Neurofibromatosis type 1-associated tumours: Their somatic mutational spectrum and pathogenesis

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Abstract

Somatic gene mutations constitute key events in the malignant transformation of human cells. Somatic mutation can either actively speed up the growth of tumour cells or relax the growth constraints normally imposed upon them, thereby conferring a selective (proliferative) advantage at the cellular level. Neurofibromatosis type-1 (NF1) affects 1/3,000–4,000 individuals worldwide and is caused by the inactivation of the *NF1* tumour suppressor gene, which encodes the protein neurofibromin. Consistent with Knudson's two-hit hypothesis, NF1 patients harbouring a heterozygous germline *NF1* mutation develop neurofibromas upon somatic mutation of the second, wild-type, *NF1* allele. While the identification of somatic mutations in NF1 patients has always been problematic on account of the extensive cellular heterogeneity manifested by neurofibromas, the classification of *NF1* somatic mutations is a prerequisite for understanding the complex molecular mechanisms underlying NF1 tumorigenesis. Here, the known somatic mutational spectrum for the *NF1* gene in a range of NF1-associated neoplasms — including peripheral nerve sheath tumours (neurofibromas), malignant peripheral nerve sheath tumours, gastrointestinal stromal tumours, gastric carcinoid, juvenile myelomonocytic leukaemia, glomus tumours, astrocytomas and phaeochromocytomas — have been collated and analysed.

Keywords: *NF1, somatic mutations, germline mutations, pathogenesis, tumorigenesis, tumour, benign, malignant*

Introduction

Neurofibromatosis type 1 (NF1) is a common autosomal dominantly inherited tumour predisposition syndrome affecting 1/3,000–4,000 individuals worldwide.[1,2] NF1 manifests a variety of characteristic features that include: hyperpigmentary abnormalities of the skin (café-au-lait macules and inguinal/axillary freckling), iris hamartomas (Lisch nodules) and the growth of benign peripheral nerve sheath tumours (neurofibromas) in the skin. Neurofibromas display many different subtypes and are associated with a variety of different clinical complications. Cutaneous neurofibromas are present in almost all adult NF1 patients.[3] Plexiform neurofibromas (PNFs), a more diffuse type of tumour, are present in 30–50 per cent of NF1 patients, and some 10–15 per cent of these benign tumours are transformed to malignant peripheral nerve sheath tumours (MPNSTs), the main cause of morbidity in NF1.[4] Other NF1-associated clinical features include: skeletal abnormalities, such as tibial bowing or pseudoarthrosis; skeletal and orbital dysplasia; ostopenia/osteoporosis; aqueduct stenosis; macrocephaly; pectus excavatum; short stature; cardiovascular malformations; learning difficulties; and attention deficit disorder.[1,5]

Cancer represents the transformation of a cell whose growth is normally tightly controlled into one that is no longer under strict regulation,

allowing the cell to multiply uncontrollably and even metastasize. This dramatic alteration in cellular control arises as a consequence of the accumulation of genetic and epigenetic changes: activated oncogenes speed up cell growth through the acquisition of gain-of-function mutations, whereas tumour suppressor genes (TSGs) promote progression by acquiring loss-of-function mutations. TSGs typically encode proteins involved in growth regulation, apoptosis initiation, cellular adhesion and DNA repair. In accordance with Knudson's two-hit hypothesis,[6] both alleles of a TSG must be inactivated for cellular transformation to occur. Typically, a patient will inherit a germline mutation in one TSG allele; a second-hit or somatic mutation then occurs post-fertilisation, thereby inactivating the remaining wild-type allele. Somatic mutation is thus a key event in cancers associated with TSG inactivation. Upon transformation, a cell may acquire many additional somatic mutations elsewhere in the genome, a few of which actively encourage tumour progression, designated as 'driver mutations', while most occur simply because of the increased number of cell replications and are usually of unknown biological consequence and so are designated as 'passenger mutations'.[7]

The *NF1* gene encodes neurofibromin, a negative regulator of the Ras/mitogen-activated protein kinase (MAPK) pathway. *NF1* is a TSG and, consistent with Knudson's two-hit hypothesis, most patients carry (in all their cells) both a normal and a dysfunctional *NF1* gene copy — the latter harbouring the inherited (germline) mutation. It may be inferred that any tumours that arise will have acquired a second, somatic 'hit' that inactivates the normal *NF1* allele, resulting in a complete loss of functional neurofibromin; a double hit ($NF1^{-/-}$) is critical for NF1 tumorigenesis to occur.[8,9] The question as to why only a few of these benign tumours eventually go on to become malignant, however, is still puzzling. Consistent with a central role for neurofibromin in cellular function, recent cancer genome sequencing studies have found that somatic *NF1* gene mutations occur not only in association with NF1, but also in a number of other common cancers.[10–16]

In the context of NF1, few genotype–phenotype correlations are evident. Indeed, marked intrafamilial variation in terms of the clinical phenotype is common.[5,17] The existence of such families is perhaps an indication of the importance of the second hit, since differences in the type and timing of somatic *NF1* mutations may help to explain the variability in patient phenotype.[18] An appreciation of the spectrum of somatic mutations in NF1-associated tumours is therefore essential if we are to understand the molecular pathways involved — itself a prerequisite for improvements in clinical treatment and the development of new therapeutics. This paper attempts to collate and review the spectrum of somatic *NF1* mutations so far reported in NF1-associated tumours, with a view to assessing how they may serve to induce tumour growth and whether or not any genotype–phenotype correlation may be discerned

The *NF1* gene: Structure and function

The *NF1* gene spans 283 kilobases (kb) of genomic DNA at 17q11.2[19] and contains 61 exons.[3,20] Neurofibromin, the 327 kDa protein encoded by the *NF1* gene, is translated from a 12 kb messenger mRNA transcript, and has a number of alternative isoforms[21–24] (reviewed by Upadhyaya[25]). Neurofibromin contains 2,818 amino acids and is expressed at low levels in all cells, with higher levels in the nervous system. It functions as a negative regulator of active Ras, and of the associated Ras/MAPK signalling pathway. Neurofibromin contains a Ras-specific GTPase activating protein (GAP)-related domain which interacts directly with Ras, resulting in a conformational change that greatly stimulates the intrinsic GTPase activity of the Ras protein, thus significantly accelerating the conversion of the active GTP-bound form of Ras into its inactive GDP-bound form and effecting a net decrease in overall mitogenic signalling in the cell. As the Ras/MAPK cascade is critical for the control of cellular growth and differentiation, a lack of functional neurofibromin results in the constitutive

activation of this central signalling pathway and in unregulated cell growth.[26]

NF1 tumour biology

A variety of benign and malignant tumours are associated with NF1 and all involve tumorigenesis of neural crest-derived cells. Several murine models of neurofibromatosis have both successfully recapitulated much of the NF1 human phenotype and shown that *NF1* is indeed a classical TSG.[27,28]

Neurofibromas exhibit extensive cellular heterogeneity, being composed of hyperproliferative Schwann cells (SCs), fibroblasts, mast cells and perineural cells. The SCs have been identified as the initiating cell type in neurofibromas and it is only in these cells that the *NF1* gene becomes biallelically inactivated.[29] SCs are also the target for various growth factors known to stimulate neurofibroma formation and growth. What is still not known, however, is the precise cell type within the SC cell lineage in which the somatic mutation occurs, the cell type which subsequently precipitates neurofibroma growth.

Cutaneous neurofibromas are thought to arise from skin-derived precursor cells (SKPs)[30] and these cells may well be under hormonal control, since most such tumours develop only during puberty.[31] Further, an increase in tumour size and number has also been noted during pregnancy, with some evidence for a postnatal decrease in tumour size.[32,33] Almost all PNFs appear congenitally and it is thought that they are induced by a somatic *NF1* mutation in SC precursors within the embryonic gestational window of 12.5–15.5 days.[34] It may be that this second hit does not render the SC precursor tumorigenic, but instead induces aberrant axonal segregation.[35] The extracellularly expressed transmembranal guidance protein, Sema4F, is strongly downregulated in neurofibromas and it has been suggested that this somehow indirectly promotes SC proliferation by rendering these cells more responsive to environmental signals, possibly through inhibition of axonal re-attachment.[36] In this way, the disruption of normal SC axonal interactions leads to neurofibroma development. An $NF1^{-/+}$ haploinsufficient cellular environment is also considered necessary, probably because of the growth advantage conferred by the signalling deficiency due to reduced neurofibromin levels. Indeed, Le *et al.*[30] found that *NF1* inactivation is necessary, although not sufficient, for neurofibroma formation, highlighting the importance of the tumour microenvironment. There is some evidence to indicate that the haploinsufficiency ($NF1^{-/+}$) of the other supporting cells (fibroblasts, mast cells and perineurial cells) cooperates in neurofibroma development.[37] Additionally, it has been shown that $NF1^{-/+}$ haploinsufficient mast cells readily migrate into preneoplastic nerves, probably in response to Kit ligand, which exhibits four-fold increased levels in nullizygous SCs as compared to normal SCs.[38,39] The molecular mechanisms underlying both PNF and cutaneous neurofibroma formation are becoming clearer, although the major details are still lacking. It would appear that the key to understanding neurofibroma formation lies in the elucidation of the precise molecular interactions of the haploinsufficient tumour microenvironment within the initial cell type harbouring the biallelically inactivated *NF1* gene.

NF1-associated tumours

Cutaneous neurofibromas and PNFs

Neurofibromas are a characteristic feature of NF1 and have a diverse clinical presentation. They are classified as grade 1 tumours by the World Health Organization; they have multiple forms and may affect nerves in any body location. Tumours derived from skin sensory nerves are designated dermal or cutaneous neurofibromas, and usually present as discrete tumours that remain associated with a single nerve ending. Approximately 20–50 per cent of cutaneous neurofibromas exhibit loss of heterozygosity (LOH) at the *NF1* locus and the majority of these lesions appear to be due to mitotic recombination.[40–42] Tumours associated with larger nerves within the skin may spread within the dermis and appear as a diffuse mass. PNFs are much larger tumours, usually associated

with major nerve trunks and nerve plexi. They are generally slow growing, may develop at both internal and external body locations and can often result in major disfigurement. PNFs occur in some 30–50 per cent of patients with NF1 and, although these tumours generally remain benign, some neurological impairment may result from their growth. Approximately 10–15 per cent of PNFs may become malignant.

While the genetic basis of neurofibroma development is still not fully understood, biallelic *NF1* inactivation does seem to be required, as all tumour cells harbour both a constitutional and a somatic *NF1* gene mutation.[5] About 70 per cent of PNFs have been reported to display LOH at the *NF1* locus;[20] however, there is no obvious correlation between the type or location of germline *NF1* mutations in NF1 patients and those of their somatic counterparts arising in their tumours.[20]

Another interesting, although as yet unexplained, observation is that a few patients mildly affected by NF1 who never develop any cutaneous neurofibromas or PNFs have been shown to carry the same germline *NF1* mutation (c.2970-2972delAAT) — namely, an in-frame 3-base pair (bp) deletion that leads to the loss of a methionine residue.[3]

MPNSTs

Cells derived from within some 10–15 per cent of PNFs may eventually undergo malignant transformation into an MPNST. MPNSTs are aggressive and highly invasive soft tissue sarcomas with an annual incidence of 0.16 per cent in NF1 patients, compared with only 0.001 per cent in the normal population,[43] and with a lifetime risk of 8–13 per cent in NF1 individuals[44,45] (reviewed by Upadhyaya[4]). This form of malignancy represents a major cause of morbidity and mortality in NF1. Malignant transformation usually appears to evolve from within a pre-existing PNF.[46] The distinction between benign PNFs and MPNSTs has been sensitively visualised by non-invasive [^{18}F]-2-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) imaging,[47] suggesting a potential role for FDG-PET-based non-invasive imaging in future diagnostic tests. The aberrant molecular pathways that underlie this malignant transformation are still largely unknown, and considerable effort is being directed towards elucidating the molecular defects involved.

NF1 patients carrying large (usually 1.4-megabase [Mb]) genomic deletions (which remove the entire *NF1* gene plus a variable number of flanking genes) have an increased risk of MPNST development in certain patient groups.[48,49] Indeed, over 90 per cent of MPNSTs have been found to harbour large *NF1* somatic deletions.[20] More recently, significantly increased frequencies (relative to the general NF1 population) of PNFs, subcutaneous neurofibromas, spinal neurofibromas and MPNSTs have also been reported in association with molecularly ascertained 1.4 Mb type-1 *NF1* deletions.[50] The MPNST-associated deletion breakpoints have been found not to involve the paralogous repetitive sequences that are involved in most germline *NF1* deletions.[18] The smallest common region of somatic deletion overlap is, however, restricted to approximately the same ~2.2 Mb interval that contains most of the genes deleted in recurrent constitutional *NF1* deletions.[51]

Although it is clear that biallelic *NF1* gene inactivation is required for transformation to occur, mutations at the *NF1* locus are insufficient to explain the process of tumorigenesis, as most benign neurofibromas also exhibit such biallelic *NF1* inactivation. The best evidence for the involvement of other loci relates to the tumour protein 53 gene (*TP53*), for which several different mutations have been found in MPNSTs that have not been reported in benign neurofibromas.[4,20,52,53] Mice with heterozygous mutations in both their *Nf1* and *Tp53* genes developed malignancy,[27,54] an indication, perhaps, that *TP53* loss is critical to transformation. The homozygous loss of the cyclin-dependent kinase inhibitor 2A gene (*CDKN2A*), which encodes p16INK4A and p14ARF, has also been associated with NF1 malignancy.[55–57] Another recent report has indicated that phosphatase and tensin homologue deleted on chromosome 10 gene (*PTEN*) dosage, and/or phosphatidylinositol 3-kinase/AKT8 virus oncogene cellular homologue (PI3K/AKT) pathway

activation, may be rate-limiting steps in NF1 malignant transformation.[58] As yet, however, no characteristic gene expression signature has been defined for MPNST development, although several cell-cycle and signalling regulation genes: — cyclin-dependent kinase inhibitor (*CDKN2A*); tumour protein 53 (*TP53*); retinoblastoma 1 (*RB1*); epidermal growth factor receptor (*EGFR*); CD44 antigen (*CD44*); platelet-derived growth factor receptor alpha (*PDGFRA*); hepatocyte growth factor (*HGF*); proto-oncogene protein (*C-MET*) and transcription factor (*SOX9*) — are frequently deregulated.[4]

Recent studies of the micro-RNA expression profile of MPNSTs have expanded the pathogenic spectrum associated with this tumour. For example, microRNA-34a (miR-34a) is downregulated in MPNSTs; this microRNA (miRNA) regulates many cell cycle genes and is also upregulated by p53, suggesting that *TP53* loss would lead to down-regulation of miR-34a and possibly several other miRNAs. This implies that this could be a critical event in malignant transformation.[59] In similar vein, miR-10b has been reported to be upregulated in SCs from NF1 tumours, while miR-10b inhibition reduced MPNST cell proliferation, migration and invasion.[60] *NF1* mRNA is also a specific target for miR-10b,[60] indicating that these miRNAs represent potential therapeutic targets.

Spinal neurofibromas

About 40 per cent of NF1 patients present with tumours involving their spinal nerves. This is especially marked in individuals affected with familial spinal neurofibromatosis (FSNF), a variant form of NF1 in which bilateral tumours involving multiple spinal nerve roots are often the only manifestation of NF1.[61–63] Patients with FSNF have been reported to be significantly more likely to harbour missense or splice-site germline mutations compared with patients with classical NF1.[64] A recent study of the *NF1* locus found LOH in eight of 22 spinal tumours analysed, with most (75 per cent) of this LOH being due to mitotic recombination rather than genomic deletions.[64]

Gastrointestinal stromal tumours (GISTs)

GISTs are the most common mesenchymal tumours of the gastrointestinal tract. Although most GISTs harbour activating somatic mutations of *KIT* and *PDGFRA*, the absence of such mutations from NF1-associated GISTs (NF1-GISTs) is probably indicative of a different pathogenetic mechanism. In NF1, the majority (60 per cent) of GISTs develop in the small intestine, whereas sporadic non-NF1 GISTs mainly involve the stomach.[65]

Somatic *NF1* mutations have been identified in the interstitial cells of Cajal (ICC) throughout the gastrointestinal tract and in NF1-GISTs lacking *KIT* or *PDGRA* mutations.[66] Increased signalling through the Ras/MAPK pathway has also been shown to occur in NF1-GISTS, as opposed to sporadic GISTs. This would seem to indicate that a decrease in neurofibromin level, in the presence of normal c-KIT and PDGFRA levels, leads to tumour formation. It also suggests that *NF1* haploinsufficiency is required for ICC hyperplasia, again demonstrating that, although a somatic *NF1* mutation is absolutely necessary, it is not sufficient to permit tumorigenesis: additional genetic events required. These observations concur with Knudson's two-hit hypothesis. Somatic inactivation of the *NF1* gene through gene deletion; intragenic deletion; and LOH through mitotic recombination have also been described.[66,67]

Gastric carcinoid

Gastric carcinoid tumours are associated with multiple endocrine neoplasia, atrophic gastritis and pernicious anaemia but are very rare in NF1.[17] LOH at the *NF1* locus has been demonstrated in a gastric carcinoid tumour derived from an NF1 patient.[67]

Juvenile myelomonocytic leukaemia (JMML)

Young NF1 patients are at particular risk of developing JMML,[68] a clonal haematopoietic disorder characterised by hypersensitivity (at least *in vitro*) to granulocyte-macrophage colony-stimulating factor (GM-CSF). Moreover, some 15–20 per cent of JMML patients harbour a somatic *NF1* inactivating

mutation, even though most exhibit no other NF1 symptoms.[69] Patients may also carry inactivating mutations of other genes, with a recent study identifying that 70–80 per cent of mutations involve genes in the Ras/MAPK pathway, including one tyrosine-protein phosphatase non-receptor type 11 (*PTPN11*), neuroblastoma RAS viral oncogene homologue (*NRAS*), and v-Ki-ras2 kirsten rat sarcoma viral oncogene homologue (*KRAS*) as well as *NF1* genes.[70] Additional somatic mutations have also been reported in the casitas B-lineage lymphoma (*CBL*) and additional sex combs-like 1 (*ASXL1*) genes.[71] In most cases, the *NF1* gene is lost either via LOH or by compound heterozygous microlesions,[72] which lead to a complete loss of neurofibromin and hyperactive signalling through the Ras/MAPK pathway. LOH may occur through 1.2–1.4 Mb interstitial deletions mediated by low copy number repeat (LCR) elements that flank the *NF1* gene.[73] LOH through uniparental interstitial isodisomy (50–52.7 Mb) of chromosome 17 through double mitotic recombination, in an as-yet-unknown initiator cell, has also been reported.[72] The rarity of such events may indicate the existence of a selective advantage, conferred upon the $NF1^{-/-}$ cells, which might explain the propensity of NF1 patients to develop leukaemia.[74]

Astrocytomas (ACs)

Optic pathway tumours or ACs are found in ~15 per cent of paediatric NF1 patients,[75] with the complete loss of neurofibromin evident in NF1-associated optic gliomas.[76] Approximately 84 per cent of NF1-associated ACs also exhibit LOH in the *NF1* region, with many tumours also exhibiting LOH of 17p, suggesting the likely role of *TP53* — or other 17p13-located genes — in AC formation.[77] As with MPNSTs, biallelic somatic *NF1* mutation in ACs is, again, apparently insufficient to induce transformation.

Phaeochromocytomas (PCs)

PCs are extremely rare tumours, with only one to six cases observed per million individuals. PCs develop from neural crest-derived chromaffin cells, and the tumour cells produce and release catecholamines, which cause hypertension and flushing. These are tumours of the adrenal medulla and are primarily associated with mutations of the Ret proto oncogene (*RET*), von Hippel-Lindau (*VHL*), succinate dehydrogenase complex, subunit B (*SDHB*), succinate dehydrogenase complex, subunit C (*SDHC*), and succinate dehydrogenase complex, subunit D (*SDHD*) genes, although LOH in the *NF1* region, as well as LOH of other loci on both 17q and 17p, have been observed.[78,79]

Glomus tumours

Glomus tumours are small (<5 mm), benign, but often very painful tumours that develop specifically within the highly innervated glomus body located at the end of each digit. These tumours appear to develop from α-smooth muscle actin-positive cells that have undergone biallelic *NF1* inactivation, resulting in increased Ras/MAPK activity.[80] The somatic *NF1* mutations often differ between glomus tumours, indicating highly specific tumorigenic events. Brems *et al.*[80] have suggested that glomus tumours, although rare, should now be recognised as an integral component of the NF1 spectrum of disease.

The somatic mutational spectrum of NF1-associated tumours

A review of all published — and the authors' many unpublished — somatic *NF1* alterations associated with NF1 tumours was undertaken to gain a better appreciation of NF1 tumorigenesis. As of July 2010, at least 577 different somatic *NF1* gene changes had been reported in different NF1-associated tumours, with more than half (323/577; 56 per cent) corresponding to LOH in the *NF1* gene region, some involving much larger regions of chromosome 17 (Table S1). The level of LOH detected also differs between cutaneous neurofibromas, PNFs and MPNSTs (40 per cent, 79 per cent and 85 per cent, respectively; Table 1). Table 2 provides the incidence of LOH in the other tumour types, where

Table 1: Contribution of LOH and *NF1* micro-lesions to the somatic *NF1* mutational spectrum in different types of NF1-associated tumour

Tumour type	LOH	Point mutations	Total
Dermal neurofibroma	144 (40%)	211 (60%)	355
Plexiform neurofibroma	67 (79%)	18 (21%)	85
Spinal neurofibroma	7 (70%)	3 (30%)	10
MPNST	55 (85%)	10 (15%)	65
Astrocytoma	18 (100%)	0 (0%)	18
GIST/gastric carcinoid	3 (38%)	5 (62%)	8
JMML	18 (95%)	1* (5%)	19
Phaeochromocytoma	10 (100%)	0 (0%)	10
Glomus tumour	1 (14%)	6 (86%)	7
Overall	323 (55%)	254 (44%)	577

* Compound heterozygous *NF1* mutations were identified in five of six haemopoietic tumours analysed. As no other normal tissues were available in these five cases, it was not possible to distinguish between the associated germline and somatic *NF1* mutations.

Table 2: Mechanistic basis of the *NF1* gene-associated LOH identified in different NF1-associated tumours

Tumour type	Tumour showing mitotic recombination (number & percentile)	Tumours with genomic deletions (number & percentile)
Dermal neurofibroma	28 (76%)	8 (24%)
Plexiform neurofibroma	11 (44%)	14 (56%)
Spinal neurofibroma	7 (88%)	1 (12%)
MPNST	5 (16%)	26 (84%)
Astrocytoma	0 (0%)	2 (100%)
GIST/gastric carcinoid	1 (50%)	1 (50%)
JMML	15 (79%)	4 (21%)
Phaeochromocytoma	0 (0%)	0 (0%)
Glomus tumour	1 (100%)	0 (0%)

Tabulated information only given for tumours in which the precise LOH mechanism was identifiable.

appropriate evidence has been obtained by multiplex ligation-dependent probe amplification (MLPA), fluorescence in situ hybridisation (FISH) etc; 78 per cent (28/36) of cutaneous neurofibromas, 44 per cent (11/25) of PNFs and 16 per cent (5/31) of MPNSTs display LOH resulting from mitotic recombination. Some 79 per cent (15/19) of the JMML samples that exhibited LOH appear to have lost the entire 17q arm through mitotic recombination, perhaps indicating a significant correlation with this tumour type.

Tumour DNA analysis has also identified 254 somatic *NF1* gene lesions, including nonsense, missense, splice site, microdeletion/microinsertions (<20 bp), indels (combined insertion–deletion events) and larger (>20 bp) deletions/insertions (Tables 3, S2). The consequences of all deletions and insertions for the reading frame were also determined, with five sequence changes being compound heterozygous *NF1* mutations found in five haemopoietic tumours; however, with no other tissue available for analysis, it was not possible to differentiate between germline and somatic *NF1* point mutations (Table S2). About 75 per cent (191/254) of the somatic mutations associated with NF1 tumours comprise mutations that are predicted to give rise to truncated proteins. Of these 191 changes, only 18 result from the insertion or duplication of bases; the remaining 173 truncations arise from deletion, nonsense mutation or frameshift events. Splice site mutations form a considerable proportion (39/254; 15.0 per cent) of the mutational spectrum, while missense changes only account for some 9.4 per cent (24/254) of the somatic *NF1* mutations.

Any attempt to make direct comparisons between the various tumour types would be unwise at this stage, owing to the paucity of somatic mutation data, especially for the less commonly encountered tumours. Table 3 nevertheless attempts to summarise the available data. The bias inherent in the data is immediately evident, with 211/254 (83 per cent) mutational changes originating from the analysis of cutaneous neurofibroma DNA. Hence, the relative frequencies of the various mutation types in cutaneous neurofibromas are essentially comparable with the germline mutational spectrum, with nonsense mutations, splice site mutations and missense

Table 3: The spectrum and percentile distribution of somatic *NF1* micro-lesions reported in different NF1-associated tumours

Tumour type	Mutation type							
	Deletion	Insertion	Indel	Nonsense	Splice site	Missense	Truncating	Total
Dermal neurofibroma	82 (39%)	15 (7%)	2 (1%)	59 (28%)	32 (15%)	21 (10%)	158 (75%)	211
Plexiform neurofibroma	6 (33%)	1 (6%)	–	7 (39%)	2 (11%)	2 (11%)	14 (78%)	18
Spinal neurofibroma	–	–	–	–	2 (66%)	1 (33%)	0	3
MPNST	7 (70%)	1 (10%)	1 (10%)	1 (10%)	–	–	10 (100%)	10
GIST/gastric carcinoid	1 (20%)	–	–	3 (60%)	1 (20%)	–	4 (80%)	5
JMML*	*	*	*	1 (100%)	*	*	1 (100%)	1
Glomus tumour	2 (33%)	1 (17%)	–	1 (17%)	2 (33%)	–	4 (67%)	6
Overall	98 (39%)	18 (7%)	3 (1%)	72 (28%)	39 (15%)	24 (9%)	191 (75%)	254

* Compound heterozygosity of *NF1* mutations in several JMML tumours cases meant it was not possible to distinguish between associated germline and somatic *NF1* mutations.

alterations found in cutaneous neurofibromas at frequencies of 28 per cent (59/211), 15 per cent (32/211) and 10 per cent (21/211), respectively (Table 3). Table 3 does, however, serve to highlight the high proportion of truncating mutations (191/254; ~75 per cent) involved in the somatic inactivation of the *NF1* gene in all tumour types, especially cutaneous neurofibromas.

An additional comparison between the frequency distributions of somatic microlesions and LOH is made in Table 1. There appears to be a marked difference between cutaneous neurofibromas, PNFs and MPNSTs, with 40 per cent, 79 per cent and 85 per cent, respectively, of somatic mutation events represented by LOH. This may be explained in part by the extent of the molecular rearrangements in each tumour type; MPNSTs, for example, would be predicted to exhibit a greater extent of genetic aberration than a benign dermal neurofibroma. The types of analyses performed, however, will have a direct influence on such conclusions, in that either microlesions or LOH may not be screened for in some studies.

In summary, the more severe MPNSTs show a greater degree of genetic abnormality than other tumour types, with LOH constituting a much more frequent event in these tumours. Further comparison within and between the rarer tumour types would not be valid, however, owing to the relative paucity of mutation data currently available for analysis.

Mutational mechanisms underlying the known somatic *NF1* gene lesions

Somatic inactivation of the *NF1* gene may result from different mutational mechanisms and may involve intragenic mutations, LOH and epigenetic modification of the promoter region. Among the 254 somatic *NF1* mutations listed in Table S2, 72 nonsense mutations were found, of which 36 involved mutations in just 15 codons in different tumours (codons 192, 304, 426, 440, 816, 1241, 1306, 1362, 1513, 1569, 1604, 1748, 1939, 1976 and 2429), with many previously reported in different tumours or different studies. Ten of these 15 different recurrent nonsense mutations involve C > T or G > A transitions within CpG dinucleotides and are compatible with the endogenous mutational mechanism of methylation-mediated deamination of 5-methylcytosine (5mC). Of these 72 nonsense mutations, 28 have also been reported as germline mutations in NF1 patients (Human Gene Mutation Database [HGMD]),[81] indicating that the same mutational mechanism is operating in both the soma and

germline. The importance of this mutational mechanism is evidenced by the finding that 12 of the 15 recurrent somatic nonsense mutations have also been reported independently in the germline (codons 192, 304, 426, 440, 816, 1241, 1306, 1362, 1513, 1569, 1748 and 2429). For the ten of these 15 nonsense mutations that correspond to C > T or G > A transitions within CpG dinucleotides, we may infer that the mutated cytosine must be methylated both in the soma and in the germline, thereby explaining the vulnerability of these sites to methylation-mediated deamination in both cell lineages.

Among the somatic *NF1* mutations listed in Table S2 are 21 different missense mutations. Of these, two (in codons 519 and 776) have been reported more than once in different tumours or different studies, although neither is compatible with methylation-mediated deamination of 5mC. Of the 21 missense mutations, only one (in codon 176) has also been reported in the germline (see HGMD). Since this Asp176Glu mutation has also been reported more than once in NF1-associated tumours, it may well be that this residue is of importance for the function of neurofibromin in both the soma and the germline. Furthermore, this residue is conserved in different species, including *Drosophila* and *Fugu*, and has not been identified in 250 unrelated normal individuals.

Nonsense mutations are not the only type of *NF1* mutation to occur recurrently in the soma. Among the somatic *NF1* microdeletions listed in Table S2 are five that have been reported more than once in different tumours (c.1888delG, c.2033delC, c.3058delG, c.4374_4375delCC and c.5731delT) with three microdeletions occurring in mononucleotide tracts (G_4, C_7 and T_3, respectively), suggestive of a model of slipped mispairing at the DNA replication fork. Importantly, c.2033delC has also been reported in the germline (see HGMD), indicating that this tetranucleotide stretch is a hotspot for mutation in both the germline and the soma. A microinsertion (c.1733insT, located within a T_6 tract) has also been found to occur recurrently in the soma but this has not so far been reported in the germline. The reader interested in a detailed comparative analysis of germline and somatic mutations in human TSGs is referred to Ivanov *et al.*[82]

NF1 gene somatic mutations in non-NF1-associated tumours

Various studies have identified somatic *NF1* gene mutations in non-NF1-associated cancers. Thus, somatic *NF1* aberrations have been identified in glioblastoma multiforme (GBM) tumours, lung adenocarcinomas, malignant breast tumours, leukaemia, ovarian serous carcinomas (OSCs) and neuroblastoma.[10–12,14–16,83] Some of the *NF1* gene changes are relatively frequent in these tumours and therefore have the potential to represent specific prognostic and diagnostic markers. For example, 23 per cent of sporadic GBM tumours harbour an inactivating *NF1* somatic mutation, and this may enable such GBM tumours to differentiate into the mesenchymal molecular subclass.[13] Similarly, in 22 per cent (9/41) of primary OSCs, an *NF1* mutation was detected, six of which exhibited biallelic inactivation.[12] Interestingly, all nine of these OSC samples also contained a *TP53* mutation, highlighting the likely involvement of this TSG in OSC pathogenesis.[12]

Given the pivotal role that neurofibromin plays in several cell signalling pathways, it is not surprising that its loss will affect distinct molecular subtypes in different cancers. Indeed, the efficacy of any future therapeutic intervention for many tumours will almost certainly hinge upon our ability successfully to identify such molecular subclasses of tumour.

Prospects for the development of new treatments/therapies

As the complex picture underlying the molecular nature of NF1 tumorigenesis becomes better defined, the treatment regimens available to patients should greatly improve. Although the future is encouraging, the optimal treatment for NF1 tumours currently rests with their surgical resection, in spite of the high chance of recurrent malignancy. Gottfried and colleagues[84] have suggested that the recruitment of supporting cells around the

neurofibroma, coupled with aberrant Remak bundles, could explain how the neurofibroma integrates into the surrounding tissue, and it is this that may lead to the surgical difficulties that often lead to tumour recurrence. Moreover, it has been suggested that surgical interference may even increase the recruitment of surrounding cell types, thereby inadvertently increasing the growth of lesions leading to the formation of new neurofibromas.[84] Surgical biopsy is therefore inherently problematic, and novel therapeutics are urgently required. Clinical and preclinical trials targeting different components of the Ras/MAPK signalling pathway and related growth factor receptors appear to be more promising. It is likely, however, that treatment with multiple drugs may be more effective for NF1 tumours.[5]

Concluding remarks

Biallelic inactivation of the *NF1* gene, resulting in the complete loss of functional neurofibromin, initiates the pathogenic process that eventually results in the formation of nerve sheath tumours. *NF1* gene inactivation may occur through relatively subtle lesions that affect just a few DNA bases, or may involve large genomic changes that affect large chromosomal regions, or even the entire chromosome 17. This review demonstrates that NF1-associated tumour types display a considerable degree of variation in terms of the level of LOH detected, with cutaneous neurofibromas, PNFs and MPNSTs. MPNSTs manifest increased levels of deletion-based LOH, whereas cutaneous neurofibromas appear to be associated with a localised deletion of the *NF1* gene through mitotic recombination (the situation in PNFs being somewhat intermediate). In MPNSTs, additional mutations at different gene loci are almost certainly involved in the progression of the tumour.

In terms of the molecular mechanisms of mutagenesis, both methylation-mediated deamination of 5-methylcytosine and slipped mispairing within polynucleotide tracts appear to be responsible for the occurrence of mutation hotspots in both the germline and the soma. For some types of tumour, there is interplay between the soma and the germline, in that the location of the germline mutation can influence the nature, frequency and location of the subsequent somatic mutation.[85,86] As yet, however, there is no evidence for this phenomenon in the context of NF1 tumorigenesis.

Although our knowledge of the role of the *NF1* gene in tumorigenesis is ever expanding, definitive markers of malignant transformation remain to be discovered. Mouse and other animal models, including zebrafish,[87] have provided new perspectives for research, with various knockout and mutagenesis studies potentiating functional studies. It is already clear that, in order to clarify the role of the *NF1* gene in NF1-associated tumours, we must improve our understanding of the significance of the somatic (second-hit) mutations. The brief assessment of the compilation of somatic *NF1* mutations in NF1-associated tumour types reported here failed to unearth any specific genotypic correlations. The limited size of the mutation dataset means that reliable conclusions are hard to draw, and that larger and better-defined patient groups will be needed, to allow more reliable comparisons to be made. Additionally, definitive prognostic markers should be identified that permit differentiation between benign neurofibromas that are likely to progress to malignancy and those that are not.

This review nevertheless emphasises that NF1 is a highly individual condition that exhibits extreme somatic mutational heterogeneity both within and between patients. These are the mutations which are ultimately responsible for the molecular changes that can lead to tumour formation. If we can come to understand how these changes bring about tumorigenesis, we shall be better placed not only with respect to the provision of genetic counselling, but also in terms of exploring new avenues for the development of new drug-based therapies.

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Table S1. Summary of germline mutations and loss of heterozygosity (LOH) in NF1-associated tumours

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
Dermal neurofibromas									
T190.2	Exon 2 and 3 deletion	Two exon deletion	Yes	E5 RFLP, 112b, IVS27A28.4, J1/J2, EVI20, IVS38GT53.0, 3′NF1, C7CT1/2 (3′UTR), EW206, EW207, D17S798, D17S1868	NF1 and 3′ flanking region	MLPA	Deletion	5/23	1
T190.6			Yes	E5 RFLP, 112b, IVS27A28.4, J1/J2, EVI20, IVS38GT53.0, 3′NF1, C7CT1/2 (3′UTR), EW206	NF1 and 3′ flanking region	MLPA	Deletion		
T190.11			Yes	E5 RFLP, 112b, IVS27A28.4, J1/J2, EVI20, IVS38GT53.0, 3′NF1, C7CT1/2 (3′UTR)	NF1 and 3′ flanking region	MLPA	Deletion		
T190.17			Yes	E5 RFLP, 112b, IVS27A28.4, J1/J2	Intragenic NF1	MLPA	Deletion		
T190.18ii			Yes	E5 RFLP	Intragenic NF1	MLPA	Deletion		
T206.1	Ex4b: c.499_502delTGTT; p.C167GnfsX9	4 bp deletion (FS)		LOH		NIA			Unpublished data, Cardiff
T206.2				LOH					
T206.3				LOH					

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
L002_3	Ex9: c.1246C > T; p.Arg416X	Nonsense	Yes	rs29001484, rs4583306, NF1 germline mutation, rs2055091, rs11869264	NF1	Array CGH	Deletion	6/28	2
L002_5			Yes	rs29001484, rs4583306, NF1 germline mutation, rs2055091, rs11869264	NF1	Array CGH	Mitotic recombination		
L002_12			Yes	rs29001484, rs4583306, NF1 germline mutation, rs2055091, rs11869264	NF1	Array CGH	Mitotic recombination		
L002 C			Yes	NS		NIA		3/38	3
T473.1A	Ex10b: c.1413-1414delAG; p.Lys471AsnfsX4	2 bp deletion (FS)	Yes	HHH202, J1J2, IVS27, EV120, IVS38	NF1	MLPA	Mitotic recombination	22/89	4
T473.1C			Yes	HHH202, J1J2, EV120	NF1	MLPA	Mitotic recombination		
T473.3			Yes	J1J2, EV120, IVS38	NF1	MLPA	Mitotic recombination		
T473.5			Yes	HHH202, J1J2, EV120, IVS38	NF1	MLPA	Mitotic recombination		
T473.7			Yes	J1J2, EV120	NF1	MLPA	Mitotic recombination		
T473.8			Yes	HHH202, J1J2, EV120, IVS38, 3′NF1, EW207, D17S949, D17S1822	NF1 and 3′ flanking region	MLPA	Mitotic recombination		
T473.10			Yes	J1J2, EV120, IVS38	NF1	MLPA	Mitotic recombination		

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
T473.14			Yes	J1J2, EV120, IVS38, 3′NF1, EW207, D17S949, D17S1822	NF1 and 3′ flanking region	MLPA	Mitotic recombination		
T473.15			Yes	J1J2, EV120, IVS38, 3′NF1	NF1	MLPA	Mitotic recombination		
T473.16			Yes	J1J2, EV120, IVS38, 3′NF1, EW207, D17S949, D17S1822	NF1 and 3′ flanking region	MLPA	Mitotic recombination		
T473.21			Yes	J1J2, EV120	NF1	MLPA	Mitotic recombination		
T473.35			Yes	EV120, IVS38	Intragenic NF1	MLPA	Mitotic recombination		
T473.30			Yes	J1J2, EV120, IVS38, 3′NF1, EW207, D17S949	NF1 and 3′ flanking region	MLPA	Mitotic recombination		
T473.32			Yes	J1J2, EV120	Intragenic NF1	MLPA	Mitotic recombination		
T473.34			Yes	EV120, IVS38	Intragenic NF1	MLPA	Mitotic recombination		
T225.1	Ex10b deletion [MLPA]	Single exon deletion		LOH		NIA			Unpublished data, Cardiff
T225.3				LOH					
T68.2	Deletion exons 10b-19b	Partial gene deletion	Yes	UT172 - I38 206,207	Exon 5-3′ region	NIA			Unpublished data, Cardiff
T68.3			Yes	UT172 - I38 206,207	Exon 5-3′ region				

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
CLJ1N	Ex13: c.2041C > T; p.Arg681X	Nonsense	Yes	NF1, D17S1800	NF1 and 3′ flanking region	NIA		32/126	5, 6, 7
CLJ2N			Yes	D17S33, D17S1294, NF1, D17S1800, D17S798, D17S250, D17S787, D17S802	Majority of 17q				
T170.3	Ex13: c.2041C > T; p.Arg681X	Nonsense		LOH		NIA			Unpublished data, Cardiff
T170.2				LOH					
ABA1N	Ex13: c.2251 + 2T > C	Splice site	Yes	DS17S1824, D17S841, D17S1294, D17S1863, NF1, D17S1800, D17S1880, D17S798, D17S250, D17S787, D17S802, D17S784, D17S928	Majority of 17q	NIA		32/126	5, 6, 7
ABA2N			Yes	DS17S1824, D17S841, D17S1294, D17S1863, NF1, D17S1800, D17S1880, D17S798, D17S250, D17S787, D17S802, D17S784, D17S928	Majority of 17q				5, 6, 7
T436	Ex17: c.2875C > T; p.Glu959X	Nonsense	Yes	IVS27, EV120, IVS38	Intragenic NF1	MLPA	Mitotic recombination	22/89	4
T439			Yes	IVS27, EV120, IVS38	Intragenic NF1	MLPA	Mitotic recombination		
T440			Yes	IVS27, EV120, IVS38	Intragenic NF1	MLPA	Mitotic recombination		

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
T444			Yes	HHH202, J1J2, IVS27, EV120, IVS38, 3′NF1	NF1 and flanking regions	MLPA	Mitotic recombination		
T446			Yes	IVS27, EV120, IVS38	Intragenic NF1	MLPA	Mitotic recombination		
T448			Yes	HHH202, J1J2, IVS27, EV120, IVS38, 3′NF1	NF1 and flanking regions	MLPA	Mitotic recombination		
T454			Yes	IVS27, EV120, IVS38	Intragenic NF1	MLPA	Mitotic recombination		
EAD1N	Ex20: c.3419C > G; p.Ser1140X	Nonsense	Yes	NS		NIA		32/126	5, 6, 7
EAD2N									
CSG3N	Ex21: c.3525_3526delAA; p.Arg1176GlufsX17	2 bp deletion (FS)	Yes	D17S1824, D17S1294, NF1, D17S1800, D17S1880, D17S798	NF1 and flanking regions	NIA		32/126	5, 6, 7
CSG38N			Yes	NF1	NF1				
CSN1N			Yes	D17S1294, NF1, D17S1880, D17S798, D17S250, D17S787, D17S784, D17S928	Majority of 17q				
CSG1N			Yes	D17S1294, NF1, D17S1800, D17S1880, D17S798, D17S250, D17S787, D17S802	Majority of 17q				

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
CSG2N			Yes	DS17S1824, D17S1294, NF1, D17S1800, D17S1880, D17S798, D17S250, D17S787, D17S802	Majority of 17q				
CSG4N			Yes	DS17S1824, D17S1294, NF1, D17S1800, D17S1880, D17S798, D17S250, D17S787, D17S802	Majority of 17q				
CSG5N			Yes	DS17S1824, D17S1294, NF1, D17S1800, D17S1880, D17S798, D17S250, D17S787, D17S802	Majority of 17q				
CSG21N			Yes	DS17S1824, D17S1294, NF1, D17S1800, D17S1880, D17S798, D17S250, D17S787, D17S802	Majority of 17q				
CSG25N			Yes	D17S1294, NF1, D17S1800, D17S1880, D17S798, D17S250, D17S787, D17S802	Majority of 17q				
CSG42N			Yes	D17S1294, NF1, D17S1800, D17S1880, D17S798, D17S250, D17S787, D17S802	Majority of 17q				

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
CSG51N			Yes	DS17S1824, D17S1294, NF1, D17S1800, D17S1880, D17S798, D17S250, D17S787, D17S802	Majority of 17q	FISH	Mitotic recombination		
CSG52N			Yes	D17S33, DS17S1824, D17S1294, NF1, D17S1800, D17S1880, D17S798, D17S250, D17S787, D17S802	Majority of 17q	NIA			
CSG62N			Yes	NS					
T177	Ex23.1: c.3916 C > T; p.Arg1306X	Nonsense		LOH		NIA			Unpublished data, Cardiff
T213				LOH					
NF44 UHG_1	Ex27a: c.4515-2A > T	Splice site	Yes	NF1 germline mutation, rs9891455	NF1	Array CGH	Deletion	6/28	2
NF44 UHG_41			Yes	rs1018190	NF1	Array CGH	Deletion		
T106.1	Ex37: c.6791dupA; p.Tyr2264X	1 bp insertion (FS)	Yes	IVS38, 3′NF1-1		NIA			Unpublished data, Cardiff
T106.5			Yes	I41 - C3	3′ UTR				
T106.6			Yes	J1J2, EVI20, I38, I41, C3C7, 206, 207					
T210.2	Ex42: c.7458delC; p.Tyr2487IlefsX5	1 bp deletion (FS)				30% WG			Unpublished data, Cardiff

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
T210.4						30% WG			
T210.8						30% WG			
T210.4						E8: 30% WG			
T210.5						E8: exon duplication			
HT1335	Ex4c: c.7237C > T; p.Gln2413X	Nonsense				E16: del			Unpublished data, Cardiff
T128.30	Ex6: c.784C > T; p.Arg262Cys	Missense	Yes		3′ UTR to 3′ region	NIA			Unpublished data, Cardiff
T192.4	Deletion of exons 6-27a [MLPA]	Multi-exon deletion		LOH: J1J2, EV120, HHH202,					Unpublished data, Cardiff
p062	Ex7: c.910C > T; p.Arg304X	Nonsense		LOH (6 samples)		Deletion (2 samples)			Unpublished data, Cardiff
p082	Ex7: c.910C > T; p.Arg304X	Nonsense		LOH (5 samples)		Deletion (5 samples)			
ACF1N	Ex7: c.910C > T; p.Arg304X	Nonsense	Yes	D17S841, D17S1294, D17S1863, NF1, D17S1880, D17S798, D17S250, D17S802, D17S784	Majority of 17q	FISH	Mitotic recombination	32/126	5, 6, 7
MIGS1N	Ex7: c.910C > T; p.Arg304X	Nonsense	Yes	D171863, NF1, D17S1800, D17S1880	NF1 and flanking regions	NIA		32/126	
CAG1N	Ex7: c.979delCinsTT; p.Leu327PhefsX3	Indel (FS)	Yes	NF1	Intragenic NF1	NIA		32/126	
CAG3N	Ex7: c.979delCinsTT; p.Leu327PhefsX3	Indel (FS)	Yes	NS		NIA		32/126	

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
T199	Ex7: c.983_984delGT; p.Cys324X	2 bp deletion (FS)	Yes	IVS12, J1J2		NIA			Unpublished data, Cardiff
NF56-2	Ex9: c.1246C > T; p.Arg416X	Missense	Yes	Pin 1, RsaI, AluI, Pin 28, 530, NF1 3′UTR, Mfd 15	NF1 and flanking regions	NIA		1/6	8
T197A	Ex10a: c.1318C > T p.Arg440X	Nonsense		LOH?					Unpublished data, Cardiff
CLT1N	Ex12a: c.1754_1757delTAAC; p.Thr586SerfsX19	4 bp deletion (FS)	Yes	D17S841, D17S1863, NF1, D17S1800, D17S1880, D17S787, D17S802	Majority of 17q			32/126	
p022	Ex12a: c.1756_1759delACTA; p.Thr586ValfsX18	4 bp deletion (FS)		LOH (9 samples)		Deletion (2 samples)			Unpublished data, Cardiff
p020	Ex13: c.2041C > T; p.Arg681X	Nonsense		LOH (2 samples)		Deletion (0 samples)			
T141.5	Ex13: c.2233delA; p.Ser745AlafsX2	1 bp deletion (FS)	Yes	202, 12b, IVS27, IVS38, 3′NF1					Unpublished data, Cardiff
p103	Ex15: c.2338A > C; p.Thr780Pro	Missense		LOH (10 samples)		Deletion (3 samples)			Unpublished data, Cardiff
T22	Ex17: c.2851-2A > G	Splice site	Yes		3′UTR to 3′ flanking regions				Unpublished data, Cardiff
NF253-UHG E	Ex17: c.2851-2A > G	Splice site	Yes	Not specific		NIA		3/38	3
L005 A	Ex18: c.3113 + 1G > A	Splice site							

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
319T1	Ex19b: c.3208C > T; p.Gln1070X	Nonsense	Yes	NF-exon5	Intragenic NF1			2/15	9
p023	Ex21: c.3525_3526delAA; p.Arg1176SerfsX18	2 bp deletion (FS)		LOH (14 samples)		Deletion (5 samples)			Unpublished data, Cardiff
p011	Ex22 : c.3826C > T; p.Arg1276X	Nonsense		LOH (5 samples)		Deletion (1 sample)			
MASG2N	Ex22: c.3870 + 1G > T	Splice site	Yes	NF1, D17S1880, D17S798, D17S250, D17S787	Majority of 17q	FISH	Mitotic recombination	32/126	5, 6, 7
T171	Ex23.2: c.4084 C > T; p.Arg1362X	Nonsense		LOH					Unpublished data, Cardiff
p104	Ex25: c.4309G > T; p.Glu1436X	Nonsense		LOH (3 samples)		Deletion (0 samples)			Unpublished data, Cardiff
p078	Ex27a: c.4537C > T; p.Arg1513X	Nonsense		LOH (6 samples)		Deletion (0 samples)			
p084	Ex27a: c.4572C > G; p.Tyr1524X	Nonsense		LOH (2 samples)		Deletion (0 samples)			
p102	Ex29: c.5242C > T; p.Arg1748X	Nonsense		LOH (5 samples)		Deletion (2 samples)			
p055	Ex30: c.5710 G > T; p.Glu1904X	Nonsense		LOH (1 sample)		Deletion (0 samples)			
EMN1N	Ex30: c.5749 + 332A > G	Splice site	Yes	NF1, D17S1800	NF1 and 3′ flanking region			32/126	5, 6, 7

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
p027	Ex33: c.6226delG; p.Ala2076GlnfsX13	1 bp deletion (FS)		LOH (1 sample)		Deletion (0 samples)			Unpublished data, Cardiff
p052	Ex37: c.6791_6792dupA; p.Tyr2264X	1 bp duplication (FS)		LOH (1 sample)		Deletion (1 sample)			
T23.6	Ex41: c.7268_7269delCA; p.Thr2423SerfsX2	2 bp deletion (FS)	Yes	EVI20, I38, I41, C3		NIA			Unpublished data, Cardiff
T100	Ex41: c.7267dupA; p.Thr2426X	1 bp duplication (FS)	Yes		I38 to 3′UTR				Unpublished data, Cardiff
T164.1	Ex41: c.7285 C > T; p.Arg2429X	Nonsense		LOH					Unpublished data, Cardiff
MAR2N	NI	NI	Yes	NF1, D17S1800	NF1 and 3′ flanking region	NIA		32/126	5, 6, 7
MOPT2N	NI	NI	Yes	D17S1824, D17S1294, D171863, NF1, D17S1800, D17S1880	NF1 and flanking regions				
NGL1N	NI	NI	Yes	D17S841, D17S1294, NF1, D17S1800, D17S1880, D17S798, D17S250, D17S802, D17S784, D17S928	Majority of 17q				
JRR2N	NI	NI	Yes	D17S1294, D17S1863, NF1, D17S1800, D17S1880, D17S798, D17S250, D17S787	Majority of 17q				

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
SLC1N	NI	NI	Yes	D17S33, DS17S1824, D17S841, D17S1294, NF1, D17S1880, D17S798, D17S250, D17S784, D17S928	Majority of 17q				
HT1377.1	NI	NI	Yes						Unpublished data, Cardiff
HT1377.2	NI	NI	Yes			NIA			
T109.4	NI	NI	Yes	I38, I41, 206	3′ region				
T167.c	NI	NI	Yes	IVS27, IVS38, 3′NF1					
T192.1	NI	NI	Yes	202, IVS12, J1J2, IVS27					
T197	NI	NI	Yes	J1J2					
T227.2	NI	NI	Yes	IVS12, J1J2					
T230.2	NI	NI	Yes	202, IVS12, IVS27					
T232.2	NI	NI	Yes	J1J2					
T241	NI	NI	Yes	J1J2					
NF253_32	NI	NI	Yes	rs1018190, rs9891455, rs8074061	NF1	Array CGH	Mitotic recombination	6/28	2
T224.1	NI	NI		LOH		NIA			Unpublished data, Cardiff
T162	NI	NI		LOH					
T172	NI	NI		LOH					
T179.1	NI	NI		LOH					

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
T204.2	NI	NI		LOH					
T224.2	NI	NI		LOH					
T173.1	NI	NI		LOH					
T179.2	NI	NI		LOH					
T1281.2	NI	NI		LOH					
T1281.4	NI	NI		LOH					
T220	NI	NI		LOH					
T221	NI	NI		LOH					
T223	NI	NI		LOH					
T258.1	NI	NI		LOH					
T258.2	NI	NI		LOH					
SCs from cutaneous neurofibromas									
T543.2	Ex4a: c.373delGinsATGTGT; p.Arg125HisfsX4	Indel (FS)	Yes	J1J2-3′NF1					Unpublished data, Cardiff
T536A	Ex40: c.7127_7258del132 [Exon 40 deletion?]	132 bp deletion (FS)	Yes	J1J2-IVS38		NIA			Unpublished data, Cardiff
T541.2			Yes	EV120-3′NF1					
T541.4			Yes	EV120-3′NF1					
T539	90kb Deletion	Genomic deletion				Duplication: Ex19b-25			Unpublished data, Cardiff

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
PNFs									
37a	Ex24: c.4268A > G; p.Lys1423Arg	Missense	Yes	HHH202, E5, I12b, EVI20,3'NF1-1 Complete gene deletion (1.4Mb)	NF1 (1.4 Mb)	MLPA	Genomic deletion	20/29	10
37b			Yes	IVS27, EVI20, IVS38, 3′NF1-1 Probable gene deletion	NF1 and 3′ flanking region	MLPA	Genomic deletion	20/29	10
T210.2 PNF	Ex42: c.7458delC; p.Tyr2486IlefsX15	1 bp deletion (FS)	Yes	LOH detected in only 30% of cells			30% whole gene deletion		Unpublished data, Cardiff
T261 PNF	Ex3: c.288 + 1 delG	1 bp deletion at a splice site	LOH IVS38						
605-1	Ex4a: c.289-2A > G	Splice site	Yes	D17S975, IVS27TG24.8, IVS27TG28.4, D17S1166, D17S1880, D17S907, D17S1788, D17S1861, D17S1809, D17S668, D17S928	NF1 and flanking regions	MLPA	Mitotic recombination	13/43	11
47 / T411	Ex4a: c.440_441GC > AA; p.Cys147X	Nonsense	LOH IVS27, IVS38, 3′NF1-1	IVS38, 3′NF1-1	NF1 and 3′ flanking region	NIA		20/29	10
8 / T328	Ex4b: c.480-2A > G	Splice site	LOH: IVS27, IVS38	IVS27, IVS38	Intragenic NF1	MLPA	Mitotic recombination	20/29	10
335-3	Ex4b: c.528T > A; p.Asp176Glu	Missense	Yes	D17S2237, IVS27TG24.8, D17S1166, D17S1800	NF1	MLPA	Genomic deletion	13/43	11

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
59	Ex6: c.752dupA; p.Asp241GlufsX7	Small Insertion (fs)	Yes	intron 38 marker 53.0	Intragenic NF1	NIA		1/38	12
T265.2	Ex9: c.1186-13delT (Pathogenicity?)	1 bp deletion within a splice site	LOH ivs27, ivs38						
374-4	Ex10a: c.1318C > T; p.Arg440X	Nonsense	Yes	IVS27TG24.8, IVS27TG28.4, D17S1166, D17S1880, D17S907, D17S1861	NF1 and 3′ flanking region	MLPA	Mitotic recombination	13/43	11
14a / T412	Ex13: c.2076C > G; p.Tyr692X	Nonsense	112B, 3′ NF1	112B, 3′ NF1-1	NF1 and 3′ flanking region	MLPA	Mitotic recombination	20/29	10
T263	Ex15: c.2326-2A > T	Splice site	LOH ivs27 [rest hom]						
22 / T394	Ex16: c.2446C > T; p.Arg816X	Nonsense	IVS27	IVS 27, EVI20	Intragenic NF1	MLPA	Mitotic recombination	20/29	10
T437.2	Ex16: c.2497delT; p.Ser833ProfsX7	1 bp deletion (FS)	1–6ex 1,2,3,4a,4b,4c, 6 deletion			NIA			
T212	Ex16: c.2705deT; p.Met902ArgfsX22	1 bp deletion (FS)					Ex1-Ex 41 deletion [variable ?]		
18 / T298	Ex18: c.3113 + 1G > A	Splice site	LOH :HHH202, IVS 27	IVS 27	Intragenic NF1	MLPA	Inconclusive	20/29	10
30 / T342	Ex19a: c.3123G > T; p.Met1041Ile	Missense	Yes	Determined by MLPA	NF1 (1.4 Mb)	MLPA	Genomic deletion	20/29	10

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
5	Ex20: c.345&_3460delCTCA; p.Leu1153MetfsX3	4 bp deletion (FS)	Yes	NF1 gene	NF1			1/3	13
23 / T373.2	Ex22: c.3826C > T; p.Arg1276X	Nonsense	WG deletion [mixed cell population]	IVS 27, IVS38	Intragenic NF1	MLPA	Inconclusive	20/29	10
452T	Ex23.2: c.4084C > T; p.Arg1362X	Nonsense	Yes	NF-exon5 RFLP, NF-(GATN)n intron 26, NF-Alu(AAAT)n(i27b), NF-EVI2B RFLP(i27b), NF-EVI2A RFLP(i27b), NF-IVSAC28.4(i27b), NF-Evi-20, NF-IVS38TG53.0, NF intron 41 RFLP, D17S57 (EW206), D17S250, D17S1301, D17S384	NF1 and 3′ flanking region	NIA		4/10	9
27 / T301	Ex23.2: c.4095C > A; p.Cys1365X	Nonsense	Yes	Determined by MLPA	Intragenic NF1	MLPA	Genomic deletion	20/29	10
T330	Ex24: c.4267A > G; p.Lys1423Glu	Missense	IVS27			NIA			
6 / T362 / T395	Ex24: c.4268A > G; p.Lys1423Arg	Missense	Yes	EW206, EW207	Intragenic NF1	MLPA	Inconclusive	20/29	
T362 PNF	Ex24: c.4268A > G; p.Lys1423Arg	Missense	LOH: HHH202, E5, 112b, EVI20, 3′NF			NIA			
T395 PNF	Ex24: c.4268A > G; p.Lys1423Arg	Missense	LOH:IVS27, EVI20, IVS38, 3′NF1-1						

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
317-1	Ex25: c.4270-2A > G	Splice site	Yes	IVS27TG24.8, IVS27TG28.4, D17S1166	NF1	MLPA	Genomic deletion	13/43	11
T393	Ex27a: c.4537C > T; p.Arg1513X	Nonsense					Whole gene deletion		
26 /T300	Ex29: c.5227_5229delGTAinsT; p.Val1743TyrfsX17	Indel (FS)	Yes	Determined by MLPA	NF1 (1.4 Mb)	MLPA	Genomic deletion	20/29	10
338-2	Ex29: c.5290delG; p.Ala1764LeufsX8	1 bp deletion (FS)	Yes	D17S783, D17S975, IVS27TG28.4, D17S1166, D17S1880	NF1 and flanking regions	MLPA	Genomic deletion	13/43	11
952-8	Ex30: c.5749 + 4delA	Splice site	Yes	D17S975, D17S1880, D17S907, D17S1788, D17S1861, D17S1809, D17S668, D17S928	NF1 and flanking regions	MLPA	Genomic deletion		
34 / T392	Ex31: c.5750_5754dupGTATT; p.Glu1919ValfsX4	5 bp duplication (FS)	Yes	EVI20, IVS38, 3′NF1-1,	NF1 and 3′ flanking region	NIA		20/29	10
21 / T357	Ex37: c.6791dupA; p.Tyr2264X	1 bp duplication (FS)	LOH : EW206	EW206	Intragenic NF1	MLPA	Mitotic recombination		
T375	Ex40: c.7237C > T; p.Gln2413X	Nonsense	**16Ex 16 deletion, ex13 & 18 also lower**			NIA			
7	Ex41: c.7285C > T; p.Arg2429X	Nonsense	Yes	HHH202, IVS27	Intragenic NF1	MLPA	Mitotic recombination		

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
15 / T407	Ex46: c.7926dupT; p.Lys2643X	1 bp duplication (FS)	LOH 3′NF1-1, EW206	3′NF1-1	NF1 and 3′ flanking region	MLPA	Inconclusive		
c1 UK / T56	Ex46: c.8035A > T; p.Thr2679Ser	Missense	LOH IVS27	IVS27	Intragenic NF1	NIA			
T408	Segmental NF1 NI	NI	LOH: IVS27, IVS38, 3′NF1-1						
T377	Segmental NF1 NI	NI	WG deletion						
39	Segmental NF1 NI	NI	Yes	Determined by MLPA	NF1 (1.1 Mb)	MLPA	Genomic deletion		
43	Segmental NF1 NI	NI	Yes	IVS27, IVS38, 3′NF1-1	NF1 and 3′ flanking region	MLPA	Mitotic recombination		
T385.1	NI	NI	Yes			NIA			Unpublished data, Cardiff
T385.2	NI	NI	LOH/del						
T316	NI	NI	Yes	LOH: HHH202, E5, 112b, EVI20,3'NF, C71/2, EW206					
76, 45-95	NI	NI	Yes	IVS27AC28.4, IVS27TG24.8, IVS38GT53	Intragenic NF1	NIA		8/14	14
x1, 47-95	NI	NI	Yes	IVS27AC28.4, M98509, IVS38GT53	Intragenic NF1				

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
x1, 27-97	NI	NI	Yes	IVS27AC28.4, M98509, IVS38GT53	Intragenic NF1				
293, 71-97	NI	NI	Yes	M98509, IVS27TG24.8, IVS38GT53	Intragenic NF1				
293, 124-98	NI	NI	Yes	M98509, IVS27TG24.8, IVS38GT54	Intragenic NF1				
290, 83-97	NI	NI	Yes	IVS27AC28.4, IVS27TG24.8, IVS38GT53	Intragenic NF1				
290, 121-98	NI	NI	Yes	IVS27AC28.4, IVS27TG24.8, IVS38GT53	Intragenic NF1				
292, 122-98	NI	NI	Yes	IVS27TG24.8, IVS38GT53	Intragenic NF1				
PD-T1	NI	NI	Yes	NF-Alu(AAAT)n(i27b), D17S1800	Intragenic NF1	NIA		4/10	9
386T	NI	NI		NF-Alu(AAAT) n(i27b), NF-IVSAC28.4(i27b), NF-Evi-20, NF-IVS38TG53.0, D17S1800	NF1				
454T-V	NI	NI	Yes	NF-Alu(AAAT)n(i27b), NF-EVI2B RFLP(i27b), NF-IVSAC28.4(i27b), NF-Evi-20, NF intron 41 RFLP, D17S57 (EW206), D17S1301	NF1				

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
NF284-1	NI	NI	Yes	Exon 28 14bp duplication marker (specific to germline lesion found)	Intragenic NF1			1/1	8
2654-97	NI	NI	Yes	Determined by FISH	Whole chromosome	FISH	Genomic deletion	1/11	15
385	NI	NI	Yes	D17S975, D17S1880, D17S907, D17S1788, D17S1861, D17S1809, D17S668, D17S928	NF1 and flanking regions	MLPA	Mitotic recombination	13/43	11
389-2	NI	NI	Yes	D17S975, D17S1307, D17S2237, IVS27TG28.4, D17S1800, D17S1880, D17S907, D17S1861, D17S1809, D17S668, D17S928	NF1 and flanking regions	MLPA	Mitotic recombination		
604-4	NI	NI	Yes	D17S1800, D17S1880, D17S907, D17S1861, D17S928	NF1 and flanking regions	MLPA	Mitotic recombination		
913-5	NI	NI	Yes	D17S2237, IVS27TG24.8, D17S1880, D17S1788, D17S1861	NF1 and 3′ flanking region	MLPA	Genomic deletion		
612-1	NI	NI	Yes	D17S1307, D17S2237, IVS27TG24.8, D17S1166, D17S1800, D17S1880	NF1 and flanking regions	MLPA	Genomic deletion		

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
337-5	NI	NI	Yes	D17S1307, D17S2237, IVS27TG24.8, D17S1166, D17S1800	NF1	MLPA	Genomic deletion		
390	NI	NI	Yes	IVS27TG24.8, IVS27TG28.4, D17S1166, D17S1800	NF1	MLPA	Genomic deletion		
49	NI	NI	Yes	HHH202, E5, 112b, EVI20, 3′NF1-1, C71/2, EW206	NF1	MLPA	Inconclusive	20/29	10
Spinal neurofibromas									
1	Ex7: c.899T > C; p.Leu300Pro	Missense	Yes	EVI20, IVS38	Intragenic NF1	MLPA	Mitotic recombination	8/22	16
7	Ex9: c.1186-13delT (Pathogenicity?)	1 bp deletion (FS)	Yes	IVS27, IVS38	Intragenic NF1	MLPA	Mitotic recombination		
3	Ex16: c.2410-2A > T	Splice site	Yes	IVS27	Intragenic NF1	MLPA	Mitotic recombination		
11.1	Ex22: c.3827G > A; p.Arg1276Glu	Missense	Yes	IVS38	Intragenic NF1	MLPA	Mitotic recombination		
11.2			Yes	Deletion of exons 13 > 16	Intragenic NF1	MLPA	Deletion		
2	Ex23.2: c.4066G > A; p.Glu1356Lys	Missense	Yes	27, 3′NF1	NF1	MLPA	Mitotic recombination		
10	Ex29: c.5242C > T; p.Arg1748X	Nonsense	Yes	112B, Alu1, J1J2 and EVI20	Intragenic NF1	MLPA	Mitotic recombination		

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
MPNSTs									
T196.20	Deletion exons 2 and 3	Two exon deletion	Yes	112b, IVS27AC28.4, EVI20(IVS27B), IVS38GT53.0 (IVS38), 3′-NF1, C7/CT1/2 (3′-UTR), EW206 (3′extragenic), EW207 (3′extragenic), D17S798	NF1 and 3′ flanking region	MLPA	Mitotic recombination	2/11	1
T196.24			Yes	NF1 exon 5, 112b, IVS27AC28.4, EVI20(IVS27B), IVS38GT53.0 (IVS38), 3′-NF1, C7/CT1/2 (3′-UTR), EW206 (3′extragenic), EW207 (3′extragenic)	NF1 and 3′ flanking region	MLPA	Genomic deletion	2/11	1
13	Deletion exons 2 and 3	Two exon deletion	Yes	Ex5, 112b, IVS27, EVI20, IVS38,C7CT, EW206, EW207,3′NF1	NF1 and 3′ flanking region	MLPA/CGH array	Genomic deletion	31/34	17
7	Ex4c: c.654 + 1G > T	Splice site	Yes	UT172, HH202, J1/J2, EVI20	NF1	MLPA/CGH array	Genomic deletion		
27	Ex8: c.1133_1136delACTG; p.Asp378AlafsX7	4 bp deletion (FS)	Yes	Ex5, J1J2,3′NF1	NF1 and 3′ flanking region	NIA			
9	Ex11: c.1713G > A; p.Trp571X	Nonsense	Yes	D17S182, 112b, J1/J2	Intragenic NF1				
10			Yes	UT172, HH202, J1/J2, EVI20, >2.2Mb	NF1	MLPA / CGH array	Genomic deletion		

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
23	Ex12a: c.1318C > T; p.Arg440X	Nonsense	Yes	HHH202, EVI20. IVS38	Intragenic NF1	NIA			
14	Ex12a: c.1754_1757delTAAC; p.Thr586ValfsX19	4 bp deletion (FS)	Yes	IVS27, 3′NF1	NF1 and 3′ flanking region	MLPA/CGH array	Mitotic recombination		
12	Ex13: c.2002-14C > G	Splice site	Yes	112b, IVS27, EVI20, IVS38, 3′NF	NF1 and 3' flanking region	MLPA/CGH array	Genomic deletion		
43	Ex13: c.2041C > T; p.Arg681X	Nonsense	Yes	Determined by MLPA	NF1	MLPA	Duplication mitotic recombination	6/25	18
15	Ex16: c.2497delT; p.Ser833ProfsX7	1 bp deletion (FS)		Intragenic Deletion (Exons 1-6) MLPA	Intragenic NF1	MLPA/CGH array	Genomic deletion	31/34	17
25	Ex16: c.2705delT; p.Met902ArgfsX22	1 bp deletion (FS)	Yes	Intragenic deletion (exons1-41) MLPA	Intragenic NF1	MLPA/CGH array	Genomic deletion		
17	Ex20: c.3457_3460delCTCA; p.Leu1153MetfsX3	4 bp deletion (FS)				NIA			
18			Yes	3′NF1	Intragenic NF1	MLPA/CGH array	Genomic deletion		
459T1	Ex21: c.3684delC; p.Asn1229MetfsX11	1 bp deletion (FS)	Yes	TP53(INTRON1), TP53(INTRON6), NF-(GATN)n INTRON26, NF-IVSAC28.4(i27b), D17S57, D17S250, D17S1301, D17S784	Whole chromosome	NIA		3/5	9

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
8	Ex22: c.3732delT; p.Thr1245Leufsx21	1 bp deletion (FS)	Yes	Int12, J1J2	Intragenic NF1			31/34	17
64	Ex23.1: c.3368 + 1delG	1 bp deletion at a splice site	Yes	Determined by MLPA	NF1	MLPA	Genomic deletion	6/25	18
56	Ex25: c.4276C > A; p.Gln1426Lys	Missense	Yes	Determined by MLPA	NF1	MLPA	Duplication mitotic recombination		
4	Ex27a: c.4537C > T; p.Arg1513X	Nonsense	Yes	IVS27b	Intragenic NF1	MLPA/CGH array	Genomic deletion	31/34	17
6	Ex28: c.5003insTG; p.Tyr1668LeufsX7	2 bp insertion (FS)	Yes	I4b, J1J2, EVI20	NF1	MLPA/CGH array	Genomic deletion		
21	Ex29: c.5234C > G; p.Ser1745X	Nonsense	Yes	Partial gene deletion	Intragenic NF1	MLPA/CGH array	Genomic deletion		
19	Ex37: c.6792C > A; p.Tyr2264X	Nonsense	Yes	I12b, IVS27, J1J2, EVI20, IVS38, C7CT	NF1	MLPA/CGH array	Genomic deletion		
24	Ex38: c.6961insC; p.Leu2321ProfsX5	1 bp duplication (FS)	Yes	Determined by MLPA	NF1	MLPA	Genomic deletion	6/25	18
1	Ex41: c.7268_7269delCA; p.Thr2423SerfsX2	2 bp deletion (FS)	Yes	Intron 41-30	Intragenic NF1	MLPA/CGH array	Genomic deletion	31/34	17
58	NI	NI	Yes	HHH202, NF1, EW206	Intragenic NF1		Genomic deletion	6/11	19

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
52	NI	NI	Yes	HHH202, NF1, EW206, EW207	Intragenic NF1		Genomic deletion		
22	NI	NI	Yes	HHH202	Intragenic NF1		Genomic deletion		
8	NI	NI	Yes	EW206, EW207	Intragenic NF1		Genomic deletion		
2	NI	NI	Yes	p144D6, pYNZ22.1, pYNH37.3, EW503	NF1 region and some 17p		Genomic deletion	5/6	20
3	NI	NI	Yes	EW503, EW301 (B), EW301 (T)	Intragenic NF1		Genomic deletion		
4	NI	NI	Yes	p144D6, pYNZ22.1, pYNH37.3, EW503, EW301 (T), pHHH202, EW207 (B), pTHH59	Whole chromosome		Genomic deletion		
5	NI	NI	Yes	p144D6, pYNZ22.1, pYNH37.3, EW503, EW301 (B), EW301 (T), pHHH202, EW207 (B)	Whole chromosome		Genomic deletion		
10	NI	NI	Yes	p144D6, pYNZ22.1,	NF1 region and some 17p		Genomic deletion		
88-3/14	NI	NI	Yes	D17S30, TP53, D17S71, D17S8, D17S57	Whole chromosome	G-banded chromosome 17 duplication	Genomic duplication	3/9	21

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
88-8	NI	NI	Yes	D17S30, D17S71	NF1	NIA			
88-18	NI	NI	Yes	D17S30, D17S71, D17S21, D17S33, EVI2B, D17S82	Whole chromosome				
1	NI	NI	Yes	DI7S5, DI7SI, DI7SI37, CRYBI, NF1, DI7S146	NF1 and flanking regions	NIA		2/5	22
4	NI	NI	Yes	DI7S34, DI7S5, DI7S146	NF1 and flanking regions				
1	NI	NI	Yes		NF1		Genomic deletion	1/1	23
1	NI	NI	Yes	NF1 alu, TP53 BHP53	Whole chromosome	NIA		3/7	24
7	NI	NI	Yes	CRYB1, NF1 alu, TP53 BHP53	Whole chromosome				
8	NI	NI	Yes	D17S4, D17S74, NF1 e.31, NF1 alu	NF1				
441T	NI	NI	Yes	TP53(INTRON6), D17S1863, D17twbch=S33, NF-IVSAC28.4(i27b), NF-Evi-20, NF-IVS38TG53.0, D17S1800, D17S73, D17S1301	Whole chromosome	NIA		3/5	9

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
396T4	NI	NI	Yes	NF-IVSAC28.4(i27b, NF-IVS38TG53.0, D17S57, D17S250, D17S1301	NF1 and 3′ flanking region				
2	NI	NI	Yes	NF1, P16, TP53	Whole chromosome	NIA		5/8	13
5a	NI	NI	Yes	NF1, P16, TP53	Whole chromosome				
5b	NI	NI	Yes	NF1, P16, TP53	Whole chromosome				
6a	NI	NI	Yes	NF1, P16, TP53	Whole chromosome				
6b	NI	NI	Yes	NF1, P16, TP53	Whole chromosome				
2	NI	NI	Yes	Total gene deletion	NF1	MLPA/CGH array	Genomic deletion	31/34	17
5	NI	NI	Yes	Total gene deletion	NF1	MLPA/CGH array	Genomic deletion		
24	NI	NI	Yes	EVI20, IVS27, IVS38	Intragenic NF1	MLPA/CGH array	Genomic deletion		
26	NI	NI	Yes	NF1 gene deletion	NF1	MLPA/CGH array	Genomic deletion		
48	NI	NI	Yes	Determined by MLPA	NF1	MLPA	Genomic deletion	6/25	18
86	NI	NI	Yes	Determined by MLPA	NF1	MLPA	Genomic deletion		

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
ACs									
T65.1	Ex24: c.4267A > G; p.Lys1423Glu	Missense	Yes	NS	NF1 3′ flanking region	NIA		1/1	25
57	NI	NI	Yes	EW206	Intragenic NF1	NIA		1/1	19
58	NI	NI	Yes	D17S1849, D17S1863, D17S1880	NF1 and 3′ flanking region	NIA		2/4	26
76	NI	NI	Yes	D17S1863, D17S1880	NF1 and 3′ flanking region				
182	NI	NI	Yes	IVS27TG24.8	Intragenic NF1	NIA		11/12	27
185	NI	NI	Yes	IVS27AC28.4, IVS38GT53, D17S804	NF1 region and some 17p				
187	NI	NI	Yes	IVS27AC28.4, IVS38GT53, D17S796	NF1 region and some 17p				
309	NI	NI	Yes	IVS38GT53, D17S796	NF1 region and some 17p				
330	NI	NI	Yes	IVS27AC28.4, IVS38GT53, D17S520, D17S796, D17S804	NF1 region and some 17p				

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
502	NI	NI	Yes	IVS27AC28.4, D17S520, D17S796	NF1 region and some 17p				
519	NI	NI	Yes	IVS27TG28.4, M98509, IVS27TG24.8, IVS38GT53	Intragenic NF1				
297	NI	NI	Yes	IVS27TG28.4, M98509, IVS38GT53	Intragenic NF1				
609	NI	NI	Yes	IVS27TG28.4, M98509	Intragenic NF1				
20954	NI	NI	Yes	IVS27TG24.8, IVS38GT53	Intragenic NF1				
20962	NI	NI	Yes	IVS27AC28.4, M98509, IVS38GT53	Intragenic NF1				
1	NI	NI	Yes	Homozygous		FISH	Unknown	3/4	28
9	NI	NI	Yes	Homozygous		FISH	Genomic deletion		
10	NI	NI	Yes	Homozygous		FISH	Genomic deletion		
Gastric carcinoid tumours									
1	Ex37: c.6841C > T; p.Gln2281X	Nonsense	Yes	IVS27TG24, D17S250	Intragenic NF1	NIA		1/1	29
GISTs									
1	Ex27a: c.4537C > T; p.Arg1513X	Nonsense	Yes	D17S841, Alu, IVS27GT, IVS27CAGT, IVS38, 3′NF1-1, 3′NF1-2	NF1 and 3′ flanking region	MLPA	Mitotic recombination	1/1	30

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
NF1-3	Ex45: c.7807delG; p.Aal2603LeufsX3	1 bp deletion (FS)	Yes	Alu, IVS27AC33.1, IVS38GT53.0, IVS27TG24.8	Intragenic NF1	Array CGH	Genomic deletion	1/7	3
JMML									
D102	Ex4b: c.574C > T; p.Arg192X	Nonsense	Yes	D17S925, D17S1800, D17S1880, D17S855, D17S1827, D17S787, D17S948, D17S784	Majority of 17q	SNP array	Mitotic recombination–UPD	4/5	31
D115	Ex13: c.2066delT; p.Val689GlyfsX59	1 bp deletion (FS)	Yes	D17S925, D17S1800, D17S1880, D17S855, D17S1827, D17S787, D17S948, D17S784	Majority of 17q	SNP array	Mitotic recombination–UPD		
D003	Ex22: c.3861_3862delCT; p.Cys1288ValfsX21	2 bp deletion (FS)	Yes	D17S925, D17S1800, D17S1880, D17S855, D17S1827, D17S787, D17S948, D17S784	Majority of 17q	SNP array	Mitotic recombination–UPD		
D126	Ex44: c.7699C > T; p.Gln2567X	Nonsense	Yes	D17S925, D17S1800, D17S1880, D17S855, D17S1827, D17S787, D17S948, D17S784	Majority of 17q	SNP array	Mitotic recombination–UPD		
1	NI	NI	Yes	D17S1975, D17S1294, UT172, NF1, D17S1800, D17S250, D17S801, D17S939, D17S836, D17S1806, D17S1822, D17S1830	Majority of 17q	FISH	Mitotic recombination–interstitial isodisomy (paternal)	10/10	32

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
2	NI	NI	Yes	D17S1975, D17S1294, UT172, NF1, D17S1800, D17S250, D17S801, D17S939, D17S836, D17S1806, D17S1822, D17S1830	Majority of 17q	FISH	Mitotic recombination–interstitial isodisomy (paternal)		
3	NI	NI	Yes	D17S1294, UT172, NF1, D17S1800, D17S250, D17S801, D17S939, D17S836, D17S1806, D17S1822, D17S1830	Majority of 17q	FISH	Mitotic recombination interstitial isodisomy (paternal)		
4	NI	NI	Yes	D17S1975, D17S1294, UT172, NF1, D17S1800, D17S250, D17S801, D17S939, D17S836, D17S1806, D17S1822, D17S1830	Majority of 17q	FISH	Mitotic recombination–interstitial isodisomy (maternal)		
5	NI	NI	Yes	D17S1975, D17S1294, UT172, NF1, D17S1800, D17S250, D17S801, D17S939, D17S836, D17S1806, D17S1822	Majority of 17q	FISH	Mitotic recombination–interstitial isodisomy (maternal)		
6	NI	NI	Yes	D17S1878, D17S33, D17S1975, D17S1294, UT172, NF1, D17S1800, D17S250, D17S801, D17S939, D17S836, D17S1806, D17S1822, D17S1830	Majority of 17q	FISH	Mitotic recombination–interstitial isodisomy (maternal)		

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
7	NI	NI	Yes	D17S1294, UT172, NF1, D17S1800, D17S250, D17S801, D17S939, D17S836, D17S1806, D17S1822, D17S1830, D17S928	Majority of 17q	FISH	Mitotic recombination–interstitial isodisomy (maternal)		
8	NI	NI	Yes	D17S1975, D17S1294, UT172, NF1, D17S1800, D17S250, D17S801, D17S939, D17S836, D17S1806, D17S1822, D17S1830, D17S928	Majority of 17q	FISH	Mitotic recombination–interstitial isodisomy (paternal)		
9	NI	NI	Yes	NF1, D17S1800	Intragenic NF1	FISH	Genomic deletion		
10	NI	NI	Yes	NF1, D17S1800	Intragenic NF1	FISH	Genomic deletion		
D419	NI	NI	Yes	D17S925, D17S1841, D17S1294, D17S1863, D17S1849, D17S1166, D17S1800, D17S1880, D17S1818, D17S855, D17S1827, D17S787, D17S948, D17S785, D17S784	Majority of 17q	MLPA	Mitotic recombination–UPD	5/10	33

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
D561	NI	NI	Yes	D17S1294, D17S1863, D17S1849, D17S1166, D17S1800, D17S1880, D17S1818, D17S855, D17S1827, D17S787, D17S948, D17S785, D17S784	Majority of 17q	MLPA	Mitotic recombination–UPD		
D378	NI	NI	Yes	D17S1294, D17S1863, D17S1849, D17S1166, D17S1800, D17S1880, D17S1818, D17S855, D17S785	Majority of 17q	Array CGH	Mitotic recombination–UPD		
D341	NI	NI	Yes	D17S1849, D17S1166, D17S1800, D17S1880	NF1 and flanking regions	Array CGH	Genomic deletion		
D566	NI	NI	Yes	D17S1849, D17S1166, D17S1800, D17S784	NF1 and flanking regions	Array CGH	Genomic deletion		
PCs									
1	NI	NI	Yes	DI7S34, DI7S137, CRYBI, NF1, DI7S4	Whole chromosome	NIA		7/7	22
2	NI	NI	Yes	CRYBI, DI7S33, NF1, DI7S55, DI7S4	NF1				
3	NI	NI	Yes	DI7S5, DI7S134, DI7S58, DI7S33	Whole chromosome				
4	NI	NI	Yes	DI7S33, NF1	Intragenic NF1				
5	NI	NI	Yes	DI7S71, NF1, DI7S226	Whole chromosome				

Continued

Table S1. Continued

Patient ID	Germline mutation	Type of germline mutation	LOH	LOH markers	Predicted extent of LOH	Evidence for genomic deletion? MLPA/CGH arrayCGH/ FISH	Probable mechanism	No. samples with LOH	Reference
6L	NI	NI	Yes	DI7S5, NF1, DI7S145, DI7S226	Whole chromosome				
6R	NI	NI	Yes	DI7S5, NF1, DI7S145, DI7S226	Whole chromosome				
1	NI	NI	Yes	TP53-BAM, TP53 AccII, NF1-AE25 (BglII) SNP, THH59-TaqI, THH59-PvuII	Majority of 17	NIA		2/7	34
1	NI	NI	Yes	NF1-AE25 (BglII) SNP, THH59-TaqI, THH59-PvuII-adrenal corticoid tumour	NF1 and 3' flanking region				
NS	NI	NI	Yes					14/21	35
Glomus tumours									
NF1-G2	Ex42: c.7395_7404del10; p.Thr2466SerfsX33	10 bp deletion (FS)	Yes	Introns 27-38	Intragenic NF1	Array CGH	Mitotic recombination	1/7	36

CGH, comparative genomic hybridisation; array CGH, high resolution CGH; FS, frame shift; NI, not informative; WG, whole gene; NA, not available; UPD, uniparental disomy; MLPA, multiplex ligation-dependent probe amplification; FISH, fluorescent *in situ* hybridisation.

Table S2. Summary of germline and somatic point mutations in NF1-associated tumours

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
Dermal neurofibromas					
T196.3	Ex 2 and 3 deleted	2 exon deletion	Ex4c: c.648dup73 p.Leu216 (through splice site)	73 bp duplication (FS)	1
T196.12			Ex4c: c.655-1G > A	Splice site	
T196.15			Ex6: c.750delT p.Phe250LeufsX30	1 bp deletion (FS)	
T196.16			Ex16: c.2534_2557del24 p.Cys845X	24 bp deletion (In-frame)	
T196.7			Ex16: c.2844delA p.Gly949AspfX3	1 bp deletion (FS)	
T196.4			Ex18: c.3047_c3048delGT p.Cys1016SerfsX4	2 bp deletion (FS)	
T196.5			Ex27a: c.4537C > T p.Arg1513X R	Nonsense	
T196.13			Ex27b: c.4743delG p.Asp1582IlefsX21	1 bp deletion (FS)	
T196.1			Ex44: c.7721_7722delAA p.Lys257Ser4fsX4	2 bp deletion (FS)	
T543.1	Ex4a: c.373delGinsATGTGT p.Arg125HisfsX22	Indel (FS)	Ex21: c.3568del80 p.Gly1190HisfsX3	80 bp deletion (FS)	Unpublished data, Cardiff
T543.3			Ex26: c.4388C > T p.Ser1463Phe	Missense	
T128.10	Ex6: c.784C > T p.Arg262Cys	Missense	Ex4b: c.574C > T p.Arg192X R	Nonsense	Unpublished data, Cardiff
T128.1			Ex8: c.1170delC p.Asp390LysfsX6	1 bp deletion (FS)	

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
T128.17			Ex10c: c.1556A > C p.Gln519Pro R	Missense	
T128.8			Ex32: c.6055_6056delTC p.Ser2019TrpfsX18	2 bp deletion (FS)	
NF29a-4	Ex6: c.801G > A p.Trp267X	Nonsense	Ex10a: c.1381C > T p.Arg461X	Nonsense	37
NF17-8			Ex10c: c.1528-14_1546del33 p.Asp510fs (through splice site)	32 bp deletion [FS]	
NF17-1			Ex10c: c.1641 + 1G > A	Splice site	
NF17-9			Ex18: c.3049C > T p.Glu1017X	Nonsense	
NF29a-7			Ex19b: c.3303_3314+7del19 p.Glu1101 (through splice site)	19 bp deletion [FS]	
NF17-15			Ex23.1: c.3916C > T p.Arg1306X R	Nonsense	
NF29a-9			Ex27b: c.4756insT p.Tyr1586LeufsX14	1 bp insertion (FS)	
NF17-18			Ex28: c.5205 + 1G > A	Splice site	
NF17-23			Ex31: c.5772_5775delTTTG p.Cys1924TrpfsX4	4 bp deletion (FS)	
NF29a-5			Ex40: c.7237_7253del17 p.Gln2413fsX2	17 bp deletion (FS)	
L-002 F	Ex9: c.1246C > T p.Arg416X	Nonsense	Ex3: c.246_247delTC p.Glu83SerfsX15	2 bp deletion (FS)	3
L-002 A			Ex5: c.655-1G > T	Splice site	
L-002 D			Ex8: c.1105C > T p.Gln369X	Nonsense	

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
L-002 E			Ex8: c.1153delC p.Arg385AlafsX2	1 bp deletion (FS)	
L-002 B			Ex22: c.3757_3764del8 p.Leu1253ThrfsX8	8 bp deletion (FS)	
NF282-1	Ex9: c.1260+1G > A	Splice site	Ex23.2: c.4021C > T p.Gln1341X	Nonsense	8
NF282-2			Ex23.2: c.4084C > T p.Arg1362X	Nonsense	
T473.6	Ex10b: c.1413_1414delAG p.Lys471AsnfsX1	2 bp deletion (FS)	Ex7: c.890delA p.Leu297SerfsX20	1 bp deletion (FS)	4
T473.12			Ex12b: c.1884insA p.Tyr628X	1 bp insertion (FS)	
T473.11			Ex16: c.2451insG p.Ser818ValfsX12	1 bp insertion (FS)	
T473.18			Ex22: c.3807insC p.Ser1270LeufsX13	1 bp insertion (FS)	
T473.20			Ex23.2: c.4087delA p.Ser1363ValfsX22	1 bp deletion (FS)	
T473.13			Ex31: c.5888A > C p.Asn1963Thr	Missense	
T473.33			Ex34: c.6478A > G p.Ser2160Gly	Missense	
T473.36			Ex38: c.6859delG p.Asp2287ThrfsX18	1 bp deletion (FS)	
T473.17			Ex40: c.7128delG p.Tyr2377ThrfsX23	1 bp deletion (FS)	
T82.3	Ex12a: c.1754_1757delTAAC p.Thr585ValfsX18	4 bp deletion (FS)	Ex16: c.2445delG p.Arg815SerfsX5	1 bp deletion (FS)	Unpublished data, Cardiff

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
T82.5			Ex35: c.6621_6625delGTGGA p.Gln2207HisfsX11	5 bp deletion (FS)	
T77.3	Ex12a: c.1783G > A p.Glu595Lys	Missense	Ex16: c.2446C > T p.Arg816X R	Nonsense	Unpublished data, Cardiff
T77.1			Ex29: c.5242C > T p.Arg1748X R	Nonsense	
T77.4			Ex31: c.5839C > T p.Arg1947X	Nonsense	
T141.4	Ex13: c.2233delA p.Ser745AlafsX2	1 bp deletion (FS)	Ex12b: c.1885G > A p.Gly629Arg	Missense	Unpublished data, Cardiff
T141.13			Ex30: c.5731delT p.Ser1911LeufsX9 R	2 bp deletion (FS)	
T133	Ex16: c.2446C > T p.Arg816X	Nonsense	Ex31: c.5897dupAC p.Glu1966HisfsX25	2 bp duplication (FS)	Unpublished data, Cardiff
T137			Ex31: c.5898dupAC p.Glu1966HisfsX25	2 bp duplication (FS)	
T437	Ex17: c.2875C > T p.Gln959X	Nonsense	Ex2: c.67A >T p.Ile23Leu	Missense	4
T441			Ex4b: c.586G > T p.Glu196X	Nonsense	
T459			Ex10c: c.1641+2T > G	Splice site	
T433			Ex10c: c.1660C > G p.Gln554Glu	Missense	
T469			Ex12a: c.1724delCACA p.Ser575X	4 bp deletion (FS)	
T468			Ex13: c.2041C > T p.Arg681X	Nonsense	
T472			Ex13: c.2088G > A p.Trp696X	Nonsense	

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
T463			Ex16: c.2410-3T > G	Splice site	
T451			Ex20: c.3449C > T p.Ser1150Leu	Missense	
T456			Ex22: c.3709-2A > G	Splice site	
T450			Ex23.2: c.4084C > T p.Arg1362X R	Nonsense	
T442			Ex27b: c.4687_4691del5 p.Phe1563GlyfsX36	5 bp deletion (FS)	
T443			Ex27b: c.4693insG p.Ala1565GlyfsX35	1 bp insertion (FS)	
T467			Ex29: c.5380C > T p.Gln1794X	Nonsense	
T457			Ex34: c.6448A > T p.Lys2150X	Nonsense	
T471			Ex38: c.6895delG p.Val2299TrpfsX8	1 bp deletion (FS)	
T434			Ex44: c.7699C > T p.Gln2567X	Nonsense	
T435			Ex44: c.7702C > T p.Gln2568X	Nonsense	
T460			Ex46: c.7924delT p.Ser2642LeufsX16	1 bp deletion (FS)	
CSG6N	Ex21: c.3525_3526delAA p.Arg1176SerfsX18	2 bp deletion (FS)	Ex4c: c.587-8del6 Splicing effect?	Intronic deletion	6, 7
CSG13N			Ex9: c.1260 + 1G > A	Splice site	
CSG48N			Ex10c: c.1604A > G p.Gln535Arg	Missense	

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
CSG29N			Ex14: c.2266C > T p.Gln756X	Nonsense	
CSG33N			Ex16: c.2816delA p.Asn939IlefsX12	1 bp deletion (FS)	
CSG19N			Ex17: c.2928del13 p.Glu977AsnfsX3	13 bp deletion (FS)	
CSG26N			Ex26: c.4514 + 1G > C	Splice site	
CSG44N			Ex31: c.5774delT p.Leu1925TrpfsX4	1 bp deletion (FS)	
CSG8N			Ex33: c.6292_6322del31 p.Arg2098PhefsX21	31 bp deletion (FS)	
CSG30N			Ex45: c.7908-2A >T	Splice site	
NF482-UHG B	Ex21: c.3525_3526delAA p.Arg1176SerfsX18	2 bp deletion (FS)	Ex4a: c.359_375del17 p.Phe120X	17 bp deletion (FS)	3
NF482-UHG C			Ex4c: c.603_621del19 p.Phe201fsX4	19 bp deletion (FS)	
NF482-UHG A			Ex8: c.1185 + 1G > A	Splice site	
NF482-UHG D			Ex14: c.2252-30_2252-6del**??**insT	Indel (FS?)	
T191.5	Ex22: c.3721C > T p.Arg1241X	Nonsense	Ex4b: c.505_524del20 p.Glu169X	20 bp deletion (FS)	Unpublished data, Cardiff
T191.9			Ex10b: c.1417delA p.Thr473GlnfsX24	1 bp deletion (FS)	
T191.1			Ex18: c.2991 + 1 G > A	Splice site	
T191.2			Ex22: c.3721C > T p.Arg1241X R	Nonsense	

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
T175.1	Ex23.2: c.4084C > T p.Arg1362X	Nonsense	Ex12a: c.1738insT p.Tyr580LeufsX7 R	1 bp insertion (FS)	Unpublished data, Cardiff
T175.2			Ex31: c.5817C > A p.Cys1939X R	Nonsense	
T209.1ii	Ex28: c.4950C > A p.Tyr1650X	Nonsense	Ex7: c.1062 + 1G > A R	Splice site	Unpublished data, Cardiff
T209.7			Ex10a: c.1318C > T p.Arg440X R	Nonsense	
T209.8			Ex15: c. 2326G > A p. Ala776Thr R	Missense? / splicing?	
T209.5			Ex25: c.4345delA p.Ser1449AlafsX12	1 bp deletion (FS)	
T209.6			Ex37: c.6790_6806del17 p.Tyr2264AspfsX8	17 bp deletion (FS)	
T506.5	Ex36: c.6756 + 2T > G	Splice site	Ex4b: c.480delG p.Arg160SerfsX5	1 bp deletion (FS)	4
T506.2			Ex6: c.731_732delAA p.Glu244ValfsX5	2 bp deletion (FS)	
T506.4			Ex17: c.2987insAC p.Val996AspfsX17	2 bp insertion (FS)	
T506.8			Ex19b: c.3306insA p.Phe1103IlefsX2	1 bp insertion (FS)	
T506.1			Ex22: c.3745_3764del20 p.Ser1249ThrfsX7	20 bp deletion (FS)	
T506.9			Ex33: c.6364del114 p.Glu2122 (through splice site)	114 bp deletion (FS)	
T506.6			Ex40: c.7127-3T > G	Splice site	

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
T106.3	Ex37: c.6791insA p.Tyr2264Xfs	1 bp insertion (FS)	Ex13: c.2033delC p.Pro678GlnfsX9 R	1 bp deletion (FS)	Unpublished data, Cardiff
T106.4			Ex26: c.4374_4375delCC p.Leu1459X R	2 bp deletion (FS)	
T175.1	Ex37: c.6792C > G p.Tyr2264X	Recurrent nonsense mutation that causes a splicing defect	Ex12a: c.1738insT p.Tyr580LeufsX7 R	1 bp insertion (FS)	Unpublished data, Cardiff
T143.2			Ex19a: c.3124delGTAGinsAT p.Val1042IlefsX16	Indel (FS)	
T143.13			Ex30: c.5731delT p.Ser1911LeufsX9 R	1 bp deletion (FS)	
T175.2A			Ex31: c.5817C > A p.Cys1939X R	Nonsense	
T541.3	Ex40: c.7127_7258del132 p.Gly2376. Is this a complete exon 40 deletion??	132 bp In-frame deletion (FS) Complete exon 40 deletion ??	Ex12b: c.1888delG p.Val630X R	1 bp deletion (FS)	Unpublished data, Cardiff
T541.1			Ex27b: c.4743insG p.Asp1582GlufsX18	1 bp insertion (FS)	
T536B			Ex40: c.7169delG p.Arg2390LysfsX6	1 bp deletion (FS)	
T210.1	Ex42: c.7458delC p.Tyr2487Ilefs	1 bp deletion (FS)	Ex7: c.1062 + 1G > A R	Splice site	Unpublished data, Cardiff
T210.6			Ex22: c.3870 + 2T > A	Splice site	
T181.3	E6-27b: Partial deletion of gene 90 kb	Partial gene deletion	Ex3: c.227insG p.Glu76GlyfsX30	1 bp insertion (FS)	Unpublished data, Cardiff

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
T211.2			Ex7: c.910C > T p.Arg304X R	Nonsense	
T211.3			Ex17: c 2855T > A p.Leu952X	Nonsense	
T34.1			Ex23.2: c 4108C > T p.Gln1370X	Nonsense	
T150.2			Ex34: c.6410delT p.Leu2137TyrfsX40	1 bp deletion (FS)	
T181.1			Ex34: c.6409_6410delTT p.Leu2137ThrfsX19	2 bp deletion (FS)	
T198			Ex42: c.7449delT p.Ala2484GlnfsX18	1 bp deletion (FS)	
C176_3	NF1 microdeletion	Genomic deletion	Ex4a: c.479 + 1G > A	Splice site	2
C174			Ex15: c.2326- ?_2409 Complete exon 15 deletion ?	Exon deletion?	
C186			Ex17: c.2990 + 1G > A R	Splice site	
C176_1			Ex28: c.4812C > G p.Tyr1604X R	Nonsense	
C176_2			Ex31: c.5927G > A p.Trp1976X R	Nonsense	
L-001 D	NF1 microdeletion	Genomic deletion	Ex4a: c.396_403del8 p.Leu134PhefsX21	8 bp deletion (FS)	3
L-001 B			Ex19a: c.3189T > A p.Cys1063X	Nonsense	
L-001 E			Ex22: c.3774G > A p.Trp1258X	Nonsense	

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
L-001 C			Ex23.2: c.4086_4092del7 p.Arg1362AlafsX20	7 bp deletion (FS)	
L-001 A			Ex28: c.5026_5032del7 p.Leu1676Alafs10	17 bp deletion (FS)	
NF96-1 E	NF1 microdeletion	Genomic deletion	Ex13: c.2050C > T p.Glu684X	Nonsense	3
NF96-1 B			Ex20: c.3330delT p.Phe1110LeufsX2	1 bp deletion (FS)	
NF96-1 A			Ex41: c.7394 + 1G > A	Splice site	
NF96-1 C			Ex42: c.7438delG p.Glu2480LysfsX22	1 bp deletion (FS)	
NF339-UHG B	NF1 microdeletion		Ex3: c.288 + 2T > G	Splice site	3
NF339-UHG C			Ex7: c.1007G > A p.Trp336X	Nonsense	
NF339-UHG D			Ex15: c.2409 + 1G > A	Splice site	
NF339-UHG A			Ex27b: c.4697T > A p.Leu1566X	Nonsense	
T49.2	Ex1-42: gene deletion	E1-42: gene deletion	Ex8: c.1177C > G p.His393Asp	Missense	Unpublished data, Cardiff
T49.8			Ex8: c.1178A > T p.His393Leu	Missense	
T49.1			Ex8: c.1181_1182delTT p.Phe394X	2 bp deletion (FS)	
T49.5			Ex16: c.2446C > T p.Arg816X R	Nonsense	

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
T49.7			Ex17: c.2953C > T p.Gln985X	Nonsense	
T49.3			Ex24: c.4114_4115delGT p.Val1372X	2 bp deletion (FS)	
T51.3	Whole gene deletion	Genomic deletion	Ex7: c.1062 + 1G > A R	Splice site	Unpublished data, Cardiff
T51.6			Ex8: c.1179_1180delCT p.Phe394LeufsX18	2 bp deletion (FS)	
T51.5			Ex11: c.1645_1646delCT p.Leu549AlafsX1	2 bp deletion (FS)	
T51.4			Ex16: c.2464G > T p.Gly822X	Nonsense	
T51.7			Ex41: c.7285C > T p.Arg2429X R	Nonsense	
T176.3	Large deletion	Genomic deletion	Ex23.2: c.4110 + 1G > C	Splice site	Unpublished data, Cardiff
T176.1			Ex28: c.4812C > G p.Tyr1604X R	Nonsense	
T176.2			Ex31: c.5928G > A p.Trp1976X R	Nonsense	
T217	Ex1: c.61-1G > C	Splice site	Ex12b: c.1900_1907del8 p.Ile634X	8 bp deletion (FS)	Unpublished data, Cardiff
T1440	Ex3: c.264_267delTACA p.Thr89Trpfs	4 bp deletion (FS)	Ex3: c.271G > A p.Glu91Lys	Missense	Unpublished data, Cardiff
T183.1	Ex4a: c.373delGinsATGTGT p.Arg125fs	Indel (FS)	Ex42: c.7449_7458del10 p.Leu2483IlefsX15	10 bp deletion (FS)	Unpublished data, Cardiff
T139	Ex4a: c.434_435delTC p.Leu145GlufsX19	2 bp deletion (FS)	Ex27a: c.4637C > G p.Ser1546X	Nonsense	Unpublished data, Cardiff

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
T108.12	Ex7: c.889-2A > G	Splice site	Ex7: c.910C > T p.Arg304X R	Nonsense	25
T199.1	Ex7: c.983_984delGT p.Cys328Xfs	2 bp deletion (FS)	Ex4b: c.528T > A p.Asp176Glu	Missense	Unpublished data, Cardiff
T374.5	Ex10a: c.1318C > T p.Arg440X	Nonsense	Ex23.1: c.3916C > T p.Arg1306X R	Nonsense	Unpublished data, Cardiff
T996	Ex10b: c.1393-32T > C	Splice site	Ex6: c.731-11 T > G	Splice site	Unpublished data, Cardiff
T227.3	Ex10b: c.1423insC p.Leu475ProfsX9	1 bp insertion (FS)	Ex15: c.2326-12C > T	Splice site	Unpublished data, Cardiff
T161.4	Ex10b: c.1466A > G p.Tyr489Cys	Missense	Ex17: c.2990 + 1G > A R	Splice site	Unpublished data, Cardiff
T161.3	Ex10b: c.1466A > G p.Tyr489Cys	Missense	Ex22 : c.3721insC p.Arg1241ProfsX7	1 bp insertion (FS)	Unpublished data, Cardiff
T214	Ex10b complete exon deletion	Single exon deletion	Ex22: c.3826C > T p.Arg1276X	Nonsense	Unpublished data, Cardiff
CLJ8N	Ex13: c.2041C > T p.Arg681X	Nonsense	Ex13: c.2246C > G p.Ser749X	Nonsense	6, 7
T170.1A	Ex13: c.2041C > T p.Arg681X	Nonsense	Ex12a: c.1797G > A p.Trp599X	Nonsense	Unpublished data, Cardiff
T1243	Ex13: c.2197_2214del17 p.Pro733fs	17 bp deletion (FS)	Ex36: c.6709C > T p.Arg2237X	Nonsense	Unpublished data, Cardiff
NF253-UHG D	Ex16: c.2850 + 2A > G	Splice site	Ex11: c.1663_1666delTTAG p.Leu555IlefsX12	4 bp deletion (FS)	3
T193	Ex17: c.2870delA p.Asp957Ilefs	1 bp deletion (FS)	Ex10a: c.1312G > T p.Glu438X	Nonsense	Unpublished data, Cardiff
L-004 D	Ex18: c.3113G > A p.Arg1038Lys	Missense	Ex27b: c.4729delA p.Thr1577LeufsX23	1 bp deletion (FS)	3
HT1359.2	Ex18: c.3113 + 1G > A	Splice site	Ex10a: c.1277G > A p.Trp426X R	Nonsense	Unpublished data, Cardiff

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
T140.4	Ex22: c.3732delT p.Thr1245LeufsX21	1 bp deletion (FS)	Ex41: c.7285C > T p.Arg2429X R	Nonsense	25
T37.1	Ex23.2: c.4084C > T p.Arg1362X	Nonsense	Ex10b : c.1467T > G p.Tyr489X	Nonsense	Unpublished data, Cardiff
T205.1	Ex24: c.4196C > A p.Ser1399X	Nonsense	Ex27a: c.4537C > T p.Arg1513X R	Nonsense	Unpublished data, Cardiff
T450.3	Ex27a: c.4537C > T p.Arg1513X	Nonsense	Ex4b: c.574C > T p.Arg192X R	Nonsense	Unpublished data, Cardiff
T209.8	Ex:28: c.4950 C > G p.Tyr1650X	Nonsense	Ex10a: c.1318 C > T p.Arg440X R	Nonsense	Unpublished data, Cardiff
NF116-UHG A	Ex28: c.5122insG p.Ala1708GlyfsX27	1 bp insertion (FS)	Ex27a: c.4537C > T p.Arg1513X R	Nonsense	3
T1308	Ex29: c.5546 + 19 T > A	Splice site	Ex22: c.3827G > A p.Arg1276Gln	Missense	Unpublished data, Cardiff
T149.5C	Ex34: c.6512delATGAGAGAinsC p.Tyr2171fs	Indel (FS)	Ex7: c.988G > A p.Ala330Thr	Missense	Unpublished data, Cardiff
T89.1	Ex37: c.6789_6792delTTAC p.Asp2264ThrfsX5	4 bp deletion (FS)	Ex12b: c.1888delG p.Val630X R	1 bp deletion (FS)	25
T106.1	Ex37: c.6791insA p.Tyr2264XfsX1	1 bp insertion (FS)	Ex13: c.2033delC p.Pro678GlnfsX9 R	1 bp deletion (FS)	25
L-004 B	Ex37: c.6791insA p.Tyr2264XfsX1	1 bp insertion (FS)	Ex23.1: c.3871_3974del103 Complete exon 23.1 deletion ?	103 bp deletion (FS)	3
T1200	Ex37: c.6791insA p.Tyr2264XfsX1	1 bp insertion (FS)	Ex16: c.2825G > T p.Ser942Ile	Missense	Unpublished data, Cardiff
CLO1N	Ex37: c.6792C > A p.Tyr2264X	Nonsense	mRNA study: Exon 4c skipped	Splice site?	6, 7

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
T1229	Ex39: c.7049_7064del16 p.Cys2350PhefsX19	16 bp deletion (FS)	Ex13: c.2203T > C p.Tyr735His	Missense	Unpublished data, Cardiff
T164.1E	Ex41: c.7285C > T p.Arg2429X	Nonsense	Ex23.2: c.4084C > T p.Arg1362X R	Nonsense	Unpublished data, Cardiff
T157.1A	Ex45: c.7907 + 3A > T	Splice site	Ex20: c.3492delC p.Ile1165SerfsX2	1 bp deletion (FS)	Unpublished data, Cardiff
T98.6	1.5Mb deletion	Genomic deletion	Ex34: c.6387A > C p.Arg2129Ser	Missense	25
T98	Complete gene deletion	Genomic deletion	Ex20: c.3457_3460del4 p.Leu1153MetfsX3	4 bp deletion (FS)	Unpublished data, Cardiff
T158.1	Complete gene deletion	Genomic deletion	Ex18: c.3058delG p.Glu1020LysfsX2 R	1 bp deletion (FS)	Unpublished data, Cardiff
CCF1N	Complete gene deletion	Genomic deletion	mRNA study: exons 12a and 12b skipped	Splice site?	5, 6
UWA128-3	NI	NI	Ex4b: c.543_546delGTAT p.Tyr182SerfsX7	4 bp deletion (FS)	38
T219.1	NI	NI	Ex9: c.1225_1226delGT p.Val409AlafsX18	2 bp deletion (FS)	Unpublished data, Cardiff
T116	NI	NI	Ex10c: c.1541_1542delAG p.Gln514ArgfsX43	2 bp deletion (FS)	25
T198.1	NI	NI	Ex10c: c.1555C > T p.Gln519X	Nonsense	Unpublished data, Cardiff
T128.17	NI	NI	Ex10c: c.1556A > C p.Gln519Pro R	Missense	25
T198.2	NI	NI	Ex12a: c.1792A > T p.Lys598X	Nonsense	Unpublished data, Cardiff
T63.2	NI	NI	Ex13: c.2088delG p.Trp696X	1 bp deletion (FS)	25

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
T146.5	NI	NI	Ex15: c.2326G > A p.Ala776Thr R	Missense/ splicing?	Unpublished data, Cardiff
T63.8	NI	NI	Ex15: c.2341_2358del18 p.His781Ala (in-frame)	18 bp deletion (in-frame)	25
T1265.2	NI	NI	Ex17: c.2851-16T > C	Splice site	Unpublished data, Cardiff
T233.1	NI	NI	Ex17: c.2879del38 p.Phe960X	38 bp deletion (FS)	
T158.2	NI	NI	Ex18: c.3058delG p.Glu1020LysfsX2 R	1 bp deletion (FS)	
T158.4	NI	NI	Ex18: c.3058delG p.Glu1020LysfsX2 R	1 bp deletion (FS)	
T192.1	NI	NI	Ex18: c.3113 + 1G > A R	Splice site	
T192.2	NI	NI	Ex18: c.3113 + 1G > A R	Splice site	
NF260-1	NI	NI	Ex22: c.3721C > T p.Arg1241X R	Nonsense	8
38	NI	NI	Ex22: c.3727_3728delCT p.Leu1243GlyfsX5	2 bp deletion (FS)	18
T94	NI	NI	Ex23.2: c.4083insT p.Arg1362SerfsX12	1 bp insertion (FS)	25
T565	NI	NI	Ex25: c. 4270-2A > G	Splice site	Unpublished data, Cardiff
T106.3	NI	NI	Ex26: c.4374_4375delCC p.Asp1460X R	2 bp deletion (FS)	25
T81.1	NI	NI	Ex27b: c.4662-5C > T	Splice site	25
T1284.5	NI	NI	Ex27b: c.4772 + 5G > A	Splice site	Unpublished data, Cardiff

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
20	NI	NI	Ex33: c.6253_6354 + 5del117 p.Val2085 (through splice site)	17 bp deletion (FS)	18
44	NI	NI	Ex40: c.7127-44_7174del92 p.Gly2376ValfsX8	92 bp deletion (FS)	18
PNFs					
45	Ex3: c.264_267delTACA p.Thr89TrpfsX8	4 bp deletion (FS)	Ex3: c.271G > A p.Glu91Lys	Missense	10
T399	Ex3: c.264_267delTACA p.Thr89TrpfsX8	4 bp deletion (FS)	Ex3: c.271G > T p.Glu91X	Nonsense	Unpublished data, Cardiff
T7	Ex4a: c.479 + 1G > A	Splice site	Ex16: c.2446C > T p.Arg816X R	Nonsense	39
19 UK	Ex7: c.910C > T p.Arg304X	Nonsense	Ex8: c.1177_1178delCA p.His393LeufsX16	2 bp deletion (FS)	Unpublished data, Cardiff
c3 UK	Ex8: c.1063-2A > G	Splice site	Ex7: c.910C > T p.Arg304X R	Nonsense	
14b	Ex13: c.2076C > G p.Tyr692X	Nonsense	Ex4b: c.532_558del27 p.Glu178 R	27 bp deletion (in-frame)	
T318	Ex13: c.2076C > G p.Tyr692X	Nonsense	Ex4b: c.532_558del27 p.Glu178 R	27 bp deletion (in-frame)	
T381.1	E18: c.3113 + 1G > A	Splice site	Ex10a: c.1277G > A p.Trp426X R	Nonsense	
T381.2			Ex18: c.3113 + 1G > A R	Splice site	
31	Ex29: c.5234C > G p.Ser1745X	Nonsense	Ex9: c.1246C > T p.Arg416X	Nonsense	
c4 UK	Ex33: c.6289_6290insA p.Leu2097fsX2	1 bp insertion (FS)	Ex27b: c.4706T > G p.Leu1569X R	Nonsense	
T155	Ex33: c.6291insA p.Leu2097XfsX9	1 bp insertion (FS)	Ex27b: c.4706T > G p.Leu1569X R	Nonsense	

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
24	Complete gene deletion	Genomic deletion	Ex4b: c.528T > A p.Asp176Glu	Missense	Unpublished data, Cardiff
T323	Complete gene deletion (1.4Mb ?)	Genomic deletion	Ex26: c.4501_4502delCT p.Leu1501PhefsX7 R	2 bp deletion (FS)	
T369	Complete gene deletion (1.4Mb ?)	Genomic deletion	Ex26: c.4501_4502delCT p.Leu1501PhefsX7 R	2 bp deletion (FS)	
c2 UK	NI	NI	Ex23.2: c.4083insT p.Arg1362SerfsX12	1 bp insertion (FS)	Unpublished data, Cardiff
42	NI	NI	Ex27a: c.4515-2A > G	Splice site	
T329 ?	NI	NI	Ex7: c.952_953delGA p.Glu318LysfsX11	2 bp deletion (FS)	
Spinal neurofibromas					
1	Ex7: c.899T > C p.Leu300Pro	Missense	Ex24: c.4111-2A > G	Splice site	16
13	1.4 Mb deletion	Genomic deletion	Ex21_22 splice site mutation?	Splice site?	
6	1.4 Mb deletion	Genomic deletion	Ex27b: c.4690A > G p.Lys1564Glu	Missense	
MPNSTs					
53	Ex4b: c.574C > T p.Arg192X	Nonsense	Ex24: c.4203insT p.Glu1402X	1 bp insertion (FS)	18
T168	Ex5: c.663G > A p.Trp221X	Nonsense	Ex34: c.6444delA p.Val2149SerfsX28	1 bp deletion (FS)	
T185	Ex6: c.773delA p.Ser259AlafsX21	1 bp deletion (FS)	Ex34: c.6410delT p.Leu2137TyrfsX41	1 bp deletion (FS)	
37	Ex16: c.2446C > T p.Arg816X	Nonsense	Ex6: c.731-5_741del19 through a splice site	19 bp deletion (FS)	

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
17	Ex20: c.3457_3460delCTCA p.Leu1153MetfsX4	2 bp deletion (FS)	Ex31: c.5789delC p.Pro1930HisfX6	1 bp deletion (FS)	17
20	1.4Mb deletion	Genomic deletion	Ex10c: c.1532delC p.Pro511GlnfsX14	1 bp deletion (FS)	17
44	Complete gene deletion	Genomic deletion	Ex16: c.2446C > T p.Arg816X R	Nonsense	18
T184	Segmental NF NI	NI	Ex27a: c.4580_4590del11 p.Pro1527GlnfsX11 R	11 bp deletion (FS)	18
11	NI	NI	Ex27a: c.4580_4590del11 p.Pro1527GlnfsX11 R	11 bp deletion (FS)	17
38	NI	NI	Ex12a: c.1831delCinsTT p.Leu611PhefsX3	Indel (FS)	18
GISTs					
NF1-1a	Ex24: c.4269 + 1G > T	Splice site	Ex29: c.5546 + 2T > A	Splice site	3
NF1-1b			Ex29: c.5242C > T p.Arg1748X R	Nonsense	
NF1-2a	Ex37: c.6791insA p.Tyr2264X	1bp insertion (FS)	Ex3: c.279T > A p.Cys93X	Nonsense	3
NF1-2c			Ex10c: c. del21	21 bp in-frame deletion	
NF1-2b			Ex45: c.7846C > T p.Arg2616X	Nonsense	

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
JMML					
D127	Ex14: c.2288_2295dupTGAGGCGC / Ex20: c.3366delT	Compound heterozygous *NF1* mutations found in blood cells	Ex14: c.2288_2295dupTGAGGCGC / Ex20: c.3366delT	Compound heterozygous *NF1* mutations found in blood cells	31
CZ051	Ex12a: c.1748A > G p.Lys583Arg / Ex13: c.2027delC p.T676TfsX11		Ex12a: c.1748A > G p.Lys583Arg / Ex13: c.2027delC p.T676TfsX11		
D530	Ex6: c.821T > G p.Leu274Arg / Ex34: c.6579 + 1G > C	With no other tissue analysed, unable to differentiate germline from somatic *NF1* mutations	Ex6: c.821T > G p.L274R / Ex34: c.6579 + 1G > C	With no other tissue analysed, unable to differentiate germline from somatic *NF1* mutations	32
SC049	Ex3: c.205-2A > G / Ex23.2: c.4084C > T p.Arg1362X		Ex3: c.205-2A > G / Ex23.2: c.4084C > T p.R1362X		
SCO87	Ex4b: c.482T > G p.Leu161X / Ex4b: c.495_498delTGTT p.T165TfsX11		Ex4b: c.482T > G p.L161X / Ex4b: c.495_498delTGTT p.T165TfsX11		
D252	NI	NI	Ex29: c.5242C > T p.Arg1748X R	Nonsense	
Glomus tumours					
NF1-G8	Ex4a: c.311T > G p.Leu104X	Nonsense	Ex44: c.7727C > A p.Ser2576X	Nonsense	36

Continued

Table S2. Continued

Patient ID	Germline point mutation	Type of germline mutation	Somatic point mutation	Effect of somatic mutation	Source
NF1-G3	Ex16: c.2546insG p.Val850SerfsX15	1 bp insertion (FS)	Ex29: c.5539_5546dup8 p.Ser1850ValfsX15	8 bp duplication (FS)	
NF1-G5	Ex27a: c.4515-2A > T	Splice site	Ex18: c.3113 + 1G > C	Splice site	
NF1-G1	mRNA study: Exon 29 partially skipped	Splice site?	Ex4a: c.403delC p.Arg135GlyfsX30	1 bp deletion (FS)	
NF1-G10a	Ex37: c.6789_6792delTTAC p.Tyr2264AspfsX5	4 bp deletion (FS)	Ex2: c.204 + 1G > A	Splice site	
NF1-G10b			Ex43: c.7600_7621del22 p.Lys2534GlyfsX8	22 bp deletion (FS)	
ACs					
	No *NF1* somatic mutations identified		No *NF1* somatic mutations identified		
Gastric carcinoid tumours					
	No *NF1* somatic mutations identified		No *NF1* somatic mutations identified		
PCs					
	No *NF1* somatic mutations identified		No *NF1* somatic mutations identified		

FS, frame shift; NI, no information; R, recurrent.

Supplementary Table References

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