Oxford University Hospitals NHS
NHS Foundation Trust

DMD Deletions and Duplications are Pathogenic..... Aren't They?

Jesse BG Hayesmoore,[1] Carl Fratter,[1] Charlotte Noakes,[1] Olivia Fox,[1] Alison Mills,[1] Joanna Roberts,[1]
Rebecca Candlin,[1] Alison Cleall,[1] Mohamed Wafik,[2] Usha Kini,[2] Deirdre Cilliers,[2] Carolyn Campbell.[1]

1: Oxford Medical Genetics Laboratories, Oxford University Hospitals NHS Trust, Oxford, UK.
2: Oxford Centre for Genomic Medicine, Oxford University Hospitals NHS Trust, Oxford, UK.

Introduction

Whole exon deletions and duplications of the *DMD* gene are the most frequent causes of Duchenne and Becker muscular dystrophies (D/BMD). When detected in males with muscular dystrophy (MD), such variants are considered to be disease-causing. Does this mean that *all DMD* whole exon deletions and duplications should be considered pathogenic? In recent years, increased usage of array CGH has led to increased detection of *DMD* deletions and duplications in probands referred with non-specific clinical phenotypes. In this poster, we describe a number of such cases, which provide evidence to suggest that not all *DMD* deletions and duplication are disease-causing. We also describe potential mechanisms for reduced pathogenicity, and describe investigations to aid classification of these variants.

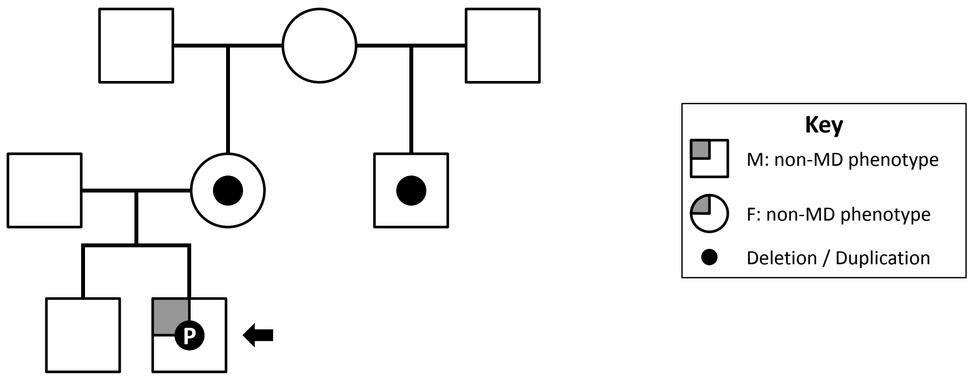
Methods

Probands with various (non-MD) clinical phenotypes were referred for array CGH testing. Array CGH analysis was undertaken using an Agilent ISCA v2 60K array. *DMD* deletions and duplications were confirmed and further characterised by multiplex ligation-dependent probe amplification (MLPA) using MRC Holland kits P034 and / or P035. A literature review to find similar cases was also undertaken.

Results

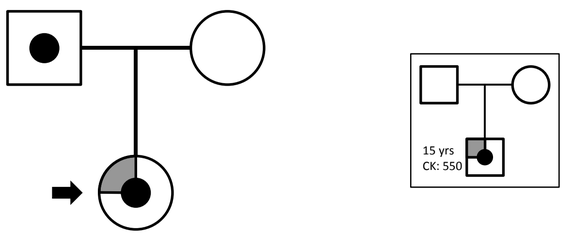
Between January 2015 and March 2017, ~4,500 probands with various clinical phenotypes were referred for array CGH testing. A total of seven (i.e. <0.2%) were found to have a deletion or duplication affecting one or more protein-coding exons of the *DMD* gene. Three of these cases are described below. Eight cases found in the literature are also summarised.

Case 1: In-Frame Duplication of *DMD* Exon 42



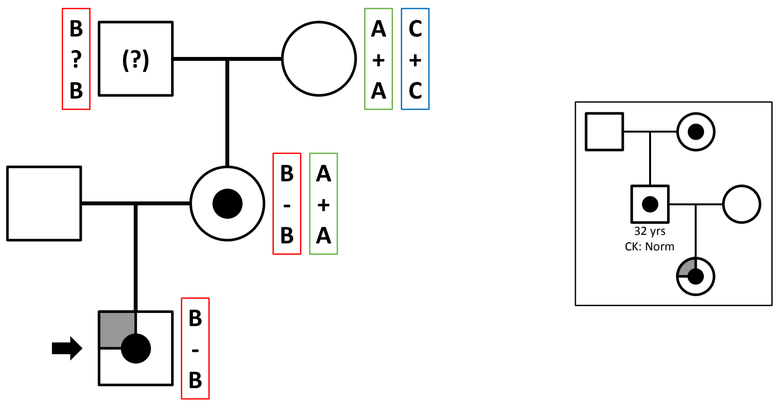
Age at Referral: <13 weeks gestation (CVS). **Referral Reason:** NT = 3.2 mm. Fetal hydrops. Potential structural abnormality. **Array Findings:** Duplication of *DMD* exon 42 (in frame). PCR studies (see Figure 1) confirmed the duplication to be in tandem (not inverted). HGVS: c.5923-13067_6117+10912dup. **Maternal Half Uncle:** 49 years. No muscular weakness. Physically active lifestyle. Duplication detected.

Case 2: Out-of-Frame Deletion of *DMD* Exon 2



Age at Referral: Five months. **Referral Reason:** Truncal hypotonia, developmental delay, frontal bossing, abnormal eye movements, delayed visual motivation. **Array Findings:** Deletion of *DMD* exon 2 (out of frame). **Other Findings:** Molar tooth sign. Compound heterozygous variants in *C5orf42* (Joubert syndrome). **Serum CK:** Not tested. **Father:** 36 years. No known muscular weakness. Deletion detected. **Inset Pedigree:** Deletion of exon 2 has been described once before.[1] The male individual was completely asymptomatic up to age 15 years. Moderately elevated CK (550 IU / l) at six years. **Other Notes:** This deletion may result in translation of a functional N-terminally truncated protein (see 'Protein Studies').[1]

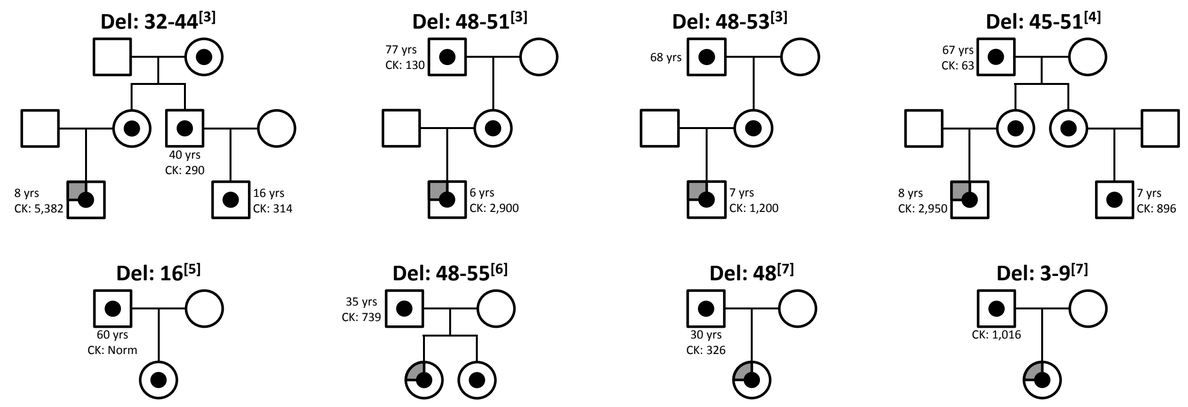
Case 3: In-Frame Deletion of *DMD* Exons 49-51



Haplotypes
Top: 5'-5n4 (intron 4).
Mid: Exons 49-51.
Bot: AFM283wg9 (intron 63).

Age at Referral: Two months. **Referral Reason:** Abnormal movements, abnormal EEG, raised ammonia. Suspected neuro-metabolic condition. **Array Findings:** Deletion of *DMD* exons 49-51 (in frame). **Other Findings:** Later genetically confirmed to have methylmalonic acidemia. **Serum CK:** Normal (130 IU / l) at referral. Moderately elevated (654 IU / l) at 11 months. **Carrier Mother:** 24 years. No known muscular problems. Serum CK mildly elevated (335 IU / l). **Maternal Grandmother:** Deletion not detected. Carries non-deletion haplotype. **Maternal Grandfather:** 43 years. No known muscular weakness. Not available for testing. Inferred to have deletion haplotype. **Inset Pedigree:** We are also aware of an exon 49-51 deletion detected in a 10-year-old female who had tiptoe walking and increased left leg reflexes.[2] The deletion was inherited from her asymptomatic 32-year-old father who had normal CK levels.

Cases From the Literature



Probands in the youngest generation were either referred for array CGH testing for a non-MD phenotype, for *DMD* testing after incidental finding of raised serum CK levels, or was a healthy volunteer for control DNA. All males with deletions had no evidence of muscular weakness. All deletions are in frame.

Pathogenicity Investigations

Given the lower prior probability of a *DMD* deletion or duplication detected in an individual without MD being disease-causing, it is important that such variants (especially duplications) are investigated further. In addition to family studies aimed at detecting the variant in healthy adult male family members, the following investigations may be useful:

(Long-Range) PCR Studies: Duplications are usually inserted in tandem with the duplicated sequence. They can be inserted without inversion or inverted with a 5' or 3' pivot (Figure 1A). Tandem duplications may be detectable by (long-range) PCR by using a forward primer targeted to the 3' end and a reverse primer targeted to the 5' end of the duplicated sequence (Figure 1A). Assuming specificity, a product should only be generated in the presence of a tandem duplication (Figure 1B). However, non-generation of a product cannot be distinguished from PCR failure; hence, non-tandem insertion cannot be assumed. Any product should be sequenced to confirm specificity. This may also reveal the breakpoints (Figure 1C).

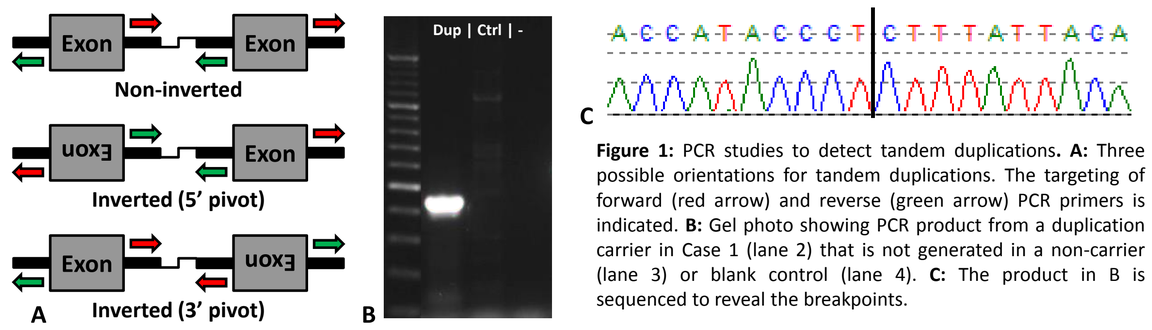


Figure 1: PCR studies to detect tandem duplications. **A:** Three possible orientations for tandem duplications. The targeting of forward (red arrow) and reverse (green arrow) PCR primers is indicated. **B:** Gel photo showing PCR product from a duplication carrier in Case 1 (lane 2) that is not generated in a non-carrier (lane 3) or blank control (lane 4). **C:** The product in B is sequenced to reveal the breakpoints.

Fluorescent *In Situ* Hybridisation (FISH): Less frequently, duplications can be inserted elsewhere in the genome. The genomic position of large duplications (e.g. >100 kb) may be determinable by FISH. For example, we have previously shown that a ~440 kb duplication of exons 45-51 was inserted in chromosome 17 (see Figure 2). Duplications inserted outside the *DMD* gene are unlikely to cause MD.

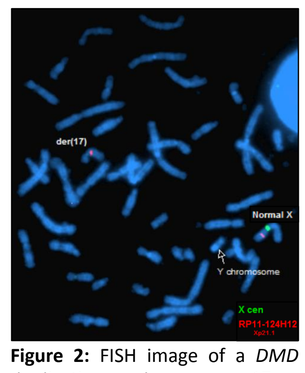


Figure 2: FISH image of a *DMD* duplication on chromosome 17.

RNA Studies: Ideally undertaken on muscle tissue (hair follicles and blood may also be used). May reveal unexpected exon skipping or intronic sequence retention. Duplicated exons may be skipped completely, consistent with a benign variant interpretation. Exon skipping or intron retention may preserve or alter the reading frame.

Protein Studies: Western blotting can detect abnormally sized proteins that may not be predictable from the mRNA sequence. For example, an exon 2 deletion (similar to Case 2) was found to result in a functional N-terminally truncated ~410 kDa protein.[1] Further studies suggested activation of an internal ribosomal entry site in exon 5 and an alternative translation start site in exon 6. Abnormal dystrophin expression and muscle fibre abnormalities can also be visualised by immunohistochemistry.

Discussion and Conclusion

DMD deletions and duplications detected in individuals without MD have a lower prior probability of being disease-causing than those detected in individuals with MD. When detected incidentally, these variants should not be assumed to be predictive of MD, but should be investigated further. This is particularly important for duplications, which may not disrupt the *DMD* gene at all. Detection of a deletion or duplication in healthy adult male family members is reassuring for a benign prognosis. However, the possibility of variable expressivity / penetrance is a significant concern, as this can be quite marked in some families.[8,9] It should also be noted that a deletion in an adult male with normal strength and normal CK levels may still confer risk of dilated cardiomyopathy later in life (e.g. >50 years).[8]

References: **[1]** Wein *et al*. (2014). *Nat Med*, 20(9): 992-1000. **[2]** Proband tested by array CGH at Bristol Genetics Laboratory. MLPA undertaken at Oxford Medical Genetics laboratory. **[3]** Melis *et al*. (1998). *Eur J Paediatr Neurol*, 2(5): 255-261. **[4]** Saengpattrachai *et al*. (2006). *Pediatr Neurol*, 35(2): 145-149. **[5]** Schwartz *et al*. (2007). *Hum Mutat*, 28(2): 205. **[6]** Cottrell *et al*. (2010). *Am J Med Genet A*, 152A(9): 2301-2307. **[7]** Nguyen *et al*. (2015). *Clin Genet*, 87(5): 488-491. **[8]** Muntoni *et al*. (1997). *Heart*, 78(6): 608-612. **[9]** Palmucci *et al*. (2000). *Neurology*, 54(2): 529-530.