109. Reynolds, JE, Fletcher JA, Lytle CH, Nie L, Morton CC, Diehl SR. Molecular characterization of a 17q11.2 translocation in a malignant schwannoma cell line. *Human Genet* 1992, **90**, 450–456.
110. Suzuki Y, Suzuki H, Takamasa K, Yoshimoto T, Shibahara S. Brain tumors predominantly express the neurofibromatosis type 1 gene transcripts containing the 63 base insert in the region coding for GTPase activating protein-related domain. *Biochem Biophys Res Commun* 1991, **181**, 955–961.
111. Uchida T, Matozaki T, Suzuki T, *et al.* Expression of two types of neurofibromatosis type 1 gene transcripts in gastric cancers and comparison of GAP activities. *Biochem Biophys Res Commun* 1992, **1**, 332–339.
112. Nur-E-Kamal MSA, Varga M, Maruta H. The GTPase-activating NF1 fragment of 91 amino acids reverses v-Ha-Ras-induced malignant phenotype. *J Biol Chem* 1993, **268**, 22331–22337.
113. Johnson MR, DeClue JE, Felzmann S, Vass WC, Xu G, White R, Lowy DR. Neurofibromin can inhibit ras-dependent growth by a mechanism independent of its GTPase-accelerating function. *Mol Cell Biol* 1994, **14**, 641–645.
114. Wiesmuller L, Wittinghofer A. Expression of the GTPase activating domain of the neurofibromatosis type 1 (*NF1*) gene in *E. Coli* and role of the conserved lysine residue. *J Biol Chem* 1992, **267**, 10207–10210.
115. Nakafuku M, Nagamine M, Ohtoshi A, Tanaka K, Toh-e A, Kaziro Y. Suppression of oncogenic ras by mutant neurofibromatosis type 1 genes with single amino acid substitutions. *Proc Natl Acad Sci USA* 1993, **90**, 6706–6710.
116. Poullet P, Lin B, Esson K, Tamanoi F. Functional significance of lysine 1423 of neurofibromin and characterization of a second site suppressor which rescues mutations at this residue and suppresses $ras2^{val-19}$-activated phenotypes. *Mol Cell Biol* 1994, **14**, 815–821.
117. Gutmann DH, Boguski M, Marchuk D, Wigler M, Collins FS, Ballester R. Analysis of neurofibromatosis type 1 (NF1) GAP-related domain by site-directed mutagenesis. *Oncogene* 1993, **8**, 761–769.

Pergamon

European Journal of Cancer Vol. 30A, No. 13, pp. 1981–1987, 1994

0959–8049/94 $7.00+0.00

0959-8049(94)00390-4

Neurofibromatosis Type 2

G. Thomas, P. Mérel, M. Sanson, K. Hoang-Xuan, J. Zucman, C. Desmaze, T. Melot, A. Aurias and O. Delattre

INTRODUCTION

TEN NEW cases of vestibular schwannomas per million inhabitants are being diagnosed each year [1, 2]. Because of its development in proximity of the cochlear branch of the eighth pair of cranial nerves, this tumour, which remains constantly benign, is a major cause of hearing loss. Close to 95% of patients with vestibular schwannomas will develop only a single schwannoma during their life time. However, for about 5% of affected patients, tumours will develop bilaterally. This bilateral development is the consequence of a genetic disease called neurofibromatosis type 2. Epidemiological studies stuggest that one person in 35 000 might be affected [3]. Here we summarise our present knowledge of this disease.

CLINICAL MANIFESTATIONS

The neurofibromatoses are autosomal dominant diseases, characterised by a predisposition to the development of nervous system tumours. They were long regarded as a single disease which could manifest with a large variety of symptoms. Currently, however, two forms of neurofibromatoses have been established. Neurofibromatosis type 1 (NF1) occurs with an incidence of 1/3000 and predisposes mainly to the development of peripheral neurofibromas, pheochromocytomas and optic nerve gliomas [4]. Alterations in a single gene localised on chromosome 17 have been shown to be causative in this disease [5] and its genetics are reviewed by Colman and Wallace in this issue. Neurofibromatosis type 2 (NF2), previously called central neurofibromatosis or bilateral acoustic neurofibromatosis (BANF), is approximately 10 times less frequent [3], and its main manifestations are clearly distinct from those of NF1.

Vestibular schwannoma is the major manifestation of the NF2 disease, being found bilaterally in 85% of gene carriers at disease onset [3, 6]. Most commonly it develops in close contact with the vestibular branch in proximity to the cochlear branch of the 8th pair of cranial nerves. The danger of this tumour arises from its development in the intracanalicular space, and subsequently in the posterior fossa; volumes that are strictly confined by bone structures. Therefore, increase in tumour volume causes progressive compression of the 8th pair of cranial nerves causing loss of hearing and tinnitus, and may later lead to compression of vital structures of the central nervous system. Less frequently, schwannomas develop in other locations, in particular in contact with the 9th cranial nerve or with spinal nerves. They may also develop from peripheral nerves and, when superficially located, can manifest as small, subcutaneous, well circumscribed tumours. The most frequent dermatological manifestations, however, are discrete hairy macules which are observed in 50%, of patients. Neurofibromas are also observed in a quarter of the patients. In contrast to NF1 patients, NF2 patients do not develop plexiform neurofibromata. Other nervous system tumours predisposed by NF2 are meningiomas, which develop in half the patients and, less frequently, ependymomas. The only non-tumoural manifestations of the NF2 disease are restricted to the eye. Indeed, the presence of opacities located centrally in

Correspondence to G. Thomas. The Authors are at the Laboratoire de Génétique des Tumeurs, CJF INSERM 9201, Institut Curie, 26 rue d'Ulm, 75231 Paris Cedex 05, France.

close proximity to the posterior capsule of the lens has been found in 40% of the NF2 patients. They can be observed early in childhood. In contrast, in the general population, less than 5% would manifest these opacities after 60 years of age. Combined pigment and retinal hamartomas and the frequent occurrence of epiretinal membranes have also been described [7].

Variability in clinical manifestations is much less pronounced in NF2 compared to NF1 patients [8]. Usually patients present with symptoms in their second and third decades [3, 9], although, on rare occasions, some gene carriers may be symptomatic as early as 1 year of age [10] or asymptomatic up to 70 years of age (Rouleau, personal communication). However, manifestations of the disease will be almost systematically observed, when they are properly searched for, after 40 years of age. Currently, the best methods to detect vestibular schwannomas and monitor their growth is with gadolinium-enhanced MRI and auditory brain stem response (ABR) testing [11].

FAMILY HISTORY

NF2 is a monogenic, autosomal dominantly inherited disease. There might be a small parental effect on severity since in two reports [3, 12] the mean age at onset ocurred at a younger age when the genetic defect was inherited from the mother rather than from the father. In the most recent study, the difference in average age of onset according to parental origin was 6.3 years ($P = 0.026$), and there was a general impression of poorer clinical course in maternal inherited cases [3].

The variability in severity of the disease phenotype is smaller among members of the same family than among genetically unrelated affected individuals. This observation had led to the proposed existence of two clinical subtypes. According to this classification, the severe type (Wishart type) has an early onset (typically before the age of 25 years), a rapid course and multiple tumours in addition to vestibular schwannomas; conversely, the mild type (Gardner type) has a late onset, a more benign course and is often restricted to bilateral vestibular schwannomas [3].

The high penetrance of the disease has allowed the documentation of the occurrence of new mutations based on clinical examination of family members. Approximately half of the registered patients are thought to have new mutations since in these cases both parents (aged over 40 years) of a single affected child do not show any manifestations of NF2. The mutation rate is not known precisely and may be between 10^{-5} and 10^{-6} per birth. In a study of 39 cases, for which the age of both parents at birth was known, the mean parental age was not significantly different from the expected age if the probability of mutation was hypothesised to be independent of parental age [3].

LOCALISATION OF THE GENETIC DEFECT LEADING TO NF2

Cytogenetic analysis and monitoring of allelic loss demonstrate that the most consistent genetic abnormality observed in meningiomas [13–19] and schwannomas [16, 20–22] is loss of chromosome 22. Application of the Knudson two-hit hypothesis to the NF2 disease suggested that the genetic lesion causing NF2 was located on this chromosome. Because NF2 is a well characterised, monogenic disease with almost complete penetrance, it was possible to perform precise cosegregation analysis of the disease phenotype with cloned polymorphic loci. This approach, applied to one large family, enabled Rouleau and associates [23] to localise the genetic defect on chromosome 22. Subsequent analyses have up to now failed to reveal genetic heterogeneity suggesting that alteration in the same gene may be responsible for a major proportion and perhaps for all NF2 families [24]. In the course of these studies, the identification of rare meiotic recombinations localised the *NF2* gene in the median portion of chromosome 22, first within a 13 cM region flanked by two probes D22S28 and D22S1 [25], then between two polymorphic loci D22S212 and D22S32 that were less than 2 cM apart ([26], Rouleau and colleagues, unpublished data). The identification of several polymorphic loci in the close vicinity of the gene has provided the necessary tools to develop reliable presymptomatic tests based on segregation analysis [26]. It has also enabled the development of positional cloning strategies which led to the identification of the *NF2* gene by two independent teams of investigators. Below, we will describe the approach that has been used by our group in collaboration with that of Rouleau.

IDENTIFICATION OF THE *NF2* GENE

A mapping panel of somatic cell hybrids allowing the precise sublocalisation of chromosome 22 cloned loci was developed and used to sublocalise over 150 probes ([27], and Delattre and colleagues, unpublished). This panel was also used to develop techniques aiming at the retrieval of cosmids located in predefined subregions from chromosome 22 specific libraries [28, 29]. This combined approach mapped 12 probes within the region bounded by D22S1 and D22S28, where the *NF2* gene had been mapped initially [25]. These probes were used to progressively expand the corresponding loci by the recurrent isolation of overlapping cosmids until the one megabase region between D22S212 and D22S32 was almost entirely cloned [30]. A highly informative microsatellite locus, D22S268, was identified in a cosmid within the region D22S212/D22S32 [31]. This marker was shown to map in close proximity to the *NF2* gene since cosegregation analyses in NF2 families failed to reveal recombination (pairwise LOD score of 14.6). Furthermore, in one NF2 family, identification of affected members that were obligate hemizygotes for this locus suggested that the NF2 disease could be associated with an interstitial germline deletion [32].

Screening for rearrangements in the D22S212/D22S32 region using pulsed-field gel electrophoresis was performed for one patient of this family and for an additional 42 unrelated NF2 propositi. Abnormal migrating bands were revealed for three individuals that were suggestive of constitutional deletions [30, 32]. For these three patients, constitutional deletions were definitively proven by fluorescent hybridisation on interphase nuclei or metaphase chromosomes of lymphoblastoid cell lines. It could further be shown that the three deletions were nested within each other. The position of the smallest one strongly suggested that the NF2 gene was 300 Kb telomeric to a recurrent chromosome breakpoint observed in Ewing's sarcoma that we had previously identified [33].

A systematic analysis of over one hundred EcoR1 fragments isolated from the cosmids located in the candidate region was undertaken [30, 33]. In particular, each EcoR1 fragment was tested for its ability to hybridise to rodent DNA and, when phylogenetic conservation was found, to detect transcripts on northern blots made with RNAs from a variety of different human cell lines. When the different data generated by this analysis were amalgamated on the map, the position of four genes became evident. The most centromeric gene was shown to be constantly rearranged in Ewing's sarcoma and was termed *EWS* [34, 35]. The closest identified gene on the telomeric side of *EWS* is expressed as a 4-kb transcript. It has recently been characterised [36]. It codes for a beta-adaptin and has been called

BAM22. The third gene has a neuronal pattern of expression and a transcript size identical to that of the neurofilament heavy chain gene, *NEFH*, a gene previously assigned to chromosome 22. This identification was confirmed. The fourth gene was detected by five different EcoR1 fragments which hybridised to transcripts of identical sizes. It was the only gene which was altered by all three deletions [30]. This gene was called *SCH* because of its putative involvement in schwannomas, the most prominent tumour predisposed by NF2. A phylogenetically conserved sequence from the *SCH* locus was used to isolate a 2.0-kb cDNA clone, N1.1, from a human fetal brain cDNA library [30]. The N1.1 cDNA was shown to be encoded by 16 exons that are spread over a region of approximately 120 Kb [30].

A similar positional cloning approach was undertaken by the group of Gusella except that an "exon trapping" strategy was used in order to identify putative transcribed sequences [37].

GERM-LINE POINT MUTATIONS

Demonstration that, on rare occasions, the *SCH* gene could be altered by large genomic deletions is not sufficient to conclude its causal involvement in the NF2 disease. In order to obtain further evidence of *SCH* alteration in NF2 patients, we chose to screen the coding sequence of the gene using denaturing gradient gel electrophoresis (DGGE). The efficiency of this method relies on its ability to discriminate between a perfectly matched double stranded DNA fragment and an almost identical fragment with one or several mismatched bases [38]. Since in a dominantly inherited disease only one of the alleles carries the causal mutation, a denaturation/renaturation cycle at the end of the PCR procedure should generate heteroduplex molecules, provided that this mutation is contained within the amplified product. Optimised experimental conditions can be determined using computer programmes which predict, as a function of the run time, the expected difference in the gradient level between a homoduplex wild-type DNA molecule and a related heteroduplex with a single-base mismatch at each selected position along the sequence. These programmes have previously been validated in a model system [39]. They were exploited to search for optimal pairs of primers that would enable detection of a single-base pair substitution anywhere in the coding sequence of the *SCH* gene or in the adjacent splicing sites [30, 40].

The screening of 91 unrelated NF2 patients revealed 32 individuals for whom one of the 16 PCR-amplified products exhibited two to four bands on the DGGE profile [30, 40]. Direct sequencing of these abnormally migrating products showed that nine of the 32 DNA variants altered either the first or the second intronic base of an intron/exon boundary. Such variation is predicted to interfere with the normal splicing process. Sequencing of the 23 other variants showed a mutation within the coding sequence. Fourteen mutations were deletions or insertions, causing translational frame shifts. Eight mutations were single nucleotide substitutions which led to nonsense mutations. Finally, a single mutation was determined to be missense resulting in a leucine to proline substitution.

Importantly, 8 patients carried a new mutation since, in each case, it was possible to show that both parents were clinically unaffected and, as shown by DGGE and sequencing, did not exhibit the mutation in their constitutional DNAs [30, 40]. These observations provided the strongest evidence of a causal relationship between the occurrence of a new mutation and the development of the disease. Taken together, these data lead to the conclusion that the *SCH* gene is the *NF2* gene.

Identification of 23 germ-line *NF2* mutations using the SSCP technique have currently been reported in the literature [37, 41–47). With four exceptions which are missense mutations, they are all predicted to lead to large interstitial deletions of peptide sequences or to synthesis of truncated proteins with a deletion of the C-terminal end. Thus, summating mutations found by DGGE and SSCP, currently a total of 55 germ-line mutations have been found, and only 5 (9%) might be missense mutations, indicating that the most frequent type of mutation leading to the NF2 disease is associated with a major alteration of the peptide sequence.

Because no genetic heterogeneity has been documented for NF2 [24], it is expected that most, if not all, mutations causing the disease should affect the chromosome 22 locus where the *NF2* gene lies. Yet, although efficient methods have been used to screen for mutations in the coding sequence of the gene and in its intron/exon boundaries [40], mutations have been found in only a third of the patients. Therefore, it may be predicted that mutations affecting either the promoter or the intronic regions of the *NF2* gene should occur frequently. In particular, large deletions such as those already observed in several patients [30, 32] may contribute to the high mutation rate observed at this locus. Interestingly, on one occasion, the *NF2* gene might have been inactivated by a t(4;22)(q12;q12qq;2) chromosome translocation [48].

SOMATIC MUTATIONS

The SSCP and DGGE strategies were also used to screen for somatic mutations in tumours known to exhibit allelic losses on chromosome 22 ([12, 14, 30, 44, 45, 47, 49, 50] Mérel and associates, submitted). Frequent mutations were only found in schwannomas and meningiomas, the two tumours that are most prominently predisposed by NF2. Interestingly, most mutations were also predictive of the synthesis of an altered protein with reduced molecular weight. All meningiomas and approximately half the schwannomas carrying a *NF2* mutation demonstrate losses of heterozygosity for chromosome 22 alleles, indicating that both copies of the *NF2* gene are inactivated in these tumours. Therefore, functional inactivation of the *NF2* gene by a two hit mechanism should operate in the tumorigenic process of both schwannomas and meningiomas. Interestingly, 40% of meningiomas did not demonstrate loss of chromsome 22 alleles nor mutation in the *NF2* gene suggesting that, in this subgroup, another mechanism, not requiring alteration of the *NF2* gene, might be triggered [14]. In this respect, it is notable that linkage studies in one family with a dominant susceptibility to the development of meningioma and ependymoma has excluded the genetic defect from a 15-cM region around the *NF2* gene, indicating that a different locus was involved [51].

In contrast screening for *NF2* gene alterations in tumours other than meningiomas and schwannomas revealed mutations only on rare occasions [41, 52, 53], suggesting that somatic alteration in the *NF2* gene might be mainly restricted to tumours predisposed by *NF2*. Surprisingly, in ependymoma, a tumour which has an increased incidence in NF2 patients and which demonstrates chromosome 22 allelic loss, the *NF2* gene, appears to be seldom somatically altered ([53], Mérel and associates, submitted).

THE PRODUCT OF THE *NF2* GENE

Sequence analysis of the N1.1 cDNA of the *SCH/NF2* gene revealed an open reading frame encoding a putative 595 amino acids protein which was named schwannomin [30]. It is highly

conserved in the mouse [54–57]. Sequence homology searches indicated it was a novel gene exhibiting significant amino acid homology with ezrin, radixin and moesin, collectively known as the ERM family [58]. Because of this homology, Trofatter and colleagues [37], who independently identified the same gene, named its product merlin (moesin-, ezrin-, radixin- like protein). The ERM family is included in the superfamily of the erythrocytic band 4.1 protein which also contains talin, three protein tyrosine phosphatases (PTP-MEG, PTP H1, PTPD1), a surface antigen of *Echinococcus multilocularis* and a protein 4.1 homologue of *Drosophila* [59–61].

Members of the ERM family are highly conserved among mammals (over 95% identity) [62] and several putative members have been found in *Drosophila* [63, 64]. They share a number of common features. They are about the same size (577–586 amino acids), with the first 300 amino acids of their sequence being very similar (over 80% identity). With the exception of the 24 antepenultimate amino acids which are identical, their C-terminal half is less homologous but is predicted to adopt in all cases an alpha helical conformation. Ezrin and moesin may form heterotypic and homotypic dimers *in vivo* [65]. It has recently been shown [66] that all three proteins bind with high affinity to a 140-kD isoform of CD44, a membrane protein implicated in lymphocyte homing and possibly involved in the metastatic process [67–70]. In model systems *in vitro*, inhibition of the expression of all three members of the ERM family by antisense oligonucleotides is necessary to induce destruction of cell–cell and cell–substrate adhesion, as well as disappearance of microvilli [71]. In fact, all three proteins are thought to be involved in several dynamic structures allowing the association of actin filaments to plasma membrane proteins [72, 73]. Indirect evidence suggests interactions of ezrin, radixin and moesin with the actin cytoskeleton probably occurs [58, 74, 75]. It has also been proposed that ezrin may interact with tubulin [76, 77]. During cytokinesis, ezrin, radixin and moesin accumulate in the cleavage furrow [75, 73].

In spite of these similarities, the members of the ERM family have distinctive features. Both ezrin and moesin have an almost exclusive tissue distribution, ezrin being present in simple or stratified epithelial cells and mesothelial cells while moesin is mostly detected in endothelial cells and in the liver cells [78]. The ERM proteins also have distinct receptor-specific patterns of phosphorylation [75, 79, 80]. The physiological role of moesin appears to be slightly different from that of ezrin and radixin since, unlike cells suppressed for ezrin or radixin expression, cells suppressed for moesin expression were able to attach and spread on a substratum [71]. In the case of ezrin and radixin, the alpha-helical structure of the C-terminal domain is interrupted by a proline-rich stretch which may be an SH3-binding domain [81, 82]. This stretch is not found in moesin [62].

The primary structure of the NF2 protein reveals several features resembling those of the ERM family. It has a comparable size (595 amino acids). Its N-terminal domain, bounded by residue 304, demonstrates approximately 60% identity with each of the three members. It is not yet known whether schwannomin binds to CD44. However, it was recently shown that a bacterial fusion protein consisting of glutathione S-transferase and schwannomin was able to bind five distinct proteins of apparent molecular mass 165, 145, 125, 85 and 70 kD [83]. These proteins have not been characterised. In particular, their relationship to CD44 has not yet been elucidated. The C-terminal domain of schwannomin may adopt the secondary structure predicted for ezrin and radixin: two alpha-helical regions separated by a small proline rich peptide sequence. However, it does not contain at its C-terminal end the stretch of 24 identical amino acids found in ezrin, radixin and moesin. In fact, in contrast to the members of the ERM family, the *NF2* gene transcript may undergo alternative splicing enabling the synthesis of two different C-terminal ends [41, 52, 56, 84]. Thus, although schwannomin resembles members of the ERM family, it is not yet known whether it shares their common properties. Ezrin, radixin and moesin have not been implicated in tumour predisposition. Moreover, although these proteins have been shown to play a crucial role in cell–cell and cell–substrate adhesion, they have not been shown to possess tumour suppressor properties. Interestingly, however, ezrin is overexpressed and has an altered cellular localisation in methylcholanthrene-induced mouse sarcoma [79].

CONCLUDING REMARKS

Although the first genetic alterations associated with tumour predispositions were identified in genes encoding transcription factors, it is now becoming increasingly evident that tumour susceptibility diseases may frequently be due to mutations altering components of the cytoskeleton and cell adhesion systems. The first indications were obtained in *Drosophila* where mutated genes, such as *lethal(1) giant larvae* or *discs-large*, known to predispose to tumour formation in the heterozygous state, were shown to encode components homologous to mammalian cadherin [85] and to Z0-1, a component of tight junctions [86], respectively. More recently, it was recognised that *expanded* codes for a putative member of the ERM family [63]. Null mutations in this gene affect cell differentiation and normal development. In particular, it causes an extra cell division in the wing primordia leading to wing overgrowth, suggesting that *expanded* could be a negative regulator of cell proliferation [63].

In humans, evidence that alteration in cell adhesion systems and signal transduction may also predispose to tumour development are also accumulating: an inherited disease, MEN2, known to predispose to tumours of the APUD system (in particular medullary thyroid carcinoma) has been shown to be caused by a mutation in the *RET* gene [87]. The product of the *RET* gene is a membrane tyrosine kinase that has an extra cellular domain with homology to cadherins. Von Hippel Lindau disease, a condition characterised by predisposition to the development of haemangioblastomas and renal tumours, is caused by the inactivation of a gene coding for a putative membrane protein which has been proposed to be involved in cell adhesion or signal transduction [88]. The colorectal cancer predisposition familial adenomatous polyposis (FAP) is due to the inactivation of a protein (APC) which has recently been shown to interact with beta-catenin [89, 90], a protein known to interact with E-cadherin a mediator of cell adhesion. It has also been proposed that APC interacts directly with microtubules [91, 92]. Similarly, neurofibromatosis type 1 is due to mutations altering a microtubule binding protein, neurofibromin [93]. Furthermore, neurofibromin contains a GTPase-activating protein (GAP)-related domain and has been shown to participate in the regulation of the p21-*ras* pathway [94]. A similar situation might be observed for tuberous sclerosis, a syndrome characterised by the development of multiple hamartomatas in many tissues. It is caused by alteration of the *TSC2* gene encoding tuberin, a protein which has a region of homology with the GTPase activating protein GAP3 [95]. Thus, together with NF2, not less than six human tumour susceptibility diseases have putatively been associated with impairment of cell adhesion and signal

transduction. There is no doubt that this figure will increase in the coming years.

Although these diseases are rare, identification of the responsible genes and of the pathways in which they are involved will have a wider relevance. It should increase our understanding of the tumorigenic mechanisms responsible for a large variety of tumour types, which in the vast majority of cases develop in the absence of a familial context. In particular, the signal transduction pathway in which schwannomin may be involved is currently entirely unknown. Its elucidation should allow us to document more precisely the mechanisms leading to schwannoma and meningioma development, two tumours which, together, account for approximately 30% of all nervous system tumours [1, 2, 96, 97].

Acknowledgements——The laboratory of the authors is supported by grants from the European Community Commissions, the Groupement de Recherches et d'Etudes sur les Génomes, the Ligue Nationale contre le Cancer, the Association pour la Recherche sur le Cancer, the Fondation pour la Recherche Médicale, the Association Francaise contre les Myopathies, the Association Neurofibromatose France. KH-X, PM and CD are recipients of fellowships from Comité Départemental de l-Yonne de la Ligue Contre le Cancer, Ministère de la Recherche et de l'Enseignment Supérieur and from Association pour la Recherche sur le Cancer respectively. We wish to thank E. Bijlsma, C. Bonnardel, P. DeJong, P. Derome, M. Desgeorges, P. Dessen, R. Fashold, G. Fisher, M. Giovannini, T. Hulsebos, P. Laurent-Puig, G. Lenoir, V.F. Mautner, E. Ollagon, J. Philippon, S. Pulst, F. Resche, G. Rouleau, L. Selleri, J.M. Sterkers, P. Vialat, F. Woimant and the NF2 patients and their families for their cooperation.

1. Acoustic Neuroma. Reprinted from *NIH Consens Dev Conf Consens Statement* 1991, **9**, 11–13.
2. Frolich AM, Sutherland GR. Epidemiology and clinical features of vestibular schwannoma in Manitoba, Canada. *Can J Neurol Sci* 1993, **20**, 126–130.
3. Evans DGR, Huson SM, Donnai D, *et al.* A genetic study of type-2 neurofibromatosis in the United Kingdom. I. Prevalence, mutation rate, fitness and confirmation of maternal transmission effect on severity. *J Med Genet* 1992, **29**, 841–846.
4. Gutman DH, Collins FS, von Recklinghausen neurofibromatosis. In *The Metabolic and Molecular Basis of Inherited Disease*, 7th edition. McGraw Hill, 1994.
5. Viskochil D, White R, Cawthon R. the neurofibromatosis type 1 gene. *A Rev Neurosci* 1993, **16**, 183–205.
6. Martuza RL, Eldridge R. Neurofibromatosis 2 (bilateral acoustic neurofibromatosis). *N Engl J Med* 1988, **318**, 684–688.
7. Landau C, Yasargil GM. Ocular fundus in neurofibromatosis type 2. *Br J Ophthalmol* 1993, **77**, 646–649.
8. Easton DF, Ponder MA, Huson SM, Ponder BA. An analysis of variation in the expression of neurofibromatosis (NF)type 1 (NF1): evidence for modifying genes. *Am J Human Genet* 1993, **53**, 305–313.
9. Evans DGR. Huson SM, Donnai D, *et al.* A genetic study of type-2 neurofibromatosis in the United Kingdom. II. Guidelines for genetic counselling. *J Med Genet* 1992, **29**, 847–852.
10. Mautner VF, Tatagiba M, Guthoff R, Samii M, Pulst SM. Neurofibromatosis in the pediatric age group. *Neurosurgery*, 1993, **33**, 92–96.
11. Tator CH. Vestibular schwannoma (acoustic neuroma): the profession is not achieving its potential for early diagnosis. *Can Med Assoc Med* 1993, **149**, 275–277.
12. Kanter WR, Eldridge R, Fabricant R, Allen JC, Koerber T. Central neurofibromatosis with bilateral acoustic neuroma: genetic clinical and biochemical distinctions from peripheral neurofibromatosis. *Neurology* 1980, **30**, 851–859.
13. Dumanski JP, Rouleau GA, Nordenskjold M, Collins VP. Molecular genetic analysis on chromosome 22 in 81 cases of meningioma. *Cancer Res* 1990, **50**, 5863–5867.
14. Ruttledge MH, Sarrazin J, Rangaratnam S, *et al.* Evidence for the complete inactivation of the NF2 gene in the majority of sporadic meningiomas. *Nature Genet* 1994, **6**, 180–184.
15. Rutledge MH, Xie YG, Han FY, *et al.* Deletion on chromosome 22 in sporadic meningioma. *Gene Chromosome Cancer* 1994, **10**, 122–130.
16. Seizinger BR, Rouleau GA, Ozelius LJ, *et al.* Common pathogenic mechanism for three tumor types in bilateral acoustic neurofibromatosis. *Science* 1987, **236**, 317–319.
17. Seizinger BR, De La Monte S, Atkins L, Gusella JF, Martuza RL. Molecular genetic approach to human meningioma: loss of genes on chromosome 22. *Proc Natl Acad Sci USA* 1987, **84**, 5419–5423.
18. Zang, KD, Singer H. Chromosomal constitution of meningiomas. *Nature* 1967, **216**, 84–85.
19. Zang KD. Cytological and cytogenetical studies on human meningioma. *Cancer Genet Cytogenet* 1982, **8**, 249–274.
20. Couturier J, Delattre O, Kujas M, *et al.* Assessment of chromosome 22 anomalies in neurinomas by combined karyorype and RFLPs analyses. *Cancer Genet Cytogen* 1990, **45**, 55–62.
21. Fontaine B, Sanson M, Delattre O, *et al*, Parental origin of chromosome 22 loss in sporadic and NF2 neuromas. *Genomics* 1991, **10**, 280–283.
22. Seizinger BR, Martuza RL, Gusella JF. Loss of genes on chromosome 22 in tumorigenesis of human acoustic neuroma. *Genomics* 1986, **4**, 1–6.
23. Rouleau GA, Wertelecki W, Haines JL, *et al.* Genetic linkage of bilateral acoustic neurofibromatosis to a DNA marker on chromosome 22. *Nature* 1987, **329**, 246–248.
24. Narod SA, Parry DM, Parboosingh J, *et al.* Neurofibromatosis type 2 appears to be a genetically homogeneous disease. *Am J Human Genet* 1992, **51**, 486–496.
25. Rouleau GA, Seizinger BR, Wertelecki W, *et al*: Flanking markers bracket the neurofibromatosis type 2 (NF2) gene on chromosome 22. *Am J Human Genet* 1990, **46**, 323–328.
26. Ruttledge MH, Narod SA, Dumanski JP, *et al.* Presymptomatic diagnosis for neurofibromatosis type 2 with chromosome 22 markers. *Neurology* 1993, **43**, 1753–1760.
27. Delattre O, Azambuja CJ, Aurias A, *et al.* Mapping of human chromosome 22 with a panel of somatic cell hybrids. *Genomics* 1991, **9**, 721–727.
28. Dejong PJ, Vokobata K, Chen C, *et al.* Human chromosome-specific partial digest libraries in human lambda and cosmid vectors (A2333). *Cytogenet Cell Genet* 1989, **51**, 985.
29. Zucman J, Delattre O, Desmaze C, *et al.* Rapid isolation of cosmids from defined subregions by differential Alu-PCR hybridization on chromosome 22 specific library. *Genomics* 1992, **13**, 395–401.
30. Rouleau GA, Merel P, Lutchman M, *et al.* Alteration in a new gene encoding a putative membrane-organizing protein causes neurofibromatosis type 2. *Nature* 1993; **363**, 515–521.
31. Marineau C, Baron C, Delattre O, Zucman J, Thomas G, Rouleau GA. Dinucleotide repeat polymorphism at the D22S268 locus. *Human Mol Genet* 1993, **2**, 336.
32. Sanson M, Marineau C, Desmaze C, *et al.* Germ-line deletion in a neurofibromatosis type 2 kindred inactivates the NF2 gene and a candidate meningioma locus. *Human Mol Genet* 1993, **2**, 1215–1220.
33. Zucman J, Delattre O, Desmaze C, *et al.* Cloning and characterization of the Ewing's sarcoma and peripheral neuroepithelioma t(11;22) translocation breakpoints. *Genes Chromosome Cancer* 1992, **5**, 271–277.
34. Delattre O, Zucman J, Plougastel B, *et al.* Gene fusion with an ETS domain caused by chromosome translocation in human tumours. *Nature* 1992, **359**, 162–165.
35. Delattre O, Zucman J, Melot T, *et al.* The Ewing family of tumours: a subgroup of small round cell tumours defined by specific chimeric transcripts. *New Engl J Med* 1994, **331**, 294–299.
36. Peyrard M, Fransson I, Xie YG, *et al.* Characterization of a new member of the human β-adaptin gene family from chromosome 22q12, a candidate meningioma gene. *Human Mol Genet* 1994, **3**, 1393–1399.
37. Trofatter JA, MacCollin MM, Rutter JL, *et al.* A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. *Cell* 1993, **72**, 791–800.
38. Abrams ES, Murdaugh SE, Lerman LS. Comprehensive detection of single base changes in human genomic DNA using denaturing gradient gel electrophoresis and a GC clamp. *Genomics* 1990, **7**, 463–475.
39. Hamelin R, Jego N, Laurent-Puig P, Vidaud M, Thomas G. Efficient screening of p53 mutations by denaturing gradient gel electrophoresis in colorectal tumors. *Oncogene*, 1993, **8**, 2213–2220.

40. Merel P, Hoang-Xuan K, Sanson M, *et al.* Screening for germ-line mutations in the NF2 gene. *Gene Chromosome Cancer* 1994, in press.
41. Bianchi AB, Hara T, Ramesh V, *et al.* Mutations in transcript isoforms of the neurofibromatosis 2 gene in multiple human tumor types: *Nature Gene* 1994, **6**, 185–193.
42. Bourn D, Carter SA, Evans DGR, Goodship J, Coakham H, Strachan T. A mutation in the neurofibromatosis type 2 tumor-suppressor gene, giving rise to widely different clinical phenotypes in two unrelated individuals. *Am J Human Genet* 1994b, **55**, 69–73.
43. Bourn D, Carter SA, Mason S, Evans DGR, Strachan T. Germ-line mutations in the neurofibromatosis type 2 tumour suppressor gene. *Human Mol Genet* 1994a, **3**, 813–816.
44. Jacoby LB, MacCollin MM, Louis DN, *et al.* Exon scanning for mutation of the NF2 gene in schwannomas. *Human Mol Genet* 1994, **3**, 413–419.
45. Lekanne Deprez RH, Bianchi AB, Groen NA. *et al.* Frequent NF2 gene transcript mutations in sporadic meningiomas and vestibular schwannomas *Am J Human Genet* 1994, **54**, 1022–1029.
46. MacCollin MM, Mohney T, Trofatter JA, Wertelecki W, Ramesh V, Gusella JF. DNA diagnosis of neurofibromatosis 2. *JAMA* 1993, **270**, 2316–2320.
47. Twist EC, Ruttledge M, Rousseau M, *et al.* The neurofibromatosis type 2 gene is inactivated in schwannomas. *Human Mol Genet* 1994, **3**, 147–151.
48. Arai E, Tokino T, Imai T, *et al.* Mapping the breakpoint of a constitutional translocation on chromosome 22 in a patient with NF2. *Gene Chromosome Cancer* 1994, **6**, 235–238.
49. Bijlsma E, Merel P, Bosch DA, *et al.* Analysis of mutations in the SCH gene in schwannomas. *Gene Chromosome Cancer* 1994, **11**, 2–14.
50. Irving RM, Moffat DA, Hardy DG, Barton DG, Xuereb JH, Maher ER. Somatic NF2 gene mutations in familial and non-familial vestibular schwannoma. *Human Mol Genet* 1994, **3**, 347–350.
51. Pulst SM, Rouleau GA, Marineau C, Fain P, Sieb JP. Familial meningioma is not allelic to neurofibromatosis 2. *Neurology* 1993, **43**, 2096–2098.
52. Arakawa H, Hayashi N, Nagase H, Ogawa M, Nakamura Y. Alternative splicing of the NF2 gene and its mutation analysis of breast and colorectal cancers. *Human Mol Genet* 1994, **3**, 565–568.
53. Rubio MP, Correa KM, Ramesh V, *et al.* Analysis of neurofibromatosis 2 gene in human ependymomas and astrocytomas. *Cancer Res* 1994, **54**, 45–47.
54. Claudio JO, Marineau C, Rouleau GA. The mouse homologue of the neurofibromatosis type 2 gene is highly conserved. *Human Mol Genet* 1994, **3**, 185–190.
55. Haase VH, Trofatter JA, MacCollins M, Tarttelin E, Gusella JF, Ramesh V. The murine NF2 homologue encodes a highly conserved merlin protein with alternative forms. *Human Mol Genet* 1994, **3**, 407–411.
56. Hara T, Bianchi AB, Seizinger BR, Kley N. Molecular cloning and characterization of alternatively spliced transcripts of the mouse neurofibromatosis 2 gene. *Cancer Res* 1994, **54**, 330–335.
57. Hynh DP, Nechiporuk T, Pulst SM. Alternative transcript in the mouse neurofibromatosis type 2 (NF2) gene are conserved and code for schwannomins with distinct C-terminal domains. *Human Mol Genet* 1994, **3**, 1075–1079.
58. Sato N, Funayama N, Nagafuchi A, Yonemura S, Tsukita S, Tsukita S. A gene family consisting of ezrin, radixin and moesin. Its specific localization at actin filament/plasma membrane association sites. *J Cell Sci* 1992, **103**, 131–143.
59. Arpin M, Algrain M. Louvard D. Membrane actin microfilament connections: an increasing diversity of players related to band 4.1. *Curr Opin Cell Biol* 1994, **6**, 136–141.
60. Fehon RG, Dawson IA, Artavanistsakonas S. A drosophila homologue of membrane skeleton protein 4.1 is associated with septate junctions and is encoded by the Coracle gene. *Development* 1994, **120**, 545–557.
61. Hundahl Moller NP, Bach Moller K, Lammers R, Kharitonenkov A, Sures I, Ullrich A. Src kinase associates with a member of a distinct subfamily of protein-tyrosine phosphatases containing an ezrin-like domain. *Proc Natl Acad Sci USA* 1994, **91**, 7477–7481.
62. Lankes WT, Schwartz-Albiez R, Furthmayr H. Cloning and sequencing of porcin moesin and radixin cDNA and identification of highly conserved domains. *Biochem Biophis Acta* 1993, **1216**, 479–482.
63. Boedigheimer M, Bryan P, Laughon A. Expanded, a negative regulator of cell proliferation in Drosophila, shows homology to the NF2 tumor suppressor gene. *Mech Develop* 1993, **44**, 83–84.
64. Edwards KE, Montague RA, Shephard S, Edgar BA, Erikson RL, Kiehart DP. Identification of Drosophila cytoskeletal proteins by induction of abnormal cell shape in fission yeast. *Proc Natl Acad Sci USA* 1994, **91**, 4589–4593.
65. Gary R, Bretscher A. Heterotypic and homotypic associations between ezrin and moesin, two putative membrane-cytoskeletal linking proteins. *Proc Natl Acad Sci USA* 1993, **90**, 10846–10850.
66. Tsukita S, Oishi K, Sato N, Sagara J, Kawai A, Tsukita S. ERM family members as molecular linkers between the cell surface glycoprotein CD44 and actin-based cytoskeletons. *J Cell Biol* 1994, **126**, 391–401.
67. Arch R, Wirth K, Hofman M, Ponta H, Marzku S, Herrlich P, Zöller M. Participation in normal immune responses of a metastasis inducing splice variant of CD44. *Science* 1992, **257**, 682–685.
68. East JA, Hart IR. CD44 and its role in tumor progression and metastasis. *Eur J Cancer* 1993, **29A**, 1921–1922.
69. Günthert U, Hofman M, Rudy W, *et al.* A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cell. *Cell* 1991, **65**, 13–24.
70. Koopman G, Heider KH, Horst E, *et al.* Activated human lymphocytes and aggressive non-hodgkin's lymphomas express a homologue of the rat metastasis-associated variant CD44. *J Exp Med* 1993, **177**, 897–904.
71. Takeuchi K, Sato N, Kasahara H, *et al.* Perturbation of cell adhesion and microvillii formation by antisense oligonucleotides to ERM family members. *J Cell Biol* 1994, **125**, 1371–1384.
72. Luna EJ, Hitt AL. Cytoskeleton–plasma membrane interactions. *Science* 1992, **258**, 955–964.
73. Sato N, Yonenura S, Obinata T, Tsukita S, Tsukita S, Radixin, a barbed end-capping actin-modulating protein is concentrated at the cleavage furrow during cytokinesis. *J Cell Biol* 1991, **113**, 321–330 (published erratum appears in *J Cell Biol* 1991, **114**, 1101–1103).
74. Algrain M, Turunen O, Vaheri A, Louvard D, Arpin M. Ezrin contains cytoskeleton and membrane binding domains accounting for its proposed role as a membrane-cytoskeletal linker. *J Cell Biol* 1993b, **120**, 129–139.
75. Frank Z, Gary R, Bretscher A. Moesin, like ezrin, colocalizes with actin in cortical skeleton in cultured cells, but its expression is more variable. *J Cell Science* 1993, **105**, 219–231.
76. Birgbauer E, Solomon F. A marginal band-associated protein has properties of both microtubules- and microfilament-associated proteins. *J Cell Biol* 1989, **109**, 1609–1620.
77. Goslin K, Birgbauer, Banker G, Solomon F. The role of cytoskeleton in organizing growth cones: a microfilament-associated growth cone component depends upon microtubules for its localization. *J Cell Biol* 1989, **109**, 1621–1631.
78. Berryman M, Franck Z, Bretscher A. Ezrin is concentrated in the apical microvilli of a wide variety of epithelial cells whereas meosin is found primarily in endothelial cells. *Cell Sci* 1993, **105**, 5949–5953.
79. Fazioli F, Wong WT, Ullrich SJ, Sakaguschi K, Appella E, Paolo Di Fiore P. The ezrin-like family of tyrosine kinase substrates: receptor-specific pattern of tyrosine phosphorylation and relationship to malignant transformation. *Oncogene* 1993, **8**, 1335–1345.
80. Krieg J, Hunter T. Identification of the two major epidermal growth factor-induced tyrosine phosphorylation sites in microvillar core protein ezrin. *J Biol Chem* 1992, **267**, 19258–19265.
81. Yu H, Chen JK, Feng S, Dalgarno DC, Brauer AW, Schrieber SL. Structural basis for the binding of proline-rich peptides to SH3 domains. *Cell* 1994, **76**, 933–945.
82. Ren R, Mayer BJ, Cicchetti P, Baltimore D. Identification of a ten-amino acid proline-rich SH3 binding site. *Science* 1993, **259**, 1157–1161.
83. Takeshima H, Izawa I, Lee PSY, Safdar N, Levin VA, Saya H. Detection of cellular proteins that interact with the NF2 tumor suppressor gene product. *Oncogene* 1994, **9**, 2135–2144.
84. Pykett MJ, Murphy M, Harnish PR, George D. The neurofibromatosis 2(NF2) tumor suppressor gene encodes multiple alternatively spliced transcripts. *Human Mol Genet* 1994, **3**, 559–564.
85. Lutzelschwab R, Klambt C, Rossa R, Schmidt O. The Drosophila melanogaster 1(2)gl gene encodes a protein homologous to the cadherin cell adhesion molecule family. *EMBO J* 1987, **6**, 1791–1797.
86. Willcott E, Balda MS, Fannings AS, Jameson B, Van Itallie C, Anderson JM, The tight junction protein Z0-1 is homologous to

the Drosophila discs-large tumor suppressor protein of septante junctions. *Proc Natl Acad Sci USA* 1993, **90**, 7834–7838.
87. Mulligan LM, Kowk JB, Healey CS, *et al.* Germ-line mutations in the RET proto-oncogene in multiple endocrine neoplasia type 2A. *Nature* 1993, **363**, 458–460.
88. Latif F, Tory K, Gnarra J, *et al.* Identification of the von Hippel Lindau disease tumor suppressor gene. *Science* 1993, **260**, 1317–1320.
89. Rubinfeld B, Souza B, Albert I, *et al.* Association of the APC gene product with B-catenin. *Science* 1993, **262**, 1731–1734.
90. Su LK, Vogelstein B, Kinzler KW. Association of the APC tumor suppressor protein with catenins. *Cell* 1993, **262**, 1734–1737.
91. Munemitsu S, Souza B, Müller O, Albert I, Rubinfeld B, Polakis P. The APC gene product associates with microtubules *in vivo* and promotes their assembly *in vitro*. *Cancer Res* 1994, **54**, 3676–3681.
92. Smith KJ, Levy DB, Maupin P, Pollard TD, Vogelstein B, Kinzler KW. Wild-type but not mutant APC associates with microtubule cytoskeleton. *Cancer Res* 1994, **54**, 3672–3675.
93. Gregory PE, Gutmann DH, Boguski M, *et al.* The neurofibromatosis type 1 gene product, neurofibromin, associates with microtubules. *Somat Cell Mol Genet* 1993, **19**, 265–274.
94. Xu G, Lin B, Tanaka K, *et al.* The catalytic domain of the neurofibromatosis type 1 gene products stimulates ras GTPase and complements ira mutants of *S. cerevisiae*. *Cell* 1990, **63**, 835–841.
95. The European Chromosome 16 Tuberous Sclerosis Consortium. Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell* 1993, **75**, 1305–1315.
96. Nakasu S, Hirono A, Shimura T, Llena JF Incidental meningiomas in autopsy study. *Surg Neurol* 1987, **27**, 319–322.
97. Rausing A, Ybo W, Stenflo J. Intracranial meningioma—a population study of ten years. *Acta Neurol Scand* 1970, **46**, 102–110.

Pergamon

European Journal of Cancer Vol. 30A, No. 13, pp. 1987–1990, 1994

0959–8049/94 $7.00+0.00

0959-8049(94)00391-2

Von Hippel-Lindau Disease

E.R. Maher

INTRODUCTION

GERMLINE MUTATIONS in the von Hippel-Lindau (*VHL*) tumour suppressor gene predispose to a variety of benign and malignant tumours which may develop during childhood or in adults. Recent advances in the molecular genetics of VHL disease culminated in the identification of the *VHL* gene which has enabled presymptomatic diagnosis by DNA analysis to be offered to VHL disease families and has provided opportunities to investigate the role of the *VHL* gene product in normal cellular growth and differentiation and the involvement of *VHL* gene mutations in human cancer.

EPIDEMIOLOGY

Although VHL disease has been considered to be rare, it is likely that diagnosis is underreported. The minimum birth incidence of VHL disease in eastern England is 1 per 36 000 [1], and the prevalence of VHL disease in southwestern Germany was estimated at 1 per 39 000 [2]. VHL disease is the commonest cause of familial renal cell carcinoma [3], and approximately 30–40% of patients with cerebellar haemangioblastoma have VHL disease (more in early onset cases) [4]. Recently, Neumann and associates [5] reported that 19% of unselected patients with phaeochromocytoma had VHL disease.

INHERITANCE AND CLINICAL FEATURES

VHL disease is inherited as a dominant trait with variable expression, and age- and tumour-dependent penetrance. The penetrance of VHL disease is almost complete by age 65 years [1], but VHL disease may present in infancy or only be diagnosed in the seventh decade [6–11]. Retinal and cerebellar haemangioblastomas are the most common presenting features of VHL disease, but renal cell carcinoma and phaeochromocytoma are the initial complications in 10 and 5% of patients, respectively (Table 1). The mean age at onset of renal cell carcinoma is later than for retinal or cerebellar haemangioblastomas, but the cumulative risks of a VHL disease patient developing a retinal angioma, cerebellar haemangioblastoma and renal cell carcinoma by age 60 years are in excess of 70% for each tumour [6]. Hence, the majority of patients with VHL disease will develop a renal cell carcinoma if they live long enough and renal cell carcinoma is the most common cause of death in VHL disease. Patients with VHL disease develop cerebellar haemangioblastomas and renal cancers at an earlier age than those who develop sporadic forms of these tumours (29 versus 48 years and 45 versus 62 years, respectively) [4], and VHL disease tumours are frequently multiple or bilateral. The earliest age at onset of renal cell carcinoma is 16 years [12].

Although phaeochromocytoma occurs in approximately 10% of patients overall [6, 11], in some families this is the most frequent complication and in others it is rare [6–8, 13]. In most VHL families with phaeochromocytoma, the predisposition to renal cell carcinoma is similar to that in families without phaeochromocytoma, but in rare families with phaeochromocytoma, renal cell carcinoma may be infrequent [13]. Recently interfamilial differences in phaeochromocytoma incidence have been correlated with the type of *VHL* gene mutation (see below). Phaeochromocytoma in VHL disease cases occurs at an earlier age than in nonfamilial cases, and is frequently multifocal (55% of cases) [5].

Pancreatic tumours in VHL disease are usually islet cell adenomas or carcinomas, which are frequently asymptomatic and detected on routine radiographic screening [14]. Although endocrine disturbances can occur, most are non-functional.

Correspondence to E.R. Maher at the University of Cambridge, Box 134, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, U.K.