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Mutation of WIF1 a potential novel cause of a Nail-Patella-like disorder

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Abstract

Purpose—Nail-Patella syndrome is a dominantly inherited genetic disorder characterized by abnormalities of the nails, knees, elbows and pelvis. Nail abnormalities are the most constant feature of Nail-Patella syndrome. Pathogenic mutations in a single gene, *LMX1B*, a mesenchymal determinant of dorsal-ventral patterning, explain approximately 95% of Nail-Patella syndrome cases. 5% of cases remain unexplained.

Methods—Here we present the exome sequencing and analysis of a four generation family with a dominantly inherited Nail-Patella-like disorder, nail dysplasia with some features of Nail-Patella syndrome, who tested negative for *LMX1B* mutation.

Results—We identify a loss of function mutation in *WIF1* (NM_007191 p.W15*), involved in mesoderm segmentation, as the suspected cause of the Nail-Patella-like disorder observed in this family.

Conclusions—Mutation of *WIF1* is a potential novel cause of a Nail-Patella-like disorder. Testing in additional patients negative for *LMX1B* mutation is needed to confirm this finding and further clarify the phenotype.

Keywords

Nail-Patella syndrome; HOOD syndrome; Fong disease; Turner-Kieser syndrome; nail dysplasia

INTRODUCTION

Nail-Patella syndrome is an autosomal dominant multi-system disorder primarily affecting skeletal and connective tissues. Primary features of the syndrome include dysplasia of the

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nails, knees and elbows. Nails are typically hypoplastic, absent, or dystrophic. Patellae are often small or shaped irregularly. Elbows abnormalities include dysplasia of the radial head and hypoplasia of the lateral epicondyle and capitellum. Joints may also swell, dislocate, display hypermobility, or display early degenerative arthritis. Iliac horns are observed radiologically in ~70% of cases. Other features include renal dysfunction and, less frequently, glaucoma. Renal dysfunction is observed in ~40% of cases, with end-stage renal diseases occurring in ~5% of cases. Glaucoma is observed more frequently and at a younger age in this population. The overall presentation can vary widely from one affected individual to another individual, with various manifestations ranging from severe to totally absent on a case by case basis. Nail changes are the most constant feature of Nail-Patella syndrome, and are observed in ~98% of cases. The incidence of Nail-Patella syndrome is thought to be 1 in 50,000 [1,2].

Loss of function mutations in *LMX1B* are the only known genetic cause of Nail-Patella syndrome. *LMX1B* is a WNT signaling responsive transcription factor crucial for mesenchymal dorsoventral patterning in developing limbs [3]. WNT signaling plays a major role in a wide variety of embryonic development processes, and it is thought that the timing and restricted expression of *LMX1B*, as well as other cooperating and compensating signaling pathways, underlie both the constraints on abnormalities typically observed in Nail-Patella syndrome, as well as the heterogeneity of clinical presentation observed from patient to patient [6]. *LMX1B* mutations account for 95% of all Nail-Patella syndrome cases [2], leaving a some cases unexplained even after comprehensive *LMX1B* mutation profiling.

Here we present the exome sequencing findings of a four generation family with a nail dysplasia with some features of Nail-Patella syndrome. Prior to referral for exome sequencing, the proband of this family was worked up for Nail-Patella syndrome, however her pelvic radiographs were negative for iliac horns, her patellas are not small on X-ray, and she tested negative for *LMX1B* mutation. Although many of the classical findings for Nail-Patella syndrome were not observed, given the similarity of the nail dysplasia observed in this family to that observed in Nail-Patella syndrome and the co-occurrence of joint laxity, the case was referred for exome sequencing for a suspected Nail-Patella-like disorder or other connective tissue disorder. Exome sequencing, bioinformatic analysis, and application of standard inheritance-based, population-based, and coding impact filters resulted in the conclusion that a loss of function mutation in *WIF1*, a WNT signaling regulator involved in mesoderm segmentation [5], is the suspected novel cause of the Nail-Patella-like disorder observed in this family.

MATERIALS AND METHODS

Study Consent

Study participants provided written informed consent under a protocol approved by the institutional review board of Scripps.

Whole Exome Sequencing, Variant Calling, and Filtration

DNA was extracted from freshly drawn blood and WES was pursued utilizing Agilent SureSelect exome hybridization followed by barcoding and sequencing of paired 100bp reads on an Illumina HiSeq2500 instrument. Read mapping and variant calling and quality filtration was performed using a BWA-GATK best practices variant quality score recalibration approach. A mean coverage of 86X per individual was achieved with 98.1% of the target exome covered by >10 reads. Variant annotation was performed using the SG-ADVISER system [6]. A series of filters was applied to derive a set of candidate disease causative variants (Table 2): (1) population-based filtration, given the rarity of this condition, removed variants present at >0.2% allele frequency in the Exome Aggregation Consortium [7], 1,000 Genomes [8], NHLBI Exome Sequencing Project [9], or Scripps Wellderly populations [10]; (3) functional impact-based filtration to remove variants that are not nonsynonymous, frameshift, inframe, nonsense, or do not affect canonical splice-site donor/acceptor sites, and (4) inheritance-based filters to remove variants that do not segregate in the family in a manner consistent with autosomal dominant inheritance.

RESULTS

Phenotype

The proband is a 45 year old female presenting with dysplastic ridged nails, joint laxity, and a history of multiple fractures with minimal trauma, a torn anterior cruciate ligament, subluxations of the left shoulder, knee and temporomandibular joints, spondylolisthesis, and arthritis. Prior to referral for exome sequencing, she was evaluated for Nail-Patella syndrome with radiographs of the knees and pelvis which documented normal sized patellas and no iliac horns. Genetic testing for pathogenic deletions, duplications, and mutations in *LMX1B* was negative (Fulgent Diagnostics).

Nail dysplasia and joint laxity co-occur in a variety of disorders, all of which are associated with additional more severe findings. A search for disorders with combined nail dysplasia (human phenotype ontology identifiers - HP:0002164) and joint laxity (HP:0001388) via the Phenomizer tool [11] returns possible diagnoses that are either severe syndromic disorders (Meier-Gorlin syndrome, Costello syndrome, chromosome 2q32-q33 deletion syndrome, Weaver syndrome, Coffin-Lowry syndrome, craniofrontonasal syndrome, and focal dermal hypoplasia), or severe bone development disorders (metaphyseal chondrodysplasia, and cranioectodermal dysplasia), as well as Nail-Patella syndrome. Thus, although many of the classical findings for Nail-Patella syndrome were not observed, we concluded the phenotype observed in the proband is a nail dysplasia with some features of Nail-Patella Syndrome, what we refer to as a Nail-Patella-like disorder.

Family history includes four generations of 14 total individuals with nail dysplasia and a variety of connective tissue concerns (Table 1, Figure 1, Figure S2–S3). All affected family members belong to the maternal lineage of the proband, including the mother, grandmother, and maternal aunts, uncles, cousins, brother and niece of the proband (see pedigree in Figure 2) – two instances of male to male transmission support autosomal dominant inheritance in the maternal lineage. To identify candidate genetic variants underlying the Nail-Patella-like

disorder observed in this four-generation family, whole exome sequencing was pursued in 5 individuals of this family, the mother-father-proband trio and two cousins (the most distantly related family members available for sequencing). Follow-up genotyping of candidate variants was performed in these individuals plus an additional 5 members of this family.

Sequencing and Candidate Variant Identification

Whole exome sequencing (~86X coverage, 98.1% of target exome covered by 10+ reads) was performed on the affected proband (ID41P), unaffected father (ID41F), affected mother (ID41M) and two affected maternal cousins (ID41K4 and ID41K5) (see *Methods* for details) (Figure 1B). Variants discovered from WES were annotated by SG-ADVISER [6]. Nonsynonymous, in-frame, frameshift, nonsense, and consensus splice site variants, at a threshold population allele frequency of <0.2%, were filtered under an autosomal dominant model of inheritance. This allele frequency threshold is 100-fold above the incidence of Nail-Patella syndrome (1 in 50,000). Twelve candidate causative variants were retained after filtration (Table 2).

Follow-up genotyping, via Sanger sequencing, for the twelve candidate variants was performed in the above mentioned individuals plus three affected children (ID41K6, ID41K7, ID41K8), an affected maternal uncle (ID41K9), and an affected maternal aunt (ID41K10) of the proband (Figure 1B). This process ultimately resulted in the retention of 3 candidate variants: KRT74 p.R379L, RDH16 p.R153G, and WIF1 p.W15* (Table 2 – bolded).

DISCUSSION

Relaxed allele frequency based filtration of coding variants inherited in autosomal dominant fashion in the study family resulted in three candidate genes for the observed Nail-Patella-like disorder; *KRT74*, *RDH16*, and *WIF1.* It should be noted that the underlying assumption leading to the identification of these three candidate variants is that the disorder is driven by a coding mutation captured by exome sequencing, and that the Nail-Patella-like disorder observed in this family is rare. No assumptions regarding the specific phenotype were included in this variant prioritization approach – i.e. variants underlying other known causes of nail dysplasia or other connective tissue disorders would have been captured by our analysis approach. Given our initial assumptions, further filtration of the candidate variants by allele frequency under the assumption of complete penetrance, and prioritization based on biological relevance of the candidate genes, results in the conclusion that mutation of *WIF1* is the suspected cause of Nail-Patella-like disorder in this family.

The candidate variant in *KRT74* can likely be removed from consideration on the basis of phenotype as well as allele frequency. *KRT74* is an epithelial keratin known to cause autosomal dominant woolly hair and autosomal recessive ectodermal dysplasia [12]. The family in this report has normal hair, teeth, skin, and sweat glands. Moreover, this particular variant in *KRT74* (rs143748352) has been observed at 0.047% allele frequency in Europeans [7], which is at approximately 25-fold excess of the incidence of Nail-Patella syndrome. Thus, under the assumption of near complete penetrance, which is appropriate given the pattern of inheritance observed in this family, the variant in *KRT74* is unlikely to

be a cause of the observed Nail-Patella-like disorder. However, we cannot fully exclude the possibility that the variant contributes to a portion of the phenotype observed in this family.

The candidate variant in *RDH16* cannot definitively be eliminated from consideration due to allele frequency but its known function does not appear to fit the observed phenotype. The variant in *RDH16* (rs372580365) has been observed at 0.006% allele frequency in Europeans [7], which is at a slight (3X) excess of the incidence of Nail-Patella syndrome – an excess which would be amplified further given 95% of cases are already explained by mutation in *LMX1B* mutation (60X excess). Moreover, *RDH16* is a liver specific retinol dehydrogenase which plays no known role in connective tissue biology. Finally, *RDH16* knockout mice have no significant abnormalities [13].

On the other hand, the variant in *WIF1* is a strong candidate as the cause of a novel Nail-Patella-like disorder. The nonsense variant (WIF1 p.W15*) observed in this gene has not been previously observed in over 100,000 genomes [7]. Thus, the frequency of this variant is consistent with a fully penetrant cause of the observed disorder. Moreover, *WIF1* is a secreted antagonist of WNT signaling, predominantly expressed at the superficial layer of epiphyseal and articular cartilage, and is known to promote chondrogenesis [14]. In mouse models of arthritis, loss of *WIF1* aggravates cartilage damage [15]. The observed nonsense mutation in *WIF1* is expected to result in the complete loss of the N-terminal signal peptide of *WIF1* (amino acids 1 – 28) and thus inhibit its secretion into the extracellular matrix where it inhibits WNT factors. Thus, loss of *WIF1* function would be expected to lead to joint cartilage abnormalities. Moreover, *WIF1* functions within the same molecular signaling network as *LMX1B* – a WNT responsive transcription factor [16].

The WNT pathway controls a variety of embryonic development processes. The specificity of WNT signaling is achieved through a variety of WNT ligands, each of which are subject to highly organized temporal and spatially restricted patterns of expression to coordinate embryonic development [17]. A complex, and not fully understood, interplay between WNT and other signaling cascades is known to mediate joint development [18]. Similarly, WNT signaling is known to play a central role in nail development [19], though the precise molecular mechanisms have not been well described. It is thought that the role of *LMX1B* as both a necessary and sufficient mediator of WNT signaling to specify dorsal limb patterning, specifically through induction by the WNT ligand WNT7A, underlies the primary abnormalities observed in Nail-Patella syndrome [16]. However, *LMX1B* is also involved in a variety of other complex WNT dependent processes, where *LMX1B* function may be compensated for by other factors, potentially leading to the phenotypic heterogeneity observed across Nail-Patella syndrome cases [4]. Similarly, *WIF1* interacts with a variety of WNT ligands, including WNT7A, and thus participates in an overlapping set of WNT signaling functions as *LMX1B* [20]. However, the details of the role of *WIF1* in embryonic and other developmental processes are not well described.

Further study of additional *WIF1* positive cases would help elucidate the role of *WIF1* mutation in Nail-Patella-like disorder, and the role of WNT signaling in nail and joint development. The phenotype in this family evolves over time. The majority of affected individuals have abnormal nails at birth but do not manifest other features until they become

competitively active at adolescence. Multiple joint dislocations and arthritis are most troublesome. Two older members of the family have developed renal issues and one has glaucoma. It is unclear if these features are related to the *WIF1* mutation or are unrelated. Longitudinal follow up of family members is clearly warranted. The complete phenotypic spectrum due to loss of *WIF1* function may be clarified if more families are reported.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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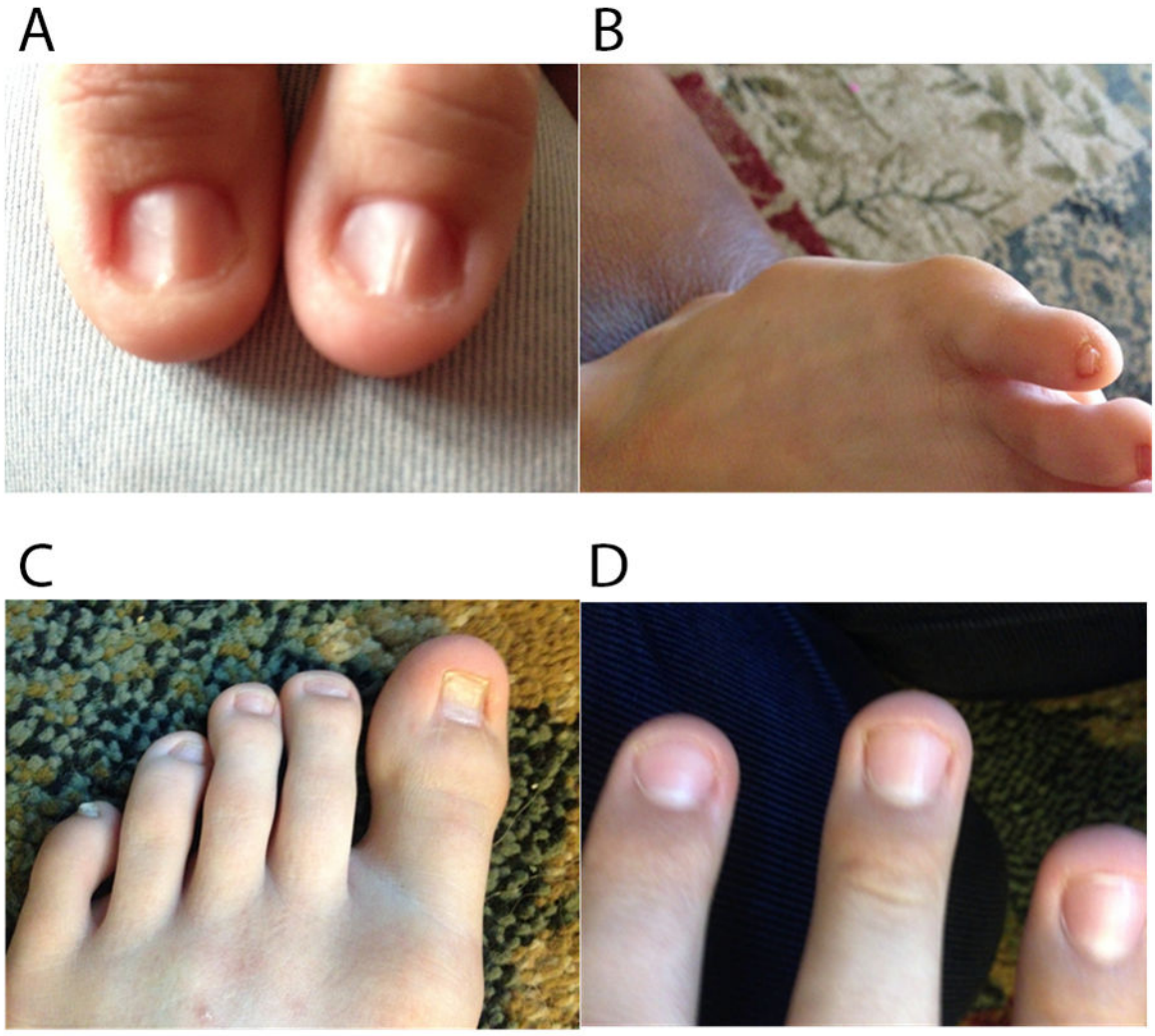

Figure 1. Images of the Proband and Children's Nail Dysplasia

A. Both thumbs of the proband (ID41P). **B.** 5th toe nail of the proband's daughter (ID41K7**).** **C–D.** Toe and finger nails of the proband's son (ID41K8). Note variable ridging, dysplasia and thin texture.

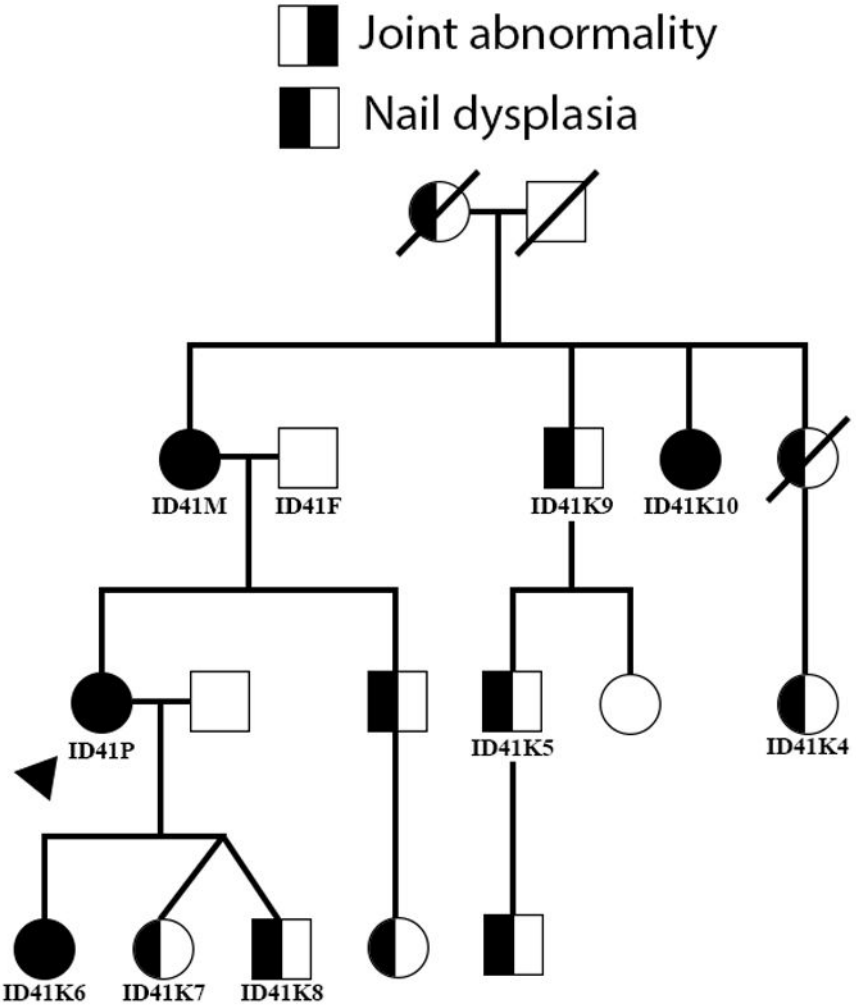

Figure 2. Family Pedigree

Pedigree of the four generation family with a dominantly inherited Nail-Patella-like disorder. The proband is indicated by the arrow. Identifiers are provided for family members that underwent genetic analysis. Corresponding identifiers can be found in tables. Not all unaffected family members are depicted. Unaffected family members not pictured include three unaffected children of the pictured brother of the proband, four unaffected children of ID41K10, and an unaffected brother of ID41K4.

Table 1

Phenotype Description

Subject	Age	Nail Dysplasia	Iliac Horns	Joint Problems	Renal Disease	Glaucoma	Other
ID41P (proband)	45	Present	Absent	lax joints, multiple subluxation, torn ACL, multiple fractures, arthritis			Irritable bowel
ID41M (mother)	73	Present	Unknown	patellar tendon rupture, 3 knee replacements, scoliosis	chronic kidney disease	Present	breast cancer, chronic cough
ID41K4 (cousin)	48	Present	Unknown				
ID41K5 (cousin)	NA	Present	Unknown				Crohn's disease
ID41K6 (daughter)	15	Present	Absent	hip dysplasia, torn ulnar collateral ligament, chronic back pain			
ID41K7 (daughter)	13	Present	Unknown	recurrent ankle sprain, microinstability at fibular fracture			
ID41K8 (son)	13	Present	Unknown	torn ankle tendon			cranial nerve palsy
ID41K9 (uncle)	NA	Present	Unknown				chronic cough
ID41K10 (aunt)	NA	Present	Unknown	multiple joint complaints, hip replacement			chronic cough
Grandmother	78	Present	Unknown	Arthritis	renal failure	Present	

All familial relations are maternal in respect to the proband.

Table 2

Inheritance of Candidate Variants

Gene	Coordinates (hg19)	Protein Impact	Allele Frequency	ID41P	ID41M	ID41F	ID41K4	ID41K5	ID41K6	ID41K7	ID41K8	ID41K9	ID41K10
DYNC2LI1	chr2:44023025 snp G>A	c.*360-1G>A	0.19%	01	01	00	01	01	00	01	00	01	01
FOXN2	chr2:48602076 snp C>T	p.L264F	<0.001%	01	01	00	01	01	00	01	00	01	01
FAT4	chr4:126370186 snp A>T	p.D2672V	0.20%	01	01	00	01	01	00	00	01	01	01
RDH16	**chr12:57348805 snp T>C**	**p.R153G**	**0.006%**	**01**	**01**	**00**	**01**	**01**	**01**	**01**	**01**	**01**	**01**
KRT74	**chr12:52962172 snp C>A**	**p.R379L**	**0.047%**	**01**	**01**	**00**	**01**	**01**	**01**	**01**	**01**	**01**	**01**
WIF1	**chr12:65514927 snp C>T**	**p.W15X**	**<0.001%**	**01**	**01**	**00**	**01**	**01**	**01**	**01**	**01**	**01**	**01**
DACT1	chr14:59113231 snp G>T	p.K630N	0.31%	01	01	00	01	01	01	01	00	00	01
EIF2AK4	chr15:40268999 ins->GACGAC	p.D736_D737dup	0.005%	01	01	00	01	01	01	01	01	01	00
PAK6	chr15:40558610 snp C>G	p.R258G	0.054%	01	01	00	01	01	01	01	00	01	00
VPS18	chr15:41191521 snp A>G	p.I169V	0.08%	01	01	00	01	01	01	01	00	01	00
ATP6V0A1	chr17:40652829 snp C>T	p.T602M	0.058%	01	01	00	01	01	00	00	00	01	01
SLC4A1	chr17:42331864 snp G>A	p.T686M	0.014%	01	01	00	01	01	00	00	00	01	01

Genotypes: 0 – reference allele, 1 – alternate allele. Variants observed in all affected family members are bolded.