

Molecular Markers in Diagnostic Paediatric Bone Lesions

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Abstract: Molecular pathology has become an essential adjunct in the realm of diagnostic histopathology. While gains in this field have been enjoyed in many areas, their impact has arguably been greatest felt in bone and soft tissue pathology. The purpose of this paper is to highlight the emerging role of molecular techniques in the context of paediatric bone pathology, discussing select syndromic and neoplastic processes affecting this demographic. To this end, we review the salient molecular advances bridging both clinical research and clinical practice.

Keywords: Bone tumours, bone lesions, paediatric, molecular markers, diagnosis.

1. INTRODUCTION

Applications in molecular biology have revolutionized the practice of pathology, enhancing not only our understanding of the pathophysiology of disease, but its diagnosis and prognosis. The first use of this term was borne from a reference to early studies in the field of heredity [1], and although many credit the term to Warren Weaver at the Rockefeller Foundation [2], it remained largely undefined for over half a century until the 1950 Harvey lecture by physicist William Thomas Astbury:

It [molecular biology] is concerned particularly with the forms of biological molecules, and with the evolution, exploitation and ramification of those forms in the ascent to higher and higher levels of organisation . . . It must at the same time inquire into genesis and function [3, 4].

Arguably, the field of soft tissue pathology has benefited most from recent advances in our understanding of the molecular basis governing many of these neoplasms [5]; the corollary applies to non-neoplastic diseases affecting skeletal bone. Decades of careful morphologic and radiologic studies have long recognized a genetic component to many osteochondrodysplasias and molecular techniques have begun to establish a basis for their pathogenesis [6]. The purpose of this brief review is to summarize some of the principal molecular attributes governing neoplasms of

paediatric bone, as well as drawing attention to recent diagnostic applications to select non-neoplastic lesions.

2. FAMILIAL SYNDROMES ASSOCIATED WITH A PREDISPOSITION TO PAEDIATRIC BONE NEOPLASMS

A large number of conditions, including those with either a sporadic and/or hereditary basis, are associated with lesions involving the bones of paediatric patients. These lesions may be neoplastic in nature, or the result of non-neoplastic processes.

By far, the most common entities causing bone lesions in the paediatric population are the skeletal dysplasias, or osteochondrodysplasias, which represent a large (>370) and heterogeneous group of conditions. Discussion of these entities is beyond the scope of this review; nevertheless, some of these disorders necessitate mention. For the remainder, it should suffice to draw attention to the classification proposed by the International Skeletal Dysplasia Society together with the Nosology Group. This classification divides these entities into 37 groups, integrating morphologic details from clinical and radiologic observations together with pathogenetic and molecular findings [7].

The following is a brief overview of some of these entities. Most can be diagnosed on a clinical and morphologic basis; however, molecular testing is possible in many cases, and may be an important consideration for patients and their family members.

2.1. Bloom Syndrome

Clinically characterised by proportional dwarfism, sun-sensitive skin and immunodeficiency, affected individuals have a predisposition to neoplasia at an early age [8, 9]. While

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the majority of neoplasms are carcinomatous or lymphoproliferative in origin, osteosarcoma has been reported in the first two decades, including one patient with a prior Wilms' tumour [9].

The *BLM* gene, situated at 15q26.1, encodes DNA helicase RecQ protein-like-3 (*RECQL3*) [10, 11]. Interestingly, two other members of the RECQL family have been linked to neoplasms of bone in childhood. The *WRN/RECQL2* gene, situated at 8p12-p11.2, encodes *RECQL2*, which is linked to Werner syndrome [12]. Mutations involving the *RECQL4* gene are associated with Rothmund-Thomson syndrome (*vide infra*) [13].

2.2. Cherubism

This condition results in bilateral bony expansion of the jaw, involving mainly the mandible and occasionally the maxilla; it usually begins in the first decade and stabilizes in the second [14, 15]. Histologically, tissue from involved sites is fibrous and osteoclast-rich; it is indistinguishable from lesions arising from hyperparathyroidism and central giant cell lesion (giant cell reparative granuloma) [16].

The trait is passed in an autosomal dominant fashion and demonstrates variable penetrance. The gene maps to 4p16.3 [17, 18], and in the majority of cases is due to mutation in the Src Homology-3 Binding Protein-2 gene *SH3BP2* [19]. In a smaller number of cases other genetic disorders have been associated with the cherubism phenotype, including neurofibromatosis type 1 [20-22] and Noonan syndrome [23-27]. It is possible other entities may also lead to this phenotype.

2.3. Diaphyseal Medullary Stenosis with Malignant Fibrous Histiocytoma

Also known as Hardcastle's Syndrome [28], diaphyseal medullary stenosis represents a rare entity and is inherited as an autosomal dominant trait with high penetrance. It tends to present in late adolescence and early adulthood [29]. The long tubular bones demonstrate diaphyseal medullary stenosis and cortical thickening, and the bone is prone to fracture. Associated with this abnormality is a high incidence of secondary malignant transformation which appears to be associated with bone infarction [29-31]. The malignant tumour associated with this disease was originally described as fibrosarcoma, this has since been changed to malignant fibrous histiocytoma (pleomorphic undifferentiated sarcoma) to reflect this tumour's aggressive course [29].

While the causative gene remains to be identified, it has been mapped to 9p21-p22 and it is associated with a loss of heterozygosity at this locus [32-34].

2.4. Enchondromatosis

Also known as multiple enchondromas, this is characterised by the development of multiple intramedullary cartilaginous neoplasms. In isolation this process is referred to as Ollier disease; when combined with the presence of multiple soft tissue haemangiomas it is called Maffucci syndrome. Neither Ollier disease nor Maffucci syndrome are familial. Both entities are associated with an increased risk of chondrosarcoma [35, 36] and osteosarcoma [35, 37, 38]. Maffucci syndrome is further associated with a remote risk of angiosarcoma [39].

Mutations in the parathyroid hormone receptor 1 gene, *PTHRI*, located at 3p21-22, have been identified as causative in a minority of patients with Ollier disease [40, 41]; these observations were not confirmed by a separate group, making this the subject of ongoing study [42]. As the cause appears to be heterogeneous, it is conceivable that mutations in other components contributing to chondrocyte differentiation (e.g., Indian Hedgehog-PTH-related protein pathway) might also contribute to enchondromatosis [43]. Of note, mutations in *PTHRI* are also known to contribute to some chondrodysplasias [44, 45].

Few reports exist of cytogenetic studies in enchondromatosis. The results of studies with enchondromas have proved inconsistent with variably normal karyotypes in a number of cases [46, 47] isochromosome of the short arm of chromosome 6 [46], t(12; 15)(q13; q26) [46], as well as more complex cytogenetic findings [47-50].

2.5. Li-Fraumeni Syndrome

This represents a heterogeneous entity both in clinical presentation, and causative gene(s). Affected individuals have a profound predisposition to malignancies at a young age; this includes osteosarcoma, soft tissue sarcomas, breast carcinoma, and an eclectic host of other neoplasms [51, 52]. The classic form of this syndrome is diagnosed in a proband with a sarcoma diagnosed before 45 years of age, *and* a first degree relative with any malignancy diagnosed before 45, *and* a first or second degree relative with a sarcoma diagnosed at any age or another form of malignancy prior to 45 years of age [53]. In Li-Fraumeni-like syndrome patients do not necessarily reach the same criteria [53, 54]. Bone tumours encountered with this syndrome are primarily osteosarcomas. While most cases are identified by means of clinical history, patients and relatives may be given the opportunity for testing by direct mutational analysis.

Classically, Li-Fraumeni syndrome is caused by mutations in *p53* on 17p13.1 [55]; a smaller number of cases have been reported as attributable to mutations in *CHK2* on 22q12.1 [56-58], although this has recently been questioned [59]. A single report of an association between Li-Fraumeni and *P16INK4A* [60] has not been confirmed by others [61]. Li-Fraumeni-like syndrome appears to be caused by mutations in *p53* in a lower percentage of cases [59, 62]; some instances have instead been shown to be due to mutations in *BRCA2* [63, 64].

2.6. Multiple Osteochondroma Syndrome

Denoted by the presence of multiple osteochondromas, these lesions can be sessile or pedunculated [65]. The diagnosis is typically made on the basis of clinical and radiologic observations.

At least three variants of osteochondroma syndrome are recognized, and multiple osteochondromas can be observed as part of other unrelated syndromes [66, 67]. Multiple osteochondroma syndrome type I is caused by mutations in *EXT1* encoding exostosin-1 at chromosome 8q24.11-q24.13 [68]. For some time interstitial deletions near this region have been recognized as associated with Langer-Giedion syndrome (trichorhinophalangeal syndrome type II), which is also associated with multiple osteochondromas [69]. The type I form of multiple osteochondroma syndrome is believed to be more severe than its other variants, and is associated with a

higher rate of malignant transformation to chondrosarcomas [67]. Multiple osteochondroma syndrome type II is caused by mutations in *EXT2* encoding exostosin-2 at chromosome 11p11-p13 [70, 71]. Deletions in this region are associated with Potocki-Shaffer syndrome, which also presents with multiple osteochondromas [72]. Multiple osteochondroma syndrome type III is caused by mutations in *EXT3* on 19p encoding an as yet unspecified protein [73].

2.7. Neurofibromatosis

Generally this is divided into type I (also known as von Recklinghausen disease and peripheral neurofibromatosis), and type II (central neurofibromatosis); additional, less well-characterised variants with overlapping features, have also been described. Type I neurofibromatosis occasionally manifests with lesions of the bones, hence it merits brief consideration. For a more comprehensive review of neurofibromatosis, the reader is referred to one of several excellent recent reviews [74, 75].

Long recognized as leading to a multitude of bone changes [76] patients with type I neurofibromatosis may exhibit short stature [75], osteopenia [77], and lesions affecting the sphenoid wing, vertebrae and tibia [78]. Neurofibromatosis may also present with a cherubism phenotype [21, 22]. From the perspective of bone tumours, patients with neurofibromatosis type I may rarely develop some of the lesions traditionally found in the soft tissues, including: neurofibroma, malignant peripheral nerve sheath tumour, fibrosarcoma and malignant fibrous histiocytoma (pleomorphic undifferentiated sarcoma) [79-81]. Ossifying fibroma [82] and osteosarcoma [83] have also been reported in patients with neurofibromatosis; however, it is difficult in some instances to know whether such an association is spurious.

Type I neurofibromatosis is caused by a mutation in *NF1*, a gene encoding neurofibromin on chromosome 17q11.2 [84-87]. Mutations in this same gene are also responsible for a variant of neurofibromatosis with a Noonan syndrome-like phenotype (Neurofibromatosis-Noonan syndrome; see below) [88, 89].

2.8. Noonan Syndrome

This represents a heterogeneous syndrome broadly characterised by multiple facial anomalies such as low-set and posteriorly rotated ears, downward slanting and hypertelorism eyes, in addition to bleeding diatheses, cardiac malformations, mental retardation, short stature and webbed neck [90].

Roughly half of cases are due to missense mutations of *PTPN11*, corresponding to chromosome 12q24.1 [91]. A smaller number of cases are attributable to mutations in *SOS1* on chromosome 2p22-p21 [92, 93]; *KRAS* on chromosome 12p12.1 [94]; and, *RAF1* on chromosome 3p25 [95]. The aforementioned mutations have also been linked to a number of entities bearing remarkable phenotypic and genotypic overlap with Noonan syndrome, including: LEOPARD syndrome [96], and cardio-facio-cutaneous syndrome [97]. For this reason, classification of Noonan syndrome and the Noonan-like syndromes may possibly evolve in the future, as a result of additional molecular characterization.

Noonan-like/multiple giant cell lesion syndrome contains phenotypic overlap with cherubism. Cases with this morpho-

logy have been linked to mutations in *SOS1* [98], and *PTPN11* [26, 27]. To date, no cases of Noonan syndrome have been associated with *SH3BP2* mutations [26]. Interestingly, Noonan-like/multiple giant cell lesion syndrome may be associated with tenosynovial giant cell tumours [99-101]. An explanation for this observation is not readily apparent. Cytogenetic studies on tenosynovial giant cell tumours are limited, but a translocation t(1; 2)(p13; q37) corresponding to the *CSF1-COL6A3* gene product has been described [102, 103]. Earlier work demonstrated several cases with t(1; 2)(p11; q35-36) and individual cases with t(1; 5)(p11; q22) and t(2; 16)(q33; q24) [104]. Deletions of 1p10-1p31.3 have also been reported [105].

2.9. Retinoblastoma

This tumour typically occurs in the first decade [106]; roughly two thirds are unilateral and one third bilateral. So-called trilateral retinoblastoma refers to the additional diagnosis of a pineoblastoma in a patient with retinoblastoma [107]. Patients with bilateral retinoblastoma have a significantly increased risk of independent secondary primary tumours, with osteosarcoma being the most common [108]; other primary bone tumours include leiomyosarcoma [109-111] and Ewing sarcoma [112-116].

Early cytogenetic evidence supported a location on chromosome 13 based on the presence of retinoblastoma in patients with 13q deletion syndrome [117, 118], and tumour cytogenetics [117]. This was subsequently found to correspond to 13q14 [119], which led to the identification and cloning of the *RB* gene [120, 121]. This gene is a tumour suppressor, binding the transcription factor *E2F* to limit progression into the cell cycle [122, 123].

2.10. Rothmund-Thomson Syndrome

This syndrome is characterised clinically by growth defects, dermatosis, premature aging and an increased risk of malignancy [124]. Patients have an increased risk of osteosarcoma and osteosarcomatosis with numerous cases identified in recent decades [125, 126]. The syndrome is caused by a mutation in *RECQL4* [13].

2.11. Werner Syndrome

Patients with this syndrome are clinically short statured, exhibit premature aging and are predisposed to neoplasia [12]. These patients are prone to an array of early malignancies, including lymphoproliferative disorders, carcinoma, meningioma, melanoma and sarcoma [127, 128]. In the bone, there is a predisposition towards osteosarcoma [128], and such cases may be associated with an atypical clinical presentation [129].

Werner syndrome is due to a mutation in *RECQL2* [12]. It is occasionally referred to as adult progeria; however, this should not be confused with Hutchinson-Gilford progeria syndrome, or atypical Werner syndrome, which are caused by mutations in the lamin A gene, *LMNA* [130].

3. NEOPLASMS OF PAEDIATRIC BONE

3.1. Benign Paediatric Bone Tumours

3.1.1. Aneurysmal Bone Cyst

Aneurysmal bone cysts (ABC) primarily occur in the first two decades [131], most often effecting the metaphysis of

long bones and the spine [132]. Aneurysmal bone cysts are benign lesions which histologically contain prominent blood-filled cavernous spaces separated by fibrous septa. The septa frequently contain parallel bands of osteoid, and lack an endothelial lining. Spindle cells admixed with osteoclast-type giant cells are also frequently observed [131]. Lesions may be primary, or secondary; while many bone lesions can be associated with secondary changes, the most frequent causes include giant cell tumour, chondroblastoma and chondromyxoid fibroma [133].

The molecular alterations in primary ABC remain to be characterised fully; nevertheless, the presence of recurrent clonal karyotypic abnormalities support a neoplastic origin [134]. Cytogenetic studies have identified frequent changes involving 17p13.2 and 16q22, including a relatively common t(16; 17)(q22; p13) translocation, suggesting loci in these areas have the potential alone, or together, to contribute to the generation of ABCs [134-136]. The t(16; 17) translocation has been shown to generate a *CDH11-USP6* fusion – drawing the cadherin 11 promotor to the ubiquitin-specific protease [137] – resulting in upregulation of USP6 [138]. The breakpoint at 17p13.2 is capable of additional gene fusions, likely via a similar mechanism, including: t(1; 17), t(2; 17), t(3; 17), t(5; 17), t(9; 17), and t(17; 17) [134, 139, 140]. Identification of the 17p13.2 breakpoint locus is amenable to detection using fluorescence *in situ* hybridization [141].

3.1.2. Bizarre Parosteal Osteochondromatous Proliferation

Also known as Nora's lesion, this represents a benign osteocartilaginous lesion arising on the bone surface, typically of the digits of the hands and feet although lesions at other locations have been described [142-145]. Tumours tend to arise in the third and fourth decades, with no apparent sex-predilection [142]. Histologically, lesions are characterised by sheets of irregular bone and cartilage interfaces, and a spindle cell proliferation [142, 146]. The cartilage is hypercellular and undergoes heterotopic calcification, and the chondrocytes may be atypical, rendering the lesion worrisome for chondrosarcoma or parosteal osteosarcoma [146, 147].

Cytogenetic studies have produced mixed observations for this tumour. There are reports of two normal karyotypes [148, 149]. A case with a ring chromosome derived from chromosome 12 has been reported [148]. Two independent groups observed balanced t(1; 17) translocations [149, 150]. Fluorescence *in situ* hybridization confirmed a 17q21 break in an additional three cases [149]. The latter observation may serve to distinguish bizarre parosteal osteochondromatous proliferation from subungual exostosis [150]; however, report of a t(1; 17) translocation, in addition to t(X; 6) translocation, in a case of subungual exostosis may limit such an application [148].

3.1.3. Central Giant Cell Lesion

Also known as giant cell reparative granuloma, this is an intraosseous lesion affecting the maxilla and mandible, typically within the first two to three decades [151]. Lesions are also recognized at other sites, most notably the small tubular bones of the hands and feet [152, 153]. Histologically, lesions comprise a mononuclear spindle-to-polygonal cell population of proliferating fibroblast-like cells, admixed with osteoclast-type giant cells [154, 155]. Central giant cell lesions have been suggested to be smaller and to contain fewer nuclei

than that of giant cell tumour of bone. Also frequently present are spicules of woven bone and haemosiderin deposition [155]. Differentiation of these lesions from hyperparathyroidism, and/or giant cell tumour of bone can at times prove challenging; the clinical presentation along with the morphology of the lesion generally points to the correct diagnosis [155, 156]. Furthermore, the expression of p63 is helpful in making the diagnosis of giant cell tumour of bone [157].

Limited cytogenetic studies have been performed on these tumours. Stable translocations of t(2; 10)(q23; q24)[158] and t(X; 4)(q22; q31.3) [159], and an unstable (8; 22) translocation [160] have been reported. A recent study identified a novel exon11 mutation in *SH3BP2*, a gene linked to cherubism, in one out of four cases of central giant cell lesion [161]. Mutational studies of other *SH3BP2* exons failed to reveal exon 10 mutations in 15 cases of central giant cell lesion and 11 cases of peripheral giant cell lesion [162]; or, exon 9 mutations in nine cases of central giant cell lesion [163], or the peripheral blood of four patients previously diagnosed with central giant cell lesion [164].

3.1.4. Chondroblastoma

Also known as epiphyseal chondromatous giant cell tumour and calcifying giant cell tumour, this is a benign neoplasm occurring predominately in the first to third decades, and affecting males slightly more than females [131, 165]. The majority of lesions occur in the epiphysis of long bones, although they may arise at almost any site of secondary ossification. Histologically the tumours are characterised by sheets of polygonal mononuclear cells with distinct cell membranes. The nuclei are round and cleaved longitudinally; mitotic activity is generally present, but not pronounced. Osteoclast-type giant cells are irregularly distributed amongst the neoplastic cells and lobules of cartilage with chicken-wire calcification are helpful morphologic features [165, 166]. These latter two features tend to decrease as the patient advances in age [131]. Expression of S100 generally allows immunohistochemical distinction from most other giant cell rich lesions.

Cytogenetics studies have yielded mixed results, including some cases with no reported karyotypic abnormalities [167-169]. Others, however, have yielded varied and complex abnormalities involving chromosomes 2, 5, 8, 11, 17 and 18 [167, 168, 170]. Breakpoints have further been identified at 2q35, 3q21-23 and 18q21 [171]. A ring chromosome 4 has also been reported [172]. At present, there are no molecular features allowing reliable confirmation of this diagnosis.

3.1.5. Chondromyxoid Fibroma

Described by Jaffe and Lichtenstein in 1948, these tumours tend to occur in the second and third decades, with no obvious sex predilection [173, 174]. Lesions predominate in the metaphysis of long bones [173, 174], but many sites can be involved [175-179]. Tumours are heterogeneous in appearance comprising lobules of myxoid material and areas of fibrous tissue. Islands of hyaline cartilage may be conspicuous, but not a predominant feature. Within the myxoid regions are medium-sized spindle-stellate cells containing eosinophilic cytoplasm. Some cells may contain enlarged, hyperchromatic and multiple nuclei, but mitotic activity is generally rare. The periphery of the lobules is typically well-demarcated and

characterised by increased cellularity. Multinucleated osteoclast-type giant cells and areas of calcification may be seen [174, 180].

Numerous cytogenetic studies have revealed non-random chromosomal rearrangements involving chromosome 6 [48, 169, 171, 181-184]. Less common rearrangements, including an unusual t(1; 5)(p13; p13) translocation [175], and insertion from the short arm of chromosome 2 to the long arm of chromosome 5 [185], have also been described. Based on their potential localization, it has been suggested that *COL10A* and *PTH/PTHrP* may be involved in the pathogenesis of these lesions [181]. Interestingly, an *in vitro* study observed less expression of *PTHrP* in chondromyxoid fibroma compared to articular cartilage [186]; whether this relates to the cytogenetic changes in this lesion remains unclear. At present, molecular studies further characterizing the aforementioned cytogenetic changes have not yet been developed.

3.1.6. Desmoplastic Fibroma

Also known as desmoid tumour of bone, this is a benign neoplasm with histological semblance to that of the soft tissue desmoid [187, 188]. Lesions most frequently present in the second and third decades, with a slight male predilection [189]. Most bones can be affected, although the more common sites include the metaphysis and/or diaphysis of long bones and the mandible. Histologically the lesion is hypocellular and composed of slender fibroblasts/myofibroblasts separated by bundles of wavy collagen. Cellular atypia and mitotic activity is generally lacking [188, 190].

Limited cytogenetic and molecular studies are published on this tumour. Trisomy 8 and trisomy 20 have been identified amongst a fraction of lesional cells [191].

3.1.7. Enchondroma

The reader is directed to above discussion of enchondromatosis.

3.1.8. Fibrous Dysplasia

Fibrous dysplasia represents a benign osteofibrous proliferation arising within the intramedullary canal. Lesions may be monostotic or polyostotic, and can occur alone or as part of a constellation of entities such as McCune-Albright (polyostotic fibrous dysplasia, cafe-au-lait spots and endocrine disorders) or Mazabraud syndromes (polyostotic fibrous dysplasia and myxomas) [192]. There is no apparent sex predilection. Histologically, lesions are characterised by a background of sheets and lobules of bland spindle cells. Occasional areas with a storiform arrangement are encountered. The nuclei tend to be oval and hyperchromatic; mitotic activity is generally minimal. Irregularly shaped trabeculae [193] of disorganized woven bone are distributed throughout the lesion and are notable for a lack of prominent osteoblastic rimming [194]. Few cytogenetic studies have been reported in fibrous dysplasia, with no apparent features consistent amongst these cases [191, 195, 196].

In both syndromic [197] and non-syndromic [198] forms of fibrous dysplasia a large percentage of cases are associated with a point mutation involving the *GNAS1* gene, located at 20q13.2. To date, activating missense mutations have been reported for codon 201 in exon 8 and codon 227 in exon 9 [198]. Mutations are believed to arise at a post-zygotic stage, thus resulting in a mosaic distribution [199]. Most methods of

detection have focused on lesional tissues; however, improvements in sensitivity can allow detection from circulating cells [199].

Detection of *GNAS1* point mutations can essentially be accomplished by one of several means. The traditional approaches have been to use allele-specific oligonucleotide hybridization [200], and mutation-specific restriction enzyme digestion on polymerase chain reaction (PCR) products [198, 201]. Other methods include direct sequencing of amplified DNA products [202], COLD-PCR [203], and pyrosequencing [204]. The latter techniques have the advantage of providing more specific information regarding the nature of the detected mutation, but perhaps the disadvantage of increased cost.

3.1.9. Langerhans Cell Histiocytosis

Also known as Langerhans cell granulomatosis, eosinophilic granuloma and histiocytosis X, this lesion represents a benign proliferation of Langerhans cells. Of note, in addition to eosinophilic granuloma, the clinical lexicon for this entity includes Letterer-Siwe disease and Hand-Schuller-Christian disease; all may exhibit prominent bone involvement [205, 206]. The majority of cases arise within the first decade, although some reports extend to the elderly; males are affected roughly twice as frequently as females. This disease can affect virtually any bone; however, it most commonly involves the skull, pelvis and femur. Histologically, cases comprise nests and sheets of round-polygonal cells with abundant pale eosinophilic cytoplasm. The nuclei are oval and contain prominent clefts and grooves; mitotic activity may be prominent. Multinucleated giant cells may be a feature. Necrosis is not uncommon. The lesion is typically associated with eosinophils, and smaller numbers of lymphocytes, plasma cell and neutrophils [131, 207].

The majority of cases of Langerhans cell histiocytosis are sporadic; however, familial clustering has been reported amongst monozygotic twins [208-211]. Cytogenetic studies are inconsistent. There are reports of normal karyotypes in 32 cases [212, 213]. A lower number of patients demonstrate cytogenetic anomalies, including a case with a paracentric inversion of chromosome 13q, and another with some cells bearing a t(7; 12)(q11.2; p13) translocation [214]. Comparative genomic hybridization in a case series revealed DNA loss in several regions, including chromosomes 1, 5, 6, 7, 9, 16, 17, and 22; deletions from arms 1p, 7p, 9p, and 22q were confirmed by loss of heterozygosity analysis [215]. Common to all cases in the latter study was a 1p35-p36.3 deletion, leading the authors to speculate this may be the site of genes contributing to the pathogenesis of Langerhans cell histiocytosis [215]. In contrast, a recent study failed to demonstrate genomic abnormalities using comparative genomic hybridization and single nucleotide polymorphism (SNP) arrays [213]. Interestingly, a role for IL-17A has recently been proposed in the pathogenesis of these lesions [216].

3.1.10. Non-Ossifying Fibroma

Also known as fibrous cortical defect, fibroxanthoma, fibrous osteomyelitis, metaphyseal fibrous defect and nonosteogenic fibroma, this represents a benign lesion involving the cortex in the metaphysis of long bones [217]. Most cases present during the first two decades with a slight male predominance [218]. While usually discovered

incidentally, pathologic fracture as the presenting symptom can occasionally occur [219]. Currently, most consider this to represent a non-neoplastic process; however, this remains to be fully established. Histologically, lesions comprise plump spindle cells arranged in a storiform pattern with variable numbers of interspersed osteoclast-type giant cells, foamy histiocytes and haemosiderin pigmentation. Mitotic activity may be noted. Multifocal non-ossifying fibromas have been reported in the setting of neurofibromatosis [220, 221] and the related Jaffe-Campanacci syndrome [222].

Limited cytogenetic information is available on these lesions. There are reports of deletion in the short arm of chromosome 4, with del(4)(p14) [185] and a translocation producing t(1; 4)(p31; q34) [223].

3.1.11. Osteofibrous Dysplasia

This represents a benign fibro-osseous proliferation characteristically affecting the cortex of the anterior tibia [131, 224]. These tumours arise in the first two decades, with a male predisposition [224]. Histologically, they are characterised by a hypocellular spindle cell proliferation admixed with curvilinear trabeculae of woven bone. The latter contains prominent osteoblastic rimming, a feature conspicuously absent in fibrous dysplasia [131, 225]. Scattered cytokeratin-positive cells are found in these tumours supporting an association with adamantinoma [225]; that being said, it is unclear if osteofibrous dysplasia-like adamantinoma always develop into the classic variant of adamantinoma [226].

Cytogenetics in a small number of cases of osteofibrous dysplasia have variably shown trisomy for chromosomes 5, 7, 8, 12, 21 and 22 [191, 226, 227]. Of note, relatively similar findings have been reported in adamantinoma, with reports of trisomy for chromosomes 7, 8, 10, 12, 13, 19 and 21 supporting an association with osteofibrous dysplasia [226, 228-231]. More complex rearrangements have also been observed in adamantinoma [228, 232, 233].

3.1.12. Subungual Exostoses

Also known as Dupuytren's subungual exostosis, these lesions lack a medullary connection to the underlying bone, thus are not true osteochondromas. Clinically these tend to be of rapid onset, associated with pain and/or ulceration [234]. Despite a broad age range, they predominate in the second decade, affecting females about twice as much as males [235]; approximately 80% occur on the dorsal-medial aspect of the great toe [234]. Histologically, these lesions demonstrate a spectrum of morphologies. Initially they are composed of a proliferation of myofibroblasts at the site of the nail bed, which undergoes enchondral ossification. In time, this yields woven, then lamellar bone which extends to the phalanx [234].

Initially thought to be reactive in nature, the presence of clonal cytogenetic abnormalities suggests a neoplastic origin [236]. Several karyotypic studies have revealed balanced translocations, including: a report of three cases with t(X; 6), with one case also containing t(1; 17) [148]; and, a single case demonstrating t(X; 6)(q25; q21), t(1; 12)(p35; q12), t(4; 5)(q33; q12) and t(6; 14)(q13; q12) [236]. An additional report contributed two additional cases with t(X; 6) translocations; based on the location of the rearrangements, the authors

propose that *COL12A1* and *COL4A5* genes contribute to the pathogenesis of these neoplasms [237].

3.2. Malignant Paediatric Bone Tumours

A number of soft tissue sarcomas that typically present in the soft tissues may also appear as primary lesions of bone. As these are frequently the subject of review, they are not discussed herein.

3.2.1. Adamantinoma

See above description of osteofibrous dysplasia.

3.2.2. Ewing Family of Tumours

Presently considered together with primitive neuroectodermal tumour [238], Ewing sarcoma affects children in the first and second decades; there is a predilection for males [131]. Tumours frequently arise in the diaphysis or metaphysealdiaphyseal regions. Histologically they comprise of sheets of small round-polyhedral blue cells which generally have a scant amount of cytoplasm. The nuclei are round-oval and mitotic activity is not abundant [131].

Few primary tumours of bone have proved more gratifying from the perspective of molecular diagnostics than Ewing sarcoma. Cytogenetic evidence initially pointed to a characteristic t(11; 22) translocation [239, 240]. The identification of other, less common, cytogenetic abnormalities continues to follow [241-250].

In the prototypic example of Ewing sarcoma, the *EWS* gene on chromosome 22q12 is paired with a member of the Ewing family of transcription factors. The most common of these is *FLI1* on chromosome 11q24, followed distantly by *ERG* on chromosome 21q22 [251]; a number of less common genes together constitute roughly 1% of the remaining cases, including *ETV1* on chromosome 7p22, *ETV4* on chromosome 17q21, *FEV* on chromosome 2q36 and *ZSG* on chromosome 22q12. Multiple breakpoints for each of the fusions result in a tremendous number of possible fusion products, thereby complicating detailed investigation into all fusion products [252]. Moreover, the rarer translocations may be highly complex and difficult to detect.

Diagnostically, the morphologic impression of Ewing sarcoma can be confirmed using cytogenetics, fluorescence *in situ* hybridization and/or reverse transcriptase-PCR (RT-PCR) based methods. Cytogenetics generally takes too long to be clinically useful [238]. Fluorescence *in situ* hybridization can be performed on metaphase spreads, and more conveniently on formalin-fixed paraffin-embedded tissue. It can be accomplished using either break-apart probes to demonstrate a chromosome break, or more specifically to identify translocation products [253, 254]. Fluorescence *in situ* hybridization for the fusion product is more labour intensive, since it also requires testing for the less common products. Unfortunately, the break-apart probe has limited application by the fact that it identifies neither the fusion partner nor the location of the breakpoint. This can be a particular problem when the gene rearrangement involves *EWS* as this gene is rearranged in several soft tissue sarcomas. However, if the FISH result is interpreted in the light of immunohistochemistry, a diagnosis can be reached in most cases. This emphasises the need to report molecular pathology with the information derived from the histopathologist.

Table 1. Summary of Markers Used for Diagnosing Primary Paediatric Bone Lesions Arising in Familial and Sporadic Settings

Disease	Cytogenetic Findings	Genetic Mutations/ Rearrangements	Testing Method
Familial syndrome			
Bloom syndrome		<i>BLM</i> mutation	DMA
Cherubism		<i>SH3BP2</i> mutation	DMA
Li-Fraumeni syndrome		<i>p53</i> mutation	DMA
Neurofibromatosis 1		<i>NF1</i> mutation	DMA
Retinoblastoma		<i>RBI</i> mutation	DMA
Sporadic disease			
Ewing sarcoma/PNET	t(2;22)(q33;q12)	<i>EWSR1/FEV</i>	Cytogenetics,
	t(7;22)(p22;q12)	<i>EWSR1/ETV1</i>	
	t(11;22)(q24;q12)	<i>EWSR1/FLI1</i>	FISH,
	t(17;22)(q12;q12)	<i>EWSR1/E1AF</i>	RT-PCR
	t(21;22)(q22;q12)	<i>EWSR1/ERG</i>	
Fibrous dysplasia (mosaic)		<i>GNAS1</i> mutation	MS-RED, DMA

Abbreviations: direct mutational analysis (DMA); fluorescence *in situ* hybridization (FISH); mutation-specific restriction enzyme digestion (MS-RED); polymerase chain reaction (PCR); primitive neuroectodermal tumour (PNET).

RT-PCR-based methods can be applied to identify directly the fusion products. This can conveniently be performed on both fresh/frozen and paraffin-embedded tissues although older tissue blocks may suffer from RNA degradation [255]. In order to detect each of the possible fusion sites, separate primer sets are necessary.

3.2.3. Conventional osteosarcoma

Also known as osteogenic osteosarcoma, this tumour primarily occurs in the second decade and occurs more often in males than females [256]. While the long bones are the most common site of involvement, this is not invariably the case. Several histological variants of conventional osteosarcoma are recognized by the World Health Organization, including osteoblastic, chondroblastic and fibroblastic [256]. Despite broad differences in cell morphology amongst the histological subtypes, as evidenced by their names, common amongst them is an atypical pattern of osteoid matrix deposition associated with malignant cells. This, for example, may take the form of a delicate filigree and/or ribbon pattern [257].

Numerous studies have investigated the cytogenetic and molecular features of osteosarcoma. While beyond the scope of the present work, it should suffice to say these changes are complex [258-260] and frequently lack a consistent abnormality [261-263]. Some of the more frequent sites of rearrangement appear to be 1p11-13, 1q10-12, 1q21-22, 1q42, 6p12-p21, 7q11, 11p15, 12p13, 14q32, 17p11-13, 19q13, and 22q11-13 [262, 264-266]. There are frequent chromosomal gains in number. Array CGH has shown gains in a number of cases in 1p36.32, 6p12.1, 6p21.1, 8q12-21.3, 8q22-q24, 8q24-23, 12q14.3, 16p13, 17p11.2-17p12; and, losses at 17p13 [267-270].

Efforts, particularly with studies integrating familial predispositions to this disease, have led to some advancement in understanding the pathogenesis of these neoplasms. At the

same time these efforts have spawned numerous molecular markers with possible prognostic significance. Despite this wealth of information, it is perhaps surprising that no single diagnostic molecular marker for osteosarcoma exists to date [271].

4. CONCLUSIONS

Tremendous gains in elucidating the molecular basis of many lesions of paediatric bone have been obtained from a multidisciplinary approach to these disorders. Important leads have been derived from investigation into familial patterns of disease inheritance and cytogenetics. Additional studies in molecular biology have extended these observations to better our understanding of the pathogenesis of many of these entities; moreover, it has provided targets for diagnostic consideration. At present, clinical markers exist to confirm the diagnosis of numerous familial patterns of disease, and a modest number of tumours affecting paediatric bone. Clearly, many exciting opportunities remain to further our understanding of the pathogenesis and diagnosis of these entities (Table 1).

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