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Performance evaluation of deleteriousness prediction methods for intronic SNVs in next generation sequences

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Introduction: Alterations in splicing sites (ss) are estimated to explain approximately 10% of human disease causal variants. Mutations outside the ss but affecting "regulatory elements" can be up to 25%. Accurate deleteriousness prediction for intronic variants is crucial for diagnostic purposes. Many deleteriousness prediction methods have been developed, but their relative values are still unclear in practical applications. We comprehensively evaluated the predictive performance of two complementary deleteriousness-scoring methods using information from real patients.

Material and Methods: We selected the dbscSNV (both ADA and RF scores) and SPIDEX algorithms, that study variants in splicing consensus regions or in regulatory regions respectively. The tools, either alone or in combination, were tested on 29294 gene intronic SNVs that have previously been characterised by ClinVar as either "pathogenic" (430) or "benign" (28864). The sensitivity, specificity and positive and negative predictive values were calculated. Moreover, we applied the algorithms to WES data from undiagnosed patients, and we analysed the mRNA sequence from genes that fitted the patient's phenotype.

Results: The highest sensitivity corresponds to dbscSNV with 96.55% while the best specificity is for SPIDEX with 95.78%. When considering the 3 scores (SPIDEX, dbscSNV ADA and RF Score), the sensitivity and specificity values were 60.7% and 94.6%. The Positive and Negative Predictive Value were 14.45% and 99.39%. The results for 20 undiagnosed cases are presented.

Conclusions: Besides the low positive predictive value, the combination of both algorithms leads less than 1% of false negatives, so their routine use can be recommended for diagnostic purposes.

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Functional and *in-silico* interrogation of rare genomic variants impacting RNA splicing for the diagnosis of genomic disorders

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Purpose: To develop a comprehensive analysis framework to identify pre-messenger RNA splicing pathogenic variants in the context of rare disease.

Methods: We ascertained variants of uncertain significance (VUS) through clinical multi-disciplinary team meetings, and defined an accurate *in-silico* strategy for the prioritization of variants impacting splicing through comparison to functional analyses. We retrospectively applied these prioritization strategies to a large cohort of 2783 individuals who had previously received genomic testing for rare genomic disorders, and assessed clinical impact.

Results: We identified 21 VUS that potentially impacted splicing, and used cell based splicing assays to identify those variants which disrupted normal splicing. We established the comparative utility of 9 *in-silico* strategies for variant prioritization. We analysed 1,346,744 variants identified through diagnostic testing for 2783 individuals, and observed that clinical impact is highly dependent on the specific incorporated *in-silico* prioritization strategy. We show that incorporation of one of these strategies into diagnostic testing would improve clarity in clinical analysis for 15% of the individuals surveyed. Prioritized variants could provide new molecular diagnoses or provide