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SHORT COMMUNICATION

Confirmation of two novel loci for infantile hypertrophic pyloric stenosis on chromosomes 3 and 5

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Infantile hypertrophic pyloric stenosis (IHPS) is a multifactorial heritable condition affecting infants in the first 3 months of life. It is characterized by hypertrophy of the pylorus resulting in blockage of the pyloric canal. Patients present with projectile vomiting, weight loss and dehydration. Five susceptibility loci have been identified through genome-wide linkage analysis and candidate gene approaches. The first genome-wide association study was recently performed and three statistically significant associations identified. Here, we report our confirmation of two of these significant results thus providing further support for new loci for IHPS on chromosome 3p25.1 and chromosome 5q35.2.
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Feenstra *et al.*[1] recently published the results of the first genome-wide association analysis of infantile hypertrophic pyloric stenosis (IHPS). They analyzed the association between disease and 523 420 single-nucleotide polymorphisms (SNPs) in 1001 IHPS cases and 2401 controls, all of Danish origin. Initial analyses identified six loci associated at $P \leqslant 1 \times 10^{-6}$, three of which were confirmed through replication analysis in a further 796 cases and 876 controls. We screened for these three SNPs (rs11712066, rs573872 and rs29784) in our resource of 301 trios as well as in a further 16 nuclear pedigrees with two affected children (none of the families included had affected parents). All samples were of northern European ancestry and have been described previously.[2,3] Genotyping was performed by KBiosciences (now part of LGC Genomics) using their proprietary KASPar technology (www.kbiosciences.co.uk). Intrafamilial association analysis was performed on these three SNPs using the PDT.[4] The PDT is a form of the transmission disequilibrium test, which compares the observed and expected transmission values of each allele or genotype from parent to child and can be applied to trios and larger pedigrees (see Supplementary Tables 1 and 2). We also performed standard χ^2-analyses on the frequency of SNP alleles and genotypes in affected children, basing expected values on the frequencies in the parental generation (see Supplementary Tables 3 and 4). Feenstra *et al.*[1] showed that the major allele (A) of rs11712066 was over-represented in cases in comparison with controls (83.1 vs 74.8%). Our analyses support this result. Intrafamilial association analysis showed overtransmission of the major allele A to affected offspring ($\chi^2_{(1\,df)} = 22.26$, $P < 0.001$) and overtransmission of the homozygous wild-type genotype AA ($\chi^2_{(2\,df)} = 39.39$, $P < 0.001$). Additional χ^2-analyses of the allele and genotype frequencies in affected children concurred with the results of the PDT as expected; the A allele and the AA genotype occur at a greater frequency than expected (allele-based analysis $\chi^2_{(1\,df)} = 11.53$, $P < 0.001$; genotype-based analysis $\chi^2_{(2\,df)} = 13.02$, $P < 0.01$). Feenstra *et al.*[1] also showed that the minor allele (A) of rs29784 was over-represented amongst cases (54.0% in cases vs 45.0% in controls). Again our analyses support this result with intrafamilial association analysis providing evidence for overtransmission of the A allele and the AA genotype (allele-based analysis $\chi^2_{(1\,df)} = 4.26$, $P < 0.05$; genotype-based analysis $\chi^2_{(2\,df)} = 7.52$, $P < 0.05$). The statistical significance of this overtransmission is lower than for rs11712066, which is probably due in large part to the more equal ratio of major to minor allele in the study population. This lower statistical significance is reflected in the allele and genotype χ^2-analyses in the affected children which are of borderline statistical significance (allele-based analysis $\chi^2_{(1\,df)} = 3.84$, $P = 0.05$; genotype-based analysis $\chi^2_{(2\,df)} = 5.36$, $P = 0.069$). However, the trend is towards over-representation of the A allele and the AA genotype in the affected children. Finally, our analyses do not support the results of Feenstra *et al.*[1] with regards to the SNP rs573872. Their analyses demonstrated a higher frequency of the minor allele (G) in the cases in comparison with the controls (28.9 vs 22.9%). Neither the PDT nor the χ^2-analyses demonstrate statistical evidence for over-representation of the G allele or GG genotype in affected individuals. Furthermore, there is not a noticeable trend in this direction; the distribution appears to be random.

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Both of the associated SNPs are intergenic; it is not the assertion of Feenstra *et al.*,[1] nor ourselves, that these SNPs are themselves likely to be causal but rather that they are acting as tagging SNPs for causal variants in nearby genes. Feenstra *et al.*[1] suggest that *MBNL1* on chromosome 3q25 and *NKX2-5* on chromosome 5q34 are the most likely functional candidates. *MBNL1* encodes the muscleblind-like splicing regulator 1. Members of the muscleblind family promote inclusion or exclusion of specific exons of different pre-mRNAs contributing to transcript complexity. MBNL1 appears to be involved in muscle differentiation. *NKX2-5* encodes the NK2 homeobox 5 transcription factor, which has been shown to have a critical role in pyloric sphincter muscle formation.[5,6] Future work will be to re-sequence these genes in individuals carrying the over-transmitted allele or genotype to determine whether either gene carries putative causal variants.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)