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Novel Gene Locus for Autosomal Dominant Left Ventricular Noncompaction Maps to Chromosome 11p15

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Background—Left ventricular noncompaction (LVNC) is a congenital unclassified cardiomyopathy with numerous prominent trabeculations and deep intertrabecular recesses in a hypertrophied and hypokinetic myocardium. It has been reported to occur in isolation or in association with congenital heart disease. Mutations in the X-linked *G4.5* gene are responsible for cases of isolated LVNC in male infants, but *G4.5* mutations were not found in patients with clinical onset of disease in adulthood. In addition, several families with LVNC and an autosomal dominant pattern of inheritance suggest genetic heterogeneity.

Methods and Results—We performed a genome-wide linkage analysis in a family with autosomal dominant LVNC and show that a locus containing the LVNC disease gene maps to chromosome 11p15. A peak 2-point logarithm of odds score of 5.06 was obtained with marker D11S902 at $\theta=0$. Haplotype analysis defined a critical interval of 6.4 centimorgan between D11S1794 and D11S928 corresponding to a physical distance of 6.8 megabases. No disease-causing mutation was identified in 2 prime positional candidate genes, muscle LIM protein (*MLP*) and *SOX6*.

Conclusions—We have mapped a locus for autosomal dominant LVNC to a 6.8-megabase region on human chromosome 11p15. Identification of the disease gene will allow genetic screening and provide fundamental insight into the understanding of myocardial morphogenesis. (***Circulation***. 2004;109:2720-2723.)

Key Words: cardiomyopathy ■ genetics ■ mapping ■ noncompaction

Left ventricular noncompaction (LVNC), also called noncompaction of the left ventricular myocardium, is a rare, unclassified cardiomyopathy.[1] It is characterized by numerous prominent trabeculations and deep intertrabecular recesses in hypertrophied and hypokinetic segments of the left ventricle.[2,3] An intrauterine arrest of myocardial development with lack of compaction of the loose myocardial meshwork is the assumed mechanistic basis.[4] By definition, isolated noncompaction of the left ventricular myocardium occurs in the absence of other structural heart disease.[5] Noncompaction of the left ventricular myocardium combined with congenital heart defects is referred to as nonisolated LVNC.[6]

There is evidence that LVNC is a genetically heterogeneous disorder. A point mutation in the *G4.5* gene* has been identified in a family with X-linked isolated LVNC (Online Mendelian Inheritance in Man [OMIM; Johns Hopkins University], No. 300183).[7] Mutations in the *G4.5* gene were also described to cause Barth syndrome[8] and other forms of infantile dilated cardiomyopathies,[9] suggesting allelism for these disorders. However, mutations in the *G4.5* gene were

not responsible in a large series of adult patients with isolated LVNC.[10] Recently, mutations in *ZASP*, a gene encoding for the Z-band alternatively spliced PDZ motif-containing protein, a component of the Z-line in skeletal and cardiac muscle, were described in patients with LVNC and left ventricular dysfunction.[11] A mutation in α-dystrobrevin in a family with LVNC and variable forms of complex congenital heart diseases has also been reported (OMIM, No. 606617).[6]

An autosomal dominant pattern of inheritance (OMIM, No. 604169) has been suggested in a number of families with LVNC.[6,10,12] We have previously described kindred LVNC-105[10] and now report a locus for autosomal dominant transmission of LVNC on chromosome 11p15 in this family. This genomic locus harbors the candidate genes coding for muscle LIM protein (MLP) and SOX6.

Methods

Clinical Evaluation

The study was carried out with the individuals of pedigree LVNC-105 as shown in the Figure. A repetition of all clinical and
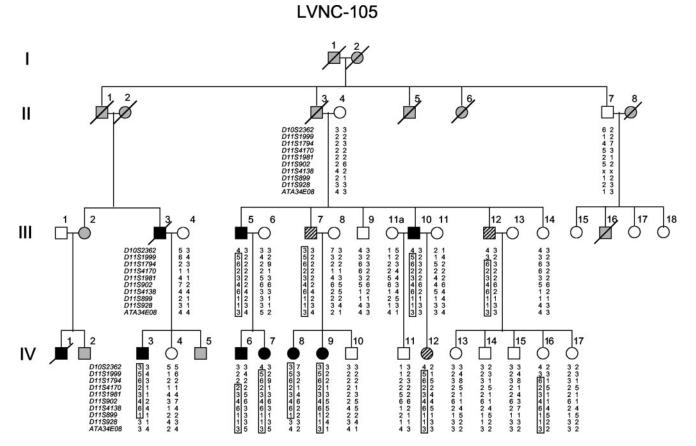
Pedigree of kindred LVNC-105 with autosomal dominant LVNC and chromosome 11p15 haplotypes. Ten microsatellite markers are presented in order from telomere (top) to centomere (bottom). Disease haplotype is boxed. Filled symbols indicate individuals with LVNC; open symbols, unaffected status; hatched symbols, individuals with a mild, partially penetrant phenotype; and shaded symbols, unknown clinical status.

echocardiographic analyses was performed at the University Hospital Zürich before the genetic analyses. Echocardiographic criteria for LVNC were as follows: The noncompacted endocardial layer is at least twice as thick as the compacted epicardial layer (ratio ≥2) and consists of prominent and excessive trabeculations and deep recesses filled with blood from the ventricular cavity as visualized by color Doppler imaging. In partially penetrant cases of LVNC, the ratio of noncompacted to compacted myocardium is <2.

Linkage Analysis

A genome-wide linkage analysis was conducted using microsatellite markers spaced at a maximum of 10 centimorgan (cM) apart according to the Marshfield Genetic Map. Two-point logarithm of odds (LOD) score calculations were performed by the LINKAGE 5.20 package, and multipoint LOD scores were calculated with the SIMWALK 2.82 program. Autosomal dominant inheritance was assumed, and LOD score calculations were performed with disease penetrance values between 0.75 and 0.90; for partially penetrant phenotypes, disease penetrance was set at 0.50.

Candidate Gene Analysis

Mutation screening of candidate genes was performed as described previously.[10] Oligonucleotide primers for polymerase chain reaction amplification of *MLP*, *SOX6*, and *ZASP* were designed according to sequences from a public database. Southern blot experiments of *MLP* exons 1 to 6 were performed according to standard protocols.

Results

Patient Characteristics

Kindred LVNC-105 has previously been described (INVM-105)[10] and was reevaluated for this study (33 subjects; 14

males, 19 females). Nine individuals were diagnosed with LVNC; 3 were found to have a partially penetrant LVNC phenotype (III-7, III-12, and IV-12); and the remaining 21 family members were classified as unaffected (see the Data Supplement for details). Reevaluation resulted in the following changes compared with the original pedigree: Individual IV-7 developed LVNC since she was last seen 3 years ago; individual III-12 was diagnosed with partially penetrant LVNC; and another parent (III-11a) was introduced. On echocardiography, 2 patients (III-7 and III-12) showed mild valvular pulmonary stenosis with mean pressure gradients of 12 and 16 mm Hg, respectively, and another 5 patients had a mild mitral valve prolapse. Patient IV-16 without LVNC had a small secundum atrial septal defect and a mild pulmonary stenosis with a mean pressure gradient of 11 mm Hg.

Linkage Results

In a genome-wide linkage analysis of LVNC in kindred LVNC-105 that used 447 highly polymorphic microsatellite markers, significant 2-point LOD scores were obtained only for markers at chromosome 11p15 (Table). The multipoint LOD score peaked at D11S902 (*Z*=5.15). LOD scores at chromosome 11p15 were robust for different values of penetrance. Haplotype analysis identified segregation of an affected haplotype in family LVNC-105 (Figure). Individual IV-16, a 14-year-old girl, carries the affected haplotype but

Two-Point LOD Scores of Chromosome 11p15 Microsatellite Markers

Marker	cM	Mb	Recombination Fraction, θ						
			0.00	0.01	0.05	0.1	0.2	0.3	0.4
D11S2362	8.9	4.9	-4.49	-3.00	-0.89	0.02	0.63	0.67	0.43
D11S1999	17.2	10.7	0.20	1.30	2.09	2.28	2.02	1.41	0.68
D11S1794	19.9	13.3	0.42	1.40	1.95	2.05	1.82	1.33	0.71
D11S4170	20.9	14.4	2.83	2.78	2.57	2.30	1.72	1.09	0.44
D11S1981	21.4	17.1	3.39	3.36	3.22	2.99	2.40	1.68	0.88
D11S902	21.4	17.5	5.06	5.00	4.73	4.34	3.41	2.31	1.10
D11S4138	21.4	17.7	3.17	3.14	2.99	2.76	2.20	1.53	0.75
D11S899	23.1	19.4	2.01	1.97	1.81	1.62	1.21	0.79	0.38
D11S928	26.3	20.1	-1.01	0.03	0.78	1.05	1.05	0.74	0.31
ATA34E08	33.0	25.9	-0.65	0.31	0.82	0.93	0.84	0.61	0.33

Microsatellite marker location (in cM) on the Marshfield sex-averaged linkage map; distances (in Mb) as shown in the Ensembl Genome Browser. Locations for multipoint calculations were adjusted according to the physical order. Disease penetrance was set at 0.90.

currently does not express the phenotype. A recombination event in affected individual IV-6 defines D11S1794 as the distal flanking marker. The proximal flanking marker, D11S928, is defined by 2 recombination events in affected individuals IV-3 and IV-8. This 6.4-cM critical interval corresponds physically to 6.8 megabases (Mb).

Haplotype analysis in 2 other kindreds (LVNC-101 and LVNC-104)[10] with autosomal dominant LVNC revealed no linkage to the 11p15 locus (data not shown), indicating that autosomal dominant LVNC is genetically heterogeneous.

Candidate Gene Analysis

Within the 6.8-Mb LVNC disease gene region, 51 putative genes are contained according to the Ensembl Human Genome Browser and 68 genes according to the National Center for Biotechnology Information (NCBI) human genome assembly. The products of most these genes are known. MLP is an important component of the cytoskeleton of cardiac and skeletal myocytes, and mutations in *MLP* cause hypertrophic or dilated cardiomyopathy.[13,14] Sequence analysis and Southern blot hybridizations of *MLP* from individuals of kindred LVNC-105 (2 affected, 1 unaffected) did not reveal any alterations. In addition, in another 38 unrelated isolated LVNC index patients, no mutations in *MLP* were detected. Another intriguing candidate gene for LVNC at chromosome 11p15 is *SOX6*, coding for a member of the SOX DNA binding protein family. *SOX6* is a candidate gene for p[100H] myopathy, heart block, and sudden neonatal death,[15] but no mutations were detected in kindred LVNC-105.

Discussion

We have demonstrated that autosomal dominant LVNC in kindred LVNC-105 is linked to chromosome 11p15. From the known or predicted genes located within the 6.8-Mb disease gene interval, *MLP* and *SOX6* were considered candidate genes for LVNC because of their cardiac expression,[15,16] the role of MLP in other myocardial disorders,[13,14] and their known biological functions in muscle development.[15,16] However, no disease-causing mutation could be detected for *MLP* or *SOX6* in kindred LVNC-105.

Like in other heart muscle disorders,[17] LVNC shows genetic heterogeneity, and mutations in *G4.5*, *ZASP*, and α-dystrobrevin have been reported.[6,7,11,18] Linkage analysis in LVNC-105 provides a novel locus for LVNC and serves as an example for the autosomal dominant form of LVNC (OMIM, No. 604169). Most patients presented with isolated LVNC, meaning the absence of any associated congenital heart anomalies. However, 2 patients with partially penetrant LVNC and mild congenital heart disease (pulmonary stenosis) were identified to carry the disease locus for autosomal dominant LVNC (III-7, III-12). These patients did not fulfill all the diagnostic criteria established for isolated LVNC because the ratio of noncompacted to compacted myocardium was <2.[2,3] However, the development of LVNC in these patients has a genetic cause and cannot be explained by the impact of a hemodynamic burden in the absence of any left ventricular inflow and outflow tract abnormalities. The autosomal dominant form of LVNC seems to comprise isolated LVNC and LVNC associated with congenital heart defects and underscores the clinical heterogeneity of this disease.

In conclusion, we have mapped a locus for autosomal dominant LVNC to chromosome 11p15 and narrowed the critical region to 6.4 cM. These findings confirm the genetic heterogeneity of this disorder. Identification of the disease-causing gene will allow genetic screening and provide fundamental insight to the understanding of myocardial morphogenesis.

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