Char Syndrome, an Inherited Disorder With Patent Ductus Arteriosus, Maps to Chromosome 6p12-p21

Masahiko Satoda, MD; Mary Ella M. Pierpont, MD, PhD; George A. Diaz, MD, PhD; Renee A. Bornemeier, MD; Bruce D. Gelb, MD

Background—Patent ductus arteriosus (PDA) is a relatively common form of congenital heart disease. Although polygenic inheritance has been implicated, no specific gene defects causing PDA have been identified to date. Thus, a positional cloning strategy was undertaken to determine the gene responsible for the Char syndrome, an autosomal dominant disorder characterized by PDA, facial dysmorphism, and hand anomalies.

Methods and Results—A genome scan was performed with 46 members of 2 unrelated families in which the disease was fully penetrant but the phenotype differed. Significant linkage was achieved with several polymorphic DNA markers mapping to chromosome 6p12-p21 (maximal 2-point LOD score of 8.39 with D6S1638 at θ=0.00). Haplotype analysis identified recombinant events that defined the Char syndrome locus with high probability to a 3.1-cM region between D6S459/D6S1632/D6S1541 and D6S1024.

Conclusions—A familial syndrome in which PDA is a common feature was mapped to a narrow region of chromosome 6p12-p21. Additional analysis with other families and polymorphic markers as well as evaluation of potential candidate genes should lead to the identification of the Char syndrome gene, which will provide insights into cardiogenesis as well as limb and craniofacial development. (Circulation. 1999;99:3036-3042.)

Key Words: ductus arteriosus, patent ■ genetics ■ genes ■ heart defects, congenital

The ductus arteriosus is the arterial connection between the pulmonary artery and the aorta that shunts blood away from the lungs during fetal life. The ductus normally constricts shortly after birth due to the postnatal drop in circulating prostaglandin E2 levels, as well as the rise in systemic PO2, which induces a cytochrome P450-dependent increase in endothelin-1 in ductal smooth muscle cells.1 Subsequent to this constriction, the ductal endothelial cells detach, the subendothelial region swells, and smooth muscle cells migrate into the region, resulting in complete ductal closure and initiating transformation of the structure into the ductal ligament.2 Failure of the duct to close results in patent ductus arteriosus (PDA), a common form of congenital heart disease (CHD). Isolated PDAs are found in 1 in 2000 full-term infants, constituting ~10% of all CHD, and are considerably more common in premature infants.3 In addition, PDAs are frequently associated with intracardiac defects, and for newborns with many forms of CHD, maintenance of ductal patency is a critical therapeutic strategy to sustain adequate pulmonary or systemic circulation before surgical intervention.

PDAs in full-term infants presumably are multifactorial in origin.4 Environmental factors have been implicated, with in utero rubella infection being the most notable example.5 Studies have revealed recurrence rates of ~3% among siblings of individuals with isolated PDA that, combined with other genetic epidemiological findings, are consistent with polygenic inheritance.4,6 To date, no genetic defect specific for PDA has been identified. A mouse model of PDA was created by targeted disruption by homologous recombination of the prostaglandin E2 receptor gene, EP2,7 but mutations in the human ortholog, which maps to chromosome 5p13, have not been reported.

One approach for understanding the genetic control of ductal development is to identify disease genes underlying mendelian disorders in which PDA is a prominent feature. Such a disorder, first described by Florence Char in 1978, is an autosomal dominant disorder characterized by PDA, facial dysmorphism, and abnormal fifth digits of the hands (MIM No. 169100).8 To identify the gene underlying this disease, a positional cloning strategy was undertaken with 2 unrelated kindreds in whom this disorder is inherited.8,9 In the present study, we report that a genome screen and haplotype analyses assigned the Char syndrome gene to a 3.1-cM region at chromosome 6p12-p21.

Methods

Two multigenerational kindreds in whom Char syndrome is inherited, as reported previously,8,9 were recruited, and their family trees

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were updated (Figure 1). Medical histories and physical examinations were obtained on available family members. Statistical comparison of the incidence of various aspects of the phenotype between the 2 families was performed with a 2×2 contingency table ($\chi^2$ distribution with 1 degree of freedom). After informed consent had been obtained, blood samples (3 to 10 mL) were drawn from these individuals for DNA isolation. Genomic DNA was prepared from pelleted leukocytes with the Puregene DNA isolation kit (Gentra Systems). Samples of genomic DNA were diluted to a concentration of 5 ng/µL and used as templates for polymerase chain reaction amplification. Genotyping with the short tandem repeats (STRs) was performed as described previously.10 Amplification products were detected by autoradiography. The genome scan was performed with the Human Mapping Panel, version 4 (Research Genetics), and additional STRs in the linkage region were determined from available human genetic maps. Genotype data were entered into the Labman11 software package, and output files were exported to the Linkage software package, version 5.1. Two-point linkage analysis was performed with the MLINK portion of Linkage 5.1. Allele frequencies for the STRs were assumed to be 1/n, where n is the number of alleles reported for a particular STR. Autosomal dominant inheritance with complete penetrance was assumed, and the frequency of the disease allele was set at 0.0001.

Results

Clinical Evaluation

Clinical findings in affected individuals from the unrelated Arkansas8 and Minnesota9 families included PDA and facial dysmorphism (Table 1). The latter included short philtrum, prominent lips, flat nasal bridge with upturned nares, mild ptosis, and low-set ears. The Arkansas kindred had more striking facial findings (Figure 2A) and abnormalities of the

<table>
<thead>
<tr>
<th>Family</th>
<th>PDA</th>
<th>Hand anomalies</th>
<th>Facial dysmorphism</th>
<th>Additional CHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arkansas</td>
<td>4/19 (21%)</td>
<td>16/18 (89%)</td>
<td>19/19 (100%)</td>
<td>1/19 (5%)</td>
</tr>
<tr>
<td>Minnesota</td>
<td>10/14 (71%)</td>
<td>0/14 (0%)</td>
<td>14/14 (100%)</td>
<td>1/14 (7%)</td>
</tr>
</tbody>
</table>
postaxial middle phalanges (Figure 2B), whereas the Minnesota kindred had a higher incidence of PDA but milder facial involvement and normal hands. The differences in the incidences of PDA and hand anomalies between these 2 families was statistically significant ($P$, 0.005 and $P$, 1.312, respectively). In addition to a PDA, 2 individuals had intracardiac cardiac defects: a small muscular ventricular septal defect in 1 affected individual in the Minnesota kindred and complex CHD with neonatal demise in an affected member of the Arkansas kindred.

Figure 2. A, Typical facial features of Char syndrome in an affected 46-year-old woman from the Arkansas family. Short philtrum, prominent lips, flat nasal bridge with upturned nares, and ptosis are evident. B, Hands of the same individual with Char syndrome. Fifth middle phalanges are absent, and fifth proximal and distal phalanges are hypoplastic.

Genetic Analysis
A genome-wide search with polymorphic STRs was conducted with genomic DNA from 28 and 18 individuals from the Arkansas and Minnesota pedigrees, respectively. Significant linkage ($LOD$ score $>3.30$) was first detected with screening marker D6S257, with which a 2-point LOD score of 3.75 at $\theta=0.10$ was generated. Subsequent analysis with additional STRs from the Génethon, Cooperative Human Linkage Center (CHLC), and Marshfield (Wis) Medical Research Foundation human genetic linkage maps in the interval near D6S257 (Figure 3) resulted in a maximal 2-point LOD score of 8.39 at $\theta=0.00$ for marker D6S1638 (Table 2). Although the threshold LOD score of 3.30 for significant linkage was exceeded by numerous STRs, only markers mapping to the interval between D6S1632 and D6S257 gave positive LOD scores at no recombinant distance. Both families contributed positively to the LOD scores within that interval and independently provided evidence for linkage (eg, LOD scores for the Arkansas and Minnesota families with D6S272 at $\theta=0.00$ were 4.00 and 3.53, respectively). These linkage results, based on assumed allele frequencies of 1/number of alleles, proved robust when allele frequencies were varied arbitrarily.

Next, haplotype analysis was performed with the genotyping data from all available individuals in both pedigrees. Affected individuals within each Char kindred shared an identical haplotype formed by 12 markers in the interval from...
D6S1541 to D6S243, and that haplotype was never observed in an unaffected individual, consistent with the assumption of complete penetrance. The Char critical region was defined by observed recombinant events in the Arkansas and Minnesota families (Figure 4). The telomeric boundary was defined by recombinant events observed in both Char kindreds. In the Arkansas family, a recombinant chromosome was observed in affected individual ARK V-15, which was then inherited by her affected son, ARK VI-6. ARK V-15 inherited alleles from her mother’s unaffected chromosome at markers D6S459 and D6S1632 but alleles from the mutant chromosome at D6S1541, D6S1566, and D6S438. This placed the telomeric boundary between D6S459/D6S1632 and D6S1541, 3 markers at no recombinant distance from one another. In the Minnesota family, unaffected individual MINN V-3 inherited affected alleles from her mother’s mutant chromosome at markers D6S271 and D6S1650 but alleles from her unaffected chromosome at D6S438 and D6S269. Because the mother of MINN V-3 was uninformative at D6S459, D6S1632, and D6S1541, positioning of the recombinant event was difficult on the autosomal recessive polycystic kidney disease (ARPKD) genetic map. However, MINN V-3 inherited the affected allele at D6S1650, a marker that colocalized with D6S459 on the Genethon genetic map, suggesting that this recombinant event occurred in the 1-cM region below D6S459/D6S1632/D6S1541. The centromeric boundary could be definitively positioned by a recombinant event observed in an affected member of the Minnesota family (MINN IV-4) who inherited maternal affected alleles as centromeric as D6S1573 but unaffected alleles at D6S257 and D6S1005. A more favorable recombinant event was observed in an unaffected member of the Arkansas family (ARK V-18), although usefulness of this information depends on the assumption of complete penetrance (consistent with the data in the present study but not completely certain because of the relatively small number of pedigrees reported with Char syndrome). He inherited alleles from the maternal mutant chromosome at D6S1024 but alleles from her unaffected chromosome at D6S1714 and D6S243. On the basis of fine genetic mapping of the ARPKD locus, D6S1024 is 1 cM centromeric to D6S1714 and D6S243 (Figure 3). Physical mapping of the region confirmed this marker order. Thus, results of the haplotyping placed the Char critical region telomeric of D6S257 with certainty and telomeric of D6S1024 with high probability.

### Discussion

Analysis of 2 unrelated families inheriting Char syndrome localized the disease critical region with high likelihood to the genetic interval between D6S459/D6S1632 and D6S1024, which spans 3.1 cM. The maximal 2-point LOD score of 8.69 achieved with D6S1638 at \( \theta = 0.00 \) was highly significant (corresponding to \( P < 5 \times 10^{-9} \)), and linkage to this locus was achieved independently with both families. This locus can be assigned to the short arm of chromosome 6 near the centromere, because that genetic interval contains the T-complex–associated testis expressed 1 (TCTE1), meprin 1α, and glutathione S-transferase-α type 1 genes, all of which have been mapped physically to 6p12-p21.14,16–18 Another trait, autosomal recessive polycystic kidney disease (PKHD1), has been mapped to this locus14,19; differences in the phenotype and mechanism of inheritance make it unlikely that PKHD1 and Char syndrome are caused by defects in the same gene. The marker order for this region was established by fine genetic mapping. The recombinant chromosome identified in ARK V-15 suggested that D6S1541 is centromeric to...
D6S459 and D6S1632, 3 STRs previously mapped at no recombinant distance. Similarly, a recombination event on the paternal chromosome inherited by ARK V-17 suggested that D6S1566 and D6S438 reside centromeric to D6S1669 and D6S452. The Char syndrome critical region can be reduced from its current size of 3.1 cM by the development of new polymorphic markers that map into the 1-cM intervals lacking markers at the centromeric and telomeric boundaries of the locus. Such new markers would permit finer definition of the recombinant chromosomes identified in the present work.

Phenotypically, Char syndrome has been notable for the autosomal dominant inheritance of PDA and facial dysmorphism. Analyses for the present study were consistent with complete disease penetrance and established that the phenotype varied, even within families. Strikingly, affected individuals in the Minnesota family had a high prevalence of PDA, mild facial dysmorphism, and normal hands, whereas those in the Arkansas family had more marked facial features and a lower prevalence of PDA, and most had the hand abnormality. Because the disease gene in both families was linked to the same region but the disease haplotypes differed, this phenotypic variability presumably resulted from different mutations to the same gene. This is analogous to findings described for the Holt-Oram syndrome in which some families have more severe cardiac defects and mild thumb abnormalities, whereas others have more marked radial ray limb defects but only secundum atrial septal defects. After identification of the Holt-Oram disease gene, TBX5, mutation analysis documented that this phenotypic heterogeneity was associated with different mutations (Reference 21; Craig T. Basson, personal communication, 1998).

Char syndrome has been reported in 3 multigenerational families in addition to the 2 used for the present study. In those 3 families, the pattern of inheritance also has been consistent with autosomal dominance, and the PDA has variably penetrant. In the family reported by Slavotinek and coworkers, an affected girl had polydactyly and syndactyly on 1 foot, and her affected mother had a bony exostosis in the same position. Both of those individuals, as well as several other affected family members, had clinodactyly of their fifth fingers. Clinodactyly, a feature noted in the Arkansas kindred, was reported in another family, although coinheritance with the Char syndrome was not well documented. PDA, inherited as an autosomal dominant trait but without facial dysmorphism or limb anomalies, has been reported in several fami-
lies.25–27 Because the Minnesota family had subtle facial features and no hand abnormalities, it remains to be determined if autosomal dominant, “isolated” PDA is part of the clinical spectrum of Char syndrome.

Two affected individuals in the present study, 1 from the Minnesota and 1 from the Arkansas family, had CHD that included intracardiac defects. In the large Char kindred reported by Davidson,23 1 of 9 affected individuals also had a small muscular ventricular septal defect. This 10-fold increase in incidence is unlikely to have occurred by chance but rather appears related to the underlying gene defect. Because the ductus arteriosus is normally patent during fetal life, this finding suggests that the Char syndrome gene has some direct role in cardiogenesis, either through its expression in the developing heart per se or in tissues such as the neural crest that migrate into the cardiac developmental field, contributing to conotruncal and sixth aortic arch development.28

Although PDA is an extracardiac defect, and the prevalence of intracardiac defects in Char syndrome was relatively low, this syndrome resembles the class of heart-hand syndromes in many respects. The identification of several syndromes with heart and extremity defects has established the connection between cardiogenesis and limb development, particularly that of the arm and hand. In addition to the Char and Holt-Oram syndromes, other examples include Tabatznik, heart-hand type III, thrombocytopenia-absent radius, ulnar-mammary (TBX3 gene), Rubinstein-Taybi (transcriptional coactivator cAMP response element binding [CREB] protein gene), and Simpson-Golabi-Behmel (glypican-3 gene) syndromes. Wilson29 correlated the occurrence of CHD with limb-patterning defects in a recent survey of mendelian syndromes and proposed the existence of a cardiomec developmental field, a region in the early embryo giving rise to both the heart and limb primordia. By analogy with other organisms such as Drosophila, the morphogenetic genes (morphogens) expressed in that field are likely to exist in a graded fashion. Therefore, the cardiac and upper-limb primordial cells, which are more proximate to one another than to the lower limb primordial cells, would be exposed to a similar environment, and a qualitative or quantitative abnormality of a morphogen might result in a heart-hand defect.

In addition to TBX3 and TBX5, other morphogenetic genes that have been shown to be expressed in the developing heart and limbs include several transforming growth factor-β superfamily members and retinoic acid receptors. None of these genes maps to the Char syndrome critical region (RXRβ, a retinoic acid receptor, maps to chromosome 6p21 but more telomerically in the HLA region).30 The genes associated with ductal constriction and obliteration, as well as other genes relevant to vascular remodeling, are also potential candidates for Char syndrome; none of the known ones, including the prostaglandin receptor genes, map to the Char syndrome critical region. Finally, a survey of known genes that have been mapped to 6p12-2p1 failed to reveal an obvious candidate for the Char syndrome gene. Therefore, future efforts to identify the gene causing Char syndrome will require further delineation of the locus by analysis of additional affected kindreds and a positional cloning approach.

Acknowledgments

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References


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