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Application of targeted next-generation sequencing for molecular diagnostics of Malignant Hyperthermia and Central Core Disease

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INTRODUCTION

Malignant Hyperthermia (MH) is a pharmacogenetic disorder of the calcium metabolism in skeletal muscles. A hypermetabolic response is triggered by exposure to anesthetic gases such as halothane, desflurane and isoflurane but also by muscle relaxants like succinylcholine. The classic signs of a MH crisis are muscle rigidity, rhabdomyolysis, tachycardia, acidosis and high fever.

Central Core Disease (CCD) is an inherited myopathy characterized by muscle weakness and wasting. The clinical presentation is highly variable and ranges from nearly unnoticed to more severe congenital forms. Most patients with CCD show also a susceptibility to MH.

Both diseases are inherited in an autosomal dominant pattern and in most cases caused by mutations in the ryanodine receptor gene (*RYR1*). More than 180 mutations in the *RYR1* gene have been identified so far and linked to MH, CCD and other neuromuscular disorders. Mutations are believed to be concentrated in two hotspot regions in the middle and at the C-terminal end of the gene. With routine molecular diagnostics, sequence analysis of the hotspots of the *RYR1* gene of MH and CCD patients identifies mutations in only ~ 25 % of cases. Thereby in a large proportion of cases the causative mutations appear to be missed. Therefore we decided to analyze all 106 exons of the *RYR1* gene in MH/CCD patients by next generation sequencing (NGS).

METHODS AND RESULTS

For target enrichment the Access Array System™ of Fluidigm was used which allows the amplification of 48 target regions for 48 samples in parallel. The 106 exons of the *RYR1* gene were amplified in 88 amplicons. Therefore we used region-specific primers with M13-overhang. Thus in a next step primers with M13-sequence, sample-specific barcodes and sequencer-specific adaptors were added (see Figure 1).

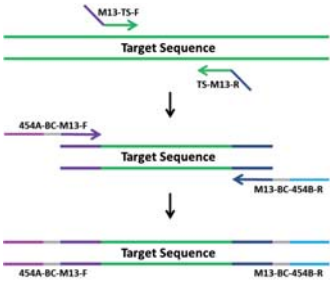

Figure 1:
Overview of the PCR: Region-specific sequences tagged with M13-overhang can be combined with second primers with M13 tag, barcode and sequencer-specific adaptors.

TS = target-specific sequence
BC = barcode sequence
454A = 454 sequencer-specific forward sequence
454B = 454 sequencer-specific reverse sequence

After sample purification and emulsion PCR sequencing was performed with the 454 GS Junior System of Roche. GenSearchNGS (PhenoSystems) was used for data analysis (see Figure 2).

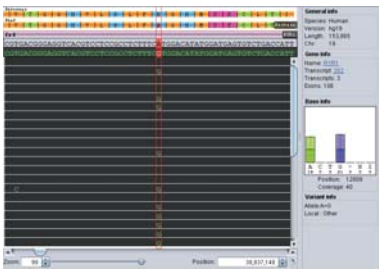

Figure 2:
Visualization of the heterozygous missense mutation c.668A>G, p.His223Arg in the *RYR1* gene causing Malignant Hyperthermia.

The whole *RYR1* gene was analyzed by NGS for 60 MH/CCD patients. Mutations in the MH/CCD hotspot regions of these patients had been excluded by Sanger sequencing before. By NGS, in more than one third of the patients (36.7 %) causative mutations could be found outside the hotspots (see Figure 3).

Exon	DNA change	Protein change	No. of patients
2	c.115G>A	p.Glu39Lys	2
6 + 66	c.488G>A +c.9718T>C	p.Arg163His +p.Cys3240Arg	1
7	c.617C>T	p.Ser206Phen	1
8	c.668A>G	p.His223Arg	1
11	c.1021G>A	p.Gly341Arg	1
14	c.1466G>T	p.Cys489Phen	1
14 + 18	c.1565A>G	p.Tyr522Cys	1
	+c.2122G>A	+p.Asp708Asn	
15	c.1654C>T	p.Arg552Trp	1
29	c.4178A>G	p.Lys1393Arg	1
34	c.5036G>A	p.Arg1679His	2
34 + 41	c.5036G>A +c.6757C>T	p.Arg1679His +p.His2253Tyr	1
41	c.6757C>T	p.His2253Tyr	1
63	c.9463G>A	p.Asp3155Asn	1
65	c.9635A>G	p.Glu3212Gly	1
71	c.10616G>A	p.Arg3539His	2
90	c.12589T>C	p.Ser4197Pro	1
98	c.14186A>C c.14189G>T	p.His4729Pro p.Gly4730Val	1 1
105	c.14970G>A	p.Met4990Ile	1

Figure 3:
The table shows the mutations found in the *RYR1* gene of 22 of all the 60 patients analyzed by NGS.
Unknown mutations are colored blue.

As the coverage between the single exons varied strongly and sometimes was very low all of the detected mutations were proved by Sanger sequencing.

CONCLUSION

The results show that obviously several additional causative mutations exist outside the known MH and CCD hotspots. For an economic diagnostic protocol, it will therefore be useful to screen the hotspot regions first, but in case of negative results to analyze also the whole *RYR1* gene by a cost-effective method like targeted NGS.