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The molecular pathology of tumours of the ear and temporal bone

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Introduction

Clinical decision making in skull base tumour surgery has never been straightforward. Management requires not only a precise knowledge of tumour anatomy and the complications of surgical removal, but also of the natural history, since many run a relatively benign and indolent course. To add to this is an increasing awareness of the importance of 'the other side' and this requires a basic understanding of cancer genetics. The occurrence of bilateral or multiple ear and temporal bone tumours is now well recognized in a variety of syndromes and these are considered in detail in this review.

Neurofibromatosis type 2 (NF2)

The NF2 gene

The neurofibromatoses consist of two clinically and genetically distinct, dominantly inherited disorders neurofibromatosis type 1 (NF1) and NF2. NF2 results from the inheritance of a mutation in a gene on chromosome 22. It has a birth incidence of one in 30–50 000 and the mutation rate within the NF2 gene is estimated at 6.5×10^{-6} (Evans *et al.*, 1992a). In approximately 50 per cent of cases of NF2 no family history can be obtained and these are believed to be a result of new germline mutations (Evans *et al.*, 1992a)

Cytogenetic studies of meningiomas first pointed to chromosome 22 as the likely site of the NF2 gene (Zang, 1982). The NF2 gene was subsequently mapped to chromosome 22 by both linkage and loss of heterozygosity analysis (Seizinger *et al.*, 1986; Rouleau *et al.*, 1987). In 1993 two groups independently isolated identical candidate NF2 genes Rouleau *et al.*, 1993; Troffater *et al.*, 1993).

The NF2 gene occupies approximately 100kb on chromosome 22q12.2. Sequence analysis reveals an open reading frame of 1785bp which encodes a protein of 595 amino acids, designated merlin or schwannomin (Rouleau *et al.*, 1993; Troffater *et al.*, 1993). The gene protein product is similar in sequence to a family of proteins including moesin, ezrin, radixin, talin and members of the protein 4.1 super-family. These proteins are involved in linking cytoskeletal components with the plasma membrane and are located in actin-rich surface projections such as microvilli.

The N-terminal region of the NF2 protein is thought to interact with components of the plasma membrane and the C-terminal with the cytoskeleton. The NF2 protein is expressed mainly in the nervous system and cells of the lens and is predominantly located in membrane ruffles and cellular protrusions (Gonzalez-Agosti *et al.*, 1996; Vaheri *et al.*, 1997).

Preliminary evidence has shown that NF2 protein over-expression can inhibit cell growth, and induces cell surface protrusions and elongation of cells (Lutchman & Rouleau, 1995; Vaheri *et al.*, 1997). To test more directly whether the gene product can function as a tumour suppressor, Tikoo *et al.* (1994) was able to reverse the malignant phenotype in *v-Ha-Ras*-transformed NIH 3T3 cells by the introduction of NF2 protein. The exact function of the NF2 protein is as yet unknown. The evidence suggests that it is involved in cell-cell or cell-matrix interactions and that it is important for cell movement, shape or communication. Its loss of function could possibly result in a loss of contact inhibition, a characteristic feature of tumour cells.

Clinical features

The mean age of onset of symptoms in NF2 is 20–22 years with almost complete penetrance by the age of 60 (Evans et al., 1992a; Parry *et al.*, 1994). Bilateral and familial vestibular schwannomas are the hallmark of NF2 and the other characteristic features are listed in Table I.

NF2 is a clinically heterogeneous condition that manifests at least two distinct forms, and has been divided into severe (Wishart) and mild (Gardner) subtypes (Figures 1, 2 and 3). This is a clinical

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TABLE I

CLINICAL FEATURES OF NF1 AND NF2. THE FREQUENCY OF CLINICAL FEATURES FOR NF2 IS FROM DATA ON 183 NF2 CASES (EVANS *et al.*, 1992, PARRY *et al.*, 1994)

	NF2		NF1
Incidence	1 in 30–50 000	%	1 in 3 500
Clinical features	Vestibular schwannoma	99	Café-au-lait (>6)
	Skin tumours	68	Axillary or inguinal freckling
	Cataracts	51	Skin neurofibromas
	Meningiomas	46	Iris hamartomas (Lisch nodules)
	Café au lait (<6)	44	Optic glioma
	Spinal tumours	35	Spinal and peripheral nerve
	Cranial N schwannoma	8	Neurofibroma
	Optic sheath meningioma	4	Skeletal dysplasia
	Retinal hamartoma	3	Macrocephaly
	Astrocytoma	3	Learning disabilities
	Ependymoma	3	
Chromosomal location	22q12.2		17q11.2

classification based on the age of onset, disease duration and number and type of tumours that develop. These subtypes tend to be consistent within families but with considerable interfamilial variations (Evans *et al.*, 1992a: Parry *et al.*, 1994).

Many cases however do not fit neatly into the 'mild' and 'severe' categories and further subdivisions have been proposed (Parry *et al.*, 1994). The possibility that some cases may represent segmental NF2, resulting from a mutation early in embryogenesis rather than in the germline has also been suggested. This has gained clinical support (Welling, 1998) and recently molecular genetic evidence, with the demonstration of somatic mosaicism in NF2 (Bourn *et al.*, 1994a; Bijlsma *et al*, 1997).

There is little variation in the clinical manifestations of NF2 between members of the same family but great differences exit between families. This suggests that there exists a relationship between phenotype and genotype and there is some molecular evidence to support this. Protein truncating mutations involving the NF2 gene are reported to be associated with the severe Wishart subtype, and a predominance of missense and splice site mutations are seen in the milder Gardner subtype (Merel *et al.*, 1995; Kluwe and Mautner, 1996; Parry *et al.*, 1996; Ruttledge *et al.*, 1996; Welling, 1998).

There is no evidence for locus heterogeneity in NF2 and it is anticipated that most, if not all cases of NF2 have mutations in the NF2 gene. However the rate of germline mutation detection is currently only 60 per cent (Bourn *et al.*, 1994b; Jacoby *et al.*, 1994; Merel *et al.*, 1995; Kluwe and Mautner, 1996).

It is estimated that half of all patients under age 20 presenting with a vestibular schwannoma have NF2. It has been suggested that all cases of vestibular schwannoma or meningioma under the age of 30; especially those with skin lesions; should be screened for the disease and followed up as at risk individuals. Currently a new NF2 family member should be followed by annual review with detailed history, neurological and slit lamp examination from the age of two to 30. Annual audiology should be introduced at age 10 and an MRI should be first done at age 16–18, if negative this is repeated again at age 30. If the risk is significantly reduced by genetic linkage or gene mutation analysis then this protocol can be altered and where NF2 excluded screening can be

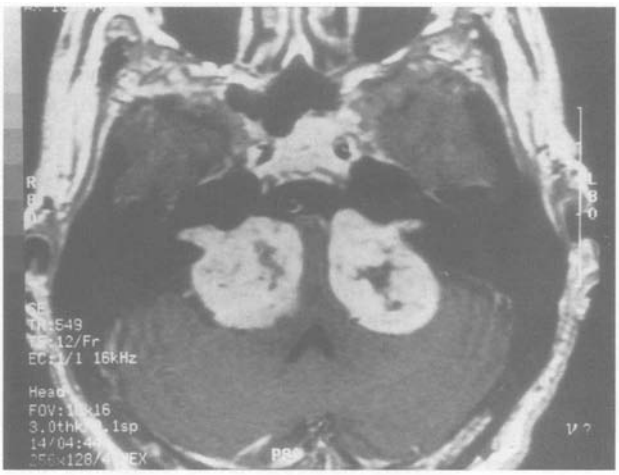

FIG. 1

A gadolinium enhanced axial T1 weighted MR scan of a case of NF2 with bilateral vestibular schwannomas and no other intracranial or spinal tumours. This represents the mild or Gardner subtype of NF2.

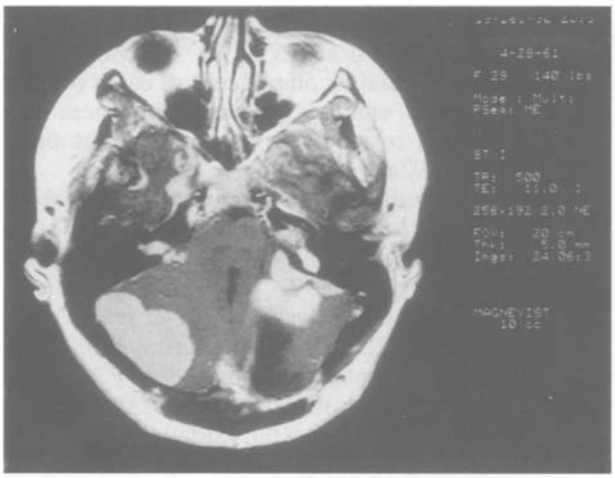

FIG. 2

A gadolinium enhanced axial T1 weighted MR scan of a case of NF2 of the severe or Wishart subtype. These are bilateral vestibular schwannomas and multiple meningiomas in the middle and posterior fossa.

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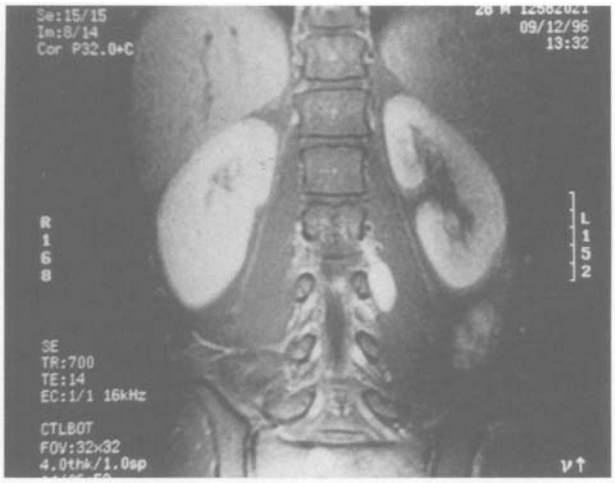

FIG. 3

A gadolinium enhanced coronal T1 weighted MR scan of the spine of an NF2 patient demonstrating multiple spinal tumours. This defines the case as a severe or Wishart subtype.

discontinued. Currently NF2 gene mutations are detected in only 60 per cent of individuals, so direct mutation analysis is not possible in all families. The alternative is to use linked markers but the results of these can be misleading as a number of mosaics have been detected making true risk assessment difficult (Bourn *et al.*, 1994a & b; Bijlsma *et al.*, 1997). If DNA analysis is inconclusive then follow-up should continue for at least five years after the onset of symptoms in the family (Evans *et al.*, 1992b).

Schwannoma

Schwannomas are nerve sheath tumours that can arise from any cranial or spinal nerve. The most common site of origin is the vestibular nerve. Vestibular schwannomas are typically benign tumours that occur at a frequency in excess of one in 100 000 per year, and will thus affect about one in 1000 people in their lifetime (Moffat *et al.*, 1995). Familial vestibular schwannomas represent approximately five per cent of cases and have only been reported in association with NF2.

A further variant is characterized by multiple spinal and cutaneous schwannomas but with no involvement of the vestibular nerve and no predisposition to meningiomas. This condition has been termed 'schwannomatosis', has an autosomal dominant inheritance and shows linkage to the NF2 gene (Evans *et al.*, 1997). Tumour tissue has demonstrated NF2 gene mutations in 75 per cent of tumours investigated, confirming that at least a proportion of 'schwannomatosis' cases are NF2 variants (Davis *et al.*, 1996). Further analysis of this condition may suggest possible genotypes that predispose to vestibular and non-vestibular tumours.

Prior to the isolation of the NF2 gene both cytogenetic and loss of heterozygosity studies of schwannomas were reported. These demonstrated that large-scale re-arrangements are common, and monosomy for chromosome 22 was the most frequent anomaly (Seiziner *et al.*, 1986; Ray *et al.*, 1987; Raggato and Cassartelli, 1989; Couturier *et al.*, 1990; Webb and Griffin, 1990; Stenman *et al.*, 1991; Bello *et al.*, 1993; Irving *et al.*, 1993). The subsequent isolation and cloning of the NF2 gene then allowed for more detailed analysis, and inactivation of the gene has been demonstrated in both familial and sporadic schwannomas (Rouleau *et al.*, 1993; Troffater *et al.*, 1993; Irving *et al.*, 1994; Welling, 1998).

The spectrum of intragenic mutations found in familial and sporadic tumours appear comparable, but the germline and somatic events are frequently dissimilar (Irving, 1996). Somatic mutations are more likely to represent large chromosome 22 deletions with the loss of one copy of the NF2 gene and splice site and missense mutations are comparatively rare. There are certain mutation 'hot spots' within the gene with the majority of reported mutations involving the first half of the gene, especially exon 2 (Irving, 1996). The frequent involvement of CpG dinucleotides has also been noted (Welling, 1998).

The available molecular genetic data has shown NF2 gene inactivation 'two hits' in 25 per cent of schwannomas and a single mutation in 50–55 per cent (Irving *et al.*, 1994; Jacoby *et al.*, 1994; Irving *et al.*, 1997a; Welling, 1998). The true figure is likely to be much higher, and Huynh *et al.* (1997) raised antibodies to the NF2 protein and demonstrated its absence in all of 29 schwannomas investigated. This suggests that the NF2 gene is inactivated in all schwannomas and is therefore a vital step in tumourigenesis.

The genotype phenotype relationship documented in NF2 might suggest a similar relationship in sporadic tumours between the NF2 gene event and behaviour. This has been investigated but so far no analogous relationship confirmed (Irving *et al.*, 1997a). Many tumours with no predicted active NF2 protein have clinical and proliferative features of a low growth potential (Irving *et al.*, 1997a). It seems likely that the NF2 gene is not the only determinant of tumourigenesis in schwannomas and other factors need to be taken into consideration. Other genes may be involved and loss of chromosomes seven, 10, 20 and Y are reported but their role in the pathogenesis of schwannomas is uncertain (Stenman *et al.*, 1991, Bell *et al.*, 1993).

Meningioma

Meningiomas account for 13–17 per cent of all intracranial neoplasms and three–12 per cent of IAC and CPA tumours (Moffat *et al.*, 1993). Hereditary meningiomas are a characteristic of a number of familial cancer syndromes although most of these occur in association with NF2. Meningiomas are observed in approximately 46 per cent of cases of NF2 (Table I) and females with NF2 have significantly more meningiomas than males (Evans *et al.*, 1995).

There are a number of reports of familial meningioma in the absence of NF2 and these do not appear to be due to the inheritance of mutations in the NF2 gene (Pulst *et al.*, 1993; Maxwell *et al.*,

1998). Meningiomas are also found in increased frequency in Werner syndrome (Goto *et al.*, 1996) and in Gorlin syndrome (Albrecht *et al.*, 1994).

Meningiomas are rare in children and adolescents and in such cases a thorough search for signs of NF2 should be undertaken. At least 20 per cent of adolescents with a meningioma have NF2 (Deen *et al.*, 1982). In addition all cases of multiple meningioma in any age group should have a diagnosis of NF2 excluded. The vast majority of meningiomas are sporadic tumours and like their hereditary counterpart they occur twice as frequently in females than males. Sporadic meningiomas show some molecular similarities with schwannomas. Both tumours demonstrate chromosome 22 deletions, monosomy for chromosome 22 being demonstrated in 61–80 per cent of sporadic meningiomas (Dumanski *et al.*, 1990; Harada *et al.*, 1996). NF2 gene mutations are detected in approximately 35 per cent of sporadic meningiomas (Harada *et al.*, 1996).

There is a relationship between histological type and NF2 gene inactivation, which has been shown to be higher in fibroblastic and transitional meningiomas as opposed to the meningothelial variant (Harada *et al.*, 1996; Wellenreuther *et al.*, 1997). The highest frequency is seen in anaplastic tumours with NF2 gene mutations detected in approximately 65 per cent (Harada *et al.*, 1996; Wellenreuther *et al.*, 1997).

Meningiomas represent a more diverse group of tumours than schwannomas. Schwannomas are invariably benign whereas five per cent of meningiomas exhibit features of malignancy. The molecular situation is more complicated with suggestions that other genes on chromosome 22 may be involved in the pathogenesis of meningioma. A recently cloned gene on chromosome 22 is a candidate meningioma locus (Lekanne Deprez *et al.*, 1995).

Mutations on other chromosomes are common in meningiomas and deletions in putative tumour suppressor genes on chromosomes one, six, nine, 10, 14, 17 and 18 have been documented (Doney *et al.*, Rempel *et al.*, 1993; Lindblom *et al.*, 1994; Weber *et al.*, 1997). Deletions of these genes appear to occur at a later stage in tumourigenesis, and in particular deletions on chromosomes 1p, 6q, 9p, 10q and 14q are associated with more aggressive atypical and malignant tumours (WHO grade II and III) (Simon *et al.*, 1995; Weber *et al.*, 1997).

NF2 gene inactivation appears to be a critical step in the development of schwannomas but this is not the case in meningiomas. Huynh *et al.* (1977) developed an antibody to the NF2 protein and detected this in three of 10 tumours suggesting that in these the NF2 gene is unaffected. This provides direct evidence that NF2 gene inactivation underlies the formation of approximately two-thirds of meningiomas.

The inactivation of the NF2 gene does not appear to influence tumour behaviour as meningiomas in NF2 are invariably benign. The NF2 gene is however the major gene implicated in the tumourigenesis of meningioma in particular the transitional and fibrous subtypes, with a significant proportion of meningiomas having normal NF2 gene function. Progression to a more aggressive tumour appears to be NF2 gene independent.

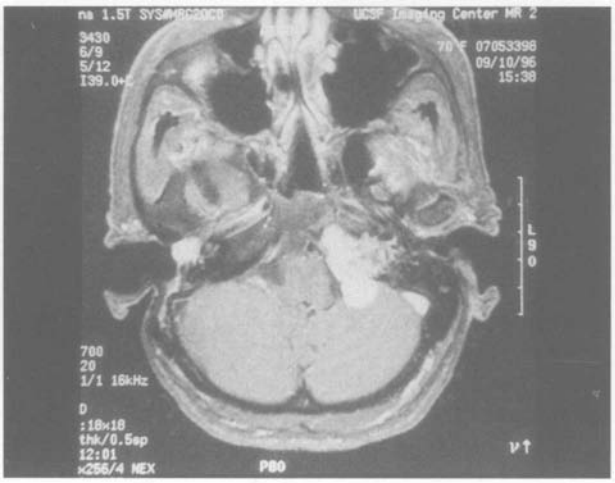

FIG. 4

A gadolinium enhanced axial T1 weighted MR scan demonstrating bilateral paragangliomas. On the left is a large jugulare with intracranial extension and on the right a recurrent glomus tympanicum tumour. This patient had no family history of similar tumours, however it is most likely that this represents a hereditary predisposition to paragangliomas.

Paraganglioma (glomus tumour)

Paragangliomas manifest as both a familial and non-familial tumour and although the true proportion of familial cases is unknown these may well represent the majority (McCaffrey *et al.*, 1994; Oosterwijk *et al.*, 1996). The true incidence of all paragangliomas is unknown but the lifetime incidence has been estimated at one in 30–100 000 (van Baars, 1982; Mariman *et al.*, 1995). There is a reported female preponderance with females being four to six times more frequency afflicted (Gulya, 1993).

Paragangliomas arise from neuroepithelioid cells of neural crest origin, and are mostly benign slow growing tumours. Hereditary paragangliomas are found predominantly in the head and neck region where they arise from glomus bodies. These are found in relation to the jugular foramen, carotid bifurcation, vagal ganglion and the middle ear (Figure 4). In a study of 38 patients from nine kindreds carotid body tumours were the commonest with 34 cases (90 per cent). Glomus jugulare or vagale tumours occurred in eight cases (21 per cent) (McCaffrey *et al.*, 1994).

Genetic linkage analysis has identified two paraganglioma (PGL) loci situated on chromosome 11, at 11q23 (PGL1) and 11q13.1 (PGL2). Linkage analysis in a large affected Dutch family initially mapped the PGL1 gene to chromosome 11q23-qter (Heutink *et al.*, 1992). The interval containing the gene was then further defined to the region 11q22.3–23.3 (Heutink *et al.*, 1994). Linkage to the PGL1 locus has also been established in three North American families (Baysal *et al.*, 1997). Anticipation in PGL1 has been

suggested but the evidence remains as yet inconclusive (Baysal *et al.*, 1997). Analysis of an unrelated Dutch pedigree has revealed the presence of linkage to a second locus also on the long arm of chromosome 11 at 11q13 (Mariman *et al.*, 1995).

All of the pedigrees investigated, both PGL1 and PGL2 linked, demonstrate autosomal dominant inheritance with maternal genomic imprinting (Heutink *et al.*, 1994; Mariman *et al.*, 1995; Baysal *et al.*, 1997). All affected individuals have inherited the disease gene from their father.

The exact mechanism of genomic imprinting at the molecular level is unknown. Both tumour suppressor and oncogene like roles have been proposed. The paraganglioma tumour gene could be a dominantly acting oncogene that is 'imprinted' and thus inactivated during female oogenesis resulting in unaffected offspring. The 'imprint' is then removed at male spermatogenesis leading to affected offspring in the following generation.

An alternative explanation is that the 'imprint' represents one of the two hits required for the inactivation of a tumour suppressor gene and loss of the normal maternally inherited gene is required for tumourigenesis (Heutink *et al.*, 1992; Mariman *et al.*, 1995). This second model is supported by tumour analysis that has demonstrated allelic loss on chromosome 11 of strictly maternal origin in five of 19 cases (Baysal *et al.*, 1997) and allelic imbalance in a further study (Devilee *et al.*, 1994). This would be consistent with the hypothesis that the tumours in the offspring remain hemizygous for the inherited paternal PGL1 mutant allele.

Genomic imprinting results in a familial syndrome that can, if the gene is passed down the maternal line, be nonpenetrant in several generations. Since glomus jugulare tumours were not clearly recognized before the 1950's this makes the diagnosis of a true sporadic tumour more difficult and some of these may in fact turn out to be hereditary. This may be especially true for the proportion of 'sporadic' tumours that are multifocal, a feature characteristic of the hereditary cancer syndromes. A thorough family history is needed before a case can be classified as a sporadic tumour.

DNA analysis based on flanking markers is now possible but is only currently useful in larger pedigrees where linkage to one of the two PGL loci can be established. In this complex disorder genetic counselling needs to take into consideration parent of origin, sex of the patient and DNA linkage (Oosterwijk *et al.*, 1996). Screening of at risk individuals is suggested every two years beginning age 16–18 with MR imaging (McCaffrey *et al.*, 1994). Head and neck paragangliomas are only rarely hormonally active but they can occasionally be associated with phaeochromocytomas. Additional screening for urinary catecholamines could be carried out and certainly should be performed if hypertension develops.

Von Hippel-Lindau Disease (VHL)

VHL is a rare hereditary cancer syndrome with an estimated birth incidence of one per 36-39 000 (Maher, 1994). It is the commonest cause of familial renal cell carcinoma. Other common associated tumours are retinal, cerebellar, medullary and spinal haemangioblastomas, phaeochromocytomas and pancreatic islet cell tumours. Cysts of the kidney, pancreas and epididymis are also occasionally observed (Maher, 1994).

Invasive temporal bone low-grade papillary adenocarcinomas have been reported in VHL. These may be bilateral and this occurrence has been observed exclusively in combination with VHL (Poe *et al.*, 1993; Pollak *et al.*, 1995; Kempermann *et al.*, 1996).

There is debate about the exact origin of these tumours and many authors believe that they originate from the endolymphatic sac (Poe *et al.*, 1993; Kempermann *et al.*, 1996). Pollak *et al.* (1995) describe a VHL case with bilateral tumours where one was removed that appeared to originate from the region of the jugular bulb and did not involve the lumen of the endolymphatic sac. Blamires *et al.* (1992) described a similar tumour with presumed origin from the choroid plexus in a VHL patient. VHL certainly predisposes to temporal bone papillary tumours, and many of these are bilateral. These may represent tumours of the endolymphatic sac, choroid plexus or originate from the mucosa of the pneumatic spaces of the temporal bone.

The gene for VHL was identified in 1993 on chromosome 3p (Latif *et al.*, 1993). Inactivation of both copies of the gene has been demonstrated in tumour tissue, suggesting that it acts as a tumour suppressor. Molecular genetic analysis has been carried out on tissue from only one papillary temporal bone tumour. This demonstrated an SSCP band shift, suggesting a mutation in exon 1 of the VHL gene (Tibbs *et al.*, 1997).

Genotype-phenotype correlation in VHL has shown that families with missense mutations have an increased incidence of phaeochromocytoma (Maher *et al.*, 1996). It appears therefore that certain mutations cause tumours in specific tissues, and ultimately it may be possible to predict from genetic testing alone which VHL patients are likely to develop temporal bone tumours.

Reports of papillary temporal bone tumours are uncommon but approximately 20 per cent of cases in the literature are associated with VHL. The true figure may be even higher (Blamires *et al.*, 1992; Poe *et al.*, 1993; Megerian *et al.*, 1995; Pollak *et al.*, 1995; Kempermann *et al.*, 1996; Tibbs *et al.*, 1997). VHL should therefore be excluded in any case of papillary temporal bone tumour.

Currently the diagnosis of VHL is based on the clinical finding of associated lesions. Either two haemangioblastomas, or a single haemangioblastoma in association with visceral involvement, or a single associated lesion in a family with VHL. Genetic analysis can be used to confirm the diagnosis in some families.

Neurofibromatosis type 1 (NF1)

Friedrick von Recklinghausen in 1882 described a clinical syndrome characterized by nerve derived tumours and this syndrome is now referred to as NF1. Characteristic clinical features are outlined in Table I. NF1 is one of the commonest human autosomal dominant diseases with an incidence of one in 3 500 (Colman and Wallace, 1994).

There is no particular predilection for ear or temporal bone lesions in NF1 but a number of cases of NF1 associated tumours involving the external auditory meatus have been described (Lustig and Jackler, 1996).

The gene for NF1 was isolated from chromosome 17q11.2 in 1990 (Viskochil *et al.*, 1990). The gene product protein 'neurofibromin' is thought to act as a negative regulator of $p21^{RAS}$ mediated signal transduction, and cellular transformation. Currently mutation detection has proved difficult in NF1 with most laboratories reporting a rate of only 10–15 per cent (Colman and Wallace, 1994). The diagnosis in the majority of cases remains based on clinical features (Table I).

Future prospects

The major contribution of molecular research so far has been in the clarification of the various hereditary cancer syndromes and a greater awareness of their manifestations amongst practitioners. The use of linked markers in affected families can detect at risk individuals and direct gene analysis has further refined this facilitating the extension of predictive testing to families where only one individual expresses the disease. Direct detection of mutations is also of value in patients in whom hereditary disease is suspected. For example, in individuals who present with a unilateral tumour at a young age, it may be possible to predict which are likely to go on to develop multiple or contralateral tumours.

The correlation of the molecular defects with tumour behaviour may allow for the identification of molecular markers of aggressivity. A genetic profile of a tumour, it is hoped, will provide a more accurate prognosis than current histopathological analysis. In vestibular schwannomas and paragangliomas this may ultimately identify those patients with slow growing or static tumours. A conservative approach could then be instituted in selected cases. In patients undergoing subtotal tumour removal, where the fate of the residual tumour is unpredictable, DNA analysis may provide predictive information on future rates of growth. Currently the methods for DNA mutation detection are costly and cumbersome, but it is hoped that improved methods including new 'chip based' DNA technologies may soon revolutionize analysis.

The future lies in working out the function of these disease genes and the intricacies of the pathways involved in growth control in specific tissues. The relationship between these and tumour behaviour may result in the identification of specific targets for novel therapeutic intervention. This will require ever-closer collaboration between clinicians and basic scientists.

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