# When Just Detecting A Variant Consistent With The Phenotype Is Not Enough!

Not uncommonly an analysts' day is challenged with a case/s where the journey to understand the origin of the detected variants is more interesting than variant detection itself! We call these cases the BRAIN TEASERS at the NCGM. Read along the brain teaser to know why:

## CASE 1:

A 2 year old male with motor developmental delay, myopathic facies, proximal contractures and distal laxity.

**Clinical suspicion** : COL6- myopathy
**Test performed** : ORION (Whole Exome Sequencing with CNV calling)
**Results** : No single nucleotide variant detected.
**CNV calling** : A heterozygous deletion of 1.6Mb ( arr[GRCh37] 21q22.3(46409764_48097372)x1 including *COL6A1* and *COL6A2* gene in chromosome 21 was detected. The same was confirmed via microarray.

**Figure 1** : Deleted region in chromosome 21.

Surprisingly, when the IGV (Integrative Genomic Viewer: a visual display of sequenced reads within the area of interest) was analyzed,*COL6A1* showed a low coverage of Exons 5-35 in the proband as compared to other reference samples

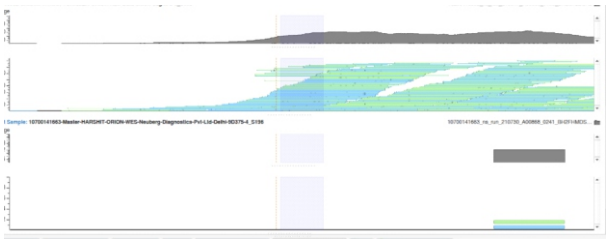

**Figure 2** : Near zero coverage of Exon 5-35.
Reference sample (Top)/ Proband sample (below)

Can a heterozygous deletion confirm a diagnosis of an autosomal recessive disorder? .

**Possibility:** Co-inheritance of a multiexonic deletion + a large copy number variant leading to homozygous loss of certain Exons in *COL6A1* causing recessive disease

- **Are exonic deletions and CNV common in COL6?**

Deletions account for <1% of cases with *COL6A1* myopathy[1]
A single case in a compound heterozygous state with a deletion on chromosome 21 and a missense mutation has been reported previously[2]

- **How do you confirm these findings?**

**Parental studies were performed:**
**a)** Microarray-CytoScan Optima:
Mother: Heterozygous carrier of 1.6kb deletion in 21q22.3 same as proband
Father: Normal
**b)** q-pcr:
Father: Heterozygous deletion carrier (Exons 5-35)
Mother: Normal

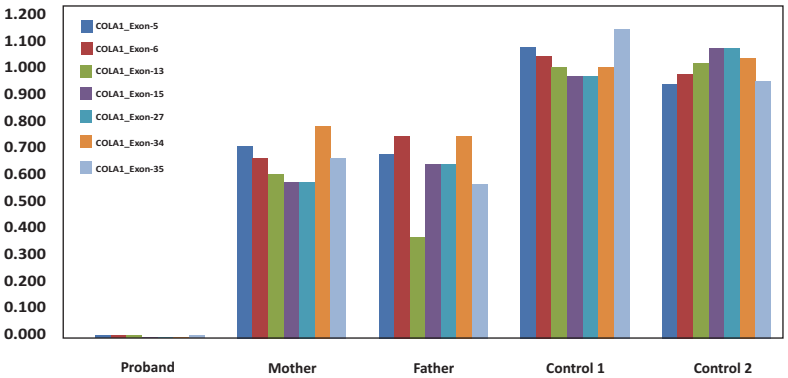


**Figure 3** :Homozygous deletion in the proband

**Conclusion:** The proband is affected with recessive *COL6A1* myopathy due to co-inheritance of a genomic deletion of 1.6kb (maternal origin) and a multi-exonic deletion in *COL6A1* (paternal origin).

## References:


1) Foley AR, MohasselP,Donkervoot S et al, COLVI-related dystrophies. Gene Reviews

2) Simsek-Kiper PO, Oguz S, Ergen FB, Utine GE, Alikasifoglu M, Haliloglu G. A Revisited Diagnosis of Collagen VI Related Muscular Dystrophy in a Patient with a Novel COL6A2 Variant and 21q22.3 Deletion. Neuropediatrics. 2020 Dec;51(6):445-449.

## Case 2:

A 4 year old male with frequent falls, calf hypertrophy, Gower's sign positive.

**Investigation** : CPK>5000IU/L
**Clinical suspicion** : Duchenne Muscular Dystrophy (DMD)
**Test requested** : *DMD*-MLPA
**MLPA Result** : A **heterozygous** deletion in Exon 16 instead of the expected **hemizygous** deletion in males

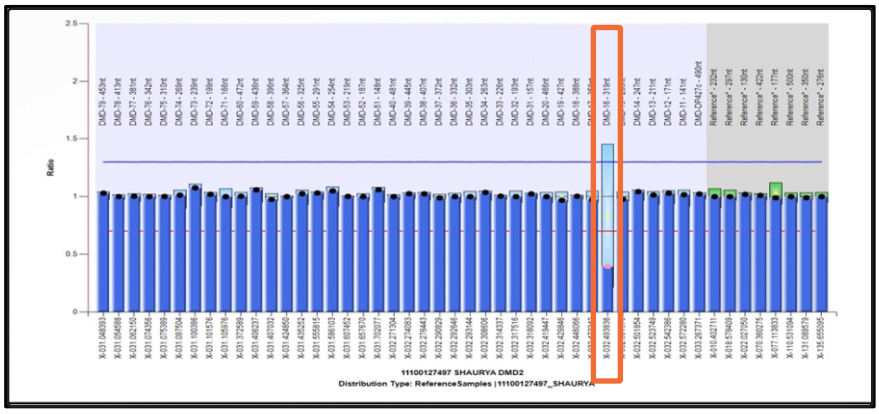


**Figure 4**: *DMD* MLPA Result

**Possibilities:**

a) A polymorphism/ variant at the MLPA probe site which is interfering with the results and leading to heterozygous deletion
b) Mosaic deletion[3]

**Further work up** : Sanger analysis of Exon 16 to detect probe site variants interfering with MLPA results
**Sanger Result** : A hemizygous likely pathogenic frameshift termination variant in Exon 16

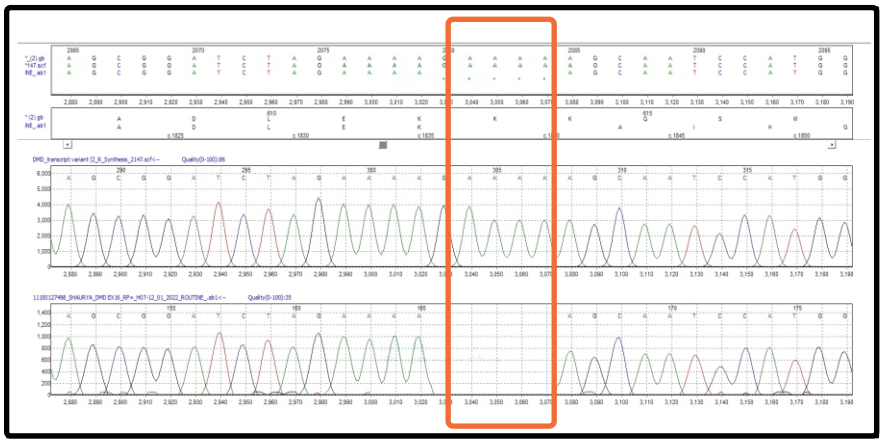

**Figure 5**: Sanger Result - Reference (top)/ Proband (below)

| | |
|---|---|
| **DMD: c.1836_1840del(p.Lys613AlafsTer20)** | **DETECTED (HEMIZYGOUS)** |

**Conclusion:** A hemizygous frameshift termination variant was present in Exon 16 in the proband. This variant interfered with the MLPA probe binding leading to a heterozygous deletion instead of the expected hemizygous deletion.

**Further recommendations:** Testing of the mother via Sanger for the frameshift termination variant

**References:**

3) Saito K, Ikeya K, Kondo E, Komine S, Komine M, Osawa M, Aikawa E, Fukuyama Y.
4) Somatic mosaicism for a DMD gene deletion. Am J Med Genet. 1995 Mar 13;56(1):80-6

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