A PDGFRα promoter polymorphism, which disrupts the binding of ZNF148, is associated with primitive neuroectodermal tumours and ependymomas

C De Bustos, A Smits, B Strömberg, V P Collins, M Nistér, G Afink

Background: Platelet derived growth factor receptor α (PDGFRα) expression is typical for a variety of brain tumours, while in normal adult brain PDGFRα expression is limited to a small number of neural progenitor cells. The molecular mechanisms responsible for the PDGFRα expression in tumours are not known, but in the absence of amplification, changes in transcriptional regulation might be an important factor in this process. Methods and results: We have investigated the link between single nucleotide polymorphisms (SNPs) within the PDGFRα gene promoter and the occurrence of brain tumours (medulloblastomas, supratentorial primitive neuroectodermal tumours (PNETs), ependymal tumours, astrocytomas, oligodendrogliomas, and mixed gliomas). These SNPs give rise to five different promoter haplotypes named H1 and H2α–δ. It is apparent from the haplotype frequency distribution that both PNET (10-fold) and ependymoma (6.5-fold) patient groups display a significant over-representation of the H2δ haplotype. The precise functional role in PDGFRα gene transcription for the H2δ haplotype is not known yet, but we can show that the H2δ haplotype specifically disrupts binding of the transcription factor ZNF148 as compared to the other promoter haplotypes. Conclusions: The specific over-representation of the H2δ haplotype in both patients with PNETs and ependymomas suggests a functional role for the ZNF148/PDGFRα pathway in the pathogenesis of these tumours.
regulation of this gene. Joosten et al. have characterised a number of polymorphic sites within the PDGFRA promoter, which can be grouped into five haplotypes (H1, H2α, H2β, H2γ, and H2δ; fig 1). These promoter haplotypes displayed large differences in their ability to drive reporter gene expression when tested in human U2-OS osteosarcoma cells. In addition, the promoter haplotypes with low transcriptional activity were under-represented, whereas those with high transcriptional activity were over-represented in patients with neural tube defects compared to a normal population. The transcription factors involved in this haplotype specific PDGFRA promoter regulation remain unknown.

In our current study we have investigated the link between PDGFRA promoter polymorphisms and brain tumour development. For this purpose we have analysed a panel of low grade astrocytomas, ependymal tumours, medulloblastomas, PNETs, mixed gliomas, and oligodendrogliomas. Our results demonstrate a characteristic over-representation of the H2 δ promoter haplotype in both PNETs and ependymomas. In addition, analysis of haplotype specific binding of transcription factors to the PDGFRA promoter demonstrated that in comparison with the other promoter haplotypes, the H2 δ haplotype promoter specifically fails to bind the transcription factor ZNF148.

METHODS

Tumour and control DNA

The tumour (and paired blood when available) samples originate from the Karolinska Hospital in Stockholm, the Uppsala University Hospital in Uppsala, and the Sahlgrenska University Hospital in Gothenburg. Classification of the tumours was performed according to the most recent WHO classification. We used the following tumour samples (mean age, range low–high): 40 medulloblastomas (10.0, 0.6–38), 12 PNETs (8.4, 0.2–23), 14 astrocytomas grade II (33.9, 9–53), 14 ependymomas (40.8, 14–72), eight myxopapillary ependymomas (39.4, 22–66), seven subependymomas (52.4, 39–69), one anaplastic ependymoma (7), 20 oligodendrogliomas (44.6, 24–73), eight anaplastic oligodendroglia (50.3, 7–73), 10 oligoastrocytomas (35.6, 11–70), and four anaplastic oligoastrocytomas (43.3, 25–64). The control group blood samples originated from 91 anonymous healthy blood donors at the Uppsala University Hospital.

The control sample blood DNA was extracted according to the protocol described by Miller et al., while tumour and paired blood DNA samples were prepared as described previously.

Ethical permission was obtained from the ethical boards of Uppsala University Hospital, Karolinska Hospital, and Sahlgrenska Hospital, as well as from the local research ethical committee at Addenbrooke’s Hospital.

PCR amplification and nucleotide sequence analysis

Samples (20–100 ng of genomic DNA were amplified in a 30 μl volume containing a primer pair (4 pmol each primer), 100 mM deoxyribonucleoside triphosphates, 1 U AmpliTaq Gold (PE Biosystems, Foster City, CA, USA), and 1×PCR buffer with 3 mM MgCl₂ (PE Biosystems). The PDGFRA promoter single nucleotide polymorphisms (SNPs) were amplified in two separate reactions with the following primer pairs (nucleotide (nt) numbering according to GenBank accession X80389): PDGFRAp-1562F: 5′-TTT GGT CCC GCT CAT TT-3′ and PDGFRAp-1340R: 5′-ATG TCA GCC TCG AAA ACA GG-3′ (nt 566–790); PDGFRAp-1110F: 5′-TCA GAG AGC GAT GAA GGT GA-3′ and PDGFRAp-759R: 5′-TGT TTT TCG GGG TTA TCT GG-3′ (nt 1021–1371).

Aliquots (10 μl) of the PCR product were checked for size by agarose gel electrophoresis and the remaining product was treated (45 min, 37°C) with 1 U exonuclease I (New England Biolabs, Beverly, MA, USA) and 2 U shrimp alkaline phosphatase (Roche, Penzberg, Germany). After an enzyme inactivation step (15 min, 80°C), 4 μl of the PCR product was directly sequenced using BigDye terminator chemistry (PE Biosystems) and one of the PCR primers. The sequencing reactions were run on an ABI 377 automated sequencer (PE Biosystems) and subsequently analysed using Sequence Navigator Version 1.01 (PE Biosystems).

Cell culture and transfections

The PDGFRA promoter-luciferase plasmids have been described elsewhere, and the β-actin promoter-LacZ plasmid was a gift from Dr R de Groot, University of Utrecht. The cell lines D324 Med and NIH3T3 were grown in Dulbecco’s modified Eagle medium and the cell line PFSK-1 was grown in RPMI with in both cases the addition of 10% fetal calf serum. The day before transfection, cells were plated in a six well dish at a cell density of 175,000/well (D324 Med), 275,000/well (PFSK-1), and 300,000/well (NIH3T3). PDGFRA promoter-luciferase plasmid DNA was isolated using the Qiagen High Speed midi-prep kit (Qiagen, Hilden, Germany). DNA from two independent isolations was used in the transfection assays. Transfections were performed using Fugene6 (Roche), following the manufacturer’s instructions. Luciferase and β-galactosidase levels were determined as described.

Electrophoretic mobility shift assay (EMSA)

Nuclear extracts from PFSK-1 cells were prepared as described. Oligonucleotides for EMSA were synthesised.

![Figure 1](http://jmg.bmj.com/)

**Figure 1** Schematic drawing of the PDGFRA promoter polymorphisms. Nine different polymorphic sites (vertical bars, numbers above refer to the position relative to the transcriptional initiation site; GenBank accession number is X80389) can be identified within the PDGFRA promoter region, of which seven are SNPs (numbered below). These polymorphisms can be grouped into five haplotypes, as indicated by the sequence. The arrows represent the location of the PCR primers used in the present analysis. Adapted from Joosten et al.
PDGFRA promoter SNPs associated with brain tumours

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Medulloblastoma</th>
<th>PNET(^1)</th>
<th>Ependymal tumour</th>
<th>Diffuse astrocytoma</th>
<th>Mixed glioma/oligodendroglioma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H1</strong></td>
<td>36 0.1978</td>
<td>18 0.2250</td>
<td>6 0.2500</td>
<td>10 0.1667</td>
<td>3 0.1071</td>
<td>18 0.2143</td>
</tr>
<tr>
<td><strong>H2α</strong></td>
<td>123 0.6758</td>
<td>44 0.5500</td>
<td>14 0.5833</td>
<td>41 0.6833</td>
<td>19 0.6786</td>
<td>55 0.6548</td>
</tr>
<tr>
<td><strong>H2β</strong></td>
<td>13 0.0714</td>
<td>0 0.1500</td>
<td>0 0.0000</td>
<td>4 0.0667</td>
<td>4 0.1429</td>
<td>9 0.1071</td>
</tr>
<tr>
<td><strong>H2γ</strong></td>
<td>7 0.0385</td>
<td>2 0.0250</td>
<td>0 0.0000</td>
<td>2 0.0333</td>
<td>2 0.0714</td>
<td>2 0.0238</td>
</tr>
<tr>
<td><strong>H2δ</strong></td>
<td>3 0.0165</td>
<td>4 0.0500</td>
<td>4 0.1667(^2)</td>
<td>3 0.0500</td>
<td>0 0.0000</td>
<td>0 0.0000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>182 1.0000</td>
<td>80 1.0000</td>
<td>24 1.0000</td>
<td>60 1.0000</td>
<td>28 1.0000</td>
<td>84 1.0000</td>
</tr>
</tbody>
</table>

Figure 2  PDGFRA promoter haplotype/genotype frequencies for the control and tumour groups. (A) Distribution of PDGFRA promoter haplotype frequencies. The haplotype frequency distribution of the PNET group differs significantly from the control group \(p = 0.014\). Within this PNET group, the H2δ haplotype is 10-fold over-represented compared to the controls \(p = 0.004\). The H2δ haplotype is also over-represented in the grade II ependymomas \(p = 0.032\). (B) Distribution of the SNPs4 genotype frequencies. The SNPs4 genotype frequency for the ependymal tumours is significantly different from the control group, with an over-representation of the AC haplotype \(p = 0.047\). This over-representation is even more apparent in the ependymal tumour group \(p = 0.007\). The H2δ homozygous genotype (AA) shows a tendency to be over-represented in PNETs \(p = 0.066\).

Table A

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Medulloblastoma</th>
<th>PNET(^9)</th>
<th>Ependymal tumour(^4)</th>
<th>Diffuse astrocytoma</th>
<th>Mixed glioma/oligodendroglioma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AA</strong></td>
<td>1 0.0110</td>
<td>2 0.0500</td>
<td>2 0.1667</td>
<td>0 0.0000</td>
<td>0 0.0000</td>
<td>0 0.0000</td>
</tr>
<tr>
<td><strong>AC</strong></td>
<td>1 0.0110</td>
<td>0 0.0000</td>
<td>0 0.0000</td>
<td>3 0.1000</td>
<td>0 0.0000</td>
<td>0 0.0000</td>
</tr>
<tr>
<td><strong>CC</strong></td>
<td>89 0.9780</td>
<td>38 0.9500</td>
<td>10 0.8333</td>
<td>27 0.9000</td>
<td>14 1.0000</td>
<td>42 1.0000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>91 1.0000</td>
<td>40 1.0000</td>
<td>12 1.0000</td>
<td>30 1.0000</td>
<td>14 1.0000</td>
<td>42 1.0000</td>
</tr>
</tbody>
</table>

Table B

as single oligonucleotides (DNA Technology, Aarhus, Denmark). They were pairwise mixed at equimolar amounts, incubated for 5 min at 100°C, and annealed using a decreasing temperature gradient. The following sequences represent only one strand of the complementary oligonucleotides (nt 1046–1065 of GenBank accession X80389): SNP4C: 5’-CTC CCC TCC CCC GCT GTC GC-3’; SNP4A: 5’-CTC CCC TCC CCC GCT GTC GC-3’; SNP4M1: 5’-CTC CCC TCA CCC GCT GTC GC-3’; SNP4M2: 5’-CTC CCC TCC CCC ACT GTC GC-3’.

A double stranded consensus SP1 oligonucleotide (5’-ATT CGA TCG GGG CGG GGC GAG C-3’) was purchased from Promega (Madison, WI, USA).

Double stranded oligonucleotides were labelled with polynucleotide kinase (New England Biolabs, Beverly, MA, USA) and \(\gamma^{32}\)P-ATP (Amersham, Upsala, Sweden). A 1–2 μl sample of nuclear extract was used in a 20 μl EMSA reaction containing 20 mM Hepes pH 7.9, 100 mM NaCl, 35 mM KCl, 5 mM MgCl\(_2\), 1 mM DTT, 10% glycerol, 1 μg poly(dI-dC),poly(dI-dC) (Amersham Biosciences, Upsala, Sweden), and 0.01 pmol labelled oligonucleotide. The reactions were incubated for 20 min at room temperature and subsequently analysed on a 4% native polyacrylamide gel.

MatInspector analysis

Analysis of the SNP4 containing sequence (nt 1046–1065 of GenBank accession X80389) for putative transcription factor binding sites was performed using Genomatix MatInspector professional release 6.2.1.\(^{10}\) We used a threshold of 0.75 for core similarity and the optimised setting for matrix similarity.
RESULTS
PDGFRA promoter haplotypes and genotypes in human brain tumours

The PDGFRA promoter region contains a number of polymorphisms, which can be organised into two subfamilies, H1 and H2, as shown in fig 1. The H2-subfamily can be further divided into four variants, thus creating a total of five different haplotypes. Since previous studies have indicated that the PDGFRA promoter haplotype distribution may be geographically specific, we first analysed the PDGFRA promoter haplotype distribution in a Swedish population. For this purpose we amplified the PDGFRA promoter region containing SNP1 through SNP6 (which enables us to distinguish all five haplotypes) from 91 control samples and determined the haplotypes by nucleotide sequence analysis. As shown in fig 2A, more than 87% of the haplotypes are either H1 or H2α, while the H2β, H2γ, and H2δ haplotypes account for small fractions of the total. The nucleotide sequence analysis of the control samples did not give any indications of the existence of additional polymorphisms in the promoter region of additional haplotypes.

Subsequent analysis of the tumour samples (fig 2A) shows that the overall haplotype distribution of PNETs deviates from the control group (Fisher exact test, \( p = 0.014 \)), with a more than 10-fold over-representation of the H2δ haplotype (Fisher exact test, \( p = 0.004 \)). The ependymomas also display a 6.5-fold over-representation of the H2δ haplotype (Fisher exact test, \( p = 0.032 \)). No H2δ haplotypes were detected among the diffuse astrocytomas or mixed gliomas/oligodendrogliomas.

The five PDGFRA promoter haplotypes can theoretically give rise to 15 different genotypes. With the exception of the rare H2δ/H2δ, H2β/H2β, H2δ/H2δ, H2γ/H2γ, and H2γ/H2δ genotypes (expected frequencies of 2.2%, 0.5%, 0.2%, 0.2%, and 0.1%, respectively, based on the control sample population haplotype frequencies), all other genotypes were detected in our control sample population (data not shown). Although our statistical analysis is hampered by the low frequencies of most genotypes (the H1/H2α/H2α/H2α/H2α/H2α genotypes make up 86.9% of all genotypes), we generally do not see any differences in genotype frequencies between tumour groups and controls (data not shown), with the exception of the H2δ-containing genotypes. The H2δ haplotype is represented by an A at the SNP4 position (fig 1). As shown in fig 2B, we do observe a significant over-representation of H2δ heterozygotes (AC) in the ependymal tumour group compared to controls (Fisher exact, \( p = 0.047 \)), which is exclusively caused by the H2δ heterozygote over-representation in the ependymomas (Fisher exact, \( p = 0.007 \)). In addition, the PNETs show a tendency of H2δ homozygote (AA) over-representation (Fisher exact, 0.066).

We have no indications that our haplotype and genotype data are affected by tumour specific deletions of PDGFRA. Analysis of the paired blood (where available) for the homozygous medulloblastoma (9 of 17) and PNET (5 of 9) samples resulted in the same haplotype/genotype as the corresponding tumour material (data not shown). Thus, in most (if not all) cases the tumour PDGFRA promoter haplotype/genotype also represents the normal genetic constitution of the patient.

Analysis of haplotype specific PDGFRA promoter activity

To investigate whether the H2δ haplotype confers a different activity on the PDGFRA promoter (as compared to the other haplotypes), we transfected all five haplotype specific PDGFRA promoter-luciferase plasmids into the medulloblastoma cell line D324 Med and the PNET cell line PFSK-1, and measured the promoter activity. As shown in fig 3, we did not see any difference in activity between the plasmids. The same result was obtained when the plasmids were transfected into the mouse fibroblast cell line NIH3T3.

Differential protein binding to the SNP4 region

The H2δ haplotype has a unique A nucleotide at the SNP4 position, compared to the C nucleotide at this position for all the other haplotypes (fig 1). We designed double stranded oligonucleotides for this region to identify possible differences in protein binding between the SNP4-C and SNP4-A(H2δ) promoter haplotype. EMSAs, using nuclear extracts from PFSK-1 cells, and an SNP4-C oligonucleotide probe, showed a distinct protein-DNA complex (arrow, fig 4A). This protein-DNA complex disappears upon competition with an unlabelled SNP4-C oligonucleotide but not with an SNP4-A(H2δ) oligonucleotide. The inability of the SNP4-A(H2δ) oligonucleotide to bind this protein is also demonstrated in fig 4B, where no DNA-protein complex can be detected with the SNP4-A(H2δ) oligonucleotide as probe. In addition, the protein-DNA complex is zinc dependent, since the zinc chelator phenanthroline completely prevents the formation of the complex (fig 4A, lanes 4 and 5).

We used the Genomatix MatInspector protocol to identify transcription factors which could potentially bind to the SNP4 containing sequence (fig 4C). Using the SNP4-C oligonucleotide sequence as input, we identified ZNF148 and NGFI-C as potential transcription factors binding to this region. Using the SNP4-A(H2δ) oligonucleotide as input sequence we only identified a potential binding site for NGFI-C. Thus, it appears that the putative ZNF148 binding factor is ZNF148. The addition of a ZNF148 antibody abolished much of the typical DNA-protein complex and also resulted in a “supershifted” complex (fig 4B, lane 4). In addition, an unlabelled oligonucleotide containing a mutation in one of the essential five cytidines in the ZNF148
binding sequence (M1, fig 4C) did not compete with the labelled SNP4-C oligonucleotide for ZNF148 binding (fig 4B, lane 5), indicating a lack of affinity of ZNF148 for the M1 sequence. An oligonucleotide containing a mutation in a non-essential nucleotide (M2, fig 4C) fully competed for ZNF148 binding (fig 4B, lane 6), indicating a strong affinity of ZNF148 for the M2 sequence. In addition, ZNF148 did not display affinity for an oligonucleotide containing a consensus SP1 sequence.

**DISCUSSION**

In this study we have examined the association of PDGFRα gene promoter polymorphisms with the occurrence of brain tumours. We initially set out to investigate the possibility of geographically specific differences in PDGFRA haplotype frequency distribution by analysing a Swedish control sample population. If we compare our data with those generated from a Dutch control population, we do see significant differences for the two major haplotypes H1 and H2α but not for any of the other haplotypes. The H1 frequency is lower (0.1978 vs 0.3052, p = 0.023, χ² test H1 vs other haplotypes) and the H2α frequency is higher (0.6758 vs 0.5714, p = 0.049, χ² test H2α vs other haplotypes) in the Swedish control group compared to the Dutch. Our Swedish H1 frequency is also much lower than a recently published H1 frequency of 0.3189 in a Hispanic population along the Texas-Mexico border. The polymorphic SNP4 nucleotide has been shaded. Also indicated are the positions of the oligonucleotide mutations M1 and M2 that have been used in the experiment shown in fig 4B. Numbers refer to the position relative to the transcription start site (GenBank accession no. X80389).

**Figure 4.** Characterisation of PDGFRA promoter haplotype specific protein binding. (A) Nuclear extracts from PFSK-1 cells were incubated with a radiolabelled SNP4-C oligonucleotide. A 200-fold molar excess of either unlabelled SNP4-C or SNP4-A oligonucleotide was added as indicated (lanes 2 and 3). In lanes 4 and 5 the nuclear extracts were incubated with either the zinc chelator phenantroline or its solvent (ethanol) as described. The arrow indicates the specific protein-DNA complex formed. (B) Nuclear extracts from PFSK-1 cells were incubated with a radiolabelled SNP4-C or SNP4-A oligonucleotide. A 200-fold molar excess of unlabelled oligonucleotides or 1 μl of a ZNF148 antibody was added where indicated. The lower arrow indicates the DNA-ZNF148 complex, while the upper arrow indicates the DNA-ZNF148-antibody complex (supershift). (C) Sequence alignment of the SNP4 oligonucleotide sequence (PDGFRA promoter) and the consensus sequences for NGFI-C (NGFIC.01) and ZNF148 (ZBP89.01) binding sites derived from the MatInspector database. Above the SNP4 oligonucleotide sequence is a more stringent ZNF148 consensus binding sequence. The polymorphic SNP4 nucleotide has been shaded. Also indicated are the positions of the oligonucleotide mutations M1 and M2 that have been used in the experiment shown in fig 4B. Numbers refer to the position relative to the transcription start site (GenBank accession no. X80389).
haplotype in the development of these tumours. The most obvious consequence of the H26 promoter haplotype would be that it affects PDGFRA transcription as compared to the other haplotypes. Although we were unsuccessful in demonstrating such a haplotype specific difference in promoter activity in transient transfection assays with promoter-reporter plasmids, we did identify a H26 promoter specific inability to bind the transcription factor ZNF148.

Haplotype specific differences in PDGFRA promoter activity have been demonstrated previously in human U2-OS osteosarcoma cells. Transfected promoter-reporter plasmids showed that the H2x and H26 promoters display much higher transcriptional activities than the L1, H2y, and H26 promoters. We do not have a good explanation as to why no haplotype specific activity difference could be demonstrated in our transfection assays. It may be that the PFSK-1 and D324 Med cells are not capable of showing ZNF148 dependent transcriptional differences, even though both PFSK-1 and D324 Med cells express both PDGFRI and intact ZNF148 (data not shown). However, the haplotype-specific transcription may require transcription factors not available in these cell lines. In addition, the assay used is not capable of showing any differences that require the environment of an intact tissue (to detect spatio-temporal and tissue specific transcriptional processes) or the placement of the promoter within the genome.

ZNF148 (also known as ZFP148, BCOL1, BERF-1, and ZBP-B9) belongs to the Krüppel-like zinc finger proteins and can either activate or repress the activity of a number of different promoters. ZNF148 is highly expressed in the neural tube during mouse embryogenesis. The in vivo function of ZNF148 in the mouse neural tube remains unknown, since mutated ZNF148 alleles are not transmitted in the germ-line, due to the requirement of two functional ZNF148 alleles for the normal development of fetal germ cells. The repressor activity of ZNF148 is often related to its inhibitory action on the activating transcription factor SP1 through competition with adjacent or overlapping binding sequences. Interestingly, a putative SP1 binding site also overlaps with the ZNF148 binding sequence in the PDGFRA promoter (data not shown), but we have no data suggesting a role for SP1 in PDGFRA transcription.

The remarkable aspect of the H26 over-representation is its tumour specificity. What do the PNETs and ependymomas have in common and what makes them different from the other tumours? In this context it is interesting to note that if we combine the haplotype distribution of the astrocytomas and oligodendrogliomas/mixed gliomas, this group is similar to the controls but significantly different from the PNETs and ependymomas (Fisher exact 5×2, p = 0.001 and p = 0.037, respectively). The H26 genotype distribution of the astrocytomas-oligodendroglias/mixed gliomas is also similar to the controls but can be clearly distinguished from the PNETs and ependymomas (Fisher exact 2×2, p = 0.029 and p = 0.007, respectively; fig 2).

One possibility would be that PNETs and ependymomas are derived from the same or similar precursor cell population. The observation that PNETs and ependymomas (but also the other brain tumours studied) frequently show PDGFRA expression may actually reflect the characteristics of their precursor cells. Although it is far from clear what the precursor cells are for these tumours, the primitive neuroectodermal characteristics of PNETs makes it likely that they are derived from neural progenitor cells. It has been shown that both cultured nestin positive neural stem cells and early neuroepithelial cells in vivo express PDGFRA.45 46 PDGF signalling is involved in neuronal differentiation, proliferation, and migration of such stem cells.47–49 Thus, it may be that the H26 haplotype promoter influences the level and/or timing of PDGFRA protein production in the normal progenitors and/or tumour precursors.

In summary, our data demonstrate that both PNET and ependymoma patients display an over-representation of the H26 PDGFRA promoter haplotype. The H26 promoter has a characteristic disruption of the ZNF148 binding site. Together, these results indicate a potential role for the ZNF148/PDGFRA pathway in the etiology of both PNETs and ependymomas. A further investigation of this pathway may lead to a better understanding of the biology of these tumours.

ACKNOWLEDGEMENTS

The authors would like to thank Gudrun Jonasdotir, Hans Garmo, and Jonas Nilsson for statistical advice, Juanita Merchant and David Law for the ZNF148 antibody, Joop van Zoelen for the promoter-lucerase plasmids, and Patrick Buckley for critically reading the manuscript.

Authors’ affiliations

C De Bustos, M Nistér, G Afink, Department of Genetics and Pathology, Uppsala University, Rudbeck Laboratory, 751 85 Uppsala, Sweden
A Smits, Department of Neuroscience, Neurology, Uppsala University, University Hospital, 751 85 Uppsala, Sweden
B Strömberg, Department of Women and Child Health, Uppsala University, University Children’s Hospital, 751 85 Uppsala, Sweden
V P Collins, Department of Pathology, University of Cambridge, Addenbrooke’s Hospital, Cambridge CB2 0QG, UK
M Nistér, G Afink, Department of Oncology-Pathology, Karolinska Institute, Cancer Center Karolinska, R8:05 Karolinska Hospital, 17176 Stockholm, Sweden

This work was supported by the Swedish Children’s Cancer Foundation (GA and MN); the Karolinska Institute Research Fund (GA); the James S. McDonnell Foundation, the Stockholm Cancer Society, and the Juvenile Diabetes Research Foundation (MN); the Samantha Dickson Research Trust, the Carly Watson Ependymoma Fund, Cancer Research UK, and the Jacqueline Seraussi Memorial Foundation for Cancer Research (VPC). CDB is supported by a fellowship from the Department of Education, Universities and Research of the Basque Government.

Competing interests: none declared

REFERENCES


A PDGFRA promoter polymorphism, which disrupts the binding of ZNF148, is associated with primitive neuroectodermal tumours and ependymomas

C De Bustos, A Smits, B Strömberg, V P Collins, M Nistér and G Afink

doi: 10.1136/jmg.2004.024034