Movement Disorders
CLINICAL PRACTICE
LETTERS: GENOTYPE AND PHENOTYPE

An Inversion Affecting the *GCH1* Gene as a Novel Finding in Dopa-Responsive Dystonia

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Dopa-responsive dystonia (DRD) is most commonly caused by autosomal dominant GTP cyclohydrolase 1 deficiency,[1] also known as Segawa disease (formerly DYT5a). Although more than 100 clinically relevant coding *GCH1* variants have been identified, a number of DRD patients lack an identifiable disease-causing variant detectable with conventional gene sequencing.[1] Some of these patients are found to have structural variants, such as deletions in the *GCH1* gene.[2,3] Here we report the first known case of DRD due to a large inversion disrupting the *GCH1* gene.

A 57-year-old Han Chinese male developed abnormal head posturing and gait disturbance with diurnal fluctuation following a trivial head injury at age 10 years. He was otherwise well with normal developmental milestones. He underwent neck muscle denervation surgery aged 15 due to deterioration in head movements, which resulted in temporary improvement. Low-dose levodopa/benserazide 200/25 mg twice daily was commenced soon thereafter due to recurrence of involuntary head movements, with marked but incomplete improvement in symptoms. At presentation to our clinic aged 42, he had cervical dystonia characterized by anterocollis, left torticollis, right laterocollis, and side-to-side head tremor. He had associated moderate bilateral bradykinesia, hypophonia, mild slowing of gait, and mildly impaired postural righting reflexes. There was no evidence of limb dystonia, tremor, rigidity, or ataxia (Video 1). He remained responsive to the same low dose of levodopa after several decades with only mild progressive deterioration in gait over 15 years of follow-up. Prior attempts to wean levodopa resulted in reversible deterioration of head movements and gait. Botulinum toxin and trihexyphenidyl were trialed with modest effectiveness but were not tolerated. There were no affected family members, including two daughters aged 11 and 14 (Fig. S1).

Magnetic resonance brain imaging was unremarkable. Blood test screening for Wilson's disease was negative. Single gene sequencing of *GCH1* and *TOR1A* did not identify a causative variant. Multiplex ligation-dependent probe amplification (MLPA) was not performed. Further genetic studies were undertaken on a research basis with written informed consent and ethics approval (2019/PID14033). Short-read whole genome sequencing (WGS) was performed as described[4] and using the ClinSV tool we detected a possible a ~5 Mb inversion on chromosome 14 disrupting *GCH1* (Fig. S2). The inversion is part of a complex structural variant (NC_000014.8:g.[55343254_55346605del;55346606_60822142inv;60822143_60823119del]). No other clinically-relevant variants were detected in the DRD-associated genes *GCH1*, *TH*, *SPR*, or other known dystonia genes (see[4]). We were unable to confirm the breakpoints using Sanger sequencing, likely because repetitive sequences in the breakpoint region hindered design of specific sequencing primers. However, targeted long-read

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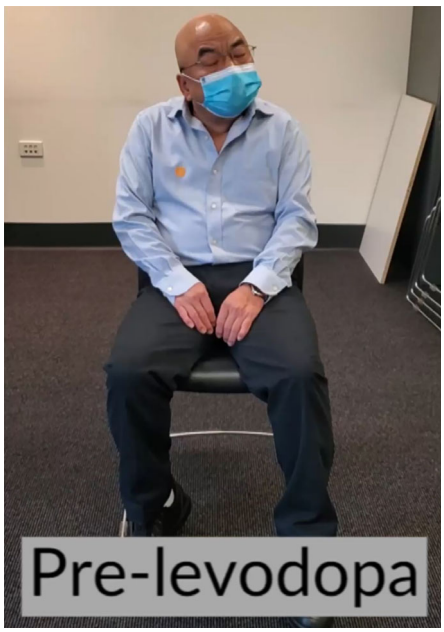

Video 1. Pre-levodopa examination shows cervical dystonia with anterocollis, left torticollis, right laterocollis, and jerky side-to-side head tremor. There is moderate bilateral bradykinesia, slowing of foot taps, and mildly reduced gait speed. Post-levodopa examination shows improvement in the dystonic neck position and tremor, bradykinesia, and walking speed.
Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/mdc3.14023

sequencing performed using Oxford Nanopore's "ReadUntil" function,[5] focusing on the candidate inversion region, confirmed the breakpoints at both ends of the putative inversion (Fig. S3). Further confirmation of the inversion was attained through Bionano optical genome mapping (OGM)[6] (Fig. 1A). Taken together, these findings confirmed the presence of a chromosomal inversion disrupting *GCH1* by displacement of the promoter and first exon (Fig. 1B). The inversion is a component of a complex structural variant belonging to the Paired-Deletion Inversion (delINVdel) subclass.[7] There are no repeat sequences implicated in recombination and so the molecular basis for generation of the variant remains unclear.

To our knowledge, this is the first report of DRD due to an inversion affecting the *GCH1* gene, which is unlikely to have been detected using traditional approaches to genetic testing. Despite the absence of a causative variant on initial standard *GCH1* gene sequencing, clues to the diagnosis of DRD included young age at onset, diurnal fluctuations, and sustained response to low-dose levodopa without development of dyskinesia. Given the high degree of clinical suspicion of DRD, we pursued short-read WGS on a research basis, which enabled identification of a complex structural variant, a component of which is an inversion impacting *GCH1*. This finding was confirmed using two orthogonal approaches, namely long read nanopore sequencing and Bionano OGM. We note studies which have used other methods (eg, fluorescence in situ hybridization, large-insert whole-genome sequencing) to detect complex chromosomal rearrangements affecting *GCH1*,[8] and also the use of long-read sequencing to resolve a complex structural variant in the *PRKN* gene.[9] MLPA has been used to detect copy

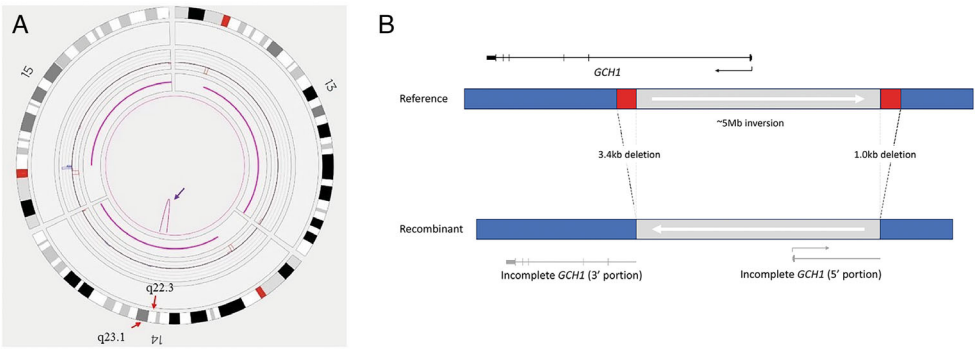

Figure 1. **(A)** Optical genome mapping (OGM) results. OGM allows us to study long DNA molecules using fluorescent labels and can be used as a tool for the detection of structural variants that are difficult to detect using other methods.[6] Chromosome 13–15 circos plot confirms an intra-chromosomal translocation event depicted by a purple arrow between the long arm q23.1 and q22.3 (highlighted by red arrows) of chromosome 14. This event coincides with the *GCH1* gene. The plot's outer to inner circles represent the chromosome cytoband, structural variation track, copy number variation (CNV) track, variant allele frequency (VAF), and translocations. **(B)** Schematic demonstrating the structural variant (NC_000014.8:g.[55343254_55346605del;55346606_60822142inv;60822143_60823119del]) affecting *GCH1*. The exonic structure of *GCH1* RefSeq transcript NM_000161.3 is shown at the top of the figure. *GCH1* is on chromosome 14 and has a negative orientation in the genome assembly, so transcription is from right to left in the figure. Reference and recombinant chromosomes are represented by colored boxes (red: deleted regions; gray: inversion, with direction change indicated by arrow; blue: regions outside of chromosomal rearrangement); note these are not drawn to scale. The left (centromere proximal) inversion breakpoint is located within *GCH1*, in the intron between the first and second exons. Consequently, the inversion displaces the promoter and first exon from the remainder of the gene, and would cause loss of function.

number variants (eg, duplications, deletions) in *GCH1*,[10] but would not have utility for detecting copy number neutral variants such as the inversion observed in our study.

For patients with a phenotype consistent with DRD in whom conventional sequencing is unrevealing, we recommend the use of WGS, nanopore long-read sequencing, and OGM as novel approaches to detect and confirm complex structural events affecting the *GCH1* gene. Clinicians should be aware that unsolved cases of DRD may be due to structural variation, such as mega-base scale inversion, affecting *GCH1*. This report highlights the value of applying emerging genomic technologies to genetically unsolved cases to detect genetic variants that are missed using conventional approaches.

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Author Roles

(1) Research project: A. Conception, B. Organization, C. Execution; (2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique; (3) Manuscript: A. Writing of the first draft, B. Review and Critique.

S.E.: 1A, 1B, 1C, 3A, 3B
A.F.: 1B, 1C, 3B
M.H.: 1B, 1C, 3A, 3B
J.C.: 1B, 1C, 3B
I.D.: 1B, 1C, 3B
I.S.: 1C, 3B
M.S.: 1C, 3B
D.Z.: 1C, 3B
R.N.: 1C, 3B
B.G.: 1C, 3B
L.W.: 1C, 3B
A.C.: 1B, 1C, 3B
D.Y.: 1C, 3B
L.I.R.: 1C, 3B
M.M.H.: 1B, 1C, 3B
V.M.H.: 1B, 1C, 3B
M.K.: 1C, 3B
K.R.K.: 1A, 1B, 1C, 3A, 3B
M.H.: 1A, 1B, 1C, 3B

Disclosures

Ethical compliance statement: Written informed consent was obtained from the patient. Ethics approval was obtained for undertaking genetic analysis on a research basis (2019/PID14033). We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

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Supporting Information

Supporting information may be found in the online version of this article.

Supplementary Figure S1. Pedigree of the family. Arrow indicates proband who underwent genetic studies, filled symbol indicates affected, squares represent males and circles represent females. d. MVA = died in motor vehicle accident. Additional family members were not available for testing.

Supplementary Figure S2. Illumina short read whole genome sequencing data indicating a structural variant (NC_000014.8:g.[55343254_55346605del;55346606_60822142inv;60822143_60823119del]) on chromosome 14 affecting *GCH1*. Chimaeric ("split") reads identified by the ClinSV tool are visualized in the IGV genome browser. To ease interpretation the alignments of segments of two representative reads are highlighted (A00488:195:HGJN7DSX2:3:2336:15573:12743 in red, and A00488:195:HGJN7DSX2:3:2160:31503:1219 in blue). Other tracks show the deletions which flank the inversion, and the exonic structure of *GCH1* transcript NM_000161.3. **(A)** The left-hand (centromere proximal) breakpoint region, associated with a 3.4 kb deletion. **(B)** The right-hand (centromere distal) breakpoint region, associated with a smaller (1.0 kb) deletion.

Supplementary Figure S3. Oxford Nanopore long read sequencing (LRS) data supporting the proposed structural variant. Chimaeric nanopore sequences are visualized in the IGV genome browser. The sequence alignments confirm the breakpoints indicated by short read sequencing analysis, and extend wide enough for a high level of confidence in read locations. **(A, B)** Inversion breakpoint regions as in Fig. S2, but in a 50 kb window.