Original Article

Maternal-Fetal Medicine®

OPEN

Detection of an 8p23.1 Inversion Using High-Resolution Optical Genome Mapping

Chunxiang Zhou, Huijun Li, Yiyan Shi, Linlin He, Honglei Duan, Jie Li*

Abstract

Objective: To evaluate the performance of optical genome mapping (OGM) in identifying an inversion located in the short arm of chromosome 8 (8p, 8p23.1), flanked by regions of complex segmental duplication (SD), using the GRCh38 and telomere-to-telomere (T2T) genome references.

Methods: We investigated a couple suspected of carrying the 8p23.1 inversion due to a terminal deletion combined with an interstitial duplication of 8p found in their abortus. OGM was performed on both individuals. The data were mapped to the current GRCh38 and the updated T2T genome references, respectively.

Results: The 8p23.1 inversion was observed in the female when mapping OGM data to the T2T assembly. In contrast, under the GRCh38 reference, the orientation between the suspected breakpoints within the SD regions could not be distinguished. Additional variants of uncertain significance were also identified in both individuals.

Conclusion: Our findings highlight the superiority of the T2T reference in recognizing structural variations involving SD regions. The enhanced SV detection using the T2T reference may contribute to a better understanding of genome instability and human diseases.

Keywords: Genome mapping; Telomere-to-Telomere reference; Segmental duplication; 8p23.1 inversion

Introduction

Inversions, as integral components of structural variations (SVs) within genomes, play a critical role in disease etiology. They can directly disrupt gene structures or regulatory sequences and indirectly predispose offspring to secondary rearrangements.[1,2] Even those inversions classified as polymorphisms have been linked to genome instability and human diseases, such as neurodevelopmental disorders.[3] Identifying inversions is essential for understanding genetic variations and associated diseases. Traditionally, inversion detection has relied on conventional cytological techniques. However, advanced technologies such as optical genome mapping (OGM), nanopore sequencing, and paired-end low-pass genome sequencing have significantly expanded our ability to identify various SVs, thereby enhancing our understanding of genomic SVs.[4–6]

Notably, the breakpoints of inversion polymorphisms are frequently located in regions of identical segmental duplication (SD), making it difficult to identify in the current human reference genome (GRCh38 and GRCh37), where SD regions are often treated with blind areas among the regions to be fully sequenced.[7,8] Within the GRCh38 and GRCh37 assembly, complex SD regions often leave gaps and provide no frame reference. The inversions flanked by these SD areas are usually missed or incorrectly presented even using the powerful technologies above.

The telomere-to-telomere (T2T) consortium recently presented a complete 3.055 billion base pair (bp) sequence of a human genome, namely, T2T-CHM13, making a novel supplement of previous unresolved areas including those within SD regions.[9–11] A study found that using the T2T reference improved the accuracy of inversion orientation representation threefold and increased sensitivity by approximately 21% compared to GRCh38.[12] This comprehensive genome reference provides a clearer view of variations involving SD regions, aiding in the prediction of genome instability and its correlation with human diseases.

In this study, OGM was utilized to investigate a couple suspected of having an inversion flanked by SD regions at chromosome 8p23.1, following the detection of a terminal deletion and an interstitial duplication at the short arm of chromosome 8 (8p) in their abortus. The inversion was confirmed in the female using the T2T genome reference but not GRCh38. Our finding highlights the superiority of the T2T reference in identifying genome inversions in clinical practice.

Clinical description

The couple, of Chinese origin, were healthy and nonconsanguineous. Their first pregnancy was uneventful until agenesis of the corpus callosum (ACC) was observed at 22 weeks during a routine ultrasound scan, confirmed by magnetic resonance imaging. The couple opted for pregnancy termination. Postnatal chromosomal microarray analysis

Obstetrics and Gynecology Medical Center, Nanjing Drum Tower Hospital, Affiliated Hospital of Nanjing Medical University, Nanjing 210008, China.

** Corresponding author: Jie Li, Obstetrics and Gynecology Medical Center, Nanjing Drum Tower Hospital, Affiliated Hospital of Nanjing Medical University, Nanjing 210008, China. E-mail: jie1967@126.com*

Maternal-Fetal Medicine (2024) 6:3

Received: 24 December 2023 / Accepted: 23 May 2024

First online publication: 1 July 2024

http://dx.doi.org/10.1097/FM9.0000000000000238

(CMA) conducted in Be Creative Lab (Beijing) Company revealed a euploid female fetus with a terminal deletion of 7.9 Mb and an interstitial duplication of 25.8 Mb at 8p, with a 4.4 Mb copy-number neutral segment between the deletion and duplication. G-banding karyotyping of the couple showed negative findings. The counseling during the last pregnancy and pre- and post-test counseling of the CMA of the abortus as well as karyotyping of the couple were conducted at different centers.

Seeking guidance for future pregnancies, the couple visited the genetic counseling clinic of Nanjing Drum Tower Hospital, affiliated with Nanjing University Medical School. Genetic counseling suggested a potential cryptic inversion at 8p23.1, based on the CMA findings. They were informed about the high prevalence of this inversion in the general population and the low recurrence risk. Preimplantation genetic testing was not recommended, but prenatal genetic testing could be considered.

Materials and methods

Optical genome mapping

Ultrahigh molecular weight (UHMW) DNA was isolated, labeled, and processed using the Bionano Genomics Saphyr® platform according to the manufacturer's protocols (Bionano Genomics, San Diego, CA) at Juno Genomics Lab (Hangzhou) Co. Ltd. UHMW DNA was isolated from peripheral blood using the SP Blood and Cell Culture DNA Isolation Kit (Bionano Genomics, San Diego, CA). The dynamic light scattering DNA Labeling Kit (Bionano Genomics) was used to fluorescently label the UHMW DNA molecules at specific sequence motifs throughout the genome with the enzyme DLE-1 (Bionano Genomics). Labeled DNA was loaded onto a Saphyr chip and imaged on the Saphyr instrument (Bionano Genomics) to collect molecules >150 kilobases (kb).

Quality control

A minimum of 320 gigabases (Gb) of data was acquired per sample. The pre-analytical quality control (QC) metrics included observable UHMW DNA viscosity/clarity during pipetting and a minimum DNA concentration of >35 ng/μL. Analytical QC metrics included an average filtered N50 > 150 kb, label density of ~15/100 kb, and effective coverage of >80× for de novo assembly generation.

Data analysis

For data analysis, Bionano Access (version 1.7), an OGM-specific structural variant analysis software available as a standard web browser application, links to bioinformatic servers running Bionano Solve (version 3.7), an automated analytical pipeline for the detection of genomic abnormalities. Single molecules were used to generate de novo assembly. SVs (insertions, duplications, deletions, inversions, fusion, and translocations) were identified based on the differences in the alignment of labels between the sample and the reference genomes. Copy number variations (CNVs) and aneuploidies were detected by alignment to the human genome GRCh38 reference. To further identify the potential SV in 8p23.1, an additional T2T genome reference was used. In addition, regions of the absence of heterozygosity (AOH) were detected based on consistent decreases in heterozygous SV calls compared to the level observed genome-wide in controls.

Ethical approval

All procedures involving human participants were conducted in accordance with institutional ethical standards, and written consent was obtained before the study. The study followed the *Declaration of Helsinki* and was approved by the ethics committee of Nanjing Drum Tower Hospital (no. 2021-462-02). Written consent for OGM testing and publication of the results was obtained.

Results

Approximately 500 Gb of data were collected from each sample. The main QC metrics of OGM are shown in Table 1. For OGM analysis on the female, mapping to GRCh38 reference did not span the flanked SDs resulting in the discontinuous maps covering 8p23.1 at these low-copy repeats (Fig. 1A). The orientation between the SDs was ambiguous, and the inversion could not be confirmed nor ruled out. By mapping OGM data to the T2T reference manually, a heterozygous inversion at 8p23.1 was determined, with start and end positions at 7,370,695 and 11,678,224, respectively (Fig. 1B). The mapping results using both references are illustrated in Figure 1C.

No SVs were identified in chromosome 8p in the male, even with manual mapping using both references (data not shown). Additional variations were found in the female (three variations) and male (four variations) when mapped to the GRCh38 reference (Table 2). According to the American College of Medical Genetics and Genomics and the Clinical Genome Resource joint consensus recommendation for CNV interpretation and reporting,[13] these variations were classified as variants of unknown significance.

Discussion

This study identified an inversion at 8p23.1 in the female by mapping OGM data to the T2T reference, highlighting the difficulty of detecting such inversions using traditional methods.[14] The findings emphasize the enhanced SV detection capability when using the OGM platform in conjunction with the T2T reference.

The rearrangement characterized by a terminal deletion and an interstitial duplication at chromosome 8p is also named 8p inverted duplication deletion syndrome (inv dup del (8p))

Table 1

Optical genome mapping quality control metrics for female and male samples.

Quality control metrics	Female	Male
Total length (≥150 Gb)	539.38	518.04
Molecule N50 (≥150 kb)	262.88	291.39
Label density (/100 kb*)	14.54	16.43
Fraction of molecules aligned	0.95	0.97
Effective coverage of reference	149.96 × †	152.14 × †

*/100 kb indicates that the measurement is normalized per 100 kilobases of the genome.

†The "x" indicates the average number of times each base in the reference genome is covered by the optical genome mapping data.

kb: Kilobase; Gb: Gigabase.

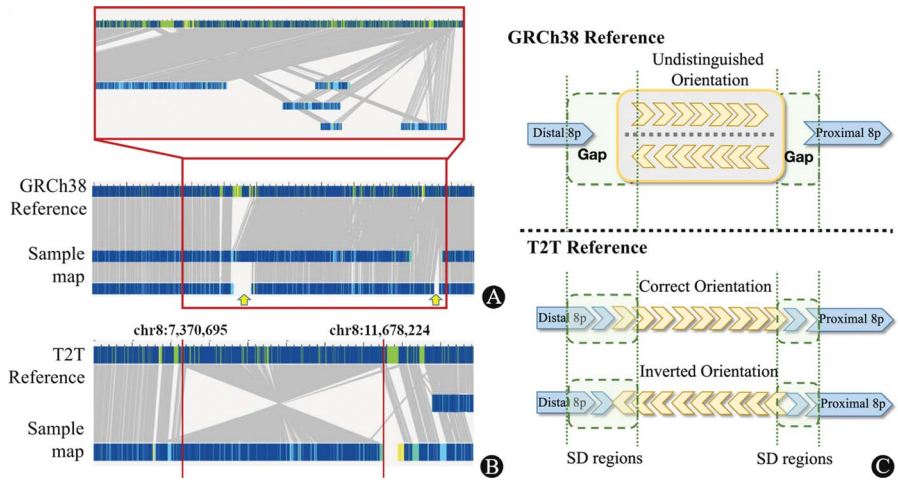

Figure 1. Optical genome mapping using different references. A Mapping to GRCh38 reference: gaps appear in two SD regions where suspected breakpoints (yellow arrows) are located. B Mapping to T2T reference: the orientation between the two SD regions is inverted, though gaps remain in the proximal SD region. C Model for distinguishing the inversion: GRCh38 reference shows gaps in SD regions, making orientation indeterminate. T2T reference reveals SD regions, allowing identification of correct or inverted orientation. SD: Segmental duplication; T2T: Telomere-to-telomere; 8p: Short arm of chromosome 8.

because the duplication is usually inverted.[14] The reported clinical features include mental retardation, intellectual disability, facial dysmorphism, structural abnormalities of the central nervous system, autism or autistic-like features, hypotonia, orthopedic abnormalities, and congenital heart defects.[15,16] Structural anomalies of central nervous system are universal in the affected individuals, with ACC or ACC hypoplasia being the most common,[16] consistent with the prenatal structural findings in the last pregnancy of the couple.

Parental 8p23.1 inversion is believed to increase the susceptibility of 8p rearrangements by mediating nonallelic homologous recombination.[14] However, parental inversion is often presumed in many studies, while few attempted to verify it. Human chromosome 8p23.1 region contains two clusters of olfactory receptor genes or defensin repeats, REPD (REPeat Distal) and REPP (REPeat Proximal), which are the distal and proximal repeats, respectively. Since the complexity of the breakpoints, studying 8p23.1 inversion is challenging, and a rate of 12%–59% in various ethnic groups has been found by fluorescent *in situ* hybridization (FISH) on around 200 individuals.[14] Besides a predisposition of the offspring rearrangement, associations between diseases and the inversion state have also been established. For instance, the presence of inversion was found to be associated with neuroticism and several risky behavior traits, as well as a lower risk of systemic lupus erythematosus.[17,18] Thus, accurate detection of the inversion also helps to further understand the correlation with these diseases. Among routine genetic testings, the inversion is undetectable by karyotyping given the low resolution. FISH is capable but special probes are needed, and it seems impracticable for wide use of FISH to detect such an inversion.

OGM has developed into a powerful method for SV detection and gave rise to an era of "next-generation cytogenetics."[6,19] By imaging very long linear single DNA molecules (median size >250 kb) labeled at specific sites, OGM can recognize nearly all types of chromosomal aberrations with non-centromeric breakpoints.[6] Given the forceful capacity of SV detection, especially for those unknown or easily overlooked ones, OGM promises to be feasible as one of the first-line diagnostic tools. However, gaps within SD and pericentromeric regions often result in missed SVs when using current genome references.[19] Because many inversions have breakpoints within SD regions and their potential influence in both genetic variations and human diseases, a more comprehensive genome is needed to fully understand these inversions. The

Table 2

Genomic variations identified in female and male samples using GRCh38 reference.

Sex	Region (GRCh38 reference)	Variation type	Zygosity	Size (bp)	OMIM genes
Female	chr11:96,122,380-96,133,009	Deletion	Heterozygous	10,630	*MAML2* (OMIM 607537)
	chr13:100,199,978-100,216,533	Deletion	Heterozygous	16,556	*PCCA* (OMIM 232000)
	chr20:25,355,373-25,370,775	Deletion	Heterozygous	15,403	*ABHD12* (OMIM 613599)
Male	chr11:78,509,518-78,522,050	Deletion	Heterozygous	12,533	*NARS2* (OMIM 612803)
	chr15:22,735,157-22,808,305	Deletion	Heterozygous	73,149	*NIPA1* (OMIM 608145)
	chr6:16,472,076-16,479,173	Deletion	Heterozygous	7098	*ATXN1* (OMIM 601556)
	chr12:20,847,759-20,861,652	Duplication	Heterozygous	13,894	*SLCO1B3* (OMIM 605495)

bp: Base pair; OMIM: Online Mendelian Inheritance in Man. *MAML2*: Mastermind like transcriptional coactivator 2; *PCCA*: Propionyl-CoA carboxylase subunit alpha; *ABHD12*: Abhydrolase domain containing 12, lysophospholipase; *NARS2*: Asparaginyl-tRNA synthetase 2, mitochondrial; *NIPA1*: NIPA magnesium transporter 1; *ATXN1*: Ataxin 1; *SLCO1B3*: Solute carrier organic anion transporter family member 1B3.

updated T2T reference makes it possible since the informative supplement about complex SD regions.[11] In a study evaluating inversions by multi technologies under T2T reference, the researchers established the association of recurrent inversions and hotspots of disease-causing rearrangements, such as a 17p11.2 inversion partially overlapping the well-known SMPLS region that potentially serve as protective effects with respect to 17p11.2 CNV formation.[3]

Our study detected the 8p23.1 inversion in the couple using OGM and evaluated the difference when mapping to GRCh38 and the updated T2T reference. Not surprisingly, when mapping the data to GRCh38 reference, the maps covering 8p were discontinuous at the two SD regions, providing no information on the orientation of the segment between the SDs. The observation coordinates another study detecting the inversion located in the same region by OGM under GRCh37 reference, which mapped 54 samples at the two SDs artificially.[20] Six out of 54 showed evidence of the polymorphic inversion, but the SV pipeline called this inversion in only one instance. Nevertheless, the only one carrying the inversion confirmed by FISH was also undistinguished even by careful analyze.[20] Together with our study, the orientation between the two SDs is illegible under the GRCh38 or GRCh37 reference. Due to the informative supplement of SD regions by the T2T reference, the mapping seemed integrated and the orientation could be determined much easier. To further verify the capacity of 8p23.1 inversion recognition as well as others with breakpoints located in similar SD regions, more data are needed to enlarge the applications in the general population.

However, the inversion was not verified by additional methods such as FISH and long range polymerase chain reaction (PCR). Similar to many other studies, we lack the specific probes for FISH analysis. Long-range PCR verification is also difficult due to the complexity of the SD region. Nevertheless, the rearrangement found in the abortus and the populating of the inversion both support the OGM finding of the inversion. The significance of our study is not only for the inversion identification in the present case. The recurrent risk is still really low although the female was identified as a carrier of the inversion. The significance of our study extends beyond this case, highlighting the potential of T2T reference to reveal SVs in previously unresolved genome regions.

Conclusion

An 8p23.1 inversion was detected using the T2T genome reference, not the GRCh38 reference, underscoring the superiority of the T2T reference in recognizing inversion polymorphisms flanked by complex SD regions. Once the T2T reference is widely used in genetic testing, comprehensive information about inversion polymorphism might help to recognize the genome events and human diseases.

Acknowledgments

We would like to thank the family for participation and for agreeing to donate their personal data.

Funding

The study was supported by funding for Clinical Trials from the Affiliated Drum Tower Hospital, Medical School of Nanjing University (2022-LCYJ-MS-06).

Author Contributions

Study design was conducted by Chunxiang Zhou. Experimental operations were performed by Chunxiang Zhou, Huijun Li, Yiyan Shi, and Linlin He. Data analysis was carried out by Chunxiang Zhou. Writing of the original draft was prepared by Chunxiang Zhou, and review and editing were done by Honglei Duan and Jie Li. Funding support was provided by Li Jie. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

None.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Campoy E, Puig M, Yakymenko I, et al. Genomic architecture and functional effects of potential human inversion supergenes. Philos Trans R Soc Lond B Biol Sci 2022;377(1856):20210209. doi: 10.1098/rstb.2021.0209.
[2] Villoutreix R, Ayala D, Joron M, et al. Inversion breakpoints and the evolution of supergenes. Mol Ecol 2021;30(12):2738–2755. doi: 10.1111/mec.15907.
[3] Porubsky D, Höps W, Ashraf H, et al. Recurrent inversion polymorphisms in humans associate with genetic instability and genomic disorders. Cell 2022;185(11):1986–2005.e26. doi: 10.1016/j.cell.2022.04.017.
[4] Yang L. A practical guide for structural variation detection in the human genome. Curr Protoc Hum Genet 2020;107(1):e103. doi: 10.1002/cphg.103.
[5] Mantere T, Neveling K, Pebrel-Richard C, et al. Optical genome mapping enables constitutional chromosomal aberration detection. Am J Hum Genet 2021;108(8):1409–1422. doi: 10.1016/j.ajhg.2021.05.012.
[6] Zhao X, Collins RL, Lee WP, et al. Expectations and blind spots for structural variation detection from long-read assemblies and short-read genome sequencing technologies. Am J Hum Genet 2021;108(5):919–928. doi: 10.1016/j.ajhg.2021.03.014.
[7] Pu L, Lin Y, Pevzner PA. Detection and analysis of ancient segmental duplications in mammalian genomes. Genome Res 2018;28(6):901–909. doi: 10.1101/gr.228718.117.
[8] Dennis MY, Eichler EE. Human adaptation and evolution by segmental duplication. Curr Opin Genet Dev 2016;41:44–52. doi: 10.1016/j.gde.2016.08.001.
[9] Nurk S, Koren S, Rhie A, et al. The complete sequence of a human genome. Science 2022;376(6588):44–53. doi: 10.1126/science.abj6987.
[10] Aganezov S, Yan SM, Soto DC, et al. A complete reference genome improves analysis of human genetic variation. Science 2022;376(6588):eabl3533. doi: 10.1126/science.abl3533.
[11] Vollger MR, Guitart X, Dishuck PC, et al. Segmental duplications and their variation in a complete human genome. Science 2022;376(6588):eabj6965. doi: 10.1126/science.abj6965.
[12] Porubsky D, Harvey WT, Rozanski AN, et al. Inversion polymorphism in a complete human genome assembly. Genome Biol 2023;24(1):100. doi: 10.1186/s13059-023-02919-8.
[13] Riggs ER, Andersen EF, Cherry AM, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen) [published correction appears in Genet Med. 2021 Nov;23(11):2230]. Genet Med 2020;22(2):245–257. doi: 10.1038/s41436-019-0686-8.
[14] Salm MPA, Horswell SD, Hutchison CE, et al. The origin, global distribution, and functional impact of the human 8p23 inversion polymorphism. Genome Res 2012;22(6):1144–1153. doi: 10.1101/gr.126037.111.
[15] Fisch GS, Davis R, Youngblom J, et al. Genotype-phenotype association studies of chromosome 8p inverted duplication deletion syndrome. Behav Genet 2011;41(3):373–380. doi: 10.1007/s10519-011-9447-4.

[16] Vibert R, Mignot C, Keren B, et al. Neurodevelopmental phenotype in 36 new patients with 8p inverted duplication-deletion: Genotype-phenotype correlation for anomalies of the corpus callosum. Clin Genet 2022;101(3):307–316. doi: 10.1111/cge.14096.
[17] Lo MT, Hinds DA, Tung JY, et al. Genome-wide analyses for personality traits identify six genomic loci and show correlations with psychiatric disorders. Nat Genet 2017;49(1):152–156. doi: 10.1038/ng.3736.
[18] Namjou B, Ni Y, Harley IT, et al. The effect of inversion at 8p23 on BLK association with lupus in Caucasian population. PloS One 2014;9(12):e115614. doi: 10.1371/journal.pone.0115614.
[19] Sahajpal NS, Barseghyan H, Kolhe R, et al. Optical genome mapping as a next-generation cytogenomic tool for detection of structural and copy number variations for prenatal genomic analyses. Genes (Basel) 2021;12(3):398. doi: 10.3390/genes12030398.
[20] Dremsek P, Schwarz T, Weil B, et al. Optical genome mapping in routine human genetic diagnostics-its advantages and limitations. Genes (Basel) 2021;12(12):1958. doi: 10.3390/genes12121958.

Edited By Yang Pan

How to cite this article: Zhou C, Li H, Shi Y, He L, Duan H, Li J. Detection of an 8p23.1 Inversion Using High-Resolution Optical Genome Mapping. Maternal Fetal Med 2024;6(3):173–177. doi: 10.1097/FM9.0000000000000238.

Erratum

Erratum to Wernicke-Korsakoff Syndrome From Hyperemesis Gravidarum. Volume 6, Issue 1, January 2024

The corresponding author, Vaishnavi Patel, wishes to update the associated email address listed on the paper. The original email address "vaishnavipatel88@gmail.com" is no longer in use, and it needs to be changed to "vaishnavipateldo@gmail.com".

Original Corresponding Author Email: vaishnavipatel88@gmail.com

Corrected Corresponding Author Email: vaishnavipateldo@gmail.com

The author apologizes for any inconvenience caused.

Reference

[1] Patel VJ, Vu J, Mercado G, Avula S, Deering S. Wernicke-Korsakoff Syndrome From Hyperemesis Gravidarum. Maternal Fetal Med 2024;6(1):54–56. doi: 10.1097/FM9.0000000000000198.

http://dx.doi.org/10.1097/FM9.0000000000000235