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CLINICAL REPORT

Detection of pericentric inversion with breakpoint in *DMD* by whole genome sequencing

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Abstract

Background: Dystrophinopathies caused by variants in the *DMD* gene are a well-studied muscle disease. The most common type of variant in *DMD* are large deletions. Very rarely reported forms of variants are chromosomal translocations, inversions and deep intronic variants (DIVs) because they are not detectable by standard diagnostic techniques (sequencing of coding sequence, copy number variant detection). This might be the reason that some clinically and histologically proven dystrophinopathy cases remain unsolved.

Methods: We used whole genome sequencing (WGS) to screen the entire *DMD* gene for variants in one of two brothers suffering from typical muscular dystrophy with strongly elevated creatine kinase levels.

Results: Although a pathogenic DIV could not be detected, we were able to identify a pericentric inversion with breakpoints in *DMD* intron 44 and Xq13.3, which could be confirmed by Sanger sequencing in the index as well as in his brother and mother. As this variation affects a major part of *DMD* it is most likely disease causing.

Conclusion: Our findings elucidate that WGS is capable of detecting large structural rearrangements and might be suitable for the genetic diagnostics of dystrophinopathies in the future. In particular, inversions might be a more frequent cause for dystrophinopathies as anticipated and should be considered in genetically unsolved dystrophinopathy cases.

KEYWORDS

chromosome inversion, Duchenne muscular dystrophy, dystrophin, genetic diagnostics, whole genome sequencing

1 | BACKGROUND

Dystrophinopathies are the most common form of X-linked recessive muscular dystrophy caused by (likely) pathogenic variants in the *DMD* gene (Koenig et al., 1987) (OMIM: * 300377). About 1:4700 new born boys suffer from dystrophinopathies (Helderman-van den Enden et al., 2013), which are mainly divided into the more severe Duchenne muscular dystrophy (DMD) and the milder Becker muscular dystrophy (BMD).

Molecularly they vary in the aspect that usually variants causing DMD destroy the open reading frame and variants causing BMD preserve the reading frame (Hoffman et al., 1988; Monaco et al., 1988). The large protein dystrophin stabilises the muscle fibre by connecting the sarcolemma with the contractile apparatus via beta-dystroglycan and actin (Ervasti & Campbell, 1993; Jung et al., 1995; Rybakova et al., 2000). The most common forms of variants in *DMD* are large deletions, followed by point mutations (especially nonsense and frameshift variants) and duplications (Aartsma-Rus et al., 2006). About 2% to 7% of patients remain without molecular diagnosis as they show no pathogenic variants in the *DMD* coding region (Dent et al., 2005; Okubo et al., 2016). Possible genetic variations in these patients could include deep intronic variants (DIVs) causing splicing effects (e.g., pseudoexons) or great structural changes (e.g., inversions). Inversions are a rare cause of DMD and pericentric inversions are even more uncommon with only a few cases published so far (Bhat et al., 2006; Saito-Ohara et al., 2002; Shashi et al., 1996; Tran et al., 2013). In some of these cases, an additional gene was presumably involved at the second breakpoint (Saito-Ohara et al., 2002; Tran et al., 2013). In general, inversions are described to cause the most complex structural rearrangements in DMD patients, for instance noncontinuous duplications or even triplications in combination with deletions (Baskin et al., 2014; Ishmukhametova et al., 2013). Those inversions have an influence on coding sequences and can be detected by standard CNV detection methods, such as MLPA, array CGH and qPCR (Baskin et al., 2014; Ishmukhametova et al., 2013; Xu et al., 2018). In rare cases, analysis of mRNA can detect inversions if they result for instance in the insertion of pseudoexons (Madden et al., 2009). To determine the inversion breakpoints is far more complicated, as they usually occur in the large *DMD* introns that are not analysed during standard diagnostics. The method of choice to detect breakpoints is long-range PCR, however, the search for the breakpoint can be time-consuming and complex (Baskin et al., 2014). A more elegant method is to sequence the complete sequences of *DMD* as recently done using commercial kits (Nallamilli et al., 2021; Xu et al., 2018). The next step would consequently be the sequencing of the whole genome sequencing (WGS), as we did in the following case.

2 | CASE PRESENTATION

Here, we present the case of a male patient who was tested for DMD for the first time in our laboratory at the age of nine. According to the attending neurologist, he was suffering from clinical distinctive signs of muscular dystrophy and strongly elevated creatine kinase levels (13,000 UI/I). His older brother is equally suffering from muscular dystrophy and in the brother's muscle biopsy a loss of dystrophin was reported. Patient's parents had given their written informed consent prior to genetic *DMD* analysis. Neither brother nor index patient showed disease-causing *DMD* variants. We performed MLPA and sequencing of the entire coding sequence of *DMD* in the index patient, but no pathogenic variant was detected. Because no RNA was available to search for variants affecting gene expression, we decided to perform WGS. We sequenced the whole genomic sequence of the patient using Nextera library preparation (Illumina, San Diego, CA, USA) and used the paired-end mode (2 × 150 bp) on a NextSeq500 (Illumina). The data of the whole genomic sequence of only *DMD* was aligned to reference sequence of GRCh37 and analysed using the software GensearchNGS (PhenoSystems, Wallonia, Belgium). All *DMD* (NM_004006.2) variants were called and eight variants that were not reported hemizygous in the genome aggregation database (Broad Institute, Cambridge, MA, USA) (Lek et al., 2016) were further analysed. All eight variants were deep-intronic variants (DIV), however no variant was predicted to be pathogenic by standard prediction tools.

Furthermore, we analysed the coverage of the *DMD* gene using GensearchNGS (PhenoSystems SA, Wallonia, Belgium). The coverage was homogenous (mean sequencing depth 11×) except for a 5.5 kb alignment gap in intron 44 (data not shown). A visualisation of the alignment at the beginning and the end of the gap showed partially misaligned reads reaching into the gap (Figure 1). Those reads were hybrid-reads consisting on one end of *DMD* intron 44 sequence and on the other end of an unknown sequence. To identify the unknown sequences, we blasted (BLAST, Basic Local Alignment Search Tool, National Library of Medicine, Bethesda, MD, USA) the unidentified sequences from the hybrid-reads of the start and the end of the alignment gap. Both mapped to a location at Xq13.3 only a few base pairs apart from each other (g.75,245,773-g.75,245,821) but in sense direction compared to the *DMD* antisense direction. The first unknown sequence from the beginning of the alignment gap received a high e-value (expect 5.1) as the sequence was quite short, whereas the other received a specific e-value (8×10^{-5}). Both are likely connected and real hits as they map to the same genomic region. Additionally, the corresponding paired-end reads at the border of the alignment gap in intron 44 mapped unusually far away, in a distance of approximately 43,000,000 bp. The addition of these paired-read distances (43,044,236 bp and 43,049,298 bp) to the chromosomal location of

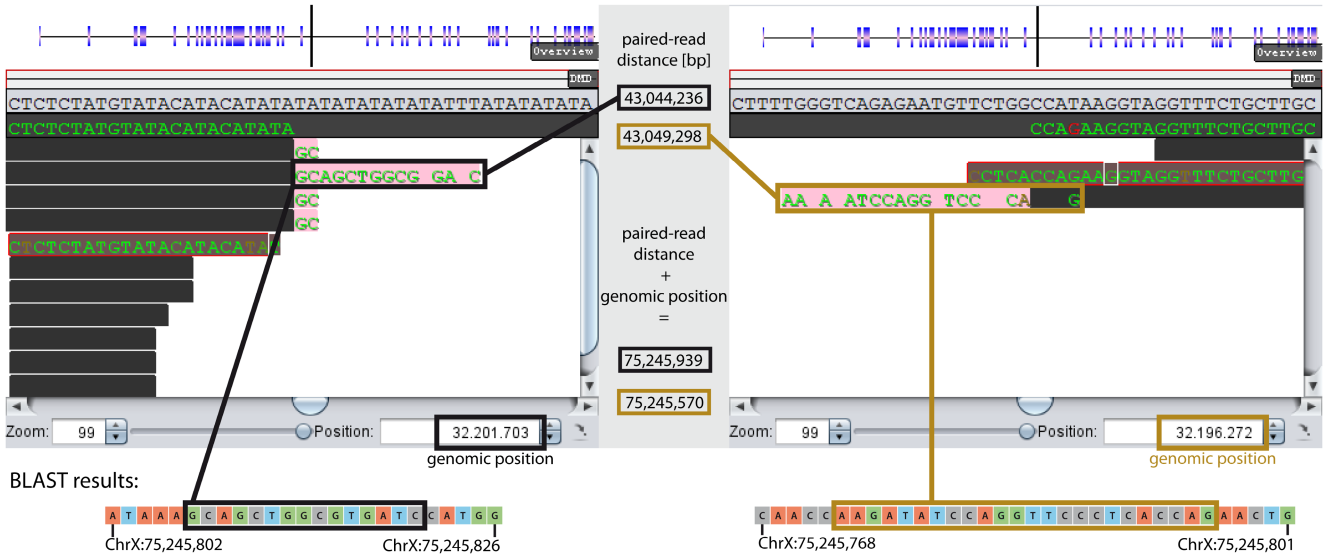

FIGURE 1 Visualisation of the alignment gap. The left picture displays the beginning of the alignment gap and the right picture the other end. On both sides, parts of the reads (black horizontal bars) reached into the gap with sequences that do not match the reference sequence (depicted in pink). These sequences (black for the beginning of the gap and beige for the end of the gap) were blasted. They matched sequences from ChrX:75,245,768 to ChrX:75,245,826. Modified from (Zaum, 2019).

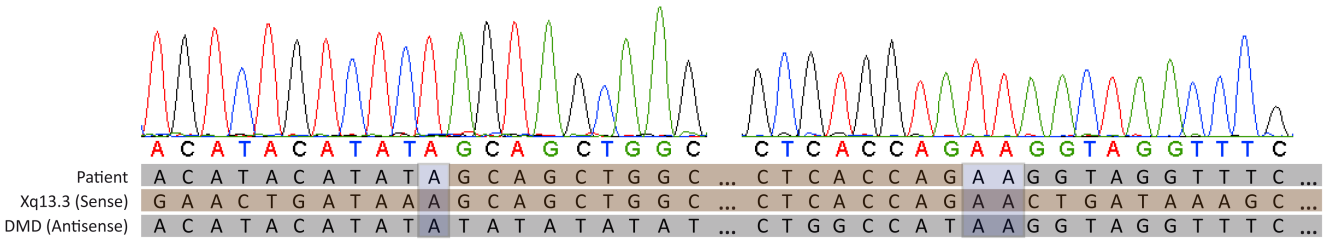

FIGURE 2 Sanger confirmation of breakpoints. Sequences form Xq13.3 are depicted in brown, sequences from *DMD* are depicted in grey. Homologies between all sequences are marked.

the reads (g.32196272 and g.32201703) is roughly equal to the chromosomal location of the blast results (g.75245773-g.75245821) (Figure 1). These results and the orientation of the sequences lead to the assumption of a possible pericentric inversion with breakpoints in the *DMD* intron 44 and the genomic region of Xq13.3 as well as a deletion of 7 bp and 5.5 kb at the first and second breakpoint, respectively. The inversion breakpoints were further verified by Sanger sequencing (Figure 2). In the patient, the sense strand of *DMD* intron 44 is disrupted by the antisense strand of Xq13.3 and vice versa (Figure 2). Conventional chromosomal analysis could also confirm the pericentric inversion (Figure S1) in the patient. His likewise affected brother and their mother were also hemizygous and heterozygous carriers of the same inversion, respectively. This large pericentric inversion (46,Y,inv(X)(p21.1q13.3)) is most likely the disease causing variant as it interrupts a large part of the C-terminal dystrophin corresponding from exon 45 to the end.

3 | DISCUSSION AND CONCLUSIONS

Using WGS, we identified a pericentric inversion with breakpoints in the large *DMD* gene and in the region Xq13.3 in a young boy suffering from dystrophinopathy who showed no mutation in the *DMD* coding region or any deletion/duplication in standard diagnostics. To the best of our knowledge, we describe the first case of a *DMD* pericentric inversion detected by WGS. A translocation including the *DMD* locus has recently been detected by WGS (Schluth-Bolard et al., 2019). The patient's breakpoint in *DMD* is located in intron 44, the largest intron of this gene (248,401 bp) and a hotspot region for deletions involving exons 45–55 (Aartsma-Rus et al., 2016). However, only one different inversion due to a breakpoint in intron 44, resulting in an intragenic inversion of exon 44 and neighbouring intronic sequences, could be identified in the literature (Oshima et al., 2009). In some cases, the breakpoints remain unknown and it seems possible

that a portion of the unsolved DMD cases is due to inversion events that might be located in intron 44 and should be further investigated.

In some published *DMD* inversions the existence of a new gene at the location of the second breakpoint is discussed (Saito-Ohara et al., 2002; Tran et al., 2013). In the case presented here, there is no known gene at the location of the second breakpoint. The breakpoint lies in a LINE repeat element (L1PA8A, RepeatMasker) (Smit et al., n.d.). However, an analysis of conservation of this breakpoint in 37 Eutheria by Ensembl (GRCh37 Release 93) (Zerbino et al., 2018) revealed a high conservation in Hominoidea, but none in the rest of the Eutheria. This could be evidence towards an involvement of this region in higher cognitive performance.

Even in well described diseases such as DMD a small portion of patients remain without molecular diagnosis. As mentioned in the introduction, inversions are a rare cause of DMD. To exclude a recurrent rearrangement, we tested 16 additional dystrophinopathy patients without pathogenic variants in the *DMD* coding region (including deletions and duplications) for the inversion with the same breakpoint 46,Y,inv(X)(p21.1q13.3), and we could not identify this structural variant in any of these patients. Despite the non-recurrence of this inversion, it is likely that large and complex rearrangements together with DIVs are a main reason for the undiagnosed DMD patients. We could show that WGS is capable of detecting chromosomal rearrangements causing DMD in detail and it is therefore a convenient method for future diagnostics in DMD as it can detect both neglected kinds of variants: complex rearrangements and deep-intronic variants. This is already occasionally done using kits that only enrich the genomic sequence of *DMD* (Nallamilli et al., 2021; Xu et al., 2018) but with decreasing prices for complete WGS this interim solution will become obsolete. Besides chromosome analysis, WGS is probably the only method that would have successfully detected this kind of pericentric inversion. Therefore, WGS is worthwhile for the future diagnostic option even of well-studied genetic diseases such as DMD as it might solve hitherto unsolved cases and it would be very interesting to follow up whether inversions are a more common cause of DMD as considered until now.

AUTHOR CONTRIBUTIONS

Molecular analysis: AZ, chromosome analysis: IN, patient information: WK, data analysis and interpretation: AZ, manuscript preparation: AZ, funding acquisition: SR and AZ, study design: SR and AZ, supervision: SR, manuscript revision: SR, WK, IN. All authors contributed to the article and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL STATEMENT

This study is a case report and informed written consent was obtained from the patient and his family to perform WGS/genetic DMD analysis in diagnostic settings and to use data for research purposes and publication.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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