Discrepancies in reporting the CAG repeat lengths for Huntington’s disease

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This study assessed the accuracy of CAG repeat number reports from local laboratories in 15 European countries within EHDN.

Background
Huntington’s disease patients who participate in the REGISTRY study may opt to have their CAG repeats in the huntingtin gene determined by a central laboratory, BioRep, based in Milan, Italy. The regenotyping results can then be compared to those obtained from local genetics laboratories in the patient’s home country.

Subjects and Methods
Blood samples from 1,326 REGISTRY participants were available for regenotyping and comparison with the original results from 121 laboratories in 15 countries. Duplicate results were compared by subtracting the BioRep result from that obtained from the local laboratory for both the disease causing (upper) and normal (lower) alleles. Discrepancies were considered in the light of acceptable measurement errors proposed by both the American College of Medical Genetics (ACMG) and the Draft European Best Practice Guidelines (BPG).

Results
For the upper allele, 49% of the results were identical and 51% were discrepant (31% by one CAG, 12% by two CAGs and 8% by three or more CAGs). The discrepancies were in both directions (55% increase and 45% decrease). When the proposed ACMG and BPG acceptable measurement errors were applied, the discrepancy rate fell from 51% to 13.3% and 9.7%, respectively. In 52 (4%) patients, the discrepancy was clinically significant. Of these, in 36 cases the repeat length moved from the reduced-penetrance range (36-39 CAGs) to the fully-penetrant range (≥ 40 CAGs) and in 11 cases from the fully-penetrant to reduced-penetrance range. A potential misdiagnosis occurred in five cases, as the change in results crossed the ≤ 35 CAGs critical boundary.

Discrepancies were noted in the results from 71% of the laboratories. Of these, 40% were outside the acceptable errors proposed by ACMG and 34% outside that proposed by BPG. Of the 121 laboratories, only 45 participated in the 2009 external quality assessments (EQA) organised by the European Molecular Genetics Quality Network. There was no correlation between the frequency of discrepancies and the time when local genotyping was performed.

To assess the reliability of the BioRep results, the DNA from 348 cases was reanalysed at an accredited laboratory in Germany. The results were identical to those of BioRep in 100% of the cases once the ACMG or BPG error rates were applied. BioRep also correctly measured the CAG repeat size in six reference samples supplied by the US National Institute for Standards and Technology.

Conclusions
This study shows that discrepancies in CAG repeat number assessment is a current and widespread problem across Europe that needs to be recognised and addressed. The authors strongly recommend that (1) laboratories provide an error rate for their measurement, (2) they participate in EQA schemes and (3) use reference materials regularly to adjust their internal standards. Knowledge of the discrepancy in CAG repeat size measurement within REGISTRY may be important for those conducting research based on CAG repeat length data pooled from multiple laboratories.