A blinded international study on the reliability of genetic testing for GGGGCC-repeat expansions in C9orf72 reveals marked differences in results among 14 laboratories


BACKGROUND
The GGGGCC-repeat expansion in C9orf72 is the most frequent mutation found in patients with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Most of the studies on C9orf72 have relied on repeat-primed PCR (RP-PCR) methods for detection of the expansions. To investigate the inherent limitations of this technique, we compared methods and results of 14 laboratories.

METHODS
The 14 laboratories genotyped DNA from 78 individuals (diagnosed with ALS or FTD) in a blinded fashion. Eleven laboratories used a combination of amplicon-length analysis and RP-PCR, whereas three laboratories used RP-PCR alone; Southern blotting techniques were used as a reference.

RESULTS
Using PCR-based techniques, 5 of the 14 laboratories got results in full accordance with the Southern blotting results. Only 50 of the 78 DNA samples got the same genotype result in all 14 laboratories. There was a high degree of false positive and false negative results, and at least one sample could not be genotyped at all in 9 of the 14 laboratories. The mean sensitivity of a combination of amplicon-length analysis and RP-PCR was 95.0% (73.9-100%), and the mean specificity was 98.0% (87.5-100%). Overall, a sensitivity and specificity of more than 95% was observed in only seven laboratories.
CONCLUSIONS
Because of the wide range seen in genotyping results, we recommend using a combination of amplicon-length analysis and RP-PCR as a minimum in a research setting. We propose that Southern blotting techniques should be the gold standard, and be made obligatory in a clinical diagnostic setting.

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