Multicentric osteolytic syndromes represent a phenotypic spectrum defined by defective collagen remodelling

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ABSTRACT

Frank-Ter Haar syndrome (FTHS), Winchester syndrome (WS) and multicentric osteolysis, nodulosis, and arthropathy (MONA) are ultra-rare multisystem disorders characterized by craniofacial malformations, reduced bone density, skeletal and cardiac anomalies, and dermal fibrosis. These autosomal recessive syndromes are caused by homozygous mutation or deletion of respectively *SH3PXD2B* (SH3 and PX Domains 2B), *MMP14* (matrix metalloproteinase 14) or *MMP2*. Here, we give an overview of the clinical features of 63 previously reported patients with *SH3PXD2B*, *MMP14* or *MMP2* mutation, demonstrating considerable clinical overlap between FTHS, WS and MONA. Interestingly, the protein products of *SH3PXD2B*, *MMP14* and *MMP2* directly cooperate in collagen remodelling. We review animal models for these three disorders that accurately reflect the major clinical features and likewise show significant phenotypical similarity with each other. Furthermore, they demonstrate that defective collagen remodelling is central in the underlying pathology. As such, we propose a nosological revision, placing these *SH3PXD2B*, *MMP14* and *MMP2* related syndromes in a novel “defective collagen-remodelling spectrum” (DECORS). In our opinion, this revised nosology better reflects the central role for impaired collagen remodelling, a potential target for pharmaceutical intervention.

KEY WORDS

Vanishing bone syndrome, MMP14, MMP2, SH3PXD2B, ECM remodelling, skeletal dysplasia, podosomes

DEFINITIONS

AR, autosomal recessive; BMD, bone mineral density; ECM, extracellular matrix; ENU, *N*-ethyl-*N*-nitrosourea; FTHS, Frank-Ter Haar syndrome; KO, knockout; MMP, matrix metalloproteinase; MONA, multicentric osteolysis, nodulosis, and arthropathy; MT-MMP, membrane-type matrix metalloproteinase; MVP, mitral valve prolapse; *Nee*, nose, eye, ear; PI3,4P2, phosphatidylinositol 3,4-bisphosphate; *Sabe*, small and bugged-eyed; SH3PXD2B, SH3 and PX Domains 2B; TKS4, Tyrosine Kinase Substrate With Four SH3 Domains; TS, Torg syndrome; VSD, ventricle septum defect; WS, Winchester syndrome.

INTRODUCTION

In 1973, Frank et al. described an 18-month-old girl with a newly identified multisystem disorder consisting of multiple skeletal anomalies, craniofacial dysmorphism, a systolic cardiac murmur and developmental delay [Frank et al. 1973]. Ter Haar et al. and later Hamel et al. reported four members of a single family with a similar phenotype [Hamel et al. 1995; Ter Haar et al. 1982]. Due to multiple affected individuals in the reported families, in addition to consanguinity, the absence of vertical transmission, and both sexes being equally affected, autosomal recessive (AR) inheritance was suggested. [Hamel et al. 1995; Ter Haar et al. 1982]. Maas et al. subsequently reported four additional patients. Based on the resemblance with the individuals described by Frank et al. and Ter Haar et al., Maas and colleagues suggested that all of these patients represent a novel entity that they named Frank-Ter Haar syndrome (FTHS, MIM 249420) [Maas et al. 2004]. Borrone et al. described two brothers with similar features [Borrone et al. 1993]. In these two patients, as well as individuals previously diagnosed with FTHS, we identified homozygous mutations in *SH3PXD2B* (MIM #613293) [Wilson et al. 2014].

Intriguingly, FTHS shows significant clinical overlap with the “vanishing bone” syndromes, a group of rare skeletal disorders characterized by excessive bone resorption [Martignetti et al. 2001; Sidwell et al. 2004; Zankl et al. 2005]. Historically, three different AR multicentric osteolytic syndromes have been distinguished: Winchester syndrome (WS, MIM #277950), multicentric osteolysis, nodulosis, and arthropathy (MONA, MIM #259600), and Torg syndrome (TS) [Evans et al. 2012; Martignetti et al. 2001; Rouzier et al. 2006; Sidwell et al. 2004; Winchester et al. 1969; Zankl et al. 2005]. These disorders typically present with swelling of the small joints of hands and feet causing deformity, with gradual involvement of more proximal

joints during early childhood [Martignetti et al. 2001; Prapanpoch et al. 1992; Sidwell et al. 2004; Winchester et al. 1969; Zankl et al. 2005]. WS, MONA and TS have been considered as distinct entities based on their supposed differences in anatomic distribution and severity of the osteolysis, in addition to associated syndromic features [Evans et al. 2012; Rouzier et al. 2006; Zankl et al. 2005]. However, sequence analysis revealed the presence of homozygous loss-of-function mutations in *MMP2* (MIM 120360) in patients with clinical diagnoses of WS, MONA, as well as TS. Consequently, it was suggested that these three syndromes constitute a spectrum [Azzolini et al. 2014; Ekbote et al. 2014; Jeong et al. 2010; Martignetti et al. 2001; Rouzier et al. 2006; Tuysuz et al. 2009; Zankl et al. 2005; Zankl et al. 2007]. Nevertheless, Evans et al. were of the opinion that the phenotype of the two patients originally reported by Winchester et al. differed from that of patients with an *MMP2* mutation [Brown et al. 1970; Evans et al. 2012; Winchester et al. 1969]. Subsequent genetic analysis of these two individuals identified a homozygous *MMP14* missense mutation (MIM 600754) [Evans et al. 2012]. In 2007, we reported two patients with a phenotype similar to that of the patients described by Borrone et al. [Borrone et al. 1993; Vanagt et al. 2004; Van Steensel et al. 2007]. However, we did not identify deleterious changes of *SH3PXD2B* or its homolog *SH3PXD2A*. Rather, we found a novel homozygous missense *MMP14* mutation in these patients [Wilson et al. 2014]. We recently reported that it mostly likely represents a hypomorphic allele [De Vos et al. 2018].

Since the first mutations were identified in FTHS, MONA and WS patients, several additional individuals with a mutation in *SH3PXD2B* or *MMP2* have been reported. Here, we give a detailed overview of the clinical features in 63 previously published patients with a homozygous *SH3PXD2B*, *MMP2* or *MMP14* mutation. We

outline the functional connections between SH3PXD2B, MMP2 and MMP14, and review current insights in the underlying pathophysiology from *in vivo* models.

DISCUSSION AND REVIEW

The clinical features of all documented individuals with a confirmed homozygous missense mutation in or deletion of respectively *SH3PXD2B* (n = 20), *MMP14* (n = 4) or *MMP2* (n = 39) are summarised in Table 1 (see Table SI for details). Most of the reported mutations are unique to a single or few families. As expected with AR inheritance, roughly half of the reported patients are male [Al Aqeel et al. 2000; Al Kaissi et al. 2011; Azzollini et al. 2014; Bader-Meunier et al. 2016; Bendon et al. 2012; Bhavani et al. 2016; Borrone et al. 1993; Castberg et al. 2013; Chang et al. 2017; Eisenstein et al. 1998; Ekbote et al. 2014; Gok et al. 2010; Hamel et al. 1995; Iqbal et al. 2010; Jeong et al. 2010; Maas et al. 2004; Martignetti et al. 2001; Mégarbané et al. 1997; Phadke et al. 2007; Pichler et al. 2016; Prapanpoch et al. 1992; Rouzier et al. 2006; Temtamy et al. 2012; Ter Haar et al. 1982; Tuysuz et al. 2009; Vanatka et al. 2010; Van Steensel et al. 2007; Wilson et al. 2014; Winchester et al. 1969; Zankl et al. 2005; Zankl et al. 2007; Zrhidri et al. 2017].

CLINICAL OVERLAP BETWEEN FTHS, MONA, AND WS

In the majority of patients, mutations of *SH3PXD2B*, *MMP14* or *MMP2* result in a phenotype primarily affecting bone, heart and skin. As mutations in *MMP14* have only been reported twice, in a total of four patients, statements concerning the effects of these mutations need to be interpreted with care.

Skeletal abnormalities—Bone mineral density (BMD) was reduced in all individuals with either an *SH3PXD2B*, *MMP14* or *MMP2* mutation in whom BMD was

assessed (Table I). Osteolysis was present in all individuals with an *SH3PXD2B* or *MMP2* mutation in which it was sought for, in addition to half of the reported patients with an *MMP14* mutation. A small majority of examined individuals with an *MMP2* mutation had a short stature, in addition to multiple individuals with an *SH3PXD2B* or *MMP14* mutation. Brachydactyly was evident in ≥50% of examined individuals with mutations in any of the three genes. Kyphoscoliosis has been reported in most examined patients with an *SH3PXD2B* or *MMP14* mutation, in addition to several individuals with an *MMP2* mutation. Other structural anomalies of the vertebral column, such as Scheuermann-like changes, are additionally present in multiple individuals with an *SH3PXD2B*, *MMP14* or *MMP2* mutation. Joint destruction, characterized by peri-articular bone loss and erosion of the of the articular surface, was present in all examined individuals with an *MMP2* mutation, in addition to half of the reported patients with an *MMP14* mutation. The majority of examined patients with an *SH3PXD2B*, *MMP14* or *MMP2* mutation had flexion deformities or contractures, most often of fingers. Finally, the anterior fontanel was found to be prominent in all patients with an *SH3PXD2B* or *MMP14* mutation in which it was examined, and open sutures were reported in most of them. These latter two features have not been reported for individuals with an *MMP2* mutation [Al Aqeel et al. 2000; Al Kaissi et al. 2011; Azzollini et al. 2014; Bader-Meunier et al. 2016; Bendon et al. 2012; Bhavani et al. 2016; Borrone et al. 1993; Castberg et al. 2013; Chang et al. 2017; Eisenstein et al. 1998; Ekbote et al. 2014; Gok et al. 2010; Hamel et al. 1995; Iqbal et al. 2010; Jeong et al. 2010; Maas et al. 2004; Martignetti et al. 2001; Mégarbané et al. 1997; Phadke et al. 2007; Pichler et al. 2016; Prapanpoch et al. 1992; Rouzier et al. 2006; Temtamy et al. 2012; Ter Haar et al. 1982; Tuysuz et al.

2009; Vanatka et al. 2010; Van Steensel et al. 2007; Wilson et al. 2014; Winchester et al. 1969; Zankl et al. 2005; Zankl et al. 2007; Zrhidri et al. 2017].

Craniofacial dysmorphism—All reported patients had some degree of craniofacial dysmorphism (Fig. 1A and Table I). Most examined individuals with an *SH3PXD2B*, *MMP14* or *MMP2* mutation had coarse facial features. Brachycephaly was present in all examined patients with an *SH3PXD2B* mutation, in addition to several patients with an *MMP2* or *MMP14* mutation. A prominent forehead and hypertelorism were present in all examined patients with an *SH3PXD2B* or *MMP14* mutation and several individuals with an *MMP2* mutation. The palpebral fissures of the majority of examined patients with an *SH3PXD2B*, *MMP14* or *MMP2* mutation were down-slanted. A thick sub-ocular fold was present in the majority of individuals with an *SH3PXD2B*, *MMP14* or *MMP2* mutation. A flat nasal bridge was observed in most examined patients with an *SH3PXD2B* mutation, but less frequently in patients with an *MMP14* or *MMP2* mutation. Most examined individuals with an *SH3PXD2B* or *MMP14* mutation had a broad mouth, as well as one third of examined patients with an *MMP2* mutation. Finally, thick lips and either a small or heavy mandible have been reported in most examined patients with an *SH3PXD2B*, *MMP14* or *MMP2* mutation [Al Aqeel et al. 2000; Al Kaissi et al. 2011; Azzollini et al. 2014; Bader-Meunier et al. 2016; Bendon et al. 2012; Bhavani et al. 2016; Borrone et al. 1993; Castberg et al. 2013; Chang et al. 2017; Eisenstein et al. 1998; Ekbote et al. 2014; Gok et al. 2010; Hamel et al. 1995; Iqbal et al. 2010; Jeong et al. 2010; Maas et al. 2004; Martignetti et al. 2001; Mégarbané et al. 1997; Phadke et al. 2007; Pichler et al. 2016; Prapanpoch et al. 1992; Rouzier et al. 2006; Temtamy et al. 2012; Ter Haar et al. 1982; Tuysuz et al. 2009; Vanatka et al. 2010; Van Steensel et al. 2007; Wilson

et al. 2014; Winchester et al. 1969; Zankl et al. 2005; Zankl et al. 2007; Zrhidri et al. 2017].

Ophthalmic anomalies—The majority of examined individuals with an *SH3PXD2B* mutation, as well as about half of the examined patients with an *MMP2* mutation had prominent eyes (Table I). Corneal opacities, traditionally associated with WS, were present in half of the reported patients with an *MMP14* mutation, in addition to several individuals with an *SH3PXD2B* or *MMP2* mutation [Al Aqeel et al. 2000; Al Kaissi et al. 2011; Azzollini et al. 2014; Bendon et al. 2012; Bhavani et al. 2016; Castberg et al. 2013; Chang et al. 2017; Eisenstein et al. 1998; Ekbote et al. 2014; Gok et al. 2010; Hamel et al. 1995; Iqbal et al. 2010; Jeong et al. 2010; Maas et al. 2004; Martignetti et al. 2001; Mégarbané et al. 1997; Phadke et al. 2007; Pichler et al. 2016; Prapanpoch et al. 1992; Rouzier et al. 2006; Temtamy et al. 2012; Ter Haar et al. 1982; Tuysuz et al. 2009; Vanatka et al. 2010; Van Steensel et al. 2007; Wilson et al. 2014; Winchester et al. 1969; Zankl et al. 2005; Zankl et al. 2007; Zrhidri et al. 2017].

Dental anomalies—Gingival hypertrophy has been reported in ≥50% of examined individuals with an *SH3PXD2B*, *MMP14* or *MMP2* mutation (Table I). Delayed dentition, attributed to gingival hypertrophy, was less frequently reported [Al Aqeel et al. 2000; Bader-Meunier et al. 2016; Bhavani et al. 2016; Borrone et al. 1993; Chang et al. 2017; Eisenstein et al. 1998; Ekbote et al. 2014; Martignetti et al. 2001; Mégarbané et al. 1997; Phadke et al. 2007; Prapanpoch et al. 1992; Temtamy et al. 2012; Tuysuz et al. 2009; Van Steensel et al. 2007; Wilson et al. 2014; Winchester et al. 1969; Zankl et al. 2007].

Cutaneous abnormalities—Although acne is the most common skin disorder in the general population, its severity in patients with an *SH3PXD2B* or *MMP14*

mutation suggests that it might be part of the phenotype (Table I). Acne has not been reported in patients with an *MMP2* mutation, possibly due to the prepubertal age at which these patients were described. Skin thickening has additionally been reported in most examined patients with an *SH3PXD2B*, *MMP14* or *MMP2* mutation. Notably, subcutaneous fibrocollagenous nodules, previously considered as typical for MONA, have been observed in patients with a mutation of either *SH3PXD2B* or *MMP14*, though are more frequent among patients with an *MMP2* mutation [Al Aqeel et al. 2000; Al Kaissi et al. 2011; Azzollini et al. 2014; Bader-Meunier et al. 2016; Bendon et al. 2012; Bhavani et al. 2016; Borrone et al. 1993; Castberg et al. 2013; Chang et al. 2017; Eisenstein et al. 1998; Ekbote et al. 2014; Gok et al. 2010; Jeong et al. 2010; Martignetti et al. 2001; Phadke et al. 2007; Pichler et al. 2016; Prapanpoch et al. 1992; Rouzier et al. 2006; Temtamy et al. 2012; Tuysuz et al. 2009; Vanatka et al. 2010; Van Steensel et al. 2007; Wilson et al. 2014; Winchester et al. 1969; Zankl et al. 2005; Zankl et al. 2007].

Cardiac anomalies—Mitral valve prolapse (MVP) and/or regurgitation have been reported in about half of the examined individuals with an *SH3PXD2B* mutation, in addition to several patients with an *MMP14* or *MMP2* mutation (Table I). Ventricle septum defect (VSD) and outflow tract anomalies have additionally been documented in patients with an *SH3PXD2B* mutation and are less frequent in individuals with an *MMP2* mutation. These cardiac anomalies caused early childhood death of multiple patients [Al Kaissi et al. 2011; Azzollini et al. 2014; Bendon et al. 2012; Bhavani et al. 2016; Borrone et al. 1993; Castberg et al. 2013; Chang et al. 2017; Ekbote et al. 2014; Gok et al. 2010; Hamel et al. 1995; Iqbal et al. 2010; Maas et al. 2004; Mégarbané et al. 1997; Pichler et al. 2016; Temtamy et al. 2012; Ter Haar et al.

1982; Tuysuz et al. 2009; Van Steensel et al. 2007; Wilson et al. 2014; Zrhidri et al. 2017].

Developmental delay—Consistent with the deformities and joint problems described above, about half of the examined patients with an *SH3PXD2B*, *MMP14* or *MMP2* mutation experienced delayed motor development (Table I). Cognitive disability has only been reported in a single patient with an *SH3PXD2B* mutation, and therefore is unlikely to be part of the phenotype [Al Aqeel et al. 2000; Al Kaissi et al. 2011; Azzollini et al. 2014; Bendon et al. 2012; Borrone et al. 1993; Castberg et al. 2013; Chang et al. 2017; Eisenstein et al. 1998; Ekbote et al. 2014; Gok et al. 2010; Hamel et al. 1995; Iqbal et al. 2010; Jeong et al. 2010; Maas et al. 2004; Martignetti et al. 2001; Mégarbané et al. 1997; Phadke et al. 2007; Pichler et al. 2016; Prapanpoch et al. 1992; Rouzier et al. 2006; Temtamy et al. 2012; Ter Haar et al. 1982; Tuysuz et al. 2009; Vanatka et al. 2010; Van Steensel et al. 2007; Wilson et al. 2014; Winchester et al. 1969; Zankl et al. 2005; Zankl et al. 2007].

The phenotypic similarities outlined above suggest that FTHS, WS and MONA could be considered as parts of a continuous spectrum. Such change in nosology reflects the central role of impaired collagen remodelling in the three disorders and is supported by the functional connections between the protein products of *SH3PXD2B*, *MMP14* and *MMP2* in collagen remodelling. These will be discussed in the following section.

MATRIX METALLOPROTEINASES

Matrix metalloproteinases (MMPs) are a family of endopeptidases that rely on a metal ion for their catalytic activity [Birkedahl-Hansen et al. 1993; Sternlicht et al. 2001]. In humans, 24 different MMPs have been identified [Strongin 2010]. While the

majority are secreted into the extracellular space, the six membrane-type (MT-) MMPs are membrane-bound [Massova et al. 1998; Page-McCaw et al. 2007; Sternlicht et al. 2001]. All known MMPs are synthesized as latent zymogens that require cleavage to achieve activity. MT-MMPs are activated intracellularly and soluble MMPs are activated after secretion [Sternlicht et al. 2001]. As their name implies, MMPs can hydrolyse almost every structural component of the extracellular matrix (ECM) [McKleroy et al. 2013]. In addition, they are able to cleave non-structural ECM components such as cytokines and growth factors, as well as cell-surface receptors [Massova et al. 1998]. Each MMP has a distinct yet overlapping substrate specificity [Birkedahl-Hansen et al. 1993]. MMPs have been shown to be involved in invasion of cells into dense matrices during developmental processes such as angiogenesis, primary bone formation and bone remodelling, as well as in pathological events such as metastasis. In both cases, MMPs facilitate invasive cell motility by degrading the surrounding ECM, including the basement membrane [Chun et al. 2004; Hiraoka et al. 1998; Hoshino et al. 2013; Krane et al. 2008; Rundhaug 2003; Sabeh et al. 2004; Sternlicht et al. 2001].

SH3PXD2B, MMP14 AND MMP2 FACILITATE INVASIVE CELL MOTILITY BY MEDIATING PODOSOME FUNCTION

MMP14 (also known as MT1-MMP) was the first membrane-bound MMP to be discovered [Sato et al. 1994]. It was shown to be widely expressed in many human tissues including bone, articular cartilage and skin. In mice, MMP14 is highly expressed in (peri-) skeletal tissues during embryonic development [Apte et al. 1997; Holmbeck et al. 1999; Kinoh et al. 1996, Rose et al. 2016]. MMP14 is tethered to the membrane by a C-terminal transmembrane domain [Koziol et al. 2012; Sato et al.

1994; Takino et al. 1995]. It is trafficked through the endoplasmic reticulum, Golgi apparatus and trans-Golgi network to specific regions of the plasma membrane involved in ECM adhesion, degradation and invasion. These include focal adhesions, as well as lamellipodia, filopodia and podosomes at the leading edge of migrating cells [Friedl et al. 2009; Koziol et al. 2012; Nakahara et al. 1997; Poincloux et al. 2009; Sato et al. 1994; Sato et al. 1997; Williams et al. 2012; Zarrabi et al. 2011]. For trafficking to podosomes, MMP14 relies on the scaffold protein Src-homology3 (SH3) And Phox-homology (PX) Domain-Containing Protein 2B (SH3PXD2B, also known as TKS4) [Buschman et al. 2009].

MMP14 can hydrolyse a vast array of substrates [Koziol et al. 2012] including fibrillar collagen [Hiraoka et al. 1998; McKleroy et al. 2013; Ohuchi et al. 1997; d'Ortho et al. 1997; Poincloux et al. 2009; Sternlicht et al. 2001]. MMP14 additionally cleaves transmembrane receptors and can thereby alter cellular behaviour [Koziol et al. 2012]. For example, digestion of β3-integrin by MMP14 was found to promote adhesion and directional migration of MCF-7 cells *in vitro* [Deryugina et al. 2000]. In addition, MMP14 can cleave and thereby activate pro-MMPs, including pro-MMP2 [Ohuchi et al. 1997; Sato et al. 1994; Takino et al. 1995]. Several animal models demonstrated an important role for MMP14-dependent collagen remodelling in the resulting phenotype (see below) [Holmbeck et al. 1999; Zhou et al. 2000; De Vos et al. 2018]. In addition, we have shown *in vitro* that MMP14's ability to activate pro-MMP2 correlates with disease severity of Winchester syndrome in humans [De Vos et al. 2018].

MMP2 is the most widely expressed metalloproteinase [Rozanov et al. 2001]. [Birkedahl-Hansen et al. 1993; Liotta et al. 1979]. It is secreted into the extracellular space as an inactive zymogen (pro-MMP2), and subsequently activated by

membrane-bound MMP14 [Emonard et al. 1992; Evans et al. 2012; Krane et al. 2008; Sternlicht et al. 2001; Takino et al. 1995]. Activated MMP2 can degrade fragments of fibrillar collagen cleaved by other MMPs such as MMP14 [McKleroy et al. 2013]. In doing so, MMP2 directly cooperates with MMP14 and augments its collagenolytic activity [Ohuchi et al. 1997; Sato et al. 1994; Takino et al. 1995]. The relevance of MMP2 activity is reflected by the overlap between the MMP14 and MMP2-related human phenotypes, as outlined above [Al Aqeel et al. 2000; Al Kaissi et al. 2011; Azzollini et al. 2014; Bader-Meunier et al. 2016; Bhavani et al. 2016; Castberg et al. 2013; De Vos et al. 2018; Eisenstein et al. 1998; Ekbote et al. 2014; Gok et al. 2010; Jeong et al. 2010; Martignetti et al. 2001; Phadke et al. 2007; Pichler et al. 2016; Rouzier et al. 2006; Temtamy et al. 2012; Tuysuz et al. 2009; Vanatka et al. 2010; Zankl et al. 2005; Zankl et al. 2007].

MMP14, MMP2 and SH3PXD2B are all present at podosomes, specialized membrane structures that are transiently formed by cell types whose roles require invasive motility, including osteoclasts, macrophages and endothelial cells. Invasive cancer cells and viral oncogene-transformed fibroblasts form similar structures called invadopodia [Buschman et al. 2009; Gawden-Bone et al. 2010; Hoshino et al. 2013; Linder et al. 2005; Murphy et al. 2011; Seano et al. 2014]. In contrast to MMP14 and MMP2, SH3PXD2B has no catalytic activity but rather helps facilitate invadopodia and podosome formation. In 2D culture, podosomes and invadopodia present as dome-shaped membrane protrusions at the surface that is in contact with the matrix or coating on which the cell is growing. Whereas podosomes are present at the leading edge of the cell and are typically 0.5-2 μm in size, invadopodia can achieve larger dimensions and are primarily found underneath the nucleus [Gawden-Bone et al. 2010; Linder et al. 2005; Murphy et al. 2011]. Despite this difference in size and

location, both have a highly similar molecular composition [Buschman et al. 2009]. Ultra-structurally, they consist of a core surrounded by the so-called ring region. The core region contains filamentous actin and associated proteins regulating actin polymerisation. The ring contains integrins, integrin-binding and signalling proteins, along with MMP14 and scaffolding proteins including SH3PXD2B and its homolog SH3PXD2A [Buschman et al. 2009; Gawden-Bone et al. 2010; Lanyi et al. 2011; Linder et al. 2005; Murphy et al. 2011]. In mice, expression of *Sh3pxd2b* was demonstrated to be highest in 11 to 17-day-old embryos. It is widely expressed in adult tissues [Buschman et al. 2009].

The formation of podosomes is a multistep process, summarized in Figure 2A and reviewed in detail elsewhere [Murphy et al. 2011; Hosino et al. 2013]. Briefly, during podosome maturation, SH3PXD2B, MMP14 and MMP2 cooperate to activate the freshly formed structure. SH3PXD2B recruits active MMP14 from intracellular stores to the nascent podosome membrane, where MMP14 subsequently cleaves secreted pro-MMP2 (Fig. 2A(iii)). Together, MMP14 and MMP2 initiate focal pericellular ECM degradation [Bendon et al. 2012; Buschman et al. 2009; Iqbal et al. 2010; Massova et al. 1998; Murphy et al. 2011; Poincloux et al. 2009].

The functional link between SH3PXD2B, MMP14 and MMP2 is further highlighted by the (partially) overlapping phenotypes of several mutant animal models that have been respectively identified or generated. Investigations utilising these animal models furthermore provided novel insights in the processes underlying the skeletal phenotype.

MUTATION OR KNOCKOUT OF MMP14 IN MICE AND ZEBRAFISH RESULTS IN PHENOTYPES THAT STRONGLY RESEMBLE WS

There are two mouse models with homozygous *Mmp14* missense mutations. The *Sabe* (small and bugged-eyed) mutation (p.R92C) arose spontaneously in the C57BL/6J background (Fig. 1B(i)), whereas the *Cartoon* mutation (p.S466P) was serendipitously recovered in an *N*-ethyl-*N*-nitrosourea (ENU) screen aimed at identifying immunological phenotypes in the C57BL/6J background (Fig. 1B(ii)) [Curtain et al. 2012; Du et al. 2013]. These different mutations in *Mmp14* result in a near identical phenotype, which is similar to that of *Mmp14* knockout (KO) mice and, importantly, shares key aspects with that of WS patients (Fig. 1B(iii)) [Gutierrez-Fernandez et al. 2015; Holmbeck et al. 1999; Zhou et al. 2000]. Interestingly, in zebrafish (*Danio rerio*), knockout of *mmp14a/b* also results in a constellation of abnormalities that strongly resembles WS including short stature, thoracic kyphosis, reduced BMD and craniofacial dysmorphology (Fig. 1C(i)) [De Vos et al. 2018]. All models have a gradually worsening phenotype encompassing short stature, progressively reduced BMD, thoracic kyphosis and craniofacial dysmorphology affecting the forehead, midface and jaw [Curtain et al. 2012; De Vos et al. 2018; Du et al. 2013; Gutierrez-Fernandez et al. 2015; Holmbeck et al. 1999; Van Steensel et al. 2007; Winchester et al. 1969; Zhou et al. 2000]. Interestingly, they also exhibit features not observed in humans, including early death due to wasting in the mice, respectively foramen magnum stenosis with spinal cord impingement in the fish [Curtain et al. 2012; De Vos et al. 2018; Du et al. 2013].

In *Mmp14* KO mice and *mmp14a/b* KO fish the early steps in osteogenesis, including the generation of cartilage *Anlagen* and initial ossification, proceed normally. However, subsequent bone remodelling is aberrant in both [De Vos et al.

2018; Holmbeck et al. 1999; Zhou et al. 2000]. Bone formation in mice and zebrafish proceeds either by endochondral (within a cartilage precursor), or intramembranous ossification (mesenchymal condensation in close association with cartilage) and these processes are conserved in humans [Bird et al. 2003; Holmbeck et al. 2006]. Knockout of *Mmp14* respectively *mmp14a/b* disturbs both of these processes, primarily by impairing the remodeling of non-mineralized collagen matrix, and thereby the timely and correct remodeling of cartilage templates [De Vos et al. 2018; Holmbeck et al. 1999; Parichy et al. 2009; Schilling 2002; Szabova et al. 2009; Witten et al. 2009; Zhou et al. 2000]. Consequently, bone formation, as well as skeletal growth, is reduced [De Vos et al. 2018; Holmbeck et al. 1999]. Increased osteoclastic bone resorption further contributes to the reduced BMD seen in mice [Holmbeck et al. 1999; Zhou et al. 2000]. In *Mmp14* KO mice, numbers of osteoclasts are increased, and osteoclastic bone resorption was observed adjacent to aberrantly remodeled bone-soft tissue interfaces [Hikita et al. 2006; Holmbeck et al. 1999; Holmbeck et al. 2006; Zhou et al. 2000]. This process has been suggested by Holmbeck and colleagues to act as a compensating mechanism, reducing the stress on (peri-) skeletal tissues caused by the impaired soft tissue remodeling, in addition to enabling continued growth up to some extent [Holmbeck et al. 1999; Holmbeck et al. 2006].

Additionally, MMP14 has been demonstrated to stimulate the osteogenic potential of murine bone marrow stem cells [Holmbeck et al. 1999; Szabova et al. 2009]. Their commitment to the osteogenic lineage over the chondrogenic and adipogenic lineage in 3D *in vitro* culture is stimulated by catalytic remodeling of the ECM by MMP14 [Tang et al. 2013]. Accordingly, mice with mesenchymal progenitor cell and skeletal stem cell-specific *Mmp*14 KO have thickened cartilage, increased

bone-marrow adiposity, and delayed membranous ossification and general osteopenia without increase in osteoclast number. Their appearance shares similarities with the *Mmp14* KO mice, including short length and brachycephaly with exophthalmos and a short snout (Fig. 1B(iv)) [Tang et al. 2013].

MOUSE SH3PXD2B MUTANTS SHARE KEY PHENOTYPIC FEATURES WITH FTHS

Murine *Sh3pxd2b* mutants recapitulate key aspects of FTHS. Moreover, they share key phenotypic features with the MMP14 mutants. Mice that lack functional Sh3pxd2b due to a truncating mutation (*Nee* (nose, eyes, ear), Fig. 1B(v)) or gene KO (Fig. 1B(vi)) are indistinguishable from their littermates at birth. After weaning, mutants develop growth retardation, craniofacial malformations that include brachycephaly, a short snout, exophthalmos, and excessive thoracic kyphosis [Dülk et al. 2016; Iqbal et al. 2010; Mao et al. 2009]. Although about 20% of *Sh3pxd2b* KO mice died of unknown causes within their first weeks, the life span of surviving KO mice, as well as that of *Nee* mutants, is unaffected [Iqbal et al. 2010]. In both mouse models, BMD was reduced and osteogenic differentiation was impaired [Dülk et al. 2016]. The mice also had various cardiac abnormalities, including septal thinning and mitral valve defects [Iqbal et al. 2010].

MMP2 KNOCKOUT MICE SHARE SOME FEATURES WITH MONA

In contrast to humans, loss of *Mmp2* in mice results in a milder and partially transient phenotype. From birth, *Mmp2* KO mice are slightly smaller, and develop a short snout and a dome-shaped skull with hypertelorism [Inoue et al. 2006; Itoh et al. 1997; Mosig et al. 2007]. However, the increased intercanthal distance and skull

height normalise over time [Mosig et al. 2007]. The BMD is generally and progressively reduced in *Mmp2* KO mice except in the calvarian bones, whose density increased at adult age [Inoue et al. 2006; Mosig et al. 2007]. Notably, *Mmp2* KO was found to impair osteoblast and osteoclast proliferation *in vitro*, but osteoblast activity intensified in *Mmp2* KO mice at later age which could, at least in part, explain the observed calvarial BMD increase [Inoue et al. 2006; Mosig et al. 2007]. Similar to the pathological fractures in several patients with MMP2 mutation, the observed osteopenia in *Mmp2* KO mice is accompanied by spontaneous tibia fractures [Bader-Meunier et al. 2016; Inoue et al. 2006; Jeong et al. 2010; Pichler et al. 2016]. Finally, adult *Mmp2* KO mice have bone erosion underlying articular cartilage destruction of the knee [Mosig et al. 2007].

MMP14 PRIMARILY ACTS THROUGH MMP2 IN HUMANS, BUT NOT IN MICE

Interestingly, the phenotype of *Mmp2*;*Mmp14* double KO mice is more severe than that of either single KO. The double KO mice are smaller than their *Mmp2* KO littermates and have generally smaller skeletal elements [Oh et al. 2004]. Epiphyseal vascular ingrowth and cartilage remodelling is impaired in the double KO mice similar to *Mmp14* KO mice, but these abnormalities are already present perinatally and only develop several weeks after birth in *Mmp14* KO mice [Oh et al. 2004]. Finally, double KO mice die perinatally due to respiratory failure, which the authors attributed to impaired skeletal muscle development [Oh et al. 2004]. Our *in vitro* results show that impaired pro-MMP2 activation plays an important role in the MMP14 mutant phenotype in humans [De Vos et al. 2018]. However, the milder and partially transient phenotype of *Mmp2* KO mice indicates that, at least in mice, MMP14 only temporarily acts through activation of MMP2 in specific tissues. Also, MMP14 might

have additional functions in mice that do not require MMP2 [De Vos et al. 2018; Holmbeck et al. 1999; Itoh et al. 2001; Itoh et al. 2006; Itoh et al. 2008; Rozanov et al. 2001].

CONCLUSION

Although the disease mechanism was not elucidated at the time, Borrone and colleagues already suspected that the simultaneous involvement of skin, joints, bone and heart pointed to involvement of ECM proteins [Borrone et al. 1993]. Indeed, mutations of *SH3PXD2B*, *MMP14* and *MMP2*, the products of which directly cooperate in collagen remodelling, were later identified in FTHS, WS and MONA patients [Evans et al. 2012; Martignetti et al. 2001; Wilson et al. 2014]. The three proteins are conserved from human to fish, and their direct functional link is reflected in considerable phenotypic overlap between FTHS, WS and MONA in humans, as well as in the corresponding animal models. These models collectively indicate that impaired degradation of collagen fibrils is central to the underlying pathology. This is reflected in the resulting phenotypes, in which collagen-rich tissues that are heavily remodelled during growth and development are primarily affected. It is generally accepted that SH3PXD2B is an upstream regulator of MMP14 membrane localisation, which in turn activates pro-MMP2 [Murphy et al. 2011; Ohuchi et al. 1997; Sato et al. 1994; Takino et al. 1995]. Multiple lines of evidence support this hierarchical order, with a key role for MMP2 in collagen remodelling in humans. Firstly, the phenotype of patients with homozygous mutation of *SH3PXD2B*, *MMP14* and *MMP2* is highly similar. Secondly, we and others have demonstrated *in vitro* that the severity of the phenotype in patients with *MMP14* mutation correlates with the level of impairment in pro-MMP2 activation [De Vos et al. 2018; Evans et al. 2010;

Van Steensel et al. 2007]. Thirdly, *Sh3pxd2b* KO mice display significant phenotypic overlap with *Mmp14* KO mice. The phenotype of *Mmp14*;*Mmp2* double KO mice is more severe, whereas KO of *Mmp2* results in a milder, partially transient phenotype. Together, these points suggest that in humans, MMP14 primarily acts through MMP2, whereas in mice MMP2 is less important. Only when MMP14 is absent does MMP2 loss have an additive effect, which is suggestive of incomplete epistasis.

In the 9th edition of the *Nosology and Classification of Genetic Skeletal Disorders*, the Fank-Ter Haar syndrome is classified under the “filamin group and related disorders”, while *MMP2* related pathology is grouped as the Torg-Winchester syndrome under the “osteolysis group” [Bonafe et al. 2015]. No *MMP14*-related skeletal disorder is included in the current edition. Given the clinical overlap and common pathology, we propose to group the *SH3PXD2B*, *MMP14* and *MMP2*-related multicentric osteolytic syndromes in the “defective collagen-remodelling spectrum” (DECORS). The central role of impaired collagen remodelling in DECORS has therapeutic implications [De Vos et al. 2018]. Bisphosphonates have previously been used to treat reduced BMD in patients with *MMP14* respectively *MMP2* mutations, with limited therapeutic benefit [De Vos et al. 2018; Phadke et al. 2007; Pichler et al. 2016; Van Steensel et al. 2007]. Bisphosphonates primarily act through inhibition of osteoclastic bone resorption, which contributes to the observed BDM reduction [De Vos et al. 2018; Drake et al. 2010]. However, as the main problem in DECORS seems to be defective collagen remodelling, we propose that any pharmacotherapy should be aimed at targeting this issue [De Vos et al. 2018]. The animal models discussed in this review, in particular the zebrafish, can be used for the development and testing of novel therapeutic strategies aimed at correcting the defective ECM remodelling [De Vos et al. 2018].

CONFLICTS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Table I – Clinical features associated with *SH3PXD2B*, *MMP14* or *MMP2* mutation.

Clinical feature		*SH3PXD2B* %	*SH3PXD2B* n/N	*MMP14* %	*MMP14* n/N	*MMP2* %	*MMP2* n/N
Craniofacial	Coarse facial features	75	12/16	100	4/4	75	25/33
	Prominent forehead	100	20/20	100	4/4	27	6/22
	Brachycephaly	100	17/17	33	1/3	42	3/7
	Hypertelorism	100	20/20	100	2/2	38	8/21
	Deep-set eyes	0	0/17	100	3/3	19	4/21
	Anti-mongoloid slant	85	12/14	100	2/2	57	12/21
	Thick sub-ocular folds	82	14/17	100	3/3	52	12/23
	Flat nasal bridge	94	16/17	50	2/4	40	9/22
	Broad mouth	77	14/18	100	3/3	34	8/23
	Thick lips	80	12/15	100	3/3	75	18/24
	Small/large mandible	84	16/18	100	4/4	78	18/23
Ophthalmic	Prominent eyes	94	17/18	0	0/2	43	10/23
	Congenital glaucoma	25	4/16	0	0/2	5	1/20
	Large cornea	62	10/16	0	0/2	0	0/21
	Corneal opacities	28	2/7	50	2/4	11	3/27
Dental	Gingival hypertrophy	100	7/7	50	2/4	50	11/22
	Delayed dentition	100	2/2	50	2/4	0	0/1
Skeletal	Short stature	75	3/4	50	2/4	60	18/30
	Prominent anterior fontanel	100	17/17	100	2/2	0	0/1
	Open sutures	6	4/6	100	1/1	0	0/4
	Kyphoscoliosis	65	11/17	67	2/3	43	7/16
	Vertebral anomalies	87	7/8	75	3/4	47	8/17
	Brachydactyly	100	20/20	50	2/4	71	15/21
	Bowing of long bones	78	7/9	33	1/3	87	7/8
	Joint destruction	0	0/2	50	2/4	100	35/35
	Osteolysis	100	7/7	50	2/4	100	36/36
	Reduced BMD	100	4/4	100	4/4	100	36/36
	Flexion deformity / contractures	69	11/16	100	3/3	96	32/33
	Talipes equinovares	67	10/15	0	0/3	16	1/6
	Starts in small joints	100	1/1	50	2/4	100	20/20
Cutaneous	Subcutaneous nodules	75	3/4	50	2/4	80	28/35
	Thickened skin	100	3/3	100	2/2	75	6/8
	Acne	25	2/8	100	2/2	0	0/19
Cardiac	MVP / regurgitation	55	11/20	100	2/2	5	1/19
	Outflow tract abnormalities	46	6/13	0	0/2	11	2/19
	VSD	35	7/20	0	0/4	22	4/18
Developmental	Cognitive disability	8	1/13	0	0/4	0	0/16
	Motor disability	56	9/16	50	2/4	14	2/14

Prevalence is shown as the number (n) or percentage (%) of patients with a feature out of the total number of individuals (N) that were examined for that feature. Details per original report are listed in Supplemental Table I [Al Aqeel et al. 2000; Al Kaissi et al. 2011; Azzollini et al. 2014; Bader-Meunier et al. 2016; Bendon et al. 2012; Bhavani et al. 2016; Borrone et al. 1993; Castberg et al. 2013; Chang et al. 2017; Eisenstein et al. 1998; Ekbote et al. 2014; Gok et al. 2010; Hamel et al. 1995; Iqbal et al. 2010; Jeong et al. 2010; Maas et al. 2004; Martignetti et al. 2001; Mégarbané et al. 1997; Phadke et al. 2007; Pichler et al. 2016; Prapanpoch et al. 1992; Rouzier et al. 2006; Temtamy et al. 2012; Ter Haar et al. 1982; Tuysuz et al. 2009; Vanatka et al. 2010; Van Steensel et al. 2007; Wilson et al. 2014; Winchester et al. 1969; Zankl et al. 2005; Zankl et al. 2007; Zrhidri et al. 2017].

Abbreviations: BMD, bone mineral density; MVP, mitral valve prolapse; NR, no record; VSD, ventricle septum defect.

Supplemental Table I – Detailed clinical features of included individuals with identified homozygous *SH3PXD2B*, *MMP14* or *MMP2* mutation.

	SH3PXD2B											*MMP14*				*MMP2*																	
	Ter Haar et al. 1982; Hamel et al. 1995	Borrone et al. 1993	Mégarbané et al. 1997	Maas et al. 2004	Iqbal et al. 2010	Bendon et al. 2012	Wilson et al. 2014	Chang et al. 2017	Zrhidri et al. 2017	Total		Winchester et al. 1969; Prapanpoch et al. 1992	Van Steensel et al. 2007	Total		Martignetti et al. 2001; Al Aqeel et al. 2000	Eisenstein et al. 1998; Zankl et al. 2007	Zankl et al 2005	Rouzier et al. 2006	Phadke et al. 2007	Tuysuz et al. 2009	Gok et al. 2010	Jeong et al. 2010	Vanatka et al. 2011	Pichler et al. 2016; Al Kaissi et al. 2011	Temtamy et al. 2012; Ekbote et al. 2014	Castberg et al. 2013	Ekbote et al. 2014	Azzollini et al. 2014	Bhavani et al. 2016	Bader-Meunier et al. 2016	Total	
Clinical feature	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**%**	**n/N**	**n/N**	**n/N**	**%**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**%**
Sex																																	
Male	2/3	2/2	1/2	0/1	4/5	2/3	1/1	1/2	0/1	**13/20**	**65**	0/2	2/2	**2/4**	**50**	1/2	0/1	0/1	0/2	1/2	1/3	2/2	0/1	0/1	0/3	1/3	1/1	0/1	1/2	10/13	0/1	**18/39**	**46**
Craniofacial																																	
Coarse facies	1/3	2/2	1/1	1/1	3/3	3/3	1/1	0/2	-	**12/16**	**75**	2/2	2/2	**4/4**	**100**	1/2	0/1	1/1	2/2	2/2	0/2	0/1	0/1	0/1	1/1	3/3	1/1	1/1	1/1	11/13	1/1	**25/33**	**75**
Prominent forehead	3/3	2/2	2/2	1/1	5/5	3/3	1/1	2/2	1/1	**20/20**	**100**	2/2	2/2	**4/4**	**100**	-	-	0/1	0/2	1/1	0/2	-	-	-	1/1	0/1	0/1	1/1	-	3/12	-	**6/22**	**27**
Brachycephaly	3/3	1/1	2/2	1/1	5/5	3/3	1/1	-	1/1	**17/17**	**100**	1/1	0/2	**1/3**	**33**	2/2	-	0/1	0/1	-	-	-	-	0/1	-	-	0/1	1/1	-	-	-	**3/7**	**42**
Hypertelorism	3/3	2/2	2/2	1/1	5/5	3/3	1/1	2/2	1/1	**20/20**	**100**	-	2/2	**2/2**	**100**	2/2	-	0/1	2/2	0/1	2/2	-	-	-	0/1	-	0/1	-	-	2/11	-	**8/21**	**38**
Deep-set eyes	0/3	0/2	0/2	0/1	0/3	0/3	0/1	0/2	-	**0/17**	**0**	1/1	2/2	**3/3**	**100**	0/2	-	0/1	0/2	0/1	0/2	-	-	-	-	-	0/1	-	-	4/12	-	**4/21**	**19**
Anti-mongoloid slant	1/3	1/1	2/2	1/1	1/3	3/3	1/1	2/2	-	**12/14**	**85**	-	2/2	**2/2**	**100**	0/1	-	1/1	2/2	0/1	1/2	-	-	-	1/1	-	1/1	-	-	6/12	-	**12/21**	**57**
Prominent eyes	3/3	-	2/2	1/1	5/5	3/3	1/1	1/2	1/1	**17/18**	**94**	-	0/2	**0/2**	**0**	2/2	-	0/1	0/2	0/1	1/2	-	-	-	1/1	2/2	0/1	-	-	4/11	-	**10/23**	**43**
Full cheeks	1/3	2/2	1/2	1/1	5/5	3/3	1/1	-	1/1	**15/18**	**83**	1/1	2/2	**3/3**	**100**	1/2	1/1	0/1	2/2	0/1	1/2	-	-	-	0/1	1/2	1/1	1/1	-	7/12	-	**15/26**	**57**
Thick subocular fold	0/3	2/2	2/2	1/1	3/3	3/3	1/1	2/2	-	**14/27**	**82**	1/1	2/2	**3/3**	**100**	1/2	1/1	1/1	1/1	1/1	1/2	-	-	-	1/1	1/1	1/1	-	-	3/12	-	**12/23**	**52**
Flat nasal bridge	3/3	1/2	2/2	1/1	3/3	3/3	1/1	2/2	-	**16/17**	**94**	2/2	0/2	**2/4**	**50**	0/1	1/1	0/1	0/2	1/1	0/2	-	-	-	1/1	-	0/1	1/1	-	5/11	-	**9/22**	**40**
Anteverted nostrils	2/2	2/2	2/2	1/1	3/5	0/3	0/1	2/2	0/1	**12/19**	**63**	1/1	0/2	**1/3**	**33**	1/2	1/1	0/1	0/2	1/1	0/2	-	-	-	-	1/1	0/1	1/1	-	7/11	-	**12/23**	**52**
Short philthrum	1/3	0/2	0/2	1/1	0/3	0/3	0/1	1/2	-	**3/17**	**18**	1/1	2/2	**3/3**	**100**	0/2	1/1	1/1	2/2	1/1	0/2	-	-	-	0/1	0/1	1/1	1/1	-	7/12	-	**14/25**	**56**
Broad mouth	1/3	2/2	0/2	1/1	5/5	3/3	1/1	-	1/1	**14/18**	**77**	1/1	2/2	**3/3**	**100**	1/2	0/1	1/1	2/2	1/1	0/2	-	-	-	0/1	0/1	0/1	-	-	3/11	-	**8/23**	**34**
Thick lips	3/3	2/2	2/2	1/1	1/3	3/3	0/1	-	-	**12/15**	**80**	1/1	2/2	**3/3**	**100**	2/2	0/1	1/1	2/2	2/2	1/2	-	-	-	-	1/1	1/1	-	-	8/12	-	**18/24**	**75**
Macroglossia	-	0/1	-	-	-	-	-	-	-	**0/1**	**0**	2/2	0/2	**2/4**	**50**	-	-	-	-	2/2	-	-	-	-	-	-	-	0/1	-	-	-	**2/3**	**66**
Prominent lower jaw	0/3	2/2	0/2	0/1	0/5	0/3	0/1	-	0/1	**2/17**	**12**	0/1	2/2	**2/3**	**67**	0/2	1/1	0/1	-	1/1	0/2	-	-	-	0/1	1/1	1/1	0/1	-	6/12	-	**10/23**	**43**
Micrognathia	3/3	0/2	2/2	1/1	5/5	1/3	1/1	-	1/1	**14/18**	**78**	2/2	0/2	**2/4**	**50**	1/2	0/1	0/1	-	0/1	2/2	-	-	-	1/1	-	0/1	1/1	-	3/12	-	**8/22**	**36**
Protruding ears	0/3	2/2	1/2	1/1	2/2	3/3	0/1	0/1	1/1	**10/15**	**67**	-	0/2	**0/2**	**0**	1/1	-	1/1	0/2	0/1	1/2	-	-	-	0/1	1/1	1/1	-	-	4/12	-	**9/22**	**40**
Ophthalmic																																	
Congenital glaucoma	1/1	-	2/2	0/1	1/5	0/3	0/1	0/2	0/1	**4/16**	**25**	-	0/2	**0/2**	**0**	0/1	0/1	-	-	-	0/2	1/1	-	-	-	0/2	-	0/1	-	0/12	-	**1/20**	**5**
Large cornea	1/1	-	1/2	0/1	5/5	0/3	0/1	2/2	1/1	**10/16**	**62**	-	0/2	**0/2**	**0**	0/1	0/1	-	-	0/1	0/2	0/1	-	-	-	0/2	-	0/1	-	0/12	-	**0/21**	**0**
Corneal opacities	-	-	2/2	0/1	-	0/3	0/1	-	-	**2/7**	**28**	2/2	0/2	**2/4**	**50**	0/1	0/1	-	0/2	0/1	1/2	0/1	0/1	0/1	-	0/2	-	0/1	1/2	1/12	-	**3/27**	**11**
Dental																																	
Broad alveolar ridges	-	-	-	1/1	3/5	-	1/1	-	-	**5/7**	**71**	-	-	**-**	**-**	-	-	-	-	-	-	-	-	-	-	-	-	0/1	-	-	-	**0/1**	**0**
Gingival hypertrophy	-	2/2	2/2	-	-	-	1/1	2/2	-	**7/7**	**100**	2/2	0/2	**2/4**	**50**	0/1	0/1	-	-	2/2	2/2	-	-	-	-	3/3	-	0/1	-	3/11	1/1	**11/22**	**50**
Delayed dentition	-	1/1	-	-	-	-	1/1	-	-	**2/2**	**100**	2/2	0/2	**2/4**	**50**	-	-	-	-	-	-	-	-	-	-	-	-	0/1	-	-	-	**0/1**	**0**
Irregularly spaced teeth	-	-	-	-	-	-	-	-	-	**-**	**-**	2/2	0/2	**2/4**	**50**	0/1	-	-	-	-	-	-	-	-	-	-	-	0/1	0/1	-	-	**0/3**	**0**
Misaligned teeth	-	-	-	-	-	-	-	-	-	**-**	**-**	1/1	1/2	**2/3**	**67**	0/1	-	-	-	-	-	-	-	-	-	-	-	0/1	0/1	-	-	**0/3**	**0**

Supplemental Table I (continued).

	SH3PXD2B											*MMP14*				*MMP2*																	
	Ter Haar et al. 1982; Hamel et al. 1995	Borrone et al. 1993	Mégarbané et al. 1997	Maas et al. 2004	Iqbal et al. 2010	Bendon et al. 2012	Wilson et al. 2014	Chang et al. 2017	Zrhidri et al. 2017	Total		Winchester et al. 1969; Prapanpoch et al. 1992	Van Steensel et al. 2007	Total		Martignetti et al. 2001; Al Aqeel et al. 2000	Eisenstein et al. 1998; Zankl et al. 2007	Zankl et al 2005	Rouzier et al. 2006	Phadke et al. 2007	Tuysuz et al. 2009	Gok et al. 2010	Jeong et al. 2010	Vanatka et al. 2011	Pichler et al. 2016; Al Kaissi et al. 2011	Temtamy et al. 2012; Ekbote et al. 2014	Castberg et al. 2013	Ekbote et al. 2014	Azzollini et al. 2014	Bhavani et al. 2016	Bader-Meunier et al. 2016	Total	
Clinical feature	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**%**	**n/N**	**n/N**	**n/N**	**%**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**n/N**	**%**
Skeletal																																	
Short stature	-	1/1	1/2	1/1	-	-	-	-	-	**3/4**	**75**	2/2	0/2	**2/4**	**50**	2/2	-	1/1	2/2	2/2	1/2	2/2	1/1	1/1	3/3	0/3	0/1	1/1	0/2	2/7	-	**18/30**	**60**
Prominent anterior fontanel	3/3	-	2/2	1/1	5/5	2/3	-	2/2	1/1	**17/17**	**100**	2/2	-	**2/2**	**100**	-	-	-	-	-	-	-	-	-	-	-	-	0/1	-	-	-	**0/1**	**0**
Open sutures	-	-	2/2	-	-	2/3	0/1	-	-	**4/6**	**67**	1/1	-	**1/1**	**100**	-	-	-	-	-	-	-	-	0/1	-	0/2	-	0/1	-	-	-	**0/4**	**0**
Kyphoscoliosis	1/3	2/2	-	0/1	4/5	1/3	1/1	2/2	1/1	**11/17**	**65**	1/1	1/2	**2/3**	**67**	-	-	0/1	2/2	-	-	-	-	1/1	-	1/1	0/1	0/1	1/2	2/7	-	**7/16**	**43**
Vertebral anomalies	-	2/2	2/2	0/1	-	-	1/1	2/2	-	**7/8**	**87**	2/2	1/2	**3/4**	**75**	0/1	-	1/1	2/2	-	-	-	1/1	1/1	-	0/2	-	0/1	-	2/7	1/1	**8/17**	**47**
Brachydactyly	3/3	2/2	2/2	1/1	5/5	3/3	1/1	2/2	1/1	**20/20**	**100**	0/2	2/2	**2/4**	**50**	2/2	-	1/1	2/2	1/1	2/2	0/1	-	1/1	1/1	2/2	0/1	-	-	3/7	-	**15/21**	**71**
Bowing of long bones	-	1/1	-	1/1	3/5	-	1/1	-	1/1	**7/9**	**78**	1/1	0/2	**1/3**	**33**	-	-	-	2/2	2/2	1/2	-	-	-	-	-	-	-	-	2/2	-	**7/8**	**87**
Limited motion	-	2/2	2/2	1/1	-	-	1/1	-	-	**6/6**	**100**	2/2	0/2	**2/4**	**50**	2/2	1/1	1/1	2/2	2/2	2/2	2/2	1/1	-	3/3	2/2	1/1	1/1	2/2	13/13	1/1	**36/36**	**100**
Joint destruction	-	0/2	-	-	-	-	-	-	-	**0/2**	**0**	2/2	0/2	**2/4**	**50**	2/2	1/1	1/1	2/2	1/1	2/2	1/1	1/1	1/1	3/3	3/3	1/1	1/1	2/2	13/13	-	**35/35**	**100**
Osteolysis	3/3	2/2	-	-	-	-	-	2/2	-	**7/7**	**100**	2/2	0/2	**2/4**	**50**	-	1/1	1/1	2/2	2/2	3/3	2/2	1/1	1/1	2/2	3/3	1/1	1/1	2/2	13/13	1/1	**36/36**	**100**
Reduced bone mineral density	-	2/2	-	1/1	-	-	1/1	-	-	**4/4**	**100**	2/2	2/2	**4/4**	**100**	2/2	-	1/1	2/2	2/2	3/3	2/2	1/1	1/1	2/2	2/2	1/1	1/1	2/2	13/13	1/1	**36/36**	**100**
Path. fractures/imp. healing	-	-	-	-	-	-	-	-	-	**-**	**-**	-	-	**-**	**-**	-	1/1	-	-	-	-	-	1/1	-	1/1	0/1	-	-	-	-	1/1	**4/5**	**80**
Arthralgia	-	-	-	-	-	-	-	-	-	**-**	**-**	1/1	0/2	**1/3**	**33**	2/2	1/1	-	-	1/1	-	0/2	-	-	1/1	1/3	-	-	1/1	10/12	-	**17/23**	**73**
Flex. deformity / contractures	-	2/2	2/2	-	2/5	1/3	1/1	2/2	1/1	**11/16**	**69**	1/1	2/2	**3/3**	**100**	2/2	1/1	-	2/2	2/2	1/2	2/2	-	-	3/3	2/2	1/1	1/1	1/1	13/13	1/1	**32/33**	**96**
Club feet	2/3	0/1	1/2	1/1	2/3	2/3	1/1	-	1/1	**10/15**	**67**	0/1	0/2	**0/3**	**0**	0/2	0/1	-	-	0/1	-	-	-	-	-	-	0/1	1/1	-	-	-	**1/6**	**16**
Enlarged metaphyses	-	-	-	-	-	-	-	-	-	**-**	**-**	1/2	0/2	**1/4**	**25**	0/2	-	-	-	-	-	2/2	-	-	1/1	-	-	-	-	-	-	**3/5**	**60**
Starts in small joints	-	-	-	1/1	-	-	-	-	-	**1/1**	**100**	0/2	2/2	**2/4**	**50**	2/2	1/1	1/1	2/2	2/2	2/2	2/2	-	1/1	1/1	2/2	1/1	1/1	1/1	-	1/1	**20/20**	**100**
Progressive to large joints	-	-	-	-	-	-	-	-	-	**-**	**-**	0/2	0/2	**0/4**	**0**	2/2	1/1	1/1	2/2	1/1	2/2	2/2	-	1/1	1/1	2/2	-	1/1	1/1	-	1/1	**18/18**	**100**
Prominent coccyx	3/3	0/1	2/2	1/1	3/5	1/3	-	2/2	1/1	**13/18**	**72**	-	0/2	**0/2**	**0**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**	**-**
Cutaneous																																	
Subcutaneous nodules	-	1/1	-	-	-	2/3	-	-	-	**3/4**	**75**	2/2	0/2	**2/4**	**50**	2/2	1/1	0/1	0/2	1/1	2/2	2/2	0/1	0/1	0/1	3/3	1/1	1/1	2/2	12/13	1/1	**28/35**	**80**
Thickened skin	-	2/2	-	-	-	-	1/1	-	-	**3/3**	**100**	-	2/2	**2/2**	**100**	2/2	-	-	1/2	-	-	-	-	-	0/1	2/2	-	-	-	-	1/1	**6/8**	**75**
Acne	-	2/2	-	-	-	0/3	0/1	0/2	-	**2/8**	**25**	-	2/2	**2/2**	**100**	0/2	-	0/1	-	0/1	0/2	-	-	-	-	-	0/1	-	-	0/12	-	**0/19**	**0**
Cardiac																																	
Myocardial damage	0/3	-	0/2	-	-	-	-	-	-	**0/5**	**0**	2/2	0/2	**2/4**	**50**	-	-	-	-	-	-	-	-	-	-	-	-	-	0/1	-	-	**0/1**	**0**
Mitral valve prolapse/regurg.	1/3	2/2	0/2	1/1	2/5	3/3	1/1	1/2	0/1	**11/20**	**55**	-	2/2	**2/2**	**100**	-	-	-	-	-	0/2	0/1	-	-	0/2	1/1	0/1	0/1	0/2	0/9	-	**1/19**	**5**
Outflow tract anomalies	1/1	0/2	0/2	0/1	1/1	2/3	0/1	2/2	0/1	**6/13**	**46**	-	0/2	**0/2**	**0**	-	-	-	-	-	1/2	0/1	-	-	0/2	0/1	1/1	0/1	0/2	0/9	-	**2/19**	**11**
Ventricle septum defect	1/3	0/2	1/2	1/1	3/5	0/3	0/1	1/2	0/1	**7/20**	**35**	-	0/2	**0/4**	**0**	-	-	-	-	-	1/2	0/1	-	-	1/2	-	1/1	0/1	0/2	1/9	-	**4/18**	**22**
Developmental																																	
Cognitive disability	0/3	0/2	0/1	1/1	-	0/3	0/1	0/2	-	**1/13**	**8**	0/2	0/2	**0/4**	**0**	0/1	0/1	0/1	0/2	0/1	0/2	0/2	0/1	0/1	0/3	0/1	0/1	-	0/2	-	-	**0/16**	**0**
Motor disability	3/3	0/2	1/1	1/1	2/3	1/3	0/1	1/2	-	**9/16**	**56**	2/2	0/2	**2/4**	**50**	1/1	-	-	-	0/1	0/2	0/2	-	-	0/3	0/1	0/1	1/1	0/2	-	-	**2/14**	**14**

A single individual (patient X-3 as reported by Ter Haar et al.) was included even though no genetic analysis was performed, as her phenotype was identical to that of her brother who had a homozygous *SH3PXD2B* mutation as identified by Iqbal et al. [Iqbal et al. 2010; Ter Haar et al. 1982]. Another patient from the same report (patient X-10 from Ter Haar et al.) was excluded, as no genetic analysis was performed and this individual is not closely related to the two other patients (7^{th} cousins). Prevalence of clinical features is shown as the number (n) or percentage (%) of patients with a feature out of the total number of individuals (N) that were examined for that feature, both per report as well as summarized per gene. Dash (-) indicates no record of that particular feature in the corresponding report. Abbreviations: flex, flexion; imp, impaired; path, pathological; regurg, regurgitation.

FIGURE LEGENDS

Figure 1 – Mutation of *SH3PXD2B*, *MMP14* and *MMP2* results in a similar phenotype in humans, mice and zebrafish. A, Mutation of *SH3PXD2B* (panel i), *MMP14* (panel ii) and *MMP2* (panel iii) in patients all result in skeletal dysplasia with significant clinical overlap and highly similar facial features (see Table 1 for details). Note the presence of a broad forehead, downslanting palpebral fissures with characteristic subocular fold, and broad mouth with full lips. **B**, murine *Mmp14* (panels i-iv) and *Sh3pxd2b* (panels v and vi) mutants share key aspects of the phenotype with patients including craniofacial malformations encompassing a relatively large neurocranium and a small snout. **C**, in zebrafish, knockout (KO) of *mmp14a/b* (3-month-old adult fish) results in a similar phenotype, including a short head with short snout and exophthalmos. Images in (A) are reproduced from Wilson et al. (i), Van Steensel et al. (ii) and Zankl et al. (iii), respectively; images in (B) are

reproduced from The Mouse Mutant Resource (The Jackson laboratory, Bar Harbor, Maine, 2007; available via www.informatics.jax.org/image/pheno/MGI: 5634011) (i), Holmbeck et al (iii), Tang et al. (iv), Mao et al. (v) and Iqbal et al. (vi); image in (C) is reproduced from De Vos et al.; all with permission [Curtain et al. 2012; De Vos et al. 2018; Holmbeck et al. 1999; Iqbal et al. 2010; Mao et al. 2009; Tang et al. 2013; Van Steensel et al. 2007; Wilson et al. 2014; Zankl et al. 2005].

Figure 2 – SH3PXD2B, MMP14 and MMP2 cooperate in podosome function. A, schematic overview of podosome formation. Binding of β1-integrin and growth factor receptors (GFR) to their respective substrates leads to the activation of the kinase SRC (panel i) [Hoshino et al. 2013; Murphy et al. 2011]. Activated SRC phosphorylates SH3PXD2B and its partners SH3PXD2A and cortactin, which are subsequently recruited to the plasma membrane at sites rich in phosphatidylinositol 3,4-bisphosphate (PI3,4P2) near focal adhesions. Here, they interact with multiple actin regulators to stimulate actin polymerization and branching during podosome assembly (panel ii) [Buschman et al. 2009; Holmbeck et al. 2003; Hoshino et al. 2013; Iqbal et al. 2010; Murphy et al. 2011]. SH3PXD2A furthermore recruits cortactin to the forming podosome, which in turn initiates the secretion of soluble pro-MMP2. SH3PXD2B recruits active MMP14 from intracellular stores to the nascent podosome membrane during podosome maturation (panel iii) [Iqbal et al. 2010; Murphy et al. 2011; Poincloux et al. 2009]. MMP14 cleaves and thereby activates secreted pro-MMP2, and together initiate focal ECM degradation [Bendon et al. 2012; Buschman et al. 2009; Massova et al. 1998; Murphy et al. 2011]. Concurrent protrusion of the podosome into the extracellular space is controlled by non-muscle myosin-II [Gawden-Bone et al. 2010]. **B**, schematic drawing of a podosome at the

ventral cell surface in 2D culture, highlighting the spatial relationship of the key players (insert). Panel (B) is adapted from Murphy et al., with permission [Murphy et al. 2011].