Successful pregnancy after prenatal diagnosis by NGS for a carrier of complex chromosome rearrangements

Jian Ou 1†, Chuanchun Yang 2†, Xiaoli Cui 2†, Chuan Chen 2, Suyan Ye 3, Cai Zhang 2, Kai Wang 2, Jianguo Chen 2, Qin Zhang 1, Chunfeng Qian 1, Guangguang Fang 3,4* and Wenyong Zhang 5,6*

Abstract

Background: The study is aimed to provide prediction for fertility risk in the setting of assisted reproduction for a woman with complex chromosomal rearrangements (CCRs).

Methods: We implemented a robust approach, which combined whole-genome low-coverage mate-pair sequencing (WGL-MPS), junction-spanning PCR and preimplantation genetic testing for aneuploidy (PGT-A) method to provide accurate chromosome breakpoint junctional sequences in the embryo selection process in the setting of assisted reproduction for a couple with recurrent abortions due to CCRs.

Result: WGL-MPS was applied to a female carrying CCRs which consisted of 9 breakpoints and 1 cryptic deletion related to fertility risks. Sequencing data provided crucial information for designing junction-spanning PCR and PGT-A process, which was performed on the 11 embryos cultivated. One embryo was considered qualified for transplanting, which carried the exact same CCRs as the female carrier, whose phenotype was normal. The amniotic fluid was also investigated by WGL-MPS and karyotyping at 19 weeks’ gestation, which verified the results that the baby carried the same CCRs. A healthy baby was born at 39 weeks’ gestation by vaginal delivery.

Conclusion(s): Our study illustrates the WGL-MPS approach combining with junction-spanning PCR and PGT-A is a powerful and practical method in the setting of assisted reproduction for couples with recurrent miscarriage due to chromosomal abnormalities, especially CCRs carriers.

Keywords: Complex chromosomal rearrangements, Breakpoints mapping, Whole-genome low-coverage mate-pair sequencing, Preimplantation genetic testing for aneuploidy, Junction-spanning PCR

Background

Complex chromosomal rearrangements (CCRs) are structural rearrangements involving three or more cytogenetic breakpoints on more than two chromosomes [1, 2]. It has been estimated that 3.5% of couples with a history of recurrent miscarriage have at least one partner who is a carrier of a chromosomal structural rearrangement [3]. The most frequent of these rearrangements is translocation. Other rearrangements include inversions, insertions, deletions, duplications, or, rarely, ring chromosomes [4]. The potential risk for chromosome imbalance in the gametes of CCRs carriers is higher than those with simple translocations, and thus contributing to higher risk of recurrent miscarriage [5]. The incidence of spontaneous abortions and abnormal pregnancy outcomes in CCR families was estimated to be 48.3 and 53.7%, respectively [6]. Almost 18.4% of all live births from CCRs carriers result in phenotypically abnormal offspring and one-half of all CCRs carriers produce offspring who are also CCRs carriers [6]. Moreover, the higher the complexity of CCRs the higher the risk for unbalanced gamete generation and hence the higher the risk for having an affected offspring [7, 8]. In order to assess the risk faced by CCRs carriers who consider
pregnancy as accurately as possible, precise characterization of CCRs is of crucial importance. Several cytogenetic and molecular methods such as G-banded metaphase spreads of cultured lymphocytes using conventional methods. The man had normal 46, XY karyotype, while the woman was found to carry complex chromosome rearrangement: a q25q28 fragment of chromosome 4 was inserted into q22 in chromosome 1, and this chromosome 4 was shifted in equilibrium with chromosome 5. The breaking points were on 4q31.1 and 1q22, respectively. Her karyotype (Fig. 1) is:

46, XX, der(1)t(1;4)(p22;q31.1), der(4)ins(5;4)(q22; q25q28)t(1;4), der(5)ins(5;4).

WGL-MPS analysis and breakpoint validation
According to the results of karyotyping analysis, there was very little possibility for her to give birth to a normal child through natural pregnancy and she faced with an increased risk for having an affected offspring.

To make sure the exact location of the breakpoint and learn more about the risks of abnormal pregnancy outcomes, WGL-MPS was performed on the woman. Her genomic DNA was extracted from peripheral blood with Qiagen DNA extraction kit and then used to construct a non-size selected mate-pair library [12] and then subjected to 50-bp-end multiplex sequencing by BGISeq-500. After removing reads containing sequencing adapters and low-quality reads, the high-quality pair-end reads were aligned to the NCBI human reference genome (hg19, GRCh37.1) using SOAP2. Only uniquely mapped reads were remained for the subsequent analysis as previously described [13, 15]. The breakpoints were validated by junction-spanning PCR as previously described [9]. The PCR primer pairs were reserved sufficiently.

Preimplantation genetic testing for aneuploidy
The woman used a long protocol, or a GnRH (Gonadotropin-releasing hormone) antagonist protocol for controlled ovarian hyperstimulation. Oocytes were retrieved 34 to 35 h after hCG injection and fertilized with intracytoplasmic sperm injection (ICSI). We obtained 20 eggs through two cycles and 15 eggs were successfully fertilized, and 11 eventually developed into blastocysts. The ovarian stimulation, oocyte retrieval and embryos culture were performed as described by Yanagimachi R, et al [16]. The trophectoderm cells from the blastocysts were obtained as described by Jian Ou, et al [17], and rinsed three time with G-MOPS (Vitrolife) medium, and then transferred to RNase–DNase-free PCR tubes (Axygen) with the minimum medium. Whole genome amplification (WGA) was performed using a QIAGEN kit. Amplification products were stored at –20 °C. To avoid contamination, this process should be all handled in a ventilation cabinet. The breakpoints validation was performed on the amplification products with the PCR primer pairs kept previously and only three embryos (including two embryos with 9 breakpoints inherited and one embryo without breakpoints) were kept for further analysis. PGT-A was done by comprehensive chromosomal screening on these three embryos [17]. An embryo was found to be a balanced euploid and transferable. After genetic counseling, the couple decided to go ahead with implantation. The HCG level was tested 14 days after the embryo transfer. Pregnancy was confirmed by fetal heartbeat on ultrasonography. Amniocentesis at 19 weeks’ gestation was performed to confirm prenatal diagnosis.

Results
In this study, we presented a unique case of a woman diagnosed with very complex chromosomal rearrangements whose corresponding breakpoints were precisely identified by WGL-MPS. We used junction-spanning PCR to verify the corresponding breakpoints of the embryos generated during assisted reproduction and further checked for aneuploidy by conventional PGT-A. After careful counseling and obtaining consent from the couple, we transplanted a screened qualified embryo and
a normal phenotype baby with the same CCRs as its mother was born. Here we describe such approach (Fig. 2) in the clinical setting.

G-banding analysis at a band resolution of \( \sim 400 \) revealed the woman to be a carrier of balanced translocation among the three chromosomes and the two breakpoints were on 4q31.1 and 1p22, respectively. However, WGL-MPS analysis indicated a far more complicated rearrangement. In summary, 9 breakpoints and a microdeletion on chromosome 1 were identified as showed in Fig. 3. Using the new nomenclature for sequenced breakpoints proposed by Ordulu [18], the formula for the chromosome translocation was thus revised as:

\[
46,XX, \text{der}(1)\text{ins}(1;4)(1\text{qter-} > 1\text{p31.1} \ (5\text{q23.3::}1\text{p31.2}) \\
4\text{q28.3-} > 4\text{qter}),\text{der}(4)t(4;1)\\n(4\text{pter-} > 4\text{q31.1}::1\text{p31.2} > 1\text{pter}), \text{der}(5)\text{ins}(5)(5\text{pter-} > 5\text{q23.3}(t(4,1)(4\text{q28.3}(\text{inv}1) \\
(p31.3::p31.2) \text{inv}1)(p31.2::p31.1)) \text{5q23.3} > 5\text{qter}).
\]

In our study, four genes, including C1orf141, IL23R, MIER1, SLC35D1 are disrupted at the deletion on 1p31.3. The IL23R gene provides instructions for making a protein called interleukin 23 (IL-23) receptor. Sequence variations in IL23R gene have also been associated with the risk of several other immune system-related conditions, like psoriasis and inflammatory bowel disease. SLC35D1 is a nucleotide sugar transporter that...
Fig. 2 Experimental operation flow chart. First, we used WGL-MPS technology to detect the CCRs in maternal chromosomes. Secondly, we used PCR to verify the corresponding breakpoints of the 11 embryos generated by serial oocyte verification. Third, we performed PGT-A testing on the selected 3 embryos, and finally obtained an embryo with the same CCRs as the mother. Finally, we transplanted a screened qualified embryo and a normal phenotype baby with the same CCRs as its mother was born.

---

Fig. 3 Reassembly of all chromosomal regions that were involved in the translocations, according to HG19 (www.genome.ucsc.edu)

| Breakpoint | chr1. 3 67416640 | chr1. 4 67632214 | chr1. 5 69057822 | chr1. 6 69473476 | chr1. 7 84663140 | chr1. 8 127939569 | chr1. 9 129698237 |
|------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| chr4. 1    134333296 | chr4. 2 139154639 | chr5. 3          | chr5. 4          | chr5. 5          | chr5. 6          | chr5. 7          | chr5. 8          | chr5. 9          |
localizes to the endoplasmic reticulum and transports both UDP-glucuronic acid and UDP-N-acetyl galactosamine. Homozygous and compound heterozygous loss-of-function SLC35D1 mutations have been reported in patients with Schneckenbecken dysplasia. On chromosome 1, the PRKACB gene encoding a catalytic subunit of cAMP-dependent protein kinase (PKA) is interrupted at the 7th breakpoint. On chromosome 4, the SLC7A11 gene is disrupted at the 2nd breakpoint. On chromosome 5, FBN2 and SLC27A6 are disrupted at the 8th breakpoint. The FBN2 gene, encoding a large protein called fibrillin-2, is annotated in OMIM to be associated with autosomal dominant congenital contractual arachnodactyly and early-onset macular degeneration. Fortunately, the woman is not affected by the 8th breakpoint, probably because the breakpoint is close to the end of FBN2 gene sequence. No other known gene is interrupted by the remaining breakpoints, which are breakpoint 1, breakpoint 5, breakpoint 6 and breakpoint 9.

Eight pairs of primers were designed according to flanking sequences of the breakpoints. The sequences of the primers were displayed in Table 1. If the breakpoints location and sequences were predicted correctly as showed in Fig. 3 and the primers were valid, the corresponding bands of the amplification products should be presented on the electropherogram.

The WGA product of trophectoderm cells from eleven embryos underwent breakpoint analysis using PCR primer pairs designed to amplify junctional sequences and three embryos (including two embryos with 9 breakpoints inherited and one embryo without breakpoints) underwent the PGT-A protocol. PGT-A showed that Embryo4 was chr16 triploid and Embryo9 had a 6q16.1(93,100,000-99,500,000) deletion (Table 2). A single euploid embryo, identified to carry all the same nine breakpoints as its mother was implanted. Prenatal diagnosis by amniocentesis and WGL-MPS was performed at 19 weeks' gestation, which revealed the fetus to be a carrier of the same complex chromosomal rearrangements and deletion as the mother. A healthy 2780 g baby was delivered at 39 weeks' gestation by vaginal delivery.

Discussion
It has previously been demonstrated that precise characterization of apparently balanced CCRs in non-affected individuals is crucial as they are likely to produce gametes with unbalanced products because of quadrivalent formations during meiosis, which usually results in reproductive failure, recurrent miscarriages or affected offspring [20, 21].

In this study, we present a rare case of a non-affected female experienced recurrent miscarriage with CCRs. The karyotyping report indicates a balanced translation between chromosome 1 and chromosome 4 and a q25q28 fragment of chromosome 4 inserted into chromosome 5q22. However, WGL-MPS utilized in this study allowed accurate reconstruction of the derivative chromosomes, and interestingly revealed a far more complex rearrangement picture compromising translocation of three fragments of chromosome 1, a fragment of chromosome 4 and a fragment of chromosome 5. It has previously been demonstrated that cryptic deletions are a common finding in “balanced” reciprocal and

| Table 1 Primer information of the breakpoints |
|---------------------------------------------|
| Primer Name | Primer pair | Sequence (5′ > 3′) | Length | Tm | GC% | Product length |
| P1 Forward primer | GGCTGGGAAGTCCAACACGA | 20 | 62.39 | 60 | 382 |
| P2 Forward primer | GGCAACCTAATCAAGTACGGAA | 22 | 58.4 | 45.45 | 471 |
| P3 Forward primer | GGGAAGAGCCTTGCTCGTA | 19 | 59.1 | 57.89 | 336 |
| P4 Forward primer | GACAAAAATGAGCAAAATGCC | 23 | 58.82 | 43.48 | 320 |
| P5 Forward primer | GACAAAAATGAGCAAAATGCC | 23 | 58.82 | 43.48 | 320 |
| P6 Forward primer | GTGTTATGTTACCTTCCGCT | 24 | 60.08 | 45.83 | 421 |
| P7 Forward primer | TTGTTATGTTACCTTCCGCT | 24 | 60.08 | 45.83 | 421 |
| P8 Forward primer | TTGTTATGTTACCTTCCGCT | 24 | 60.08 | 45.83 | 421 |
complex chromosome rearrangements, which may explain the clinical phenotypes in many cases [20]. The woman in this case carried CCRs and had already experienced two miscarriages. Due to high degree of her CCRs, there was very little possibility for her to give birth to a normal child through natural pregnancy and she faced with an increased risk for having an affected offspring. After consulting with her physicians, the couple decided to go through the assisted reproduction procedure. Because of CCRs, breakpoints need to be accurately determined before transplantation, and embryos that do not have breakpoints or carry breakpoints like the mother need to be kept. The embryos retained in the above screening should be tested by PGT-A to screen out those with abnormal chromosomal structure and number. If this woman and her child reproduce in the future, they need assisted reproduction and do the above corresponding tests to screen for appropriate embryos. Our case demonstrated that WGL-MPS method combining with junction-spanning PCR and PGT-A could be a powerful and practical tool in the process of risk assessment and embryo selection for couples with recurrent miscarriage due to chromosomal abnormalities.

Precise identification of the breakpoints has been one of the most interesting and technically challenging field in cytogenetics for investigating the possible genotype and phenotypic outcomes of carriers of chromosomal rearrangements. Conventional techniques, such as in situ hybridization with fluorescent dye-labelled bacterial artificial chromosome clones and DNA array hybridization combined with chromosome sorting have been adopted to characterize the chromosome breakpoints to the kilobase level [22–25]. However, these techniques are laborious and expensive. In the recent years, massive parallel sequencing has been developed to accurately detect the breakpoints, but this technique is highly dependent on prior knowledge of the affected G-band region. In our study, we developed a practical solution which could rapidly localize the cryptic breakpoints to individual genes, and substantially improve the prediction of the fertility risks and phenotypic outcomes and timely inform antenatal medical care within a time frame that allows for clinical action. In addition, our approach which could precisely identify the breakpoints down to nucleotide level, can better assess the genotypic and phenotypic consequences of chromosomal abnormalities.

Conclusions

Accurate breakpoints mapping is the key to provide prediction for fertility risk, genetic counseling, and fertility guidance for couples who carry CCRs. In this study, a robust approach, whole-genome low-coverage mate-pair sequencing (WGL-MPS), was applied to a female CCRs carrier without taking advantage of the result of G-banding, precisely revealed 9 breakpoints and 1 cryptic deletion related to fertility risks, and provided crucial information for the PGT-A process. Junction-spanning PCR and PGT-A were performed on the 11 embryos cultivated and only one embryo was considered qualified which carried the exactly same CCRs as the female carrier, whose phenotype was normal. The amniotic fluid was also investigated by WGL-MPS, which verified the baby carried the same CCRs as the female carrier.

| Code  | Break(1–3) | Break(2–6) | Break(5–9) | Break(8–7) | Break(8–1) | Break(2–5) | Break(4–7) | Break(6–9) | PGT-A result | Result |
|-------|------------|------------|------------|------------|------------|------------|------------|------------|--------------|--------|
| Embryo1 | √          | √          | √          | √          | √          | √          | √          | √          | normal       | OK     |
| Embryo2 | ×          | √          | ×          | √          | ×          | ×          | ×          | ×          | NA           | Failed |
| Embryo3 | ×          | ×          | √          | ×          | √          | ×          | ×          | ×          | NA           | Failed |
| Embryo4 | √          | ×          | √          | ×          | ×          | ×          | ×          | ×          | chr16 triploid | Failed |
| Embryo5 | ×          | ×          | √          | ×          | ×          | ×          | ×          | ×          | NA           | Failed |
| Embryo6 | √          | ×          | √          | ×          | ×          | ×          | ×          | ×          | NA           | Failed |
| Embryo7 | ×          | ×          | √          | ×          | ×          | ×          | ×          | ×          | NA           | Failed |
| Embryo8 | ×          | ×          | √          | ×          | ×          | ×          | ×          | ×          | 6q16.1 del   | Failed |
| Embryo9 | ×          | ×          | √          | ×          | ×          | ×          | ×          | ×          | NA           | Failed |
| Embryo10 | √          | ×          | √          | ×          | ×          | ×          | ×          | ×          | NA           | Failed |
| Embryo11 | ×          | ×          | √          | ×          | ×          | ×          | ×          | ×          | NA           | Failed |

Acknowledgements

The authors thank The Affiliated Suzhou Hospital of Nanjing Medical University for providing samples and ethical approval and Technology-CheerLand Institute of Precision Medicine for providing research equipment.

Authors’ contribution
Jian Ou - made substantial contributions to conception and design, acquisition of data; been involved in drafting the manuscript and given final approval of the version to be published. Chuanchun Yang - made substantial
contributions to conception and design and modify the manuscript. Xiaoli Cui-made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data; been involved in drafting the manuscript. Chuan Chen and Jianguo Chen-made substantial contributions to biological experiments and acquisition of experimental data. Suyan Ye-made substantial contributions to revise the manuscript critically for important intellectual content. Cai Zhang and Kai Wang - investigated and read relevant background documents; been involved in revising the manuscript critically for important intellectual content. Qin Zhang and Chunfeng Qian - acquisition of sample data; been involved in drafting the manuscript. Guangguang Fang made substantial contributions to revise the manuscript critically for important intellectual content and given final approval of the version to be published. Wenyong Zhang made substantial contributions to conception and design; been involved in revising the manuscript critically for important intellectual content and given final approval of the version to be published.

Funding
This study was supported by the Industrial Technology Innovation Project of Suzhou City (SYS201566), Jiangsu Province “13th Five-Year” Youth Medical Person Program (QMRC2016246) and Special Fund for Science and Technology Innovation and Industrial Development in Dapeng New District, Shenzhen (YL201800201).

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
All procedures performed in the study involving samples were in accordance with the ethical standards of The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Center for Reproduction and Genetics, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Suzhou 215002, China. 2CheerLand Biological Technology Co., Ltd, Shenzhen 518000, China. 3Shenzhen Dapeng New District Maternity & Child Health Hospital, The First Affiliated Hospital of Shenzhen University, Shenzhen, China. 4Shenzhen Dapeng New District Maternity & Child Health Hospital, The Affiliated Suzhou Hospital of Suzhou City (SYS201566), Jiangsu Province “13th Five-Year” Youth Medical Person Program (QMRC2016246) and Special Fund for Science and Technology Innovation and Industrial Development in Dapeng New District, Shenzhen. 5Shenzhen Dapeng New District Maternity & Child Health Hospital, The First Affiliated Hospital of Shenzhen University, Shenzhen, China. 6School of Medicine, Southern University of Science and Technology-CheerLand Institute of Precision Medicine, Shenzhen, China. 7School of Medicine, Southern University of Science and Technology, Shenzhen, China.

Received: 7 November 2019 Accepted: 10 February 2020
Published online: 29 February 2020

References
1. Pai GS, G.H. Thomas, W. Mahoney, and B.R. Migeon, Complex chromosome rearrangements: Report of a new case and literature review. Clin Genet. 1980;18:436–44.
2. Kliczkowska A, Fryns JP, Van den Bergh H. Complex chromosomal rearrangements (CCR) and their genetic consequences. J Genet Hum. 1982;30:199–214.
3. Stephenson MD. Frequency of factors associated with habitual abortion in 197 couples. Fertil Steril. 1996;66:24–9.
4. Sierra S, Stephenson M. Genetics of recurrent pregnancy. loss. Semin Reprod Med. 2006;24:17–24.
5. Escudero T, Estop A, Fischer J, Munne S. Preimplantation genetic diagnosis for complex chromosome rearrangements. Am J Med Genet A. 2000;146A:1662–9.
6. Gorski JL, Kistenmacher ML, Punneth HH, Zackai EH, Emanuel BS. Restrictive errors for carriers of complex chromosome rearrangements: analysis of 25 families. Am J Med Genet. 1988;29:247–61.
7. Pellestor F, Anahory T, Lefort G, Puechhartier J, Liehr T, Hedon B, Sarda P. Complex chromosomal rearrangements: origin and meiotic behavior. Hum Reprod Update. 2011;17:476–94.
8. Madan K. Balanced complex chromosome rearrangements: reproductive aspects. A review. Am J Med Genet A. 2012;158A:947–63.
9. Aristidou C, et al. Accurate breakpoint mapping in apparently balanced translocation families with discordant phenotypes using whole genome mate-pair sequencing. PLoS One. 2017;12:e016935.
10. Chen W, et al. Breakpoint analysis of balanced chromosome rearrangements by next-generation paired-end sequencing. Eur J Hum Genet. 2010;18:39–43.
11. Le Scouarret S, Gribble SM. Characterising chromosome rearrangements: recent technical advances in molecular cytogenetics. Heredity (Edinb). 2012;108:75–85.
12. Luo A, et al. Maternal interchromosomal insertional translocation leading to 1q43-q44 deletion and duplication in two siblings. Mol Cytogenet. 2018;11:24.
13. Dong Z, et al. A robust approach for blind detection of balanced chromosomal rearrangements with whole-genome low-coverage sequencing. Hum Mutat. 2014;35:625–36.
14. Yao H, et al. Breakpoints and deleted genes identification of ring chromosome 18 in a Chinese girl by whole-genome low-coverage sequencing: a case report study. BMC Med Genet. 2016;17:49.
15. LI L, et al. Mapping breakpoints of a familial chromosome insertion (18,7) (q22.1; q62.2q21.11) to DPP9 and CACNA2D1 genes in an azoospermic male. Gene. 2014;457:43–9.
16. Yanagimachi R. Intracytoplasic injection of spermatooza and spermatogenic cells: its biology and applications in humans and animals. Reprod BioMed Online. 2005;10:247–88.
17. Ou J, et al. Identification of small segmental translocations in patients with repeated implantation failure and recurrent miscarriage using next generation sequencing after in vitro fertilization/Intracytoplasic sperm injection. Mol Cytogenet. 2015;8:105.
18. Orduz Z, et al. Describing sequencing results of structural chromosome rearrangements with a suggested next-generation cytogenetic nomenclature. Am J Hum Genet. 2014;94:695–709.
19. Toufaily MH, Roberts DJ, Westgate MN, Holmes LB. Triploidy: variation of phenotype. Am J Clin Pathol. 2016;145:86–95.
20. De Gregori M, et al. Cryptic deletions are a common finding in “balanced” reciprocal and complex chromosome rearrangements: a study of 59 patients. J Med Genet. 2007;44:750–62.
21. Aleksandrova N, et al. Comparison of the results of preimplantation genetic screening obtained by a-CGH and NGS methods from the same embryos. Gynecol Endocrinol. 2016;32:1–4.
22. Baronchelli S, et al. Investigating the role of X chromosome breakpoints in premature ovarian failure. Mol Cytogenet. 2012;5:32.
23. Scott SA, Cohen N, Brandt T, Warburton PE, Edelmann L. Large inverted repeats within Xp11.2 are present at the breakpoints of isodicentric X chromosomes in Turner syndrome. Hum Mol Genet. 2010;19:3383–93.
24. Fonseca AC, Bonaldi A, Bentola DR, Kim CA, Otto PA, Vianna-Morgante AM. The clinical impact of chromosomal rearrangements with breakpoints upstream of the SOX9 gene: two novel de novo balanced translocations associated with acampomelic campomelic dysplasia. BMC Med Genet. 2013;14:50.
25. Vergult S, et al. Mate pair sequencing for the detection of chromosomal aberrations in patients with intellectual disability and congenital malformations. Eur J Hum Genet. 2014;22:652–9.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.