practice in genetic diagnosis. WES sequences ~95% of the genome; specifically, it examines the protein-coding exons of ~20,000 genes. Although exons encompass less than 2% of the genome, they contain >85% of all disease causing pathogenic variants [11]. WES has recently become an accepted test in the neonatal, pediatric and adult populations. Postnatal WES in patients with a suspected genetic abnormality who remain undiagnosed with traditional methods has a diagnostic yield of 25-30% [12]. In recent years, WES is being used for identify pathogenic variants in fetuses with structural abnormalities [13]. The ability to diagnose pathogenic single nucleotide variants prenatally has been limited to a small number of genetic diseases with recognizable phenotypes [14]. Various single gene disorders present prenatally and are undiagnosed with traditional cytogenetic techniques, many of which may be diagnosed using WES.

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All patients that are considered for WES should receive pre- and post-test counseling from a provider with genetics expertise [15]. As WES is a newer modality for prenatal testing and studies are ongoing for its clinical utility, The American College of Medical Genetics and Genomics (ACMG) and Society of Maternal-fetal Medicine (SMFM) do not recommend routine use for prenatal diagnosis [16]. In those cases in which other approaches to diagnosis are uninformative, it may be appropriate to offer WES. Sequencing of the whole exome and protein-coding regions is well justified as an efficient approach to diagnose variants underlying rare genetic conditions. It can improve the accuracy of prognosis, prenatal counseling and pregnancy management, while reducing the diagnostic testing that occurs postnatally. This may result in a reduction of the costs of molecular, imaging and other investigations. It can also improve early postnatal management in cases of life threatening neonatal conditions, such as Bartter syndrome [13,17,18].

CASE REPORT

We report on a 33 year old Arabic woman in her third pregnancy (G3P2002). She had a history of gestational diabetes, which was being treated with Metformin. She did not report any significant family history. Fetal anatomy survey at 23 weeks revealed an enlarged fetal bladder (**Figure 1**); amniotic fluid was assessed as normal. Due to the enlarged fetal bladder, a follow up ultrasound was performed at 27 weeks gestation; this revealed a normally grown fetus and polyhydramnios (amniotic fluid volume (AFI) 44 cm, with the deepest fluid pocket of 14 cm) in the absence of any other structural abnormality. Patient presented at 31 weeks with abdominal discomfort and fetus was then noted to be growth restricted (EFW 1369 g, <5th percentile) with normal Doppler studies and AFI 65 cm. After discussion of therapeutic options, serial amnioreductions were performed, removing approximately 3 L of amniotic fluid each time.

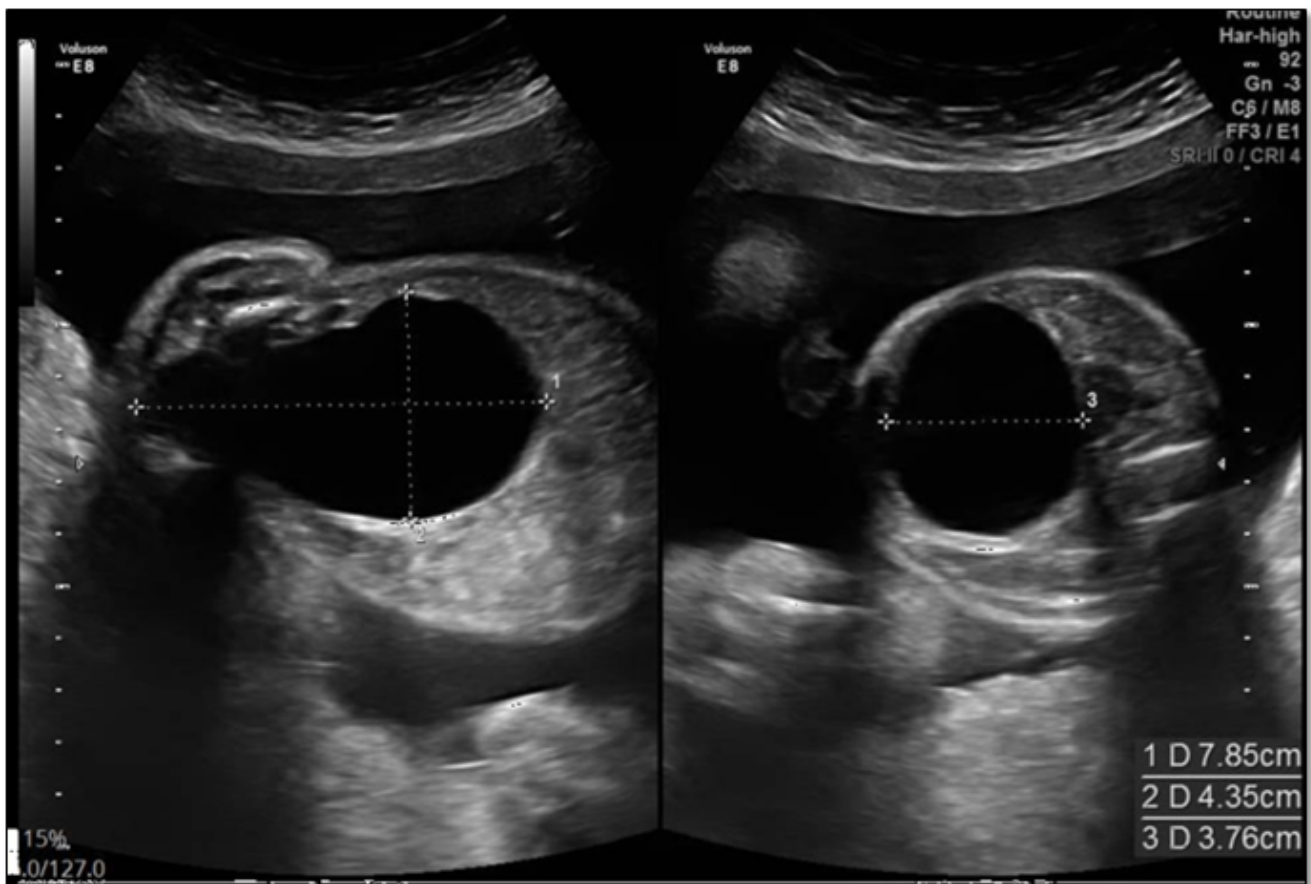


Figure 1. Ultrasound demonstrating enlarged fetal bladder at 31 weeks of gestation.

Fetal karyotype performed on cultured amniocytes revealed normal male chromosomes (46, XY). Chromosomal microarray analysis (180K SNP-aCGH array) revealed no clinically significant copy number variants; however, the

SNP array discovered several regions of homozygosity encompassing 12.1% of the genome, indicating inheritance from a shared ancestor. A family history was further reviewed with the patient after these findings and she

reported being double-first cousins with her husband (**Figure 2**). A fetal MRI was also performed to assess the upper airway of the fetus, which appeared normal. The

pregnancy continued with weekly Doppler studies and bi-weekly non-stress tests.

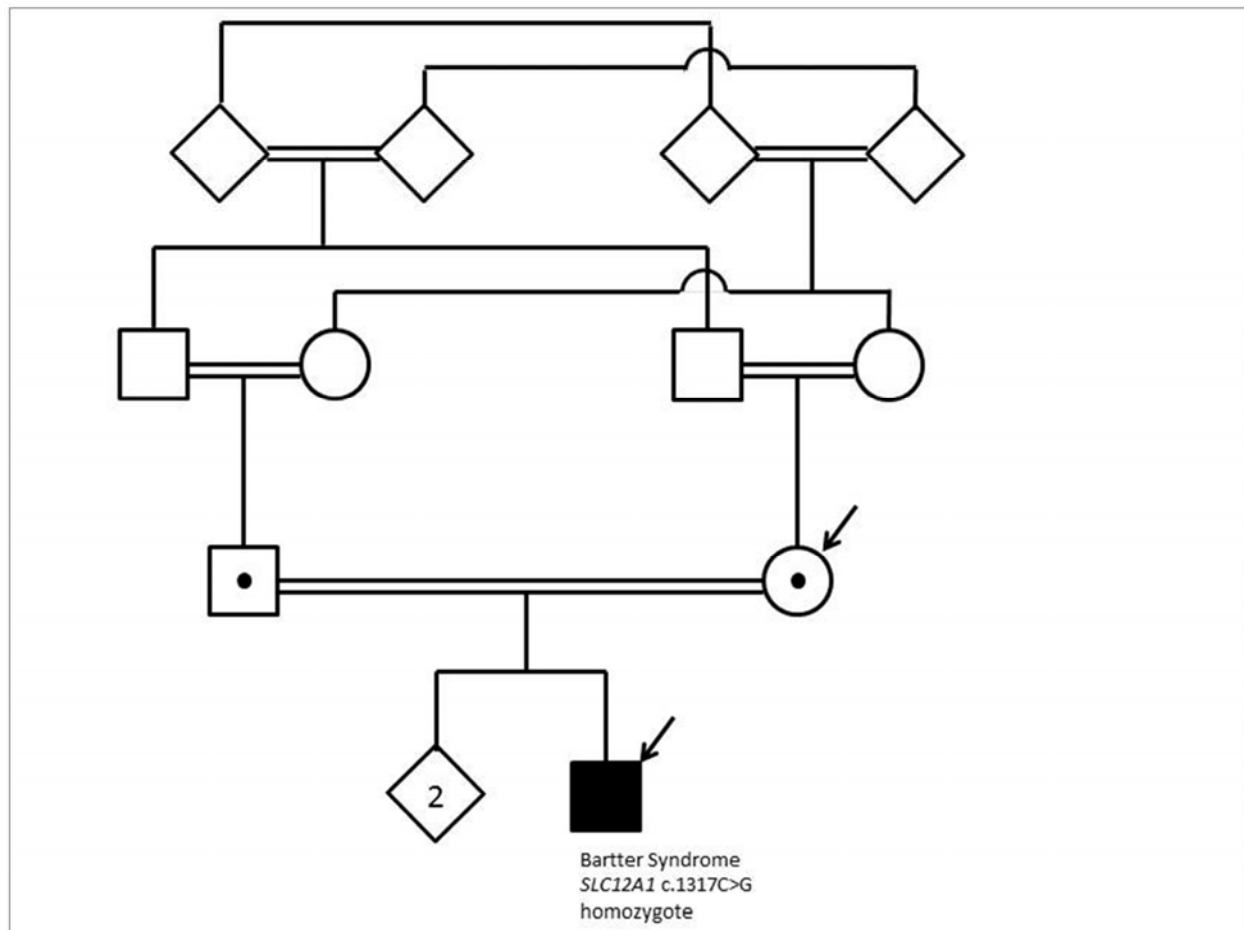
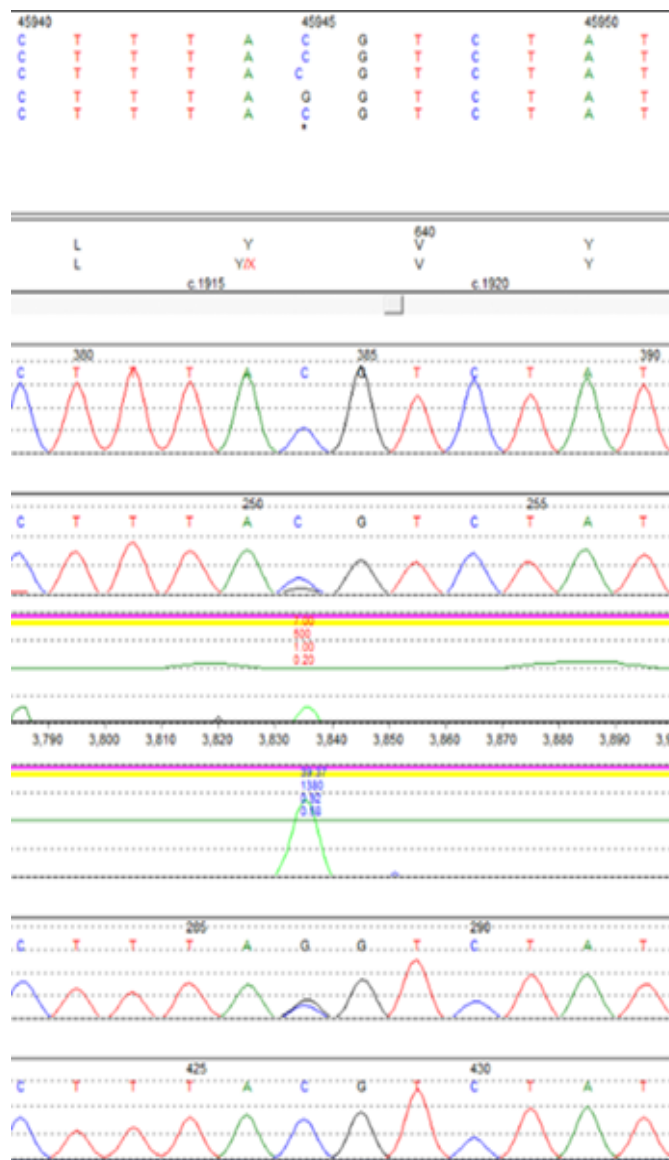


Figure 2. Pedigree of proband's family, indicating at least double-first-cousin relationship in parents as a result of multiple generations of similar consanguineous marriages.

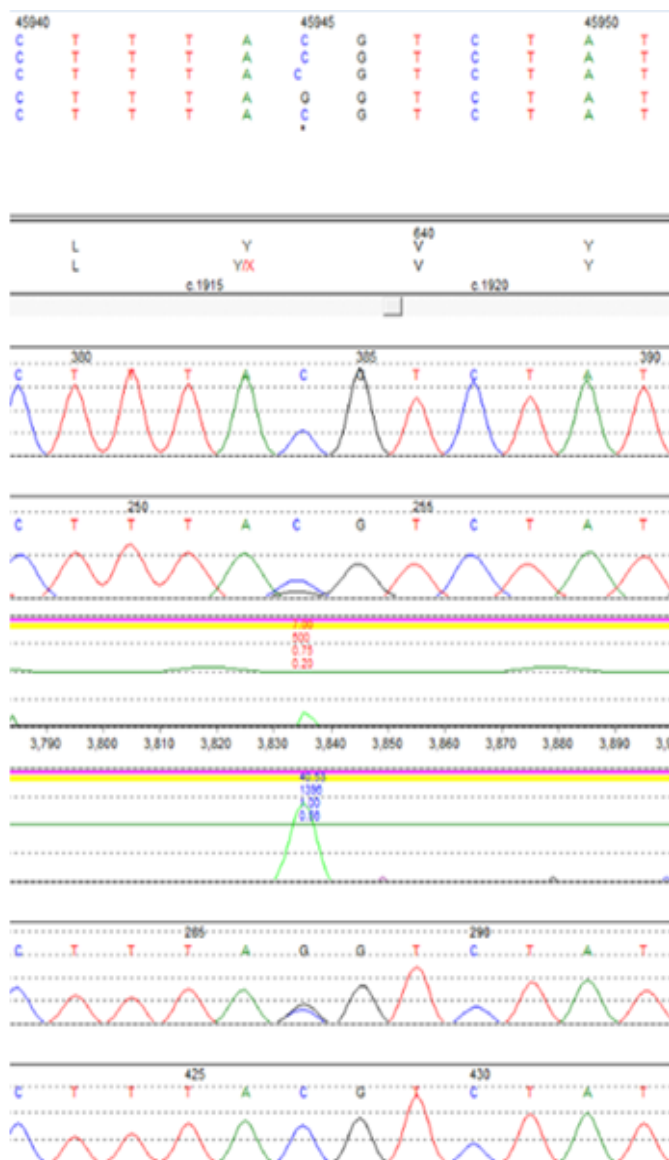
At 32 weeks of gestation, the umbilical artery demonstrated intermittent absent end diastolic flow and the patient received two doses of betamethasone. A repeat Cesarean section delivery was performed secondary to fetal bradycardia at 33 weeks of gestation. The patient delivered a male neonate with a birth weight of 1590 g and APGAR scores of 8 at 1 min and 9 at 5 min of age. The neonate was noted to have polyuria and severe electrolyte disturbances soon after birth (urine output 8 mL/kg/h, sodium 151, potassium 6.3, anion gap 26). These abnormalities were thought to be secondary to immature fetal kidneys, which were expected to normalize over the next several days. The neonate was started on feeds and maintenance fluid, supplementing with sodium and potassium; the urine output

normalized by 2 weeks of age. The neonate was discharged home at 6 weeks of life.

The infant presented to the hospital at 2 months of age with failure to thrive. The Pediatric Genetics service was consulted, as the etiology of the polyuria and electrolyte imbalances was not identified. Expedited whole exome sequencing revealed homozygosity for a truncating loss of function variant, c.1917C>G (p.Y639X) in the SLC12A1 gene (MIM 600839), consistent with the diagnosis of autosomal recessive Bartter Syndrome Type 1 (**Figure 3**). The region of chromosome 15q21.1 containing this gene was inherited from a common ancestor per microarray results (**Figure 4**). No other pathogenic, likely pathogenic, or variants of unknown significance were reported.



A)



B)

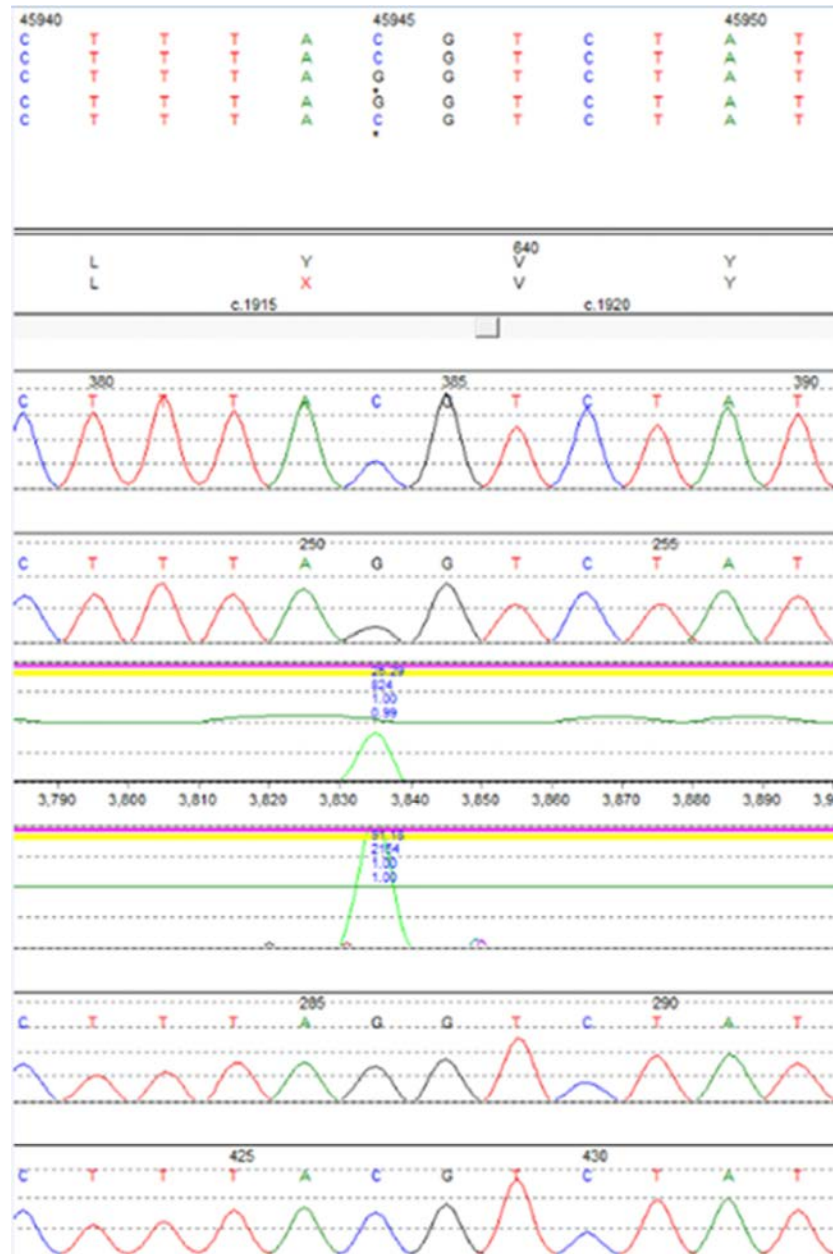


Figure 3. Sanger tracings from the trio show a homozygous G at the cDNA location c.1917 C>G in the proband. Both parents are heterozygous carriers for G on one allele and C on the second allele. **A)** Mother **B)** Father **C)** Proband.

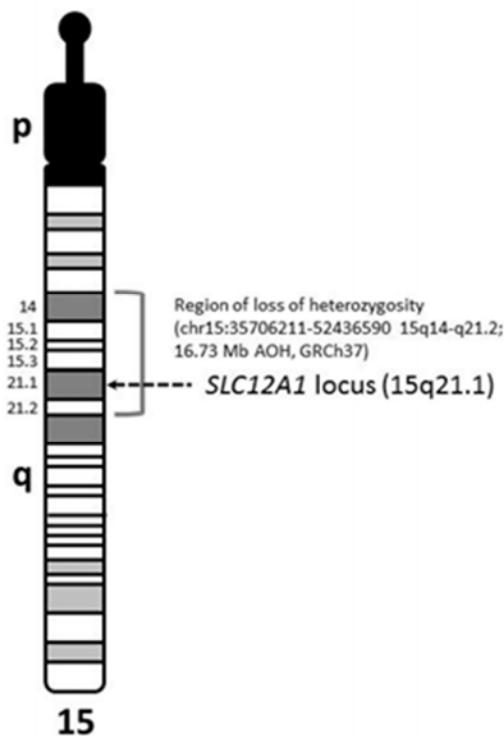


Figure 4. Ideogram of chromosome 15, indicating approximate region of loss of heterozygosity per SNP-oligonucleotide microarray on cultured amniocytes and approximate location of the SLC12A1 locus implicated in Bartter syndrome.

DISCUSSION

Bartter syndrome is a rare inherited renal tubular disorder associated with hypokalemic alkalosis. Hyperreninemic hyperaldosteronism with normal blood pressure, increased excretion of urinary prostaglandin E and hyperplasia of the juxtaglomerular apparatus are other features of this syndrome [19]. The antenatal form of this disorder is a severe condition that manifests as unexplained severe polyhydramnios with its associated complications of preterm labor and delivery, postnatal dehydration and electrolyte disturbances. It can lead to significant morbidity and mortality if undiagnosed and untreated, with some women considering termination of pregnancy. Due to the severity of this condition, we advocate prenatal diagnosis for prenatal and postnatal management.

There is inconsistency of amniotic fluid biochemistry and hormone levels in the diagnosis of Bartter syndrome [20-23]. In fact, controversial results of amniotic fluid electrolytes have been reported, especially elevated chloride levels in several case reports [24,25]. In contrast, a study of 16 cases of Bartter syndrome revealed no significant differences in amniotic fluid chloride levels [22]. Furthermore, elevated amniotic fluid aldosterone levels have also been described previously [20], while more recent evidence demonstrates

that amniotic fluid aldosterone levels cannot be used for prenatal diagnosis of Bartter syndrome [26]. This case study highlights the difficulty of antenatal diagnosis of Bartter syndrome with traditional genetic methods and the opportunity to diagnose it prenatally using WES. Recent professional position statements recommend utilizing prenatal WES in cases of at least one major anomaly suggestive of a possible genetic etiology with a normal microarray. This case highlights the potential utility of WES in the setting of unexplained polyhydramnios with normal fetal anatomy and nondiagnostic microarray to aid in the diagnosis of antenatal Bartter Syndrome. Additionally, we highlight the usefulness of WES in cases where microarray identifies regions of homozygosity by descent indicating an increased risk for a single-gene recessive condition. Further studies examining the clinical utility and cost-benefit ratio of prenatal WES in cases such as ours are clearly needed before expanded recommendations for utilization can be made.

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