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A search for the gene(s) predisposing to idiopathic clubfoot

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To the Editor:

Idiopathic clubfoot is one of the most common serious congenital deformities of the musculoskeletal system. A genetic component to the etiology of idiopathic clubfoot is suggested by the 33% concordance of identical twins (1). Four complex segregation analyses of idiopathic clubfoot populations have been performed (2-5), and all support a single, major gene effect as explaining a large portion of the liability to having a clubfoot. Therefore, we performed a genome-wide linkage screen in a large family to attempt to localize a predisposing gene.

The family employed in the genome screen for linkage consisted of four generations, with 13 individuals affected with clubfoot and 41 available unaffected members. We assessed the statistical power available to LOD score linkage analysis using the SLINK software routines. We determined that the mean value of the maximum ELOD of 3.85 could be obtained using our family.

We used the DNA pooling and shared segment analysis strategy described by Sheffield et al. (6) to screen the genome using the Cooperative Human Linkage Center/Marshfield Screening set 8 short tandem repeat DNA polymorphisms. DNA from all the affected pedigree members was pooled and a second DNA pool of unaffected members of the family was created. Four individual affected members were selected who had the minimum of genetic sharing possible to search for coinherited alleles. The four individual affected members and the two pooled DNA samples were assayed with each marker. Visual examination determined the predominant allele in both the affected and unaffected DNA pools. We developed a hierarchical scoring system for candidates with the best candidate markers having a single allele that was shared by all four affected individuals as well as being the dominant allele in the pooled DNA of affected family members and not the dominant allele in the pooled DNA of unaffected family members. Based on this hierarchical ranking, we prioritized primers for individual genotype testing against the entire family.

We ran a complete family analysis on 77 markers in areas of interest. Eight regions gave LOD scores greater than 1 and two of these had scores above 2 (position 38.3 on chromosome 3 LOD 2.18 at 0.1 and 55.3 on chromosome 13 with LOD 2.00 at 0). Both of these loci had nearby markers and also generate LODs above 1.0 as well. We identified no regions of linkage that met criteria for significant LOD scores (<3.0).

Two biologically plausible candidate genes are present near the markers with maximum LOD scores: one on chromosome 3 and one on 13 (Table 1) *Wnt7* is a gene encoding a secreted protein that stimulates LMX-1 to confer dorsal patterning in the developing limb ectoderm (7). That a major axis/patterning gene could cause such a subtle disorder such as clubfoot is possible. For example, mutations in the *HOXD 13* gene cause both synpolydactyly and hand-foot-genital syndrome. The second candidate gene resides on chromosome 13 - LM07. The function of this gene is unknown, but many splice variants have an F-box domain, which are

involved in protein-protein interactions. High expression has been found in skeletal muscle and moderate expression in the spinal cord, rendering it a plausible candidate for affecting limb development.

Weeks and Lathrop (8) have suggested that mapping susceptibility loci in disorder of complex inheritance may be complicated by high population frequency, incomplete penetrance, phenocopies, genetic heterogeneity, possible epistasis, and/or pleiotropy. Our study utilized one large family. This approach minimized many of the potential problems especially that of genetic heterogeneity, but limited our power to identify a susceptibility gene with a modest effect. If one branch of our family or even one person is a phenocopy, linkage analysis might fail to localize a true susceptibility locus. Similarly if a single individual chosen as one of the critical four affected individuals employed in the DNA pooling/shared segment analysis strategy was a phenocopy, we might fail to identify a susceptibility locus.

Coupling candidate gene evaluation to linkage localization is the quickest way to move from gene map to gene and mutation identification. This approach is limited by how well candidates can be selected and the broad interval that must be evaluated when the disease is complex, penetrance is incomplete, and the LOD or equivalent score is low. The biological relevance of a candidate gene is perhaps the most difficult challenge to overcome, but the history of success using this approach forces its continued application (9,10).

The strong evidence for a genetic contribution to the liability for idiopathic clubfoot from complex segregation analyses supports the continued search for causative gene(s). Identification of such gene(s) will contribute to our understanding and treatment of clubfoot as well as the determinants of normal limb growth and development. The results of this study can be combined with new studies to identify regions of higher statistical significance and can be exploited for new candidate gene identification as new genes are mapped to our best regions, new functions are found for known genes that would be consistent with clubfoot or animal models found and their genes mapped to syntenic sites found in this report.

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References

1. Idelberger K. Die Ergebnisse der Zwillingsforschung beim angeborenen Klumpfuß. *Brerh Dtsch Orthop Ges* 1939;33:272–276.
2. Wang JH, Palmer RM, Chung CS. The role of major gene in clubfoot. *Am J Hum Genet* 1988;42(5):772–776. [PubMed: 3358425]
3. Yang H, Chung CS, Nemecek RWA Genetic analysis of clubfoot I Hawaii. *Genetic Epidemiol* 1987;4:299–306.
4. Rebbeck TR, Dietz FR, Murray JC, et al. A single-gene explanation for the probability of having idiopathic talipes equinovarus. *Am J Hum Genet* 1993;53(5):1051–1063. [PubMed: 8213831]
5. de Andrade M, Barnholtz JS, Amos CI, et al. Segregation analysis of idiopathic talipes equinovarus in a Texan population. *Am J Med Genet* 1998;79(2):97–102. [PubMed: 9741466]
6. Sheffield VC, Pierpont ME, Nishimura D, et al. Identification of a complex congenital heart defect susceptibility locus by using DNA pooling and shared segment analysis. *Hum Mol Genet* 1997;6(1):117–121. [PubMed: 9002679]
7. Tickle C. Genetics and limb development. *Dev Genet* 1996;19:1–8. [PubMed: 8792604]
8. Weeks DE, Lathrop GM. Polygenic disease: methods for mapping complex disease traits. *Trends Genet* 1995;11(12):513–519. [PubMed: 8533170]

9. Jezewski PA, Vieira AR, Nishimura C, et al. Complete sequencing shows a role for MSX1 in non-syndromic cleft lip and palate. *J Med Genet* 2003;40(6):399–407. [PubMed: 12807959]
10. Zuccherro TM, Cooper ME, Maher BS, et al. Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate. *N Engl J Med* 2004;351(8):769–780. [PubMed: 15317890]

Table 1

LOD scores of markers and their position

Marker	Chromosome	Gen Map cM	Physical location	2 pt LOD score	Theta
D3S1259	3	36.7	11973681-12173924	-1.70	0.10
D3S3610	3	37.2	12880656-13080996	1.28	0.10
D3S2403	3	37.2	13047397-13247709	0.90	0.20
D3S3608	3	38.3	13679236-13679541	2.18	0.10
D3S2403	3	38.3	13147397-13147709	0.51	0.10
D3S2385	3	38.5	13753945-13954287		
WNT 7			13835079-13896325		
D3S3613	3	41.6	15236926-15437204		
D13S800	13	55.3	72672650-72873041	2.00	0.00
LM07			75230575-75430743		
D13S317	13	63.9	81520034-81720315	0.00	0.50
D13S1818	13	67.7	88392785-88593286	1.20	0.10