

# Identification of recurrent regions of chromosome loss and gain in vestibular schwannomas using comparative genomic hybridisation

C Warren, L A James, R T Ramsden, A Wallace, M E Baser, J M Varley, D G Evans

*J Med Genet* 2003;40:802–806

See end of article for authors' affiliations

Correspondence to: Professor D Gareth Evans, Academic Unit of Medical Genetics and Regional Genetics Service, St Mary's Hospital, Manchester M13 0JH, UK; gareth.evans@mmc.nhs.uk

Revised version received 23 May 2003  
Accepted for publication 9 July 2003

**Background:** Schwannomas are benign tumours of the nervous system that are usually sporadic but also occur in the inherited disorder neurofibromatosis type 2 (NF2). The *NF2* gene is a tumour suppressor on chromosome 22. Loss of expression of the *NF2* protein product, merlin, is universal in both sporadic and NF2 related schwannomas. The GTPase signalling molecules RhoA and Rac1 regulate merlin function, but to date only mutation in the *NF2* gene has been identified as a causal event in schwannoma formation.

**Methods:** Comparative genomic hybridisation (CGH) was used to screen 76 vestibular schwannomas from 76 patients (66 sporadic and 10 NF2 related) to identify other chromosome regions that may harbour genes involved in the tumorigenesis.

**Results:** The most common change was loss on chromosome 22, which was more frequent in sporadic than in NF2 related tumours. Importantly, eight tumours (10%) showed gain of copy number on chromosome 9q34. Each of the two NF2 patients who had received stereotactic radiotherapy had non-chromosome 22 changes, whereas only one of eight non-irradiated NF2 patients had any chromosome changes. Three tumours had gain on 17q, which has also been reported in malignant peripheral nerve sheath tumours that are associated with neurofibromatosis type 1. Other sites that were identified in three or fewer tumours were regions on chromosomes 10, 11, 13, 16, 19, 20, X, and Y.

**Conclusions:** These findings should be verified using techniques that can detect smaller genetic changes, such as microarray-CGH.

Schwannomas are benign tumours arising from Schwann cells, which form the myelin sheaths surrounding the peripheral nervous system. About 95% of vestibular schwannomas are sporadic and unilateral, and about 5% occur in the inherited disorder neurofibromatosis type 2 (NF2), an autosomal dominant disorder with an incidence of approximately 1 in 40 000 live births.<sup>1</sup> Bilateral vestibular schwannomas are pathognomonic for NF2, and people with NF2 are also predisposed to schwannomas in other locations, and to other nervous system tumours such as meningiomas and ependymomas.<sup>2–4</sup>

In 1986, the *NF2* gene was mapped to chromosome 22, and in 1993, two groups independently isolated the gene and named its encoding protein merlin or schwannomin.<sup>5–6</sup> The *NF2* gene is a tumour suppressor, as corroborated by studies showing complete inactivation of the gene in more than 60% of schwannomas, either by mutation in both alleles or by loss of one allele and mutation in the other.<sup>7</sup> The *NF2* gene is inactivated in both familial and sporadic schwannomas,<sup>5–8</sup> suggesting that a common pathogenic mechanism is involved. Point mutations are the most usual constitutional *NF2* mutations in NF2 patients, whereas small deletions are the main causal event in unilateral sporadic vestibular schwannoma.<sup>9</sup> Several studies have documented genotype–phenotype correlations in NF2. For example, patients with nonsense or frameshift mutations generally have severe disease, whereas missense mutations usually cause mild disease.<sup>10–13</sup>

Regardless of mutation type or allelic loss, the loss of expression of the *NF2* protein, merlin, appears to be universal in schwannomas. Merlin has high homology with the ERM proteins (ezrin, radixin, and moesin) and shares an amino-terminal domain.<sup>14</sup> However, there are differences in the carboxy-terminal domain, suggesting that merlin may have functions that are distinct from the other ERM proteins.<sup>14–15</sup>

The ERM proteins accumulate below the plasma membrane in structures such as microvilli, membrane ruffles, and cell–cell contact sites, and function as membrane organisers and linkers to the cytoskeleton.<sup>15</sup> Merlin also processes these functional properties,<sup>16</sup> and like the ERM proteins is predominantly located in membrane ruffles and cellular protrusions. In addition to binding to the cytoskeleton, ERM proteins and merlin associate with cell surface glycoproteins, such as CD44. The formation of the ERM/CD44 complex is regulated by Rho mediated signal transduction in vivo,<sup>17</sup> and the small GTPase signalling molecules RhoA and Rac1 are involved in merlin function.<sup>18</sup> Rac/Cdc42 signalling is linked to merlin by p21 activated kinase, affecting its activity and localisation.<sup>19</sup>

These cell biology studies have provided a greater understanding of the role of merlin in regulating cell growth, but to date only the *NF2* gene has been identified in the actual pathogenesis of the transformed Schwann cell. In addition to the *NF2* gene on chromosome 22, cytogenetic studies suggest that chromosomes 11, 13, and 19 could be involved in vestibular schwannoma tumorigenesis,<sup>20–21</sup> but no consistent regions of interest have been identified through the analysis of a large number of tumours.

Comparative genomic hybridisation (CGH) is a rapid method for analysing DNA copy number losses and gains across the genome in a single experiment.<sup>22</sup> In this study, we used CGH to screen a large number of vestibular schwannomas to determine common regions of loss or gain on the genome, which could be sites of a secondary genetic alteration in this tumour.

## METHODS

Seventy six primary vestibular schwannoma tumours were removed from 76 patients (age range 10 to 73 years) at the otolaryngology department of the Manchester Royal

Infirmery, Manchester, England. Of these, 10 were from NF2 patients. Directly after removal, the tumour was snap frozen in liquid nitrogen and stored for DNA extraction. All studies were carried out with the approval of all appropriate ethics review committees.

### DNA extraction

The tumour was powdered on ice using a pestle and mortar in 0.5 ml of 2× lysis buffer (154 mM NH<sub>4</sub>Cl, 9.5 mM KHCO<sub>3</sub>, 2 mM EDTA). When the mixture was homogeneous, the solution was made up to a volume of 5 ml using 2× lysis buffer and mixed on a rotator for 10 minutes at 4°C. The suspension was centrifuged for 10 minutes at 1400 *g* and 4°C, after which the supernatant was removed and the pellet resuspended in 5 ml 2× lysis buffer. Centrifugation was repeated and the supernatant removed. The resulting pellet was then resuspended in 0.5 ml salt/EDTA buffer (75 mM NaCl, 25 mM EDTA), with 50 µl 10% sodium dodecyl sulphate (SDS) and 1.7 µl of 20 mg/ml proteinase K and incubated overnight at 55°C. The DNA was then extracted using phenol saturated with 100 mM Tris-HCl (pH 8.0) and precipitated with ice cold absolute ethanol. DNA was removed and redissolved in TE buffer (Tris HCl 10 mM plus EDTA 1 mM).

### Comparative genomic hybridisation

Comparative genomic hybridisation (CGH) was done as described previously.<sup>23</sup> Briefly, 1 µg of normal reference DNA (human male chorionic DNA, Sigma, Poole, UK), was labelled by nick translation with SpectrumRed-dUTP (Vysis, London, UK) and 1 µg of high molecular weight tumour DNA with FluoroGreen-dUTP (fluorescein-11-dUTP, Amersham International, Amersham, Buckinghamshire, UK). The reference and test DNA, along with 50 µg of Cot-1 DNA (Life Technologies, Paisley, Strathclyde, UK), were then hybridised onto normal male metaphase chromosomes, prepared as described previously.<sup>24</sup> The hybridisation was done at 37°C in an atmosphere of 50% formamide/2× SSC (NaCl/sodium citrate) and left for a minimum of 24 hours. For each batch of hybridisations, a control consisting of normal male DNA against normal female DNA was used to set the fluorescence ratio thresholds for each set of experiments in the batch. Results were visualised using a fluorescence microscope and image analysis was done with a Vysis QUIPS workstation.<sup>25</sup>

### Loss of heterozygosity analysis

In a subset of 33 tumours, loss of heterozygosity (LOH) was tested using the microsatellite markers D22S275, NF2CA3, D22S268, and D22S280. NF2CA3 is located within intron 1 of *NF2*, and D22S275, D22S268, and D22S280 are tightly linked to *NF2*. For the markers D22S275, NF2CA3, and D22S268, LOH was determined on fluorescently labelled polymerase chain reaction (PCR) products. The lymphocyte and tumour DNA pairs were amplified at the same time and electrophoresed on the same Genescan gels. Fragment sizing and comparison between lymphocyte and tumour DNA results were carried out using the Genescan version 2.1.1 fragment analysis program. A diminution in relative allele signal strength between lymphocyte and tumour DNA of more than 30% was taken to indicate LOH. For the marker D22S280, LOH was determined by comparison of lymphocyte and tumour. PCR amplifications were electrophoresed on non-denaturing gels and visualised by silver staining. The fluorescence thresholds were set as previously described,<sup>23, 26</sup> with a FluoroGreen:SpectrumRed ratio between 1.15 and 1.18 to identify gains in chromosome copy number, and less than 0.80 to identify loss of copy number in tumour material.

### Indices of tumour proliferation rate

In a subset of 29 tumours, tumour proliferation rate was estimated using monoclonal antibodies to Ki-67 and PDGF. Ki-67 and PDGF immunostaining was done on 4 µm thick sections cut from formalin fixed or paraffin embedded tumour samples using the streptavidin–biotin complex peroxidase technique.<sup>27</sup> To calculate the proliferation indices, the area with the most staining was selected, at least 300 nuclei were counted, and the index was the percentage of positive nuclei. In the Ki-67 proliferation staining, there were few positive nuclei, indicating that most of the cells had entered the S phase of the nuclear division cycle. In the PDGF staining, none of the tumours had diffuse strong positivity.

The association between tumour diameter and genetic changes was evaluated using multiple predictor linear regression analysis. There were 70 tumours with information on tumour diameter and genetic changes (nine NF2 related and 61 sporadic vestibular schwannomas). The association between proliferation indices and the number of genetic changes was evaluated using Pearson's *r* correlation coefficient.

### RESULTS

Changes were detected in 30 of the 76 tumours (43%), of which 18 (60%) showed the expected loss in chromosome 22 (table 1). Surprisingly, a gain in copy number on chromosome 9q34 was detected in eight tumours (10%). Other changes were much less common: gains in chromosomes 10, 16, 17, and 19 (three tumours each) and 18 (one tumour); and losses in chromosomes Y (three tumours) and X, 21, 13, and 11 (one tumour each). Thus, aside from loss of chromosome 22, gain of copy number was more common (21 tumours) than loss of copy number (seven tumours). There were no marked differences between male and female subjects in the proportions with genetic changes, or the number of genetic changes, or loss of chromosome 22—consistent with epidemiological data indicating a lack of sex difference in vestibular schwannoma formation.<sup>28</sup>

Twenty one tumours had either a single loss or gain of copy number in one area only, with no other detectable changes. This was associated with loss of chromosome 22: 15 of 18 tumours with loss in chromosome 22 had no other observed event. Nine tumours had more than one change, including six with gain on chromosome 9q34 and eight with loss or heading toward loss on chromosome 22 ("heading toward loss" refers to individual metaphase spreads showing loss on chromosome 22, but when taken as an average, the overall fluorescence ratio is on the boundary of normal).

Three of the 33 tumours that were analysed by LOH for the *NF2* allele were uninformative for all three markers. Sixteen (53%) of the 30 tumours that could be assessed by both CGH and LOH had loss of the *NF2* locus. Ten had both LOH and loss or partial loss using CGH, six had LOH only, and 14 had no apparent LOH. Four of the 10 NF2 related vestibular schwannomas had LOH that was not detected by CGH, whereas 10 of 12 sporadic tumours had LOH that was detected by CGH. In the 10 NF2 related tumours, only three showed changes on CGH—none of which was loss of chromosome 22—and two of these three had been irradiated. The constitutional *NF2* mutation was known for all 10 NF2 patients (data not shown). These mutations were three large deletions detected by fluorescent in situ hybridisation (FISH) (one uninformative for LOH and the remaining two not showing LOH) and seven truncating mutations (one uninformative for LOH and four of the remaining six showing LOH).

In the multiple predictor linear regression model, two covariates were informative predictors of maximum tumour diameter: age at presentation and gain of chromosome 9q34.



**Table 2** Results of multiple predictor linear regression with maximum tumour diameter as the outcome

Covariate	B	SE <sub>B</sub>	p Value
<i>NF2 related vestibular schwannoma and sporadic vestibular schwannoma</i>			
Age at presentation (per year)	-0.04	0.01	<0.001
Gain of chromosome 9q34	-1.16	0.48	0.017
<i>Sporadic vestibular schwannoma only</i>			
Age at presentation (per year)	-0.04	0.01	0.004
Gain of chromosome 9q34	-1.25	0.52	0.019

These covariates were informative predictors when NF2 related and sporadic vestibular schwannomas were considered in aggregate, and also when only sporadic tumours were considered (table 2). After accounting for age at presentation, tumours with gain on chromosome 9q34 were an average of 1.2 cm in diameter smaller than tumours without detectable gain on chromosome 9q34. The model  $r^2$  for all tumours was 0.25; logarithmic transformation of tumour diameter did not improve model fit. Loss of chromosome Xq24-25 occurred in one patient with a 6 cm diameter tumour; this genetic change contributed to the model but was excluded as an outlier. There were not significant correlations between the number of non-chromosome 22 changes and either Ki67 or PDGF (Ki67: Pearson's  $r^2 = 0.06$ ,  $p = 0.19$ ; PDGF: Pearson's  $r^2 = 0.01$ ,  $p = 0.61$ ).

## DISCUSSION

This is the first large scale analysis of vestibular schwannoma tumours by CGH. We found that 43% of primary vestibular schwannoma tumours had a change in copy number (loss/gain). Not surprisingly, the predominant alteration was loss on chromosome 22, detected in 24% of tumours. As 40–60% of the tumours would be expected to have inactivating *NF2* mutations,<sup>10–13</sup> this highlights one of the shortcomings of CGH. CGH is specifically designed to identify regions of amplification or deletion, but the technique only detects changes greater than 5 to 10 Mb.<sup>29</sup> Regions smaller than 5 to 10 Mb, and aberrations that do not change copy number (such as translocations and inversions), will not be detected. This may explain the lack of detectable loss on chromosome 22 in the 10 NF2 related samples, including four in which LOH was detected.

Nonetheless, LOH was found in 10 of 12 sporadic vestibular schwannomas, similar to the percentage in sporadic meningiomas using CGH.<sup>30</sup> This suggests that chromosome 22 losses in NF2 related tumours are smaller than those in sporadic tumours, where total loss of chromosome 22 may be more common. Alternatively, the NF2 related tumour samples may have lost the wild-type allele, but duplicated the mutant allele. If true, this suggests that there may be a different mechanism for inactivation of the second *NF2* allele in NF2 related from sporadic vestibular schwannomas. Interestingly, the three NF2 related tumours with changes showed gain rather than loss of copy number: two tumours with gains on 9q34 and on 16q23-qter, and one with multiple gains on 10q26, 17q23-qter, and chromosome 19.

Some of these changes have previously been reported in vestibular schwannomas.<sup>21</sup> Gain on 17q is interesting because a similar finding has been reported in malignant peripheral nerve sheath tumours (MPNST). MPNSTs occur in NF1 and are also thought to arise from the Schwann cell. Gain on 17q

in conjunction with gain on 7p15-21 is associated with a significantly lower survival rate in NF1.<sup>31</sup>

Other common regions of loss and gain in both vestibular schwannoma tumours and MPNSTs are gain on 9q34, loss on 13q21-22, and loss on 11q (a larger region of loss on 11q occurs in MPNSTs, but it includes the 11q21-22 region which is lost in vestibular schwannomas).<sup>32</sup> These similarities could suggest that there are common pathways between the two tumour types that arise from clinically distinct diseases. However, this is not apparent from current knowledge of the cell biology of the *NF1* and *NF2* gene products. Neurofibromin acts as a GTPase activating protein in Schwann cells,<sup>33</sup> so that loss of neurofibromin leads to increased Ras activity and increased cell growth.<sup>34</sup> Conversely, merlin suppresses the Ras induced transformation of fibroblasts.<sup>35</sup>

An unexpected finding in this study was that 10% of the vestibular schwannomas had gain of 9q34. This gain is commonly seen in a variety of solid tumours, including colorectal carcinomas,<sup>36</sup> prostate cancer (stage pT2NO),<sup>37</sup> sporadic parathyroid adenomas,<sup>38</sup> metastatic lung adenocarcinomas,<sup>39</sup> T cell non-Hodgkin's lymphomas,<sup>40</sup> and childhood adrenal cortical tumours.<sup>23</sup> Using NCBI locus link, we identified a number of interesting genes in this region, including Ral guanine nucleotide dissociation stimulator (*RALGDS*) and *Netrin G2*. *RALGDS* is particularly active on the RAS related family members of ral A and ral B GTPases.<sup>41</sup> This is interesting because the ERM proteins (of which merlin is a member) have been linked to the Rho-GTPase signalling network,<sup>42</sup> and merlin itself has been postulated to act upstream of Rho and Rac in human fibroblasts.<sup>18</sup> *Netrin G2* is predicted to produce proteins related to cell signalling/communication, nucleic acid management, or cell structure/motility.<sup>43</sup> This also has interesting implications for merlin and its proposed function as a membrane organiser/linker to the cytoskeleton. *Netrin G2* products may influence pathways involving merlin, and consequently its role as a tumour suppressor.

Mathematical modelling of vestibular schwannoma development in NF2 patients suggests that more than two hits may be necessary for the development of this tumour in NF2.<sup>44</sup> A potential third genetic "hit" in vestibular schwannoma is also consistent with the *NF2* mouse model, which requires genetic alteration in addition to bi-allelic inactivation of *NF2* for tumorigenesis of schwannomas and other tumours.<sup>45</sup> A gene on 9p may well be a candidate for the site of a third mutational event. It is intriguing that gain on 9q34 was significantly associated with tumour diameter. This observation merits further investigation, although tumour diameter is not a good surrogate for tumour growth rate.<sup>46</sup> The two tumours with gain on 10q26 had been clinically classified as "fast growing."

Two NF2 patients had undergone radiation treatment. It is intriguing that each had CGH changes (and one had the most chromosomal aberrations), compared with one in eight of the NF2 patients who did not have radiation treatment. NF2 patients have lower success rates with stereotactic radiosurgery, and there is a significant risk of malignant transformation after such treatment.<sup>47</sup> Additional study of a larger number of postirradiated tumours is needed to characterise genetic damage to vestibular schwannomas after radiotherapy.<sup>48</sup>

## Conclusions

We have identified various regions other than chromosome 22 that may be involved in Schwann cell tumorigenesis. Gain on 9q34, although found in only 10% of tumours, may be more prevalent given the limitations of CGH as a diagnostic tool. Gain on 9q34 has been identified in other tumours,

which gives credence to its involvement in the progression of a Schwann cell to a schwannoma. Our findings should be verified using techniques that can detect smaller genetic changes, such as microarray-CGH, and perhaps in conjunction with a more detailed study of the chromosome 9q34 region.

#### Authors' affiliations

**C Warren, L A James, J M Varley**, Cancer Research UK Department of Cancer Genetics, The Paterson Institute for Cancer Research, Manchester, UK

**R T Ramsden**, ENT Department, Manchester Royal Infirmary, Manchester

**A Wallace, D G Evans**, Academic Unit of Medical Genetics and Regional Genetics Service, St Mary's Hospital, Manchester

**M E Baser**, Los Angeles, California, USA

#### REFERENCES

- 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**:841-6.
- Evans DGR, Huson SM, Donnai D, et al. A clinical study of type 2 neurofibromatosis. *Q J Med* 1992;**84**:603-18.
- Parry DM, Eldridge R, Kaiser-Kupfer MI, et al. Neurofibromatosis 2 (NF2): clinical characteristics of 63 affected individuals and clinical evidence for heterogeneity. *Am J Med Genet* 1994;**52**:450-61.
- Mautner V-F, Lindenau M, Baser ME, et al. The neuroimaging and clinical spectrum of neurofibromatosis 2. *Neurosurgery* 1996;**38**:880-6.
- Rouleau GA, Merel P, Lutchman M, et al. Alteration in a new gene encoding a putative membrane-organizing protein causes neuro-fibromatosis type 2. *Nature* 1993;**363**:515-21.
- Trofatter JA, MacCollin M, 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.
- Jacoby LB, MacCollin M, Barone R, et al. Frequency and distribution of NF2 mutations in schwannomas. *Genes Chromosomes Cancer* 1996;**17**:45-55.
- Jacoby LB, MacCollin M, Louis DN, et al. Exon scanning for mutation of the NF2 gene in schwannomas. *Hum Mol Genet* 1994;**3**:413-19.
- Welling DB. Clinical manifestations of mutations in the neurofibromatosis type 2 gene in vestibular schwannomas (acoustic neuromas). *Laryngoscope* 1998;**108**:178-89.
- Merel P, Hoang-Xuan K, Sanson M, et al. Screening for germline mutations in the NF2 gene. *Genes Chromosomes Cancer* 1995;**12**:117-27.
- Parry DM, MacCollin MM, Kaiser-Kupfer MI, et al. Germ-line mutations in the neurofibromatosis 2 gene: correlations with disease severity and retinal abnormalities. *Am J Hum Genet* 1996;**59**:529-39.
- Rutledge MH, Andermann AA, Phelan CM, et al. Type of mutation in the neurofibromatosis type 2 gene (NF2) frequently determines severity of disease. *Am J Hum Genet* 1996;**59**:331-42.
- Evans DGR, Trueman L, Wallace A, et al. Genotype/phenotype correlations in type 2 neurofibromatosis (NF2): evidence for more severe disease associated with truncating mutations. *J Med Genet* 1998;**35**:450-5.
- Tsukita S, Yonemura S, Tsukita S. ERM (ezrin/radixin/moesin) family: from cytoskeleton to signal transduction. *Curr Opin Cell Biol* 1997;**9**:70-5.
- Vaheri A, Carpen O, Heiska L, et al. The ezrin protein family: membrane-cytoskeleton interactions and disease associations. *Curr Opin Cell Biol* 1997;**9**:659-66.
- Sainio M, Zhao F, Heiska L, et al. Neurofibromatosis 2 tumour suppressor protein localizes with ezrin and CD44 and associates with actin-containing cytoskeleton. *J Cell Sci* 1997;**110**:2249-60.
- Hirao M, Sato N, Kondo T, et al. Regulation mechanism of ERM (ezrin/radixin/moesin) protein/plasma membrane association: possible involvement of phosphatidylinositol turnover and Rho-dependent signaling pathway. *J Cell Biol* 1996;**135**:37-51.
- Pelton PD, Sherman LS, Rizvi TA, et al. Ruffling membrane, stress fiber, cell spreading and proliferation abnormalities in human schwannoma cells. *Oncogene* 1998;**17**:2195-209.
- Xiao GH, Beeser A, Chernoff J, et al. p21-activated kinase links Rac/Cdc42 signaling to Merlin. *J Biol Chem* 2002;**277**:883-6.
- Sarlomo-Rikala M, El-Rifai W, Lahinen T, et al. Different patterns of DNA copy number changes in gastrointestinal stromal tumours, leiomyomas and schwannomas. *Hum Pathol* 1998;**29**:476-81.
- Antinheimo J, Sallinen SL, Sallinen P, et al. Genetic aberrations in sporadic and neurofibromatosis 2 (NF2)-associated schwannomas studied by comparative genomic hybridization (CGH). *Acta Neurochir (Wien)* 2000;**142**:1099-105.
- Kallioniemi A, Kallioniemi OP, Sudar D, et al. Comparative genomic hybridization for molecular cytogenetic analysis for solid tumors. *Science* 1992;**258**:818-21.
- James LA, Kelsey A, Birch JM, et al. Highly consistent genetic alterations in childhood adrenocortical tumours detected by comparative genomic hybridisation. *Br J Cancer* 1999;**81**:300-4.
- Mitchell ELD, Jones D, White GRM, et al. Determination of the gene order of three loci, CD2, NGFB, and NRAS at human chromosome band 1p13 and refinement of their localisation at the sub-band level by fluorescence in situ hybridisation. *Cytogenet Cell Genet* 1995;**70**:183-5.
- James LA, Mitchell ELD, Menasce L, et al. Comparative genomic hybridisation of ductal carcinoma in situ of the breast: identification of regions of DNA amplification and deletion in common with invasive breast carcinoma. *Oncogene* 1997;**14**:1-7.
- James LA. Comparative genomic hybridisation as a tool in tumour cytogenetics. *J Pathol* 1999;**187**:385-95.
- Hsu SM, Raine L, Fanger H. The use antiavidin antibody and avidin-biotin-peroxidase complex in immunoperoxidase techniques. *Am J Clin Pathol* 1981;**75**:816-21.
- Evans DG, Blair V, Strachan T, et al. Variation of expression of the gene for type 2 neurofibromatosis: absence of a gender effect on vestibular schwannomas, but confirmation of a preponderance of meningiomas in females. *J Laryngol Otol* 1995;**109**:830-5.
- Forozan F, Karhu R, Kononen J, et al. Genome screening by comparative genomic hybridization. *Trends Genet* 1997;**13**:405-9.
- Khan J, Parsa NZ, Harada T, et al. Detection of gains and losses in 18 meningiomas by comparative genomic hybridization. *Cancer Genet Cytogenet* 1998;**103**:95-100.
- Schmidt H, Wurl P, Taubert H, et al. Genomic imbalances of 7p and 17q in malignant peripheral nerve sheath tumours are clinically relevant. *Genes Chromosomes Cancer* 1999;**25**:205-11.
- Mechtersheimer G, Otano-Joos M, Ohl S, et al. Analysis of chromosomal imbalances in sporadic and NF1-associated peripheral nerve sheath tumours by comparative genomic hybridization. *Genes Chromosomes Cancer* 1999;**25**:362-9.
- Kim HA, Rosenbaum T, Marchionni MA, et al. Schwann cells from neurofibromin deficient mice exhibit activation of p21<sup>ras</sup>, inhibition of cell proliferation and morphological changes. *Oncogene* 1995;**11**:325-35.
- Reed N, Gutmann DH. Tumorigenesis in neurofibromatosis: new insights and potential therapies. *Trends Mol Med* 2001;**7**:157-62.
- Tikoo A, Varga M, Vijaya R, et al. An anti-ras function of neurofibromatosis type 2 gene product (NF2/Merlin). *J Biol Chem* 1994;**269**:23387-90.
- Knosel T, Petersen S, Schwabe H, et al. Incidence of chromosomal imbalances in advanced colorectal carcinomas and their metastases. *Virchows Arch* 2002;**440**:187-94.
- Wolter H, Gottfried HW, Mattfeldt T. Genetic changes in stage pT2NO prostate cancer studied by comparative genomic hybridization. *BJU Int* 2002;**89**:310-16.
- Garcia JL, Tardio JC, Gutierrez NC, et al. Chromosomal imbalances identified by comparative genomic hybridization in sporadic parathyroid adenomas. *Eur J Endocrinol* 2002;**146**:209-13.
- Goeze A, Schlun K, Wolf G, et al. Chromosomal imbalances of primary and metastatic lung adenocarcinomas. *J Pathol* 2002;**196**:8-16.
- Renedo M, Martinez-Delgado B, Arranz E, et al. Chromosomal changes pattern and gene amplification in T cell non-Hodgkin's lymphomas. *Leukemia* 2001;**15**:1627-32.
- Albright CF, Giddings BW, Liu J, et al. Characterization of a guanine nucleotide dissociation stimulator for a ras-related GTPase. *EMBO J* 1993;**12**:339-47.
- Matsui T, Maeda M, Doi Y, et al. Rho-kinase phosphorylates COOH-terminal threonines of ezrin/radixin/moesin (ERM) proteins and regulates their head-to-tail association. *J Cell Biol* 1998;**140**:647-57.
- Nagase T, Nakayama M, Nakajima D, et al. Prediction of the coding sequences of unidentified human genes. XX. The complete sequences of 100 new cDNA clones from brain, which code for large proteins in vitro. *DNA Res* 2001;**8**:85-95.
- Woods R, Friedman JM, Evans DGR, et al. Exploring the "2-Hit Hypothesis" in NF2: tests of 2-hit and 3 hit models of vestibular schwannoma development. *Genet Epidemiol* 2003;**24**:265-72.
- Giovannini M, Robanus-Maandag E, van der Valk M, et al. Conditional biallelic NF2 mutation in the mouse promotes manifestations of human neurofibromatosis type 2. *Genes Dev* 2000;**14**:1617-30.
- Moyhuddin A, Yokura EA, Evans DG, et al. Is clinical growth index a reliable predictor of tumour growth in vestibular schwannomas? *Clin Otolaryngol* 2003;**28**:85-90.
- Baser ME, Evans DGR, Jackler RK, et al. Malignant peripheral nerve sheath tumours, radiotherapy and neurofibromatosis 2. *Br J Cancer* 2000;**82**:998.
- Shin M, Ueki K, Kurita H, et al. Malignant transformation of a vestibular schwannoma after gamma knife radiosurgery. *Lancet* 2002;**360**:309-10.