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Supporting Data

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Paroxysmal Cranial Dyskinesia and Nail-Patella Syndrome Caused by a Novel Variant in the *LMX1B* Gene

Sara Bech, MD, PhD,[1*]
Annemette Løkkegaard, MD, PhD,[1]
Troels T. Nielsen, MSc, PhD,[2] Anne Nørremølle, MSc, PhD,[3]
Sabine Grønborg, MD,[4,5] Lis Hasholt, DMSc,[3]
Gudrun K. Steffensen, MD,[6] Gabor Graehn, MD,[7]
Jess H. Olesen, MSc,[5] Niels Tommerup, DMSc,[3]
Yuan Mang, PhD,[3] Mads Bak, MSc, PhD,[5]
Jørgen E. Nielsen, MD, PhD,[2,3] Hans Eiberg, MSc,[3] and
Lena E. Hjermind, MD, PhD[2,3]

[1]*Department of Neurology, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark* [2]*Danish Dementia Research Centre, Department of Neurology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark* [3]*Department of Cellular and Molecular Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark* [4]*Department of Pediatrics, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark* [5]*Department of Clinical Genetics, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark* [6]*Department of Nephrology, Sygehus Lillebaelt, Kolding Sygehus, Kolding, Denmark* [7]*Department of Nephrology, Hospital of Southern Denmark, Sønderborg, Denmark*

ABSTRACT: Background: In a Danish family, multiple individuals in five generations present with early-onset paroxysmal cranial dyskinesia, musculoskeletal abnormalities, and kidney dysfunction.
Objective: To demonstrate linkage and to identify the underlying genetic cause of disease.
Methods: Genome-wide single-nucleotide polymorphisms analysis, Sequence-Tagged-Site marker analyses, exome sequencing, and Sanger sequencing were performed.
Results: Linkage analyses identified a candidate locus on chromosome 9. Exome sequencing revealed a novel variant in *LMX1B* present in all affected individuals, logarithm of the odds (LOD) score of $z = 6.54$, predicted to be damaging. Nail-patella syndrome (NPS) is caused by pathogenic variants in *LMX1B* encoding a transcription factor essential to cytoskeletal and kidney growth and dopaminergic and serotonergic network development. NPS is characterized by abnormal musculoskeletal features and kidney dysfunction. Movement disorders have not previously been associated with NPS.
Conclusions: Paroxysmal dyskinesia is a heretofore unrecognized feature of the NPS spectrum. The pathogenic mechanism might relate to aberrant dopaminergic circuits.

Key Words: dyskinesia; paroxysmal dyskinesia; dystonia; nail-patella syndrome

***Correspondence to:** Dr. Sara Bech, Department of Neurology, Bispebjerg Hospital, University of Copenhagen, Nielsine Nielsens Vej 7, opg. 3, 2. Floor, DK-2400 Copenhagen, Denmark;
E-mail: sbwbech@dadlnet.dk

Hans Eiberg and Lena E. Hjermind contributed equally to this work.

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Paroxysmal dyskinesia (PxD) was first described in 1940 by Mount and Reback[1] and was initially termed paroxysmal dystonic choreoathetosis. PxDs are clinically and genetically heterogeneous movement disorders characterized by recurrent attacks of abnormal movements, mainly dystonia or chorea, without loss of consciousness. They have traditionally been grouped phenomenologically according to precipitating factors into kinesigenic, nonkinesigenic, and exercise induced. With an increasing number of underlying genetic causes being identified, significant genotypic and phenotypic variability is becoming evident, hence challenging traditional classification.[2]

The nail-patella syndrome (NPS) is an autosomal dominantly inherited disorder caused by variants in *LMX1B* (NPS [MIM: 161200]), encoding the transcription factor *LMX1B*, known to be important for normal cytoskeletal and kidney development.[3,4] It has also been demonstrated that *LMX1B* is a key regulatory gene in neuronal development of, for example, serotonergic and dopaminergic neurons in the mammalian CNS,[5-8] and it plays an important role in the development of dopaminergic circuits.[9] Clinical hallmarks of this syndrome are morphological changes to nails, joints, bones, and kidney parenchyma. Renal disease, manifesting as proteinuria progressing to a form of glomerulonephritis, nephritic syndrome, or other manifestations of nephropathy, is a common feature. Neurological symptoms in the form of polyneuropathy and increased prevalence of epilepsy have been noted.[10,11] In recent years, behavioral and psychiatric symptoms in patients with *LMX1B* variants have been reported, indicating that the disorder may be more heterogeneous than previously thought.[12,13] Movement disorders have so far not been associated with NPS.

We present a large multigenerational Danish family with cranial PxD triggered by wind, touch, and water (Supporting Information Video S1) and features of NPS (Supporting Information Video S2) with several affected individuals in each generation, in which we demonstrated linkage to the 9q32-34.11 region. By exome sequencing we identified a novel missense variant within *LMX1B* that segregates with the disorder in the family.

Subjects and Methods

Subjects

Thirteen members of a five-generation Danish family were examined. Medical files and biological samples of several additional family members were collected (Fig. 1, pedigree). Informed consent was obtained from all living family members prior to diagnostic workup, including blood and tissue sampling. Parents' consent was obtained for the children.

Methods

Linkage analysis using single-nucleotide polymorphisms (SNP6) and Sequence-Tagged-Site (STS) marker analyses to identify the candidate region was performed for 23 family members. It was followed by exome sequencing of one affected individual and subsequent Sanger sequencing of the identified novel variant in all available family members. Protein expression analysis was performed in two patients. To predict pathogenicity, we analyzed the variant using Ensembl Variant Effect Predictor (VEP; https://www.ensembl.org/info/docs/tools/vep/index.html). For details, see Supporting Information in Appendix S1.

Brain magnetic resonance imaging (MRI), dopamine transporter–single-photon emission computed tomography (DaT-SPECT), and cerebrospinal fluid (CSF) analyses were performed in selected individuals. For details, see Supporting Information in Appendix S1.

Results

Clinical Features

For the clinically affected family members in five generations, the overriding debilitating symptom was PxD with onset in infancy or early childhood, manifesting as involuntary recurrent contractions around the nose and eyes, often spreading to the oropharyngeal area. It could be objectified as blepharospasms and concomitant dystonic frowning of the face (forehead, nose, mouth, and occasionally throat) precipitated by sudden sensory triggers toward the face, such as wind, moving of hair across the face, or water to the face (see Supporting Information Videos S1 and S2), rendering the affected functionally blind for the duration of the paroxysm, which could last from seconds to minutes, reoccurring several times daily. For some, symptoms were grave already in infancy, complicating breastfeeding. Many of the affected used sensory tricks to alleviate the unpleasant muscle contractions, such as pressing against both sides of the nose or under the nose. A partial effect of carbamazepine, clonazepam, and baclofen was observed in some family members. The PxDs were constant throughout life, although varying in severity over time, as well as between individuals, but greatly affecting activities of daily living. All dyskinesia-affected family members also exhibited musculoskeletal features of NPS and kidney symptoms to varying degrees.

Musculoskeletal features in variant carriers included proximal muscle hypotrophy of the arms, limitation of elbow extension, recurring shoulder dislocations, hypoplastic patellae, patella displacement or subluxation requiring surgery, and musculoskeletal pain. Affected individuals also demonstrated proteinuria, progressing to kidney disease of heretofore unknown cause in some. Several were under the care of nephrologists because of recurring or persisting proteinuria and hypertension. Individual II-7 was the first Dane to commence peritoneal dialysis as a result of kidney failure. He subsequently underwent three kidney transplants (because of complications) and died at the age of 61 years in kidney failure. Individuals IV-5, IV-6, and V-3 had NPS features, but no dyskinesia. No clinical information was available for the proband's great-grandmother (I-2). For an overview of genotypes and phenotypes in affected and nonaffected individuals, see Table 1.

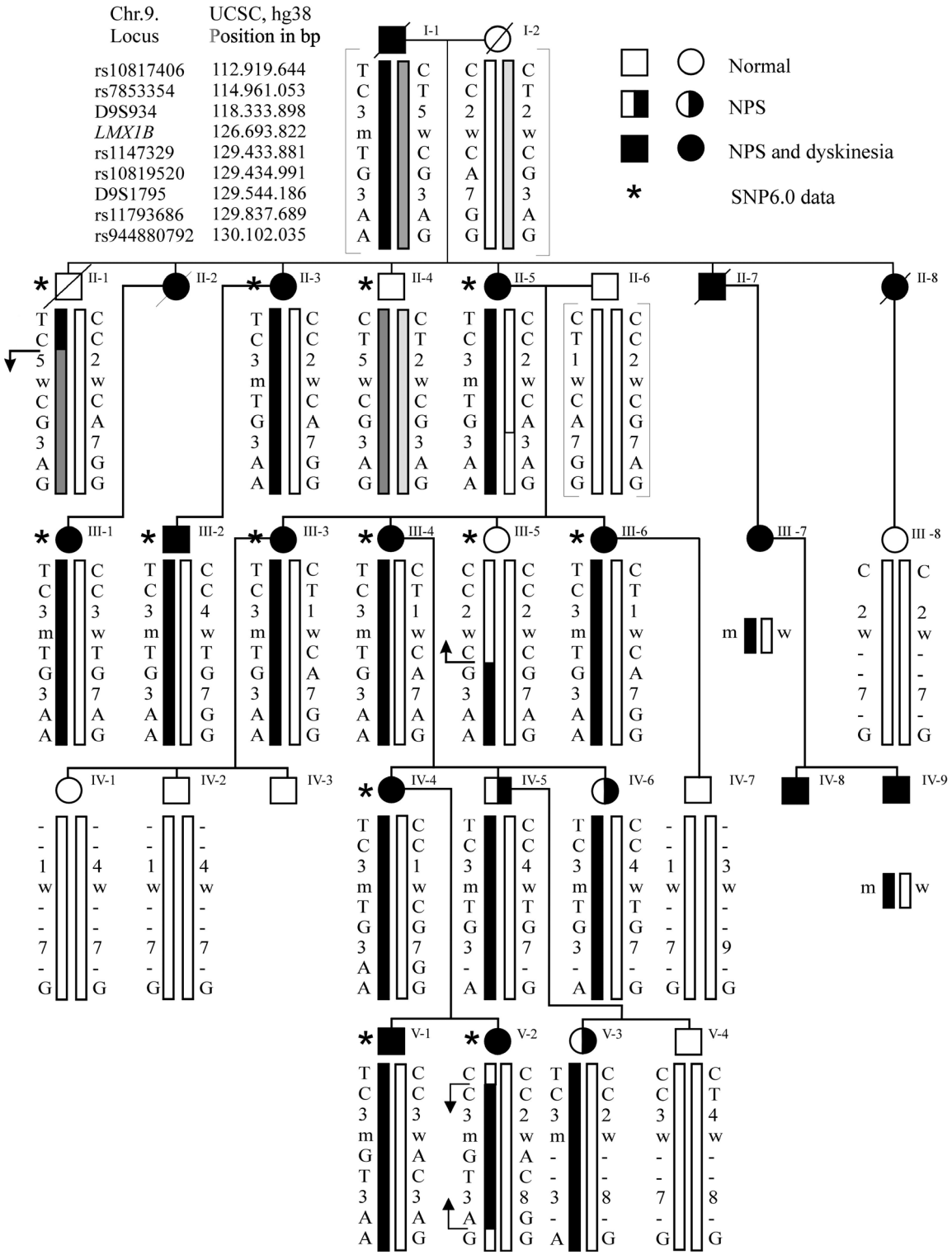

FIG. 1. Pedigree. All black indicates the presence of nail-patella syndrome (NPS) musculoskeletal features *and* paroxysmal dyskinesia (PxD); black and white indicates musculoskeletal phenotype only. Asterisks (*) indicate availability of SNP6.0 data. The haplotypes for Individuals I-1, I-2, and II-6 are inferred.

TABLE 1. Genotypes and phenotypes of affected and nonaffected individuals

Case no.	I-1	II-1	II-2	II-3	II-4	II-5[a]	II-7	II-8	III-1	III-2	III-3[a]	III-4[a]	III-5	III-6[a]	III-7[a]	III-8	IV-1	IV-2	IV-4[a,b]	IV-5[a]	IV-6[a]	IV-7	IV-8[a]	IV-9[a]	V-1[a]	V-2[a]	V-3[a]	V-4[a]
Genotype	v/w	w/w	NIA	v/w	w/w	v/w	NIA	NIA	v/w	v/w	v/w	v/w	v/w	v/w	w/w	w/w	w/w	w/w	v/w	v/w	v/w	w/w	NIA	v/w	v/w	v/w	v/w	w/w
SNP6.0 data	–	+	–	+	+	+	–	–	+	+	+	+	+	+	–	–	–	–	+	–	–	–	–	–	+	+	–	–
Paroxysmal dyskinesia	+	–	+	+	–	+	+	+	+	+	+	+	–	+	–	–	–	–	+	–	–	–	+	+	+	+	–	–
Nail-patella syndrome	+	–	+	+	–	+	+	+	+	+	+	+	–	+	–	–	–	–	+	+	+	–	+	+	+	+	+	–
Dyskinesia time of onset	Childhood	NA	Childhood	Childhood	NA	Childhood	Childhood	Childhood	Childhood	Childhood	Childhood	Childhood	NA	Childhood	NA	NA	NA	NA	Childhood	NA	NA	NA	Childhood	Childhood	Infancy	Infancy	NA	NA
Proteinuria	NA	NA	NA	+	NA	+	+	+	+	+	+	+	–	+	–	+	–	–	+	+	+	–	+	+	+	+	–	–
Kidney disease	NA	–	–	+	–	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Headache[c]	NA	+	+	+	NA	+	NA	+	+	+	+	+	+	+	–	–	–	–	+	+	–	–	–	–	–	–	–	–
Depression	NA	NA	+	+	NA	–	NA	+	+	NA	–	–	+	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–
MRI brain	–	–	–	–	–	–	–	–	–	–	N	N	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Dat-SPECT	–	–	–	–	–	–	–	–	–	–	N	N	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
CSF	–	–	–	–	–	–	–	–	–	–	Abn	Abn	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Abbreviations: –, absent; +, present; Abn, abnormal; CSF, cerebrospinal fluid; DaT-SPECT, dopamine transporter–single-photon emission computed tomography; MRI, magnetic resonance imaging; N, normal; NA, not applicable; NIA, no information available; v, variant; w, wild type.

[a]Physically examined.

[b]Proband.

[c]Headaches described as "migrainous."

Paraclinical Data

Brain MRI and DaT-SPECT for Individuals III-3 and III-4 were normal. For CSF results, see Supporting Information in Appendix S1.

Linkage and Sequencing Analysis

LOD (logarithm of the odds) score calculations using the SNP6.0 (Affymetrix) microarray data from 13 family members detected a maximum multipoint LOD score of $z = 2.1$. The candidate region was a 14.7-Mb region from 114.96 to 129.43 Mb on chromosome 9 (University of California, Santa Cruz [UCSC], hg38) flanked by the SNP loci rs7853354 and rs10819520 (Supporting Information Figures S1 and S2). All other regions in the genome were excluded by a negative LOD score of $z \leq -2$.

SNP and STS analyses in 10 additional family members and a recalculation gave a maximum LOD score of $z = 6.54$ between the disease-segregating haplotype and NPS features in the family (Fig. 1). PxD was associated with the same haplotype as NPS features, but was nonpenetrant in Individuals IV-5, IV-6, and V-3 (in Fig. 1). The LOD score between the candidate haplotype and PxD was calculated to be $z = 3.84$ for an affected-only model.

Exome sequencing of Individual III-6 revealed a C>G transition at position chr9: 126,693,572 (hg38). Sanger sequencing confirmed the presence of this novel variant in all other individuals with PxD and NPS features in the family, as well as in three individuals with the musculoskeletal features, but no dyskinesia (Fig. 1), indicating incomplete penetrance of the dyskinesia feature. The transition (CAG > GAG) results in a Q-to-E amino acid change at position 264 located within the Homeobox domain of the protein. VEP analyses using eight different tools showed the variant to be "damaging" or "deleterious" (for details of results from prediction tools, see Supporting Information in Appendix S1). The variant has not previously been reported.

Discussion

Variants in *LMX1B* have previously been established to be causative in NPS. Here, we report on the finding of a novel variant in *LMX1B*, predicted to be disease causing, that segregates with PxD in a multigenerational Danish family with musculoskeletal features characteristic of NPS. The dyskinesia manifests as brief, dystonic contractions in the cranial area triggered by sensory stimuli and as such is viewed as a paroxysmal non-kinesigenic dyskinesia (PKND). It differs from classic PKND, which usually affects limbs, is not stimulus sensitive, and is much longer in duration. This is the first time that a movement disorder has been associated with NPS. The resulting amino acid change is located within the Homeobox domain of the protein. In their 2005

genotype-phenotype study of patients with NPS, Bongers et al[14] found that those with pathogenic variants in the Homeobox domain of the gene had significantly more frequent and higher values of proteinuria compared with those with variants in the LIM domains. López-Arvizu et al[12] found no significant correlation between *LMX1B* variants and predicted protein status and the presence or degree of neuropsychiatric symptoms in their NPS patient cohort. Whether the location of this novel *LMX1B* variant predicts the phenotype in this family is unclear.

PxD is present along with NPS features in all variant carriers but three. The musculoskeletal features seen in the affected family members are all in line with the classic descriptions of NPS phenotype, varying from mild to more severe. In their clinical review of 123 British patients with NPS, Sweeney et al,[11] for the first time, described neurological features as a part of the phenotypic spectrum, including sensory disturbances and epilepsy occurring with a prevalence far surpassing what is reported in the general population. Bongers et al[14] described sensorineural hearing loss in patients with NPS. It was hypothesized that this could be explained by the known role of LMX1B in neuronal development and migration, as previously shown in mice. In this same vein, we hypothesize that the amino acid change resulting from this novel variant likely results in aberrant regulation of dopaminergic cell development, and subsequent disruption of dopaminergic function in nigrostriatal pathways ensues and is the underlying cause of PxD in this family. The lack of dyskinesia as part of the phenotype in the three individuals in the fourth and the fifth generations indicates reduced penetrance of the dyskinesia trait, most likely because of a so far unknown modifier effect. This would be consistent with the intrafamilial phenotypic variability previously described in other *LMX1B* genotypes. Further investigations are needed to understand the nature of modifier effects.

Several family members with NPS and PxD also suffer, or suffered, migrainous headaches and depression. This is noteworthy because both of these conditions have previously been reported in relation to NPS. However, they are also quite common in the general population and known to be multifactorial, and because they are also reported in nonvariant carriers in the second, third, and fourth generations, no conclusions can be drawn with regard to a potential association with the family's *LMX1B* genotype.

Conclusions

We present a large, five-generation Danish family with cranial PxD and NPS cosegregating with a novel variant in *LMX1B* where the clinical picture in affected individuals is dominated by short-lasting dystonic contractions around the nose and eyes, often spreading to the oropharyngeal area and triggered by sensory stimuli. This is the first time that a movement disorder has been associated with NPS. We conclude that PxD is a hitherto unrecognized part of the clinical spectrum of NPS. Our findings highlight the importance of looking for other, nonneurological features in patients presenting with dyskinesias because they might be part of a broader syndrome. ■

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Supporting Data

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