Neurofibromatosis type 1

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Abstract | Neurofibromatosis type 1 is a complex autosomal dominant disorder caused by germline mutations in the *NF1* tumour suppressor gene. Nearly all individuals with neurofibromatosis type 1 develop pigmentary lesions (café-au-lait macules, skinfold freckling and Lisch nodules) and dermal neurofibromas. Some individuals develop skeletal abnormalities (scoliosis, tibial pseudarthrosis and orbital dysplasia), brain tumours (optic pathway gliomas and glioblastoma), peripheral nerve tumours (spinal neurofibromas, plexiform neurofibromas and malignant peripheral nerve sheath tumours), learning disabilities, attention deficits, and social and behavioural problems, which can negatively affect quality of life. With the identification of *NF1* and the generation of accurate preclinical mouse strains that model some of these clinical features, therapies that target the underlying molecular and cellular pathophysiology for neurofibromatosis type 1 are becoming available. Although no single treatment exists, current clinical management strategies include early detection of disease phenotypes (risk assessment) and biologically targeted therapies. Similarly, new medical and behavioural interventions are emerging to improve the quality of life of patients. Although considerable progress has been made in understanding this condition, numerous challenges remain; a collaborative and interdisciplinary approach is required to manage individuals with neurofibromatosis type1 and to develop effective treatments.

Neurofibromatosis type 1 is an autosomal dominant disorder that affects multiple organ systems and has a wide range of variable clinical manifestations. It is one of three conditions described under the broad heading of the 'neurofibromatoses'; the other two, neurofibromatosis type 2 and schwannomatosis, are clinically and genetically distinct from neurofibromatosis type 1.

The defining feature of neurofibromatosis type 1 is the neurofibroma, a nerve sheath tumour that forms in intimate association with spinal, peripheral or cranial nerves (FIG. 1). Other manifestations include pigmentary abnormalities, low-grade gliomas and skeletal dysplasias, as well as the involvement of numerous other organ systems (FIG. 2). The condition is gradually progressive over the lifetime of an individual, although the specific manifestations, rate of progression and severity of complications vary widely. At present, no definitive treatment is available, and clinical management is typically limited to surveillance and symptomatic treatment, usually surgical, for specific complications.

The gene responsible for neurofibromatosis type 1 (*NF1*, which encodes neurofibromin) was identified in 1990 (REF. 1), and its function and role in the formation of tumours and the other manifestations of neurofibromatosis type 1 have been under intensive study. With an increasing understanding of the mechanisms that underlie the pathogenesis of neurofibromatosis type 1 clinical features, numerous targeted therapies have emerged, which are now being evaluated in preclinical models and in phase II clinical trials. This is a time of great hope for individuals with neurofibromatosis type 1, which is bolstered by the emergence of new treatments that aim to improve their quality of life (QOL).

This Primer discusses the pathophysiology of neurofibromatosis type 1, including the mechanisms underlying the development of the associated clinical manifestations, as well as its epidemiology, diagnosis and management, in addition to highlighting QOL issues faced by individuals with this condition.

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Article number: 17004
doi:10.1038/nrdp.2017.4
Published online 23 Feb 2017

Epidemiology

The average global prevalence of neurofibromatosis type 1 is ~1 case per 3,000 individuals[2], although prevalence estimates vary by country and range from 1 case per 960 individuals in Israel to 1 case per 7,812 individuals in Russia[2]. Although these variable prevalence estimates could represent different rates of the disorder in distinct populations, whether they are due to true differences is challenging to prove. True differences in prevalence might result from founder effects or factors that influence *de novo* mutation rates that also vary by population, such as older paternal age[3–6]

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or ethnicity[7–9]. Importantly, regardless of the population, 50% of cases of neurofibromatosis type 1 are familial (inherited) and the remainder arise from a *de novo* *NF1* mutation[10,11].

The life expectancy of individuals with neurofibromatosis type 1 is reduced by ~8–21 years[2,12–15], and an excess of deaths occurs in younger individuals (<40 years of age), compared with the general population; the most common cause of early death is malignant neoplasm[2,13,14,16]. Individuals have an increased risk for malignant and non-malignant conditions compared with the general population (BOX 1).

Epidemiological research on neurofibromatosis type 1 is faced with many challenges, including the lack of population-based registries that record patients in most countries. As a result, many studies have relied on death certificates and non-population-based cohorts to estimate risks; however, these methods can create biased estimates. In addition, few studies have gone beyond descriptive epidemiology to identify the risk factors for the medical and social issues associated with neurofibromatosis type 1. The increasing availability of electronic health data will help facilitate research aimed at further defining the aetiology of health complications in individuals with neurofibromatosis type 1.

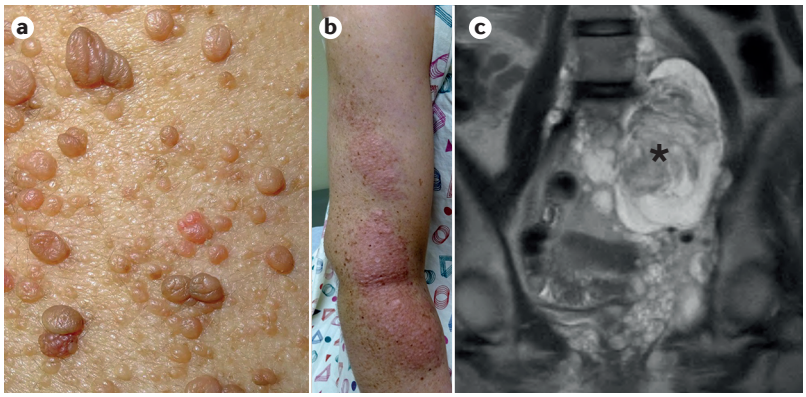

Figure 1 | **Benign peripheral nerve sheath tumours.** One of the main features of neurofibromatosis type 1 is the development of nerve sheath tumours (neurofibromas). Neurofibromas can grow as discrete nodules, which can be found on the skin as dermal neurofibromas (part **a**). Neurofibromas can also involve multiple branches of larger nerves — referred to as plexiform neurofibromas — which can be seen on the skin (part **b**) or can be internal. Part **c** shows an MRI scan of an intra-abdominal plexiform neurofibroma (asterisk).

Mechanisms/pathophysiology

Genetics

Neurofibromatosis type 1 is an autosomal dominant genetic condition, such that all people with a germline *NF1* mutation have this disease. However, patients can show extreme variability in their clinical features, even in individuals from the same family with an identical germline *NF1* mutation. In addition to the more common generalized neurofibromatosis type 1, some people harbour features of neurofibromatosis type 1 that are restricted to one segment of their body ('segmental' or mosaic neurofibromatosis type 1), which probably arises from a somatic mutation in *NF1* that occurs during fetal development. In each of these situations, the *NF1* mutation is thought to result in loss of neurofibromin function from that mutated allele.

The complexity of molecular testing to identify causative mutations in *NF1* is related to the large size of the gene (~60 exons), the relative lack of mutation hotspots and the diversity of the pathogenetic mutations. A multi-step approach is required, with analysis of blood genomic DNA and mRNA, as well as fluorescent *in situ* hybridization testing for whole *NF1* deletions[17]. This strategy identifies >95% of causative mutations, but in people with segmental neurofibromatosis type 1, analysis of affected tissues is necessary as the *NF1* mutation is not usually detected in the blood.

To date, >7,000 people with neurofibromatosis type 1 have undergone genetic testing, and >3,000 different germline *NF1* mutations have been identified. Although genotype–phenotype correlations are uncommon in neurofibromatosis type 1, three well-established correlations have been identified. Individuals with 1.4 Mb deletions that encompass the entire *NF1* gene typically show facial dysmorphism, reduced intellectual abilities and an increased incidence of cancer[18]. In addition, ~1% of people with neurofibromatosis type 1 have mutations that affect codon 1809 (REF. 19) and typically present with café-au-lait macules (CALMs), short stature and pulmonic stenosis, but lack externally visible plexiform or dermal neurofibromas. In addition, another mutation has been associated with an absence of neurofibromas[20].

The precise mechanisms underlying the development of the clinical manifestations of neurofibromatosis type 1 can vary, such that some manifestations result from haploinsufficiency of *NF1*, whereas others require biallelic *NF1* inactivation or the addition of modifying factors, such as hormones or other genetic alterations. For example, biallelic *NF1* inactivation is required for the development of CALMs and neurofibromas, but cooperating genetic alterations, such as *TP53* mutation, are required for the formation of malignant peripheral nerve sheath tumours (MPNSTs).

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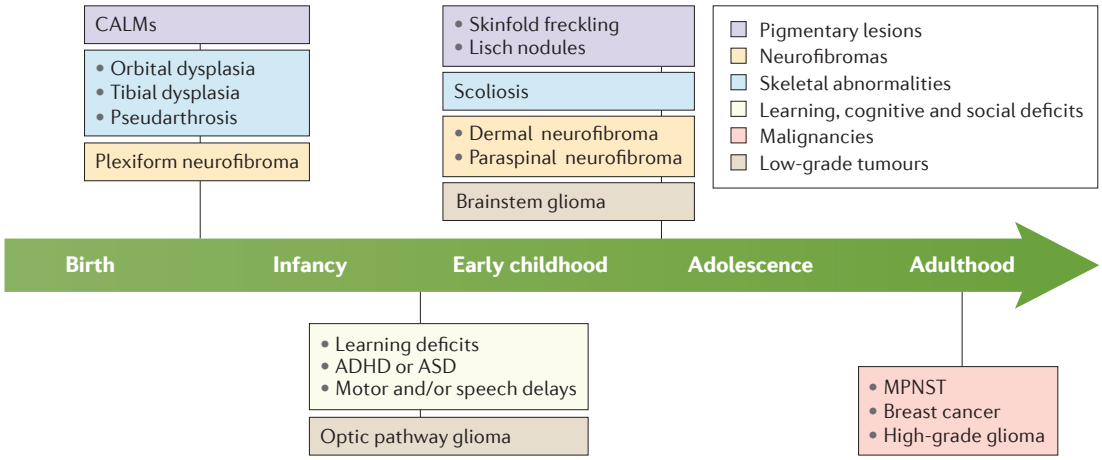

Figure 2 | **Development of clinical features of neurofibromatosis type 1.** The timing of the clinical manifestations and the severity of features can vary between individuals. The skeletal abnormalities include scoliosis and long bone dysplasia; although not part of the diagnostic criteria, scoliosis is the most common skeletal manifestation of neurofibromatosis type 1 and is found in up to 30% of children. Dysplasia of a long bone is found in ~2% of children with neurofibromatosis type 1. Behavioural manifestations can include cognitive impairment (observed in ~80% of children), attention-deficit/hyperactivity disorder (ADHD; a prevalence of ~30–50% in children[124]) and autism spectrum disorder (ASD; ~40% of children with neurofibromatosis type 1 show features associated with ASD and 13% have autism[125]). Tumours can include malignant peripheral nerve sheath tumours (MPNSTs) and gliomas. The lifetime risk of developing MPNSTs associated with neurofibromatosis type 1 is between 8% and 16%[143,144]; these tumours are the most common in the third decade of life, but can occur at any age. Between 15% and 20% of children with neurofibromatosis type 1 will develop an optic pathway glioma, which is symptomatic in 7–10% of individuals[111], whereas 5% of patients have brainstem gliomas. Other, albeit rare, manifestations include juvenile xanthogranuloma, pheochromocytomas and gastrointestinal stromal tumours. CALM, café-au-lait macule.

NF1 *gene and neurofibromin*

With the discovery of *NF1* in 1990 (REFS 21,22), it became possible to envision a future in which treatments might emanate from a deeper understanding of the function of neurofibromin[1]. Neurofibromin is expressed in many cell types, including neurons, glial cells, immune cells, endothelial cells and in cells of the adrenal medulla, but probably functions differently in distinct cell types. Close examination of the predicted amino acid sequence of neurofibromin revealed that a small 300-residue domain of neurofibromin was structurally similar to a family of proteins that function as negative regulators of the RAS proto-oncogene. These proteins, termed GTPase-activating proteins, inactivate RAS by accelerating the conversion of active GTP-bound RAS to the inactive GDP-bound form (FIG. 3). In this manner, loss of neurofibromin expression, as seen in tumours associated with neurofibromatosis type 1, is predicted to lead to increased cell growth and survival through hyperactivation of RAS[23,24]. RAS then transmits its growth-promoting signal through the AKT–mechanistic target of rapamycin (mTOR) and MEK–extracellular signal-regulated kinase (ERK) effector pathways[25,26].

Moreover, the identification of *NF1* facilitated the generation of several *Nf1* genetically engineered mouse strains (including tissue-specific conditional *Nf1* knockouts and mice heterozygous for *Nf1* mutations) that are crucial to defining the pathogenesis of neurofibromatosis type 1-related problems, as well as to the discovery and evaluation of potential treatments for children and adults with this disease[27]. As such, loss of neurofibromin has a wide range of pathological consequences, including the formation of pigmentary lesions, tumours and skeletal abnormalities. The use of *Nf1*-knockout mouse strains has elucidated some of the mechanisms underlying these abnormalities. However, no single mouse model exhibits all or most of the features of human neurofibromatosis type 1.

Clinical manifestations

Pigmentary lesions. The most common non-neoplastic manifestations are the pigmentary features (FIG. 4), of which CALMs are the best studied. CALMs consist of a dense population of melanocytes with biallelic *NF1* inactivation[28]. These melanocytes are responsive to growth factors, such as hepatocyte growth factor and KIT ligand (also known as stem cell factor), that activate receptor tyrosine kinase signalling and cell growth (FIG. 3). Melanocytes derived from CALMs show increased proliferation *in vitro* compared with melanocytes derived from individuals who do not have neurofibromatosis type 1 (REF. 29). In mice, melanocytes emanate from a common precursor cell shared with Schwann cells, that is, the neoplastic cells in peripheral nerve sheath tumours, which suggests a common embryological origin.

Skeletal abnormalities. The proper establishment and maintenance of bone require a coordinated interplay between bone-resorbing cells (osteoclasts) and bone-forming cells (osteoblasts). The defects in bone observed in individuals with neurofibromatosis type 1 are due to the loss of both copies of *NF1* in osteoclasts and/or osteoblasts, as first demonstrated in samples from

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individuals with tibial dysplasia and neurofibromatosis type 1 (REF. 30). Using several *Nf1*-conditional knockout mouse strains used to model tibial bowing, dystrophic scoliosis or kyphosis[31], or impaired tibial union[32], increased osteoclast and impaired osteoblast function have been demonstrated[33,34].

In mice, osteoblast dysfunction following *Nf1* loss results in an increased generation of pyrophosphate, which inhibits bone mineral (hydroxyapatite) production and bone mineralization, causing reduced bone density and a higher risk of bone fracture. In addition, *Nf1*-conditional knockout mice have reduced bone morphogenetic protein 2 (BMP2)-induced osteoprogenitor differentiation into osteoblasts and impaired bone remodelling[35]. On the basis of these observations, preclinical studies have shown that defects in bone remodelling and bone mineral production were corrected by asfotase-α enzyme (a pyrophosphate inhibitor)[35]. Conversely, impaired neurofibromin function in osteoblasts causes increased proliferation, cyclic AMP (cAMP)-dependent transcription factor ATF-4-dependent collagen synthesis, reduced senescence and higher telomerase activity, which ultimately lead to disrupted bone maintenance[36]. Although these findings seem contradictory, *Nf1*-deficient osteoblasts promote osteoclast migration and maturation into active osteoclasts through cytokines (for example, osteopontin), which creates a dysfunctional cycle of bone formation and destruction.

Behavioural abnormalities. Elements of the cognitive and behavioural deficits observed in children with neurofibromatosis type 1 have also been modelled in mice with a heterozygous mutation in *Nf1* (*Nf1*$^{+/-}$ mice), which exhibit defects in hippocampal spatial learning and deficits in attention and social behaviour[37].

The defects in hippocampal spatial learning in *Nf1*$^{+/-}$ mice are due to enhanced RAS activity, which causes enhanced γ-aminobutyric acid (GABA)-mediated inhibitory neurotransmission[37]. As such, pharmacological reduction of RAS activity decreases the levels of GABA and ameliorates these spatial learning defects[38]. How neurofibromin or RAS controls GABA transmission is unknown, but the mechanism might reflect disrupted expression of proteins associated with glutamate and GABA neurotransmission[39]. In addition, the defects in hippocampal spatial learning in *Nf1*$^{+/-}$ mice reflect impaired neurofibromin function in inhibitory interneurons, rather than astrocytes or excitatory neurons[40]. Moreover, both attention[37] and social behaviour[41] in mouse models of neurofibromatosis type 1 reflect RAS control of GABA neurotransmission. In this regard, the defects in social learning were shown to reflect increased serine/threonine-protein kinase PAK1 activation of MEK, which results in increased glutamate and GABA neurotransmission in the amygdala[41].

In light of the finding of biallelic inactivation of *NF1* in bone and pigmentary lesions associated with neurofibromatosis type 1, neurons generated from induced pluripotent stem cells from patients with neurofibromatosis type 1 showed germline *NF1* mutations associated with reductions in neurofibromin expression ranging from <25% to >75%[42]. Examination of these neurons demonstrated that the levels of neurofibromin positively correlated with dopamine levels, which suggests that changes in dopaminergic signalling might have a role in the behavioural manifestations of neurofibromatosis type 1. Following biallelic *Nf1* loss in neurons in the brain, mice exhibit defects in hippocampal learning[43] and attention system function[44], reflecting reduced dopamine signalling and attenuated neurofibromin control of dopamine homeostasis. Importantly, these defects were corrected by treating mice with medications that increase the levels of dopamine in the brain (for example, methylphenidate).

Neurofibromas. The most common tumours in children and adults with neurofibromatosis type 1 are peripheral nerve sheath tumours, which include neurofibromas (dermal and plexiform neurofibromas) and MPNSTs[45] (FIG. 1).

Dermal neurofibromas are thought to arise from skin-derived precursor cells. In mice, biallelic *Nf1* loss in skin-derived precursor cells results in dermal neurofibroma formation, but only in females where it is accelerated and enhanced by pregnancy[46]. By contrast, dermal neurofibromas occur equally in men and women with

Box 1 | **Risk of cancer and other disorders in individuals with neurofibromatosis type 1**

The cumulative risk of malignancy by 50 years of age in individuals with neurofibromatosis type 1 has been estimated as 20–39%[144,204], with a lifetime cancer risk of ~60%[144]. Specifically, the risk of malignancy is increased by 2–5-fold in individuals, relative to the general population, and individuals have a 50-fold increased risk for high-grade tumours[205]. In addition, individuals with neurofibromatosis type 1 have exceptionally high risks for malignant brain tumours (~40-fold increased risk of high-grade glioma), endocrine cancers (>74-fold increased risk for adrenal cancer) and connective tissue malignancies (>1,000-fold increased risk for malignant peripheral nerve sheath tumour)[144,204,206]. The risk of benign tumours affecting the central nervous system has also been reported to be significantly increased[206], with optic pathway gliomas found in ~15% of individuals with neurofibromatosis type 1 (REFS 207,208). In addition, the risk for early-onset breast cancer (<40–50 years of age) is increased by ~4–11-fold[144,204,209,210]. Increased risk of other malignancies, including buccal cavity, pharyngeal, oesophageal, skin (melanoma), thyroid and ovarian cancer, has been described, but replication in additional studies will be required to firmly establish these risks. Children with neurofibromatosis type 1 have an increased risk of leukaemia[163]; the relative risk for acute lymphocytic leukaemia is 5.4 compared with a relative risk for non-Hodgkin lymphoma of 10. Excesses in the prevalence of cardiovascular[2,12] and respiratory[2,14] disease, as well as reduced prevalence of diabetes mellitus[13,14,211] and deaths by other causes (for example, suicides and accidental injuries)[2,13,14] have been reported. In addition, several studies support increased risks for multiple sclerosis[211,212], epilepsy[211,213], learning disabilities[120,121] and sleep disorders[214,215]. Paediatric patients often exhibit craniofacial and dental abnormalities.

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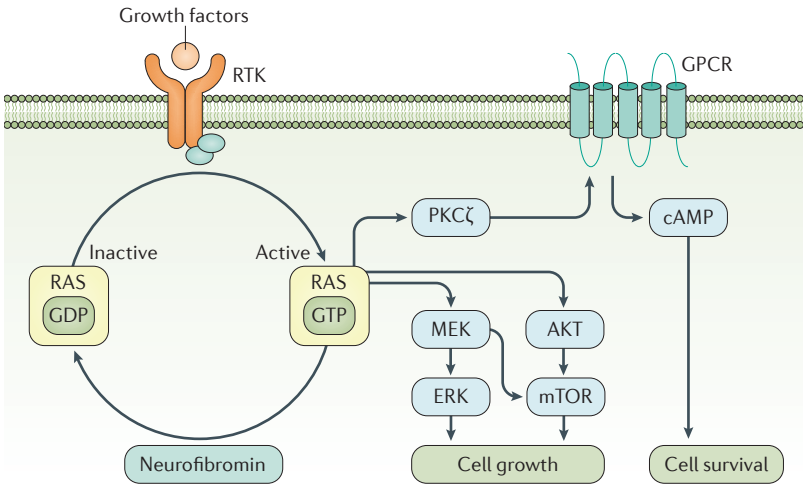

Figure 3 | **Neurofibromin signalling pathway.** Neurofibromin regulates cell growth and survival through several downstream signalling effectors by accelerating the conversion of active GTP-bound RAS to its inactive GDP-bound form. RAS signalling can be activated by receptor tyrosine kinases (RTKs) following the binding of growth factors (such as hepatocyte growth factor and KIT ligand), which results in increased AKT and/or MEK activity and, subsequently, greater cell proliferation and/or survival. In addition, RAS controls the generation of cyclic AMP (cAMP) through protein kinase C-ζ (PKCζ) following the activation of G protein-coupled receptors (GPCRs). Depending on the cell type, RAS signalling might differentially use these downstream effectors, resulting in different cellular consequences. ERK, extracellular signal-regulated kinase; mTOR, mechanistic target of rapamycin.

neurofibromatosis type 1. Although the role of gonadal sex hormones in the development of dermal neurofibromas is unclear, varying effects of oestrogen and progesterone have been reported in one explant model system[47]. With the development of tissue culture models of human dermal neurofibromas[48], these findings can now be formally studied.

More is known about plexiform neurofibromas, as mouse strains with biallelic *Nf1* loss in Schwann cell progenitors generate plexiform neurofibromas that are histologically similar to those found in human tumours[49–52]. The responsible Schwann cell precursors express neuromodulin (encoded by *Gap43*)[52] and myelin proteolipid protein[53]. Like human plexiform neurofibromas, the tumours in mice contain distinct cell types, including mast cells, macrophages, fibroblasts, neurons and Schwann cells (FIG. 5).

The importance of the tumour microenvironment has been demonstrated in small-animal experiments, which showed that *Nf1*-mutant mast cells[54] respond to KIT ligand secreted by *Nf1*-deficient Schwann cells, and increase their proliferation and migration. The recruitment of mast cells into the developing neurofibroma[55] results in the release of transforming growth factor-β (TGFβ), which leads to increased *Nf1*-deficient Schwann cell growth[56] and increased synthesis of collagen to establish a rich extracellular matrix. Importantly, these bone marrow-derived mast cells are necessary for tumour formation and tumour maintenance, based on bone marrow transplantation experiments[54]. Other cell types, including endothelial cells and fibroblasts, are similarly recruited through mechanisms that remain to be elucidated.

This elaborately choreographed cellular circuit of paracrine signalling and growth factor induction resulted in the discovery of imatinib, which blocks the KIT ligand receptor, as a potential therapy for patients with plexiform neurofibromas[57]. Other cell types, including macrophages, might also have active roles in tumour initiation or continued growth[58]. In addition to targeting Schwann cell and mast cell interactions, inhibiting the RAS downstream signalling pathways that are hyperactivated in *Nf1*-null neoplastic Schwann cells (for example, MEK and mTOR) have been successful in preclinical trials[59,60] and are being evaluated in human clinical trials.

Modelling of MPNSTs in mice has revealed that biallelic *Nf1* inactivation is not sufficient for tumour formation and requires additional cooperating genetic changes, including loss of *Trp53*, *Pten* (which encodes phosphatase and tensin homologue)[61,62] or *Suz12*, which encodes a member of Polycomb repressive complex 2 (PRC2). As such, mice with heterozygous mutations in both *Trp53* and *Nf1* develop MPNSTs[63], but complete *Trp53* or *Pten* loss might not be required for tumour formation[64,65]. In addition to this cooperating genetic change, inappropriate expression of growth factor receptors (such as the epidermal growth factor receptor)[66] and chemokines (such as stromal cell-derived factor 1)[67] create new autocrine circuits that decrease the dependence of the cancer cells on their microenvironment and facilitate self-propagating cell growth.

That *SUZ12* is a co-deleted gene in MPNSTs in individuals with neurofibromatosis type 1 was instrumental in demonstrating that loss of SUZ12 expression potentiates RAS-driven transcription through its effects on chromatin[68,69]. PRC2 increases chromatin methylation and limits transcription, such that loss of PRC2 function results in acetylation, bromodomain protein 4 (BRD4) binding and increased transcription. This requirement for BRD4 opened the door to the use of BRD4 inhibitors in combination with pharmacological agents that block RAS or RAS effector signalling for the treatment of MPNSTs.

Optic pathway glioma. These benign brain tumours in children with neurofibromatosis type 1 are composed of several cell types, including astrocytes, oligodendrocytes, neurons, microglia and progenitor or stem cells[70]. In contrast to peripheral nerve sheath tumours, benign brain tumours are rarely biopsied, resulting in fewer insights into the human tumours.

Numerous mouse strains of *Nf1* optic pathway glioma have been generated over the past 15 years by inducing biallelic *Nf1* loss in glial progenitor cells[71] during embryonic development in *Nf1*$^{+/-}$ mice. The responsible glial progenitor cells reside in the third ventricle[72–74] or in other brain regions[74]. Similar to plexiform neurofibromas, optic pathway gliomas exhibit a strong growth dependence on cells in the tumour microenvironment, specifically microglia, which facilitate optic pathway glioma formation[75] and growth by sustaining their proliferation[76–78]. Microglia, like mast cells, drive optic pathway glioma growth in mice through the secretion of cytokines (such as CC-chemokine ligand 5 (CCL5)

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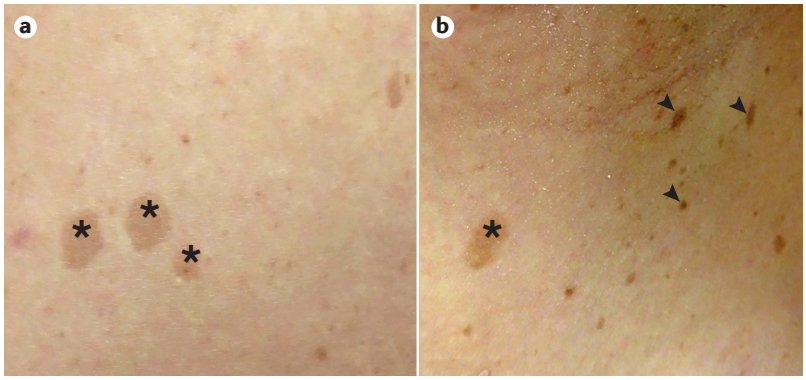

Figure 4 | **Pigmentary features of neurofibromatosis type 1.** Individuals with neurofibromatosis type 1 can present with several pigmentary features, including café-au-lait macules (asterisks in part **a** and part **b**) and freckling in the axilla (arrowheads in part **b**).

or CXC-chemokine ligand 12 (CXCL12)) that recruit additional microglia and increase *Nf1*-deficient astroglial cell proliferation and survival in the tumour[79]. Furthermore, microglia produce neurotoxins that lead to damage of optic nerve axons, culminating in retinal ganglion cell loss and impaired visual acuity. This dependence on the tumour microenvironment suggests that adjuvant therapies might include agents that silence the pro-tumoral aspects of microglia function (FIG. 6).

In addition to microglia, optic pathway gliomas in mice are maintained by low-grade glioma cancer stem cells[42], which have unique molecular properties relevant to the design of biologically targeted therapies. In this regard, optic pathway glioma growth requires the downstream activation of mTOR[25,80,81] through either the AKT or MEK pathways following RAS activation[82], which is enhanced with *Nf1* loss and can be further increased in conjunction with other cooperating genetic mutations (for example, heterozygous *Pten* loss[83]). In this regard, inhibition of mTOR reduces optic pathway glioma size and proliferation[82,84], but this response is dependent on continued drug administration, which perhaps reflects the tumoristatic properties of these compounds or the evolution of optic pathway glioma cancer stem cells, resulting in attenuated sensitivity to these therapies[42].

Although optic pathway glioma growth is an essential component of cancer clinical trials, children with neurofibromatosis type 1 rarely die from these tumours; however, some manifest considerable morbidity owing to impaired vision. In *Nf1*-mutant mouse strains, reduced vision is due to progressive disruption of retinal ganglion cell axons[85], followed by retinal ganglion cell death and loss[86], and impaired visual acuity[87]. One factor associated with reduced visual acuity in both mice and humans with mutations in *NF1* is sex; girls with optic pathway gliomas are 5–10-times more likely to lose vision and require treatment than boys[88]. In addition, in *Nf1*-mutant mice with optic pathway gliomas, only females develop impaired visual acuity as a result of retinal ganglion cell loss[87]. The responsible aetiology is currently being studied and probably reflects gonadal sex hormone or sex chromosome differences[89].

Another factor that could contribute to differences in optic pathway glioma formation associated with neurofibromatosis type 1 is the underlying germline *NF1* mutation. For example, the generation of *Nf1*-mutant mice with different germline mutations, as seen in individuals with neurofibromatosis type 1, showed that mutations can have variable effects on optic pathway glioma formation and retinal pathology[90]. Moreover, the type of germline *NF1* mutation and the influence of sex hormones can influence the cAMP pathway, which is regulated by neurofibromin and maintains retinal ganglion cell survival. Neurofibromin can positively regulate cAMP levels through RAS[91], leading to reduced levels of cAMP in retinal ganglion cells in *Nf1*-mutant mice. Importantly, treatment of *Nf1*-mutant mice with drugs that increase the levels of cAMP (such as rolipram or lovastatin) can rescue the retinal ganglion cell death induced by optic pathway gliomas[86,92], suggesting another adjuvant therapeutic approach to consider for human optic pathway glioma clinical trials.

Diagnosis, screening and prevention

As previously mentioned, the manifestations of neurofibromatosis type 1 are widespread and can involve multiple organ systems, such that individuals have an increased risk for other medical problems. The main clinical presentations (FIG. 2) are reflected in the diagnostic criteria established by the 1987 NIH Consensus Development Conference[93] (BOX 2).

Usually, the disorder can be diagnosed by assessing the individual's family history and by physical examination. However, the diagnosis can be problematic in young children (<6 years of age) or in children with non-familial disease who have CALMs as their only clinical feature; these children might require genetic testing to confirm a diagnosis of neurofibromatosis type 1. In a recent analysis of 71 individuals <20 years of age with six or more CALMs and no non-pigmentary manifestations, 47 were discovered to have neurofibromatosis type 1, 6 had Legius syndrome and 18 harboured no disease-causing variants[94]. On the basis of a retrospective assessment, most children with six or more CALMs will fulfil the diagnostic criteria for neurofibromatosis type 1 by 6–8 years of age[95,96]. Individuals without an affected parent and with localized clinical features are likely to have segmental neurofibromatosis type 1 (REF. 97).

Genetic testing

Most experts recommend that genetic testing be contemplated at any age in individuals who present with an unusual neurofibromatosis type 1 phenotype (for example, those with multi-level symmetrical spinal nerve root neurofibromas, with or without other manifestations of generalized neurofibromatosis type 1 (REF. 98)) and in people >8 years of age who do not fulfil the diagnostic criteria after early childhood. Genetic testing might also help to resolve the uncertainty of diagnosis in young children with multiple CALMs only and no family history of similar features. In addition, genetic testing to assess for the differential diagnosis of

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Legius syndrome (see 'Differential diagnosis') is merited in families with CALMs and skinfold freckling who do not have other diagnostic features of neurofibromatosis type 1 (REF. 94).

Differential diagnosis

Disorders that have overlapping features with neurofibromatosis type 1 should be considered in the differential diagnosis, which can include Legius syndrome, skin hyperpigmentation, mismatch repair and overgrowth syndromes and from tumours that are misidentified as neurofibromas (lipomas). Mutations in *SPRED1* cause Legius syndrome, which is characterized by CALMs, skinfold freckling, learning difficulties and macrocephaly, but not neurofibromas, Lisch nodules or central nervous system tumours[99]. Genetic testing can help to differentiate between a diagnosis of neurofibromatosis type 1 and Legius syndrome. Importantly, neurofibromatosis type 1 is clinically and genetically distinct from other rare tumour predisposition conditions, such as neurofibromatosis type 2 (which is caused by mutations in *NF2*)[100] and from schwannomatosis (which is associated with mutations in *SMARCB1* or *LZTR1*)[101,102].

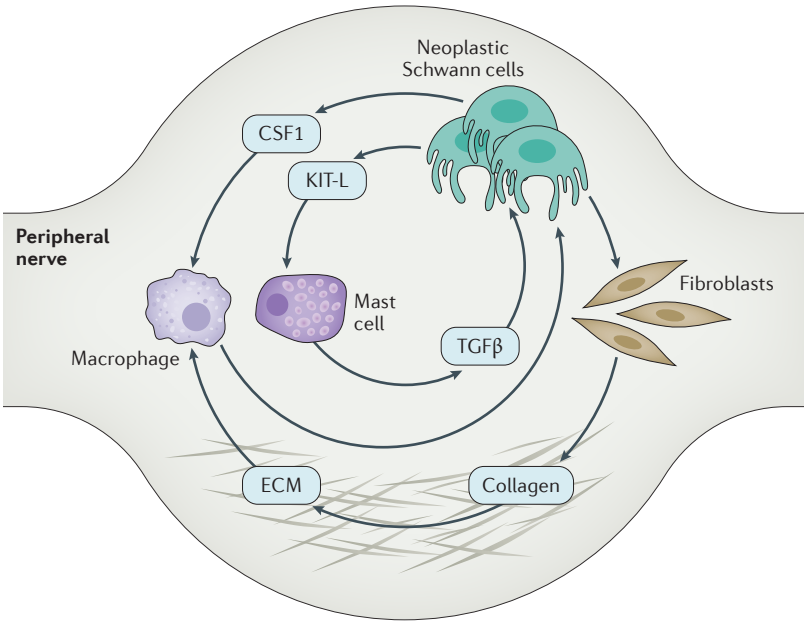

Figure 5 | **Pathogenesis of plexiform neurofibromas.** The complex interplay between neoplastic cells (Schwann cells) and non-neoplastic cells (macrophages, mast cells and fibroblasts) dictates the development and growth of plexiform neurofibromas. The secretion of KIT ligand (KIT-L) by *Nf1*-deficient Schwann cells increases the proliferation and migration of *Nf1*-mutant mast cells[54], which, when recruited into the developing neurofibroma[55], result in transforming growth factor-β (TGFβ) release, increased *Nf1*-deficient Schwann cell growth[56] and the establishment of a rich extracellular matrix (ECM). Importantly, the bone marrow-derived mast cells are necessary for tumour formation and tumour maintenance[54]. Other cells in the tumour microenvironment, such as macrophages, might have active roles in tumour initiation or continued growth[58]. The mechanisms underlying the recruitment of other cell types, including endothelial cells and fibroblasts, to the developing neurofibroma remain to be elucidated. Fibroblasts can also produce collagen and other ECM proteins to further support the growth of plexiform neurofibromas. Each cell type (mast cell, macrophage, fibroblast and Schwann cell) and acellular component (KIT-L, macrophage colony-stimulating factor 1 (CSF1), TGFβ and the ECM) is a potential target for therapeutic drug design.

Prenatal diagnosis

As an autosomal dominant genetic disorder, neurofibromatosis type 1 is fully penetrant without asymptomatic carriers or skipped generations[103]. The risk of having a child with generalized neurofibromatosis type 1 is 50% if one of the parents has the condition, but is ~5% for individuals with segmental disease[97]. Prenatal and pre-implantation genetic testing is available when the causative *NF1* mutation has been identified in the parent, but cannot predict clinical disease severity[104]. Three notable exceptions to the lack of genotype–phenotype correlations exist: families who have either a 3 base pair in-frame deletion in exon 17 (c.2970–2972 delAAT) or missense mutations involving codon 1809 (REFS 19,20) tend to have mild phenotypes without neurofibromas; by contrast, individuals with microdeletions that remove the entire *NF1* gene and numerous neighbouring genes have a more severe phenotype, with early development of neurofibromas, an increased risk of cancer, substantial cognitive impairment and facial dysmorphism[105].

Monitoring for complications

Children. Experts have consistently recommended that all children with neurofibromatosis type 1 should be evaluated yearly in a multidisciplinary clinic with rapid and seamless access to the full complement of medical subspecialists necessary to cover the widespread manifestations of this disease. In the United States, children ≤10 years of age should have complete annual ophthalmological examinations to assess for signs of an optic pathway glioma[106], as the appearance of *de novo* optic pathway glioma after 10 years of age is unusual[107]. Although data are not sufficient to firmly support this recommendation, the authors have endorsed periodic ophthalmological evaluations at increasing intervals after 10 years of age[108]. Yearly measurements of weight and height should be recorded and plotted on standardized growth charts to identify accelerated linear growth, which is the earliest manifestation of precocious puberty. Blood pressure measurements should be obtained at each clinical visit to identify early signs of renovascular hypertension[109]. Although vasculopathy can occur in virtually any artery in individuals with neurofibromatosis type 1, the renal artery is most commonly affected. In addition, the spine should be examined each year for signs of scoliosis, and infants should be closely examined for signs of pseudarthrosis. As learning disabilities, attention-deficit/hyperactivity disorder (ADHD) and social perception problems (such as in autism spectrum disorder) are perhaps the most devastating complications of this condition in childhood, screening for these manifestations using established clinical assessment instruments is crucial, especially as early treatment with stimulants and/or appropriate academic interventions can result in improvements in academic performance, self-esteem and behaviour in affected individuals.

However, in the United Kingdom, published guidelines recommend that all children with uncomplicated disease should be assessed yearly, ideally by a paediatrician who can facilitate coordinated care[110]. In addition,

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yearly ophthalmological screening until 8 years of age is recommended, with annual screening by an orthoptist (optometrist) until 16 years of age and every 2 years thereafter.

Routine 'screening' neuroimaging of asymptomatic children with neurofibromatosis type 1 would be important if it led to early detection of an optic pathway glioma and early initiation of therapy to preserve vision. A longitudinal study of children with neurofibromatosis type 1 failed to identify any tumours in which early detection altered the clinical course[111]. Optic pathway gliomas can develop in young children with neurofibromatosis type 1 shortly after normal neuroimaging, which suggests that screening is of little value[112,113]. For this reason, the National Neurofibromatosis Foundation Optic Pathway Task Force recommended against routine screening neuroimaging of children with neurofibromatosis type 1 (REF. 108). However, some centres prefer to use screening neuroimaging up to 15 months of age to identify retrochiasmal optic pathway gliomas before detectable visual loss[114], as tumours in these locations carry a worse prognosis[115].

Adults. Adults with neurofibromatosis type 1 require education about potential disease complications, as well as access to reliable online information (see the Children's Tumour Foundation (http://www.ctf.org), the Washington University NF Center (http://nfcenter.wustl.edu) and the Neuro Foundation (http://www.nfauk.org)).

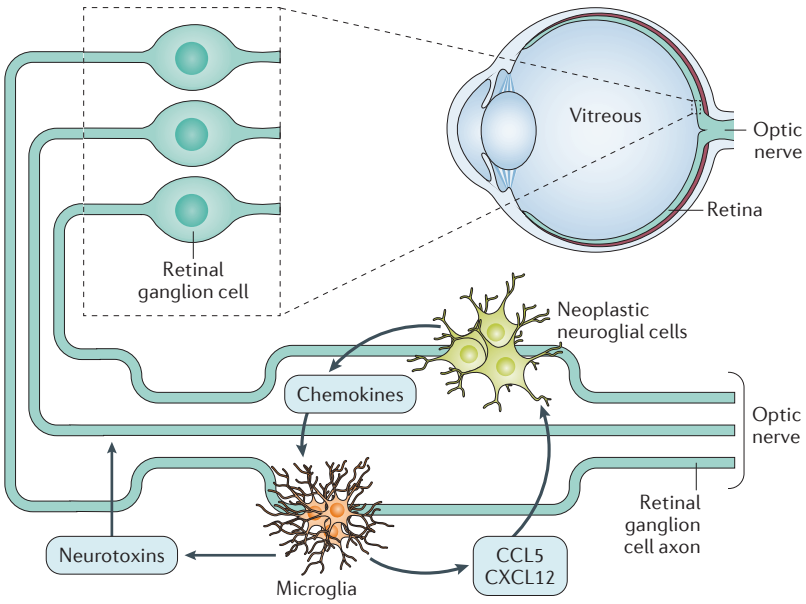

Figure 6 | **Pathogenesis of optic pathway gliomas.** The choreographed relationship between neoplastic and non-neoplastic cell types in the optic nerve underlies gliomagenesis, tumour maintenance and glioma-associated vision loss. In this model, *NF1*-deficient neuroglial cells (such as glioma stem cells and astrocytes) attract microglia through the elaboration of chemokines, which, in turn, produce growth factors and other chemokines (for example, CC-chemokine ligand 5 (CCL5) and CXC-chemokine ligand 12 (CXCL12)) that increase neuroglial cell proliferation. In addition, microglia are responsible for the production of neurotoxins (for example, IL-1β), which damage optic nerve axons, culminating in retinal ganglion cell loss, retinal nerve fibre thinning and impaired visual acuity. Similar to plexiform neurofibromas, each cell type and acellular component becomes a potential target for therapeutic drug design.

Annual assessments by clinicians who are familiar with neurofibromatosis type 1 are advocated, whereas rare or potentially life-threatening complications are best managed by expert multidisciplinary teams that can provide diagnosis and lifelong surveillance and care[110].

Individual surveillance should include the clinical assessment of neurofibromas. Whole-body MRI performed between 16 and 18 years of age is advocated by some centres to determine neurofibroma load and to assess the size and extent of individual tumours, although this is not part of routine care[116]. Most neurofibromatosis specialists recommend that individuals should be given advice on bone health, vitamin D supplementation (owing to the increased risk of pathological fractures) and blood pressure monitoring (owing to the increased risk of hypertension). Evaluation of psychological well-being should include assessment of cognition and literacy as well as employment status. Visual assessment with driving fields is advised, particularly in geographical regions where driving is essential. Finally, women with neurofibromatosis type 1 would benefit from regular breast self-examinations, and, if necessary, follow-up with mammography or MRI of the breast in women <40 years of age, because of the increased risk of breast cancer[2].

Management

The goal of management of neurofibromatosis type 1 in the United States and in the United Kingdom is the early detection of potential treatable complications[110].

Skeletal abnormalities

Scoliosis. In individuals with neurofibromatosis type 1, scoliosis (FIG. 7) is categorized as either dystrophic or non-dystrophic. Non-dystrophic scoliosis is similar to idiopathic scoliosis and is more common than dystrophic scoliosis in individuals with neurofibromatosis type 1. By contrast, dystrophic scoliosis, which is usually recognizable in early childhood, results from primary bone dysplasia and typically causes a sharply angulated curve that spans several vertebral bodies[117]. Dystrophic scoliosis generally requires corrective surgery to fuse the abnormal vertebral bodies; this is often performed at a younger age and at smaller angles than is seen in individuals with non-dystrophic scoliosis. In addition, scoliosis can reflect the presence of paraspinal neurofibromas, which can cause vertebral erosion. Many children with non-dystrophic scoliosis can be managed expectantly or with bracing to prevent progression.

Osteopenia or osteoporosis. Individuals with neurofibromatosis type 1 also have an increased incidence of osteopenia (that is, a reduction in bone mineral density), which is considered a precursor for osteoporosis. In addition, both children and adults have a greater risk of fracture in non-dysplastic bones, which might be associated with deregulated osteoclast function or low levels of vitamin D[118]. For those individuals with low levels of vitamin D, supplementation is recommended (ClinicalTrials.gov identifier: NCT01968590).

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Box 2 | **1987 NIH Consensus Development Conference diagnostic criteria***

Neurofibromatosis type 1 can be diagnosed if an individual presents with two or more of the following features:

- Six or more café-au-lait macules of ≥5 mm in diameter before puberty or ≥1.5 mm in diameter after puberty
- Axillary or inguinal skinfold freckling
- Two or more dermal neurofibromas or one plexiform neurofibroma
- Two or more iris hamartomas (Lisch nodules)
- An optic pathway glioma
- A distinctive long bone dysplasia involving the sphenoid wing or thinning of the long bone cortex with or without pseudarthrosis
- A first-degree relative with neurofibromatosis type 1

*See REF. 93.

Tibial dysplasia. Dysplasia of a long bone (most commonly involving the tibia, but can occur in virtually any long bone) is characterized by congenital bowing (FIG. 7). Bowing of long bones can produce a visible deformity and weakened bone that is predisposed to fracture[118]; as such, bracing is recommended to prevent bone fracture. When fractures do occur, the failure of primary union of the separate bone elements following a fracture can create a 'false joint' or pseudarthrosis. The rigid stabilization of the fractured bone using combinations of bone grafting, intramedullary rod placement or an Ilizarov external fixation is necessary to promote proper bone alignment and healing[119]. Currently, a multicentre study is underway to determine the use of BMP2, which can correct defects in bone remodelling and bone mineral production in *Nf1*-mutant mouse strains, to promote bone healing in individuals undergoing pseudarthrosis repair (NCT02718131).

Behavioural deficits

Cognitive impairment in children with neurofibromatosis type 1 encompasses a reduction in average IQ (~85) and is associated with learning disability and attentional problems[120,121]. In addition, executive dysfunction, reduced working memory, literacy problems and visual spatial difficulties have been reported. Impaired sustained and divided attention and reduced response inhibition are also observed[122,123], and children can develop ADHD[124]. Children with ADHD associated with neurofibromatosis type 1 have more difficulty in following complex verbal instructions, learning to read and performing math than typical-developing peers[124]. Paediatric patients can also have features associated with autism spectrum symptomatology, but a reduction in the overall male predominance relative to idiopathic autism is apparent in individuals with neurofibromatosis type 1 (REFS 125,126). The early recognition of behavioural and learning difficulties in children is essential to facilitate timely intervention; these difficulties are managed in a similar manner as in individuals without neurofibromatosis type 1, requiring both pharmacological and non-pharmacological interventions. Many adults with neurofibromatosis type 1 also have problems with numeracy and literacy and exhibit social difficulties, which require ongoing support.

Preclinical research in *Nf1* genetically engineered mice has demonstrated that statins, dopamine uptake inhibitors or lamotrigine (a sodium channel blocker) can ameliorate these cognitive deficits. These data have led to clinical trials in children with neurofibromatosis type 1 (NCT02256124 and NCT00169611)[38,43,127], but trials of statins did not improve learning (NCT00853580)[128–131]. Results from clinical trials assessing the use of dopamine uptake inhibitors or lamotrigine are pending.

Peripheral nerve sheath tumours

Neurofibromas. Neurofibromas present as dermal (cutaneous or subcutaneous), spinal nerve root, diffuse plexiform, nodular plexiform tumours or as neurofibromatous neuropathy[132]. Dermal neurofibromas usually develop in late adolescence, but can occasionally form in younger children. The common complaints of individuals with dermal neurofibromas include itching, stinging, pain, tenderness, bleeding and cosmetic problems. In most neurofibromatosis specialty clinics, the management of dermal neurofibromas involves surgical removal, laser ablation for small lesions, electrodessication, emollients (moisturizers), camouflage make-up and psychological support.

Discrete subdermal neurofibromas and spinal neurofibromas can cause pain or neurological deficits (including sensory and motor loss). When unclear, discrete subdermal neurofibromas should be differentiated by biopsy from glomus tumours (that is, tumours that form under the nail bed), which cause sensitivity to cold and lancinating pain when bumped[133].

Paraspinal neurofibromas located on the upper cervical spinal nerves can cause spinal cord compression, possibly because the second cervical nerve root is vulnerable to repeated low-grade trauma as it emerges from the neural foramen and courses over the superior aspect of the second cervical lamina[134]. Spinal cord compression can be present even in the absence of neurological signs and symptoms. Management of paraspinal neurofibromas includes surgery, the need for which should be decided on the basis of a progressive neurological deficit and the risk of permanent neurological deficit (such as paralysis or urinary sphincter disturbance).

Plexiform neurofibromas are typically congenital and many of these tumours are indolent. Faster tumour growth has been reported in children and adolescents with plexiform neurofibromas, in addition to those with large tumour volumes. Over 50% of individuals with neurofibromatosis type 1 have internal tumours, such that volumetric whole-body MRI is used in some centres to assess the tumour burden and growth[116]. Although these more diffuse tumours are benign, they can cause considerable morbidity, including pain, disfigurement, neurological deficit, difficulties with swallowing and breathing, potentially life-threatening haemorrhage and the risk of malignant transformation[135]. Pain management and the excision of surgically amenable tumours are currently the mainstay of treatment for associated morbidity or tumour progression. However, several biologically targeted therapies (such as mTOR inhibitors, imatinib and selective MEK inhibitors) that inhibit the pathways responsible for

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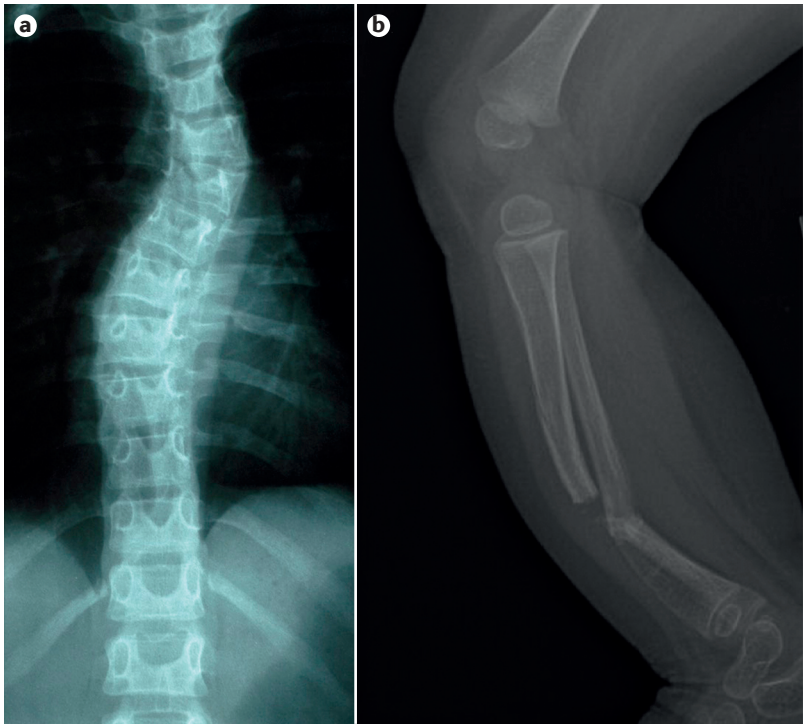

Figure 7 | **Skeletal defects in neurofibromatosis type 1.** Individuals with neurofibromatosis type 1 can present with a range of skeletal defects, including dystrophic scoliosis (part **a**) and tibial dysplasia (part **b**), which can be detected by radiographic imaging.

tumour growth have been evaluated in clinical trials based on reductions in tumour size in *Nf1* preclinical mouse models (NCT01140360, NCT00634270, NCT01365468, NCT01402817, NCT01275586, NCT02101736, NCT02390752, NCT01412892 and NCT01673009)[57,136]. By contrast, clinical trials with farnesyltransferase inhibitors, multi-kinase inhibitors and anti-fibrotic agents have not been successful (NCT00076102, NCT00727233 and NCT00754780)[137]. However, a phase I trial of oral selumetinib was well tolerated and showed an 8–39% decrease in plexiform neurofibroma volume in all 11 individuals evaluated[60,138]. A phase II trial of selumetinib in children and adults with plexiform neurofibromas associated with symptomatic neurofibromatosis type 1 is now underway (NCT01362803).

Neurofibromatous neuropathy is an indolent length-dependent sensorimotor axonal neuropathy that can cause pain, weakness, muscle wasting, numbness and tingling[132]. The diagnosis of neurofibromatous neuropathy can be confirmed by neurophysiology (nerve conduction) testing. Individuals with neurofibromatous neuropathy are usually mildly affected, but this manifestation can be associated with MPNSTs, and long-term follow-up is essential[139,140].

Atypical neurofibromas. Histology of some symptomatic neurofibromas reveals hypercellularity and atypical nuclei, but few mitoses and no necrosis[141]. These atypical neurofibromas exhibit molecular changes that are also found in MPNSTs and are regarded as pre-malignant[142]. Treatment includes complete excision and clinical surveillance.

MPNSTs. Although MPNSTs associated with neurofibromatosis type 1 can appear spontaneously, they usually arise within pre-existing plexiform neurofibromas[143,144]. Risk factors for MPNSTs include a large internal neurofibroma burden or numerous subdermal neurofibromas, atypical neurofibromas, neurofibromatous neuropathy, previous treatment with radiotherapy, a personal or family history of MPNSTs and microdeletions of the *NF1* locus[145]. Attention to individuals with symptomatic plexiform neurofibromas or those presenting with one or more of the above risk factors is important, but serial screening with MRI and fluorodeoxyglucose (FDG)-PET/CT is not indicated. The importance of patient education in recognizing important symptoms and seeking prompt specialist advice cannot be overemphasized.

The symptoms of MPNSTs overlap with those of benign symptomatic plexiform neurofibromas, but MPNSTs should be suspected in hard, rapidly growing neurofibromas that cause persistent or nocturnal pain, or a neurological deficit. Blind biopsy can miss the site of malignancy in heterogeneous lesions, and MRI-guided or ^{18}F-FDG-PET-guided biopsy is advocated. Similarly, MRI can delineate the site and extent of the lesion; however, imaging with PET is the most sensitive and specific non-invasive diagnostic tool for MPNSTs[146].

MPNSTs can exhibit clinical heterogeneity, and low-grade MPNSTs treated appropriately are compatible with long-term survival. Conversely, some high-grade tumours metastasize widely and have a poor prognosis[145]. The aim of treatment is complete excision of MPNSTs with tumour-free margins. In some cases, isolated lung metastases can also be managed surgically. Neoadjuvant chemotherapy (administration of chemotherapy before surgery) with an anthracycline (such as doxorubicin) and ifosfamide can be used to reduce the size of tumours and facilitate surgical removal[147,148]. The combination of an anthracycline and ifosfamide and other drugs can be delivered as adjuvant chemotherapy, but this remains controversial[149]. In individuals with metastatic MPNSTs, outside of a clinical trial, an anthracycline is often used as front-line therapy to prolong patient survival. Newer neoadjuvant and combination therapies, including those derived from preclinical mouse experiments, are currently in clinical trials.

Brain tumours

Glioma. Gliomas can develop in any location in the central nervous system in individuals with neurofibromatosis type 1 and are usually classified as WHO grade I gliomas (pilocytic astrocytomas). The majority of pilocytic astrocytomas in individuals with neurofibromatosis type 1 are indolent neoplasms; more-aggressive behaviour has been associated with symptomatic tumours, presentation after 8 years of age and gliomas that are not contained within the optic pathway[150].

Focal areas of signal intensity on T2-weighted fluid-attenuated inversion recovery (FLAIR) MRI sequences are detected in most children with neurofibromatosis type 1 and sometimes persist into adulthood (FIG. 8). These T2-hyperintensities typically arise in the basal

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ganglia, cerebellum, brainstem and optic pathway, where they do not enhance following gadolinium administration, lack architectural distortion (mass effect) and are typically iso-intense on T1-weighted MRI[151]. In some cases, these lesions can be mistaken for glioma, and neuroimaging should be reviewed by experienced neuroradiologists to prevent an incorrect diagnosis. Many gliomas do not require treatment and are followed by annual MRI surveillance in most centres. Children with tumours that cause neurological signs or symptoms might require shunting, surgical resection or chemotherapy.

Optic pathway gliomas. Between 15% and 20% of children with neurofibromatosis type 1 will develop an optic pathway glioma (FIG. 8); African-American children with neurofibromatosis type 1 have a lower incidence of optic pathway glioma than white children with neurofibromatosis type 1. In addition, the prevalence of visual impairment is higher in girls with optic pathway glioma associated with neurofibromatosis type 1 than in boys[87,152].

Although symptomatic tumours have been identified in older individuals, the greatest risk for the development of an optic pathway glioma is during the first 6 years of life. Optic pathway gliomas associated with neurofibromatosis type 1 generally present in one of three ways: the rapid onset of proptosis (protrusion of the eye) accompanied by moderate-to-severe visual loss in the affected eye, abnormal ophthalmological examinations without any visual symptoms, or signs of precocious puberty. Toddlers and preschool children rarely complain of visual loss, even when this is severe; as such, annual eye examinations are imperative in children with neurofibromatosis type 1. Ophthalmological signs of optic pathway glioma can include an afferent pupillary defect, optic nerve atrophy, papilloedema (swelling of the optic disc), strabismus (misalignment of the eyes) or defects in colour vision[106].

Paediatric individuals presenting with chiasmal optic pathway gliomas can present with signs of precocious puberty, which most commonly manifests as accelerated linear growth due to inappropriate growth hormone production. These children commonly have normal ophthalmological examinations[153]. The use of standardized paediatric growth charts is crucial for detecting the first hint of accelerated growth, which can then be prevented with the use of a luteinizing hormone-releasing hormone agonist to enable preservation of adult height, as well as prevention of its associated psychosocial ramifications.

Once identified, optic pathway gliomas should be followed by serial MRI and ophthalmological examinations, typically every 3 months for the first year, to assess for tumour progression. Many optic pathway gliomas associated with neurofibromatosis type 1 remain quiescent or grow slowly only to stop subsequently[154]. In the past, treatment was initiated only after the demonstration of clear radiographic progression of disease. However, more recently, the focus of treatment has turned to the preservation of vision, and, in this regard, a prospective multicentre study is underway to identify predictors of visual deterioration. When indicated, treatment using chemotherapy (carboplatin and vincristine) is the mainstay of initial therapy[155]. Radiation therapy is not appropriate for optic pathway gliomas associated with neurofibromatosis type 1 because of an increased risk for the development of vasculopathy and secondary malignancies[156,157]. Clinical trials assessing the use of mTOR and MEK inhibitors for the treatment of optic pathway gliomas are in progress (NCT01158651 and NCT02285439).

Brainstem gliomas. Brainstem gliomas (FIG. 8) tend to arise in slightly older children and are generally indolent[158]. These tumours might cause symptoms as a result of ventricular obstruction, but they are often discovered following neuroimaging for non-related reasons, and subsequently remain static[159]. The distinction between brainstem gliomas and T2-hyperintensities on MRI, which are common in individuals with neurofibromatosis type 1 and often disappear over time, can be problematic. However, the use of defined radiographic criteria for tumours (for example, a mass with gadolinium enhancement and associated T2-hyperintensity, or a discrete T2-hyperintensity that is T1-hypointense with associated mass effect or architectural distortion on MRI) should distinguish between these entities.

Other malignancies

Pheochromocytoma. The mean age at presentation for individuals with pheochromocytoma associated with neurofibromatosis type 1 is ~42 years of age. Approximately 84% of individuals with pheochromocytoma have solitary adrenal tumours, 9.6% have bilateral adrenal tumours and 6% have tumours in the abdominal sympathetic chain, the organ of Zuckerkandl or the bladder[160]. Symptoms of pheochromocytoma can include hypertension, sweating or flushing[161]. In individuals with neurofibromatosis type 1, 11.5% of pheochromocytomas are malignant and often present with distant metastases. When suspected, pheochromocytomas are diagnosed by assessing the levels of plasma free metanephrines and MRI, combined with functional imaging using ^{123}I-tagged metaiodobenzylguanidine[162]. Surgical resection is the standard treatment for these tumours.

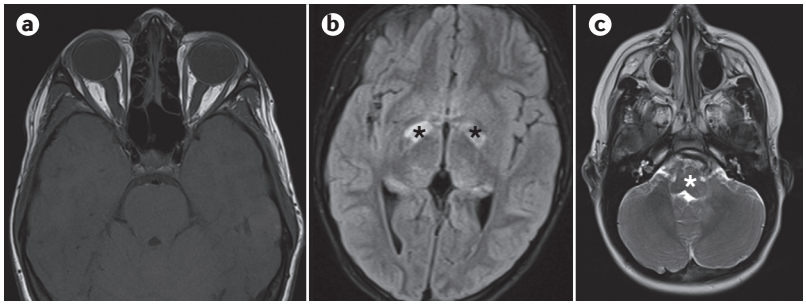

Figure 8 | **Central nervous system abnormalities. a** | Bilateral optic pathway gliomas. **b** | T2-weighted fluid-attenuated inversion recovery (FLAIR) lesions within the basal ganglia (asterisks). **c** | Brainstem glioma (asterisk).

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Leukaemia. Children with neurofibromatosis type 1 are predisposed to the development of juvenile chronic myelogenous leukaemia and juvenile myelomonocytic leukaemia[163]. However, these cancers are rare and accurate risk estimates are not available. Management is similar to that for leukaemias arising in the general population, including bone marrow transplantation and chemotherapy.

Gastrointestinal stromal tumours. Gastrointestinal stromal tumours can present with signs of intestinal obstruction, abdominal pain, gastrointestinal bleeding, or as incidental findings on abdominal imaging or at autopsy[164]. Gastrointestinal stromal tumours associated with neurofibromatosis type 1 do not typically harbour mutations in *KIT* and *PDGFRA*, which are commonly associated with sporadic gastrointestinal stromal tumours, and also tend to occur at an earlier age, are located more distally in the gastrointestinal tract and have lower mitotic indices than sporadic tumours[165]. Treatment of gastrointestinal stromal tumours associated with neurofibromatosis type 1 is complete surgical resection; however, individuals with higher-risk lesions (for example, those of larger size or with a higher mitotic index) might be treated with adjuvant imatinib[166].

Other manifestations

Juvenile xanthogranuloma. Juvenile xanthogranulomas (yellowish papules, typically <1 cm in diameter that are usually found on the head or trunk[167]) are commonly observed in children with neurofibromatosis type 1. Although a rare association between juvenile xanthogranulomas and the development of juvenile myelomonocytic leukaemia has been documented in the general population[168], this has not been validated in individuals with neurofibromatosis type 1, and most clinicians do not obtain screening haematological assessments following initial diagnosis of these lesions. Unless juvenile xanthogranulomas are located in the eye, where they could result in hyphema (that is, blood collection in the eye), they are innocuous, do not require treatment and generally regress spontaneously.

Vasculopathy. Vasculopathy associated with neurofibromatosis type 1 can affect any arterial vessel, leading to systemic hypertension secondary to renal artery stenosis[169], cerebrovascular events[170] or peripheral vascular insufficiency[171]. Both the appearance of new lesions and the progression of pre-existing ones have been described. Characteristic pathological changes have been described in all layers of the vascular wall that ultimately lead to narrowing of the arterial lumen.

Renal artery vasculopathy should be considered in any adult with neurofibromatosis type 1 who has refractory hypertension and in paediatric patients with hypertension, prompting an evaluation by selective angiography of the renal arteries. If the blood pressure cannot be controlled with oral antihypertensives in children or adults, percutaneous transluminal angioplasty can be performed and repeated if initially unsuccessful. Cerebral vasculopathy is rare in individuals with neurofibromatosis type 1, but can result in post-stenotic proliferation of small capillaries, termed moyamoya disease. This condition is diagnosed by cerebral angiography and is usually treated by direct revascularization or indirectly with encephalo-duro-arterio-synangiosis[172].

Quality of life

The complications of neurofibromatosis type 1 can substantially affect QOL[173,174]. Studies are ongoing to identify the affected QOL domains and the predictors of impaired QOL, and clinical trials are now using outcome measures to evaluate the effects of therapies on aspects of QOL.

In children and adolescents with neurofibromatosis type 1, research findings indicate reduced QOL relative to population norms[173,175–177], and parent-reported ratings tend to yield lower scores in several domains than child self-report[173,176,178]. One factor that can contribute to poorer QOL is greater disease severity. Children and adolescents with moderate-to-severe complications of neurofibromatosis type 1, including the presence of skeletal manifestations and plexiform neurofibromas[179], have lower overall QOL scores than those with mild or no complications[177,180]. More frequent pain is related to greater functional disability[175], and higher pain interference in daily activities is associated with poorer overall QOL[180]. In addition, children and adolescents with more visible complications, such as cutaneous signs and plexiform neurofibromas, have worse QOL in selected emotional domains[176]. Social–emotional functioning, as assessed by parental reports of anxiety, depression and social stress, predicts QOL[175,180] and mediates the effects of pain interference on QOL[180]. Cognitive impairments associated with neurofibromatosis type 1, which might underlie some social–emotional difficulties[181], are also associated with poorer QOL[179]. Other predictors of selected QOL domains include socioeconomic status, a family history of neurofibromatosis type 1 and family cohesion[176–178]. Taken together, a range of disease, cognitive, social–emotional, and environmental and/or family factors contribute to the QOL of children and young adults with neurofibromatosis type 1, supporting the use of the biopsychosocial model[182] to better understand these complex interactions and design multidisciplinary interventions (FIG. 9).

In adults with neurofibromatosis type 1, studies consistently report reduced general QOL relative to population norms across physical, emotional, role functioning and social domains[174,183,184]. More severe complications of neurofibromatosis type 1 (REF. 183), poorer health[185], worse headache[186] and bodily pain[187], as well as older age[188] have been shown to negatively affect QOL. Higher self-perceived disease visibility is associated with worse skin disease-specific QOL[183,184,189], which might be mediated by perceived body image[190]. The emotional aspects of QOL are particularly affected[188,189], such that depressive symptoms are frequently reported in individuals with neurofibromatosis type 1 and strongly predict poorer overall QOL[191]. In addition, attention and learning problems are predictors of mental health difficulties in adults with neurofibromatosis type 1 (REFS 187,188).

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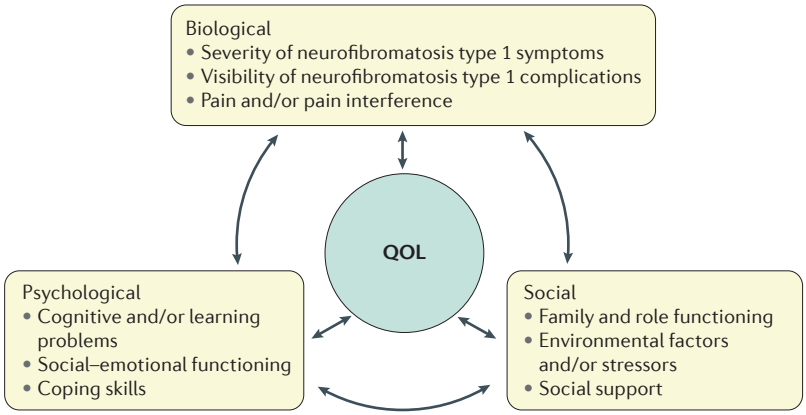

Figure 9 | **Biopsychosocial factors affecting quality of life in neurofibromatosis type 1.** Individuals with neurofibromatosis type 1 have reduced quality of life (QOL) in several domains. Complex interactions between biological, psychological and social factors influence QOL in these individuals, as shown in this biopsychosocial model.

QOL measures are being incorporated as secondary outcomes in clinical trials evaluating targeted therapies designed to reduce tumour growth and as primary outcomes in behavioural interventions devised to improve patient coping. Indeed, patient-reported outcomes are feasible and provide unique information beyond tumour response in clinical trials[136,192,193]. For example, the treatment of neurofibromatosis type 1-associated plexiform neurofibromas with sirolimus (also known as rapamycin; a mTOR inhibitor) reduced pain in several children[194] and improved emotional and school QOL domains in small samples[192,193]. On the basis of preclinical studies in mice, RAS pathway inhibitors that decrease depressive-like behaviour and improve learning and/or memory[195] might underlie such behavioural changes in children with neurofibromatosis type 1. In addition, selumetinib (a MEK inhibitor) reduced plexiform neurofibroma volumes and decreased anecdotal reports of tumour-related pain[138], which is currently being further evaluated using objective patient-reported outcome pain measures in a phase II trial. Initial behavioural interventions, such as Acceptance and Commitment Therapy, resulted in a reduction of symptoms and improved QOL[196,197]. As only limited patient-reported outcome measures exist that are validated for neurofibromatosis type 1 (REF. 198), various tools are being used to assess QOL, making it difficult to compare results across studies[173]. However, the Response Evaluation in Neurofibromatosis and Schwannomatosis International Collaboration is working to achieve consensus on the most appropriate outcome measures to assess aspects of QOL in clinical trials[199].

Outlook

Mechanisms of disease

Despite major advances in understanding the pathogenesis of neurofibromas, many unanswered questions remain regarding the events that facilitate tumorigenesis and the origin of other non-tumour lesions. For example, the mechanisms underlying the recruitment of cells without biallelic *NF1* mutations (harbouring only the germline *NF1* mutation) to neurofibromas and the time points at which the 'second-hit' *NF1* mutations occur are not known. In addition, why plexiform neurofibromas grow in childhood, but dermal neurofibromas occur after puberty, as well as what influences the time and location of neurofibromas have not been fully elucidated. Moreover, the mechanisms underlying the development of large internal and spinal tumour burdens in individuals with some missense mutations who harbour few, if any, dermal tumours[98] are poorly understood. Neurofibromin is a large protein and can potentially interact with many other cellular proteins and subcellular structures; however, the role of these interactions in the pathogenesis of neurofibromatosis type 1 and whether these interactions can present additional therapeutic targets are unclear. Similar questions remain unanswered for other manifestations of neurofibromatosis type 1, such as optic pathway gliomas, cognitive and behavioural problems, and bone defects.

New treatments

Given the potential for major morbidity, and even mortality, as a result of neurofibromatosis type 1, in addition to the stress of progressive disfigurement and the lifelong uncertainty as to future manifestations, a strong impetus exists to develop new approaches to treatment. The natural history of some features associated with neurofibromatosis type 1, such as optic pathway gliomas and plexiform neurofibromas, suggests that novel therapeutic approaches might emerge from targeting the mechanisms responsible for tumour initiation and maintenance. However, the complexity and variability of the phenotypes observed in people with neurofibromatosis type 1 present challenges that require careful consideration before any kind of 'cure' can be considered.

Whether all manifestations of neurofibromatosis type 1 will respond to the same approach to treatment is unknown. Although strong evidence supports that lack of neurofibromin activity in Schwann cells leads to increased RAS signalling, other mechanisms might account for the other manifestations of this disorder, such as cognitive deficits. In addition, the optimal time to initiate treatment in individuals is uncertain; whether plexiform neurofibromas should be treated at diagnosis, which would require the use of toxic treatments in young children, or whether treatment should be delayed until they are symptomatic, is a topic of discussion. Whether treatment at a single point in time permanently can prevent the growth of tumours or whether there is a need for continued treatment is unknown, as is the extent of treatment-related toxicity accepted by patients. It is likely that the acceptability of adverse effects of drug treatment is different when one is treating a life-threatening malignancy, compression of the spinal cord, disfigurement or moderate cosmetic impairment. Finally, how to accurately identify individuals whose tumours require treatment requires establishment.

Most approaches to treatment tested to date have targeted the RAS signalling pathway, as this controls the proliferation of cells within neurofibromas, optic pathway gliomas and MPNSTs[200]. Mouse models that show several features of neurofibromatosis type 1 have been used for preclinical testing of various treatments[201], and several promising therapies, especially the use of MEK

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inhibitors for the treatment of plexiform neurofibromas, have emerged from these studies[60]. Although phase II clinical trials conducted in individuals with neurofibromatosis type 1 have revealed encouraging results, no FDA-approved drug with proven benefits has yet emerged. The most promising drug tested so far, selumetinib, leads to a partial reduction of plexiform neurofibroma tumour volume, but not complete tumour regression. MPNSTs are refractory to treatment despite multiple clinical trials with various regimens, and to date, no treatments have been found that improve cognitive function other than the use of standard treatments for ADHD.

Whether any single therapy will be sufficient to treat any one, never mind all, of the manifestations of neurofibromatosis type 1 remains unclear. Given the partial responses seen so far by targeting the RAS pathway, combinations of therapeutic agents might be needed, both to target different disease mechanisms and to avoid the development of treatment resistance. Similarly, it is likely that different molecular subsets of tumours exist, each with their own individual drug sensitivities. Moreover, targeting specific *NF1* mutations to restore neurofibromin function, as has been successful in cystic fibrosis, might also have a role in future treatments. Longer-term prospects might include gene editing, gene replacement strategies or immunomodulation, particularly for malignant lesions.

Clinical outcomes

Although astonishing progress has been made in understanding neurofibromatosis type 1 over the past 25 years, that this disease will remain a clinical entity for some time to come is likely. Genetic testing permits the prenatal diagnosis of neurofibromatosis type 1 for pregnancies that are known to be at risk, but not all couples are interested in prenatal testing[202]. While the field works towards effective treatments, much can be done to improve clinical outcomes and QOL in individuals. Individuals are challenged by a lack of access to health care providers with experience in managing the condition. Although consensus guidelines have been developed for some of the manifestations associated with neurofibromatosis type 1 (REFS 110,203), evidence-based guidelines are lacking, which results in varying approaches to management. Although some complications are too rare to have a robust evidence base, others, such as optic pathway gliomas, plexiform neurofibromas, dermal neurofibromas and learning disabilities, might be amenable to evidence-based reviews. Patient advocacy groups have played an important part in supporting research and educating both health care providers and patients. With the increasing availability of personal computing devices and wearable technologies, opportunities might arise to actively engage individuals in helping to manage their own care and in data-sharing both with health care providers and within the patient community.

In summary, the care of individuals with neurofibromatosis type 1 brings to the fore many of the complex issues of modern medicine, such as access to care, coordination of multiple specialties, balancing proactive disease surveillance with not overwhelming a patient with tests and medical appointments, and tempering hope for new treatments with avoiding untested claims. As such, the management of children and adults with neurofibromatosis type 1 represents a team effort, including both medical specialists and patients, and the development of effective treatments, given the complexity of this disorder, will similarly require cross-disciplinary collaboration and teamwork.

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Acknowledgements
This work was partially funded by a grant from the US Department of Defense (W81XWH-12-1-0155 to B.R.K.).

Author contributions
Introduction (B.R.K.); Epidemiology (K.J.J.); Mechanisms/pathophysiology (D.H.G.); Diagnosis, screening and prevention (R.E.F.); Management (R.H.L. and R.E.F.); Quality of life (P.L.W.); Outlook (B.R.K.); Overview of Primer (D.H.G.).

Competing interests
The authors declare no competing interests.

How to cite this article
Gutmann, D. H. *et al.* Neurofibromatosis type 1. *Nat. Rev. Dis. Primers* **3**, 17004 (2017).

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