European Journal of Human Genetics (1998) 6, 345–349

http://www.stockton-press.co.uk/ejhg

ORIGINAL PAPER

Linkage analysis in two large Italian pedigrees affected with nail patella syndrome

Salvatore Melchionda[1], Marco Seri[2], Massimo Carella[1], Maria Rosaria Piemontese[1], Xiao-xiao Zhang[2], Leopoldo Zelante[1], Giovanni Romeo[3] and Paolo Gasparini[1]

[1]*Servizio di Genetica Medica, IRCCS Ospedale Casa sollievo della Sofferenza, San Giovanni Rotondo, Foggia, Italy*
[2]*Laboratorio di Genetica Molecolare, Istituto G. Gaslini, Genova, Italy*
[3]*International Agency on Cancer Research, Lyon, France*

Nail patella syndrome (NPS) or osteo-onychodysplasia, is an autosomal dominant disorder characterised by nail dysplasia, absent or hypoplastic patellae, iliac horns and nephropathy. Previous studies have demonstrated linkage of the nail patella locus to the ABO and adenylate kinase loci on human chromosome 9q34. Recently, informative recombination events placed the NPS locus within a 1–2 cM interval within D9S60 and the *AK1* gene. We describe here linkage analysis performed in two large Italian pedigrees with 10 and 11 members affected, respectively. A set of highly informative markers have been analysed and the allele segregation in the two families confirmed the linkage to chromosome 9. The presence of three recombination events allows definition of the critical region with a centrometric boundary between markers D9S1881 and D9S1840 and a telomeric boundary between markers D9S315 and D9S290.

Keywords: nail patella syndrome; linkage analysis; 9q34

Introduction

The nail patella syndrome (NPS), or hereditary osteo-onychodysplasia, is an autosomal dominant disorder first reported by Little in 1897.[1] The disease was characterised by skeletal abnormalities as well as other mesenchymal tissues. The most common features of the syndrome are represented by nail dysplasia, absent or hypoplastic patellae, iliac horns, small head of the radius, 'cloverleaf' pigmentation at the inner margin of the irides and, in 48% of the cases, nephropathy.

The NPS was one of the first disorders in humans for which a linkage relationship was established. In particular, linkage between NPS and ABO blood group was found in 1955 by Renwick and Lawler[2] and with the adenylate Kinase 1 (*AK1*) gene in 1969 by Schleutermann *et al*.[3] However, despite the early establishment of linkage, the precise location of the *NPS* gene on chromosome 9 has only recently been reported.[4–6]

We assembled two large Italian pedigrees in which the NPS segregates in 10 and 11 affected individuals respectively. In order to define the critical region of the NPS, we performed linkage studies using a set of highly informative markers from the region spanning the NPS locus.

Correspondence: Dr Marco Seri, Laboratorio di Genetica Molecolare, Istituto G. Gaslini, Largo G. Gaslini n. 5, 16148 Genova Quarto, Italy. Tel: +39 10 5636532; Fax: +39 10 3779797; E-mail: genetica@unige.it
Received 26 August 1997; revised 9 December 1997; accepted 17 December 1997

Material and Methods

Family 1

This family is composed of 24 subjects in three generations (Figure 1a). Ten patients and six healthy relatives were analysed for linkage studies. Among the patients, the phenotype appeared quite variable. All patients shared nail dysplasia while flexion deformity of the elbow was evident in subjects II.2, II.5, II.6, II.9, III.1, III.5, and III.7. Coxa plana was diagnosed in patients II.5 and II.6 while lumbar lordosis was evident in II.5, II.6, and III.5. Hypoplastic and dislocated patellae were present in patient III.5 who also showed spondylolystesis. Iliac horns were found only in II.6 and III.5 subjects. Renal function is at the moment normal in all patients.

Family 2

In this three-generation family (Figure 1b), 11 affected and 16 unaffected individuals were available for study. A large phenotypic variability is present in the family. In individual II.27 the only sign of the disease is the presence of iliac horns revealed by a radiological examination. Individual II.22 has only dysplastic nails while her daughter III.23 presents displacement of the hypoplastic patellae, angular deformity of the legs and short stature. No abnormal renal function is present in this pedigree.

DNA extraction

Blood samples were obtained from members of the two families and DNA was extracted using standard protocols.

Genotyping

The following dinucleotide repeats on 9q33-34 were typed in the two NPS families: D9S117, D9S282, D9S1881, D9S1840, D9S1829, D9S1825, D9S1789, D9S1821, D9S112, D9S315, AK1, D9S290, D9S61. The primers were synthesised by an ABI 392 machine, labelled with FAM, TET or HEX fluorescent dies, and purified with OPC columns (ABI, Perkin-Elmer) according to the manufacturer's conditions. PCR was carried out under standard conditions. PCR product was loaded in an automated sequencer ABI mod 373A and allele size defined using the Genescan software.

Linkage analysis

Classic pairwise lod score analysis was carried out in the two pedigrees using the programs included in the LINKAGE package.[7] The analysis was performed modelling the NPS as a fully penetrant dominant disorder with an estimated disease allele frequency of 0.0001. Haplotype analysis was performed in order better to characterise the meiotic recombinants and define the critical region for the disease gene.

Results

Table 1 reports the results of the pairwise linkage analysis for the NPS locus with the chromosome 9q34 markers in the two families. The order of the markers is the result of Généthon map,[8] integrated map from the Whitehead Institute (http://www-genome.wi.mit.edu/), chromosome 9 report[6] and our data.

Combined Lod scores >3.00 at $\theta = 0.00$ were obtained with markers D9S117, D9S282, D9S1840, D9S1829, D9S1825, D9S1789, D9S112, D9S315, AK1, D9S290, D9S61. In particular, the maximum Lod scores of 7.11, 6.99, 6.10, 7.05 were obtained respectively for markers D9S1829, D9S1825, D9S1789, D9S315 in the critical region. Haplotypes constructed for the chromosome 9q markers tested in the two families are reported in Figure 1.

Three recombination events have been detected in the two families. In the first family, the presence of one recombination event in the affected individual II.2 places the telomeric boundary of the NPS locus distal to marker D9S315. In addition, a second recombination event detected in the healthy individual II.11 places the NPS locus proximal to D9S290. Thus the telomeric boundary of the NPS region is defined by markers D9S315 and D9S290. In the second family, a recombination event in the healthy individual III.22 defines the centromeric boundary between markers D9S1881 and D9S1840. Thus, the region containing the *NPS* gene includes the following markers: D9S1840, D9S1829, D9S1825, D9S1789, D9S1821, D9S112, D9S315.

Discussion

The results of the present work confirm linkage of NPS to loci in 9q34 in two large Italian families. In particular, pairwise Lod scores >3.00 at $\theta = 0.00$ were obtained with markers D9S117, D9S282, D9S1840, D9S1829, D9S1825, D9S1789, D9S112, D9S315, AK1, D9S290, D9S61. The presence of three different recombination events allows the *NPS* gene to be located between markers D9S1881 and D9S290, confirming the smallest critical region recently reported by McIntosh *et al*.[5,6] The maximum Lod scores obtained with markers D9S1829, D9S1825, D9S1789, D9S315 located in the critical region, also confirmed previous data.[5] This region spans about 3–4 cM[8] on the genomic DNA and includes a minimum of seven loci (D9S1840, D9S1829, D9S1825, D9S1789, D9S1821, D9S112, D9S315). Preliminary physical mapping in this region suggests that the interval containing the *NPS* gene is 2.5–3 Mb in size.[5]

When we also compared our data concerning the limits of the NPS interval with those reported by McIntosh *et al*[5] we obtained similar results. The marker D9S1881 defines the centromeric limit of the NPS critical region in the two works. With regard to the distal limit, McIntosh *et al*[5] positioned the NPS critical

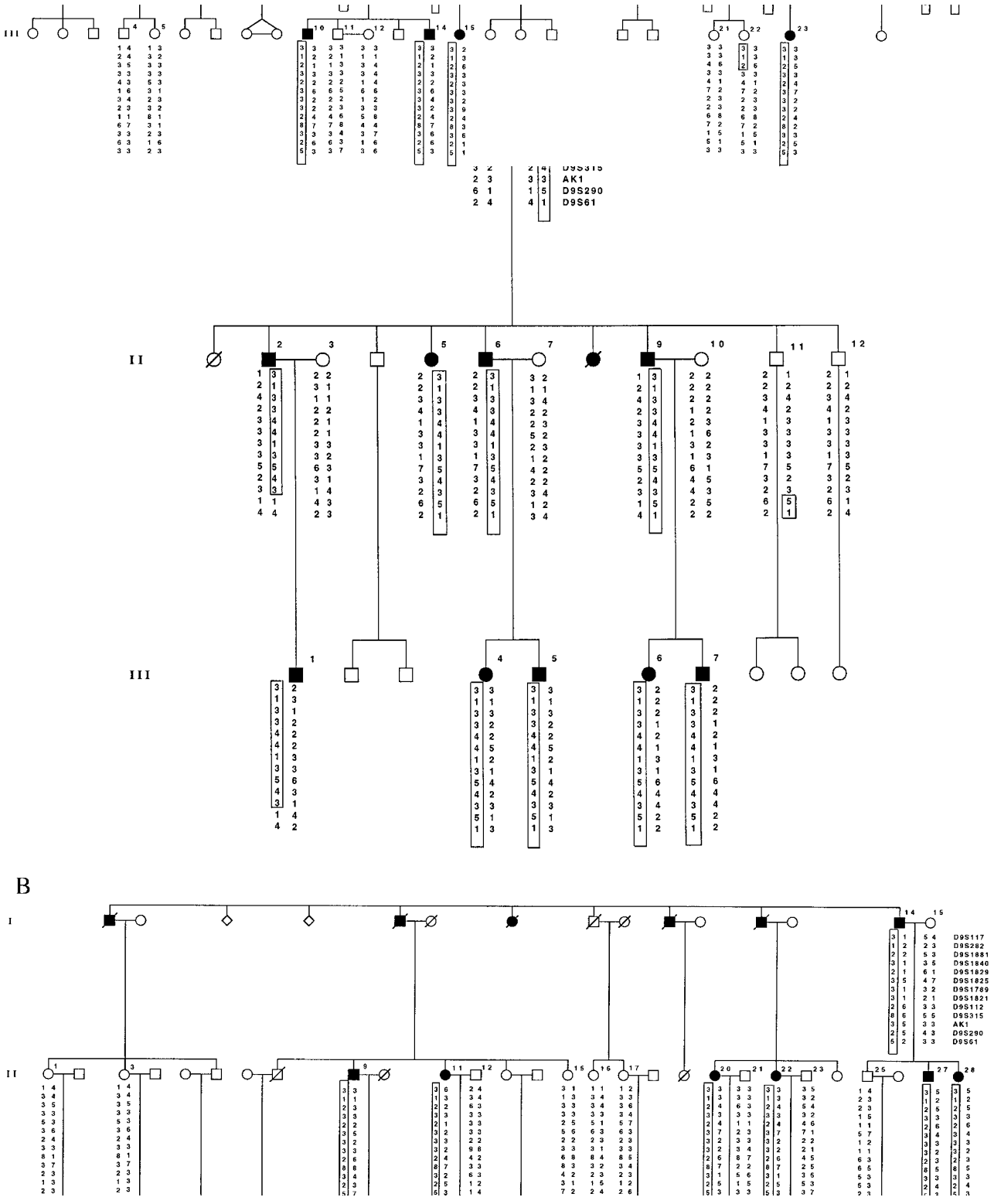

Figure 1 *Pedigrees of the two Italian NPS families studied showing the haplotypes for the chromosome 9 markers analysed. The haplotype segregating with the disease is boxed.*

Table 1 Pairwise lod scores between NPS and chromosome 9 markers

Marker and family	*0.000*	*0.010*	*0.050*	*0.100*	*0.200*	*0.300*	*0.400*	θ_{max}	Z_{max}
D9S117									
1	3.010	2.962	2.765	2.507	1.944	1.309	0.607		
2	3.152	3.096	2.863	2.559	1.906	1.192	0.455		
	6.162	6.058	5.628	5.006	3.850	2.501	1.062	0.000	6.160
D9S282									
1	3.010	2.962	2.765	2.507	1.944	1.309	0.606		
2	–∞	0.694	1.145	1.140	0.840	0.439	0.086		
	3.010	3.656	3.910	3.647	2.784	1.748	0.692	0.039	3.920
D9S1881									
1	1.204	1.173	1.049	0.889	0.562	0.257	0.054		
2	–∞	0.735	1.216	1.236	0.947	0.529	0.168		
	1.204	1.908	2.265	2.125	1.509	0.786	0.222	0.050	2.260
D9S1840									
1	3.010	2.962	2.765	2.507	1.944	1.309	0.606		
2	0.764	0.749	0.686	0.600	0.411	0.217	0.059		
	3.774	3.711	3.451	3.107	2.355	1.526	0.665	0.000	3.770
D9S1829									
1	3.010	2.962	2.765	2.506	1.944	1.309	0.606		
2	4.103	4.033	3.743	3.364	2.545	1.641	0.689		
	7.113	6.995	6.508	5.870	4.489	2.950	1.295	0.000	7.110
D9S1825									
1	3.010	2.962	2.765	2.506	1.944	1.309	0.606		
2	3.985	3.915	3.628	3.256	2.460	1.596	0.708		
	6.995	6.877	6.393	5.762	4.404	2.905	1.314	0.000	6.990
D9S1789									
1	3.010	2.962	2.765	2.506	1.944	1.309	0.606		
2	3.090	3.023	2.751	2.400	1.662	0.902	0.245		
	6.100	5.985	5.516	4.906	3.606	2.211	0.851	0.000	6.100
D9S1821									
1	0.602	0.593	0.557	0.510	0.408	0.292	0.158		
2	1.934	1.901	1.766	1.589	1.204	0.787	0.368		
	2.536	2.494	2.323	2.099	1.612	1.079	0.526	0.000	2.530
D9S112									
1	0.602	0.593	0.557	0.510	0.408	0.292	0.158		
2	4.086	4.011	3.704	3.305	2.443	1.499	0.548		
	4.688	4.604	4.261	3.815	2.851	1.791	0.706	0.000	4.680
D9S315									
1	3.010	2.962	2.765	2.506	1.944	1.309	0.606		
2	4.049	3.979	3.691	3.315	2.504	1.612	0.675		
	7.059	6.941	6.456	5.821	4.448	2.921	1.281	0.000	7.050
AKI									
1	0.602	0.593	0.557	0.510	0.408	0.292	0.158		
2	3.684	3.614	3.327	2.957	2.181	1.368	0.583		
	4.286	4.207	3.884	3.467	2.589	1.660	0.741	0.000	4.280
D9S290									
1	–∞	–1.326	–0.070	0.348	0.562	0.497	0.298		
2	3.885	3.817	3.537	3.168	2.368	1.487	0.587		
	3.885	2.491	3.467	3.516	2.930	1.984	0.885	0.078	3.540
D9S61									
1	–∞	–1.325	–0.069	0.348	0.562	0.498	0.298		
2	4.557	4.479	4.158	3.738	2.834	1.838	0.789		
	4.557	3.154	4.089	4.086	3.396	2.336	1.087	0.072	4.140

region proximal to *AK1* gene. Our data show recombination events with marker D9S290 in the first family, whilst markers D9S1821, D9S112 and AK1 are not informative. Since D9S315 is the first non-recombinant distal marker, the telomeric limit of the NPS region is defined by markers D9S315 and D9S290. D9S315 and AK1 are reported to be included in a same contig.[6] The physical order of markers D9S1821, D9S112, D9S315 and AK1 could be important to improve the characterisation of the distal limit of the NPS interval.

Many genes have been localised to 9q34[9] but have yet to be associated with specific genetic disorders. As discussed by McIntosh *et al*,[5] two of these are particularly interesting as possible candidate genes for NPS. The first, the transforming growth factor-β (TGF-β) type I receptor gene (*TGFBR1*) previously mapped in 9q34,[10] can be considered as candidate gene for its control in collagen synthesis and bone growth. The second, the pre-B cell leukemia transcription factor 3 gene (*PBX3*), which seems to interact with other transcription factors in the control of gene expression,[11] has been recently excluded as candidate gene on the basis of its physical location.[6]

Moreover, in the genomic region here defined, the search for expressed sequences at 'The Human Genome Map' site (http://www.ncbi.nlm.nih.gov/Science96/)[12] shows that at least 94 ESTs are included within this interval. These ESTs represent potential new candidates genes. They have already been collected in order to integrate the map of the critical region and to identify the *NPS* gene by the candidate gene approach. In addition, the presence in this interval of one of the genes specifically expressed in human keratinocytes[13] can represent an indication for other candidate genes.

Acknowledgements

This work was supported by the Italian Ministry of Health.

References

1 Little EM: Congenital absence or delayed development of the patella. *Lancet* 1897; **2**: 781.
2 Renwick JH, Lawler SD: Genetical linkage between the ABO and nail-patella loci. *Ann Hum Genet* 1955; **19**: 312–331.
3 Schleutermann DA, Bias WB, Murdoch JL, McKusick VA: Linkage of the loci for nail-patella syndrome and adenylate kinase. *Am J Hum Genet* 1969; **21**: 606–613.
4 Campeau E, Watkins D, Rouleau GA *et al*: Linkage analysis of the nail-patella syndrome. *Am J Hum Genet* 1995; **56**: 243–247.
5 McIntosh I, Clough MV, Schaffer AA *et al*: Fine mapping of the Nail-Patella syndrome locus at 9q34. *Am J Hum Genet* 1997; **60**: 133–142.
6 McIntosh I, Clough MV, Schaffer AA, Francomano CA, McCormick MK: Fine mapping of the Nail-Patella syndrome locus and integration of new markers into the 9q34 map. *Ann Hum Genet* 1996; **61**: 216–217.
7 Ott J: *Analysis of Human Genetic Linkage* 2nd edn. The Johns Hopkins University Press: Baltimore, 1992.
8 Dib C, Faure S, Fizames C *et al*: A comprehensive genetic map of the human genome based on 5.264 microsatellites. *Nature* 1996; **380**: 152–154.
9 Pericak-Vance MA, Bale AE, Haines JL *et al*: Report on the fourth international workshop on chromosome 9. *Ann Hum Genet* 1995; **59**: 347–365.
10 Johnson DW, Qumsiyeh M, Benkhlifa M, Marchuk DA: Assignment of human transforming growth factor b type I and type III receptor genes (*TGFRB1* and *TGFRB3*) to 9q33-34 and 1p32-p33, respectively. *Genomics* 1995; **28**: 356–357.
11 Monika K, Galili N, Nourse J, Saltman D, Cleary ML: *PBX2* and *PBX3*, new homeobox genes with extensive homology to the human proto-oncogene *PBX1*. *Mol Cell Biol* 1991; **11**: 6149–6157.
12 Schuler GD, Boguski MS, Stewart EA *et al*: A gene map of the human genome. *Science* 1996; **274**: 540–546.
13 Konishi K, Morishima Y-i, Ueda E *et al*: Cataloging of the genes expressed in human keratinocytes: analysis of 607 randomly isolated cDNA sequences. *Biochem Biophys Res Commun* 1994; **202**: 976–983.