

## REVIEW

# *INPPL1* gene mutations in opsismodysplasia

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The *INPPL1* (inositol polyphosphate phosphatase-like 1) gene encodes the inositol phosphatase, SHIP2 (for src homology 2 domain-containing inositol phosphatase 2). SHIP2 functions to dephosphorylate, and negatively regulate, the lipid second messenger phosphatidylinositol (3,4,5)P<sub>3</sub>. SHIP2 has been well studied in the area of insulin resistance and obesity but has roles in cancer and other disorders. Recently, it was reported that mutations in *INPPL1* cause opsismodysplasia, a rare, autosomal recessive severe skeletal dysplasia. This review focuses on the mutations associated with opsismodysplasia and explores the role of *INPPL1*/SHIP2 in skeletal development.

*Journal of Human Genetics* (2017) 62, 135–140; doi:10.1038/jhg.2016.119; published online 6 October 2016

## INTRODUCTION

Skeletal dysplasias are a large family of disorders characterized by disturbances of cartilage and bone growth and development leading to shape and size abnormalities of the skeleton. The family is markedly heterogeneous with 456 clinically distinct disorders caused by mutations in 226 genes.<sup>1</sup> The chondrodysplasias are a subset of skeletal dysplasias and result from the abnormal growth of cartilage of the long bones, which typically manifests as disproportionate short stature. Opsismodysplasia (OPS) (OMIM 258480) is a rare skeletal chondrodysplasia primarily characterized by growth plate defects and delayed bone maturation. Clinical features at birth are variable but include short limbs, small hands and feet, macrocephaly with an anterior fontanel and facial dysmorphism including prominent brow ridge, depressed nasal bridge, anteverted nose and relatively long philtrum (<http://omim.org/entry/258480>). Typical radiographical features include short long bones with markedly delayed epiphyseal mineralization, metaphyseal cupping, short metacarpals and phalanges, and severe platyspondyly.<sup>2–17</sup> Radiographs of an individual with severe OPS are shown in Figure 1. Recurrence in the siblings and consanguinity suggested an autosomal recessive mode of inheritance. In 2013, it was reported that OPS is caused by homozygous or compound heterozygous mutations in the *INPPL1* (inositol polyphosphate phosphatase-like 1) gene encoding the SHIP2 protein (for src homology 2 domain-containing inositol phosphatase 2).<sup>4,6,7,9,17</sup> However, not all patients have *INPPL1* variants suggesting that OPS exhibits genetic heterogeneity.<sup>4,7</sup>

## INOSITOL POLYPHOSPHATE PHOSPHATASE-LIKE 1/SRC HOMOLOG 2 DOMAIN-CONTAINING INOSITOL PHOSPHATASE 2

The *INPPL1* gene is 14.3 kb in size, composed of 28 exons and located on human chromosome 11q13.4. The *INPPL1*/SHIP2 open reading frame comprises 1258 amino acids with a predicted molecular weight

of 113 kDa. In addition to a catalytic domain, SHIP2 contains several motifs known to be involved in protein–protein interactions and has been shown to function as a docking protein for a variety of intracellular molecules.<sup>18,19</sup> These include an N-terminal SRC homology 2 (SH2) domain, potential phosphotyrosine binding sites containing the consensus NPxY, a sterile alpha male domain and C-terminal proline-rich domains with potential for SH3 binding (Figure 2).

SHIP2 functions to dephosphorylate the lipid second messenger phosphatidylinositol 3,4,5-triphosphate (PI(3,4,5)P<sub>3</sub>). Phosphatidylinositol (PI) is a membrane phospholipid that can be reversibly phosphorylated at the 3, 4 and 5 positions through the action of PI kinases and PI phosphatases. SHIP2 is one of 10 mammalian inositol polyphosphate 5-phosphatase that dephosphorylates PI(3,4,5)P<sub>3</sub> at the 5'-position of the inositol ring to PI(3,4)P<sub>2</sub>.<sup>20,21</sup>

SHIP2 is prominent in a range of human diseases with most research focused on diabetes and cancer (reviewed by Suwa *et al.*<sup>22</sup>). Single-nucleotide polymorphisms in *INPPL1* gene are associated with susceptibility to type 2 diabetes and metabolic syndrome in Japanese, Chinese and European populations.<sup>23–27</sup> *In vitro* studies suggested that several of these variants increase SHIP2 levels<sup>24,26</sup> or enhance Akt pathway activation,<sup>27</sup> the major downstream target of SHIP2. The role of SHIP2 in diabetes is an active area of research because of the possibility for developing small-molecule inhibitors that modulate SHIP expression or activity for the treatment of diabetes.<sup>27–30</sup>

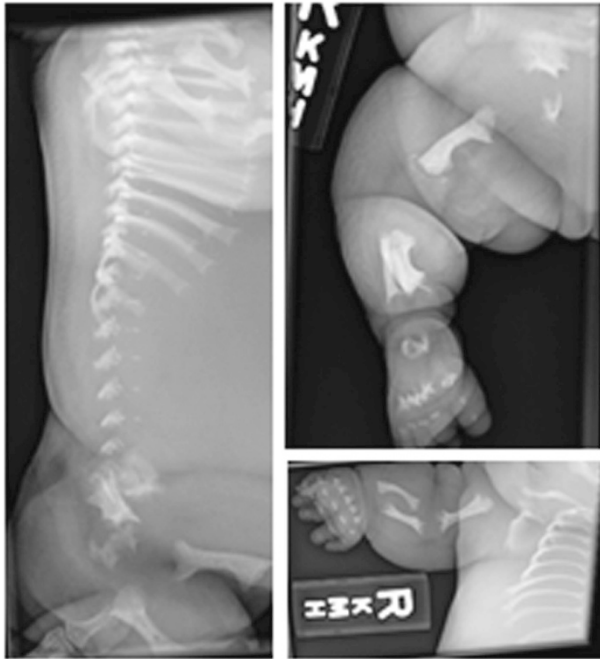
For many cancers, increased SHIP2 expression is associated with cancer progression and is being explored as a clinical biomarker for these cancers.<sup>31–35</sup> However, SHIP2 may not be a universal marker for cancer as in gastric cancer the reverse occurs where suppression of SHIP2 is associated with tumorigenesis.<sup>36</sup> On the basis of these studies, efforts are also underway to develop anticancer drugs with the goal of pharmacological inhibition of SHIP2 activity.<sup>37</sup>

Clearly, SHIP2 has multiple, and sometimes opposite, roles in the growth and function of cells depending on cell type and pathology. In

the majority of these conditions, the pathology is associated with enhanced SHIP2 expression. The remainder of this review will focus on the emerging role of SHIP2 mutations in skeletal development, which appears to be associated with the loss of SHIP2 function.

### MUTATIONS IN INPPL1

Overall, 25 mutations spread throughout the molecule have been identified in 20 families; with 3 nonsense mutations, 1 in-frame mutation, 7 missense mutations, 9 frameshift mutations and 5 splice

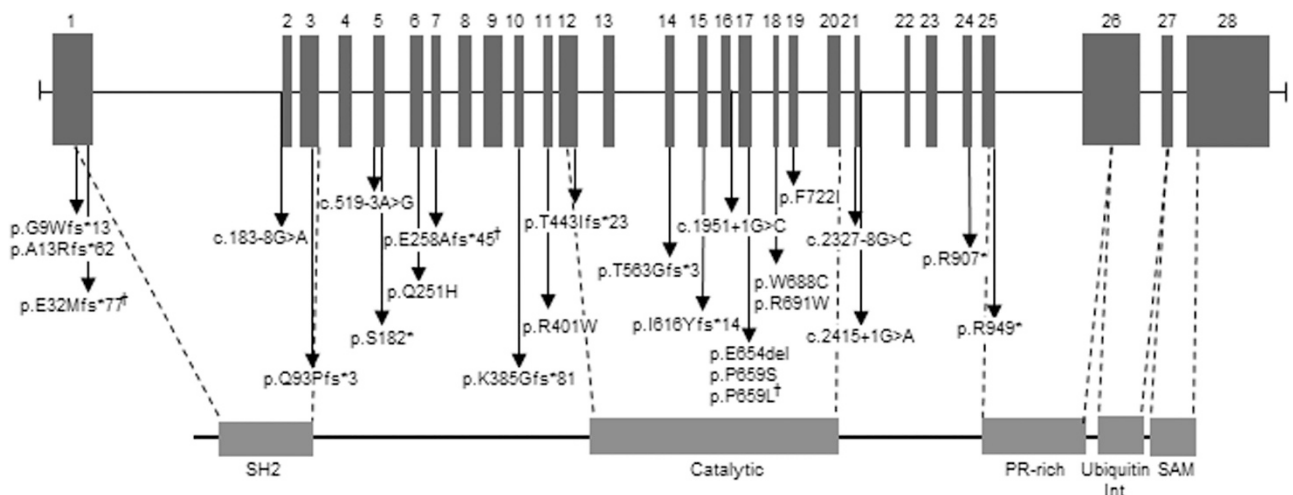


**Figure 1** Radiographs of a severely affected opsimodysplasia fetus at 31 weeks gestation.<sup>56</sup> Left, lateral view of spine showing severe platyspondyly. Top right, lateral view of right leg showing bowed femur and flared metaphyses. Lower right, anteroposterior view of right arm showing undermineralized bones of the hand with shortened metacarpals and phalanges, and bowing of the humerus, radius and ulna.

site mutations reported (Table 1).<sup>4,6,7,9,16,17</sup> The SHIP2 domain structure and location of mutations are shown in Figure 2.

The nonsense and frameshift mutations lead to premature stop codons and are expected to result into a truncated protein lacking the proline-rich domain and the sterile alpha male domain and, for most of them, the truncated protein is expected to also lack the catalytic domain. To minimize the potentially deleterious effects of truncated protein exerting a dominant-negative effect on cells and tissues, the vast majority of premature stop codons undergo a process called 'nonsense-mediated mRNA decay'.<sup>38</sup> This is a cell surveillance system that serves to specifically degrade mRNA for the mutant allele containing the premature stop codon leaving the non-mutated allele intact. With the OPS mutations, premature stop codons on both alleles would result in no production of SHIP2 protein. One individual had an in-frame mutation that resulted in the deletion of glutamine at amino acid 654 in the catalytic domain that is predicted to be deleterious for the protein by the PROVEAN algorithm.<sup>39</sup> This glutamine residue is conserved between other inositol-5-phosphatase family members. Seven missense mutations have been identified and all are pathogenic according to Sift and PolyPhen pathogenicity algorithms.<sup>40,41</sup> Significantly, the proline 659, the tryptophan 688 and the arginine 691 are highly conserved among the inositol-5-phosphatase family members highlighting the notion that their substitution has a deleterious effect on protein function.

All patients reported in the literature have mutations in the homozygous state or are compound heterozygous suggesting that SHIP2 needs to be disabled for complete penetrance of the phenotype to occur. No parents of affected individuals have features of OPS suggesting that 50% levels of wild-type SHIP2 is sufficient to protect from the effects of the mutant allele. The majority of mutations (17/25) either create a stop codon directly or, for the frameshift and splice-site mutations, are predicted to result in a downstream stop codon. These are expected to result in a null for the affected allele by nonsense-mediated mRNA decay. However, even if these mutant transcripts escaped nonsense-mediated mRNA decay the resulting truncated protein would likely not have significant function. Six out of seven missense mutations are located in the catalytic domains and presumably inactivate the phosphatase function of SHIP2.



**Figure 2** Genomic structure of inositol polyphosphate phosphatase-like 1 and location of mutations involved in opsimodysplasia. 1 mark indicates recurrent mutation.

**Table 1** *Inpp1* mutations and outcome in patients with opsismodysplasia

Mutation type	Status	Nucleotide change	AA change	Location	Domain	Consequence	N	Recurrence	CS	Outcome	References
Frameshift Missense	He	c.24-39 del c.753 G>C	p.Gly9Trp p.Gln251His	Exon 1 Exon 6	–	Stop codon –	2	1 family	No	Alive	4
Frameshift Frameshift	He	c.35dup c.1687-1691 del	p.Ala13Arg p.Thr563Gly	Exon 1 Exon 14	– Catalytic domain	Stop codon Stop codon	1	1 family	No	Death (newborn)	4
Frameshift	Ho	c.94-121 del	p.Glu32Met	Exon 1	SH2 domain	Stop codon	3	2 families	Yes	Death (stillborn; newborns)	6
Splice site	Ho	c.183-8G>A		Intron 1		New acceptor site, in frame	1	1 family	Yes	Alive	9
Frameshift	He	c.276-280 del	p.Gln93Pro	Exon 3	SH2 domain	Stop codon	1	1 family	No	Alive	6
Missense		c.1975 C>T	p.Pro659Ser	Exon 17	Catalytic domain	–					
Splice site	Ho	c.519-3A>G		Intron 4		New acceptor site, stop codon	1	1 family	Yes	Alive	6
Nonsense	Ho	c.545 C>A	p.Ser182	Exon 5	–	Stop codon	1	1 family	Yes	Alive	4
Frameshift	Ho	c.768-769 del	p.Glu258Ala	Exon 7	–	Stop codon	1	1 family	No	Alive	4
Frameshift	He	c.768-769 del	p.Glu258Ala	Exon 7	–	Stop codon	1	1 family	No	Alive	4
Splice site		2415+1G>A		Intron 21	–	Suppression donor site, stop codon					
Frameshift	He	c.1150-1151 del	p.Lys385Gly	Exon 10	–	Stop codon	2	1 family	No	Termination of pregnancy (17 weeks); death (stillborn)	17
Splice site		c.2327-1G>C		Intron 20		Skipping exon 21, stop codon					
Missense	He	c.1201 C>T	p.Arg401Trp	Exon 11	Catalytic domain	–	2	1 family	No	Alive; termination of pregnancy (22 weeks)	6
Missense		c.2164 T>A	p.Phe722Ile	Exon 19	Catalytic domain	–					
Frameshift	He	c.1328 delins TA	p.Thr443Ile	Exon 12	Catalytic domain	Stop codon	2	1 family	No	Termination of pregnancy (15 weeks; 29 weeks)	6
Missense		c.2064 G>T	p.Trp688Cys	Exon 18	Catalytic domain	–					
Frameshift	Ho	c.1845 dupT	p.Ile616Tyr	Exon 15	Catalytic domain	Stop codon	3	1 family	Yes	Termination of pregnancy (14, 15 and 29 weeks)	6
Splice site	Ho	c.1951+1G>C		Intron 16		Suppression donor site, stop codon	1	1 family	Yes	Alive	6
In frame	Ho	c.1960-1962 del	p.del Glu	Exon 17	Catalytic domain	–	1	1 family	No	Alive	7
Missense	Ho	c.1976 C>T	p.Pro659Leu	Exon 17	Catalytic domain	–	4	2 families	Yes/?	Alive	4,16
Missense	Ho	c.2071 C>T	p.Arg691Trp	Exon 18	Catalytic domain	–	1	1 family	Yes	Termination of pregnancy	4
Nonsense	Ho	c.2719C>T	p.Arg907	Exon 24	–	Stop codon	1	1 family	Yes	Alive	6
Nonsense	Ho	c.2845 C>T	p.Arg949	Exon 25	SH3 domain	Stop codon	2	1 family	Yes	Termination of pregnancy; death (15 months)	6

Abbreviations: AA, amino acid; CS, consanguinity; He, heterozygous; Ho, homozygous; *Inpp1*, inositol polyphosphate phosphatase-like 1; N, number of patients affected.

There does not appear to be a clear genotype–phenotype correlation at the present time and additional mutations need to be described to identify a correlation, if one exists.

Of all the surviving subjects described in the literature, the oldest is now 24 years old.<sup>42</sup> This individual is compound heterozygous for a frameshift and the single-missense mutation (p.Gln251His) located outside the catalytic domain. It would be interesting to establish the level of SHIP2 catalytic activity in this patient. Indeed, even a small amount of enzyme function may be enough to ameliorate the severity

of the phenotype. It is also possible that other genetic factors modify severity of the phenotype in this individual.

Due to the extreme demineralization and renal phosphate wasting seen in one family with two severely affected OPS children carrying the same homozygous missense mutation in exon 17 of *INPPL1* (p. Pro659Leu), bisphosphonate therapy was used to improve bone mineral density.<sup>16</sup> Both children responded well to treatment with the more severely affected child markedly improving lumbar and hip Z-score. After 7 years of pamidronate treatment this child is now 10

years of age and is no longer wheelchair-bound suggesting that bisphosphonates may be useful for OPS patients with severe undermineralization.

It is unknown why some OPS patients have phosphate wasting but it is worth noting that mutations in another member of the inositol polyphosphate 5-phosphatase family, *INPP5F*, have been involved in the oculocerebrorenal syndrome of Lowe that is characterized by phosphate wasting.<sup>43</sup>

Recently, a homozygous *INPPL1* variant was described in Schneckenbecken dysplasia (SD), a severe, autosomal recessive, perinatal lethal dysplasia.<sup>44</sup> SD presents some features of OPS notably very-short long bones and platyspondyly, but is distinguished from other severe skeletal dysplasias by the presence on radiographs of a medial projection from the ilia that resembles the shape of a snail. SD demonstrates locus heterogeneity and had previously been shown to be caused by mutations in *SLC35D1*, encoding an ER-resident sugar transporter essential for glycosaminoglycans synthesis.<sup>45,46</sup> However no evidence suggests that SHIP2 could be involved in this process and the identification of *INPPL1* in additional SD families is needed to confirm a role for SHIP2 in SD.

#### MECHANISM OF ACTION IN SKELETAL DEVELOPMENT

Single-nucleotide polymorphisms in SHIP2 are associated with insulin resistance and the development of type 2 diabetes, and there is an emerging role for SHIP2 in cancer where elevated SHIP2 levels are associated with poor prognosis in certain cancers (for review see Suwa *et al.*<sup>22</sup>). The majority of these single-nucleotide polymorphisms when tested functionally appear to increase SHIP2 mRNA or protein expression, or activation of Akt, a critical downstream effector. On the other hand, skeletal disease seems to result from the loss of SHIP2 activity due to catalytic domain mutations or no SHIP2 protein due to premature stop codons. Indeed, several *INPPL1* mutations are located within the catalytic domain suggesting that the phosphatase activity of SHIP2 is critical for normal chondrogenesis further suggesting that knocking out the catalytic function is sufficient to cause OPS. This is consistent with what is known about PI signaling pathways. SHIP2 dephosphorylates lipid second messenger PI (3,4,5), also known as PIP3, to PIP2. PIP3 is known to participate in bone formation via its role in Akt signaling where it is able to recruit PDK1, which in turn activates Akt.<sup>47</sup> Akt and its downstream effectors have critical roles in endochondral ossification; *Akt1* knockout mice are smaller and show delayed secondary ossification<sup>48</sup> and double *Akt1/Akt2* knockout mice display severe dwarfism and delayed bone ossification among other defects.<sup>49</sup> Inactive or absent SHIP2 would serve to increase PIP3 levels and promote signaling through Akt and other downstream effectors suggesting the downregulation of the PIP3-Akt1 pathway is not the cause of the skeletal pathology characteristic of OPS.

Histological studies on bone from affected OPS individuals illustrate the negative effects of SHIP2 mutations on developing cartilage revealing a disorganized growth plate with a lack of columnar organization in the proliferative zone and a reduced hypertrophic zone with fewer hypertrophic chondrocytes.<sup>5,6,12</sup> However, knockout mice studies provide little in the way of new insights. *Inpp1l* is expressed at embryonic day E14.5 in the developing limb. Mice deficient for *Inpp1l* have multiple developmental defects including a resistance to dietary obesity and subtle skeletal phenotype. *Inpp1l*<sup>-/-</sup> mice have diminished growth and craniofacial abnormalities, however, these are mild in severity compared with humans with null SHIP2 mutations. Both sexes showed diminished longitudinal growth compared with wild-type littermates although this was more

pronounced in males compared with females. The only skeletal abnormality detected by imaging was a shortened facial profile. No mineralization defects were apparent and the mice developed to adulthood.<sup>50</sup> It is not clear why the lack of *Inpp1l/Ship2* does not reflect the dramatic mineralization defect seen in humans and result in a milder phenotype overall. However, several mouse models show a disorganized growth plate including the conditional *Piga* knockout mouse. *Piga* encodes the catalytic subunit of an essential enzyme in the glycosylphosphatidylinositol anchor pathway. Mice lacking *Piga* expression in hindlimbs present with a disorganization of the growth plate and a delay in ossification due to the defect of cell polarity at embryonic day E14.5.<sup>51</sup> Interestingly, several studies suggest that SHIP2 is involved in cell polarity.<sup>52,53</sup> Production of PI (3,4)P2 at cell-extracellular matrix contacts of epithelial cells by SHIP2 is necessary for the recruitment of Dlg1, master regulator of basolateral polarity.<sup>52</sup> Also SHIP2 interacts directly with RhoA, one of the major regulators of front-rear polarity in glioma cells.<sup>53</sup> One hypothesis is that loss of SHIP2 dysregulates cell position and organization of growth plate chondrocytes leading to undermineralization.

Several studies suggest a role for SHIP2 in fibroblast growth factor (FGF) signaling pathways. Morpholino knockdown of maternal *Ship2a* in zebrafish leads to dorso-ventral patterning defects in the early embryo due to expanded expression of FGF-mediated outputs including FGF-dependent gene expression and MAPK signaling.<sup>54</sup> The authors conclude that modulation of FGF signaling may be a principal function of SHIP2 in mammals. In humans, elevated FGF signaling via gain-of-function mutations in FGFR3 leads to achondrodysplasia (most common form of dwarfism), hypochondroplasia and thanatophoric dysplasia. Disturbances in FGFR3 signaling disrupt the MAPK ERK and p38 pathways, which are important for regulating post-embryonic skeletal development (for review see Narayana and Horton<sup>55</sup>). These data suggest a role for SHIP2 in FGF signaling, which opens up the exciting possibility of utilizing therapies used in the treatment of FGFR3-related disorders for OPS. For example, C-type natriuretic peptide, which antagonizes the MAP kinase pathway downstream of FGFR3 analogues, has been used to treat achondroplasia.

#### FUTURE RESEARCH DIRECTIONS

The finding that SHIP2, an inositol phosphatase previously known to be involved in metabolic processes, is a major regulator of extracellular matrix mineralization was an unexpected observation and one that is poorly understood. Future efforts should be directed toward investigating an early growth plate phenotype in SHIP2 knockout mice with attention paid to early growth plate organization and cell polarity, and subsequent mineralization. In cell culture systems using chondrocyte- and osteoblast-related cells lines, it should be possible to define the mechanism of SHIP2 action in skeletally relevant setting. For example, specific SHIP2 inhibitors currently in clinical trials for the metabolic effects of SHIP2 perturbations could be used to define the downstream consequences of SHIP2 deficiency. This could be complemented using CRISPR-Cas9 gene editing technologies to specifically delete or modify the *INPPL1* gene. The CRISPR-Cas9 method is well suited for investigating single-gene defects because of the relative ease and speed in generating gene variants.

The ultimate goal is to understand mechanisms that are responsible for the mineralization defect and to translate these insights into new treatments for OPS and potentially other conditions of undermineralization.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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