

# Whole exome sequencing for prenatal diagnosis of CHARGE syndrome: a case report

**Irene WY LOK**<sup>1</sup> MBChB, MRCOG, FHKAM (O&G)

**CW KONG**<sup>1</sup> MBChB, MSc in Medical Genetics, MRCOG, FHKAM (O&G), Cert HKCOG (MFM)

**Anita SY KAN**<sup>2,3</sup> MBBS, MPH(HK), MRCOG, FHKCOG, FHKAM (O&G)

**KS YEUNG**<sup>4</sup> PhD

**Mullin HC YU**<sup>4</sup> MSc

**Brian HY CHUNG**<sup>4</sup> MBBS(Hons, HKU), MSc(Genomics and Bioinformatics, CUHK), MD(HKU), DCH(Ireland), MRCPCH (UK), FHKAM(Paediatrics), FRCPC(HK), FCCMG(Clinical Genetics, Canada)

**William WK TO**<sup>1</sup> MBBS, MPH, M Phil, MD, Dip med, FHKAM (O&G), Cert HKCOG (MFM)

<sup>1</sup> Department of Obstetrics and Gynaecology, United Christian Hospital

<sup>2</sup> Department of Obstetrics and Gynaecology, Queen Mary Hospital

<sup>3</sup> Prenatal Diagnostic Laboratory, Tsan Yuk Hospital

<sup>4</sup> Department of Paediatrics and Adolescent Medicine, The University of Hong Kong

We report a prenatal case of CHARGE syndrome with multiple fetal structural abnormalities detected on ultrasonography despite normal karyotype and chromosomal microarray results. Whole exome sequencing of the fetus identified a pathogenic, *de novo* mutation in *CHD7*, and hence CHARGE syndrome was molecularly confirmed. The challenges in prenatal diagnosis of CHARGE syndrome by clinical features are discussed, as are the usefulness and limitations of whole exome sequencing in prenatal diagnosis.

*Keywords: CHARGE syndrome; Prenatal diagnosis; Whole exome sequencing*

## Case presentation

In October 2018, a 34-year-old Chinese woman was referred to United Christian Hospital for multiple fetal structural abnormalities detected on morphology scan. She was gravida 2 with one previous normal full-term vaginal delivery. Her family history was unremarkable. Her second-trimester biochemical Down syndrome screening was negative, with a calculated risk of 1 in 49000. Ultrasonography at 22 weeks detected multiple fetal abnormalities, including Dandy walker variant anomaly, median cleft lip, hypoplastic left heart syndrome, and absent stomach bubble (Figure 1). Amniocentesis followed by rapid aneuploidy detection by quantitative fluorescent polymerase chain reaction showed normal copy numbers of chromosomes 13, 18, and 21. The patient opted for termination of pregnancy at 22 weeks and 4 days of gestation in view of fetal multiple congenital anomalies before karyotype and chromosomal microarray results were available. The termination was uneventful.

Karyotyping showed 46,XY but chromosomal microarray result was normal. Autopsy of the fetus revealed cerebellar vermis hypoplasia, median cleft lip and palate, aorta isthmic hypoplasia, absent right brachiocephalic vein and artery, and clinodactyly of right fifth finger (Figure 2). The heart valves and the four cardiac chambers were unremarkable. Chona were patent and the oesophagus

and stomach were normal. In view of multiple fetal abnormalities despite negative karyotype and chromosomal microarray results, whole exome sequencing (WES) was performed, and a NM\_017780.4:c.2959C>T:p.(Arg987Ter) mutation in exon 12 of *CHD7* was identified. Parental analysis showed that neither parent carried the variant, indicating the variant was *de novo* in origin. According to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines for interpretation of sequence variants<sup>1</sup>, the mutation was pathogenic and indicated CHARGE syndrome. Sanger sequencing was performed for validation. The risk of recurrence in future pregnancy was 1% to 2% owing to the risk of gonadic mosaicism<sup>2</sup>.

## Discussion

CHARGE syndrome (OMIM number 214800) is a rare, usually sporadic disorder caused by loss-of-function mutations in *CHD7*, which is of autosomal dominant inheritance<sup>3</sup>. Loss-of-function mutation refers to mutation that results in a premature stop of the transcription of the gene and a non-functional truncated protein. The CHARGE acronym summarises the features commonly found postnatally: Coloboma of eye, Heart defects, Atresia choanae, Retardation of growth, Genital abnormalities,

*Correspondence to: Irene Wing Yi LOK*

*Email: happyah2@hotmail.com*

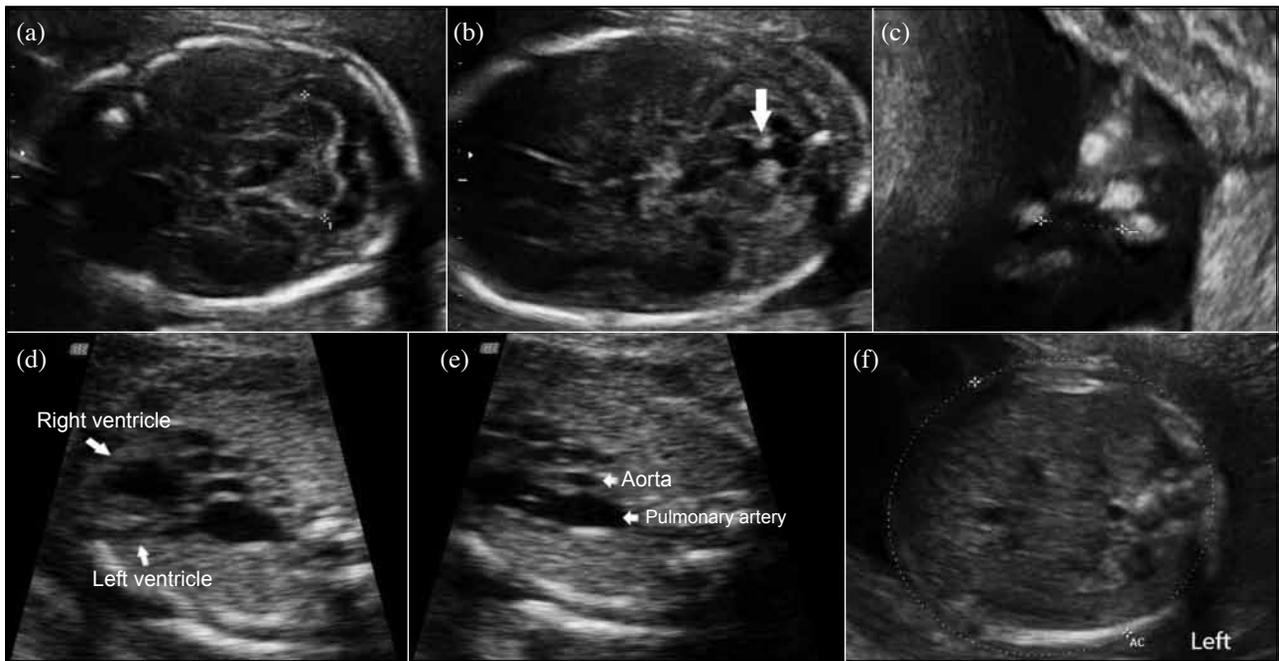


Figure 1. Prenatal ultrasound at 22 weeks' gestation showing (a) normal cerebellar hemispheres and posterior fossa (transcerebellar diameter=2.28 cm), (b) a defect in the inferior cerebellar vermis (arrow) that communicates with the 4th ventricle signifying Dandy walker variant anomaly, (c) median cleft lip (size=1.00 cm). (d) Four-chamber view and (e) three-vessel view showing hypoplastic left heart with narrow aorta and (f) absent stomach bubble on transverse view of the abdomen.



Figure 2. Abortus showing (a) median cleft lip and palate, (b) external ear abnormality, (c) clinodactyly of right fifth finger.

and Ear abnormalities/deafness. Its incidence ranges from 1 in 8500 to 10000 live births<sup>4,5</sup>. The phenotypic presentations are highly variable and involve multi-organ systems. Orofacial cleft, oesophageal atresia, and limb defects are common features. Based on the frequency and specificity of a distinct set of anomalies, all four major criteria (coloboma, choanal atresia, characteristic ear abnormalities, cranial nerve dysfunction) or three major and three minor criteria (genital hypoplasia, developmental delay, cardiovascular malformations, growth deficiency, orofacial cleft, tracheoesophageal fistula, distinctive face) must be exhibited in order to fulfil the diagnosis of CHARGE syndrome<sup>5</sup>. Major criteria also include cranial nerve anomalies, including weak chewing or sucking, facial palsy, sensorineural hearing loss, balance vestibular problems, and swallowing problems. Among patients with CHARGE syndrome, 92% exhibit at least one cranial nerve anomaly and 72% more than one. Isolated cranial nerve

involvement is rare<sup>6</sup>.

Prenatal diagnosis of CHARGE syndrome is challenging as prenatal ultrasound may not be able to diagnose coloboma or choanal atresia, and growth retardation only arises postnatally. Moreover, cranial nerve dysfunction and mental retardation cannot be assessed before birth. A case series identified three constant features in all 10 fetuses: bilateral and asymmetric external ear abnormalities, semicircular canal hypoplasia or agenesis, and arhinencephaly (lack of olfactory tracts); intrauterine growth retardation was never observed<sup>7</sup>. The case series subsequently expanded to include 40 cases and identified some novel features in fetuses that differed from living affected patients. Features such as coloboma, developmental delay, genital anomalies, and growth retardation were uncommon or missed in fetuses, and 16 of the 40 cases would have been missed if postnatal

CHARGE diagnostic criteria were used. Thus, criteria for diagnosing fetal CHARGE syndrome should include at least four of the six major criteria (external ear anomalies, heart defects, semicircular canal agenesis/hypoplasia, arhinencephaly, coloboma, and choanal atresia or cleft) or three major and two of eight minor criteria (central nervous system anomalies, limb anomaly, genital anomalies, thymic hypoplasia/agenesis, polyhydramnios, renal anomaly, skeletal anomaly, and oesophageal anomalies), and absence of intrauterine growth retardation<sup>8</sup>.

Establishing the diagnostic criteria for fetal CHARGE syndrome aids prenatal detection and hence proper counselling. Although our case had some major features (cleft lip/palate and congenital heart defect) and minor features (vermis hypoplasia and limb anomalies), neither the clinical nor the pathological features documented were sufficient to fulfil the diagnostic criteria of fetal CHARGE syndrome. One reason is that major features such as arhinencephaly and semicircular canal agenesis are not routinely examined histopathologically. This highlights the importance of detailed fetal autopsy including neuropathological examination for diagnosis. Focused ultrasonography and fetal magnetic resonance imaging are recommended for assessment of external ear abnormalities, choanal atresia, semicircular canal agenesis, and arhinencephaly. However, expertise is often not readily available in routine practice.

Next-generation sequencing is a high-throughput sequencing technology that sequences DNA in a massively parallel manner. It can be classified into three categories: targeted gene panels, WES, and whole genome sequencing (WGS). WES sequences the protein-coding part of the genome, which represents 1.5% to 2% of the genome (about 30 megabases). WGS sequences every nucleotide in the whole genome, which is equivalent to approximately 3.3 gigabases, and covers non-coding and inter-genic regions. Next-generation sequencing is widely used for diagnosing complex diseases that involve a large number of genes. WES/WGS is more cost-effective than sequencing individual genes sequentially. Over 85% of known disease-causing mutations are found in exome, and therefore WES is a reasonable approach for diagnosing some diseases to reduce cost and data storage. However, WES may miss a pathogenic variant in a non-coding region of the genome. WGS may be preferable to WES when the cost decreases and more information about the role of non-coding DNA in human diseases becomes available. However, WGS may unexpectedly cover many variants of uncertain significance that makes clinical interpretation more challenging. Sanger

sequencing, which is first-generation DNA sequencing technology, has >99.99% accuracy for most genes sequenced and remains the gold standard for diagnosis. Therefore, Sanger sequencing is generally performed to confirm any variant reported as pathogenic by WES or WGS as secondary validation<sup>9,10</sup>.

Conventional prenatal cytogenetic test of karyotype allows low-resolution detection of chromosomal abnormalities. Although chromosomal microarray analysis offers higher detection rate of copy number variants, it still cannot detect point mutations and small insertion-deletion mutations that cause >4600 known single gene disorders and others yet to be characterised. Some phenotypes can be caused by mutations in different genes. Our case is an example of CHARGE syndrome overlapping with DiGeorge syndrome, VACTERL association, renal coloboma, and Feingold or anophthalmia-oesophageal-genital syndromes. Owing to limitations of prenatal imaging and the fact that intellectual disability, minor birth defects, and dysmorphic features can only be ascertained after birth, comprehensive, unbiased genetic diagnosis prenatally using next-generation sequencing is needed<sup>11</sup>.

Although WES is an invaluable tool for genetic diagnosis in paediatrics, it is still not widely adopted in prenatal diagnosis. WES is useful in prenatal cases with multiple fetal anomalies identified by ultrasound but without a particular syndrome being diagnosed. A local study evaluated the usefulness of WES in prenatal diagnosis of fetuses with structural anomalies detected on ultrasound but with normal chromosomal microarray results<sup>12</sup>. 33 families were recruited to undergo trio-based WES. Pathogenic mutations were identified in 9.1% of fetuses, including mutations in *DNAH11*, *RAF1*, and *CHD7* genes, which were associated with primary ciliary dyskinesia, Noonan syndrome, and CHARGE syndrome, respectively. Variants of uncertain significance were detected in 18.2% of fetuses. In a prospective multicentre study of 34 units in the United Kingdoms, 610 fetuses with structural anomalies after exclusion of aneuploidy and large copy number variants were analysed by trio-based WES<sup>13</sup>. A pathogenic genetic variant was identified in 8.5% of fetuses; the variant was present in 15.4% of fetuses with multisystem anomalies, 11.1% of fetuses with cardiac anomalies, and 15.4% of fetuses with skeletal anomalies. And 3.9% of fetuses were found to have a variant of uncertain significance. WES is useful to diagnose monogenetic disease in fetuses with structural anomalies despite normal cytogenetic findings. In 2018, the International Society for Prenatal Diagnosis, the Society for Maternal Fetal Medicine, and the Perinatal

Quality Foundation published a joint position statement and recommended the use of diagnostic genome wide sequencing for evaluation of fetuses with single major anomaly or with multiple organ system anomalies that are suggestive of a possible genetic aetiology, but with uninformative chromosomal microarray results. Nevertheless, the routine use of diagnostic prenatal sequencing cannot be supported until more validation studies are available. Currently, WES and WGS are ideally performed in the research setting<sup>14</sup>.

WES aids a definite genetic diagnosis so that proper counselling on fetal prognosis can be provided and appropriate management plan can be arranged. In addition, WES enables estimation of the risk of recurrence in future pregnancy so that future reproductive decision including preimplantation genetic testing and early prenatal diagnosis can be discussed. However, there are limitations and ethical considerations for prenatal WES. The cost of WES may be a financial burden to parents. There are time constraints from sample retrieval to obtaining genetic results and the time limit on gestation for termination of pregnancy. Women may have unrealistically high expectations of test performance and may be disappointed or falsely reassured conversely when no causative mutations are discovered.

## References

- Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405-24. [Crossref](#)
- Lalani SR, Hefner MA, Belmont JW, et al. CHARGE Syndrome. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. *GeneReviews®* Seattle: University of Washington; 1993-2019.
- Charge syndrome. Online Mendelian Inheritance in Man (OMIM). Available from: <https://www.omim.org/entry/214800>. Assessed 10 April 2019.
- Issekutz KA, Graham JM Jr, Prasad C, Smith IM, Blake KD. An epidemiological analysis of CHARGE syndrome: preliminary results from a Canadian study. *Am J Med Genet A* 2005;133A:309-17. [Crossref](#)
- Blake KD, Davenport SL, Hall BD, et al. CHARGE association: an update and review for the primary pediatrician. *Clin Pediatr (Phila)* 1998;37:159-73. [Crossref](#)
- Blake KD, Hartshorne TS, Lawand C, Dailor AN, Thelin JW. Cranial nerve manifestations in CHARGE syndrome. *Am J Med Genet A* 2008;146A:585-92. [Crossref](#)
- Sanlaville D, Etchevers HC, Gonzales M, et al. Phenotypic spectrum of CHARGE syndrome in fetuses with CHD7 truncating mutations correlates with expression during human development. *J Med Genet* 2006;43:211-7. [Crossref](#)
- Legendre M, Gonzales M, Goudefroye G, et al. Antenatal spectrum of CHARGE syndrome in 40 fetuses with CHD7 mutations. *J Med Genet* 2012;49:698-707. [Crossref](#)
- Rizzo JM, Buck MJ. Key principles and clinical applications of “next-generation” DNA sequencing. *Cancer Prev Res (Phila)* 2012;5:887-900. [Crossref](#)
- Rehm HL, Bale SJ, Bayrak-Toydemir P, et al. ACMG clinical laboratory standards for next-generation sequencing. *Genet Med* 2013;15:733-47. [Crossref](#)
- Johnson K, Eason J. Prenatal diagnosis of genetic disorders. *Obstet Gynaecol Reprod Med* 2018;28:308-13. [Crossref](#)
- Leung GKC, Mak CCY, Fung JLF, et al. Identifying the genetic causes for prenatally diagnosed structural congenital anomalies (SCAs) by whole-exome sequencing (WES). *BMC Med Genomics* 2018;11:93. [Crossref](#)
- Lord J, McMullan DJ, Eberhardt RY, et al. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. *Lancet* 2019;393:747-57. [Crossref](#)
- International Society for Prenatal Diagnosis; Society for Maternal and Fetal Medicine; Perinatal Quality Foundation. Joint Position Statement from the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF) on the use of genome-wide sequencing for fetal diagnosis. *Prenat Diagn* 2018;38:6-9. [Crossref](#)
- van den Veyver IB, Eng CM. Genome-wide sequencing for prenatal detection of fetal single-gene disorders. *Cold Spring Harb Perspect Med* 2015;5.pii: a023077. [Crossref](#)

WES may incidentally reveal gene mutations that are unrelated to the initial indications for the test including unexpected childhood disorders, cancer-susceptibility genes, and adult-onset disorders. Variants of uncertain significance is also found at relatively high incidence and their significance on the future outcome of the baby can be difficult to determine<sup>15</sup>. Therefore, comprehensive pre- and post-test counselling from an expert in genetics is crucial when offering the test.

## Conclusion

WES is useful to aid in prenatal diagnosis of CHARGE syndrome. WES has an increased diagnostic yield for the genetic diagnosis of fetal structural anomalies when cytogenetics or chromosomal microarray analysis showed normal results. However, the cost and turnaround time of WES is a concern. Variants of uncertain significance and incidental findings of other genetic diseases are major challenges for applying WES in prenatal diagnosis. Appropriate case selection is crucial to maximise its benefit in prenatal diagnosis.

## Declaration

The authors have no conflict of interest to disclose.