

Measurement methods and accuracy in copy number variation: failure to replicate associations of beta-defensin copy number with Crohn's disease.

ABSTRACT

The copy number variation in beta-defensin genes on human chromosome 8 has been proposed to underlie susceptibility to inflammatory disorders, but presents considerable challenges for accurate typing on the scale required for adequately powered case-control studies. In this work, we have used accurate methods of copy number typing based on the paralogue ratio test (PRT) to assess beta-defensin copy number in more than 1500 UK DNA samples including more than 1000 cases of Crohn's disease. A subset of 625 samples was typed using both PRT-based methods and standard real-time PCR methods, from which direct comparisons highlight potentially serious shortcomings of a real-time PCR assay for typing this variant. Comparing our PRT-based results with two previous studies based only on real-time PCR, we find no evidence to support the reported association of Crohn's disease with either low or high beta-defensin copy number; furthermore, it is noteworthy that there are disagreements between different studies on the observed frequency distribution of copy number states among European controls. We suggest safeguards to be adopted in assessing and reporting the accuracy of copy number measurement, with particular emphasis on integer clustering of results, to avoid reporting of spurious associations in future case-control studies.

Keyword: Copy number variations (CNVs); Paralogue ratio test (PRT); Crohn's disease; Inflammatory disorder.