NGS panel for diagnostics of myofibrillar myopathies

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INTRODUCTION

Myofibrillar myopathies (MFMs) are clinically characterized by a slowly progressive weakness of distal and proximal muscles with usually late onset and typical morphological alterations involving the Z-disk of muscle fibres and producing typical cytoplasmic inclusions.

Known causative genes of MFMs are *DES*, *CRYAB, MYOT, LDB3* (*ZASP*), *FLNC* and *BAG3*. In addition, mutations in *FHL1, DNAJB6* and *TTN* have also been reported as causative for MFM but can also cause other subtypes of myopathies.

METHODS

We developed a NGS panel with eight MFM genes and applied it to 36 patients clinically and histopathologically diagnosed as MFM. The coding exons of the known causative genes of MFM were included in the panel except for the very large *TTN* gene. NGS data were analysed with GenSearch NGS (PhenoSystems) and variants confirmed by Sanger sequencing. Twenty-four patients were analysed by this panel. In 12 additional patients, only *FLNC* was sequenced by this method since mutations in all the other genes had previously been excluded by Sanger sequencing. Exon 343 of *TTN* (NM_001267550) comprising a mutation hot spot for MFM was screened separately by Sanger analysis in all 36 patients.

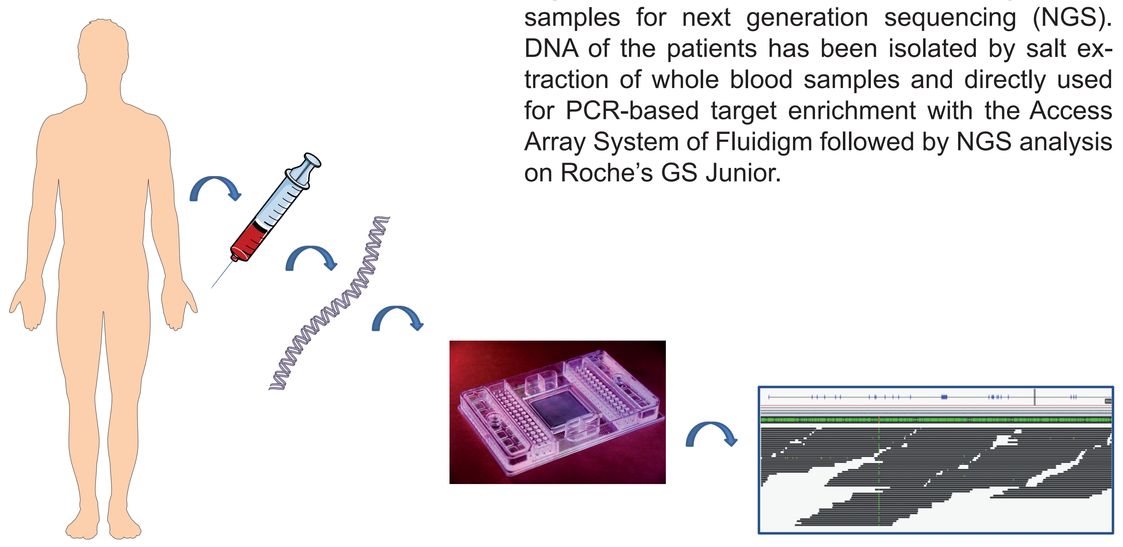

Figure 1: Overview on the processing of the samples for next generation sequencing (NGS). DNA of the patients has been isolated by salt extraction of whole blood samples and directly used for PCR-based target enrichment with the Access Array System of Fluidigm followed by NGS analysis on Roche's GS Junior.

RESULTS

As a result, five of the patients were diagnosed with a causative heterozygous mutation (table 1). The variants in the *DES* and the *ZASP* gene are well-known MFM causing mutations. The mutations in *BAG3* and *TTN* have not been described before.

Table 1: Overview on the causative mutations found in five of the analysed patients. Prediction was performed with MutationTaster, PolyPhen-2 and SIFT.

Patient	Gene	Variant	Reference	MAF	Prediction
7, 17	*DES*	c.1049G>C, p.Arg350Pro	rs57965306, CM051448	-	disease causing
10	*ZASP*	c.494C>T, p.Ala165Val	rs121908334, CM050285	-	disease causing
23	*BAG3*	c.626C>A, p.Pro209Gln	-	-	disease causing
27	*TTN*	c.95351C>T, p.Ala31784Val	-	-	disease causing/ possibly damaging

Bioinformatic tools uniformly classify them as disease causing and further histopathological and clinical examinations of the patients confirmed their causality (data not published). Additionally, in case of the *BAG3* variant a C>T (Pro>Leu) exchange at the same position is known as causative for MFM (rs121918312).
Nine further variants could not reliably be classified as pathogenic or polymorphism and were categorised as unclassified variants (UVs; table 2).

The functional effect is predicted disease causing in case of a *TTN* and a *FLNC* variant and inconsistently in the rest of cases. All of the variants have been found before with a MAF < 1 % but some MAF information is only based on data of the NHLBI exome sequencing project (ESP) which cannot reliably exclude causative mutations of late onset diseases like MFMs. The causality of the UVs thus remained uncertain.

Table 2: Overview on the unclassified variants (UVs) with a minor allele frequency (MAF) < 1 %. Prediction of missense variants was performed with MutationTaster, PolyPhen-2 and SIFT. MAF information is based on dbSNP and NHLBI exome sequencing project (ESP) data (italic letters).

Patient	Gene	Variant	dbSNP	MAF	Prediction
2	*TTN*	c.95297C>T, p.Ser31766Phe	rs191484894	0.2 %	disease causing
23	*ZASP*	c.664G>A, p.Ala222Thr	rs139922045	< *0.1 %*	inconsistently
24	*CRYAB*	c.324+13T>G	rs370222107	< *0.1 %*	
1	*FLNC*	c.1549+15C>A	rs181134489	0.3 - *0.5* %	
1	*FLNC*	c.3721C>T, p.Arg1241Cys	rs146953558	*0.1* - 0.6 %	inconsistently
14	*FLNC*	c.4022G>A, p.Arg1341Gln	rs149641783	0.2 %	inconsistently
27	*FLNC*	c.4737+9_10del	-	< *0.1 %*	
31	*FLNC*	c.5578C>T, p.Arg1860Cys	rs181067717	0.1 - *0.6* %	disease causing
18	*FLNC*	c.6595G>A, p.Gly2199Arg	rs368977589	< *0.1 %*	inconsistently

CONCLUSION

In total, five pathogenic mutations and nine UVs were found with this small panel for MFMs comprising eight genes and a hot spot *TTN* exon. Especially in case of the 48 exons spanning *FLNC* gene this study showed the benefit of the capacity of NGS as several variants could be identified outside the mutation hot spot in exon 48.

The genes *DNAJB6* and *FHL1* provided no variants during this study of 36 patients. In summary, the panel has proven to be a fast and practical method for the diagnostic screening of several MFM genes in parallel.

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