REPORT

Linkage of Monogenic Infantile Hypertrophic Pyloric Stenosis to Chromosome 16p12-p13 and Evidence for Genetic Heterogeneity

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Infantile hypertrophic pyloric stenosis (IHPS) is the most common form of bowel obstruction in infancy. The disease affects males four times more often than females and is considered a paradigm for the sex-modified model of multifactorial inheritance. However, pedigrees consistent with autosomal dominant inheritance have also been documented. We analyzed a 3-generation family with IHPS including 10 affected individuals (5 males and 5 females) and mapped the underlying disease locus to chromosome 16p12-p13 (LOD score 3.23) by using a single-nucleotide polymorphism–based genomewide scan. The analysis of 10 additional multiplex pedigrees yielded negative or nonsignificant LOD scores, indicating the presence of locus heterogeneity. Sequence analysis of candidate genes from the chromosome 16 disease interval excluded the presence of pathogenic mutations in the *GRIN2A* and *MYH11* genes.

Infantile hypertrophic pyloric stenosis (IHPS [MIM #179010]) is an inherited form of bowel obstruction affecting up to 8 in 1,000 newborns. The disease is characterized by a marked hypertrophy of the pylorus smooth muscle, which leads to a blockage of the gastric outlet and provokes increasingly severe episodes of projectile vomiting. Effective IHPS treatment requires surgical intervention to relieve the bowel blockage.[1]

IHPS has been associated with a number of inherited syndromes (e.g., Smith-Lemli-Opitz and Cornelia de Lange syndromes) and with a variety of chromosomal abnormalities.[2–5] Nonsyndromic IHPS shows familial aggregation, and the classic studies of Carter and Evans[6] defined the disease as a paradigm for the multifactorial, sex-modified threshold model of inheritance.

The pylorus hypertrophy underlying IHPS is thought to result from a failure to relax the sphincter smooth muscle. It has been proposed that disease susceptibility may be associated with a diminished production of nitric oxide, the main mediator of smooth-muscle relaxation in the gastrointestinal tract.[7] In particular, *NOS1* (MIM *163731), the gene encoding neuronal nitric oxide synthase, has been implicated in the disease pathogenesis by expression studies, animal models, and genetic analyses of small IHPS data sets.[8–10]

Despite well-documented evidence of multifactorial inheritance of IHPS, multigeneration families consistent with autosomal dominant transmission of the disease have also been described.[11,12] We report here a linkage analysis of several extended pedigrees and the identification of the first locus for monogenic IHPS.

As part of our ongoing research on common and complex forms of IHPS, we ascertained a data set of nuclear and multiplex pedigrees through collaborations with several centers for pediatric surgery (Great Ormond Street Hospital, The Royal London Hospital, and St George's Hospital, London; and Karolinska Institute, Stockholm). Three northern European families (IHPS021, IHPS036, and IHPS078; fig. 1)—including more than six affected individuals and presenting with a disease-transmission pattern consistent with autosomal dominant inheritance—were selected for this study. In all patients, IHPS was diagnosed according to standard clinical criteria and was confirmed during pyloromyotomy. All individuals who participated in the study granted their informed consent. Ethical approval was obtained from the Ethics Committee of University College London Hospital and from the relevant committees of all hospitals involved in the family-ascertainment effort.

We first excluded linkage to *NOS1* in the three extended pedigrees by genotyping a set of intragenic microsatellite markers (*NOS1a, NOS1b,* and *NOS1e29*; primer sequences and cycling conditions are available on request). Parametric linkage analysis was performed using Merlin 1.0,[13] under the assumption of autosomal dominant inheritance with reduced penetrance. Negative LOD scores were observed in all three families for 60% and 95% penetrance values (table 1).

Having excluded linkage to *NOS1,* we undertook a genomewide linkage scan of family IHPS036, which included the largest number of affected individuals. A total of 16 individuals (the 9 living affected individuals and 7 unaffected relatives; fig. 1) were genotyped using Illumina Linkage Panel IV, which consists of 5,850 SNP markers spaced at an average distance of 0.64 cM. Four micrograms of genomic DNA from each individual was analyzed on a BeadArray platform (Illumina), by use of GoldenGate assay (Illumina) reagents. Both parametric and nonparametric linkage analyses were performed using Merlin 1.0.[13] On the basis of the IHPS transmission pattern observed

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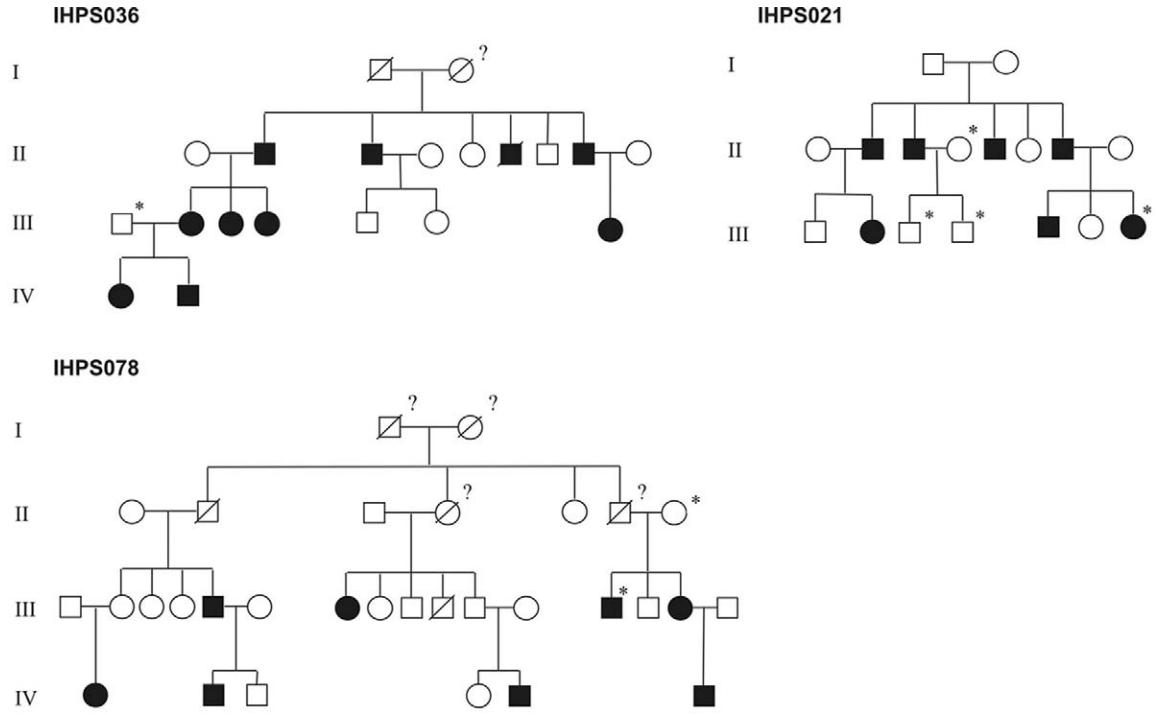

Figure 1. The three multigeneration IHPS pedigrees. Biological samples were obtained from all living individuals, except those marked with an asterisk (*). No samples were obtained from any of the deceased family members. Question marks (?) indicate individuals whose disease status could not be determined.

in family IHPS036, the parametric analysis of the genome scan was implemented assuming autosomal dominant inheritance with 95% penetrance. The disease-allele frequency and phenocopy rate were set at 0.001 and 0.0001, respectively.

Multipoint LOD scores >3 were observed at a single genomic region (in bold italics in table 2), on chromosome 16p12-p13 (maximum LOD 3.23; fig. 2*A*). A more detailed analysis of this locus was undertaken, and LOD scores ⩾3 were observed across penetrance values ranging from 82% (maximum LOD 3.01) to 100% (maximum LOD 3.31). NPL analysis also confirmed linkage to this interval (NPL $Z_{\text{mean}} = 9.5$; $P < .00001$). Segregation analysis identified a risk haplotype that was shared by all affected individuals (fig. 2*B*) and was not found in any of the unaffected subjects. Critical recombination events that occurred in two

Table 1. Linkage Analysis of the *NOS1* Genomic Region in Three Multigeneration Pedigrees, with 60% or 95% Penetrance

Marker (Position)	Multipoint LOD Score for Pedigree					
	IHPS021		IHPS036		IHPS078	
	60%	95%	60%	95%	60%	95%
NOS1a (5′ UTR)	−2.08	−1.93	−4.6	−5.9	−5.0	−4.5
NOS1b (intron 12)	−2.08	−1.93	−4.5	−5.8	−5.7	−5.2
NOS1e29 (3′ UTR)	−2.08	−1.93	−4.0	−4.8	−5.0	−4.5

Table 2. Summary of Results of Genomewide Scan

Chromosome	LOD Score	
	Minimum	Maximum
1	−12.2	1.5
2	−12.6	−3.4
3	−13.7	−3.1
4	−12.5	−3.4
5	−9.9	1.9
6	−13.6	−4.3
7	−11.2	1.9
8	−12.3	−.8
9	−9.9	−2.8
10	−11.1	1.9
11	−13.2	−4.8
12	−12.0	−3.5
13	−13.9	−1.0
14	−10.9	−3.0
15	−11.2	−3.1
16	−11.3	***3.2***
17	−13.8	−5.5
18	−12.0	−1.4
19	−11.7	−2.1
20	−10.6	.6
21	−9.9	−2.7
22	−11.8	−4.0

NOTE.—Multipoint LOD score >3 is indicated by bold italics.

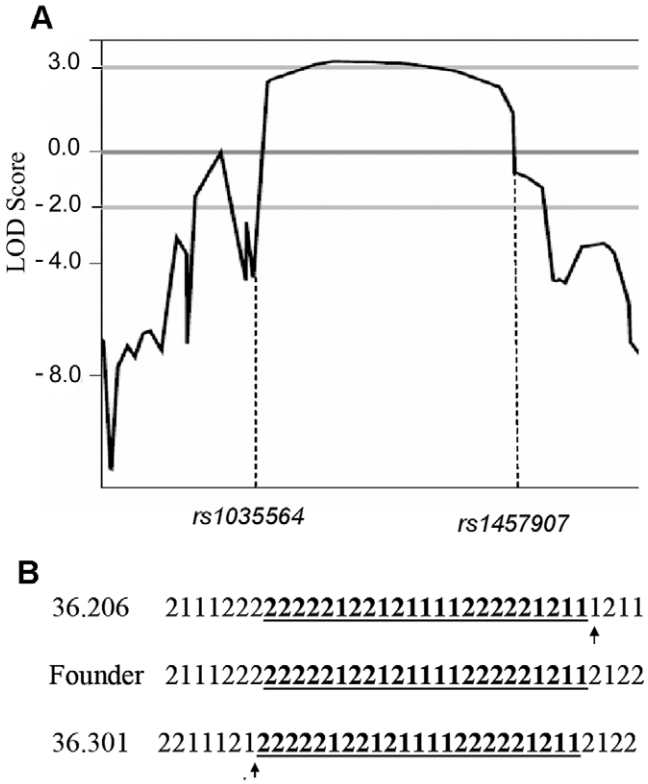

Figure 2. Mapping of the IHPS disease locus to chromosome 16p. *A,* Output of chromosome 16 multipoint LOD score analysis. *B,* Definition of the disease interval by means of haplotype analysis. The middle row (Founder) shows the SNP haplotype segregating in the affected individuals from family IHPS036. The top and bottom rows show the recombining haplotypes observed in subjects 36.206 and 36.301. Arrows indicate the sites of recombination, and the genomic segment shared by all affected individuals is underlined.

patients allowed us to define a 23-cM disease interval delimited by SNPs *rs1035564* and *rs1457907* (fig. 2*B*).

Three microsatellite markers spanning the linkage interval (*D16S3052, D16S3075,* and *D16S405*; primer sequences and cycling conditions are available on request) were typed in the two remaining extended families (IHPS021 and IHPS078). We also analyzed an additional eight northern European multiplex pedigrees, which we selected from a data set previously ascertained by Chung et al.,[8] on the basis of their negative LOD scores at the *NOS1* locus. The chromosome 16p microsatellite markers yielded negative LOD scores in all examined families (tables 3 and 4), with the exception of a single pedigree (with four affected individuals), which yielded positive but nonsignificant LOD scores (family IHPS005). Altogether, these results indicated that the chromosome 16p linkage is specific to family IHPS036 and hindered a further refinement of the disease locus.

The IHPS interval identified by the genomewide scan spans 11 Mb. The high marker density of the Illumina Linkage Panel IV provided us with well-defined boundaries for the disease interval, eliminating the need for further fine mapping. The distal end of the minimal IHPS region is delimited by a recombination between *rs1035564* and *rs933478*; these two markers are only 173 kb apart, and no transcript has been mapped between them. Likewise, the recombination defining the proximal boundary of the IHPS locus occurred between two markers (*rs1457907* and *rs936347*) that are 75 kb apart and encompass no genes.

The 11-Mb disease locus includes a total of 65 genes. We investigated the function and expression patterns of all transcripts by mining the Ensembl, UniGene, and GeneCards databases. We prioritized for mutational analysis the *MYH11* (MIM *160745) and *GRIN2A* (MIM *138253) genes, since they encode proteins with important roles in smooth-muscle relaxation. *MYH11* encodes the heavy chain of myosin XI, the molecular motor that powers smooth-muscle contraction.[14] Four myosin isoforms are generated by alternative splicing of *MYH11* and are differentially expressed in various smooth-muscle cell types.[15] *GRIN2A* encodes the 2A subunit of ionotropic glutamate receptors of the *N*-methyl-D-aspartate subtype. These are ligand-gated ionic channels that mediate the influx of calcium into neurons, a crucial signal in the regulation of smooth-muscle relaxation.[16]

We examined all coding and UTR exons, as well as exon-intron junctions, in both candidate genes. Purified PCR products (primer sequences and cycling conditions are available on request) were sequenced using an ABI 3100 Genetic Analyzer (Applied Biosystems). Nucleotide changes were identified by visual inspection of chromatograms. Several known SNPs were identified in both genes, but no pathogenic mutation was observed.

With this study, we mapped the first locus for monogenic IHPS through a SNP-based genome scan of a multigeneration pedigree. The sex-segregation male:female ratio in family IHPS036 is 1:1. In our experience, the IHPS male sex bias tends to be less pronounced in familial cases (1.8:1 in our data set; table 3), which suggests that the effect of the underlying mutations is unlikely to be mod-

Table 3. Characteristics of the 10 Additional IHPS Pedigrees Used in Linkage Analysis

Characteristic	No. of Subjects in Pedigree										Total No. of Subjects
	IHPS005	IHPS007	IHPS009	IHPS020	IHPS021	IHPS027	IHPS035	IHPS057	IHPS078	IHPS146	
Affected (male:female ratio)	4 (1:1)	5 (4:1)	5 (5:0)	4 (3:1)	6 (2.5:1)	4 (1:4)	4 (1:3)	4 (1:1)	7 (1.6:1)	4 (4:0)	47 (1.8:1)
Unaffected	3	3	10	7	7	4	3	8	10	4	59

Table 4. Linkage Analysis of Chromosome 16p Microsatellite Markers in 10 Additional IHPS Pedigrees, with 60% or 95% Penetrance

	Multipoint LOD Score for Pedigree																			
	IHPS005		IHPS007		IHPS009		IHPS020		IHPS021		IHPS027		IHPS035		IHPS057		IHPS078		IHPS146	
Marker	60%	95%	60%	95%	60%	95%	60%	95%	60%	95%	60%	95%	60%	95%	60%	95%	60%	95%	60%	95%
D16S3052	−1.9	−.6	−4.5	−5.1	−.4	−.6	−1.2	−.02	−5.9	−6.5	−3.1	−4.3	−2.2	−1.4	−5.1	−3.8	−8.1	−5.7	−.1	−.2
D16S3075	.2	.2	−6.4	−7.0	−.4	−.6	−.6	−.02	−5.5	−3.4	−2.9	−3.4	−.3	−1.0	−1.3	−1.2	−9.8	−9.1	−.2	−.3
D16S405	.7	.6	−6.4	−7.0	−.2	−.4	−1.3	−.02	−4.7	−.6	−2.6	−3.0	.4	−.3	−1.8	−1.1	−7.1	−3.2	−2.4	−1.6

ified by sex-specific factors (e.g., hormone levels). Conversely, we did not observe any differences in the clinical phenotype of family IHPS036 compared with the phenotypes of other familial or sporadic cases. The analysis of an additional 10 families yielded negative or nonsignificant LOD scores, indicating that the chromosome 16 locus is specific to the highly penetrant mutation segregating in family IHPS036.

The observation of genetic heterogeneity in a Mendelian subtype of a complex disease is not uncommon, and several genes are known to be mutated in the monogenic forms of breast cancer,[17] Alzheimer disease,[18] and maturity-onset diabetes of the young.[19] In the case of IHPS, the presence of genetic heterogeneity is consistent with the complexity of the molecular pathways underlying smooth-muscle relaxation.[20] Given the high number of proteins involved in these signaling cascades, it is reasonable to hypothesize that IHPS may be caused by mutations occurring in different genes and segregating with various degrees of penetrance.

We sequenced the two most prominent candidate genes from the chromosome 16p linkage interval but did not observe any pathogenic mutation. The identification of heterozygous genotypes at a number of SNP loci also rules out the possibility that the disease is caused by the deletion of either *MYH11* or *GRIN2A*. We cannot exclude the occurrence of a mutation in the promoter regions of either gene. However, the high penetrance of the defect segregating in family IHPS036 argues against the hypothesis of a subtle regulatory mutation. It is also worth mentioning that a recent report of dominant *MYH11* mutations in families with thoracic aortic aneurism[21] makes this gene a less likely candidate for IHPS.

The linkage interval identified in family IHPS036 contains at least 21 anonymous cDNAs (Ensembl v37 Feb 2006), which we are currently characterizing using a range of bioinformatic tools. We also plan to integrate results obtained *in silico* with those of laboratory-based expression analyses; as more information on the functions and expression patterns of these transcripts becomes available, additional IHPS candidate genes should emerge. Advances in the field of smooth-muscle physiology could also help to identify additional chromosome 16p genes that may be involved in the relaxation of the pylorus sphincter.

The identification of a disease locus is an important step toward a better understanding of IHPS molecular pathogenesis. Although our findings indicate that chromosome 16p is unlikely to be a common IHPS locus, the analysis of additional family data sets will be needed to establish how frequently this locus segregates with the disease. The eventual identification of the disease gene will also allow an assessment of the contribution of chromosome 16p mutations to sporadic IHPS.

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Web Resources

The URLs for data presented herein are as follows:

Ensembl, http://www.ensembl.org/Homo_sapiens/index.html
GeneCards, http://www.genecards.org/index.shtml
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for IHPS, *NOS1, MYH11,* and *GRIN2A*)
UniGene, http://www.ncbi.nlm.nih.gov/UniGene/

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