Linkage analysis of infantile pyloric stenosis and markers from chromosome 9q11–q33: no evidence for a major gene in this candidate region

E Chung, R Coffey, K Parker, P Tam, M E Pembrey, R M Gardiner

Abstract
A genetic component in the aetiology of infantile pyloric stenosis (PS) is well established. Segregation analysis is compatible with a multifactorial sex modified threshold model of inheritance but a major gene of low penetrance has not been excluded. PS has been reported to occur in 57% (four of seven) of cases with duplication of chromosome 9q11–q33. Twenty families with PS were studied using genetic markers at loci D9S55, D9S111, D9S15, D9S12, D9S56, D9S59, and ASS from this region of chromosome 9. Pairwise lod scores of 2–2 were obtained with all these markers at recombination fractions greater or equal to 0.04 under both autosomal dominant and autosomal recessive models of inheritance. This provides evidence against the existence of a major locus predisposing to PS within chromosome 9q11–q33. (J Med Genet 1993;30:393–5)

Infantile pyloric stenosis (PS) is the commonest condition requiring surgical intervention in the first year of life. Its incidence is estimated to be 1 to 5/1000 live births in Britain.12 Mortality was high until successful treatment by pyloromyotomy was developed by Rammstedt in 1911. Clinically it is characterised by projectile vomiting, visible gastric peristalsis, and a palpable pyloric tumour. Diagnosis is usually made clinically, supported on occasion by examination with ultrasound or barium contrast.

The aetiology of PS is entirely unknown though both genetic and environmental factors have been implicated. Its familial incidence was first reported by Cockayne and Penrose.14 This has been confirmed by subsequent family studies5–7 but the exact inheritance mechanism has not been accurately defined.5,7,9 Segregation analysis is best explained by a multifactorial sex modified threshold model of inheritance,5–10 but it is compatible with a single major dominant gene of low penetrance with a multifactorial background.5,11–12 The nature of the environmental factors concerned has remained unclear.

PS has been described in association with Smith-Lemli-Opitz syndrome and various chromosomal disorders, including Edward's syndrome (trisomy 18), Down's syndrome (trisomy 21), and Turner's syndrome (XO). Chromosome 9 is suspected as a possible location of a PS locus because of the reported association of PS and duplication of chromosome 9q11–q33.13–15 PS was reported to occur in four of seven (57%) of the cases with this duplication.16 Association of a phenotype with chromosomal duplication can be a guide to the location of causative gene loci, as illustrated by the identification of a locus for familial Alzheimer's disease on chromosome 21.17,18 Chromosome 9 is therefore a candidate region for the location of a gene predisposing to PS.

The existence of a comprehensive high resolution map of the human genome has rendered disease with complex inheritance amenable to linkage analysis. Following ascertainment of a suitable family resource, we are undertaking a systematic genome search to identify any major gene predisposing to PS by linkage analysis. The strategy involves analysis of candidate regions such as chromosome 9q in the first instance. We present the results of linkage analysis of 20 families and seven markers from this region of chromosome 9.

Materials and methods
FAMILIES
Twenty families with multiple members affected with PS were ascertained from a variety of sources, including families of probands identified in previous studies such as those of Carter.1 Blood was obtained for DNA studies from 207 family members, 65 of whom were affected (46 males, 19 females). At least three affected subjects existed in 13 of the 20 families (figure). Diagnosis of PS was made according to standard criteria. It was confirmed at laparotomy in all but five of the affected subjects; these five were treated medically. One was diagnosed clinically and confirmed by barium studies. The other four were diagnosed on clinical examination alone. They belong to family 7 in which there are a total of five affected subjects in over two generations.

DNA STUDIES
Genomic DNA was extracted from leucocytes using a salt precipitation technique.18 Family members were phenotyped at (CA)n, repeat microsatellite marker loci on human chromosome 9: D9S55, D9S111, D9S15, D9S12, D9S56, D9S59, and ASS. Alleles at these loci were amplified using the polymerase chain reaction (PCR). Products were separated by 6% polyacrylamide gel electrophoresis and detected by autoradiography.20

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Results
Pairwise log scores between the PS trait and the seven marker loci are shown in the table. The recombination fractions at which log score = -2 are also shown. Family 8 was uninformative for all the seven markers and family 7 and 20 were uninformative for locus D9S55.

The pairwise log score was = -2 at @ greater or equal to 0.04 for all marker loci. Our data provide evidence against the existence of a major locus predisposing to IHPS over the whole region from D9S55 (9p12) to ASS (9q34.1) for the AD model. For the AR model, the area of exclusion did not include regions of 5 cm between loci D9S56 and D9S59 and between loci D9S59 and ASS.

Results of the HOMOG analysis showed no evidence of heterogeneity (data not shown).

Discussion
The genetic basis of PS has been extensively investigated since its familial occurrence was first reported by Cockayne and Penrose\(^1\) over 50 years ago. A genetic contribution to its aetiology has been firmly established, but neither the exact mechanism of inheritance nor the gene products involved have been elucidated.

New methods for the investigation of inherited diseases known only by their phenotype, so called linkage analysis and ‘positional cloning’ strategies, have allowed major advances in our understanding of single gene disorders such as cystic fibrosis and Duchenne muscular dystrophy. However, most common familial human diseases, including PS, show complex inheritance. Application of linkage analysis to these complex traits raises numerous difficulties which have been widely discussed.\(^2\)\(^3\)

Several features of PS render it potentially more tractable to linkage analysis than other diseases with complex inheritance. The condition is clinically homogeneous with a simple well defined phenotype and diagnosis of affected subjects at operation is unequivocal. This reduces the risk of genetic heterogeneity and misdiagnosis. Ascertainment of a substantial family resource is facilitated by the high incidence, early presentation, and non-lethal nature of PS. By studying such high density families, in which there are mostly three or more affected subjects over several generations, it can be anticipated that the incidence of phenocopies is reduced and there is enrichment for cases with a major genetic contribution to aetiology.

Linkage analysis has been carried out using the lod score method. In the absence of definitive data from segregation analysis, arbitrary assumptions are necessary concerning inheritance parameters such as mode of inheritance, penetrance, and phenocopy rate. The most important assumption concerns the segregation of a locus with a major effect on the phenotype. Segregation analysis is compatible with a single major dominant gene of low penetrance. If inheritance is truly polygenic, it is unlikely that linkage to an individual locus can be detected.

Values for penetrance and phenocopy rate significantly affect the results of linkage analysis. If an adequate degree of incomplete pene-
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**Pairwise lod scores between the PS trait and chromosome 9 marker loci. Penetrance 0·60 for male, 0·15 for female. A, autosomal dominant model of inheritance. B, autosomal recessive model of inheritance.**

<table>
<thead>
<tr>
<th>Marker locus</th>
<th>0.00</th>
<th>0.05</th>
<th>0.10</th>
<th>0.20</th>
<th>0.30</th>
<th>0.40</th>
<th>θ at lod = -2</th>
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<tbody>
<tr>
<td>A PS-D9S5S</td>
<td>-12.015</td>
<td>-4.300</td>
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<td>-0.285</td>
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<td>-4.836</td>
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<td>-0.092</td>
<td>0.051</td>
<td>0.01</td>
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<td>PS-D9S15</td>
<td>-9.246</td>
<td>-3.230</td>
<td>-1.313</td>
<td>0.093</td>
<td>0.380</td>
<td>0.267</td>
<td>0.07</td>
</tr>
<tr>
<td>PS-D9S12</td>
<td>-9.895</td>
<td>-3.565</td>
<td>-1.355</td>
<td>0.146</td>
<td>0.413</td>
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<td>0.07</td>
</tr>
<tr>
<td>PS-D9S26</td>
<td>-9.077</td>
<td>-3.614</td>
<td>-1.562</td>
<td>0.078</td>
<td>0.255</td>
<td>0.051</td>
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<td>PS-D9S39</td>
<td>-10.307</td>
<td>-4.687</td>
<td>-2.625</td>
<td>-0.751</td>
<td>-0.053</td>
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<td>PS-ASS</td>
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<td>-4.385</td>
<td>-1.835</td>
<td>-0.652</td>
<td>-0.138</td>
<td>0.026</td>
<td>0.09</td>
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<tr>
<td>B PS-D9S5S</td>
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<td>PS-D9S11</td>
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<td>-1.768</td>
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<td>PS-D9S15</td>
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<td>-3.630</td>
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<td>PS-D9S26</td>
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<td>PS-D9S36</td>
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<td>PS-D9S59</td>
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<td>0.198</td>
<td>0.141</td>
<td>0.05</td>
</tr>
</tbody>
</table>

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