Nail-Patella Syndrome: evidence for genetic heterogeneity

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F. Petit[1,2], F. Escande[1], A. Jourdain[1], C. Baumann[3], F. Fellmann[4], D. Lacombe[5], S. Marlin[6], S. Odent[7], N. Porchet[1,2], M. Holder-Espinasse[1,2,8], S. Manouvrier-Hanu[1,2]

[1] CHRU Lille, France
[2] Université Lille Nord de France
[3] CHU Paris, Hôpital Robert Debré, France
[4] CHU Lausanne, Suisse
[5] CHU Bordeaux, France
[6] CHU Paris, Hôpital Armand-Trousseau, France
[7] CHU Rennes, France
[8] Guy's Hospital, London, United Kingdom

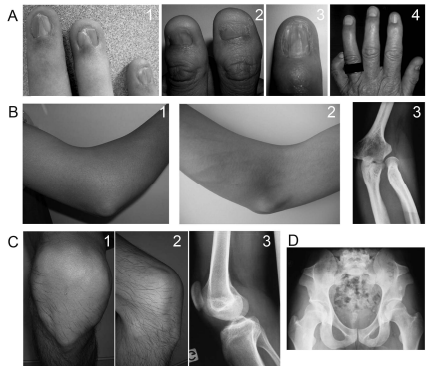
INTRODUCTION

Nail patella syndrome (NPS) is a hereditary osteo-onychodysplasia with autosomal dominant inheritance. Prevalence is estimated at 1/50 000. This affection comprises characteristic skeletal anomalies (nail dysplasia, hypoplastic or absent patella, elbow dysplasia, iliac horns. See Figure 1.) frequently associated with ocular or renal involvement. NPS results from mutations in the *LMX1B* gene, localized in 9q34 and spanning approximately 95 kb. This gene encodes a transcription factor belonging to the LIM homeodomain protein family. This protein plays a crucial role in the dorso-ventral polarisation of the limbs.

Mutations responsible for haploinsufficiency of *LMX1B* are identified in 85-90% of patients. In about 10-15% of patients, no mutation is identified in *LMX1B*. To our knowledge, the hypothesis of a genetic heterogeneity has never been studied in this disease.

We study 4 families affected with typical NPS for whom routine screening did not identify a mutation in *LMX1B* (exons and flanking introns sequencing, MLPA). We performed NGS for the whole *LMX1B* gene, revealing no mutation in coding or non-coding sequence. In one family, results provide clues for non-linkage at 9q34, confirming the likelihood of a genetic heterogeneity.

Figure 1: Typical clinical features in NPS patients: (A) Nail dysplasia: more severe dysplasia on the ulnar side, triangular lunula. (B) (1-2) Elbow dysplasia associated with imitation of the extension. (3) Elbow X-ray showing dysplastic dislocated radial head and hypoplasia of the capitellum. (C) Patellar dysplasia. (1-2) Subluxed patella on knee flexion. (3) Knee X-ray showing lateral and superior displacement of hypoplastic patella. (D) Pelvis X-ray showing typical iliac horns.

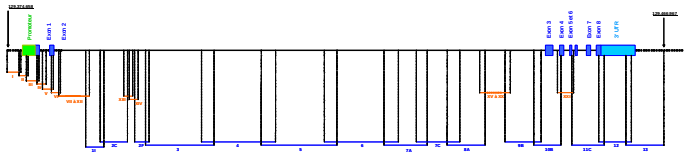


MATERIALS AND METHODS

Patients : Four families affected with typical Nail-Patella Syndrome and without *LMX1B* anomaly (exons and flanking introns Sanger sequencing, MLPA and array-CGH 44K Agilent) were recruited from the « Centre de référence des anomalies du développement et syndromes malformatifs » of Lille University Hospital.

Target sequencing: We designed 15 long-range PCR of approximately 10 kb covering almost entirely the *LMX1B* gene, for pyrosequencing using GS454 Junior (Roche®) technology (Figure 2, Amplicons 1I to 13). Bioinformatic analysis was performed by the GenSearch Software with the following parameters: minimal coverage 5X, minimal variant frequency 10%. Regions uncovered by these amplicons were Sanger sequenced (Figure 2, Amplicons I to XXI). Five affected patients (patients 1 and 2 are issued from the same family) and 3 unaffected individuals were studied.

Figure 2: Whole LMX1B coverage by next generation sequencing (Amplicons 1I to 13)and Sanger sequencing (Amplicons I to XXI).

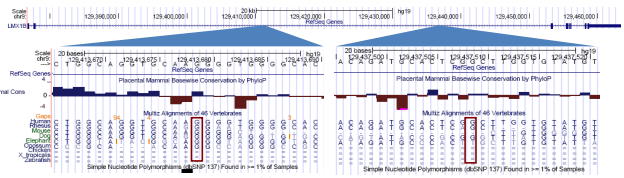


RESULTS

Number of variants identified by whole *LMX1B* sequencing in 5 NPS patients (single nucleotide variations and indels). Results after successive filtering (1/allele frequency between 20-80%; 2/variants identified in controls; 3/ dbSNP135, rs frequency>5%) and number of remaining variants validated by Sanger sequencing, segregating with the phenotype in the family and absent in 100 control chromosomes.

Patient	Number of variants	Filter 1 All. Freq. 20-80%	Filter 2 Controls	Filter 3 dbSNP135	Validated by Sanger	Absent in 100 control chromosomes
1	388	125	16	12	0	0
2	458	132	28	10	0	0
3	462	152	23	7	4	1
4	491	177	32	20	5	0
5	357	167	26	15	2	1

For two families, one single nucleotide polymorphism was remaining after the filtering. Both variants were localized in *LMX1B* intron 2, which spans ~75kb, at long-distance from the exon-intron boundaries. The wild type nucleotides seem to be conserved among species, but are not within an evolutionary conserved region.



For one family (Patients 1 and 2), SNPs segregation analysis show no linkage in *LMX1B* locus (9q34), suggesting that this gene is not directly implicated in the phenotype.

DISCUSSION

LMX1B is the major gene responsible for Nail-Patella syndrome, 85 to 90% of patients harboring a mutation responsible for haploinsufficiency. We studied 4 families affected with NPS for whom routine screening did not identify a mutation in *LMX1B*. We performed whole *LMX1B* next generation sequencing in 5 patients and found 2 intronic single nucleotide variants in 2 families. These variants lye between the large intron 2, at long-distance from the exon-intron boundaries, thus suggesting that an consequence on its splicing is unlikely. Additionally, the regions concerned are not evolutionary conserved, and no binding-site for transcriptions factors are predicted in silico. Assuming these data, these are likely to be rare non-pathogenic variants. Furthermore, segregation of SNPs in one of the families (patients 1 and 2) shows no linkage in 9q34, suggesting a genetic heterogeneity. *LMX1B* transcript expression studies and whole exome sequencing are in progress to further understand the molecular mechanisms involved in these families affected with Nail Patella Syndrome.

The authors declare no conflict of interest.