Hereditary Tumor Syndromes and Gliomas

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Abstract Several congenital syndromes caused by germline mutations in tumor suppressor genes predispose to the development of glial tumors. In the last few decades our knowledge about the molecular functions of these genes and the pathogenesis of hereditary tumor syndromes has greatly increased. The most common syndromes are the neurofibromatoses (type 1 and type 2) and the tuberous scleroses complex. There are interesting overlaps in the molecular pathogenesis. Deregulation of Ras or downstream Ras pathways including MEK/ERK and AKT/mTOR plays an important role in these three syndromes. Other rare syndromes include Li-Fraumeni, melanoma-astrocytoma, and Turcot syndrome involving cell cycle regulators and DNA repair genes. The genes and pathways involved in the pathogenesis of these syndromes also play an important role in the development of sporadic tumors. Therefore research on hereditary syndromes contributes substantially to our understanding of tumor formation.

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5.1 Neurofibromatosis

5.1.1 Historic Aspects

Descriptions of patients with characteristic features of neurofibromatosis go back to the second century (Huson and Hughes 1994). Friedrich Daniel von Recklinghausen coined the term "neurofibromatosis" (1882). In the second half of the twentieth century the clinical difference between a "peripheral" (NF1) and a "central" (NF2) form of neurofibromatosis was established. After the identification of the *NF1* and the *NF2* genes in the early 1990s, the two forms were recognized as distinct genetic entities.

5.1.2 Neurofibromatosis Type 1

Synonyms: von Recklinghausen's disease, Watson disease, peripheral neurofibromatosis.

Neurofibromatosis type 1 (NF1) is an autosomal dominant familial tumor syndrome affecting 1 in 3,500 individuals. It is characterized by multiple benign tumors and a predisposition to malignant neoplasms. The most consistent features of NF1 are café-au-lait spots and dermal and plexiform neurofibromas. Furthermore, patients develop different tumors of the

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Table 5.1 Diagnostic criteria for NF1

The presence of two or more of the following signs identify the NF1 patient:	
1.	Six or more café-au-lait patches, diameter greater than 5 mm in prepubertal and over 15 mm in postpubertal individuals
2.	Two or more neurofibromas of any type or one plexiform neurofibroma
3.	Axillary and/or inguinal freckling
4.	Glioma of the n. opticus
5.	A distinctive osseous lesion, such as dysplasia of the sphenoid wing, thinning of the long bone cortex, with or without pseudarthrosis
6.	A first-degree relative (parent, sibling, or offspring) with NF1 according to the above criteria 1–5

central nervous system (pilocytic astrocytomas WHO grade I, but also glioblastomas WHO grade IV). Additional manifestations of NF1 are bone deformities (scoliosis, macrocephaly, pseudarthrosis), small stature, encroachment of central nervous system functions such as intellectual properties, changes of personality structure, and vascular malformations particularly fibromuscular hyperplasia (Bader 1986).

The variable expressivity of the symptoms results in a complex clinical picture. The guidelines for the diagnosis of NF1 have been established by an NIH consensus development conference statement and are listed in Table 5.1. The frequency of selected NF1-associated symptoms is given in Table 5.2.

The increased risk for malignancies is of special clinical importance because these tumors are the major cause of early death in NF1 patients (Friedman 1999). Malignancies include malignant peripheral nerve sheath tumor (MPNST), triton tumor, rhabdomyosarcoma, acute myeloid leukemia, and malignant astrocytoma (Huson and Hughes 1994).

Table 5.2 Frequency of characteristic symptoms in NF1-patients

Symptoms	Frequency
Neurofibromas postpubertal	90%
thereof leading to neurological deficits	10%
Cognitive and social problems	50%
Plexiform neurofibromas	30%
Scoliosis	30%
Pilocytic astrocytomas	15%

5.1.2.1 Molecular Genetics

The basis of inheritance in NF1 is a germline mutation in the *NF1* tumor suppressor gene. The *NF1* gene was isolated using positional cloning (Cawthon et al. 1990b; Viskochil et al. 1990). It maps to the chromosomal region 17q11.2. Germline alterations in both parental alleles have never been seen and the intrauterine lethality of mouse embryos with biallelic germline mutation suggests prenatal lethality of biallelic *NF1* deficiency in humans too (Jacks et al. 1994).

The *NF1* gene spans at least 335 kb containing 60 exons with an 8,457-bp open reading frame that codes for 2,818 amino acids (neurofibromin type I). It belongs to the group of giant genes according to the classification of McKusick. Exon 27b of *NF1* carries three embedded genes: *EVI2A* (ecotropic viral integration site 2A), *EVI2B* (ecotropic viral integration site 2B), and *OMG* (oligodendrocyte myelin glycoprotein). All three genes are encoded in reverse direction to the *NF1* sense strand (Cawthon et al. 1990a, 1991; Viskochil et al. 1991; Shen et al. 1996; Habib et al. 1998). At least 12 *NF1* pseudogenes

are distributed on different human chromosomes; however, none of these pseudogenes contains sequences beyond exon 29. The extensive size of the *NF1* gene may contribute to the high rate of spontaneous mutations being the cause of disease in approximately 50% of the patients. *NF1* mutations affect all regions of the gene without significant hotspots. The majority of the mutations lead to a truncated protein (about 80%) and only a small proportion code for missense mutations (10%). With respect to genotype–phenotype correlation, it has been reported that large deletions (up to 1.5 Mb genomic DNA) of the *NF1* gene are associated with an earlier age of onset of cutaneous neurofibromas, learning disability, dysmorphic features, and developmental delay (Castle et al. 2003). In addition, a recent study reported on 21 unrelated probands with the same *c*.2970–2972 delAAT (p.990delM) germline mutation but without cutaneous or plexiform neurofibromas (Upadhyaya et al. 2007).

5.1.2.2 Molecular Pathogenesis

The *NF1* gene product, neurofibromin, is expressed ubiquitously with the highest levels in the central and peripheral nervous systems, in leukocytes, and the adrenal gland (DeClue et al. 1991; Gutmann et al. 1991; Daston et al. 1992). There are at least five human isoforms. All but neurofibromin type I are generated by alternative splicing of four exons: 9a, 10a-2, 23a, and 48a. Neurofibromin type I does not contain any of these exons (Nishi et al. 1991; Gutman et al. 1993; Danglot et al. 1995; Kaufmann et al. 2002). The molecular weight of human neurofibromin is 250–280 kDa in SDS page. Neurofibromin localizes mainly to the cytoplasm, but it has been found in the nucleus and a nuclear localization signal of neurofibromin encoded by exon 43 of the *NF1* gene has been reported (Vandenbroucke et al. 2004).

There are only a few putative functional domains within neurofibromin: RasGAP, SEC14-like, and a pleckstrin homology (PH)-like domain. Beside these, a cysteine/serine-rich domain (CSRD) upstream of RasGAP has been described (Fahsold et al. 2000).

5.1.2.3 Neurofibromin and Ras Proteins

The monomeric GTP/GDP-binding proteins of the Ras superfamily are functionally active in the GTP-bound form. Guanine exchange factors (GEFs) promote the switch from the inactive GDP-form to the active GTP-form. The active GTP form is localized in the membrane and has a low intrinsic GTPase activity. The physiological inactivation is enhanced by up to five orders of magnitude by GTPase-activating proteins (GAPs). Neuro-fibromin belongs to the specific GAPs of the subfamily of Ras proteins. Due to its function the corresponding domain of neurofibromin is called the “GAP-related domain” (GRD). This is the best studied region of the *NF1* gene. The domain shares high homology to related domains of other GAPs and to IRA1 and IRA2, proteins with inhibitory effect on Ras in *Saccharomyces cerevisiae*. Significant homology can be observed between *NF1* and *IRA1* in regions that extend beyond the GAP-related domain. Neurofibromin exerts its activity on H-Ras, K-Ras (viral Harvey and Kirsten murine sarcoma oncogenes), N-Ras (human neuroblastoma oncogene), R-Ras, as well as Tc21 (R-Ras2). Multiple activators such as hormones, cytokines, growth factors, extracellular matrix proteins, or antigens in T-cell activation can affect GTP-Ras formation. Some heterotrimeric G proteins are also able to activate Ras proteins. There are at least seven different effectors of GTP-Ras proteins initiating different signal cascades, which in the end lead to differences in gene expression. One major signal cascade which has been shown to play a critical role in cell proliferation is activated by interaction of active Ras with Raf serine/threonine kinase. Raf serine/threonine-kinase phosphorylates a sec-

ond kinase, the MAP kinase/ERK kinase (MEK). MEK phosphorylates ERK family members. Phosphorylated ERK phosphorylates a number of other proteins like other kinases (S6 kinase) and transcription factors, such as CREB (Grand and Owen 1991; Boguski and McCormick 1993; Macara et al. 1996).

Loss of functional neurofibromin can favor the active status of Ras and therefore continuously stimulate the Raf-MEK-ERK pathway leading to cell proliferation.

Another cascade which is triggered by activated Ras leads to the activation of phosphoinositide 3-kinase (PI3K), followed by phosphorylation of protein kinase AKT (also known as protein kinase B). AKT has the ability to inactivate the hamartin/tuberin complex by phosphorylation. The consequence of hamartin/tuberin inhibition is the activation of the small GAP Rheb (Ras homologue enriched in brain) which activates the kinase serine/threonine target of rapamycin (TOR or mTOR) (Pan et al. 2004). Evidence for an activation of mTOR in NF1-associated tumors has been reported and neurofibromin-dependency of the mTOR pathway could be demonstrated in cell culture systems (Dasgupta et al. 2005; Johannessen et al. 2005).

Thus, Ras proteins influence in a cell type-specific manner a diversity of cell processes such as proliferation, migration, differentiation, apoptosis, and senescence.

The SEC14 domain is found in secretory proteins and in lipid-regulated proteins and may play a role in co-regulating Ras GTPase activity (Aravind et al. 1999; D'Angelo et al. 2006; Welti et al. 2007). There is evidence that neurofibromin may exhibit Ras-modulating effects independent of its GAP activity by participating in the rearrangement of cytoskeletal components (Corral et al. 2003). A recent publication reveals the ability of neurofibromin to bind caveolin (Cav-1), a membrane protein, which is known to regulate signaling molecules like Ras, protein kinase C, and growth factor receptors. The fact that missense mutations occur in potential caveolin-binding sites speaks in favor of a role of caveolin in neurofibromin function (Boyanapalli et al. 2006).

5.1.2.4 Neurofibromin and Adenylate Cyclases

Neurofibromin function seems to be involved in the cAMP protein kinase A (PKA-) pathway. There is evidence from *Drosophila* models that neurofibromin is involved in activation of adenylate cyclases (AC) (Guo et al. 1997, 2000; The et al. 1997). Lower neuropeptide- and G protein-stimulated AC activity in *NF1*$^{-/-}$ than in *NF1* $^{+/-}$ mouse brains has been found, indicating that neurofibromin regulates AC activity also in mammals (Tong et al. 2002). Recently two *NF1*-dependent adenylate cyclase pathways in *Drosophila* brain have been described (Hannan et al. 2006). On the other hand, a threefold increase of cAMP levels in Schwann cells from *NF1*-null mice compared to wild-type has been found arguing for an antagonistic role of neurofibromin at cAMP accumulation (Kim et al. 2001). An increased baseline level of cAMP has also been seen in neurofibromin-deficient astrocytes, but it could be demonstrated that inactivation of neurofibromin in astrocytes results in reduced cAMP generation in response to pituitary adenylate cyclase-activating polypeptide (PACAP), attenuated calcium influx, and Rap1 activation (Dasgupta et al. 2003). In this context is has to be noted that cAMP exhibits mitogenic effects in Schwann cells, whereas increased cAMP levels in astrocytes lead to a growth inhibitory signal (Dugan et al. 1999; Kim, Ratner et al. 2001). Thus the role of neurofibromin in AC activity seems to be cell type specific and coupled to an antiproliferative effect.

5.1.2.5 NF1 and Astrocytomas

NF1 is associated with a highly increased occurrence of pilocytic astrocytomas (PA) WHO grade I (15–20% of patients). Preferential

localizations are the optic tracts (opticus glioma) and the brainstem.

According to the classical "two-hit" hypothesis for the inactivation of tumor suppressor genes, several studies could prove that NF1-associated PA harbor a somatic mutation ("second hit") in the NF1 gene (von Deimling et al. 1993; Gutmann et al. 2000; Kluwe et al. 2001). Furthermore, lack of neurofibromin expression has been found along with elevated levels of Ras-GTP and activation of the Raf/MAPK and PI3K/AKT pathways in an NF1-associated PA (Lau et al. 2000). The suggested role of neurofibromin in NF1-associated PA gave rise to the question of whether it is of same importance in the pathogenesis of histological identical sporadic pilocytic astrocytomas. It has been shown that NF1 gene mutations occur at low frequency in sporadic PA and that the NF1 expression is increased (approximately 10- to 20-fold) in sporadic PA compared to normal brain (Platten et al. 1996; Wimmer et al. 2002). These data argue against neurofibromin loss of function as a typical molecular event in the pathogenesis of sporadic PA. PAs were analyzed for activation of Ras and Ras mutations. While only 1 of 21 tumors harbored an oncogenic K-Ras mutation, all tumors demonstrated activation of the Ras pathway (Sharma et al. 2005). Recently a gene expression profile in NF1-accociated PA distinct to that of sporadic cases has been found (Sharma et al. 2007).

Thus, it can be concluded that NF1-associated and sporadic pilocytic astrocytomas both share a hyperactivation of the Ras pathway, but that the underlying molecular events are different. The increased expression of *NF1* in sporadic PA is most probably the result of a positive feedback regulation by activated Ras.

Using a mouse model in which the mice lack *NF1* function in the central nervous system (CNS), global reactive gliosis in the adult murine brain and an increased proliferation of glial progenitor cells could be determined. Additionally, the mice developed enlarged optic nerves and some of them developed optic pathway gliomas (Zhu et al. 2005b).

The results of epidemiological studies revealed that NF1 patients also have an increased risk for malignant gliomas (Blatt et al. 1986; Rasmussen et al. 2001). In a mouse model of NF1-associated malignant gliomas all mice lacking *TP53* in the germline and *NF1* function in CNS cells and all mice with compound heterozygosity for *TP53* and *NF1* in CNS cells developed malignant astrocytomas (grade II astrocytomas to grade IV glioblastomas). Mice lacking *NF1* in CNS cells and heterozygosity for *TP53* rarely developed CNS tumors (1/18). It can be concluded that *TP53* loss prior to or concomitant with *NF1* loss (Ras activation) is required for effective malignant tumor formation (Zhu et al. 2005a) in this model.

5.1.3 Neurofibromatosis Type 2

Neurofibromatosis type 2 (NF2) is a dominantly inherited familial tumor syndrome affecting 1 in 40,000 individuals predisposing to benign and, less frequently, malignant neoplasms. The most important diagnostic feature of NF2 is the development of bilateral vestibular schwannomas. Further frequent tumors include meningiomas, astrocytomas, and ependymomas. Due to the multiplicity and the unfavorable tumor sites in patients with NF2, schwannomas in the cerebellopontine angle, and spinal ependymomas, the clinical presentation is often much more severe than might be anticipated from the histological analysis of the lesions.

The guidelines for the diagnosis of NF2 are listed in Table 5.3. The frequency of selected NF2-associated symptoms is given in Table 5.4.

5.1.3.1 Molecular Genetics

The basis of inheritance in NF2 is a germline mutation in the *NF2* tumor suppressor gene, located in chromosome region 22q12.2 (Rouleau et al. 1993; Trofatter et al. 1993). It is phylo-

Table 5.3 Diagnostic criteria for NF2

The following are diagnostic:		
1.		Bilateral vestibular schwannomas; or
2.		A first-degree relative with NF2, and either
	(a)	A unilateral vestibular schwannoma or
	(b)	Two of the following: meningioma, schwannoma, glioma, posterior subcapsular lens opacity, or cerebral calcification; or
3.		Two of the following
	(a)	Unilateral vestibular schwannoma
	(b)	Multiple meningiomas
	(c)	Either schwannoma, glioma, neurofibroma, posterior subcapsular lens opacity, or cerebral calcification

Table 5.4 Frequency of characteristic symptoms in NF2 patients

Tumors or symptoms	Frequency
Spinal tumors	92%
Bilateral vestibular schwannomas	81%
Ophthalmologic abnormalities	62%
Skin schwannomas	59%
Cerebral meningiomas	58%
Cranial nerve tumors	48%
Abdominal calcification	10%
Peripheral neuropathy	10%

genetically highly conserved. Mice with homozygous *NF2* germline mutations are not viable (McClatchey et al. 1997). The *NF2* gene spans 119 kb containing 17 exons. Most of the mutations lead to a truncated protein due to a high rate of nonsense mutations (34%). Missense mutations occur in about 7% of cases (http://neurosurgery.mgh.harvard.edu/NFclinic/NFresearch.htm). There is no direct correlation between geno- and phenotype, but statistically protein truncating mutations are more often associated with a severe clinical course than missense mutations. Remarkably big deletions of the *NF2* gene have been observed in patients with a milder phenotype (Bourn et al. 1994; Parry et al. 1996; Ruttledge et al. 1996; Evans et al. 1998; Lopez-Correa et al. 2000).

5.1.3.2 Molecular Pathogenesis

The *NF2* gene product, merlin or schwannomin, is expressed in most human tissues including the brain. Two isoforms spanning either exons 1–15 and 17 or exons 1–16 are known. Isoform I or "NF2–17" lacks exon 16 and isoform II or "NF2–16" contains exon 16. Merlin exhibits homology to the protein 4.1 family including ezrin, moesin, and radixin (ERM proteins). These proteins share the FERM (four-point one, ezrin, radixin, moesin) domain at the amino terminus (Rouleau et al. 1993; Trofatter et al. 1993). There are two other obvious functional domains, a coiled-coil region and a short carboxy terminal domain. Merlin isoform I and the ERM proteins might exist in two different conformations. The amino- and the carboxy terminus can bind to each other (folded conformation). Phosphorylation near the carboxy terminus inhibits head-to-tail folding and thereby leads to an open configuration (Gary and Bretscher 1995; Matsui et al. 1998). Merlin isoform II is not able to form an intramolecular association and exists in a constitutively open conformation (Sherman et al. 1997; Gonzalez-Agosti et al. 1999). Merlin has many properties in common with the ERM proteins, but shows a unique tumor suppressor function. Finding specific interaction partners of merlin, which do not interact in the same manner with ERM proteins, could be a way

to understand its tumor suppressor function. The fact that missense mutations occur in the FERM domain of merlin argues for merlin-specific protein–protein interactions and specific functions. In addition the FERM domain of merlin shows significant differences to ERM proteins. Beside structural similarities, merlin's C-terminal domain lacks the F-actin-binding ability of the ERM proteins. However, merlin can instead bind F-actin with its FERM domain (Xu and Gutmann 1998; Brault et al. 2001; James et al. 2001). Several other merlin-interacting proteins have been identified: examples are beta-spectrin II (Scoles et al. 1998; Neill and Crompton 2001), solute carrier family 9 (sodium/hydrogen exchanger) (Murthy et al. 1998), schwannomin-interacting protein 1 (Goutebroze et al. 2000), beta 1 integrin (Obremski et al. 1998), CD44 (Sainio et al. 1997; Morrison et al. 2001), hepatocyte growth factor-regulated tyrosine kinase substrate (Scoles et al. 2000), Rho GDP dissociation inhibitor (Maeda et al. 1999), syndecan-binding protein (Jannatipour et al. 2001), paxillin (Fernandez-Valle et al. 2002), and RIb subunit of the PKA (Bretscher et al. 2002; Gronholm et al. 2003). Many of these proteins are plasma membrane-associated proteins or proteins with adaptor function connecting membrane proteins to cytoskeletal components.

Merlin was found to mediate contact inhibition of cell proliferation. At high cell density, merlin is hypo-phosphorylated and active in inhibiting cell growth in response to hyaluronate (HA), a component of the extracellular matrix. This function is dependent on interactions with CD44, a transmembrane HA receptor. At low cell density, merlin is phosphorylated, forms a complex with ezrin and moesin, which is associated with CD44, and does not show growth inhibitory activity (Morrison et al. 2001).

Mitogen-activated protein kinases (MAPK or ERKs), which are downstream targets of active Ras, play a well-known role in regulation of cell proliferation and differentiation (Winston and Hunter 1995; Marshall 1996). Merlin was shown to exert anti-Ras activity (Tikoo et al. 1994; Kim et al. 2002; Lim et al. 2003). The exact mechanism mediating this effect is not known. In recent years there has been progress in understanding by which means merlin is able to influence these signaling pathways. Adaptor protein paxillin binds directly to merlin and mediates the localization of merlin to the plasma membrane, where it associates with beta 1 integrin and erbB2. Paxillin allows the binding of Rho-GTPase regulators and effectors as well as kinases and phosphatases at beta 1 integrin-dependent contacts. It recruits PAK to focal complexes (Fernandez-Valle, Tang et al. 2002). Merlin has an inhibitory function on activated kinase PAK1, a critical mediator of the Rac/Cdc42 signaling pathway. The inhibitory function is mediated by a direct interaction between merlin and PAK1 (Kissil et al. 2003). It was observed that merlin is able to inhibit the Ral guanine nucleotide dissociation stimulator (RalGDS), a downstream molecule of Ras, via direct interaction (Ryu et al. 2005). In a recent study it has been shown that merlin displays an inhibitory effect on the growth hormone-stimulated activation of the Raf-ERKs pathway by binding to growth factor receptor-bound protein 2 (Grb2) (Lim et al. 2006). The nucleotide exchange factor son of sevenless homolog (Sos) may bind the Grb2 SH3 domain, and the formation of an EGFR/Sos/Grb2 complex is associated with Ras activation (Buday 1999). The protein magicin is able to interact with merlin as well as Grb2 and is capable of forming a complex with these proteins (Wiederhold et al. 2004). Another recent study reports evidence for an inhibitory role of merlin in activation of Ras and Rac (Morrison et al. 2007). Merlin was found to bind PIKE-L (PI3K enhancer), a GTPase that binds to PI3K and triggers its activation. Merlin was shown to compete with PI3K for binding to PIKE-L, thereby inhibiting activation of the PI3K-AKT pathway (Rong et al. 2004). It has been shown

that the protein kinase AKT directly binds to and phosphorylates merlin on residues Thr 230 and Ser 315, thereby abolishing merlin's head-to-tail folding and promoting its degradation by ubiquitination (Tang et al. 2007). Another study describes the direct interaction of merlin with the eukaryotic initiation factor 3 (eIF3) p110 subunit (eIF3c). The FERM domain of merlin was shown to bind the C-terminal half of eIF3c. Increased expression of eIF3c elevated cell proliferation and merlin was effective at inhibiting cellular proliferation when eIF3c levels were at their highest (Scoles et al. 2006).

These observations show that merlin plays a role in modulating receptor–cytoskeleton linkage as well as in signaling to the cytoskeleton affecting cell growth and adhesion.

5.1.3.3 NF2 and Tumors

Merlin is nearly absent in tumors from NF2 patients. In addition to the inherited defect, the second allele of the *NF2* gene has been inactivated – usually by a deletion including major portions of chromosome 22. This is consistent with the "two-hit" hypothesis by Knudson explaining the high incidence of tumors in patients who have inherited a mutation in a tumor suppressor gene. Somatic *NF2* gene mutations are observed to a high degree in those sporadic tumor types that characterize the NF2 tumor syndrome. Sporadic tumors with *NF2* mutations include schwannoma, meningioma (with transitional and fibroblastic variants being more often affected than meningotheliomatous meningiomas), and ependymomas in spinal localization usually in adult patients. NF2 patients typically develop multiple meningiomas and the tumors occur at a younger age than in the general population. Meningiomas in NF2 are often recurrent but the frequency of atypical or anaplastic meningiomas is not increased in NF2 (Antinheimo et al. 1997).

5.2 Tuberous Sclerosis Complex

Tuberous sclerosis complex (TSC) is an autosomal-dominant inherited syndrome affecting 1 in 6,000 to 10,000 individuals. The disease is characterized by the development of different types of benign hamartomas involving the CNS, skin, kidney, and heart. The majority of hamartomas associated with TSC are extremely rare in the general population and are therefore highly diagnostic for TSC (Table 5.5). The clinical picture is variable, making a clinical diagnosis difficult in some cases.

Criteria for TSC have been established. Criteria for definite TSC: either two major features or one major feature plus two minor features. Criteria for probable TSC: One major plus one minor feature. Criteria suggestive of TSC: either one major or two or more minor features (Roach et al. 1998). CNS manifestations are cortical tubera, subependymal nodules, and subependymal giant cell astrocytomas and the majority of patients (78–92%) have epileptic seizures. Mental retardation can be part of the syndrome (Kwiatkowski and Short 1994). An increased risk for malignancies exists only for kidney tumors (malignant angiolipoma or renal cell carcinoma) with a lifetime risk of 2–3% (Cook et al. 1996; Al-Saleem et al. 1998).

5.2.1 Molecular Genetics

TSC is caused by mutations in one of two different genes: *TSC1* located on chromosome 9q34 and *TSC2* on chromosome 16p13.3 (European Chromosome 16 Tubererous Sclerosis Consortium 1993; van Slegtenhorst et al. 1997). Families with TSC carry germline mutations in *TSC1* and *TSC2* in 50% of cases. There is a high rate of spontaneous mutations representing 65–85% of all cases. Spontaneous cases are more often due to a *TSC2* germline mutation (65%). The *TSC1* gene has 23 exons and encodes

Table 5.5 Diagnostic criteria for TSC

Major features	Minor features
Facial angiofibromas or forehead plaque	Multiple randomly distributed pits in dental enamel
Nontraumatic ungual or periungual fibroma	Hamartomatous rectal polyps
Hypomelanotic macules (3 or more)	Bone cysts
Shagreen patch (connective tissue nevus)	Cerebral white matter radial migration lines[a]
Cortical tuber[a]	Gingival fibromas
Subependymal nodule	Nonrenal hamartoma
Subependymal giant cell astrocytoma	Retinal achromic patch
Multiple retinal nodular hamartomas	"Confetti" skin lesions
Cardiac rhabdomyoma, single or multiple	Multiple renal cysts
Lymphangioleiomyomatosis[b]	
Renal angiomyolipoma[b]	

[a]When cerebral cortical tuber and cerebral white matter migration lines occur together, they should be counted as one rather than two features of TSC (**Adapted from Tuberous Sclerosis Consensus Conference; Roach et al. 1998)**

[b]When both lymphangioleiomyomatosis and renal angiomyolipomas are present, other features of tuberous sclerosis are required for definite diagnosis

for the 130-kDa protein hamartin. The *TSC2* gene has 42 exons and encodes for the 198-kDa protein tuberin. Nearly all germline mutations of *TSC1* are protein truncating, whereas 20% of those in *TSC2* are missense mutations (Cheadle et al. 2000).

5.2.2 Molecular Pathogenesis

Hamartin and tuberin bind to each other and form a stable complex. This explains the similarity of clinical symptoms in two genetically distinct diseases. Hamartin/tuberin interacts with the AKT and the mTOR pathway. Upon growth factor simulation, receptor tyrosine kinases recruit type Ia phosphoinositide 3-kinase (PI3K) to the cell membrane followed by the formation of phosphatidylinositol-3,4,5-trisphosphate. Thereby the kinase AKT (PKB) localizes to the membrane where it is phosphorylated and activated amongst others by the mTOR-rictor complex at S473 and by PDK1 at T308 (Vanhaesebroeck and Alessi 2000; Sarbassov et al. 2005). Active AKT phosphorylates several proteins (e.g., the FOXO family of transcription factors, BAD, GSK3) including tuberin. At least five sites of tuberin can be phosphorylated by AKT (Dan et al. 2002; Manning and Cantley 2003; Downward 2004). In analogy to neurofibromin and Ras, the hamartin/tuberin complex acts as a specific GAP for Rheb (Ras homolog enriched in brain). Loss of hamartin/tuberin function results in increased levels of Rheb-GTP which in turn plays a central role in the activation of mTOR (mammalian target of rapamycin) kinase (Garami et al. 2003; Inoki et al. 2003a; Zhang et al. 2003). mTOR forms two complexes: mTORC1 (with raptor and GβL) and mTORC2 (with rictor and GβL). mTORC1 phosphorylates ribosomal S6 kinases (S6K1 and S6K2) and the eukaryotic initiation factor 4E (eIF4E)-binding protein (4E-BP1), which is (upon other events) necessary for their activation. These targets affect cell growth, proliferation, and survival (Raught et al. 2004; Richardson et al. 2004). Both hamartin and tuberin have multiple phosphorylation sites and phosphorylation of the different sites has different effects on the activity of the hamartin/tuberin complex. Whereas phosphorylation by AKT has an inhibitory influence, the phosphorylation by the energy-sensitive AMP-activated protein kinase (AMPK) results in an enhanced Rheb-GAP activity of hamartin/tuberin (Inoki et al. 2003b). Mutations in the tumor suppressor gene *LKB1* are associated with the Peutz-Jeghers syndrome, for which gastrointestinal

hamartomas are characteristic. *LKB1* encodes for an AMPK-activating kinase. Its loss also leads to elevated mTOR activity (Corradetti et al. 2004; Shaw et al. 2004).

It has been demonstrated that the MAPK/ERK1/2 pathway can activate mTOR via hamartin/tuberin inhibition by phosphorylation (Roux et al. 2004; Ma et al. 2005).

5.2.3 Subependymal Giant Cell Astrocytomas

Subependymal giant cell astrocytomas (SEGA) are tumors with large cells exhibiting morphological and immunohistochemical properties of astrocytes and neurons. Mutational analysis revealed that cells in SEGA harbor "two-hits" in *TSC1* or *TSC2* consistent with Knudson's theory. An activation of the mTOR pathway can be found by immunohistochemical staining of phosphorylated S6 (Chan et al. 2004). Furthermore, in SEGA high levels of AKT and ERK1/2 phosphorylation have been found indicating an involvement of these pathways in tumor formation (Han et al. 2004).

5.3 Li-Fraumeni and Li-Fraumeni-Like Syndrome

This rare cancer-predisposing syndrome was described by Li and Fraumeni in 1969. A wide range of tumors may occur, with typical entities being premenopausal breast cancer (24%), sarcomas (bone sarcomas 12.6%; soft tissue sarcomas 11.6%), brain tumors (12%), adrenal cortex cancer, and acute leukemia (Kleihues et al. 1997). Two different syndromes are distin-guished. The classic Li-Fraumeni syndrome (LFS) and the Li-Fraumeni-like syndrome (LFL) (Table 5.6)

The mean age of onset of brain tumors in LFS is 25 years. Brain tumors are mainly astrocytic tumors (64%) followed by medulloblastomas/PNET and choroid plexus tumors (together 25%). Other entities occur at a lower frequency (Kleihues, Schauble et al. 1997). There are different descriptions of LFS families with a high incidence of CNS tumors (Dockhorn-Dworniczak et al. 1996; Lynch et al. 2000). In 71–77% of classic LFS and in 22–40% of LFL the underlying molecular genetic event is a germline mutation of the *TP53* gene. The majority of the mutations are missense mutations and occur within exons 5–8 (Institute Curie Database). The gene encodes for the p53 protein, which is a central checkpoint protein in the cell cycle and has an essential role in promoting DNA damage repair and apoptosis thereby possessing tumor suppressor function (Vousden and Lu 2002). Furthermore some p53 mutants are believed to acquire oncogenic properties (Frazier et al. 1998; Sigal and Rotter 2000; Vikhanskaya et al. 2007). Despite intensive efforts in mutation analysis it is not possible to detect a *TP53* germline mutation in all LFS or LFL patients, indicating that there are alternative molecular alterations. Heterozygous germline mutations in the *hCHK2* gene, which encodes a G_2 checkpoint control protein, were found in patients with LFS/LFL (Bell et al.

Table 5.6 Diagnostic criteria for LFS and LFL

Li-Fraumeni syndrome is defined as: Proband with a sarcoma < 45 years of age plus a first-degree relative with any cancer < 45 years of age plus an additional first- or second-degree relative in the same lineage with any cancer < 45 years of age or a sarcoma at any age. The Li-Fraumeni-like syndrome is defined as: Proband with any childhood tumor or a sarcoma, brain tumor or adrenocortical tumor < 45 years of age plus a first- or second-degree relative in the same lineage with a typical LFS tumor at any age and an additional first- or second-degree relative in the same lineage with any cancer < 60 years of age.

1999; Varley 2003). However, there are numerous LFS/LFL families for which the underlying germline mutation remains unidentified. Some candidate genes like *MDM2* (Birch et al. 1994), *PTEN, CDKN2* (Burt et al. 1999) (Brown et al. 2000; Portwine et al. 2000), *Bcl10* (Stone et al. 1999), and *TP63* (Bougeard et al. 2001) could be excluded.

5.4 Melanoma–Astrocytoma Syndrome

In 1990 Kaufman et al. described a family with cutaneous malignant melanoma or cerebral astrocytoma, or both, in eight members over three generations (Kaufman et al. 1993). Others reported on families in which several members developed malignant melanoma, dysplastic nevi, astrocytoma in all grades, benign, or malignant schwannoma, neurofibroma, or meningioma (Azizi et al. 1995; Bahuau et al. 1997). The chromosomal region 9p21 has been identified as a locus for predisposition to malignant melanoma (Kamb et al. 1994a). There are three candidate genes in this region: *CDKN2A* (encodes p16 protein), *CDKN2B* (encodes p15 protein), and the gene encoding p14ARF. The protein p14ARF is encoded by an alternative exon 1 (1ß) and exon 2 of the *CDKN2A* gene. Controlled by its own promoter, exon 1ß is spliced to *CDKN2A* exon 2 in an alternate reading frame to that of the p16 protein (Kamb et al. 1994b; Stone et al. 1995).

The function of both p15 and p16 is to prevent progression in the cell cycle through the G_1 restriction point through inhibition of CDK4/CDK6 in the retinoblastoma pathway (Roussel 1999). MDM2 binds to p53 and promotes its degradation by the ubiquitin pathway (Oliner et al. 1992; Weber et al. 1999). MDM2 is also able to inactivate the retinoblastoma protein (Rb) (Xiao et al. 1995). P14ARF binds to MDM2 triggering the sequestering of MDM2. Thereby, no binding of MDM2 to p53 or Rb is possible, resulting in p53 activation.

In two melanoma–astrocytoma families large germline deletions of 9p21 which involve *CDKN2A* and *CDKN2A* exon 1ß have been described (Bahuau et al. 1998). In a family with melanomas, neurofibromas, and multiple dysplastic nevi, splice site mutations were detected. The mutations appear to result in transcripts which lack exon 2, encoding for both p16 and p14 proteins (Petronzelli et al. 2001; Prowse et al. 2003). Other families showed some features of the melanoma–astrocytoma syndrome and a germline deletion of exon 1ß of the *CDKN2A* gene. The deletion identified did not appear to disrupt the function of the p16 protein (Randerson-Moor et al. 2001).

It may be assumed that functional loss of both the p16 and p14ARF tumor suppressor genes or of p14ARF alone might be the predisposing factor in these families.

5.5 Turcot Syndrome

Turcot syndrome is defined as the occurrence of multiple colorectal adenomas and/or colorectal adenocarcinoma in combination with a primary brain tumor. Most cases of Turcot syndrome occur in patients with the familial adenomatous polyposis or hereditary non-polyposis colorectal carcinoma syndromes. Brain tumors are typically astrocytomas including glioblastomas or medulloblastomas (together 95% of brain tumors). Two main phenotypes can be distinguished. One involves development of thousands of polyps in the colon and medulloblastoma, and the other one shows few polyps but development of colorectal carcinoma and glial brain tumors. These two groups seem to be associated with different genetical alterations. The group of patients with numerous polyps and medulloblastomas often harbor a germline mutation in the adenomatous polyposis coli (*APC*) gene on chromosome 5q21 (Hamilton et al. 1995). The other group of patients with occurrence of glial

brain tumors (mainly glioblastomas) has mutations in the DNA mismatch repair (MMR) genes *hMSH2, hMLH1*, or *hPMS2* (Lucci-Cordisco et al. 2003). Indeed there are also reports about patients with Turcot syndrome who developed both glioblastoma and medulloblastoma (McLaughlin et al. 1998).

5.6 Familial Gliomas

Families have been described that do not suffer from one of the discussed syndromes, but in which the frequency of gliomas is increased.

The pattern of tumor occurrence is different from most familial cancers. There is no involvement of multiple generations or occurrence at an unusually early age. The prognosis for affected patients is as for typical high-grade astrocytomas (Grossman et al. 1999).

Using segregation analysis, both autosomal recessive as well as multifactorial mendelian models have been proposed, while a model postulating a purely environmental cause was rejected (de Andrade et al. 2001; Malmer et al. 2001). Investigations of candidate genes for familial gliomas included *TP53, PTEN, CDKN2A*, and *CDK4*. *TP53* was found to harbor a germline mutation in a patient with familial glioma that did not meet all the criteria of Li-Fraumeni syndrome (Tachibana et al. 2000; Paunu et al. 2001).

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