Exome Sequencing Identifies *INPPL1* Mutations as a Cause of Opsismodysplasia

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Opsismodysplasia (OPS) is a severe autosomal-recessive chondrodysplasia characterized by pre- and postnatal micromelia with extremely short hands and feet. The main radiological features are severe platyspondyly, squared metacarpals, delayed skeletal ossification, and metaphyseal cupping. In order to identify mutations causing OPS, a total of 16 cases (7 terminated pregnancies and 9 postnatal cases) from 10 unrelated families were included in this study. We performed exome sequencing in three cases from three unrelated families and only one gene was found to harbor mutations in all three cases: inositol polyphosphate phosphatase-like 1 (*INPPL1*). Screening *INPPL1* in the remaining cases identified a total of 12 distinct *INPPL1* mutations in the 10 families, present at the homozygote state in 7 consanguinous families and at the compound heterozygote state in the 3 remaining families. Most mutations (6/12) resulted in premature stop codons, 2/12 were splice site, and 4/12 were missense mutations located in the catalytic domain, 5-phosphatase. INPPL1 belongs to the inositol-1,4,5-trisphosphate 5-phosphatase family, a family of signal-modulating enzymes that govern a plethora of cellular functions by regulating the levels of specific phosphoinositides. Our finding of *INPPL1* mutations in OPS, a severe spondylodysplastic dysplasia with major growth plate disorganization, supports a key and specific role of this enzyme in endochondral ossification.

Opsismodysplasia (OPS [MIM 258480]) is a rare chondrodysplasia, first described in 1977 by Zonana et al.[1] and coined as "opsismodysplasia" (from "opsismos," Greek for "late") by Maroteaux et al. in 1984.[2,3] To date, 30 cases have been reported and recurrence in sibs and/or consanguinity have suggested an autosomal-recessive mode of inheritance.[4] The disorder is characterized by pre- and postnatal micromelia with extremely short hands and feet. The main radiological features are severe platyspondyly, squared metacarpals, major delay in skeletal ossification, and metaphyseal cupping. The outcome is more variable than initially thought, ranging from severe prenatal findings to late survival.[4]

In the international nosology for skeletal dysplasias,[5] OPS belongs to the group of severe spondylodysplastic dysplasias (group 14). This group also includes (1) achondrogenesis type 1A (ACG1A [MIM 200600]) due to *TRIPP11* mutations (MIM 604505) and distinct by poor ossification of vertebral bodies and skull, (2) Schneckenbecken dysplasia (MIM 296250) due to *SLC35D1* mutations (MIM 610804) and characterized by a snail-like appearance of the ilia, (3) spondylometaphyseal dysplasia (SMD) Sedaghatian type (MIM 250220), a less severe condition, characterized by laciness of the iliac wings, and finally (4) fibrochondrogenesis (FCG [MIM 228520]), the molecular basis of which remains unknown.

In order to identify the mutations causing OPS, a total of 16 cases from 10 unrelated families were included in this study. Among them, seven were terminated pregnancies (14–29 weeks of gestation) and nine were postnatal cases (birth to 19 years). Recurrency was observed in 5/10 families and consanguinity in 7/10. Inclusion criteria were (1) major delay in epiphyseal ossification, (2) platyspondyly, (3) metaphyseal cupping, and (4) very short metacarpals and phalanges (Figure 1). The clinical details are summarized in Table 1. Histological study of the femoral growth plate performed in the three cases from family 5 and in the prenatal case from family 3 (Table 1) showed similar disorganization of the growth plate with absence of columnar arrangement of proliferative cells and reduced hypertrophic zone with small number of hypertrophic chondrocytes (Figure 1A, III and IV). In two postnatal cases (Table 1, families 3 and 7), the phosphocalcic work up was normal (including blood levels of creatinine, calcium, phosphorus, thyroxin, thyrotropin, 25-hydroxyvitaminD, 1,25-dihydroxyvitaminD, parathyroid hormone, and urinary levels of creatinine, calcium, and phosphorus).

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http://dx.doi.org/10.1016/j.ajhg.2012.11.015.

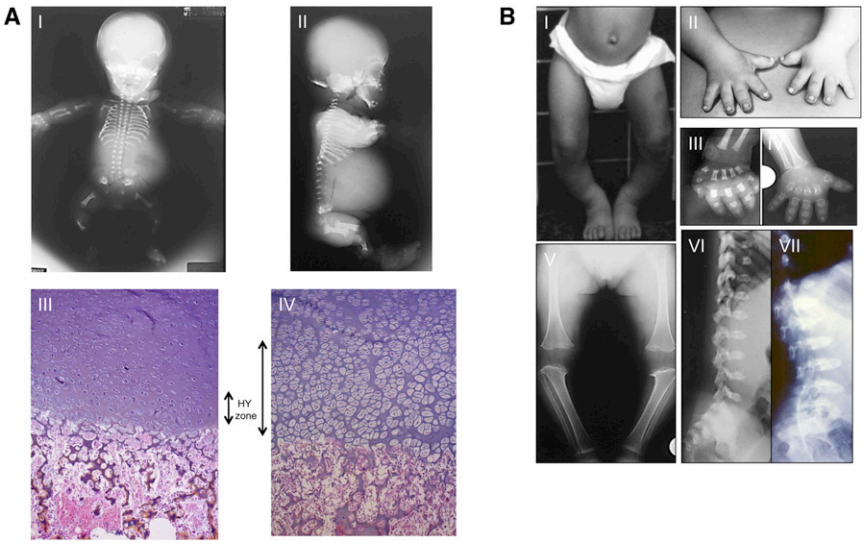

Figure 1. Clinical, Radiological, and Histological Features of OPS Cases
(A) Antenatal radiological and histological features in two OPS cases from family 5. Skeleton X-rays (I, II) in case 1 showing hypoplastic vertebral bodies, very short metacarpals, horizontal acetabular roof, and metaphyseal irregularities. Growth plate study at the femoral head in sib 2 (15 WG, III) showing nearly absent columnar organization and reduced hypertrophic zone (HY zone) with small reduced number of hypertrophic chondrocytes compared to wild-type growth plate (15 WG, IV).
(B) Clinical and radiological findings in case 1 (family 3) at 4 years (I, II, IV, V, VII) and 2 years (III, VI) showing lower limb valgus deformity (I), extremely short hands (II), short metacarpal with metaphyseal cupping and dysplastic carpal ossification (III, IV), severe epiphyseal delay and metaphyseal cupping around the knee (V), and severe platyspondyly (VI, VII).

Informed consent for participation and sample collection were obtained by protocols approved by the Necker Hospital ethics board committee. We first excluded the genes involved in the lethal spondylodysplastic group by direct sequencing, namely *SBDS* (MIM 607444) involved in some cases of spondylometaphyseal dysplasia Sedhagatian type,[6] *SLC35D1*,[7] and *TRIP11*.[8] We then decided to undertake an exome capture-sequencing in three OPS cases from families 1–3.

Exome capture was performed at the French National Sequencing Institute (CNG) with the SureSelect Human All Exon kit (Agilent Technologies).[9] Single-end sequencing was performed on an Illumina Genome Analyzer IIx (Illumina), generating 72-base reads. For sequence alignment, variant calling, and annotation, the sequences were aligned to the human genome reference sequence (hg18 build), via BWA aligner.[10] Downstream processing was carried out with the Genome Analysis Toolkit (GATK),[11] SAMtools,[12] and Picard Tools. Substitution calls were made with GATK Unified Genotyper, whereas indel calls were made with a GATK IndelGenotyperV2. All calls with a read coverage $\leq 2\times$ and a Phred-scaled SNP quality of ≤ 20 were filtered out. All the variants were annotated with an in-house developed annotation software system. We first focused our analyses on nonsynonymous variants, splice acceptor and donor site mutations, and coding indels, anticipating that synonymous variants were far less likely to be disease causing (Table S1 available online). We also defined variants as previously unidentified if they were absent from both control populations and data sets including dbSNP129, the 1000 Genomes Project, and in-house exome data.

Based on the recessive mode of inheritance of OPS, only one gene was found to harbor mutations in all three cases (Table S1) and was therefore selected. This gene, inositol polyphosphate phosphatase-like 1 (*INPPL1*), is also referred to as *SHIP2,* for SH2 (Src homology 2)-domain-containing inositol phosphatase (MIM 600829). Indeed, exome analysis detected five *INPPL1* mutations in the three individuals and was present at the homozygote state in case 1 and compound heterozygote state in cases 2 and 3. These results were confirmed by Sanger sequencing. *INPPL1* (RefSeq accession number NM_001567.3) is composed of 28 coding exons and encodes a protein of 1,258 amino acids characterized by a N-terminal SH2 domain, a conserved catalytic 5-phosphatase domain, a C-terminal proline-rich region with consensus sites for SH3 domain interactions, a ubiquitin interacting motif, and a sterile alpha motif (SAM) (Figure 2). Subsequent screening of the 28 *INPPL1* coding exons in the remaining cases led to identification of seven additional mutations. Altogether, we identified a total of 12 distinct *INPPL1* mutations in the 10 families (Figure 2, Table 2). Among them, 2/12 were nonsense mutations (c.2845C>T [p.Arg949*], c.2719C>T [p.Arg907*]) and 4/12 were frameshift mutations (c.276_280del [p.Gln93Profs*3], c.1845dupT [p.Ile616Tyrfs*14], c.94_121del [p.Glu32-Metfs*77], c.1328delinsTA [p.Thr443Ilefs*23]) located in regions encoding the SH3 binding, SH2, or 5-phosphatase domains. They were expected to result in a truncated protein with no prolin-rich and SAM domains, which are crucial for protein-protein interaction. In addition, 2/12 were splice-site mutations (c.519−3A>G, c.1951+1G>A). Alamut Splicing Predictions, via bioinformatic analysis SSF, MaxEnt, NNSPLICE, and HSF, predicted a new acceptor site in c.519−2, generating a frameshift of two additional bases followed by a premature stop codon, and the suppression of the donor site in c.1951, generating a premature stop codon. Finally, 4/12 were missense mutations (c.1975C>T [p.Pro659Ser], c.1201C>T [p.Arg401Trp], c.2164T>A [p.Phe722Ile], c.2064G>T [p.Trp688Lys]) located in the 5-phosphatase domain. These mutations cosegregated with

Table 1. Clinical Features of the Ten OPS Families

	Ethnic Origin	CS	Prenatal Findings	Parameters at Birth	Facial Features	Respiratory Insufficiency	Short Hand, Feet	Other	Outcome
Family 1, Sib 1	Arab Moslem	1st cousin	short long bones (25 WG)	W 2,700 g; L 41.5 cm; HC 32.5 cm	hypertelorism, high forehead, short nose, long philtrum, large fontanelle	–	yes	–	died at 15 months
Family 1, Sib 2	Arab Moslem	1st cousin	short long bones	?	?	–	yes	–	termination of pregnancy
Family 2 (male)	Somalian	no	?	?	coarse face, hypertelorism, high forehead, short nose, large fontanelle	lung infections	yes	–	4 years old; L, W ≤5 SD, HC P3
Family 3, Sib 1 (male)	French/Algerian	no	ureteral dilatation	W 3,400 g; L 49 cm; HC 36 cm	coarse face, high forehead, short nose, long philtrum, large fontanelle	–	yes	paraplegia after surgery for scoliosis	19 years old; H 140 cm; W 36 kg
Family 3, Sib 2 (male)	French/Algerian	no	polyhydramnios, short femora (22 WG), narrow thorax, short hands, feet	W 440 g; L 19.5 cm (22 WG)	high forehead, short nose	lung hypoplasia	yes	–	termination of pregnancy (22 WG)
Family 4 (male)	Portuguese	1st cousin	short limbs	W 3,300 g; L 45 cm; HC 38 cm	hypertelorism, frontal bossing, short nose, long philtrum, macrostomia, wide fontanelle	yes; narrow thorax	yes	cervical kyphosis, scoliosis	19 years
Family 5, Sib 1 (male)	French	no	short limbs (24 WG), short hands, narrow thorax	W 1,280 g; L 33 cm; HC 29 cm (29 WG)	coarse face, high forehead, short nose, long philtrum	pulmonary hypoplasia	yes	–	termination of pregnancy (29 WG)
Family 5, Sib 2 (female)	French	no	hygroma, short limbs (14 WG), narrow thorax	W 30 g; L 9.5 cm (15 WG)	–	–	–	–	termination of pregnancy (15 WG)
Family 5, Sib 3 (female)	French	no	hygroma, narrow thorax, short limbs	W 40 g; L 8.5 cm (14 WG)	–	–	–	–	termination of pregnancy (14 WG)
Family 6 (male)	Arab (Saudi)	1st cousin	short limbs	?	hypertelorism, midface hypoplasia, wide fontanelle, broad forehead	chronic lung disease	yes	tricuspide and mitral valve prolapse	3.5 years old
Family 7 (male)	Algerian	2nd cousin	–	W 3120 g; L 34 cm; HC 34 cm	high forehead, short nose with anteverted nares	–	yes	scoliosis, lower limb varus deformity	9 years old; H 115 cm (≤4 SD); W 24.9 kg; HC 55.5 cm
Family 8, Sib 1 (female)	French	no	short femora (24 W), short hands, narrow thorax	W 1,400 g; L 29 cm; HC 31 cm	coarse face, high forehead, short nose	pulmonary hypoplasia	yes	–	termination of pregnancy (29 WG)
Family 8, Sib2 (female)	French	no	hygroma, IUGR	L 9 cm (15 WG)	–	–	yes	–	termination of pregnancy (15 WG)

(*Continued on next page*)

Table 1. *Continued*

	Ethnic Origin	CS	Prenatal Findings	Parameters at Birth	Facial Features	Respiratory Insufficiency	Short Hand, Feet	Other	Outcome
Family 9 (male)	Brazil	1st cousin	short limbs, polyhydramnios	W 1,455 g; L 32 cm; HC 29 cm	brachycephaly, midface hypoplasia, broad forehead, short nose, long philtrum, retrognathia, low-set ears	pulmonary hypoplasia	yes	short neck, narrow thorax, prominent calcaneus	stillborn (30 WG)
Family 10, Sib 1 (male)	Brazil	7th cousin	short limbs, narrow thorax, polyhydramnios	W 2,620 g; L 40 cm; (35 WG)	brachycephaly, midface hypoplasia, broad forehead, short nose, low-set ears	yes	yes	absence right kidney (prenatal US)	died at 5 days
Family 10, Sib 2	Brazil	7th cousin	short limbs, narrow thorax, platspondyly	W 2,420 g; L 35.5 cm; HC 36 cm (36 WG)	brachycephaly, midface hypoplasia, broad forehead, short nose, low-set ears	yes	yes	–	died at 30 min

Abbreviations are as follows: CS, consanguinity; WG, weeks of gestation; L, length; W, weight; HC, head circumference; IUGR, intrauterine growth retardation; ?, unknown; –, no.
Families 3–5 have been previously published in Cormier-Daire et al.[4] and family 4 was previously published in Santos et al.[17]

Figure 2. Localization of *INPPL1* Mutations Identified in OPS Individuals

the disease, were present at the heterozygote state in the parents, were considered as pathogenic in the PolyPhen and Sift database, and were absent from alleles in 200 ethnicity-matched controls.

Here, we report *INPPL1* mutations in ten unrelated families of opsismodysplasia. All cases clearly fulfilled the diagnostic criteria for OPS but were variable in severity. Indeed, prenatal findings detected in four families led to early termination of pregnancies (especially in recurrent sibs) in 7/16 cases and hygroma, short long bones, short extremities, and narrow thorax were consistently observed. Four children died early (stillborn at 30 weeks of gestation to 15 months of age). The five remaining cases ranged in age from 3 to 19 years old and had normal cognitive development, severe short stature (<4 SDS), lower limb deformity, and severe scoliosis with atlanto axial instability (at least in one case).

Most mutations (6/12) resulted in premature stop codons, 2/12 were splice-site mutations, and 4/12 were missense mutations located in the catalytic domain, 5-phosphatase, presumably responsible for impaired catalytic activity.

INPPL1 belongs to the inositol-1,4,5-trisphosphate 5-phosphatase family, a family of signal-modulating enzymes that govern a plethora of cellular functions by regulating the levels of specific phosphoinositides. Growth factor or insulin stimulation induces a canonical cascade resulting in the transient phosphorylation of phosphatidylinositol (PtdIns) (4,5)P(2) by PI3K (phosphoinositide 3-kinase) to form PtdIns(3,4,5)P(3), which is rapidly dephosphorylated either by phosphatase and tensin homolog (PTEN) back to PtdIns(4,5)P(2) or by the inositol polyphosphate 5-phosphatases (5-ptases) generating PtdIns(3,4)P(2). Ten mammalian 5-ptases have been identified. Their gene-targeted deletion in mice has revealed that these enzymes regulate haemopoietic cell proliferation, synaptic vesicle recycling, insulin signaling, endocytosis, vesicular trafficking, and actin polymerization.[13] More specifically, INPPL1 has been implicated in the negative regulation of insulin signaling and glucose homeostasis in specific tissues.[14] SNP analysis in the Japanese population have suggested that *INPPL1* polymorphisms

Table 2. ***INPPL1*** **Mutations in Families in Opsismodyplasia**

Family	Ethnic Origin	CS	No. of Affected Children	Nucleotide Change	Status	Amino Acid Change	Location	Domain
1	Arab Moslem	yes	2	c.2845C>T/	ho	p.Arg949*	Ex25	SH3 binding domain
2	Somalian	no	1	c.276_280del	he	p.Gln93Profs*3	Ex3	SH2 domain
				c.1975C>T	he	p.Pro659Ser	Ex17	5phophatase domain
3	French/Algerian	no	2	c.1201C>T	he	p.Arg401Trp	Ex11	5phophatase domain
				c.2164T>A	he	p.Phe722Ile	Ex19	5phophatase domain
4	Portuguese	yes	1	c.2719C>T	ho	p.Arg907*	Ex24	/
5	French	yes	3	c.1845dupT	ho	p.Ile616Tyrfs*14	Ex15	5phophatase domain
6	Arab (Saudi)	yes	1	c.519-3A>G	ho	?	In4	/
7	Algerian	yes	1	c.1951+1G>A	ho	?	In16	/
8	French	no	2	c.1328delinsTA c.2064G>T	he he	p.Thr443Ilefs*23 p.Trp688Lys	Ex12 Ex18	5phophatase domain 5phophatase domain
9	Brazil	yes	1	c.94_121del	ho	p.Glu32Metfs*77	Ex1	SH2 domain
10	Brazil	yes	2	c.94_121del	ho	p.Glu32Metfs*77	Ex1	SH2 domain

Abbreviations are as follows: Cs, consanguinity; ho, homozygote; he, heterozygote; /, mutation not localized in a known protein domain.

are associated with a predisposition to insulin resistance.[15] Moreover, animal models lacking *Inppl1* had increased glucose intolerance and insulin sensitivity. However, *Inppl1*$^{-/-}$ mice were viable and had normal glucose and insulin levels but were highly resistant to weight gain, suggesting that *Inppl1* mediates obesity resistance.[16] In the four survivors from our series, no insulin resistance was reported and length and weight were both $\leq$4 SD.

Recent studies have suggested additional noncatalytic properties of INPPL1 that may act as a docking protein for a large number of proteins including cytoskeletal, focal adhesion, or scaffold proteins, phosphatases, and tyrosine kinase-associated receptors, like EGF receptor. Moreover, loss of INPPL1 in zebrafish led to an increased and expanded expression of outputs of FGF-mediated signaling.[13]

The finding of *INPPL1* mutations in OPS, a severe spondylodysplastic dysplasia with major growth plate disorganization, supports a key and specific role of this enzyme in the endochondral ossification process, through either its role in postranslational modifications (phosphorylation or ubiquitination) or its interaction with specific protein network. We conclude that *INPPL1* mutations are responsible for OPS. Ongoing studies will hopefully lead to an understanding of the specific role of this enzyme in the ossification process.

Supplemental Data

Supplemental Data include one table and can be found with this article online at http://www.cell.com/AJHG/.

Acknowledgments

We thank all families for their contribution to this work. We thank the GIS Maladies Rares for the funding of the exome project.

Received: October 12, 2012
Revised: November 12, 2012
Accepted: November 28, 2012
Published: December 27, 2012

Web Resources

The URLs for data presented herein are as follows:

Alamut, http://www.interactive-biosoftware.com/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org/
Pfam, http://pfam.sanger.ac.uk/
Picard, http://picard.sourceforge.net/
Polyphen, http://genetics.bwh.harvard.edu/pph/index.html
RefSeq, http://www.ncbi.nlm.nih.gov/RefSeq
SIFT, http://sift.bii.a-star.edu.sg/
UniProt, http://www.uniprot.org/

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