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Detection of SMA by discrimination of *SMN1* and *SMN2* using long-read sequencing (ONT) and artificial intelligence (AI)

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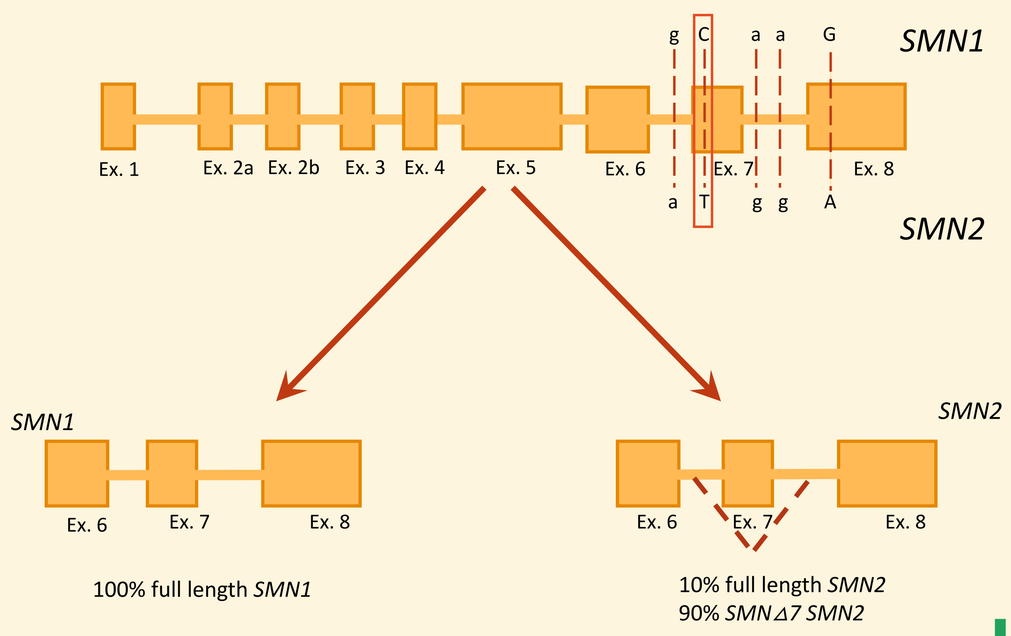
ABSTRACT

SMA, a progressive, recessive neuromuscular disease with varying presentations of onset and severity, is caused by bi-allelic mutations in the *SMN1* gene (deletion of the gene in 95% of the cases). The severity is determined by the number of *SMN2* copies. *SMN1* and *SMN2* only have 5 different nucleotides in the whole sequence.
Due to its high clinical and genetic heterogeneity and low prevalence (1/10,000 births), diagnosis and treatment are highly challenging.

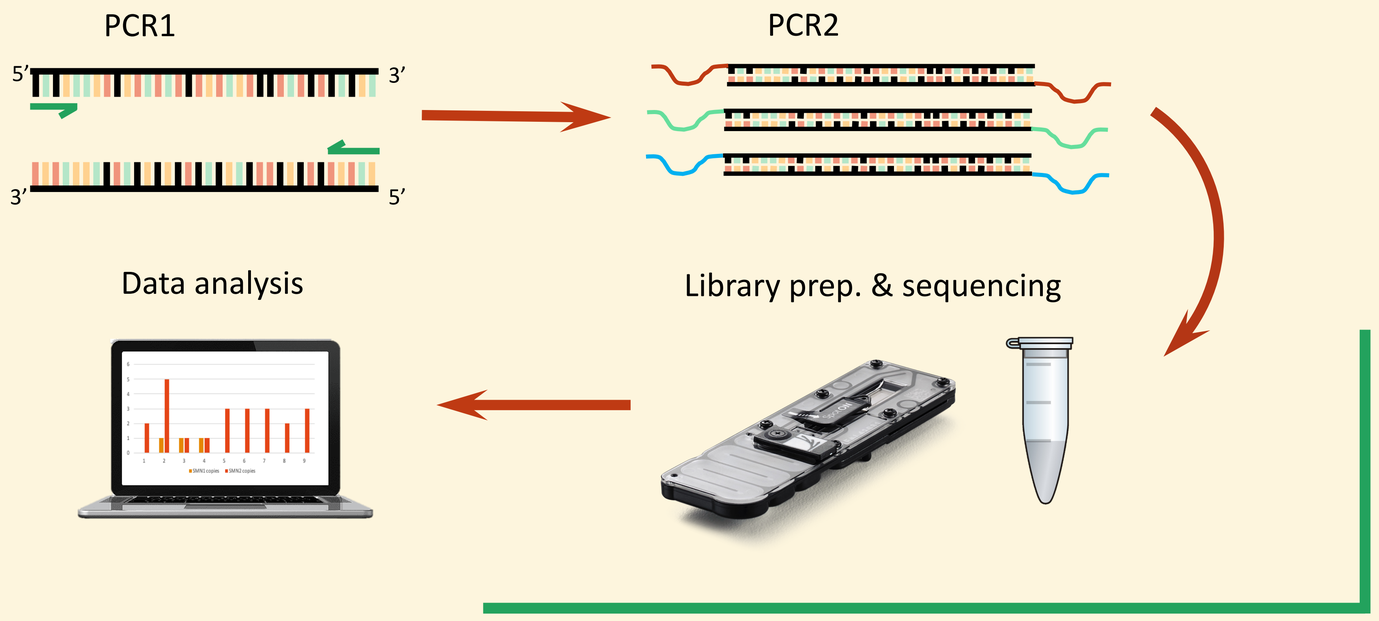
Diagnosis is usually made using RT-PCR for *SMN1* (and sometimes *SMN2*) after clinical symptoms suggest the condition. However, molecular genetic testing is needed to confirm the positive result. This procedure is costly, slow, and inefficient, as many of the clinical symptoms overlap with other neuromuscular diseases (DMD, BMD, or multiple sclerosis), increasing the misdiagnosis rate.

Our proposed solution combines targeted ONT sequencing and our Phivea® platform to discriminate between *SMN1* and *SMN2,* as well as the number of copies per gene. We also identify a point mutation (C>T) that usually occurs in *SMN1* and leads to exon 7 skipping, suggesting that our approach might be able to improve diagnostic rates for SMA.

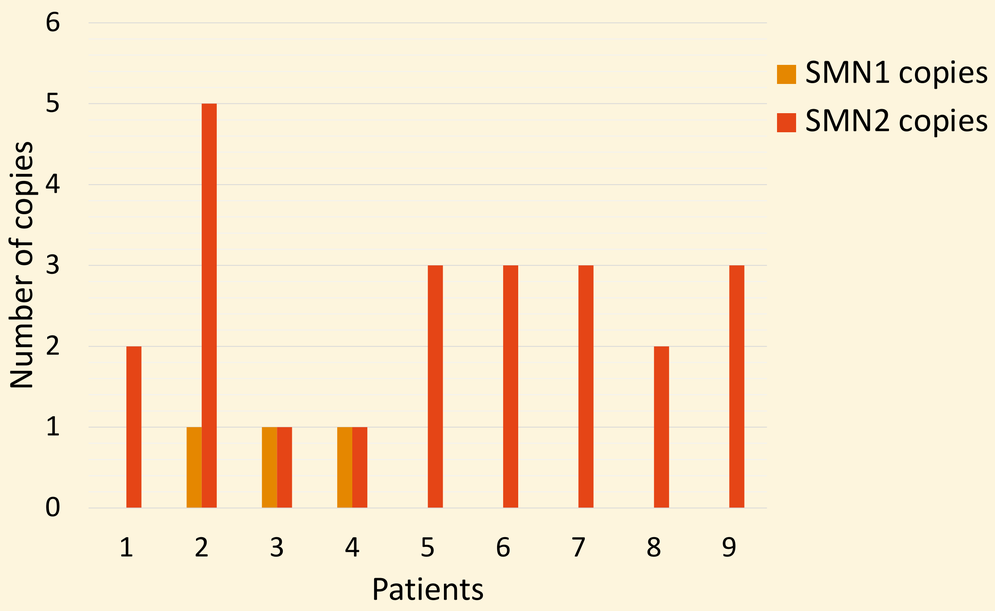


MATERIALS & METHODS

- → **PCR1:** Targeted DNA amplification of intron 6 to exon 8 for *SMN1* and *SMN2* by specific primers. Amplification of an endogenous control.
- → **PCR2:** Amplification for the attachment of barcodes
- → **Library preparation and sequencing:** Done following the Oxford Nanopore Technologies protocol and loaded into a FLO-MIN106 (R9.4.1) flow cell and sequenced in GridION x5
- → **Data analysis:** Base called fastq files are run through our Phivea® Platform. Demultiplexing of the data is performed live giving an automated and real-time classification of the data.



RESULTS



Patient	1	2	3	4	5	6	7	8	9
SMN1	0	1	1	1	0	0	0	0	0
SMN2	2	5	1	1	3	3	3	2	3
Result	SMA	Carrier	Carrier	Carrier	SMA	SMA	SMA	SMA	SMA

The number of *SMN1* and *SMN2* copies are normalized based on the number of reads mapped to the endogenous control gene

CONCLUSIONS

- → Our technology can discriminate *SMN1* and *SMN2* based on the 5 different nucleotides after reads are correctly mapped to the reference sequence.
- → The test is reliable for identification of point mutations.
- → The results are consistent with the report from Coriell Institute for Medical Research
- → Knowing the number of *SMN2* copies gives a more accurate diagnosis, since the greater the number of copies, the milder the severity of the disease.
- → Further testing with more samples needs to be done

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