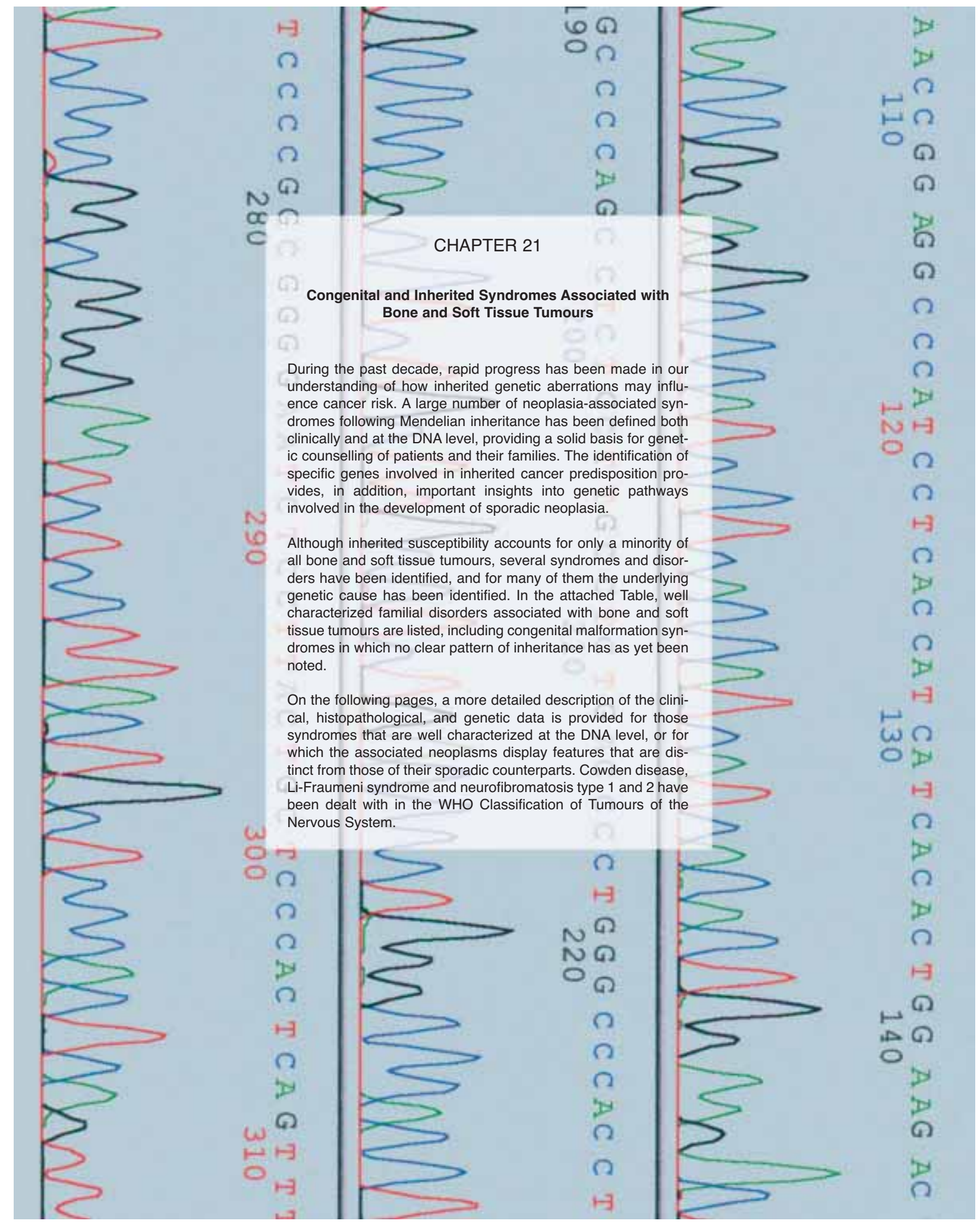

CHAPTER 21

Congenital and Inherited Syndromes Associated with Bone and Soft Tissue Tumours

During the past decade, rapid progress has been made in our understanding of how inherited genetic aberrations may influence cancer risk. A large number of neoplasia-associated syndromes following Mendelian inheritance has been defined both clinically and at the DNA level, providing a solid basis for genetic counselling of patients and their families. The identification of specific genes involved in inherited cancer predisposition provides, in addition, important insights into genetic pathways involved in the development of sporadic neoplasia.

Although inherited susceptibility accounts for only a minority of all bone and soft tissue tumours, several syndromes and disorders have been identified, and for many of them the underlying genetic cause has been identified. In the attached Table, well characterized familial disorders associated with bone and soft tissue tumours are listed, including congenital malformation syndromes in which no clear pattern of inheritance has as yet been noted.

On the following pages, a more detailed description of the clinical, histopathological, and genetic data is provided for those syndromes that are well characterized at the DNA level, or for which the associated neoplasms display features that are distinct from those of their sporadic counterparts. Cowden disease, Li-Fraumeni syndrome and neurofibromatosis type 1 and 2 have been dealt with in the WHO Classification of Tumours of the Nervous System.

Table 21.01
Congenital syndromes associated with bone and soft tissue tumours.

OMIM[a]	Disorder[b]	Inheritance	Locus[c]	Gene	Bone and soft tissue tumours
103580	Albright hereditary osteodystrophy	AD	20q13	*GNAS1*	Soft tissue calcification and osteomas
153480	Bannayan-Riley-Ruvalcaba syndrome	AD	10q23	*PTEN*	Lipomas, haemangiomas
130650	Beckwith-Wiedemann syndrome	Sporadic/AD	11p15	Complex, incl. *CDKN1C* and *IGF2*	Embryonal rhabdomyosarcomas, myxomas, fibromas, hamartomas
210900	Bloom syndrome	AR	15q26	*BLM*	Osteosarcomas
160980, 605244	Carney complex	AD	17q23-24 2p16	*PRKAR1AK* -	Cardiac and other myxomas, melanocytic schwannomas
112250	Diaphyseal medullary stenosis with malignant fibrous histiocytoma	AD	9p21-22	-	Malignant fibrous histiocytomas of bone
151623	Li-Fraumeni syndrome	AD	17p13 22q11	*TP53* *CHEK2*	Osteosarcomas, rhabdomyosarcomas and other soft tissue sarcomas
151800	Lipomatosis, symmetrical	Sporadic	-	-	Lipomas, lipomatosis of the head and neck
166000	Maffucci syndrome	Sporadic	-	-	Enchondromas, chondrosarcomas, spindle cell haemangiomas, haemangiomas, angiosarcomas
-	Mazabraud syndrome	Sporadic	20q13	*GNAS1*	Polyostotic fibrous dysplasia, osteosarcomas, intramuscular myxomas
174800	McCune-Albright syndrome	Sporadic	20q13	*GNAS1*	Polyostotic fibrous dysplasia, osteosarcomas
133700, 133701	Multiple osteochondromas, non-syndromic	AD	8q24, 11p11-12	*EXT1* *EXT2*	Osteochondromas, chondrosarcomas
228550	Myofibromatosis	AR	-	-	Myofibromas
162200	Neurofibromatosis type 1	AD	17q11	*NF1*	Neurofibromas, malignant peripheral nerve sheath tumours
101000	Neurofibromatosis type 2	AD	22q12	*NF2*	Schwannomas
166000	Ollier disease (enchondromatosis)	Sporadic	3p21-22	*PTHR1*	Enchondromas, chondrosarcomas

OMIM[a]	Disorder[b]	Inheritance	Locus[c]	Gene	Bone and soft tissue tumours
167250; 602080	Paget disease of bone, familial	AD	18q21 5q31 5q35	*TNFRSF11A* - -	Osteosarcomas
176920	Proteus syndrome	Sporadic	-	-	Lipomas
180200	Retinoblastoma	AD	13q14	*RB1*	Osteosarcomas, soft tissue sarcomas
601607	Rhabdoid predisposition syndrome	AD	22q11	*SMARCB1*	Malignant rhabdoid tumours
268400	Rothmund-Thomson syndrome	AR	8q24	*RECQL4*	Osteosarcomas
180849	Rubinstein-Taybi syndrome	AD	16p13	*CREBBP*	Myogenic sarcomas
138000	Venous malformations with glomus cells	AD	1p21-22	-	Glomus tumors
277700	Werner syndrome	AR	8p11-12	*WRN*	Various bone and soft tissue sarcomas

a OMIM = entry number in McKusick's Online Mendelian Inheritance in Man {1376}.
b Syndromes associated with tumours affecting only the skin or parenchymatous organs are not included.
cAD = autosomal dominant; AR = autosomal recessive.

Familial adenomatous polyposis

M. Nilbert
C.M. Coffin

Definition

Familial adenomatous polyposis (FAP) is characterized by the development of multiple colorectal polyps, which are premalignant lesions with a strong tendency to progress into carcinomas. Gardner syndrome, characterized by colorectal polyps as well as extracolonic manifestations such as dental abnormalities, osteomas, epidermoid cysts and desmoid tumours, was initially considered a separate entity, but has now been recognized as a variant of FAP. FAP is caused by mutations in the adenomatosis polyposis coli (*APC*) gene on chromosome 5.

OMIM number 175100

Synonyms

Bussey-Gardner polyposis, adenomatous polyposis coli, familial polyposis coli, familial multiple polyposis, etc.

Incidence

Estimates of the incidence of FAP vary between 1/7,000 and 1/30,000 {1033}. Whereas dental abnormalities and osteomas occur in more than half of the patients, desmoid tumours and epidermoid cysts develop in a minority of the patients. Overall, FAP accounts for less than 1% of all colorectal cancers.

Diagnostic criteria

The diagnosis of FAP requires 1) at least 100 colorectal adenomas or 2) a germline, disease-causing mutation of the *APC* gene or 3) a family history of FAP and at least one of the following: epidermoid cysts, osteomas or desmoid tumour. Other types of extracolonic manifestations are associated with FAP, including adenomatous polyps of the upper gastrointestinal tract, congenital hypertrophy of the retinal pigment epithelium (CHRPE), an increased risk of hepatoblastoma and tumours of the endocrine system, most commonly papillary carcinoma of the thyroid. Furthermore, an association with brain tumours, especially medulloblastomas, occurs in the Turcot syndrome, which in two-thirds of the cases is caused by *APC* mutations. In familial infiltrative fibromatosis (OMIM No. 135250), which is also caused by germline mutations of *APC*, there is an inherited predisposition to desmoid tumours, but only few or no colonic polyps.

Fig. 21.01 Epidermoid cyst on the dorsal surface of the hand of a patient with familial adenomatous polyposis.

Clinical features

Colorectal adenomas usually develop into endoscopically detectable lesions at 10-20 years of age and increase in number and size over time. Untreated FAP patients develop colorectal cancer at a median age of about 40 years. FAP patients should be screened with endoscopy with 1-2 year intervals from 10-15 years of age up to 40 years of age and prophylactic colectomy is performed when adenomas are detected. Extraintestinal manifestations, in particular epidermoid cysts, dental abnormalities, osteomas and CHRPE often preceed the development of adenomas and may serve as clinical markers of FAP.

Bone and soft tissue tumours

The description of Gardner syndrome in the 1950's highlighted the association of familial polyposis coli with a spectrum of extracolonic manifestations, including lesions of soft tissue and bone {766-769}. The most commonly encountered bone and soft tissue lesions are osteomas, cortical thickening of bone, epidermoid cysts, and desmoid-type fibromatoses {766,768,1544,1606,1705}. In addition to these lesions, a variety of other soft tissue masses have been clinically described, with varying extents of pathological analysis. These include ill defined connective tissue masses, "lipomas" {1705}, "fibrous dysplastic lesions" {1544}, "familial infiltrative fibromatosis" {1913}, fibromatous mesenteric plaques {363}, juvenile nasopharyngeal angiofibroma {784}, Gardner fibroma {2227}, and rhabdomyosarcoma {84}.

The association of desmoids, including those with childhood onset, with adenomatous polyposis of the coli is now well recognized {175,312,361,362,566,768, 769,1032,1068,1913}. The incidence of desmoid tumours in patients with polyposis has been estimated to be around 10%. Pathological features of desmoid-type fibromatosis are described elsewhere in this book (see page 83). Particular *APC* mutation types are associated with a higher frequency of desmoid tumours {175, 312, 859, 931, 957, 1015,1047,1137,1286,1685,1799,1993}. The Gardner fibroma {2227}, described elsewhere in this book (see page 76), is similar to the fibromatous mesenteric plaques reported in patients with adenomatous polyposis coli {363}. These lesions are associated with development of desmoid-type fibromatosis in the same site, either following surgery or de novo {361,2227}. Recognition of the Gardner fibroma in childhood can serve as the sentinel event for diagnosis of adenomatous polyposis of the colon {2227}. Juvenile nasopharyngealangiofibroma has also been reported in association with adenomatous polyposis of the colon {8,10,784}. However, some have questioned whether this association is coincidental or whether it is actually related to another alteration of the *APC* gene {850}. Rhabdomyosar-coma has been reported in rare instances in individuals or families with adenomatous polyposis of the colon {84,1299}, but it is unclear whether this is a sporadic occurrence or another syndromic

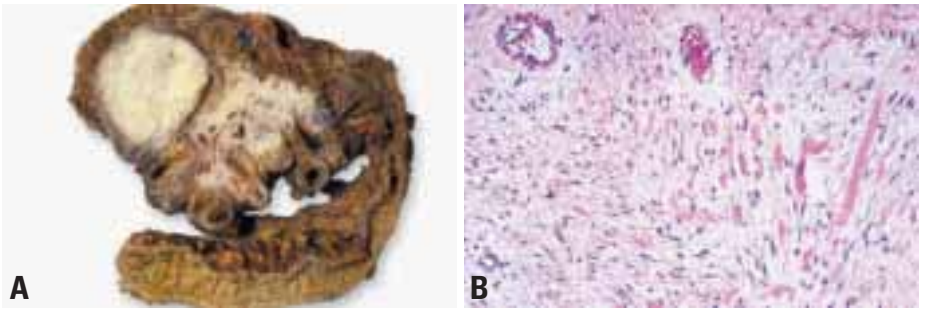

Fig. 21.02 Mesenteric fibromatosis (desmoid tumour) in a patient with FAP. **A** The lesion entraps loops of small intestine. **B** Histopathology is dominated by collagen bands and small vessels.

manifestation.

Bone lesions associated with adenomatous polyposis of the colon are entirely benign and are viewed as dysplasias. Multiple osteomas formed by membranous ossification, especially of calvarial and mandibular surfaces, characterize the "ivory exostosis" of Gardner syndrome {285, 331, 1075, 1690}. Histologically, the Gardner osteoma is a nodular excrescence of mature lamellar bone involving the cortical surface, especially the outer table of the skull, the mandibular cortex, or rarely other sites. Like desmoid fibromatosis, particular *APC* gene mutations are associated with more severe osseous manifestations {451, 1180, 2080}. Diffuse craniofacial sclerotic bone changes and dental malformations are also encountered. The bony lesions of adenomatous polyposis of the colon do not evolve into other benign neoplasms, such as osteoblastomas, or into malignant lesions.

Genetics

Germline mutations of the *APC* gene is the only identified cause of FAP. FAP is autosomally dominantly inherited with an almost complete penetrance. However, at least one-fifth of the patients lack a family history and are thus assumed to carry de novo mutations of the *APC* gene {204}.

Gene structure

The *APC* gene was in 1986 localized to 5q21-22 through observation of a patient with polyposis and a constitutional interstitial deletion of 5q followed by an establishment of linkage to this locus in several FAP kindreds {940, 1241}. The *APC* gene was isolated in 1991 and was found to be mutated in the germline of patients with FAP {840, 1123}. The gene spans 120 kb, is composed of 15 coding exons and contains an open reading frame of 8,538 bp. Several alternatively spliced forms of *APC* with different 5´regions have been identified.

Gene expression

The 2,843 amino-acid APC protein is ubiquitously expressed in most normal tissues with the highest expression found in the central nervous system. APC is a multifunctional protein with several functional domains through which APC exerts its main function as a negative regulator of the Wnt signalling pathway {312,693,921,1819}. Normal Wnt signalling inhibits the function of glycogen synthase 3ß (GSK3B), dephosphorylates axin / conductin and thereby targets ß-catenin for degradation. ß-catenin is involved in the cytoskeletal organisation with microtubule binding and in cell adhesion through interaction with E-cadherin. *APC* mutations, presumably trough loss of binding sites and degradation sites for ß-catenin lead to intracellular accumulation of ß-catenin, which is transferred to the nucleus and through interaction with transcription factors of the TCF/LEF family regulates expression of downstream target genes such as *MYC* and *CCND1* {2011, 2104}. The C-terminal mediates binding to microtubule-associated proteins of the EB1/RP1 family. Truncated APC thereby promotes chromosomal instability through disrupted interaction between the kinetochores and the spindle microtubules {693}.

Mutations

Analyses of the *APC* gene in patients with FAP reveal mutations in about 80% of the kindreds examined, and the remaining patient are likely to carry *APC* gene mutations leading to large deletions or impaired protein expression. Over 95% of the mutations identified result in protein truncation, which largely result from nonsense point mutations or deletions causing frameshifts. Genotype-phenotype correlations exist;

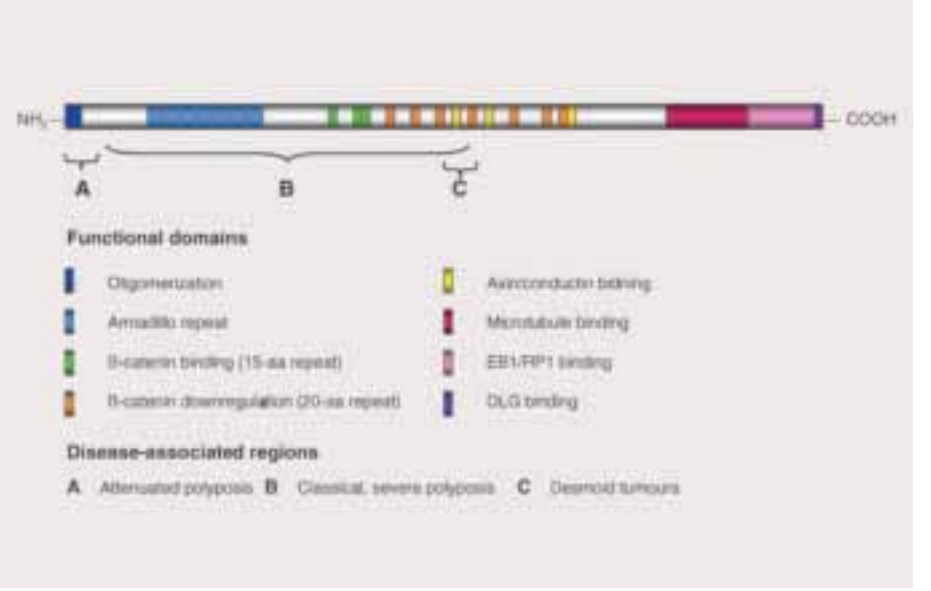

Fig. 21.03 Functional and disease-related domains of the *APC* gene. ß-catenin binding is achieved through the 15-amino acid and 20-amino acid repeat-containing regions and the C-terminal of APC which interacts with microtubule-associated proteins of the EB/RP family and with DLG, a human homologue of the Drosophila discs large tumour suppressor protein. Mutations between codons 1403 and 1578 have been associated with the extracolonic manifestations, e.g. desmoid tumours.

truncating mutations in the 5´ end of the gene have been associated with attenuated FAP, mutations in the central region of gene, including the mutational hotspot at codon 1309, are associated with multiple polyps at young age, and mutations between codons 1444 and 1578 are associated with an increased incidence of desmoid tumours {451, 1124, 2011}. However, patients with identical mutations can develop dissimilar clinical features and the genotype clinically serves as a risk determinant rather than as an absolute predictor of the extent of the disease.

Beckwith-Wiedemann syndrome

M. Mannens

Definition
The Beckwith-Wiedemann syndrome (BWS) is a complex overgrowth disorder caused by a number of genes that are subject to genomic imprinting. A high incidence of solid childhood tumours, including rhabdomyosarcoma, is seen in patients that present with BWS.

OMIM number 130650

Synonyms
EMG syndrome (Exomphalos-Macroglossa-Gigantism syndrome), WBS (Wiedemann-Beckwith syndrome).

Incidence
The syndrome occurs with an estimated incidence of 1:13,700 and most cases (85%) are sporadic.

Diagnostic criteria
Patients can be classified as having BWS according to the clinical criteria proposed by Elliot or DeBaun {479, 580} although cases of BWS are known that do not comply with either set of criteria. Elliot classifies patients as BWS when they present with three major features or two major features plus three or more minor features (major features: anterior abdominal wall defects, macroglossia and pre- and/or postnatal growth > 90th centile; Minor features: ear creases or pits, naevus flammeus, hypoglycaemia, nephromegaly and hemihypertrophy). DeBaun is less strict in his classification i.e. two or more of the five most common features (macroglossia, birth weight > 90th percentile, hypoglaecemia in the first month of life, ear creases/pits and abdominal wall defects).
BWS can be diagnosed in the laboratory by chromosome banding analysis (< 5%) or DNA-diagnostics. The current major test involves methylation assays or loss of imprinting (LOI) studies at the RNA level. The majority of cases (50-80%) demonstrates aberrant methylation of *KCNQ1OT1*, with or without aberrant methylation of *IGF2/H19*. These latter cases often show uniparental disomy (UPD), in a mosaic form, for 11p15, which explains this aberrant methylation. However, the majority of cases with *KCNQ1OT1* defects and some cases with *H19/IGF2* defects have no UPD 11p15. Therefore, an imprinting switch can be assumed involving an imprinting centre, analogous to the Prader-Willi and Angelman syndromes. The current data are most compatible with two distinct imprinting centres for either *KCNQ1OT1* or *IGF2/H19*. *CDKN1C* mutation analyses might be considered, especially in familial cases of BWS. The increased tumour risk for BWS patients seems to be associated with UPD in general and *H19* methylation defects in particular. *KCNQ1OT1* methylation defects only seem to be a favourable prognostic factor since tumours are not, or only very rarely associated with this group of patients. Recurrence risks for a second pregnancy can be assessed with UPD studies. In case of a UPD in a mosaic form, there is no increased recurrence risk for BWS in a second pregnancy since the genetic defect occurred post-fertilisation.

Clinical features
The BWS is a disorder first described by Beckwith in 1963 at the 11th annual meeting of the Western Society for Pediatric Research. Later, Wiedemann and Beckwith described the syndrome in more detail {149, 2266}. BWS is characterized by a great variety of clinical features, among which are abdominal wall defects, macroglossia, pre- and postnatal gigantism, earlobe pits or creases, facial nevus flammeus, hypoglycemia, renal abnormalities and hemihypertrophy.

Tumours
BWS patients have a risk of 7.5% for the development of (mostly intra-abdominal) childhood tumours. Tumours most frequently found are Wilms' tumour, adrenocortical carcinoma, embryonal rhabdomyosarcoma, and hepatoblastoma. Also myxomas, fibromas, and chest wall hamartomas have been reported to occur at increased frequencies.

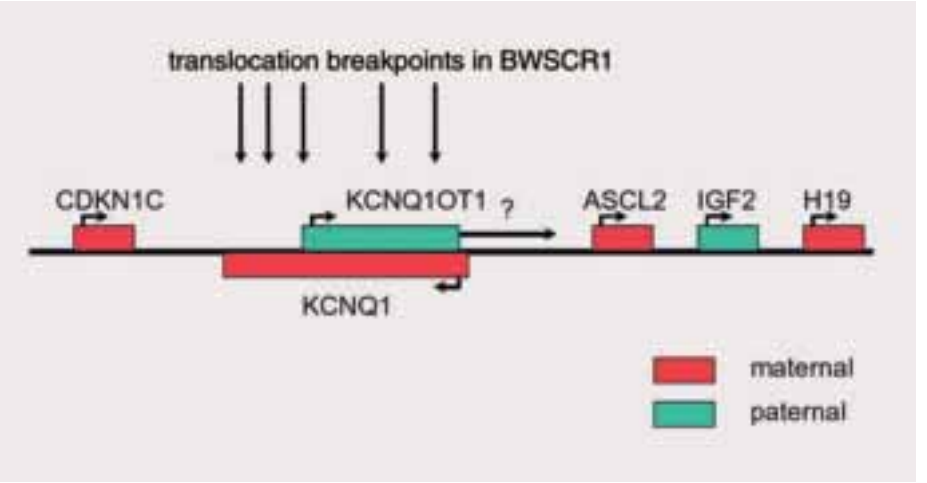

Fig. 21.04 Imprinted genes on 11p15 involved in BWS. The parental expression (imprinting) of these genes is indicated.

Genetics

BWS is caused by genetic changes in chromosome band 11q15, as shown by linkage studies, and the detection of chromosome abnormalities, LOI, and gene mutations. The syndrome is subject to genomic imprinting since maternal transmission seems to be predominant. In addition, chromosomal translocations are of maternal origin, duplications and UPD of paternal origin. All hitherto known causative genes are imprinted. The translocation breakpoints on chromosome 11 map to three distinct regions within 11p15.3-pter. Beckwith-Wiedemann syndrome chromosome region 1 (BWSCR1) near *INS/IGF2*, BWSCR2 5 Mb proximal to BWSCR1, and BWSCR3 2 Mb even more proximal {967}. This already points to genetic heterogeneity but also at the clinical level there seems to be heterogeneity. Chromosomal translocations in BWSCR1 and BWSCR3 are associated with the classical BWS phenotype and BWSCR2 with minor BWS features but pronounced hemihypertrophy. BWSCR 1 and BWSCR2 have been cloned and genes isolated from these regions were shown to be involved in the development of this disorder. All genes involved are subject to genomic imprinting {1326, 2023}.

BWSCR1

This region consists of a number of imprinted genes. All known translocation breakpoints disrupt *KCNQ1*, a gene coding for a potassium channel in-volved also in inherited cardiac arrhythmia syndromes. This imprinted gene, however, is most likely not directly involved in BWS. A gene transcribed in the antisense orientation of *KCNQ1* clearly is. This gene, *KCNQ1OT1*, shows aberrant methylation in 50-80% of BWS cases. It does not code for a protein and may function through its RNA. *CDKN1C* is an inhibitor of cyclin-dependent kinases. Heterozygous mutations have been identified in about 20% of BWS patients in two studies. Others, however, have not been able to confirm this mutation frequency. Although not a major cause of BWS, it is possible that in certain countries, e.g., in Asia, the mutation frequency is elevated. In addition, it has been reported that this gene is more frequently involved in familial cases of BWS. *CDKN1C* mouse models revealed some of the clinical BWS features such as omphalocele and renal adrenal cortex anomalies. In humans, *CDKN1C* also seems to be more frequently associated with abdominal wall defects. Another strong candidate for involvement in the aetiology of BWS is *IGF2*. Mouse models overexpressing Igf2 displayed a phenotype overlapping with the BWS phenotype. Loss of *IGF2* imprinting is often seen in BWS patients. Down-stream from *IGF2* lies *H19*, again a non-coding gene. The expression of *IGF2* and *H19* seems to be linked. *H19* is important for the maintenance of the imprinting status of *IGF2*. Mouse studies underline the link between *IGF2* and *H19* expression and overgrowth phenotypes were found. *H19* loss of imprinting is frequently seen in BWS cases although not always in combination with *IGF2* LOI. Finally, a gene called *ASCL2* is localized to the 11p15-imprinted region. Although no direct involvement in the BWS aetiology is known, this gene might account for the fact that most, if not all, BWS cases with UPD present in a mosaic form. The mouse homologue codes for a transcription factor, which is expressed during early mouse development and is essential for the development of the placenta. Therefore, also in humans, complete lack of expression might be lethal.

BWSCR2

Two patients define this second chromosomal region, one of whom developed a Wilms tumour {34}. Both translocations in 11p15.4 disrupt a paternally imprinted zinc-binding finger gene *ZNF215*. Parts of the 3' end of this gene are transcribed from the antisense strand of a second zinc-finger gene, *ZNF214*. Although putative mutations in these genes in other sporadic BWS cases were found, their involvement in BWS needs to be further elucidated by functional studies.

More detailed information on the structure and expression of genes involved in BWS could be found at:
http://www.infobiogen.fr/services/chromcancer/Kprones/BeckwithWiedemannID10037.html
{1325}.

Enchondromatosis: Ollier disease and Maffucci syndrome

F. Mertens
K. Unni

Definition

Ollier disease is a developmental disorder characterized by the occurrence of multiple cartilaginous masses, particularly affecting the short and long tubular bones of the limbs. When cutaneous, soft tissue or visceral haemangiomas are also present, the disorder is referred to as Maffucci syndrome.

OMIM number 16600L {1376}

Synonyms

Ollier disease is also referred to as multiple enchondromas or dyschondroplasia.

Incidence

Rare, but exact incidence is unknown. Enchondromatosis has been described in many different ethnic groups, and there is no significant gender bias.

Diagnostic criteria

The diagnosis is based on the roentgenographic appearance and clinical features. No distinctive genetic or biochemical marker for either Ollier disease or Maffucci syndrome has as yet been identified.

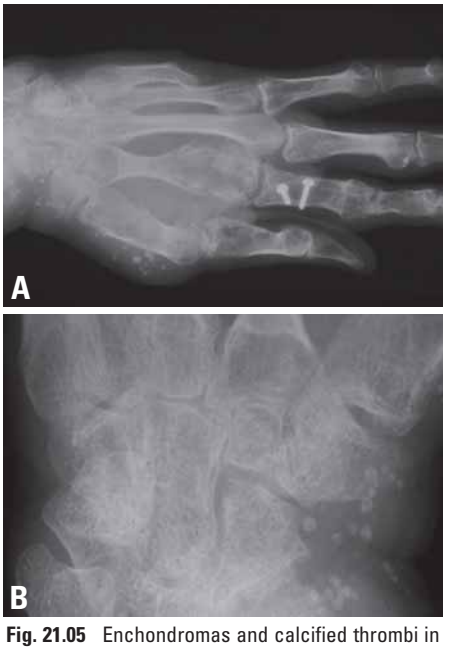

Fig. 21.05 Enchondromas and calcified thrombi in soft tissue haemangiomas in the left hand of a patient with Maffucci syndrome.

Clinical features and tumours

Ollier disease usually manifests already in early childhood, commonly presenting as swelling of the fingers. Enchondromas in the metaphyseal regions of long bones may also result in deformity and limb asymmetry, as well as pathological fractures. Although careful examination will reveal that the vast majority of patients have bilateral enchondromatosis, there is a tendency for one side of the body to be more severely affected. The extent of a patient's orthopedic complications, which is highly variable and difficult to predict, is largely dependent on the number and skeletal distribution of enchondromas.

The enchondromas primarily affect the short and long tubular bones of the extremities, but flat bones, such as the pelvis and ribs, may be involved. The craniofacial bones and vertebrae, however, are usually spared. With few exceptions, the enchondromatous lesions stop growing at puberty. Continued or renewed growth in adults should raise the suspicion of malignancy. Whereas sarcomatous transformation of solitary enchondromas is rare, patients with Ollier have a markedly increased risk, ranging from 15 to 30%, of developing malignant bone tumours, in particular chondrosarcomas {1274,1901}. Some patients even develop multiple sarcomas {303}.

Most patients with Maffucci syndrome present at birth or in early childhood with cavernous haemangiomas, varying in size from a few millimetres to several centimetres, that are typically located in the dermis or subcutaneously on the distal parts of the limbs. However, haemangiomas may also be found in internal organs. In addition, spindle cell haemangioma, a vascular lesion with a high propensity for local recurrence but no potential for metastasis, is overrepresented among patients with Maffucci syndrome {639,1688}.

The skeletal features in Maffucci syndrome are indistinguishable from those in Ollier disease, but the risk of developing chondrosarcoma is possibly even higher among patients with Maffucci syndrome, with incidence figures reaching 20-30% in some series {1067,2055}. An increased incidence has also been suggested for other malignancies, including angiosarcomas, brain tumours, and tumours of the hepatobiliary system {538, 1901}, as well as certain benign tumours. In both forms of enchondromatosis, careful surgical and orthopedic intervention may avoid or minimise deformities. Furthermore, all patients should be instructed to pay close attention to signs or symptoms heralding malignant transformation.

The more widespread the disease, the greater is the likelihood for malignant transformation {538}. The prognosis for patients developing secondary chondrosarcoma is similar to that for patients with sporadic chondrosarcomas, and depends on tumour size and location, and histological malignancy grade {1230}.

Roentgenographic features

Roentgenographic features of Ollier disease and Maffucci syndrome are similar except for the presence of phleboliths in the soft tissue haemangiomas in the latter condition. The cartilage present has expansile masses at the metaphyseal region with calcification in the form of longitudinal striation.

Fig. 21.06 Multiple enchondromas causing swelling and angular deformity in the left hand of a patient with Ollier disease.

Microscopic features
The cartilage in enchondromas is present as well circumscribed nodules in the medullary cavity and occasionally on the surface. The matrix does not show myxoid change. The lesion is hypercellular and the chondrocyte nuclei are enlarged and irregular.

Genetics
Most cases of enchondromatosis are sporadic, but families with multiple affected members have been reported, possibly suggesting autosomal dominant inheritance with reduced penetrance {1376}. Molecular genetic analysis of a high grade chondrosarcoma from a patient with Ollier disease revealed loss of heterozygosity for the chromosomal bands harbouring the *RB1* and *CDKN2A* tumour suppressor genes as well as TP53 overexpression, but none of these changes were found in tissue from an enchondroma {243}.
Recently, a study of patients with Ollier disease revealed mutations of the *PTHR1* gene, encoding a receptor for parathyroid hormone and parathyroid hormone-related protein (PTH/PTHrP), in two of six cases; in one as a germline mutation, and in one as a somatic mutation in enchondroma tissue {968}. The detected mutation, resulting in an R150C substitution in the extracellular domain of PTHR1, was shown to cause increased cAMP signalling, which is analogous to the situation in Jansen metaphyseal chondrodysplasia (OMIM 156400), an autosomal dominant disorder sharing some radiographic and histological features with Ollier disease. The hypothesis that a mutant PTH/PTHrP receptor could delay the differentiation of proliferating chondrocytes by constitutively activating Hedgehog signalling {1885} was further substantiated by studies of transgenic mice carrying the same R150C *PTHR1* mutation {968}. The R150C substitution could not be detected in a series of 50 sporadic chondrosarcomas {968}.

McCune-Albright syndrome

M.M. Cohen, Jr.
G.P. Siegal

Definition
McCune-Albright syndrome (MAS) is a sporadically occurring disorder consisting of polyostotic fibrous dysplasia, café-au-lait spots, and hyperfunctioning endocrinopathies. The syndrome is caused by mutations in the *GNAS1* gene.

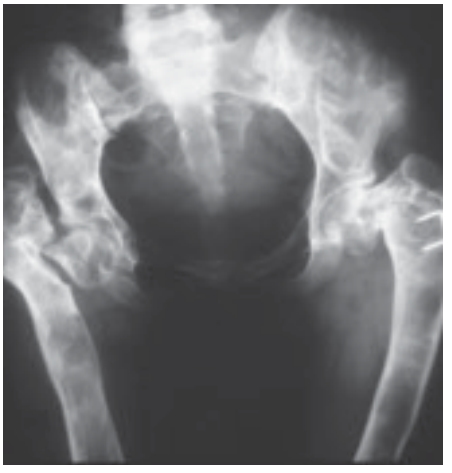

Fig. 21.07 Fibrous dysplasia in Albright syndrome.

OMIM number 174800L

Incidence
No accurate incidence has ever been determined for MAS. Fibrous dysplasia may occur without MAS and the overwhelming majority of these cases are monostotic. Polyostotic fibrous dysplasia occurs much less frequently and about 3% of the these cases represent MAS {382,383}.

Diagnostic criteria
Polyostotic fibrous dysplasia, café-au-lait spots, and hyperfunctioning endocrinopathies {31-33,1375}.

Clinical features
Cardinal features include café-au-lait spots, polyostotic fibrous dysplasia, multiple endocrinopathies including sexual precocity, pituitary adenoma, and hyperthyroidism. There is high expression of the *FOS* proto-oncogene in cells populating the bone marrow spaces. Many other abnormalities are found with low frequency: gastrointestinal polyps; hyperplasia of the thymus, spleen, and pancreatic islet cells; hepatobiliary disease; cardiac disease; failure to thrive; metabolic acidosis; abnormalities in serum electrolytes, glucose, or insulin

Table 21.02
GNAS1 mutations in solitary, sporadic neoplasms.

Neoplasm
Osteosarcoma
Pituitary adenoma
Thyroid adenoma
Thyroid carcinoma
Parathyroid adenoma
Leydig cell tumour
Ovarian cyst
Intramuscular myxoma*
Breast carcinoma

* Mazabraud syndrome, the combination of polyostotic fibrous dysplasia and intramuscular myxomas, is also caused by *GNAS1* mutations. From Cohen {382}

Table 21.03
Mutations in the *GNAS1* gene.

Disorder	Exon	Nucleotide Change	Amino Acid Substitution
McCune-Albright syndrome	8 8	C → T G → A	Arg201Cys Arg201His
Polyostotic fibrous dysplasia	8	C → T	Arg201Cys
Monostotic fibrous dysplasia	8 8	C → T G → A	Arg201Cys Arg201His
Panostotic fibrous dysplasia	8	C → A	Arg201Ser
Solitary pituitary adenoma	8 8 8 9 9	C → T G → A C → A A → G G → T	Arg201Cys Arg201His Arg201Ser Gln227Arg Gln227His

From Cohen and Howell {383}

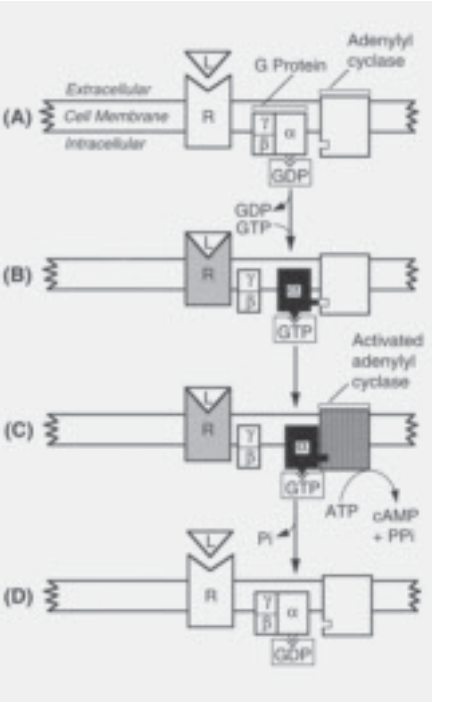

Fig. 21.08 (A) G protein composed of α , β, and γ subunits. This is the inactive form. B) Ligand (L) binding produces conformational change in receptor (R) and guanosine diphosphate (GDP) is replaced by guanosine triphosphate (GTP), resulting in dissociation of the α subunit. (C) Binding of α subunit to adenylyl cyclase activates 3',5'-cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). (D) Hydrolysis of GTP to GDP by GTPase, causing dissociation of the α subunit from adenylyl cyclase and binding to the β and γ subunits, the inactive form. Ligand binding causes repetition of the cycle {383}.

levels; hyperphosphaturic hypophosphatemia; osteo-sarcoma (4%); developmental delay; microcephaly; and sudden or premature death {302,382,383, 392,1936}.

Bone and soft tissue tumours

As noted above, one of the primary pathological conditions which defines MAS is polyostotic fibrous dysplasia. Other benign lesions associated with this condition include mucoceles of the head and neck {547,745}, simple (unicameral) bone cysts {1001,1129} and aneurysmal bone cysts {76, 1288,1759}. Perhaps the best known concordance is with soft tissue, usually intramuscular, myxomas, known as the Mazabraud syndrome {2108}. Interestingly, activating mutation in the *GNAS1* gene have been detected in myxoma cells {1605}, but not in leukocytes or fibroblasts, from patients with Mazabraud syndrome.

Malignant bone tumours have also been associated with the fibrous dysplasia seen in MAS. Osteosarcoma, and possibly also conventional and dedifferentiated chondrosarcoma, appear to occur with increased frequency {212, 872, 932, 1282, 1630, 1725, 1823}. Although other sarcomas, including fibrosarcoma and malignant fibrous histiocytoma, have been linked to fibrous dysplasia {1822}, these have not been reported in patients with MAS.

Individuals with MAS are also susceptible to endocrine tumours, including adrenocortical and pituitary tumours {1133,1637}.

Genetics

McCune-Albright syndrome (MAS) is caused by mutations in the *GNAS1* gene located in chromosome band 20q13. *GNAS1* (guanine nucleotide-binding protein, α-stimulating activity polypeptide 1) encodes the G-protein α stimulatory subunit ($G_s\alpha$), a component of heterotrimeric G-protein complexes.

Gene function

G proteins (guanine nucleotide proteins) are a family of molecules composed of three subunits designated α, β, and γ. The function and specificity of each G protein is determined by the α subunit, which is unique for each type. The β and γ subunits tend to be more homogeneous. Like all G proteins, the inactive form of $G_s\alpha$ contains bound GDP (guanosine diphosphate). A GPCR (G protein-coupled receptor) facilitates the exchange of bound GTP (guanosine triphosphate) for GDP producing the active form {382,383}.

Adenylyl cyclase is activated following ligand-binding to G-protein-coupled receptor. Ligand-binding (B) produces a conformational change in the receptor and GDP is replaced by GTP, which results in dissociation of the a subunit. Binding of the active form of the α subunit to adenylyl cyclase (C) activates this enzyme, resulting in the formation of cAMP from ATP. Hydrolysis of GTP to GDP is catalysed within seconds by the intrinsic GTPase (guanosine triphosphatase) activity of $G_s\alpha$ which causes dissociation of the a subunit from adenylyl cyclase and binding to the β, and γ subunits, resulting in the inactive form {382,383}.

Mutations

MAS, polyostotic fibrous dysplasia (PFD), monostotic fibrous dysplasia (MFD), and solitary pituitary adenoma (PA) have the same causal genesis – a ligand-independent, activating *GNAS1* mutation in the a subunit of stimulatory G protein ($G_s\alpha$). Mutations are located near the site which interacts with the γ-

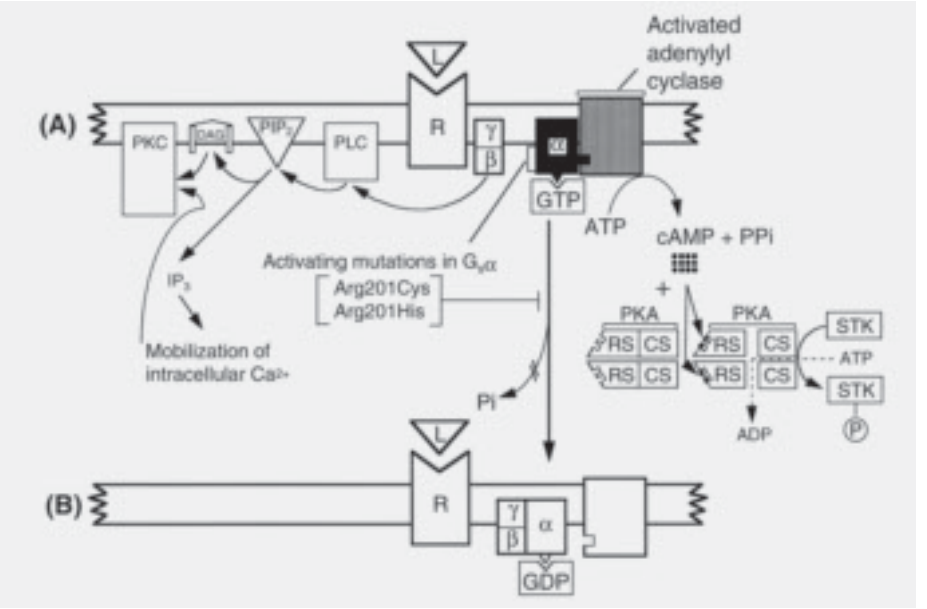

Fig. 21.09 (A) Activating mutations (Arg201Cys or Arg201His) in the gene encoding the α subunit of stimulatory G protein ($G_s\alpha$), causing inappropriate stimulation of adenylyl cyclase interfering with hydrolysis of GTP by GTPase to GDP. The PKA pathway (protein kinase A or cAMP-dependent protein kinase pathway) is shown on the right. The PKC pathway (protein kinase C pathway) is shown on the left. Because the α subunit ($G_s\alpha$) cannot dissociate from adenylyl cyclase, cAMP is overproduced which, in turn, overactivates the PKA pathway. PKA is composed of two regulatory subunits (RS) that have binding sites for cAMP, and two catalytic subunits (CS) that, when dissociated, phosphorylate serine/threonine kinases (STK). The dissociated βγ subunit overactivates the PKC pathway. PLC (phospholipase C) cleaves PIP2 (phosphatidylinositol bisphosphate) into two intracellular messengers: DAG (diacylglycerol) and IP3 (inositol trisphosphate). The latter triggers the release of sequestered calcium ions (Ca2+) which together with DAG activate PKC {383}.

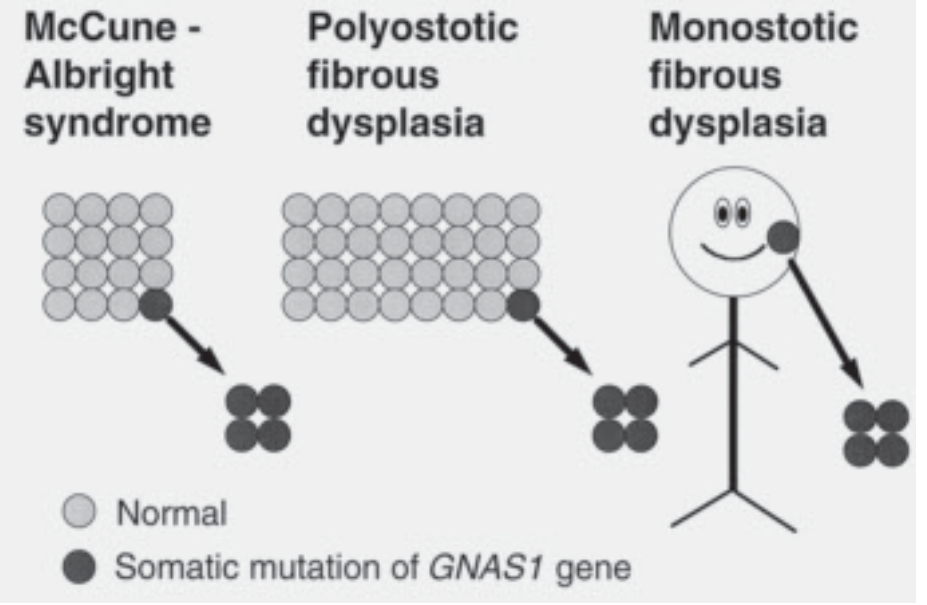

Fig. 21.10 How mutations cause McCune-Albright syndrome, polyostotic fibrous dysplasia, and monostotic fibrous dysplasia depend on when during embryonic development or during postnatal life the mutation occurs. Somatic mutation in a small cell mass is likely to result in McCune-Albright syndrome. Mutation in a larger cell mass may result in polyostotic fibrous dysplasia. A mutation in postnatal life – during infancy, childhood, or adult life – may result in monostotic fibrous dysplasia {383}.

phosphate of GTP, thus interfering with hydrolysis of GTP to GDP. Because $G_s\alpha$ cannot dissociate from adenylyl cyclase and bind to $G_\beta\gamma$, adenylyl cyclase remains active, producing increased cAMP activity which results in the pathology of MAS, PFD, MFD, and PA {382,383,1934,1936,1937,2230}. *GNAS* mutations have also been recorded in various solitary tumours {382}.

MAS, PFD, MFD, and PA occur sporadically. Mutations in the *GNAS1* gene occur postzygotically in a somatic cell. Clinical manifestations are variable in distribution and appearance. More generalized vs. more localized expression depends on (a) how small or how large the cell mass is during embryogenesis when the mutation occurs, and (b) where in the cell mass the mutation occurs {382, 383}.

GNAS1 mutations for MAS, PFD, MFD, and PA are of the gain-of-function type. It should be carefully noted that *GNAS1* mutations of the loss-of-function type are found in endocrine disorders characterized by hormone resistance, such as type 1a pseudohypothyroidism, glucocorticoid deficiency, and nephrogenic diabetes insipidus {1934}.

Multiple osteochondromas

J.V.M.G. Bovée
P.C.W. Hogendoorn

Definition

Multiple osteochondromas (MO) is an autosomal dominant condition. It is genetically heterogeneous and is caused by mutations in one of the *EXT* genes.

OMIM numbers

According to the gene involved, the following OMIM numbers have been assigned:

EXT1	133700
EXT2	133701
EXT3	600209
TRPS2 / Langer Giedion syndrome	150230
Potocki-Shaffer syndrome	601224

Synonyms

EXT, diaphyseal aclasis, (multiple hereditary) osteochondromatosis, multiple cartilaginous exostoses, hereditary multiple exostoses.

Incidence

The solitary (sporadic) form of osteochondroma is approximately 6 times more common than the occurrence within the context of MO. The incidence of MO is approximately 1:50,000 persons within the general population {1887}. Males are more often affected (male: female ratio 1.5:1) {1236, 2265}, due in part to an incomplete penetrance in females {1236}. Approximately 62% of the patients with multiple osteochondromas have a positive family history {1236}.

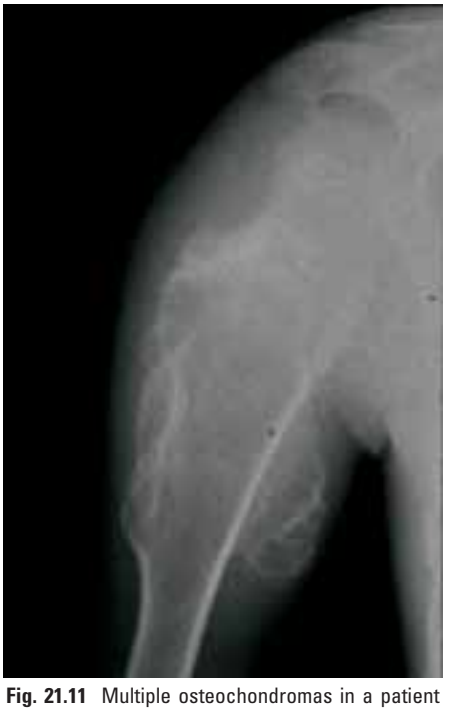

Fig. 21.11 Multiple osteochondromas in a patient with hereditary multiple osteochondromas.

Diagnostic criteria

A diagnosis of multiple exostoses can be made when radiologically at least two osteochondromas of the juxta-epiphyseal region of long bones are observed {1236}. MO is diagnosed in case of a positive family history and/or a proven germline mutation in one of the *EXT* genes.

Clinical features

Osteochondromas develop and increase in size in the first decade of life, ceasing to grow when the growth plates close at puberty. They are pedunculated or sessile (broad base) and can vary widely in size. The majority are asymptomatic and located in bones that develop from cartilage, especially the long bones of the extremities, predominantly around the knee. The number of osteochondromas may vary significantly within and between families. In addition, in the majority of MO patients bone remodelling defects are observed resulting in deformities of the forearm (shortening of the ulna with secondary bowing of radius) (39-60%) {1887, 1929}, inequality in limb length (10-50%) {1887, 1929}, varus or valgus angulation of the knee (8-33%) {1887, 1929}, deformity of the ankle (2-54%) {1887, 1929} and disproportionate short stature (37-44%) {1236, 2265}. It has long been thought that these abnormalities are the result of skeletal dysplasia, although recent evidence indicates that osteochondromas are neoplastic (see chapter 10), and it has been suggested that the growth retardation in MO may result from the local effects of enlarging osteochondromas {1717}. Moreover, the severity of angular deformity was found to be correlated with the number of sessile osteochondromas {309}.

The most important complication of MO is malignant transformation of an osteochondroma, which is estimated to occur in 0.5-3% of MO patients {815, 1236, 1695, 1887, 2265}. The suspicion of secondary chondrosarcoma is indicated by growth of the tumour after puberty, the presence of pain, or a thickness over 1 cm of the cartilaginous cap in adults. The size of the cartilaginous cap can be well established with T2-weighted MR imaging. There are no universally accepted guidelines for surveillance of individuals with MO so far.

Other complications of the osteochondromas include osseous and cosmetic deformities, fracture, bursa formation, arthritis (14%) {2265}) and impingement on adjacent tendons, nerves (23%) {2265}, vessels (11%) {2265} or spinal cord (<1%) {2187, 2265}.

Bone and soft tissue tumours

Hereditary osteochondromas and secondary peripheral chondrosarcomas developing within the cartilaginous cap of hereditary osteochondromas are histopathologically similar to their sporadic counterparts. Morphologically two types of osteochondroma can be recognized: broad based sessile cases with irregular cartilaginous linings and those with a well defined cartilaginous cap. Both may occur within and outside the context of MO. Malignant transformation of osteochondroma leads to a secondary peripheral chondrosarcoma in 94% of the cases {2276}. Very rare cases of other sarcomas developing in osteochondroma have been described, most often in solitary cases of osteochondroma {56, 1214, 1576, 1902, 1968, 2181} including osteosarcomas, and spindle cell sarcomas {1214,1356}. These tumours develop in the stalk of the osteochondroma, in contrast to secondary peripheral chondrosarcomas, which develop in the cap of the pre-existing osteochondroma. A few cases of MO

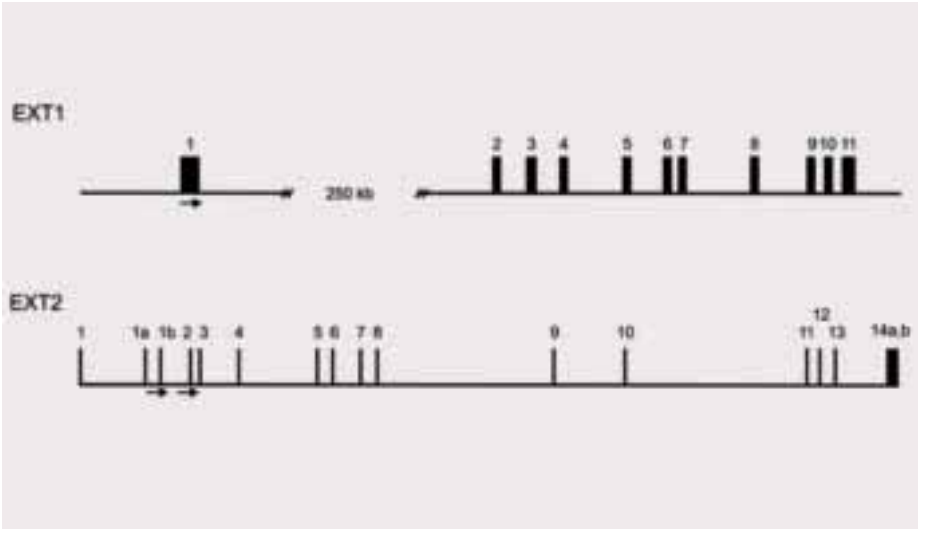

Fig. 21.12 Genomic structure of the *EXT1* and *EXT2* genes.

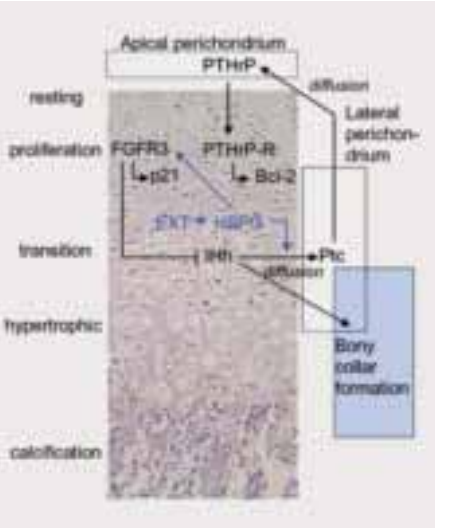

Fig. 21.13 706 Hypothesized function of EXT within the normal early embryonic growth plate.

patients have been reported to develop other sarcomas as well {239, 2139}. These osteosarcomas and spindle cell sarcomas (malignant fibrous histiocytomas and fibrosarcomas) display an indistinguishable phenotype from their non osteochondroma-related counterparts. Even more rare is the occurrence of "conventional" dedifferentiated peripheral chondrosarcoma, in which case the osteochondroma gives rise to peripheral low grade chondrosarcoma that in turn "dedifferentiates" into a high grade sarcoma that may appear as fibrosarcoma, malignant fibrous histiocytoma or osteosarcoma {183}. No soft tissue neoplasms are described within the context of MO.

Genetics

MO is a genetically heterogeneous disorder for which two genes, *EXT1* and *EXT2* located respectively at 8q24 and 11p11-p12, have been isolated {20, 395, 2031, 2310}. Additional linkage to chromosome arm 19p has been found, suggesting the existence of an *EXT3* gene {1229}. Loss of heterozygosity however is absent at this locus {236, 924, 1760} and the gene has never been identified. Three new genes, *EXTL1*, *EXTL2* and *EXTL3* have been identified based on their homology with the *EXT1* and *EXT2* genes {2180, 2283,2309}. However, no association with disease has been documented.

Both *EXT* genes are involved in a contiguous gene deletion syndrome. Patients carrying a deletion of 8q24 demonstrate the Langer-Giedion syndrome (LGS or trichorhinophalangeal syndrome type II (TRPS2; OMIM 150230), which is characterized by craniofacial dysmorphism and mental retardation in addition to multiple osteochondromas {975,1297,1298,1491}. LGS is due to loss of functional copies both of the *TRPS1* gene, encoding a zinc-finger protein {1491}, and the *EXT1* gene at 8q24 {975,1298}. Trichorhinophalangeal syndrome type I (TRPS1) (OMIM 190350) is similar to LGS although multiple osteochondromas are absent. Patients carrying a deletion of 11p11.2-p12 demonstrate Potocki-Shaffer syndrome (proximal 11p deletion syndrome {2307}, DEFECT11, 11p11.2 contiguous gene deletion syndrome). These patients demonstrate enlarged parietal foramina, multiple osteochondromas, and sometimes craniofacial dysostosis and mental retardation {134,1721}. The syndrome is caused by deletion of *EXT2* and probably of *ALX4*; haploinsufficiency of the latter was shown to potentially cause enlarged parietal foramina {134, 2303}.

Gene structure

The *EXT1* gene was identified by positional cloning {20}. The gene is composed of 11 exons, and spans approximately 350 kb of genomic DNA {1296}. The cDNA has a coding region of 2238 bp {20}. The promoter sequence is characteristic of a housekeeping gene {1296}. A mouse-homologue is found on mouse chromosome 15 with a very high level of sequence homology {1266, 1279}. Additional homologues have been identified in Caenorhabditis elegans {369} and Drosophila melanogaster {156}.

The *EXT2* gene was also identified by positional cloning {2031,2310} and contains 16 exons, two of which (1a and 1b) are alternatively spliced {369}. The gene spans approximately 108 kb of genomic DNA {369}. The cDNA consists of approximately 3 kb, defining a single open reading frame of 2154 bp. The mRNA demonstrates alternative splicing {2031,2310}. A highly significant similarity with the *EXT1* gene product has been found, especially in the carboxy terminal region {2031,2310}. Homologues are found on mouse chromosome 2 {369, 2032} and in Caenorhabditis elegans {369}.

Gene expression

Both *EXT1* and *EXT2* mRNA is ubiquitously expressed {20, 2031, 2310}. A high level of expression of *Ext1* and *Ext2* mRNA has been found in developing limb buds of mouse embryos {1265, 2032} and expression was demonstrated to be confined to the proliferating and prehypertrophic chondrocytes of the growth plate {2030}. The gene products, exostosin-1 (*EXT1*) and exostosin-2 (*EXT2*), are endoplasmic reticulum localized type II transmembrane glycoproteins which form a Golgi-localized hetero-oligomeric complex that catalyzes heparan sulphate (HS) polymerisation

Table 21.04
The *EXT* gene family.

Gene	Chromosomal localization	Associated disease	Function: *glycosyltransferase activity involved in heparan sulphate (HS) biosynthesis:*
EXT1	8q24 {907}	MO	HS chain elongation {1267, 1372, 1954}
EXT2	11p11-p12 {977,961}	MO	HS chain elongation {1267, 1372, 1954}
EXTL1	1p36.1 {971}	Unknown	HS chain elongation {1108}
EXTL2	1p11-p12 {976}	Unknown	HS chain initiation {1127}
EXTL3	8p12-p22 {966}	Unknown	HS chain initiation and elongation {1108}

{1267,1372,1373,1954}. Heparan sulphate proteoglycans (HSPG) are large macromolecules composed of heparan sulphate glycosaminoglycan chains linked to a protein core. Four HSPG families have been identified: syndecan, glypican, perlecan and isoforms of CD44. HSPGs are required for high-affinity binding of fibroblast growth factor to its receptor {1275}. Furthermore, an *EXT1* homologue in Drosophila (tout-velu, Ttv) has been shown to be required for diffusion of an important segment polarity protein called Hedgehog (Hh) {156, 2107, 2126}, a homologue of mammalian Indian Hedgehog (IHh). It is therefore hypothesized that *EXT* mutations affect FGF and IHh signalling within the normal growth plate.

Mutations

The *EXT1* gene was reported to show linkage in 44%-66% of the MO families {1235, 1761}, whereas *EXT2* would be involved in 27% {1235}. Germline mutations of *EXT1* and *EXT2* in MO patients have been studied extensively in Caucasian as well as Asian populations {2306} (For overview see also: The human gene mutation database Cardiff www.hgmd.org {1176}). In *EXT1*, mutations are more or less randomly distributed over the first 6 exons, while the last 5 exons, containing the conserved carboxyterminal region, contain significantly less mutations {2306}. Similarly, in *EXT2* most mutations are found in the first exons. No mutational hotspots are found {2306}. Approximately 80% of the mutations are either non-sense, frameshift, or splice-site mutations leading to premature termination of EXT proteins {714,1656, 1761,1917,2308,2313}. The majority of missense mutations also lead to defective EXT protein function {340}.

Loss of the remaining wildtype allele has been demonstrated {238}, indicating that the *EXT* genes act as tumour suppressor genes. The limited number of genotype-fenotype correlational studies performed so far provide no uniform data {309,714}. The risk of malignant transformation would be higher in patients carrying *EXT1* mutations {714}.

Retinoblastoma syndrome

W.K. Cavenee
T. Hadjistilianou
O. Bögler
I.F. Newsham

Definition

Retinoblastoma (RB) is a malignant tumour originating from the embryonic neural retina. Familial retinoblastoma is typically bilateral, caused by a germline mutation in the *RB1* tumour suppressor gene and is often associated with the development of second site primary tumours, including osteosarcoma, fibrosarcoma, chondrosarcoma, Ewing sarcoma, pinealoblastoma, epithelial tumours, leukaemia, lymphoma, mela-noma and brain tumours.

OMIM number 180200 {1376}

Synonym

Retinoblastoma / osteogenic sarcoma syndrome.

Incidence

Retinoblastoma, the most common intraocular tumour of children, has a worldwide incidence between 1/3500 and 1/25000 with no significant differences between the sexes or races {28, 147,511,1856}.

Diagnostic criteria

Presentation is a white, pink-white, or yellow-white pupillary reflex termed "leukocoria'' resulting from replacement of the vitreous by tumour, or by a large tumour growing in the macula {718}. Another common symptom, strabismus (exotropia or esotropia), can occur alone or associated with leukocoria. Less frequent presenting signs include a red, painful eye with secondary glaucoma, low-vision orbital cellulitis, unilateral mydriasis, and heterochromia {2346}. The tumour can be difficult to differentiate from a variety of simulating lesions such as persistent hyperplastic primary vitreous, retrolental fibroplasia, Coats disease, Toxocara canis infection, retinal dysplasia, or chronic retinal detachment {582,976}. These can be distinguished using CT, MRI, ultrasonography or fine-needle aspiration biopsy and a careful history of the family and affected child {582}.

Clinical features

Retinoblastoma can be unifocal or multifocal. In bilateral cases, one eye is usually in a more advanced stage, while the contralateral eye has one or more tumour foci. The average age at diagnosis is 12 months for bilateral and 18 months for unilateral cases, with 90 percent of the cases diagnosed before the age of 3 {29, 1157,1860,2123}. Retinoblastoma can be a part of the 13q-deletion syndrome in association with moderate growth and mental retardation, broad prominent nasal bridge, short nose, ear and dental abnormalities, and muscular hypotonia {38,717}.

Trilateral retinoblastoma describes the association between bilateral retinoblastoma and midline brain tumours, usually in the pineal region {554}. Pineal tumours resembling well differentiated retinoblastomas are also called ectopic retinoblastoma. CT scanning and MRI have reduced the misinterpretation of pineal tumours as intracranial spread of retinoblastoma {2346}. This is clinically important since ectopic intracranial retinoblastoma requires therapy to the whole neuraxis as well as high-dose equivalent radiotherapy to the primary tumour.

Pathology of retinoblastoma

Retinoblastoma occurs as a mass between the choroid and retina (exophytic) or bulge from the retina toward the vitreous (endophytic). Most advanced tumours show both patterns of growth. The tumour is histologically characterized by rosettes and fleurettes, which are believed to represent maturated or differentiated neoplastic cells. Rosettes are spherical structures (circular in section) of uniform cuboidal or short columnar cells arranged about a small round lumen (Flexner-Wintersteiner rosette) or without any lumen (Homer-Right rosette). The latter also appears in other neuroectodermal tumours such as medulloblastoma. Fleurettes are arranged with short, thin stromal axes surroun-

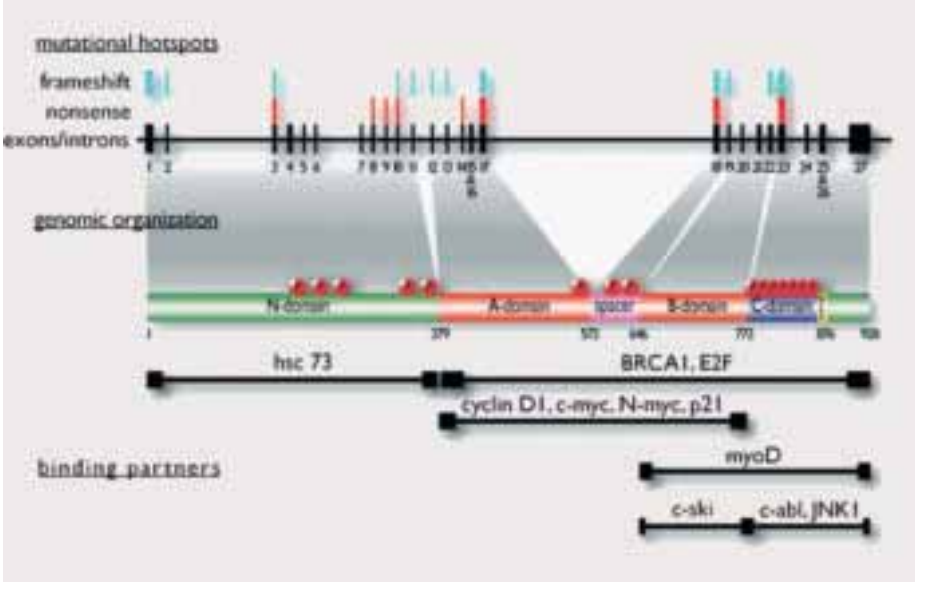

Fig. 21.14 Genomic and protein domain organization of the 105kD retinoblastoma protein. Mutational hotspots for frameshift and nonsense mutations are identified above individual exons. Examples of some of the known cellular binding proteins and their region of interaction are depicted below the protein domains. Sites of phosphorylation are also noted.

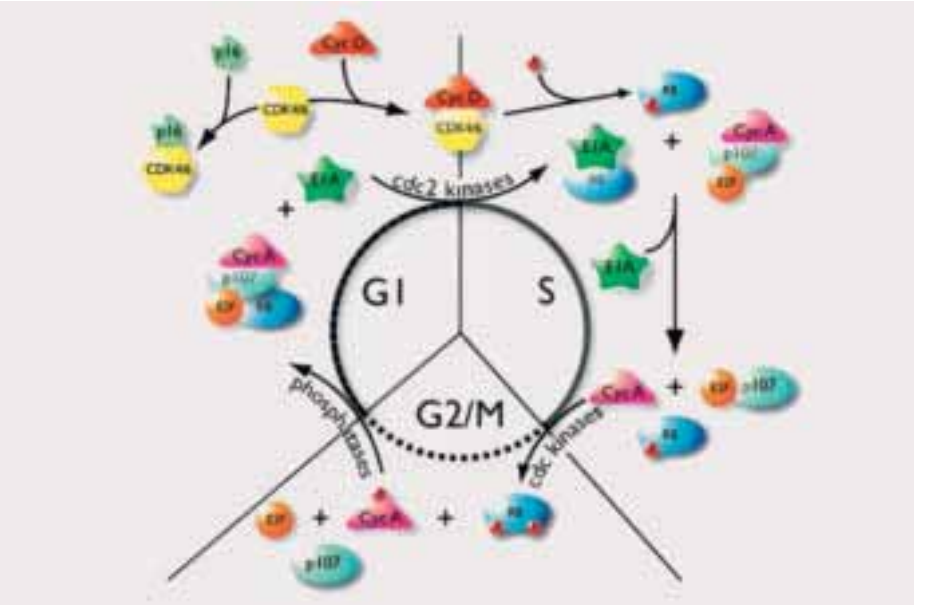

Fig. 21.15 Regulation of the cell cycle through oscillating phosphorylation of the p105 retinoblastoma protein.

ded by differentiated neoplastic cells with their apical part facing the externum. Tumours can be necrotic, with surviving cells around blood vessels, creating ``pseudo-rosettes.'' Calcified foci and debris from nucleic acids can be found in necrotic areas giving rise to basophilic vessel walls {29, 1860, 2123}.

Growth patterns and other histological parameters are not useful for determining prognosis. The degree of differentiation and number of mitoses show a weak correlation. Stronger relationships exist with invasion of the choroids, optic nerves and sclera. Progressive invasion of the eye coats, even in the horizontal plane, is highly informative for determining prognosis {1157, 2123}.

Bone and soft tissue tumours

Second-site primary malignant tumours refer to nonmetastatic tumours arising in "disease-free'' patients treated for initial disease. Tumours associated with retinoblastoma include osteosarcoma, fibrosarcoma, chondrosarcoma, epithelial malignant tumours, Ewing sarcoma, leukaemia, lymphoma, melanoma, brain tumours, and pinealoblastoma {12, 502, 550, 1793, 1837}. These second tumours are classified into five groups: (a) tumours in the irradiated area, (b) tumours outside and remote from the irradiated area, (c) tumours in patients not receiving radiotherapy, (d) tumours unable to be determined as primary or metastases, and (e) tumours in members of retinoblastoma families who were free of retinal tumours. Two important observations have emerged from analysing these patients: (a) the great majority of children in whom second neoplasms develop have or will have bilateral retinoblastoma, and (b) the incidence of second neoplasms in this group was similar whether they received radiation or not. Osteogenic sarcomas are the most frequent second site neoplasms in all the published series {12, 336a, 502, 550, 1720a, 1723a, 1793, 1837}.

Genetics

Retinoblastoma has served as the prototypic example of a genetic predisposition to cancer. It is estimated that 60 percent of cases are nonhereditary and unilateral, 15 percent are hereditary and unilateral, and 25 percent are hereditary and bilateral. In the latter two types, autosomal dominant inheritance with nearly complete penetrance is observed. Analysis of such cases by epidemiological / cytogenetic {716, 941, 1145, 2006, 2017, 2044}, molecular genetic {317, 318} and molecular biological {558, 969} methods suggests as few as two required stochastic mutational events in the *RB1* locus for tumour formation. The first mutation can be inherited through the germ line or somatically acquired, whereas the second occurs somatically in either case. *RB1* locus inactivation is also found in non-hereditary retinoblastoma {552}, osteogenic and other sarcomas occurring as second primary tumours in retinoblastoma patients and some primary sarcomas in the absence of retinoblastoma involvement {724, 882}.

Gene structure and expression

The *RB1* locus in chromosome band 13q14.1 {317, 716, 2006, 2044} encompasses 200 kb of genomic DNA organized into 27 exons {228, 744, 1233}. The 105 kD RB1 protein is ubiquitously expressed in normal human and rodent tissues, including brain, kidney, ovary, spleen, liver, placenta, and retina. RB1 is differentially phosphorylated {1234}, with the unphosphorylated form predominantly found in the G1 stage of the cell cycle, and an initial phosphorylation occurring at the G1/S boundary {284, 482}. Viral proteins bind the p105RB protein {481, 564, 2262} using regions necessary for their transforming function. Over 100 intracellular pRB binding proteins have also been identified including E2F transcription factors, tumour suppressor BRCA1 and the RB-like proteins p107 and p130 {1508}. Complexing of the two latter factors also oscillates in a cell-cycle-dependent manner linking the tumour-suppressing function of RB1 with transcriptional regulation.

Mutations

Mutations that result in loss of RB1 function have been described for retinoblastoma patients and their tumours at the DNA, RNA, and protein levels. RB1 alterations have also been detected in a variety of clinically related second-site primary tumours including osteosarcoma, as well as other non-secondary tumours such as breast and small-cell lung carcinoma. Detection of *RB1* mutations provides for accurate prenatal risk assessment {319, 970, 2267, 2318}.

Rothmund-Thomson syndrome

N.M. Lindor

Definition

Rothmund-Thomson syndrome (RTS) is a constellation of various skin abnormalities, skeletal defects, juvenile cataracts, premature ageing, and a predisposition to osteosarcoma, skin cancer, and other tumours. At least a subset of cases are caused by inherited mutations in the *RECQL4* helicase gene.

OMIM number 268400

Synonym

Poikiloderma congenitale.

Incidence

RTS is a rare, autosomal recessive disorder. The exact incidence is unknown, but more than 250 cases have been reported in the world literature from a variety of ethnic backgrounds. A slight male preponderance (M:F = 2:1) has been reported {2212}.

Diagnostic criteria

Specific criteria for the diagnosis of RTS have not been established. The diagnosis is based upon clinical findings, the identification of *RECQL4* in a subset of cases, and laboratory tests that can exclude some other, similar disorders.

Fig. 21.16 Osteosarcoma of the rib in a patient with Rothmund-Thomson syndrome.

Clinical features

The cardinal feature of RTS is a sun-sensitive erythematous rash that typically appears during the first 6 months. It usually starts in the face and then spreads to the buttocks and extremities. With time, the rash enters a chronic phase resulting in skin atrophy, telangiectasias, and marbleized mixed hyper- and hypopigmentation (poikiloderma) {1735, 2198, 2199, 2212}. Other features associated with RTS include short stature (~2/3 of the cases), premature greying and loss of hair (50-65%), sparse eyebrows/lashes (60-75%), juvenile cataracts (7-50%), photosensitivity (35%), radial ray anomalies (>20%) and other bony abnormalities, dystrophic nails and teeth, hypogonadism, and hypersensitivity to cytotoxic drugs and radiotherapy {1735, 2198, 2199, 2212}. RTS does not seem to be associated with intellectual or immunological impairment. There are no specific or consistently identifiable laboratory features in RTS. There have been several reports of acquired, clonal somatic mosaicism for chromosome abnormalities, especially trisomies, isochromosomes, and translocations frequently involving chromosome 8, often found in fibroblast cultures {1269}. There is no evidence of mismatch repair deficiency in the form of tumour microsatellite instability, as seen in tumours associated with the hereditary non-polyposis colon cancer syndrome, due to germline mutations in genes of the DNA mismatch repair complex). Furthermore, there is no increase in chromosomal sister-chromatid exchange rates (as seen in Bloom syndrome), no excess of bleomycin-induced chromosome breakage (as seen in ataxia telangiectasia), and no chromosomal radial formation with mitomycin-C exposure (as seen in Fanconi anaemia). Ultraviolet sensitivity studies have yielded inconsistent results.

Bone and soft tissue tumours

Osteosarcomas, involving any bone and especially in non-common sites, have been reported to occur in up to one third of the patients, with a median age of diagnosis at 11.5 years {2212}. Also cutaneous malignancies, in particular squamous cell carcinomas, have been reported to be overrepresented in RTS {1735, 2212}.

Genetics

At least a subset of the cases of RTS are caused by mutations in the *RECQL4* (also known as *RECQ4*) helicase gene in chromosome band 8q24.3 {1128}. Only a small number of patients has as yet been investigated, with mutations being detected in approximately 40% of the cases {749}. The *RECQL4* gene has a predicted protein product of 1208 amino acids. It is highly expressed in the thymus and testis with low levels of intranuclear expression in multiple other tissues. *RECQL4* mutation analysis is available only in specialized centres. Mutations have included frameshift mutations, nonsense mutations, and deletions including part of the consensus helicase domain. This gene is homologous to the genes that cause Bloom syndrome and Werner syndrome, which might explain some of the clinical overlap {749}.

Werner syndrome

R.J. Monnat, Jr.

Definition

Werner syndrome (WS) is a rare, autosomal recessive genetic instability syndrome and is caused by mutations in the *WRN* gene. Affected patients develop a prematurely aged appearance in the second and third decades of life, and are at increased risk of developing both neoplastic and non-neoplastic diseases. Tumours include soft tissue sarcomas, thyroid carcinoma, malignant melanoma, meningioma, haematological neoplasms, and osteosarcoma. The most common causes of death are cancer and atherosclerotic cardiovascular disease.

OMIM number 277700

Synonym

Progeria of the adult.

Incidence

WS patients have been identified worldwide {819}. Estimates of the frequency or prevalence of WS, obtained by case counting and from consanguinity data, range from 1/22,000 to $1/10^6$ (reviewed in {1883}). The frequency of WS in different countries is strongly influenced by the presence of founder mutations and the frequency of consanguinity or inbreeding. The range of frequency estimates also undoubtedly reflects the variable and delayed development of the WS clinical phenotype {604, 819}, with consequent underdiagnosis.

Clinical features and diagnostic criteria

The most consistent clinical findings develop after age 10. These include bilateral cataracts, dermatological pathology resembling scleroderma, short stature and premature greying and loss of scalp hair {604,819}. There may be affected siblings as well as evidence of parental consanguinity (3rd cousin or closer). Additional, less consistent findings include diabetes mellitus, hypogonadism, osteoporosis, soft tissue calcification, premature atherosclerotic cardiovascular disease, high pitched, 'squeeky', or hoarse voice and flat feet.

A definite diagnosis can be established on clinical grounds when all of the consistent features and at least two additional findings are present. Additional diagnostic aids include evidence of elevated 24 hr urinary hyaluronic acid secretion; loss of WRN protein from fibroblasts or peripheral blood lymphocytes; and mutations in the *WRN* gene on chromosome arm 8p.

A clinical scoring system has been devised to identify more reliably definite, probable or possible WS patients. Additional information on this scoring system and the clinical diagnosis of WS can be found on the International Registry of Werner Syndrome Web site:
www.pathology.washington.edu/research/werner/registry/diagnostic.html

Table 21.05
Histopathological spectrum of neoplasia in Werner syndrome.
A wide spectrum of neoplasms has been identified in Werner syndrome (WS) patients, who are clearly at elevated risk of developing one or more of the neoplasms listed in the left column ('frequent'). These neoplasms represent 71% of all neoplasms reported in WS patients. WS patients may be at elevated risk of developing neoplasms listed in the right column, although the number of affected patients is too small in most cases to firmly establish this suspicion. A total of 257 neoplasms were represented in this analysis {820, 1494} (Y. Ishikawa, personal communication). The percentage of neoplasms from this analysis in each column or tumour type is indicated in parentheses.

Frequent (71%)	Less common (29%)
Soft tissue sarcomas (15.5% of cases) malignant fibrous histiocytoma leiomyosarcoma fibrosarcoma malignant schwannoma synovial sarcoma rhabdomyosarcoma	**Non-melanoma skin cancer (5.8%)**
Thyroid carcinomas (14%) follicular papillary anaplastic	**Hepatobiliary carcinomas (5.3%)** hepatocellular cholangiocarcinoma gallbladder
Malignant melanoma (12.6%) acral lentigenous melanoma mucosal malignant melanoma	**Genito-urinary (4.8%)** bladder carcinoma uterine/ovarian carcinoma renal cell carcinoma prostate carcinoma seminoma
Meningioma (11.1%) benign multiple / malignant	**Gastro-intestinal carcinoma (4.3%)** gastric oesophagus pancreas colon
Haematological (11.1%) acute myelogenous leukaemias (M1-5) erythroleukaemia (M6) megakaryocytic leukaemia (M7) myelofibrosis/myelodysplasia aplastic anaemia	**Breast carcinoma (3.9%)**
Osteosarcoma (6.3%)	**Oro-pharyngeal carcinoma (2.4%)**

Neoplastic disease spectrum

WS patients are at increased risk of developing both sarcomas and epithelial neoplasms {820, 1494}. The elevated risk of neoplasia is selective, and includes the following neoplasms in order of decreasing frequency: soft tissue sarcomas, thyroid carcinoma, meningioma, malignant melanoma, malignant or pre-neoplastic haematological disease and osteosarcoma. Many other neoplasms, including common adult epithelial malignancies, have been observed in WS patients. However, it is not clear whether the risk of developing these neoplasms is elevated above population controls. This histo-pathological spectrum of neoplasms overlaps with, though is distinct from, that observed in patients with two other RecQ helicase deficiency syndromes, Bloom syndrome and Rothmund-Thomson syndrome {1494}.

Several features of neoplasia in WS patients indicates that this human RecQ helicase deficiency syndrome is a heritable cancer predisposition: patients develop neoplasms at a comparatively early age; often have unusual sites of presentation (e.g., osteosarcoma of the patella) or less common histopathologic subtypes (e.g., follicular as opposed to papillary thyroid carcinoma); and can have multiple concurrent or sequential neoplasms, e.g., thyroid carcinoma and osteosarcoma. Estimates of the increased risk of neoplasia in WS patients range from 30-fold elevated overall lifetime risk across all tumour types to 1000-fold elevated risk for acral lentigenous melanoma.

Soft tissue sarcomas that have been identified in WS patients include malignant fibrous histiocytoma, malignant peripheral nerve sheath tumour, fibrosarcoma, rhabdomyosarcoma, lipo-sarcoma, and synovial sarcoma. Three histological subtypes of thyroid carcinoma have been reported in WS patients (follicular, papillary and anaplastic), with a predominance of the less common follicular variant. There has been no reported case of medullary thyroid carcinoma in a WS patient. The risk of malignant melanoma is confined almost exclusively to the relatively rare variants that arise on the palms and soles (acral lentigenous melanoma) or in mucosa of the nasal cavity or esophagus. Melanoma risk is most clearly elevated in Japanese WS patients {820}.

The spectrum of haematological disease in WS includes acute myelogenous leukaemia (M1-5), erythroleukaemia (M6) and megakaryocytic leukaemia (M7); atypical leukemia arising in the context of myelodysplasia; and the pre-malignant conditions myelodysplasia, myelofibrosis, and aplastic anaemia. The elevated risk of developing marrow-associated pre-malignant or malignant disease may be related to the progressive accumulation of genetic damage in bone marrow cell lineages {1509}.

Genetics

WS is an autosomal recessive disease: no cases are known to have been acquired or to have been caused by other agents. WS constitutes, together with Bloom syndrome and Rothmund-Thomson syndrome, a group of inherited human genetic instability / cancer predisposition syndromes that result from loss of function of a human RecQ helicase protein.

Gene structure and expression

The *WRN* gene consists of 35 exons in a 165 kb region of chromosome region 8p11-12 {2331}.

Two stable RNAs are encoded by the *WRN* gene, and the shorter, of 5.8 kb, is ubiquitously expressed at varying levels in many cell types, tissues and organs {2331}. The 162 kDa WRN protein is readily detectable in cell lines and tissue samples from normal individuals and heterozygous carriers of single mutant copies of the *WRN* gene by Western blot analysis {1510}. No systematic study of the level of expression of WRN protein as a function of cell type or of development has as yet been published. The WRN protein encodes both DNA helicase and exonuclease activities {1931}, and is likely to play an important physiologic role in homologous recombinational repair in human somatic cells {1728}.

Mutations

WS is an autosomal recessive disease, and thus patients have mutations in both *WRN* alleles. Virtually all of the *WRN* patient mutations thus far identified truncate the *WRN* open reading frame, lead to protein reduction or loss from patient cells and thus can be detected by Western blot analysis {821,1510}. Further mutation characterization can be performed by a combination of mutation-specific allele identification and / or DNA sequencing. Mutation analysis can be especially helpful in the diagnosis of WS in young patients, where the diagnosis is suspected but the clinical phenotype may be incompletely developed. A HUGO Locus-Specific *WRN* Mutational Database summarizes patient mutation data and mutation designations, polymorphism data, and related clinical data and cross-references these to the primary literature (www.pathology.washington.edu/research/werner/ws_wrn.html) {1511}. Additional information on *WRN* mutation analysis for the purpose of confirming a diagnosis of Werner syndrome can be obtained through the International Registry of Werner Syndrome Web site (www.pathology.washington.edu/research/werner/registry/diagnostic.html).