

1 Introduction

Dysferlinopathy encompasses a spectrum of autosomal recessive muscular dystrophies that result from the absence of the dysferlin protein, which is encoded by the *DYSF* gene and crucial for skeletal muscle membrane fusion and repair. Major types of dysferlinopathies include limb-girdle muscular dystrophy type 2B (LGMD2B), Miyoshi myopathy (MM), and distal myopathy with anterior tibial onset (DMAT). Dysferlinopathy diagnosis is complicated by clinical heterogeneity and overlapping clinical features with other muscular dystrophies, requiring confirmation by molecular testing. Several small scale next-generation sequencing (NGS) studies have been conducted with a small sample size and lack of copy number variant (CNV) analysis. Large scale molecular studies are needed to better understand the genetic basis and complex variant spectrum of dysferlinopathy.

2 Methods

A comprehensive analysis of both sequence and copy number variants was conducted in a total of 686 individuals with at least 1 reported variant in the *DYSF* gene using different NGS molecular assays, including a neuromuscular gene panel, exome sequencing, and genome sequencing. Sequencing was performed on genomic DNA isolated from various sample types by 2x150 bp reads on an Illumina NGS platform at a mean coverage of 80X for panels and exome sequencing and 40X for genome sequencing. Sequence variants (SNVs) were assessed by our proprietary analysis and interpretation pipeline, Ordered Data Interpretation Network (ODIN). CNV analysis was completed using Biodiscovery's NxClinical software (BioDiscovery, El Segundo, CA). All variants were classified according to ACMG/AMP guidelines.

3 Results

A molecular diagnosis was established in 110 cases (16.0%) by identifying pathogenic (P) or likely pathogenic (LP) variants in the *DYSF* gene (Fig. 1A). Out of 110 cases, 75 (68.2%) individuals underwent NGS panel testing and 35 (31.8%) individuals underwent exome or genome testing (Fig. 1A). Probands were compound heterozygous for two LP/P variants in 56 (50.9%) cases and homozygous for an LP/P variant in 54 (49.1%) cases (Fig. 1B).

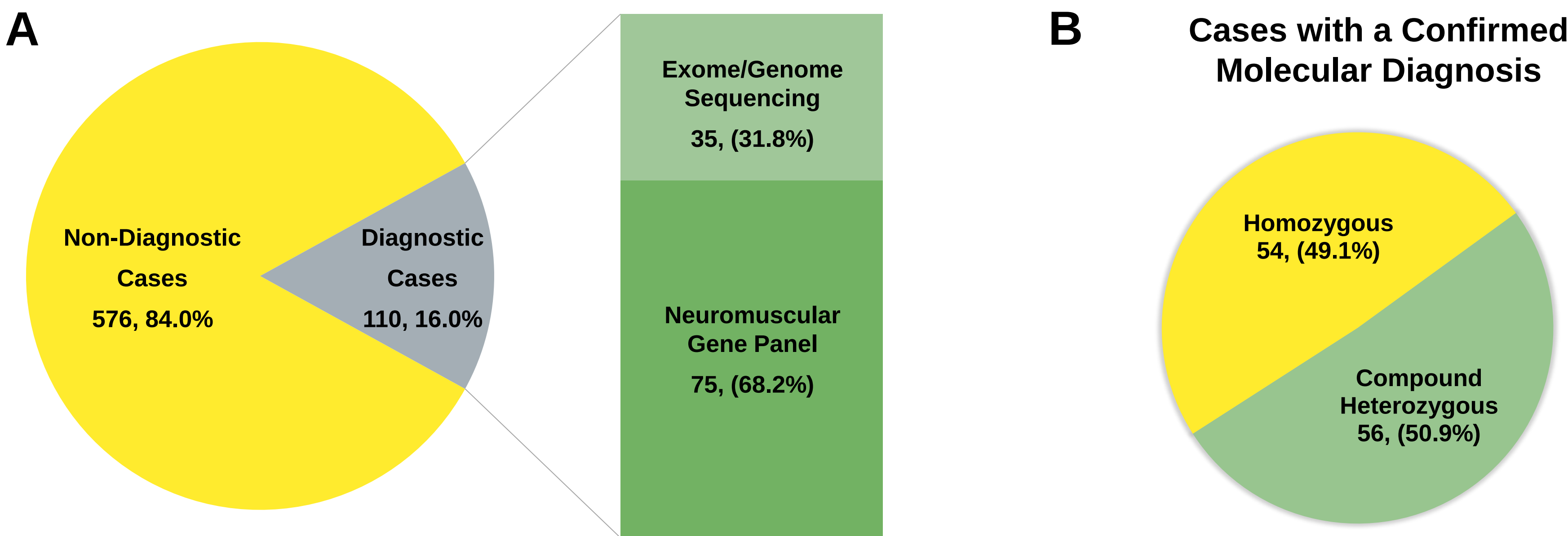


Figure 1. (A) Summary of molecular assay and (B) zygosity of variants identified in diagnostic dysferlinopathy cases.

An additional 24 (3.5%) cases were compound heterozygous for an LP/P variant and a variant of uncertain significance (VOUS) and 12 (1.7%) cases were homozygous for a VOUS, of which 33 probands had a strong clinical suspicion of dysferlinopathy (Fig. 2A). Two VOUS were identified in 30 cases; although only 8 probands presented with features consistent with dysferlinopathy (Fig. 2A). A single LP/P variant or VOUS was reported in 45 (6.6%) and 465 (67.8%) cases, respectively (Fig 2B). CNVs were identified in 7 cases, including 4 intragenic deletions and 3 duplications (Table 1, Fig. 3).

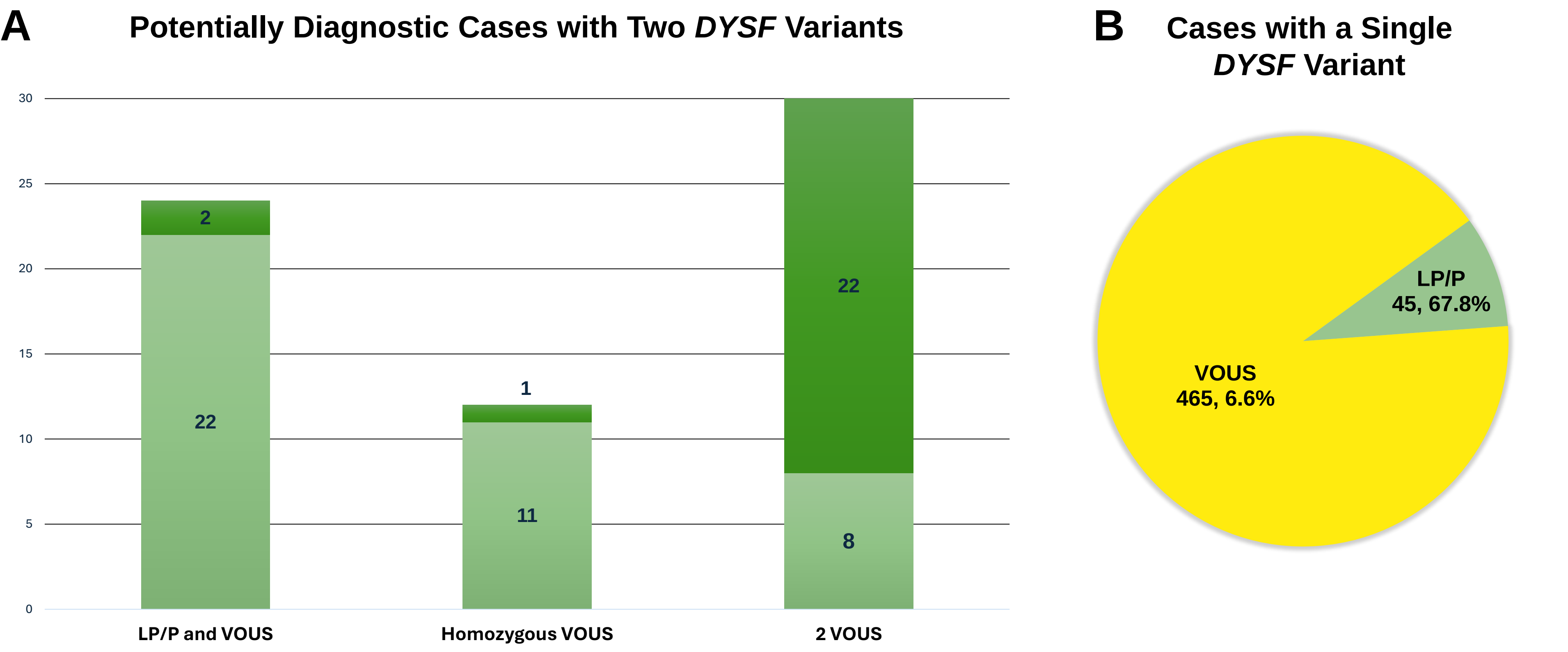


Figure 2. (A) Variant pathogenicity in cases with 2 *DYSF* variants in individuals with high clinical suspicion of dysferlinopathy (light green) and those either suspected of having a different neuromuscular disorder or lacking clinical information (dark green). (B) Variant pathogenicity in cases with a single *DYSF* variant.

Table 1. <i>DYSF</i> copy number variants identified in this cohort.						
#	CNV	Zygosity	Classification	SNV	Zygosity	Classification
Diagnostic cases						
1	2.1 kB loss <i>DYSF</i> exon 52	Heterozygous	Pathogenic	<i>DYSF</i> c.1931-2del	Heterozygous	Likely Pathogenic
2	186 bp loss <i>DYSF</i> exon 53	Heterozygous	Pathogenic	<i>DYSF</i> c.5767+1G>A	Heterozygous	Pathogenic
Potentially diagnostic cases						
3	3.5 kB loss <i>DYSF</i> exons 2-3	Heterozygous	Pathogenic			
4	28.3 kB gain <i>DYSF</i> exons 13-17		Pathogenic	<i>DYSF</i> c.2643+1G>A	Heterozygous	Pathogenic
5	1.3 kB loss <i>DYSF</i> exon 34	Heterozygous	Pathogenic			
6	85.0 kB gain <i>DYSF</i> exons 10-35		Pathogenic			
7	17.8 kB gain in <i>DYSF</i> exons 13-17		Pathogenic	<i>DYSF</i> c.2643+1G>A	Heterozygous	Pathogenic

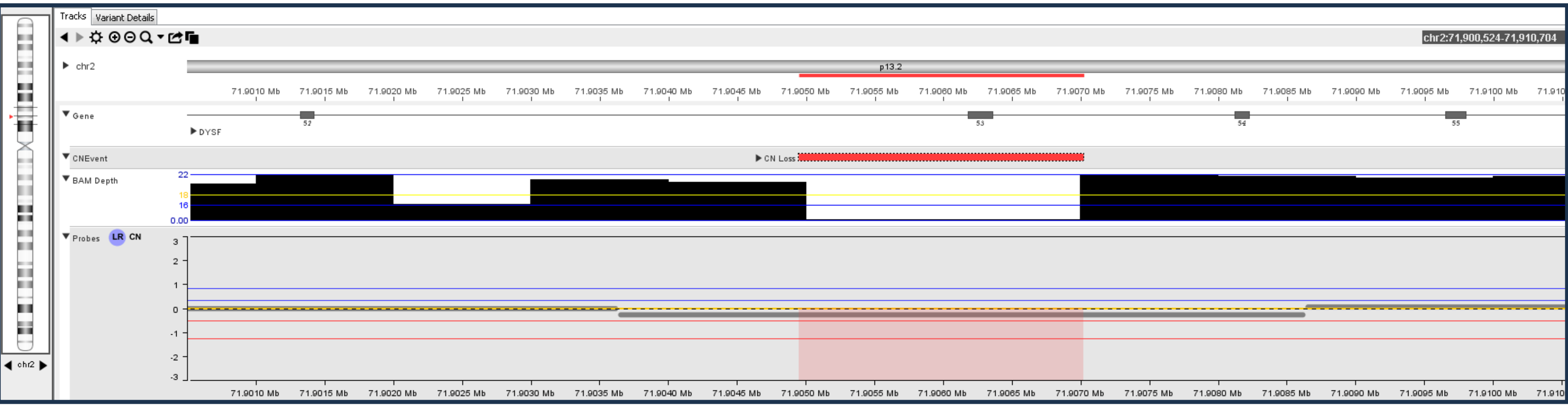


Figure 3. NGS-based copy number analysis. An approximately 2.1 kb copy number loss of *DYSF* exon 52 was identified in an 18-year-old male with clinical suspicion of dyferlinopathy with a history of limb-girdle muscular dystrophy and hyperCKemia.

4 Conclusions

Overall, this comprehensive analysis of both SNVs and CNVs greatly helped in establishing a molecular diagnosis and better understanding the complex variant spectrum of dysferlinopathies. In total, 439 unique SNVs were identified in this cohort, including 357 missense, 2 nonsense, 33 frameshift, and 39 splice variants, as well as 6 in-frame insertions or deletions. Three common, pathogenic variants, c.4253G>A, c.2643+1G>A, and c.5979dup, were each identified in more than 15 cases. Deep intronic variants in the *DYSF* gene were identified in a total of 7 cases. CNVs were identified in total of 7 cases.