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Review

SHP2 sails from physiology to pathology

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ABSTRACT

Over the two past decades, mutations of the *PTPN11* gene, encoding the ubiquitous protein tyrosine phosphatase SHP2 (SH2 domain-containing tyrosine phosphatase 2), have been identified as the causal factor of several developmental diseases (Noonan syndrome (NS), Noonan syndrome with multiple lentigines (NS-ML), and metachondromatosis), and malignancies (juvenile myelomonocytic leukemia). SHP2 plays essential physiological functions in organism development and homeostasis maintenance by regulating fundamental intracellular signaling pathways in response to a wide range of growth factors and hormones, notably the pleiotropic Ras/Mitogen-Activated Protein Kinase (MAPK) and the Phosphoinositide-3 Kinase (PI3K)/AKT cascades. Analysis of the biochemical impacts of *PTPN11* mutations first identified both loss-of-function and gain-of-function mutations, as well as more subtle defects, highlighting the major pathophysiological consequences of SHP2 dysregulation. Then, functional genetic studies provided insights into the molecular dysregulations that link SHP2 mutants to the development of specific traits of the diseases, paving the way for the design of specific therapies for affected patients. In this review, we first provide an overview of SHP2's structure and regulation, then describe its molecular roles, notably its functions in modulating the Ras/MAPK and PI3K/AKT signaling pathways, and its physiological roles in organism development and homeostasis. In the second part, we describe the different *PTPN11* mutation-associated pathologies and their clinical manifestations, with particular focus on the biochemical and signaling outcomes of NS and NS-ML-associated mutations, and on the recent advances regarding the pathophysiology of these diseases.

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1. SHP2, a protein tyrosine phosphatase with pleiotropic functions

1.1. Structure, activity and regulation

The SH2 domain-containing tyrosine phosphatase 2 (SHP2) is a ubiquitous protein tyrosine phosphatase (PTP) with conserved structure and function from worms to mammals. With its close relative SHP1, it forms the family of SH2-domain containing, non receptor PTPs, which display the particular feature of being recruited by phosphotyrosines while dephosphorylating other phosphotyrosines. SHP2/Shp2 proteins and their orthologs in non vertebrates (Corskrew/CSW in flies and PTP-2 in worms) are encoded by the *PTPN11/Ptpn11* genes in Mammals, *ptpn11a/b* in

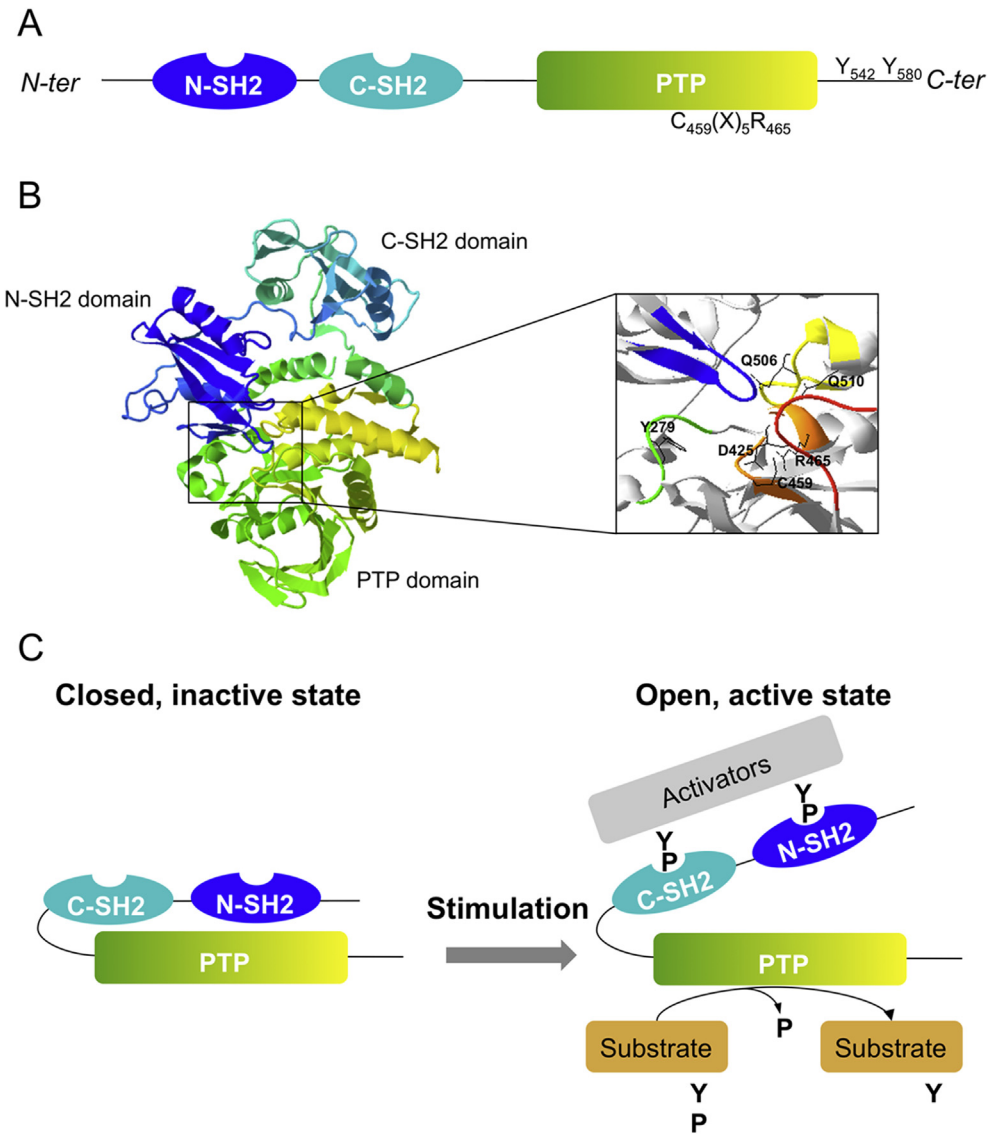

Fig. 1. SHP2 structure and function. A – Structural organization of SHP2, showing its SH2 domains, its PTP catalytical domain and its regulatory phosphotyrosine residues. B – 3D structure of SHP2, highlighting the different domains and loops involved in SHP2 activity and regulation (adapted from www.rcsb.org/pdb, PDBID: 4DGP, (Yu et al., 2013b)). Magnification shows the different loops walling the catalytical domain (D'E loop in blue, pY loop in green, WPD loop in red, Q loop in yellow, P loop in orange). Lateral chains of key residues are depicted. Please note that orientation has been slightly changed to make all residues visible. C – Mechanism of SHP2 activation: in the resting state, inhibitory intramolecular interactions between the N-SH2 and the PTP domains keep SHP2 in a closed, inactive state. Upon stimulation with growth factors/hormones, SH2 domains bind to tyrosine phosphorylated upstream activators, thereby releasing inhibitory constraints. SHP2 is then in an open, active conformation and can dephosphorylate its substrates.

D. rerio, *Csw* in *D. melanogaster* and *ptp-2* in *C. elegans*. The human gene is divided into 16 exons and produces a broadly expressed 7 kb transcript that contains a 1.779 bp open reading frame encoding a 593 aa protein. Little is known about promoter or regulatory sequences, and transcriptional regulation (Grossmann et al., 2010).

From a structural point of view, SHP2 is composed of two tandem SH2 domains at the N-terminal level (N-SH2 and C-SH2), a central PTP domain carrying the catalytical activity, and a C-terminal tail displaying regulatory properties (Neel et al., 2003) (Fig. 1A). SH2 domains mediate SHP2's interaction with phosphotyrosine – containing activators (e.g. tyrosine kinase receptors, docking proteins including Insulin Receptor Substrate 1, IRS1, GRB2-Associated Binding Protein 1, GAB1…) bearing monopartite binding sites for the N-SH2 domain (e.g. Y1009 on Platelet-Derived Growth Factor Receptor, PDGFR) or bipartite binding sites (e.g. Y1172/Y1222 on IRS1, Y627/Y659 on GAB1) (Cunnick et al., 2001; Ekman et al., 2002; Noguchi et al., 1994). They are also involved in regulatory intramolecular interactions with the PTP and C-terminal domains (Hof et al., 1998). SHP2's PTP domain encompasses 4 loops (P-loop, pY-loop, WPD-loop, and Q-loop) that wall the active site pocket and play specific roles in SHP2 activity. Inside the pY-loop, the Y279 defines catalytical site depth and therefore contributes to substrate specificity. The P-loop carries the PTP signature motif ($C459(X)_5R465$) that contains key residues for catalysis: the R465 stabilizes the phosphotyrosine substrate–enzyme complex and the C459 triggers a nucleophilic attack on the phosphorus atom, thereby transferring the phosphate group from the substrate to the enzyme. Hydrolysis of the resulting thiophosphoryl enzyme intermediate and release of the dephosphorylated substrate then occur, which involve several key residues (N425 of the WPD loop, Q506/510 in the Q-loop) (Barford and Neel, 1998; Hof et al., 1998; Zhang, 2003) (Fig. 1B). The C-terminal tail carries serine and tyrosine phosphorylation sites as well as a prolin rich domain that can mediate interaction with SH3 domain-containing proteins, although its precise role remains unclear. Two tyrosine residues (Y542 and Y580) can be phosphorylated in response to some growth factors (e.g. PDGF or Fibroblast Growth Factor, FGF), creating binding sites for adaptor proteins, such as GRB2 (Araki et al., 2003). While this SHP2/GRB2 interaction seems dispensable in some conditions, these phosphorylated residues have also been known to mediate activating intramolecular interactions with the SH2 domains (see below).

Crystal structure resolution of SHP2, together with biochemical experiments, has unraveled the mechanisms of SHP2 activation (Barford and Neel, 1998; Hof et al., 1998). At the basal state, part of the N-SH2 domain (D'E loop) penetrates into the catalytic cleft and physically occludes the active site, thereby preventing the access of SHP2's substrates to the catalytic pocket. This intramolecular interaction keeps the phosphatase into a closed, auto-inhibited conformation. When the SH2 domains bind to specific phosphotyrosine motifs borne by SHP2′ upstream signaling partners, these inhibitory interactions are released. The phosphatase is then in an open conformation that allows the positioning of SHP2 substrates into the catalytic site and their dephosphorylation (Fig. 1C). Through this molecular switch, SHP2 is supposed to be active only when recruited to its signaling partners. Supporting this view, phosphatase assays, using recombinant or immunoprecipitated SHP2, have shown that it has a very low basal catalytic activity that is clearly enhanced in the presence of phosphopeptides with affinity for its N-SH2 domain, following deletion of its N-SH2 domain or when specific residues located at the interface between the N-SH2 and the PTP domains are mutated (see chapter II) (Barford and Neel, 1998; Cunnick et al., 2001; O'Reilly et al., 2000). To add some complexity into SHP2 regulation, it has been proposed that intramolecular interaction between the phosphorylated Y542 and Y580 and the SH2 domains can activate the phosphatase in the absence of upstream regulator (Araki et al., 2003; Lu et al., 2001). However, this hypothesis remains controversial, since some studies did not find any role of these phosphorylation events on SHP2 activity or function (Feng et al., 1993; Lu et al., 2001; O'Reilly et al., 2000). Interestingly, it has been recently shown that GRB2 binding to phosphorylated Y542/Y580 can modulate their interaction with SHP2's SH2 domains, thereby playing a regulatory role on SHP2 activity (Sun et al., 2013). Inhibitory mechanisms have also been described, notably through reactive oxygen species (ROS)-dependent reversible oxidation of the catalytical cystein within the phosphatase motif (Jang et al., 2014; Kwon et al., 2005), and by threonine and serine phosphorylation events within the C-terminal domain (Peraldi et al., 1994).

1.2. Molecular roles of SHP2

1.2.1. Regulation of intracellular signaling pathways

Appropriate cell signaling relies on a delicate balance of phosphorylation events that must be fine-tuned in term of space, time, duration, and intensity. Because PTPs counteract the positive signal emanating from tyrosine kinases, they are generally seen as negative regulators of cell signaling. However, SHP2 is one of the rare PTPs displaying rather positive function in signal transduction. Indeed, the best-established function of SHP2 is to promote, in response to a wide range of agonists and in various cell types, the activation of the RAS/Mitogen-Activated Protein Kinases (MAPK) Extracellular-Regulated Kinases 1/2 (ERK1/2) pathway, a canonical signaling cascade that plays key roles in various cellular processes, including proliferation, survival, differentiation, migration, or metabolism (Fig. 2). Thus, downstream from many tyrosine kinase receptors, SHP2 is necessary for maintaining MAPK activation, which is even blunted in some cases in the absence of functional SHP2, although it can also reportedly display negative actions in this same pathway (Bennett et al., 1994; Cunnick et al., 2000, 2002; Dance et al., 2008; Saxton et al., 1997; Tang et al., 1995). The key role of SHP2 in this signal transduction pathway is so important that MAPK-hyperactivating mutations of SHP2 are responsible for human diseases (see chapter 2) (Tartaglia et al., 2011).

While the signal-enhancing function of SHP2 in RAS/MAPK activation is clearly established, the molecular mechanisms involved remain incompletely understood. Firstly, it has been proposed that SHP2, through its phosphorylated Y542/Y580, could function as a docking protein for the GRB2/SOS complex, thereby promoting ERK1/2 activation (Bennett et al., 1994; Li et al., 1994; Miura et al., 2013; Vogel and Ullrich, 1996). However, upon some stimuli, SHP2 is not phosphorylated or does not recruit GRB2 (Araki et al., 2003), while its phosphatase activity remains necessary for RAS/MAPK activation (Deb et al., 1998; Maroun et al., 2000; Noguchi et al., 1994), implying the involvement of a phosphorylated substrate, the dephosphorylation of which could promote RAS/MAPK activation. Such substrates could be:

- SPRY/Sprouty, a negative regulator of RAS, which dephosphorylation by SHP2 can promote GRB2/SOS -dependent activation of RAS (Hanafusa et al., 2004; Tefft et al., 2005, 2002),
- RASA/p120 RASGAP-binding sites containing proteins: several studies have reported that SHP2 can dephosphorylate tyrosine kinase receptors (e.g. PDGFR, Torso) or adaptor proteins (e.g. GAB1) on specific phosphotyrosines that mediate the recruitment of p120 RASGAP, the GTPase-activating protein that triggers the return of RAS to the GDP-bound, inactive state. This results in the exclusion of RASGAP from signaling complexes, thereby promoting RAS activation (Agazie and Hayman, 2003;

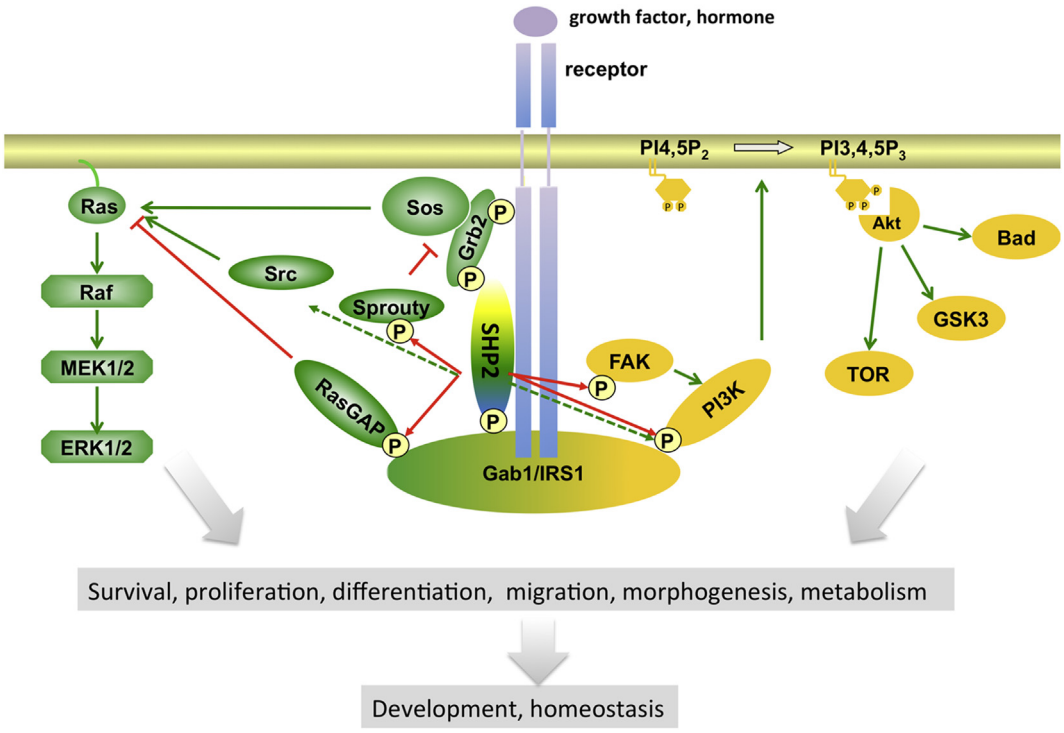

Fig. 2. Role of SHP2 in the activation of RAS/MAPK and PI3K/AKT signaling pathways downstream from tyrosine kinase receptors (TKR). Following ligand binding on its cognate receptor, this latter autophosphorylates, creating binding sites for adaptor proteins like GRB2. This allows recruiting SOS, the RAS GTPase exchange factor, to the plasma membrane. Once activated, GTP-bound, RAS initiates the RAF1/MEK1/2/ERK1/2 cascade. On the other side, class Ia PI3K are mobilized through binding to different adaptor proteins (GAB1, IRS1). They phosphorylate their substrates (e.g. $PI4,5P_2$) to generate D3-phosphoinositides (e.g. $PI,4,5P_3$), which mediate the recruitment and/or activation of various proteins, notably AKT. ERK1/2 and AKT activate various effectors, thereby regulating numerous cellular processes. SHP2 can activate the RAS/MAPK pathway and inhibit the PI3K/AKT cascade through different mechanisms (see text for details). Green and red arrows indicate activating and inhibiting effects, respectively.

Bard-Chapeau et al., 2006; Cleghon et al., 1998; De Rocca Serra-Nedelec et al., 2012; Montagner et al., 2005),

- SRC kinases: SHP2 can promote SRC-dependent MAPK activation, by negatively regulating the recruitment of CSK, the kinase that phosphorylates the inhibitory Y527 of SRC (Ren et al., 2004; Yang et al., 2006; Zhang et al., 2004b).

SHP2 is also known to regulate, *in vitro* and *in vivo*, the Phosphoinositide 3-Kinase (PI3K)/AKT pathway, a fundamental cascade with key functions, among others, in cell survival, proliferation, migration, morphogenesis, and metabolism. However, its effects appear to be stimulus- and cell context-dependent. Indeed, Zhang et al. have shown that SHP2 inhibits AKT activation in response to Epidermal Growth Factor (EGF) but promotes this same pathway under PDGF or Insulin-like Growth Factor 1 (IGF1) stimulation in the same cell type (Zhang et al., 2002), while both signal-enhancing and inhibiting functions have been reported under insulin stimulation (Myers et al., 1998; Ouwens et al., 2001; Ugi et al., 1996). SHP2's activating effects have been associated to increased IRS1 phosphorylation, implying an indirect process (Ugi et al., 1996). In contrast, a direct molecular mechanism has been proposed to explain SHP2's negative role in the PI3K/AKT pathway. Indeed, SHP2 can dephosphorylate binding sites for the p85 regulatory subunit of PI3K (Y-X-X-M motif) borne by GAB1 or IRS1, thereby dampening down PI3K recruitment and activation (Fig. 2) (Myers et al., 1998; Ouwens et al., 2001; Zhang et al., 2002). Importantly, this regulation may have a major physiological outcome since hyperactivation of PI3K-downstream targets has been linked to SHP2 mutations-associated genetic disease (Edouard et al., 2010; Marin et al., 2011; Tajan et al., 2014) (see chapter 2).

Besides its roles in Ras/MAPK and PI3K/AKT pathways, SHP2 has been involved in the regulation of many other signaling actors, notably the JAK/STAT (Janus Kinase/Signal Transducer and Activator of Transcription) modules, various kinases (e.g. AMP-activated Protein Kinase, AMPK; Target of Rapamycin, TOR; c-Jun N-terminal Kinase, JNK), or cytoskeleton-associated proteins (e.g. PXN/paxillin, Rho-associated Kinase (ROCK)), although the precise molecular mechanisms involved remain incompletely understood (Fukunaga et al., 2000; Huang et al., 2012; Langdon et al., 2012; Xu and Qu, 2008; Zito et al., 2007).

1.2.2. *Non signaling functions*

In addition to its cytoplasmic functions in signal transduction, nuclear and mitochondrial roles of SHP2 have been proposed. Indeed, the group of Hatakeyama has described a nuclear localization of SHP2, which is regulated by physical interaction with the YAP/TAZ (Yes-associated protein/Transcriptional co-activator with PDZ-binding motif) transcriptional coactivators (Tsutsumi et al., 2013). Within the nucleus, SHP2 can dephosphorylate a protein called parafibromin, which thus shifts from an anti-oncogenic, epigenetic-driven activity, to a pro-oncogenic, WNT/CTNNB1 (β-catenin)-promoting function (Takahashi et al., 2011). Moreover, in 2004, SHP2 was the first tyrosine phosphatase to be identified in the mitochondria (Salvi et al., 2004). In recent studies, expression of hyperactive mutants of SHP2 (leukemia-associated mutant E76K, Noonan syndrome-causing mutant D61G) induced an increase in mitochondria respiration and ROS overproduction. SHP2 could trigger a decoupling activity within the mitochondria and/or alter proton-pumping activity of the OxPhos complexes, since Zheng et al. found a reduced ADP:O_2 ratio in SHP2 E76K-expressing cells

Table 1
Mouse models of *PTPN11* invalidation. The invalidation strategy, the targeted tissue/cell and the resulting phenotype are indicated.

Target tissue or cell/ construct	Phenotype	Signaling defect	References
Ubiquitous/*Ptpn11*$^{-/-}$	Embryonic lethality.		(Saxton et al., 1997; Yang et al., 2006)
Ubiquitous (inducible)/ *Ptpn11*$^{fl/fl}$ × Ub-ERT2-cre	Altered hematopoiesis, weight loss, skeletal abnormalities, premature death, orofacial cartilage malformation.		(Bauler et al., 2011; Kamiya et al., 2015)
Skeletal and cardiac muscle/ *Ptpn11*$^{fl/fl}$ × αMHC-cre or Mck-cre	Dilated cardiomyopathy. Insulin resistance, glucose intolerance. Decreased myofibers size and number.	↘Erk1/2. ↗ RhoA, Akt, STAT3, Erk5	(Fornaro et al., 2006; Kontaridis et al., 2008; Princen et al., 2009)
Mesenchymal stem cells/ *Ptpn11*$^{fl/fl}$ × Prx1-cre	Postnatal growth retardation. Limb and chest deformations, cranial abnormalities. Lack of subcutaneous adipose tissue.	↘ Erk1/2/Akt in bone	(Lapinski et al., 2013)
Chondrocytes (postnatal)/ *Ptpn11*$^{fl/fl}$ × Col2a1-CreERt2	Kyphosis, scoliosis. Metachondromatosis.	↘ Erk1/2 in cartilage	(Kim et al., 2013; Kim et al. 2014; Bowen et al., 2014)
Mesenchymal progenitors, osteoclasts/ *Ptpn11*$^{fl/fl}$ × Cathepsin K-cre	Metachondromatosis.	↘ FGFR/MEK/Erk1/2	(Yang et al., 2013)
Neural crest/ *Ptpn11*$^{fl/fl}$ × Wnt1-cre	Altered Schwann cell development. Peripheral nerve hypomyelination. Enteric nervous system defect. Craniofacial defects. Arteries malformations.	↘ Erk1/2 (neural crest) ↘ FAK, Erk1/2 (Schwann cells)	(Grossmann et al., 2009; Nakamura et al., 2009)
Embryonic cortex/ *in utero* shRNA electroporation	↘ neurogenesis.↗ gliogenesis.		(Gauthier et al., 2007)
Central nervous system/ *Ptpn11*$^{fl/fl}$ × Nestin-cre	Altered corticogenesis and cerebellum development. ↘ neurogenesis. ↗ gliogenesis.Early post natal lethality.	↘ SDF-1α-induced Erk1/2/ ↗ SDF-1α-induced FAK signaling	(Ke et al., 2007; Hagihara et al., 2009)
Telencephalon progenitors and oligodendrocytes/ *Ptpn11*$^{fl/fl}$ × Olig2-cre	↘ oligodendrocyte progenitors, hypomyelinisation.	↘ Erk1/2 in ventricular zone	(Ehrman et al., 2014)
Post migratory neural crest/ *Ptpn11*$^{fl/fl}$ × Postn-cre	Altered sympathetic cardiac inervation, bradychardia.	↘ Erk1/2	(Lajiness et al., 2014)
Post-mitotic forebrain neurons/ *Ptpn11*$^{fl/fl}$ × CaMKIIα-Cre	Obesity. Leptin resistance. Impaired glucose metabolism. Liver steatosis	↘ Erk1/2. ↗ JAK2/STAT3	(Zhang et al., 2004)
Neurons/ *Ptpn11*$^{fl/fl}$ × CRE3	Obesity. Diabetes. Hyperphagia. Insulin and leptin resistance. Vasculitis. Nephropathy.		(Krajewska et al., 2008)
Propiomelanocortin neurons/ *Ptpn11*$^{fl/fl}$ × POMC-Cre	Obesity. Impaired glucose metabolisms. Leptin resistance. Decreased EE		(Banno et al., 2010; do Carmo et al., 2014)
Chimeric mice/ *Ptpn11*$^{-/-}$/RAG-2 blastocyst	Absence of erythroid and myeloid progenitors. Skeletal, intestinal, pulmonary, cutaneous defects.		(Qu et al., 2001)
Hematopoietic stem cells/ *Ptpn11*$^{fl/fl}$ × Mx1-cre	Hematopoietic stem cells and progenitors apoptosis (all lineages).	↘ SCF- and TPO-induced Erk1/2/Akt in Lin^{-} cells	(Chan et al., 2011)
T cells/ *Ptpn11*$^{fl/fl}$ × Lck-cre	Altered T cells maturation/differentiation.	↘ Erk1/2 in T cells	(Nguyen et al., 2006)
T cells/ *Ptpn11*$^{fl/fl}$ × CD4-cre	Stimulation of melanoma progression. Increased release of pro-inflammatory cytokines.		(Zheng et al., 2013)
Mastocytes/ *Ptpn11*$^{fl/fl}$ × Mcpt5-cre	Decreased mastocytes survival. Altered SCF-induced chimiotactism.	↘SCF-induced Akt/Erk1/2/ Lyn	(Sharma et al., 2012; Sharma et al. 2014)
Megacaryocytes Platelets/ *Ptpn11*$^{fl/fl}$ × PF4-cre	Decreased megacaryocyte ploidy. Macrothrombocytopenia, increased platelet clearance and CLEC-2 hypersensitivity.	↘TPO- and αIIbβ3-induced Erk1/2. ↘ fibrinogen-induced SFK	(Mazharian et al., 2013)
Hepatocytes/ *Ptpn11*$^{fl/fl}$ × Alb-cre	Altered liver regeneration. ↗ tumor progression/inflammation. Chronic hepatobiliary disorders. Improved insulin sensitivity and glucose tolerance. Obesity resistance. Increased EE.	↘ FGF19-induced Erk1/2, p90RSK, PKC ↗ STAT3 ↗insulin-induced PI3K/ Akt ↘ insulin-induced Erk1/2	(Bard-Chapeau et al., 2006; Matsuo et al., 2010; Bard-Chapeau et al., 2011; Nagata et al., 2012; Li et al., 2014)
Intestine epithelial/ *Ptpn11*$^{fl/fl}$ × Villin-cre	Severe colitis, immune cell infiltration. Decreased goblet cells. Increased intestine permeability.	↗ Stat3, NF-κB Active K-Ras rescue	(Coulombe et al., 2013; Heuberger et al., 2014; Yamashita et al., 2014)
Lung epithelial cells/ *Ptpn11*$^{fl/fl}$ × SPC-rtTA × (tetO)7-CMV-cre	Pulmonary fibrosis.	↘ FGF-induced Erk1/2	(Zhang et al., 2012)
Mammary gland/ *Ptpn11*$^{fl/fl}$ × MMTV-cre	Impaired lobulo-alveolar outgrowth and lactation.	↘ STAT5/↗ STAT3	(Ke et al., 2006)
Kidney/ *Ptpn11*$^{fl/fl}$ × Hoxb7-cre	Rudimentary kidneys (impaired cortex and medulla, reduced numbers of tubules and glomeruli). Renal failure. P1 lethality.		(Willecke et al., 2011)
Germline cells/ *Ptpn11*$^{fl/fl}$ × Vasa-cre	Impaired spermatogenesis.	↘ bFGF- and GDNF-induced Erk1/2	(Puri et al., 2014)
Adipose tissue/ *Ptpn11*$^{fl/fl}$ × Adiponectin-Cre or aP2-cre	No phenotype (adiponectin). Severe lipodystrophy. Impaired adipogenesis. Low blood pressure. Compensatory erythrocytosis. Premature death (aP2).	↘ p38 (aP2)	(Bettaieb et al., 2011; He et al., 2012)
Pancreas/ *Ptpn11*$^{fl/fl}$ × Pdx1-cre	Defective glucose-stimulated insulin secretion. Impaired glucose tolerance.		(Zhang et al., 2009)

and expression of hyperactive mutants of SHP2 was associated to a decreased membrane potential. However, the precise role of SHP2 on ATP production and on mitochondrial enzyme expression/activity remains unclear (Lee et al., 2010; Xu et al., 2013; Zheng et al., 2013). Besides mitochondria function, Duarte et al. have also shown that SHP2 could trigger mitochondria fusion, which could also promote mitochondria energy metabolism (Duarte et al., 2012).

1.3. Physiological functions of SHP2

1.3.1. Developmental roles

The pleiotropic roles of the SHP2, combined with its ubiquitous expression, confer key functions on SHP2 in numerous physiological processes and in organism patterning during embryo development. This is notably illustrated by the impact of global SHP2 invalidation in various animal models. Thus, SHP2′ global loss-of-expression/function revealed its requirement for development, whatever species is considered (*C. elegans*, fly, zebrafish, xenopus, mouse…). This conserved role appears to be related to its capability to promote the Ras/MAPK pathway. Indeed, in *C. elegans*, PTP-2 is required for oogenesis while fly CSW controls the development of terminal embryonic structures as well as R7 photoreceptor development, both processes being RAS-dependent (Allard et al., 1996; Gutch et al., 1998; Herbst et al., 1996; Perkins et al., 1996, 1992). In zebrafish and in xenopus, SHP2 downregulation/loss-of-function severely alters embryo development, due to pleiotropic defects associated with ERK hypoactivation (Bonetti et al., 2014b; Jopling et al., 2007; Stewart et al., 2010; Tang et al., 1995). These crucial developmental roles of SHP2 were also found in mammals. Indeed, in the mid-90's, mice harboring a deletion of exon 2 and 3, which results in a truncated SHP2 missing its N-SH2 domain, died *in utero* during gastrulation (Saxton et al., 1997). Ten years later another knock out strategy, resulting in a complete loss of SHP2 expression, showed an even stronger phenotype, the embryos dying as soon as the peri-implantation from a massive death of blastocyst internal mass cells, trophoblastic cells apoptosis, and failure to produce trophoblastic stem cells (Yang et al., 2006). Further studies on embryonic stem cells (ESC) revealed that SHP2 knock down in murine and human ESC inhibited their differentiation into all three germ layer cell lineages (ectoderm, endoderm and mesoderm) while improving their self-renewal potential. This effect was associated with sustained expression of pluripotent ESC marker genes (Nanog, Oct3/4, Rex1 and Sox2) and dysregulation of ERK and STAT3 signaling pathways (Wu et al., 2009). Consistent with this notion, the role of SHP2 in controlling the switch from self-renewal to differentiation was then extended to many stem cells and progenitors, conferring on SHP2 important roles in the morphogenesis and function of numerous organs and tissues (Chan et al., 2011; Ehrman et al., 2014; Heuberger et al., 2014; Nabinger and Chan, 2012; Puri et al., 2014; Yang et al., 2013; Zhou et al., 2015; Zhu et al., 2011).

Because the early lethality of total SHP2 knock out precludes further study regarding SHP2 functions, many tissue-specific knockouts of SHP2 have thus been developed, which unraveled numerous roles of SHP2 in tissue/organ development and/or homeostasis, including bone, nervous system, heart, muscle, blood, kidney, lung, liver, mammary gland, intestine, adipose tissue, and spermatogonia. These effects have often been associated with ERK1/2 hypoactivation, although other dysfunctions have also been reported (Table 1).

Importantly, some of these developmental functions of SHP2 may participate, when altered, to the pathogenesis of SHP2-associated diseases, in particular growth, skeletal and craniofacial defects, cardiopathies, cognitive defects or hematological diseases:

- SHP2 invalidation in neural crest, mesenchymal stem cells or chondrocyte progenitors led to severe bone and cartilage defects (skull, limb, and chest abnormalities, scoliosis, kyphosis, osteopetrosis…), which have been associated, when tested, to MAPK and/or AKT hypoactivation (Bowen et al., 2014; Kim et al., 2013, 2014; Lapinski et al., 2013; Nakamura et al., 2009a; Yang et al., 2013). Moreover, in an inducible mouse model, allowing SHP2 inactivation during adulthood, Bauler et al. observed severe skeletal abnormalities, demonstrating key functions of SHP2 in bone and cartilage development in adults in addition to its embryonic roles (Bauler et al., 2011). These defects have been notably ascribed to a negative and positive role of SHP2 on chondrocyte and osteoblast differentiation, respectively. Further analysis of this mouse model recently revealed orofacial cartilage malformations associated with ERK hypoactivation. Interestingly, this phenotype has been ascribed to a defect in ciliogenesis, the number of primary cilia and the expression levels of intraflagellar transports being significantly decreased (Kamiya et al., 2015). As we will see in the second part, this critical role of SHP2 in bone homeostasis is further highlighted by the fact that skeletal abnormalities, facial dysmorphia, and cartilaginous tumors are found in human diseases caused by SHP2 dysregulation (for recent review, see (Kamiya et al., 2014)).
- in skeletal muscle, SHP2 invalidation decreases myofiber size and number, highlighting a role of SHP2 in myotubes formation and skeletal muscle growth (Fornaro et al., 2006; Kontaridis et al., 2004). These functions could be particularly important in heart physiology, as two mouse models with cardiomyocyte- or myocyte-targeted SHP2 knock-outs develop severe dilated cardiomyopathy associated with ERK hypoactivation and PI3K hyperactivation (Kontaridis et al., 2008; Princen et al., 2009). For extensive review of the role of SHP2 in cardiac physiology and diseases, see (Lauriol et al., 2014).
- a number of studies have also defined SHP2 as necessary for proper development of the central nervous system. Indeed, it is involved in cortex and cerebellum patterning and controls neural progenitors' fate, by promoting ERK-dependent neurogenesis while inhibiting STAT3-dependent gliogenesis (Gauthier et al., 2007; Hagihara et al., 2009; Ke et al., 2007). Moreover, SHP2 is also required for proper commitment of Schwann cell and oligodendrocyte progenitors (Ehrman et al., 2014; Grossmann et al., 2009).
- SHP2 is highly expressed in hematopoietic cells and is critically involved in the development of all blood cell lineages. Indeed, its inactivation results in a severe loss of erythroid, myeloid, and lymphoid populations, highlighting a critical role for SHP2 in hematopoietic stem cells (HSC) survival and differentiation (Chan et al., 2011, 2006; Qu et al., 2001). Moreover, SHP2 also plays complex roles in T lymphocyte differentiation and TCR signaling (Nguyen et al., 2006; Salmond et al., 2005), in mastocyte survival and chemotaxis (Sharma et al., 2014, 2012), and in megakaryocytes development and platelets production (Mazharian et al., 2013). These functions have been causally linked to hematological disorders associated to SHP2 mutations.

1.3.2. Roles in organism homeostasis

In addition to its pleiotropic developmental roles, evidence has been accumulated over previous years to confer on SHP2 key functions in homeostasis maintenance, notably in metabolism regulation.

Firstly, a large body of data obtained over the two past decades has defined a central place for SHP2 in energy balance regulation, notably through its capability to regulate the action of leptin, a hormone notably secreted by adipose tissue that provides

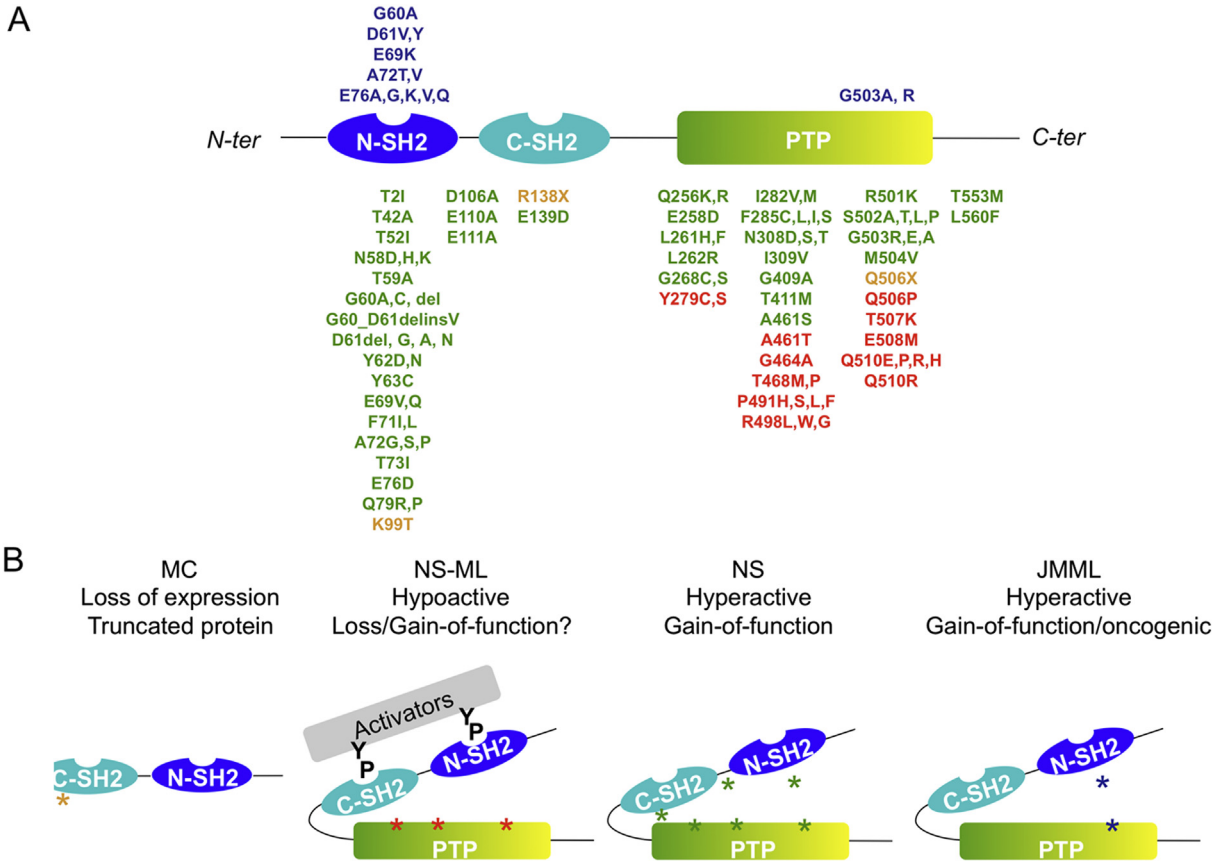

Fig. 3. SHP2 mutations in human diseases. A – The main mutations responsible for JMML (in blue), NS (in green), NS-ML (in red), and MC (in orange) are represented on the SHP2 structure. For MC, note that only missense and nonsense mutations are shown, other mutations (frameshifts, deletion in the non coding sequence…) being responsible for MC (adapted from NSEuroNet Database, www.nseuronet.com and www.ncbi.nlm.nih.gov/clinvar/PTPN11). B – Functional consequences of SHP2 mutations. MC-associated mutations result in truncated protein or loss of expression. NS-ML-causing mutations reduce the catalytical activity of SHP2 but may increase its binding affinity for its activator and/or favor its open conformation. NS- and JMML-linked mutations increase SHP2's enzymatic activity and/or abrogate the inhibitory constraints, thereby activating/hyperactivating the phosphatase.

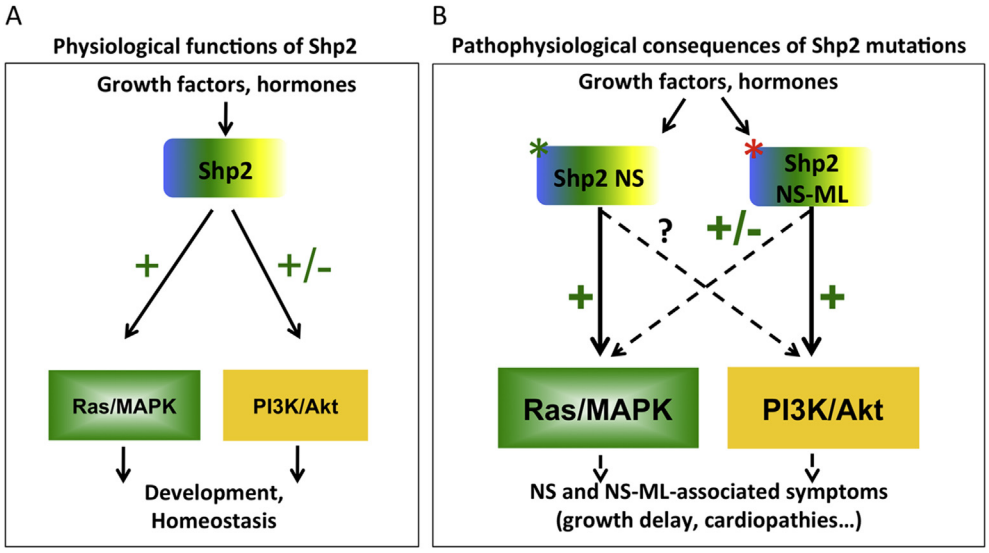

Fig. 4. Model for physiological and pathophysiological roles of SHP2. A – In physiological conditions, SHP2 participates in the activation of the RAS/MAPK cascade, while it can inhibit or activate the PI3K/AKT pathway. This combined regulation is necessary for proper development and homeostasis. B – NS-causing SHP2's mutants hyperactivate the RAS/MAPK pathway while the NS-ML-associated mutants hyperactivate the PI3K/AKT pathway. These signaling imbalances are linked to the development of some symptoms of the diseases (cardiopathies, cranio-facial defects, growth delay). NS-ML-linked mutants can also positively or negatively impact the RAS/MAPK pathway, through mechanisms that are poorly understood.

anorexigen cues at the central level and also stimulates energy expenditure (EE). Indeed, several studies have pinpointed that SHP2 directly binds activated leptin receptor (LEPR) and regulates various leptin-evoked signaling pathways (e.g. JAK2/STAT3, RAS/MAPK) (see (Allison and Myers, 2014) for recent review). Consistent with this, neuronal SHP2 inactivation results in obesity, diabetes and leptin resistance, as a consequence of hyperphagia and/or decreased EE (do Carmo et al., 2014; Krajewska et al., 2008) while mice expressing a hyperactive mutant of SHP2 in forebrain neurons displayed an inversed phenotype (He et al., 2012a). However, an opposite role for SHP2 in EE control has also been suggested. Indeed, liver-specific SHP2 knock out mice display increased EE, which correlated with higher mitochondrial respiration in hepatocytes, and are resistant to High-Fat Diet (HFD)-triggered weight and fat gain (Nagata et al., 2012).

Secondly, it is clearly established that SHP2 is involved in the maintenance of physiological glucose levels, an essential process for proper energy homeostasis given that all living cells use glucose as the main or only source of energy. In particular, SHP2 has been described as having combined actions on signaling, physiological effects and biosynthesis of insulin, which plays a central role in glycaemia regulation through its hypoglycaemic effects (stimulation of glucose transport, use and storage, inhibition of hepatic glucose production) on insulin-sensitive tissues (liver, muscle, and adipose tissue). Thus, as mentioned above, SHP2 is recruited in response to insulin stimulation and plays complex roles in insulin signaling (RAS/MAPK activation, PI3K/AKT activation/inhibition, recruitment of negative regulators of insulin signaling) (Fukunaga et al., 2000; Maegawa et al., 1996; Mussig et al., 2005; Myers et al., 1998; Ouwens et al., 2001; Ugi et al., 1996). In terms of insulin-regulated biological/cellular processes, SHP2 is necessary for MAPK-driven proliferation and promotes glucose transport, while it plays a negative role on protein and glycogen synthesis, possibly through an inhibitory action on PI3K signaling (Bifulco et al., 2002; Chen et al., 1997; Hausdorff et al., 1995; Milarski and Saltiel, 1994; Myers et al., 1998; Ouwens et al., 2001; Ugi et al., 1996). *In vivo*, initial studies using *Ptpn11*$^{+/-}$ mice demonstrated no haploinsufficiency in term of metabolism regulation (Arrandale et al., 1996; Saxton et al., 1997), and overexpression of a dominant negative mutant of SHP2 in several metabolic tissues resulted in glucose intolerance and insulin resistance. However, at odds with almost all models of SHP2 inactivation, this mutant surprisingly induced ERK1/2 hyperactivation and PI3K/AKT hypoactivation (Maegawa et al., 1999). SHP2 targeted invalidation in various metabolic tissues then provided key insights into the physiological role of SHP2 in glycaemia control. Thus, its hepatic disruption resulted in improved glucose tolerance and insulin sensitivity, and concomitant PI3K hyperactivation and MAPK hypoactivation (Matsuo et al., 2010). This phenotype translated into protection from insulin resistance upon a high fat diet, which could directly arise from improved insulin sensitivity but could also indirectly result from the reduced weight/fat gain developed by those mice (Nagata et al., 2012). In contrast, mice with muscle invalidation of SHP2 developed, in addition to their cardiopathy, insulin and glucose intolerance, with reduced glucose uptake in skeletal muscle, through mechanisms that are poorly understood (Princen et al., 2009). How SHP2's functions in adipose tissue participate in glucose homeostasis remains unclear, since the impact of SHP2 invalidation in adipose tissue development and/or function, and subsequent consequences on glucose metabolism, varied depending on the mouse model used (Bettaieb et al., 2011; He et al., 2012b). Finally, mice with pancreas-targeted SHP2 deletion displayed reduced glucose-induced insulin secretion and consistent glucose intolerance, as a result of defective glucose-evoked PI3K/AKT/FOXO1 and MAPK signallings and impaired expression/activity of PDX1, a major activator of insulin expression (Zhang et al., 2009a). Given the divergent roles of SHP2 depending on the considered tissue, which might result from different signaling pathways involved or distinct regulatory processes, studies in integrated models with inducible loss of function of SHP2 are still warranted to understand its physiological role in glucose homeostasis. Interestingly, it was recently shown that the systemic heterozygous expression of a SHP2 mutant with reduced phosphatase activity resulted in improved glucose tolerance, low insulinemia, and increased insulin tolerance. Consistent with this, but at odds with SHP2 knock-out models, a hyperactivation of both ERK1/2 and AKT was found in insulin-sensitive tissues (Tajan et al., 2014).

Finally, important roles for SHP2 in lipid metabolism have also been reported. Indeed, aP2-mediated SHP2 invalidation resulted in marked reduction of white adipose tissue as a result of impaired adipogenesis, supporting previous studies *in vitro* that showed a role of SHP2 in promoting Peroxisome Proliferator-Activated Receptors gamma (PPARγ)-dependent adipogenesis. While aP2 SHP2$^{-/-}$ mice displayed severe lipodystrophia, decreased leptin levels, reduced glycaemia and insulinemia, and low blood pressure leading to premature death, it has to be noted that another mouse model with a more adipose tissue-specific, adiponectin-driven deletion of SHP2 did not display such alterations, unveiling discrepancies depending on the targeting strategy and specificity (Bettaieb et al., 2011; He et al., 2012b; Uehara et al., 2007).

Moreover, in several of the above-described models, SHP2 loss of function was found to impact lipid metabolism. Generally speaking, when SHP2 loss is beneficial for glucose homeostasis (e.g. better glucose tolerance in SHP2^{liver} mice), lipid catabolism (e.g. reduced triglyceride (TG) levels) is improved (Matsuo et al., 2010), while when it is detrimental (e.g. insulin resistance in SHP2^{muscle} mice), TG levels are raised (Princen et al., 2009). Whether these alterations directly result from SHP2 inactivation or are a secondary consequence of the primary metabolic phenotype is difficult to ascribe. In addition, as expected, the development of obesity or lipodystrophy correlated with hepatic steatosis, hypertriglyceridemia and hypercholesterolemia, and *vice versa* (Banno et al., 2010; He et al., 2012a, 2012b; Krajewska et al., 2008; Nagata et al., 2012; Zhang et al., 2004a). More direct evidence for a role of SHP2 in lipid metabolism came from two recent studies, which demonstrated that SHP2 controls the expression levels of fatty acid metabolism-regulating enzymes, notably by promoting Acyl-CoA synthetase 4 (ACSL4) expression and triggering Fatty Acid Synthase (FAS) ubiquitinylation and proteasome-dependent degradation (Cooke et al., 2011; Yu et al., 2013a).

Given the numerous roles of SHP2 in energy metabolism regulation, one may wonder whether it could participate in the occurrence of obesity and/or associated disorders (e.g. insulin resistance, type II diabetes). Supporting this hypothesis, overexpression, and possibly hyperactivation, of SHP2 have been observed in the liver and muscle of obese and/or diabetic mice (Ahmad and Goldstein, 1995a,b; Bettaieb et al., 2011; Bonini et al., 1995a, 1995b; Nagata et al., 2012). In addition, Nagata et al. showed that hepatic SHP2 levels fluctuated between starved and fed states (Nagata et al., 2012). More importantly, *PTPN11* polymorphisms have been linked to lipid plasma levels (Jamshidi et al., 2007; Jia et al., 2013; Lu et al., 2008) and genetic variants of *PTPN11* have been recently associated to metabolic syndrome (Kraja et al., 2014).

2. Mutations of SHP2 and pathophysiology

Over the two past decades, a growing interest has centered on SHP2 with the discovery that gain-of-function (GOF) or loss-of-function (LOF) mutations of *PTPN11* (MIM *176876) result in several human diseases. Indeed, missense mutations have been

causally linked to two related polymalformative syndromes, called Noonan syndrome (NS, MIM ♯163950) and Noonan syndrome with multiple lentigines (NS-ML, MIM ♯151100, formerly called LEOPARD syndrome). These diseases are associated with multiple congenital abnormalities (cranio-facial dysmorphia, cardiopathies, growth delay, mental retardation, skin defects…), illustrating SHP2's pleiotropic roles in organism patterning. With related diseases, notably Costello syndrome (MIM ♯218040), Cardio-Facio-Cutaneous syndrome (MIM ♯115150), type I Neurofibromatosis (MIM ♯162200), and Legius syndrome (MIM ♯611431), they form the family of neuro-cardio-facio-cutaneous syndromes, also called Rasopathies named after the mutated genes encoding actors of the RAS/MAPK signaling cascade. In addition, LOF mutations of *PTPN11* have been recently identified as the cause for metachondromatosis (MC; MIM ♯156250), a rare disease characterized by cartilaginous tumors. Moreover, the identification of sporadic GOF mutations in various leukemias, and to a lesser extent in solid tumors, led to define *PTPN11* as a genuine proto-oncogene.

2.1. Somatic mutations and tumorigenesis

As mentioned in the first chapter, SHP2 is a key regulator of proliferation and survival signaling pathways (e.g, RAS/MAPK, PI3K/AKT), the dysregulation of which has been causally linked to tumor development. Consistently, somatic point mutations of *PTPN11* have been identified as the main cause (35% of cases) of juvenile myelomonocytic leukemias (JMML, MIM ♯607785), a rare and aggressive myeloid malignancy of early childhood, associated with excessive monocytic and macrophagic proliferation (Loh et al., 2004a; Tartaglia et al., 2003). JMML-associated SHP2 mutations mainly hit residues within the N-SH2 and PTP domains involved in the auto-inhibitory constraint, resulting in an increased basal activity (Keilhack et al., 2005; Niihori et al., 2005; Tartaglia et al., 2003) (Fig. 3). Expression in hematopoietic cells of such GOF mutants (D61Y, E76K) promoted cell-cycle progression and survival of hematopoietic progenitors *in vitro*, two phenomena that are associated with PI3K/AKT and RAS/MAPK hyperactivation, and resulted in lethal myeloproliferative disorder *in vivo* (Chan et al., 2009; Mohi et al., 2005; Xu et al., 2011; Yang et al., 2008). Interestingly, in support of a role of RAS/MAPK hyperactivation in JMML development, activating NRAS (OMIM *164790) and KRAS (OMIM *190070) mutations as well as inactivating neurofibromin (NF1, a RASGAP, OMIM *613113) mutations have been found in patients with JMML (de Vries et al., 2010; Loh et al., 2004b; Side et al., 1998; Tartaglia et al., 2003). Excessive dephosphorylation of STAT3 has also been proposed to promote leukemogenesis (Zhang et al., 2009b). In addition to JMML, SHP2 mutations are found in other myeloid neoplasms (acute myeloid leukemia, OMIM ♯601626; chronic myelomonocytic leukemia, ♯OMIM 607785, myelodysplastic syndrome, OMIM ♯614286; acute B lymphoblastic leukemia, OMIM ♯613065). Of note, while the frequencies of such neoplasms are substantial in children, they are almost non-existent for adults with the same myeloid disorders (Hugues et al., 2005). SHP2 mutations also occur, albeit rarely, in solid tumors (neuroblastoma, OMIM ♯256700; lung carcinoma, OMIM ♯211980) (for review, see (Grossmann et al., 2010)). Moreover, there is much non genetic evidence for SHP2 involvement in various cancers (Aceto et al., 2012; Gomes et al., 2013; Gu et al., 2014; Hu et al., 2014; Liu et al., 2011; Sausgruber et al., 2014; Xu et al., 2005), so that its gene has been defined as a *bona fide* proto-oncogene, although, in exceptions to the rule and as a token of SHP2's duality, it can also act as a tumor suppressor in the liver and cartilage (Bard-Chapeau et al., 2011; Yang et al., 2013).

2.2. Germline inactivating mutations and metachondromatasis

Metachondromatosis (MC; MIM ♯156250) is a rare autosomal dominant disease characterized by the combination of multiple enchondromas in iliac crests and metaphyses of long bones, and osteochondromas lesions on hands and feet, notably on digits. Although MC-associated benign tumors can cause various adverse effects, these are most often asymptomatic and can even spontaneously regress (Bowen et al., 2011). In 2010, genetic analyses led to the identification of heterozygous LOF mutations (frameshifts, nonsense or splice site mutations) within the *PTPN11* gene in most of patients with MC, associated with loss-of-heterozygosity within the tumor, in accordance with Knudson two-hits hypothesis (Bowen et al., 2011; Sobreira et al., 2010) (Fig. 3). Consistent with this, while *Ptpn11*$^{+/-}$ mice do not develop bone tumors, SHP2 total invalidation in chondrocytes or in mesenchymal progenitors resulted in cartilaginous tumors (Bowen et al., 2014; Kim et al., 2014; Yang et al., 2006, 2013). According to Bowen et al., osteochondromas could be caused by disorganized growth and delayed differentiation of chondrocytes, and enchondromas could arise from ectopic chondrogenesis of cells on the bone surface. Interestingly, both mechanisms may result from RAS/MAPK hypoactivation, this latter being measured in SHP2-deficient chondrocytes or mesenchymal progenitors as well as in cartilaginous tumors, and ERK1/2 being positive regulators of chondrocyte terminal differentiation.

2.3. Germline point mutations and RASopathies

Point mutations of *PTPN11* (MIM *176876) are the main causes of NS and NS-ML, being found in 50% of NS cases and more than 80% of NS-ML. So far, a dozen genes (*PTPN11* (MIM *176876), *SOS1* (MIM *182530), *SOS2* (MIM *601247), *KRAS* (MIM *190070), *NRAS* (MIM *164790), *RAF1* (MIM *164760), *BRAF* (MIM *164757), *MEK1* (MIM *176872), *SHOC2* (MIM *602775), *CBL* (MIM *165360), *RIT1* (MIM *609591), *LZTR1* (MIM *600574)) have been linked to NS pathophysiology (Aoki et al., 2013; Rauen, 2013; Roberts et al., 2013; Tartaglia and Gelb, 2010; Yamamoto et al., 2015) and mutations of *RAF1* and *BRAF* have been reported in non *PTPN11* cases of NS-ML (Koudova et al., 2009; Pandit et al., 2007; Sarkozy et al., 2009). In this review, we will describe the clinical presentation of both syndromes and focus on the pathogenetics of *PTPN11*-associated NS/NS-ML.

2.3.1. Noonan syndrome (NS)

2.3.1.1. Clinical presentation. Named after Jacqueline Noonan who first described it in 1968, NS is a quite frequent genetic disease with an estimated prevalence between 1/1000 and 1/2500 living births (Noonan, 1968). It is characterized by a phenotypical triad associating growth retardation, facial dysmorphic features, and cardiopathy (i.e. pulmonary valve stenosis, PVS, and hypertrophic cardiomyopathy, HCM). Many other developmental abnormalities (skeletal, neurodevelopmental, cutaneous…) can also be found in patients with NS. Because of the heterogeneity of NS presentation among patients and phenotypic redundancy with other Rasopathies, NS can be hard to diagnose and differentiate from other related syndromes (Roberts et al., 2013; Tartaglia et al., 2011, 2010). Patients with NS have various facial abnormalities (hypertelorism, ptosis, downslanting palpebral fissures, low-set posteriorly rotated ears, and short/webbed neck) that evolve with age. A cardinal NS feature is post-natal growth retardation, hitting more than 70% of patients. Prenatal growth is usually normal with normal birth weight and body length. During early childhood, failure to thrive can be linked to feeding difficulties, justifying enteral nutrition in 20% of infants. During childhood, the mean height in both sexes

follows the third percentile until the age of puberty, after which the mean height decreases below the normal range due to delayed puberty and poor pubertal growth spurts. The final height is about 2 standard deviations (SD) in male and female (Malaquias et al., 2012; Ranke et al., 1988; Romano et al., 2010; Witt et al., 1986). Abnormalities of the growth hormone (GH)/IGF-I axis, a major regulator of post-natal growth, have been suggested to explain growth retardation. Indeed, normal-to-elevated serum GH levels associated with low serum IGF-I levels are found in NS patients, suggesting GH insensitivity (GHI) at the post-receptor level (Noonan and Kappelgaard, 2014). Interestingly, in addition to short stature, Malaquias et al. also reported low Body Mass Index for NS patients (−0.9 SD for men, −0.5 SD for women) (Malaquias et al., 2012). This is correlated with a lower prevalence of being overweight and obesity when compared with the general population (Binder et al., 2012). Impaired bone homeostasis, notably decreased mineralization associated with increased resorption, has also recently been reported (Choudhry et al., 2012; Stevenson et al., 2011). With about 80% of patients displaying congenital heart defects, NS is the second most frequent syndromic cause of congenital cardiopathy after trisomy 21. While the most common cardiac anomalies are PVS (50–60%) and HCM (20%), many other lesions can be found including septal or valve defects, aortic coarctation or patent arterial duct (Prendiville et al., 2014). Given the high prevalence of cardiac involvement, electrocardiograms and echocardiographies are consistently performed for patients with NS. NS is also associated with cancer predisposition. Thus, a higher incidence of JMML, with frequent neonatal manifestations, has been described in *PTPN11*-associated NS, in particular for patients carrying mutation on the D61 and T73 residues (Strullu et al., 2014). Moreover, in a recent study, Kratz et al. quantitatively assessed the occurrence of childhood cancers and determined that the cancer risk in patients with NS was increased 8-fold (Kratz et al., 2015). Numerous other defects, including hematological, lymphatic, psychomotor, neurological, neurosensorial, urogenital, or integumental abnormalities have also been reported in this disease (Tartaglia et al., 2011, 2010; Tartaglia and Gelb, 2010).

2.3.1.2. NS pathogenetics: consequences of SHP2 mutations. Structural, biochemical, and functional data have allowed understanding of the consequences of NS-associated mutations of SHP2 on its structure/activity (Darian et al., 2011; Keilhack et al., 2005; Martinelli et al., 2012, 2008; Qiu et al., 2014; Tartaglia et al., 2010; Tartaglia and Gelb, 2010). Most of the mutations (e.g. D61G, D61Y, E76K) are concentrated in mutational hotspots within or close to the N-SH2 and PTP domains, and hit residues involved in the autoinhibitory interaction, thereby opening the molecule and resulting in increased phosphatase activity (Darian et al., 2011; Keilhack et al., 2005; Niihori et al., 2005; Tartaglia et al., 2006). As for other mutations borne by SH2 domains (T42A, D106A or E139D), they result in increased affinity of SH2 domains for phosphotyrosines, so that corresponding mutants are more sensitive and activable. Another kind of mutation, located between the SH2 domains, affects N-SH2 domain flexibility and interaction capacity with the PTP domain (Martinelli et al., 2008; Qiu et al., 2014). Therefore, all SN-associated mutations of SHP2 are GOF mutations, favoring its open conformation and resulting in its hyperactivation, albeit milder than JMML-causing mutations (Tartaglia et al., 2006) (Fig. 3). This difference in strength may explain why SN-, but not JMML-, associated mutations are germline. Supporting this view, heterozygous expression of JMML-causing mutants is embryonic lethal (Chan et al., 2009; Xu et al., 2011). Coherent with the signal-enhancing role of SHP2 on the RAS/MAPK pathway, various functional analyses have demonstrated that hyperactivating, NS-associated mutations of SHP2, induce a hyperactivation of ERK1/2 both *in vitro* and *in vivo*, in different cell types, in the basal state as well as under stimulation by several agonists, although the molecular mechanisms involved are still poorly described (Araki et al., 2004; Bonetti et al., 2014a; Eminaga and Bennett, 2008; Fragale et al., 2004; Krenz et al., 2005; Oishi et al., 2006). More interestingly, studies in animal models of NS led to the demonstration that ERK1/2 dysregulation was causally linked to the different clinical traits of the disease (see below). Whether NS-causing SHP2 mutants disrupt other signaling pathways, notably the PI3K/AKT cascade, remains to be established. Moreover, given the existence of some genotype/phenotype correlations between *PTPN11* and non *PTPN11* cases of NS, the contribution of other dysregulated signaling pathways, already suggested in different animal models of the disease, remains to be established, notably for clinical traits that may be more specifically associated to SHP2 mutations (Oishi et al., 2006; Paardekooper Overman et al., 2014) (Fig. 4).

2.3.1.3. Pathophysiology of SHP2 mutation-linked NS. Better understanding of the pathophysiology of NS came with the development of animal models mimicking the human disease. Indeed, global or targeted expression of SHP2 mutants (transgenic or knock-in models) in flies, zebrafish and mice, resulted in the development of a disease phenotype, allowing the pathophysiological mechanisms to be deciphered, underlying the development of the main clinical traits of NS.

2.3.1.3.1. Cardiopathies. The physiopathology of SHP2 mutation-driven cardiac diseases in NS has been extensively studied in animal models of the diseases (Lauriol et al., 2014). Mice or zebrafish expressing NS-causing SHP2 variants developed cardiac defects and abnormal valve development (Araki et al., 2009, 2004; Bonetti et al., 2014a; Jopling et al., 2007; Krenz et al., 2008). Using advanced targeted knock-in approaches in various cardiac cells, Araki et al. nicely demonstrated that cardiac developmental alterations arose from enhanced endocardial-mesenchymal transformation of endocardial cells (Araki et al., 2009). Moreover, impaired elongation of heart tube, defective heart laterality and cardiomyocyte migration were evidenced in zebrafish expressing NS-associated SHP2 mutants (Bonetti et al., 2014a). Interestingly, in mice embryos, endocardial cushions displayed enhanced ERK1/2 activation, suggesting that ERK1/2 hyperactivation could participate in NS pathophysiology. Several studies then confirmed this hypothesis, showing that SN mutants-driven cardiac defects could be rescued by genetic or pharmacological inhibition of the RAS/MAPK pathway (Araki et al., 2009; Bonetti et al., 2014a; Krenz et al., 2008; Nakamura et al., 2007).

2.3.1.3.2. Growth retardation and craniofacial defects. All mice models of SHP2 mutation-driven NS developed so far, including knock-in mice for D61G and N308D mutations as well as transgenic mice with neural crest cell-targeted expression (Wnt1Cre) of the Q79R variant, display short stature (Araki et al., 2004, 2009; Eminaga and Bennett, 2008; Fragale et al., 2004; Krenz et al., 2005; Nakamura et al., 2009b). A study in $SHP2^{D61G/+}$ mice revealed that growth retardation appears postnatally, the animals displaying normal weight and length at birth (De Rocca Serra-Nedelec et al., 2012). Interestingly, a hyperphosphorylation of ERK1/2 was measured in several developing tissues and early treatment with a selective MEK inhibitor significantly improved growth velocity in $SHP2^{D61G/+}$ and Wnt1Cre:Q79R mice (De Rocca Serra-Nedelec et al., 2012; Nakamura et al., 2009b). Consistent with this finding, pharmacological inhibition of the Ras/MAPK pathway also improved growth in other NS mouse models (Chen et al., 2011; Wu et al., 2011). Regarding the underlying mechanisms, it has been shown that growth delay in $SHP2^{D61G/+}$mice came along, as for NS patients, with reduced IGF-I levels, which were partially normalized upon chronic MEK inhibition (De Rocca

Serra-Nedelec et al., 2012). Moreover, RAS/MAPK hyperactivation at the bone/cartilage levels could participate in growth plate dysfunction and subsequent growth retardation, as already demonstrated for other short stature-associated diseases, notably achondroplasia (Yamashita et al., 2014).

NS mouse models, as well as zebrafish expressing NS mutants of SHP2, all display craniofacial defects that are reminiscent of facial dysmorphia in patients (Araki et al., 2009, 2004; Jopling et al., 2007; Nakamura et al., 2009b). Interestingly, this abnormality could be reverted in NS mice by MEK inhibition during gestation, supporting a role for ERK1/2 hyperactivation in promoting craniofacial defects in NS (Nakamura et al., 2009b).

2.3.1.3.3. Other defects. Little is known about the pathophysiology of other clinical traits associated with SHP2 mutations-driven NS. Concerning NS-associated JMML, RAS/MAPK and PI3K/AKT hyperactivation, as well as STAT3 hypophosphorylation, have been measured in NS-mutant expressing hematopoietic cells, but the respective contribution of these dysregulations to leukemogenesis has not been precisely assessed (Araki et al., 2004; Zhang et al., 2009b). Constitutive expression of NS mutants in transgenic fruit flies resulted in a developmental phenotype, ranging from embryonic lethality to wing vein abnormalities depending on the allele strength (Oishi et al., 2006). Moreover, Pagani et al. nicely showed that overexpression of several NS-associated SHP2 mutants in central nervous system in drosophila resulted in long term memory (LTM) deficit, an effect that was ascribed to sustained MAPK activation, which impaired the spacing effect necessary for LTM induction (Pagani et al., 2009). Recently, Lee et al. demonstrated that $SHP2^{D61G/+}$, and to a lesser extent $SHP2^{N308D/+}$, mice displayed impaired spatial learning and memory and defective synaptic plasticity, which were associated with increased ERK1/2 phosphorylation in hippocampus. Importantly, MEK inhibition, but also lovastatin treatment, which can dampen down basal RAS/MAPK activation by decreasing RAS isoprenylation, rescued this phenotype (Lee et al., 2014). Expression of NS-associated SHP2 mutants has been also correlated with mitochondrial dysfunction, which could participate in the development of some traits of the disease, although this has not been demonstrated so far (Lee et al., 2010).

Altogether, the current data converges on a critical role of ERK1/2 hyperactivation in NS pathogenesis. Supporting this view, non *PTPN11* cases of SN are all associated, so far, with mutations of genes encoding actors or regulators of the RAS/MAPK cascade that result in its hyperactivation (Roberts et al., 2013; Tartaglia et al., 2010). This notion could be extended to almost all Rasopathies cases, leading to the concept of RAS/MAPK inhibition as a potent therapeutic option. Importantly, proof-of-concept studies have already been performed to define statins, through their inhibitory effect on RAS isoprenylation, as promising therapeutic strategies to alleviate Rasopathies' symptoms (Lee et al., 2014; Li et al., 2005; Mainberger et al., 2013).

2.3.2. Noonan syndrome with multiple lentigines

2.3.2.1. Clinical presentation. NS-ML, formerly named LEOPARD syndrome with reference to the major symptoms of the disease (multiple Lentigines, ECG conduction abnormalities, Ocular hypertelorism, Pulmonic stenosis, Abnormal genitalia, Retardation of growth, and sensorineural Deafness), is also a polymalformative autosomal dominant genetic disease. Phenotypically very close to NS, it has distinctive features, notably cutaneous defects and deafness. Much rarer than NS, NS-ML has a prevalence of less than 10.000 live births, but misdiagnosis may occur given the phenotypical redundancy with other Rasopathies (Sarkozy et al., 2008). NS-ML shares with NS the same phenotypical triad. However, although it is difficult to draw definite conclusions given the small number of cases, facial dysmorphism and growth retardation seem to be milder. According to Digilio et al., weight and height at birth are usually in the normal range, although one third of newborns have higher-than-normal weight (Digilio et al., 2006). Growth retardation, defined as height SD below −2, may only affect about 30% of patients with SN-ML, thus being less prominent than in NS (Carcavilla et al., 2013; Sarkozy et al., 2008). Although there is no definite data concerning body composition in SN-ML patients, a recent study in a small cohort has reported that most of patients with NS-ML have lower-than-average BMI (Tajan et al., 2014). Concerning heart defects, contrary to NS, the vast majority of NS-ML patients (80%) display HCM, making it a cardinal feature of NS-ML (Limongelli et al., 2007; Sarkozy et al., 2004, 2008). NS-ML-associated HCM in general affects the left ventricle, with outflow obstruction for 40% of cases, and can be congenital or develop during childhood. Cardiological follow-up is required given the risk of heart failure or sudden death (Lauriol et al., 2014; Limongelli et al., 2007, 2008; Woywodt et al., 1998). Lentigines are a major feature of NS-ML, found in more than 90% of cases (Martinez-Quintana and Rodriguez-Gonzalez, 2012). They can be present at birth but usually appear in mid-childhood, mostly on the face, neck, and upper body, their number increasing up to several thousand by the time patients reach puberty. “Café-au-lait” spots are also present for half of patients, and more rarely hypo-pigmented lesions (Sarkozy et al., 2008). Various other abnormalities have been described in NS-ML, including skeletal defects (pectus carinatum or excavatum), genital impairment, mild mental retardation or learning disabilities, and neurosensorial deafness. Tumor predisposition has also been suggested, although the low number of cases prevents absolute conclusion (Choi et al., 2003; Laux et al., 2008; Seishima et al., 2007; Ucar et al., 2006).

2.3.2.2. NS-ML pathogenetics: consequences of SHP2 mutations. Assessing the structural and functional outcomes of NS-ML causing mutations of SHP2 has also been central to the understanding of NS-ML pathophysiology. Remarkably, all mutations are located within the PTP domain, and hit conserved residues that are necessary for PTP activity (e.g. Y279 that defines substrate specificity, T468 in the signature motif) (Lauriol and Kontaridis, 2011; Sarkozy et al., 2008). Coherently, biochemical assessment, using different methodological approaches, revealed that all NS-ML mutations collapsed SHP2's basal and induced phosphatase activity (Edouard et al., 2010; Hanna et al., 2006; Kontaridis et al., 2006; Marin et al., 2011; Martinelli et al., 2008; Tartaglia et al., 2006; Yu et al., 2013b). However, in contrast to “true” catalytically-inactive mutants (e.g. SHP2 C459S), NS-ML mutants retain some catalytic activity, albeit low, and are stimulatable, features that may play key roles in NS-ML pathogenesis (Oishi et al., 2008; Yu et al., 2013b). Further data argues against a simple haplo-insufficiency being the cause of NS-ML. First, $SHP2^{+/-}$ mice do not develop any developmental defect reminiscent of NS-ML clinical presentation (Saxton et al., 1997). Second, in contrast to MC, none of the SN-ML mutation results in SHP2 loss of expression, and MC and NS-ML are clinically distinct diseases (Bowen et al., 2011), providing strong evidence that the mutants associated with NS-ML cannot be merely LOF. On the other hand, NS-ML mutants may have a dominant negative effect over the WT form of SHP2. Indeed, structural modelization showed that NS-ML associated mutations, notably Y279C and T468M mutations, favor SHP2′ open conformation and association with its upstream partners (Kontaridis et al., 2006; Qiu et al., 2014). As a consequence, NS-ML SHP2 mutants display increased binding capacity to their upstream effectors when compared to WT SHP2. Thus, by interacting with their activators (e.g. IRS1, GAB1) more rapidly, more sustainably and more “sensitively” (i.e. for lower agonist doses) than WT SHP2, NS-ML-

associated SHP2 mutants have a binding advantage that could prevent WT SHP2 recruitment and activation (Kontaridis et al., 2006; Martinelli et al., 2008; Yu et al., 2013b).

The demonstration that NS-ML SHP2 mutants have reduced activity and dominant negative effect over the WT SHP2 obviously raised the question about how NS- ans NS-ML mutants, while displaying opposite biochemical effects, can result in related syndromes. Answering this question requires understanding of the functional consequences of these mutations, which largely remains to be explored and is a matter of debate (Edouard et al., 2007). Indeed, the first studies revealed that overexpression of several NS-ML mutants in HEK cells or in zebrafish embryos resulted in reduced ERK1/2 activation in response to several agonists (Kontaridis et al., 2006; Qiu et al., 2014; Stewart et al., 2010). Moreover, in the first mouse model of NS-ML, heterozygously carrying that Y279C mutation, Marin et al. also observed a hypoactivation of ERK1/2 in the heart, following treatment with different agonists (Marin et al., 2011). On a mechanistic point of view, this effect could result from the above-described preferential recruitment of a catalytically-impaired variant of SHP2, which could prevent WT SHP2 from activating RAS/MAPK (Kontaridis et al., 2006; Qiu et al., 2014). However, different studies have highlighted that NS-ML-associated mutants of SHP2 could have a dominant positive effect on RAS/MAPK activation. Indeed, in flies and zebrafish, expression of NS-ML mutants resulted in ERK1/2 hyperactivation and, interestingly enough, in a comparable phenotype as animals expressing NS mutants (ectopic wing veins development in flies, defective heart displacement in zebrafish) (Bonetti et al., 2014a; Oishi et al., 2006; Oishi et al., 2008). Moreover, at odds with the aforementioned report, Yu et al. described that expression of NS-ML mutants in HEK cells resulted in ERK1/2 hyperactivation in response to EGF (Yu et al., 2013b). These apparent discrepancies might be explained, *ceteris paribus*, by the difference in agonist dose or in mutants' expression level. Insulin-evoked MAPK activation was also enhanced in mouse embryonic fibroblasts expressing NS-ML mutants (Tajan et al., 2014). Moreover, an original model of induced pluripotent stem cells (iPSC)-derived cardiomyocytes from NS-ML patients also displayed MEK1 basal hyperactivation (Carvajal-Vergara et al., 2010). Further supporting a dominant positive effect of NS-ML SHP2 mutants on RAS/MAPK, a basal hyperactivation of ERK1/2 has been observed in heart extracts from mice overexpressing the Q510E mutant of SHP2 and a hyperactivation of MAPK was measured in several tissues of $SHP2^{T468M/+}$ mice following insulin injections (Schramm et al., 2012; Tajan et al., 2014). Given the positive role of SHP2 in RAS/MAPK activation, how its catalytically impaired derivatives can hyperactivate this signaling pathway remains a burning question. Yu et al. recently proposed a model to explain this GOF effect of NS-ML mutants on MAPK activation, in which their agonist hypersensitivity could compensate for their residual activity, and even confer on them signal-enhancing properties (Yu et al., 2013b). Although additional investigations are required, this hyperactivation of the RAS/MAPK, at least upon some conditions, could explain the phenotypical similarities between NS-ML and NS, and other Rasopathies.

Regarding the PI3K/AKT cascade, it has been shown in transfected cells, in cutaneous fibroblasts from patients, as well as in cardiomyocyte cell line or in newborn rat cardiomyocytes, that NS-ML mutants induce a hyperactivation of the PI3K pathway in response to EGF, as revealed by the hyperactivation of both AKT and its downstream targets Glycogen synthase kinase 3 (GSK3), mechanistic Target of Rapamycin (MTOR), RPS6KB1/p70S6K, or Ribosomal protein S6 (Edouard et al., 2010; Ishida et al., 2011; Schramm et al., 2013, 2012). These findings have been confirmed *in vivo*, a hyperphosphorylation of AKT and/or of its targets being measured in the heart of NS-ML mutant-expressing zebrafish (Bonetti et al., 2014a), and in hypertrophic heart and in insulin-sensitive tissues of mouse models of NS-ML (Marin et al., 2011; Schramm et al., 2012; Tajan et al., 2014). Regarding the underlying mechanisms, a hyperphosphorylation of FAK has been described, that could trigger PI3K/AKT activation (Ishida et al., 2011). Conversely, it has been proposed that SN-LM mutants may be defective in dephosphorylating PI3K-binding sites, which combined with their dominant negative effect on the recruitment of WT SHP2, may result in sustained PI3K activation (Edouard et al., 2010; Hanna et al., 2006). Importantly, this hyperactivation of the PI3K pathway has been causally linked to NS-ML pathophysiology, notably to the HCM that preferentially associates with NS-ML, suggesting that this signaling characteristic could explain the particular symptoms of NS-ML (see below) (Fig. 4).

2.3.2.3. Pathophysiology of SHP2-associated NS-ML. As for SN, the development of animal models provided key insights into SN-ML pathophysiology.

2.3.2.3.1. Cardiopathies. The pathophysiological link between NS-ML-causing SHP2 mutants and NS-ML-associated cardiac defects has been explored *in vivo* and *in vitro*. Zebrafish expressing NS-ML mutants developed similar cardiac defects as NS mutant-expressing animals (Bonetti et al., 2014a; Jopling et al., 2007; Stewart et al., 2010). In contrast, knock-in mice heterozygously expressing the Y279C or the T468M variant of SHP2, as well as mice with cardiomyocyte-specific overexpression (β-MHC (Myosin Heavy Chain) promoter) of the Q510E mutant, all developed HCM, likely with prenatal onset (Marin et al., 2011; Schramm et al., 2012; Tajan et al., 2014). In zebrafish models, while heart dysfunction was initially associated with ERK1/2 hypoactivation, a recent study demonstrated that a treatment with a MEK inhibitor could rescue the cardiac phenotype in zebrafish expressing NS-ML-associated SHP2 mutants, suggesting that hyperactivation of the RAS/MAPK pathway could participate in NS-ML-associated cardiopathy (Bonetti et al., 2014a; Stewart et al., 2010). These apparent discrepancies could find an explanation in the fact that altering positively or negatively the fine-tuned regulation of the MAPK pathway during heart development might result in similar defects, given that a precise level or duration of MAPK activity is required to elicit a specific proliferative or pro-differentiation response. Further supporting a role for RAS/MAPK hyperactivation in NS-ML-associated cardiopathy, Schramm et al. reported that MEK inhibition could partially revert the hypertrophic phenotype of cardiomyocytes expressing the Q510E mutant of SHP2. However, given the absence of MAPK hyperactivation in their model, the authors ascribed this effect to crosstalks between RAS/MAPK and PI3K/MTOR pathways (Schramm et al., 2013). Indeed, several studies have identified a causative role for PI3K hyperactivation in NS-ML-associated HCM. Overexpression of NS-ML mutants of SHP2 in chicken embryos, cardiomyocyte cell line or isolated newborn rat cardiomyocytes resulted in PI3K-dependent hypertrophy of cardiac cells (Edouard et al., 2010; Ishida et al., 2011; Schramm et al., 2013). Moreover, in $SHP2^{Y279C/+}$ and in transgenic bMHC-Q510E SHP2 mice, HCM development correlated with AKT and/or MTOR hyperactivation, probably as a result of sustained PI3K activation, and could be alleviated by chronic treatment with Rapamycin (Marin et al., 2011; Schramm et al., 2012). These studies led to the concept that NS-ML-driven hyperactivation of the PI3K/AKT/TOR pathway could promote HCM, and, importantly, that rapamycin may be used to efficiently cure this cardiopathy. More importantly, a very recent study revealed that a 12 week-everolimus treatment in a NS-ML infant with rapidly progressive HCM could improve cardiac function, although no reversion of the severe hypertrophy was observed, suggesting that such therapy could potently alleviate

NS-ML-associated HCM if started early to avoid irreversible cardiac remodeling (Hahn et al., 2015).

2.3.2.3.2. Growth retardation, craniofacial defects, and other features. NS-ML mouse models, as well as zebrafish expressing NS-ML mutants of SHP2, display similar craniofacial defects as NS models, suggesting that these features could arise from RAS/MAPK hyperactivation, although this has not been proved to date (Jopling et al., 2007; Marin et al., 2011; Tajan et al., 2014). In addition, moderate growth retardation was documented in two mice models of NS-ML, heterozygously carrying the Y279C or the T468M mutations, but the molecular mechanisms involved have not been described yet (Marin et al., 2011; Tajan et al., 2014). Since RAS/MAPK hyperactivation has been causally linked to growth retardation in NS and other diseases, an explanation for this phenotypic variation could be that, in growth-controlling cells or systems, RAS/MAPK could be differentially disrupted by NS-ML mutants. Moreover, and interestingly, it has to be noticed that patients suffering from other syndromes with mosaic hyperactivation of the PI3K/AKT signaling pathway display segmental overgrowth, in support of the well-known growth-promoting effect of the PI3K/AKT pathway. Therefore, one may hypothesize that NS-ML-associated PI3K hyperactivation could soften growth delay in NS-ML when compared with NS, and other Rasopathies (Lindhurst et al., 2012, 2011; Riviere et al., 2012).

Besides established features, the recent metabolic characterization of SHP2$^{T468M/+}$ mice revealed a lean phenotype, associating reduced adiposity and improved carbohydrate metabolism, which could be reverted by MEK inhibitor and rapamycin treatment, respectively. Defective adipogenesis, increased energy expenditure and mitochondria activity/biogenesis, as well as improved insulin sensitivity have been proposed as causal events to this phenotype (Tajan et al., 2014). Whether this metabolic phenotype is, at least in part, a common trait of Rasopathies or a particular feature of NS-ML, and whether such metabolic imbalances participate in the development of other traits of the diseases remain to be established.

Therefore, in light of the current knowledge, *PTPN11*-associated NS-ML could be caused by the combined dysregulation of the RAS/MAPK and the PI3K/AKT pathways. Whether NS-ML is linked to RAS/MAPK hypo-or hyperactivation still requires investigation. While MAPK hypoactivation has been reported in NS-ML, some evidence exists and some mechanisms have been proposed in favor of RAS/MAPK hyperactivation, which would also be expected to explain the clinical similarities with other Rasopathies and with *BRAF/RAF1*-associated NS-ML, which are all caused by activating mutations of the RAS/MAPK pathway (Pandit et al., 2007; Sarkozy et al., 2009; Tidyman and Rauen, 2009). Besides this, PI3K/AKT/MTOR hyperactivation has been causally linked to NS-ML pathogenesis and pinpoints rapamycin as an effective treatment of HCM in SHP2-dependent NS-ML. Of note, whether PI3K/AKT/TOR hyperactivation is a hallmark of this particular condition remains an open question. If so, it could explain some specific symptoms, such as increased HCM risk but also lentigines or increased insulin sensitivity, these latter also being found in some PI3Kpathies (Bauer and Stratakis, 2005; Pal et al., 2012). Conversely, it remains to be understood how NS-ML-associated BRAF/RAF1 mutations, which may only affect MAPK signaling, result in similar phenotypes as those caused by SHP2 mutations. However, since MAPK and PI3K are intimately linked, one may also consider that joined dysregulation of both pathways, to various levels, could be a common feature in Rasopathies, since PI3K/AKT hyperactivation has also been described in Costello syndrome or type 1 Neurofibromatosis (Dasgupta et al., 2005; Rosenberger et al., 2009).

To conclude, SHP2 is a multifaced protein that plays multiple roles in organism homeostasis and physiology, notably through its dual activity on the RAS/MAPK and the PI3K/AKT signaling pathways. Additional non signaling fields of action (e.g. mitochondria activity, regulation of epigenetic modulators), as well as regulatory events, will certainly add some more complexity into SHP2's global function, even if possible. Consistent with such pleiotropic effects, genetic mutations that alter SHP2's function/activity have major pathological consequences. While the underlying molecular and pathophysiological mechanisms start to be uncovered and open interesting therapeutic perspectives, many questions remain unanswered regarding the precise impact of the mutations on SHP2-dependent signaling cascades as well as other functions, and the relative contribution of the different mutation-driven alterations on diseases development and progression. Moreover, besides genetic diseases, it is likely that acquired SHP2 deregulation can participate in other pathologies (cancer, cardio-vascular diseases, diabetes…), which will broaden its pathophysiological spectrum. Future work will certainly shed some light on these unsolved issues.

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