mucositis in conjunction with neutropenia can be identified, prophylactic oral penicillin or benzylpenicillin introduced early in the course of the fever may be of benefit. This needs to be assessed prospectively in a randomised control trial.

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Expression of HLA-D Subloci DR and DQ by Breast Carcinomas is Correlated with Distinct Parameters of Favourable Prognosis

Christina A. Brunner, Josef M. Gokel, Gert Riethmüller and Judith P. Johnson

The expression of HLA-D region products HLA-DR, DQ and DP by primary breast carcinomas was examined for its relationship to standard prognostic parameters. A positive correlation was found between the expression of HLA-DR and the differentiation state of the tumour ($P = 0.02$) and the expression of progesterone receptors ($P = 0.002$), two parameters which are associated with good prognosis and with each other. No correlation was seen between these parameters and the expression of HLA-DQ or HLA-DP. In contrast, tumour diameter was inversely correlated with the expression of HLA-DQ ($P = 0.0004$) although no association was observed between this parameter and HLA-DR expression. Essentially all HLA-DQ positive tumours had a diameter of less than 2 cm although these represented only 50% of the tumours of this size examined. These data show that in breast carcinomas HLA class II expression is correlated with several distinct parameters of good prognosis and suggest that HLA-DQ expression may define a subtype of T1 tumours.
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INTRODUCTION

THE HLA-D class II antigens of the major histocompatibility complex (MHC) are highly polymorphic cell surface molecules which control immune recognition through the presentation of foreign antigens to regulatory T cells [1–3]. While constitutive expression of HLA-class II molecules is restricted to B cells, myelomonocytic cells and their precursors, these molecules can be induced on many other cell types by lymphokines [4]. Class II molecules are also found on some types of malignant tumours where their expression has often been shown to be of prognostic relevance. Depending on the type of tumour, the expression of HLA class II antigens can be associated with either good or poor prognosis. Thus, expression of HLA-D region molecules by cutaneous melanoma increases in freqeuncy with tumour progression and is associated with the early occurrence of metastases [5, 6]. In contrast, HLA-class II expression on larynx carcinomas is associated with highly differentiated tumours which have a good prognosis [7]. In some solid tumours, such as colorectal carcinomas, the expression of class II molecules does not appear to have any prognostic significance [8].

Although previous investigations have shown that HLA-D region products are expressed by breast carcinomas, no conclusions could be drawn on the relationship of this to tumour stage or prognostic parameters [9–13]. Given the fact that 20–30% of women with stage I disease develop metastases, it is important to try to establish additional prognostic markers. In the study presented here, the expression of the HLA-D region molecules HLA-DR, HLA-DQ and HLA-DP on primary breast carcinomas has been examined for its correlation with histopathological G-grading, hormone receptor expression, tumour size and lymph-node status, parameters important for predicting disease free interval and overall survival.

Correspondence to J.P. Johnson.
C.A. Brunner, G. Riethmuller and J.P. Johnson are at the Institute for Immunology and J.M. Gokel is at the Institute of Pathology, University of Munich, Goethestrasse 31, 8000 Munich 2, Germany.
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MATERIALS AND METHODS

Specimens

Tumour samples were obtained from 66 patients ranging in age from 34–90 years. 15 patients were premenopausal. The 66 tumours studied included 59 infiltrating ductal carcinomas (19 carcinoma scirrhous, 26 carcinoma simplex, 2 mucinous carcinoma, 4 comedocarcinomas, 6 undifferentiated carcinomas, 2 papillary carcinomas), 2 non-invasive ductal carcinomas and 5 infiltrating lobular adenocarcinomas. Histopathological G-grading was available for 58 tumours. Hormone receptor levels were determinated in 62 patients and clinical staging according to the international TNM system was also available for 65 patients.

The tissue specimens were snap frozen with carbon dioxide or isopentane cooled over liquid nitrogen and stored at −80°C. Serial cryostat sections (5–7 μm) were cut, dried overnight at room temperature and stored at −80°C until use.

Immunohistochemistry

The sections were stained using a 3-step indirect immunoperoxidase procedure [14]. Briefly, sections were incubated with the monoclonal antibody (MAb) (50–100 μl per section) washed in phosphate buffered saline pH 7.4 (PBS) and then incubated with peroxidase-conjugated rabbit anti-mouse antiserum (Dakopatts, Hamburg) diluted in 20% human serum. After washing in PBS, sections were incubated with peroxidase-conjugated swine anti-rabbit antiserum (Dakopatts) diluted in 20% human serum. The peroxidase reaction was developed in 1 mmol/l 3-amino-9-ethylcarbazol (Sigma, St Louis) in 0.1 mol/l acetate buffer pH 4.9 containing 0.00015% H_2O_2. The sections were washed in acetate buffer, counterstained in Mayer's haemalaun and mounted. Tumours were considered positive when more than 10% of the tumour cells were stained. The degree of reactivity was estimated from an examination of the entire section using an amplification of 40×. Serial sections stained with a MAb directed to the common leukocyte antigen CD45 were used to determine the location of infiltrating leukocytes and isotype controls were used to rule out endogenous peroxidase activity and Fc receptor binding. The extent of mononuclear infiltrate was estimated from the CD45 stained sections according to the following criteria: slight = tumours containing isolated CD45 positive cells comprising less than 5% of the total cells in the section and with no secondary lymphoid follicles; medium = CD45 positive cells comprising less than 30% of the total cells and with secondary lymphoid follicles; and heavy = samples with a greater degree of infiltrate.

Steroid hormone receptor assay

Preparation of the cytosol fraction and measurement of oestrogen receptor (ER) and progesterone receptor (PR) by the standard charcoal–dextran method were performed as previously described [15]. Concentrations equal or greater than 10 fmol/mg for ER and PR were considered positive.

Monoclonal antibodies

The specificity of the MAbs used in this study are summarised in Table 1.

Statistical analysis

The Fisher exact probability test was used for statistical comparison of the HLA-class II patterns, histopathological G-grading, hormone receptor levels and TNM-staging.

Table 1. Monoclonal antibodies used in this study

Ref.	MAb*	Isotype	Specificity	Working concentration	Source
16,17	L243	IgG2	DR α	S 1:50	ATCC
17	L227	IgG1	DR βI	S	ATCC
18	TÜ22	IgG1	DQ	S	A.Ziegler
19	Leu10	IgG1	DQ except DQ2	P 1:100	BD
20	B7.21	IgG	DP	S	F.Bach
21	BBM.1	IgG2b	β2 micro	S	ATCC
22	W6/32	IgG2a	HLA-A,B,C	S 1:100	ATCC
	T29/33		CD45	A 1:1000	Hybritech

* All antibodies are directed against non-polymorphic determinants except for Leu10. S = culture supernatant, P = purified antibody, A = ascites, ATCC = American Type Culture Collection, Rockville, BD = Becton–Dickinson.

RESULTS

Expression of HLA-class II subloci and relation to the degree of mononuclear cell infiltrate

The expression of HLA-DR and HLA-DQ products was analysed on 66 carcinomas, and 40 were also examined for HLA-DP. To differentiate tumour cells from infiltrating leukocytes, serial sections were stained with anti-HLA-D MAb and anti-CD45 (the common leukocyte antigen T200) and were carefully compared. The intratumour mononuclear cells were consistently stained with anti-DR and anti-DQ MAbs, and served therefore as an internal positive control on all sections. An example of the staining with anti-CD45 and anti-HLA-DR is shown in Fig. 1. While only isolated cells in the connective tissue are stained with anti-CD45 (Fig. 1a), the tumour cell nests are stained with anti-HLA-DR as can be seen in the boxed area (enlarged) in Fig. 1b.

Two MAbs were used to estimate DR and DQ expression. No differences were found between the staining intensity of the two anti-HLA-DQ MAbs. L243 which reacts with DR α chain [17] showed a similar pattern to L227 which recognises the product of the DR βIII gene [23] although in some tumours L243 staining was slightly broader.

HLA-DR was expressed by 57.6% of the tumours examined, HLA-DQ by 21.2% and HLA-DP by 17.5%. In most cases HLA-DR reactivity was heterogeneous, with both strongly stained and completely negative areas. In positive tumours, the extent of reactivity ranged from 15–95% of the tumour cells. HLA-DQ reactive tumours generally had fewer positive cells and in all cases a heterogeneous staining pattern was observed. The HLA-DQ reactive regions were in most cases also HLA-DR positive. The HLA-DP reactivity was highly heterogeneous. It sometimes resembled the HLA-DQ or the HLA-DR pattern and sometimes only isolated cells were stained. HLA-DQ and HLA-DP positive tumours were always HLA-DR positive although HLA-DQ and DP appeared to be independently expressed. Thus 44.4% of the DQ positive tumours were DP negative and 14.3% of the DP positive tumours were DQ negative. 27 tumours were analysed for HLA-class I or β_2-microglobulin expression and of these, 29.6% were negative. The HLA class I/β_2 negative tumours were also negative for class II expression.

A significant correlation was observed between the presence of a heavy mononuclear cell infiltrate and HLA-DR expression ($P = 0.0106$). However, tumours with a low degree of infiltration were evenly divided between HLA-DR positive and

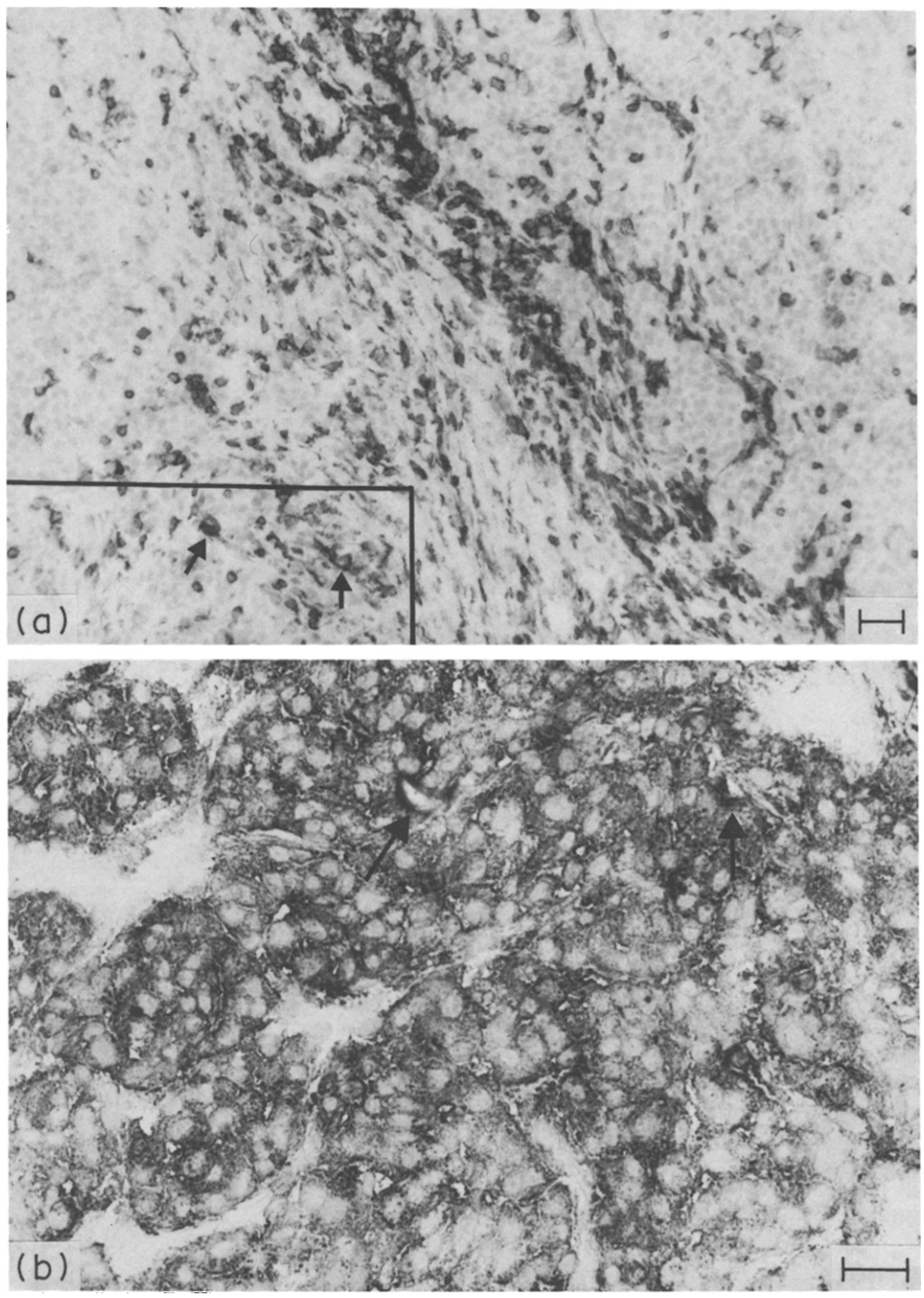

Fig. 1. Comparison of anti-HLA-DR and anti-CD45 reactivity. Serial sections of an invasive ductal carcinoma of the breast stained by MAb T29/33 (a) and by MAb L227 (b). For orientation blood vessels are marked by arrows. Figure 1b is an enlargement of the boxed area in a. (Scale bar = 50 μm.)

negative (Fig. 2) and the majority of the HLA-DQ positive tumours had a strong cellular infiltrate (Fig. 2). No significant correlation was observed between the extent of mononuclear cell infiltration and any of the prognostic parameters investigated. In addition no significant correlation was found between HLA-DP expression and prognostic parameters.

Correlation between HLA-class II expression and the differentiation state of the tumour

Although only one well differentiated tumour (GI) was examined, 39 tumours were characterised as moderately differentiated (GII) and 18 as poorly differentiated (GIII). A statistically significant correlation was found between the expression of HLA-DR and the differentiation state of the tumour, the positive tumours being the most differentiated ($P = 0.02$, Fig. 3). When the HLA-DR positive tumours were subdivided into HLA-DQ positive and HLA-DQ negative tumours, no correlation between expression of this locus and differentiation state was observed (Table 2). No significant correlation was found between HLA-class I expression and tumour differentiation stage (Table 2).

Correlation between HLA-class II expression and hormone receptor expression

A significant correlation was also found between HLA-DR expression and expression of ER and PR (Fig. 4). Although

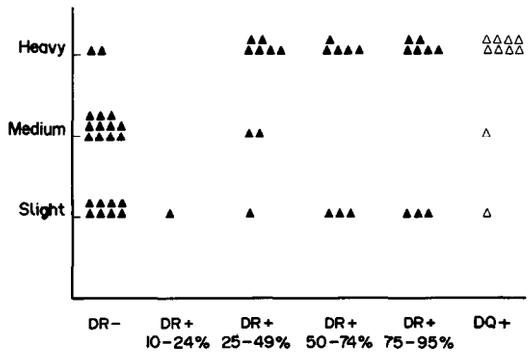

Fig. 2. Expression of HLA-DR and HLA-DQ in relation to the extent of mononuclear cell infiltrate (n = 48 tumours). Each tumour is denoted by a triangle. The open triangles represent DR+DQ+ tumours.

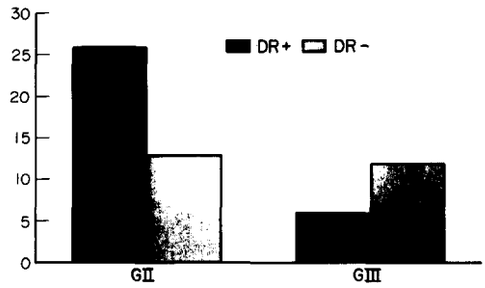

Fig. 3. Correlation of HLA class II expression with tumour differentiation state. GII, n = 39 tumours; GIII, n = 18 tumours. The number of tumours is shown on the y axis.

ER and PR are frequently coexpressed, examination of these receptors separately indicated that the correlation is primarily between HLA-DR and PR expression (P = 0.002). Neither HLA-DQ nor HLA-class I showed significant correlation to receptor expression (Table 2).

Correlation between HLA-class II expression and tumour diameter

No correlation was observed between HLA-DR expression and tumour diameter. However, there was a highly significant inverse correlation between this parameter and the expression of HLA-DQ (P = 0.0004, Fig. 5). Although virtually all HLA-DQ positive tumours were smaller than 2 cm, they represented only 50% of the T_1 tumours in this study.

Table 2. P values for correlation between HLA expression and prognostic parameters

	HLA-DR	HLA-DQ	HLA-ABC
G-grading	0.02	0.44	0.11
ER	0.03	0.52	0.41
PR	0.002	0.42	0.23
T_1 vs. T_{2-4}*	0.44	0.0004	0.17
N_0 vs. $N_{>1}$†	0.84	0.28	ND

* Tumour diameter (TNM classification), † number of involved lymph nodes (TNM classification). ND = not done.

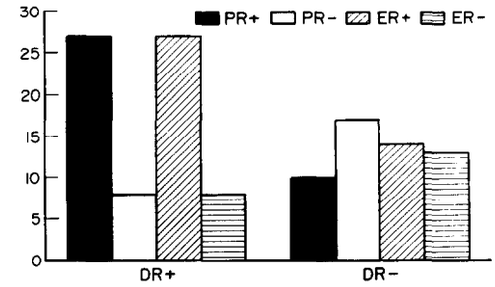

Fig. 4. Correlation of HLA class II expression with oestrogen and progesterone receptor expression. DR− n = 27 tumours; DR+ n = 35 tumours.

No correlation was observed between lymph node status and HLA-class II expression (N_0 vs. N_1 Table 2). Although the patients have only been followed up for 2 years (2–34 months; S.D. 8.94) metastases have been diagnosed in 14 patients and local tumour recurrence in 4. Surprisingly, 3 of the 4 patients who developed local recurrence had class II positive (HLA-DR+DQ−) primary tumours as compared with 5 of the 14 patients with metastases. None of the patients with HLA-DQ positive tumours had developed metastases or local recurrence. A longer follow-up period will be needed to determine if there is any correlation between HLA-DR and HLA-DQ expression and local recurrence or metastases.

DISCUSSION

In breast carcinoma the number of positive lymph-nodes, tumour diameter, state of differentiation and progesterone receptor status are the main parameters used to predict disease-free interval and overall survival [24, 25]. More recently, amplification of the c-*erb*B-2 gene in invasive ductal carcinomas [26] and high levels of cathepsin D [27] have been shown to be associated with poor overall survival.

The results presented here document a statistically significant positive correlation between the expression of the HLA-D region molecules by breast carcinomas and certain parameters predictive of good prognosis. Expression of HLA-DR is associated with a more differentiated state of the tumour and with the expression of progesterone receptors, two parameters which are associated with each other [28]. In addition a highly significant correlation between expression of HLA-DQ and small tumour diameter was observed in the absence of any significant association between this parameter and HLA-DR expression.

The expression of class II antigens on breast carcinoma cell lines can be induced by lymphokines [29]. As these tumours are generally infiltrated by mononuclear cells, lymphokine induc-

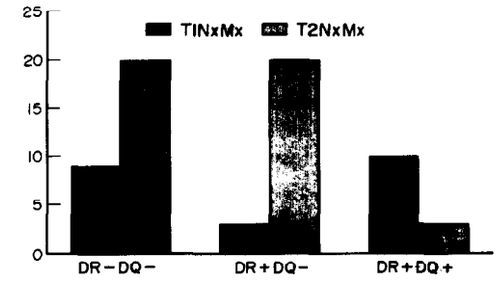

Fig. 5. Correlation of HLA class II expression with tumour diameter. DR−DQ−, n = 29 tumours; DR+DQ−, n = 23 tumours; DR+DQ+, n = 13 tumours.

tion may in part explain the expression of class II antigens by the tumour cells. A significant correlation was observed between the presence of a heavy mononuclear cell infiltration and HLA-DR expression by the tumour cells although the degree of cellular infiltration did not correlate with hormone receptor expression, lymph node metastases or tumour size. 50% of the tumours with only slight mononuclear infiltration were nevertheless HLA-DR positive, suggesting that the degree of infiltration alone cannot be the decisive factor. Although one cannot rule out the role of a particular constellation of lymphokines, produced by a small select cell population, the hormonal environment of the breast may also play a role in HLA-DR expression. In fact prolactin has been shown to induce class II expression in mammary tissue of mice and guinea pigs *in vivo* [30] and in the MCF-7 breast carcinoma cell line *in vitro* [31]. Prolactin may also lead indirectly to PR expression since it increases ER levels on breast cancer cells and ER stimulation leads to induction of PR [32]. Consistent with these observations, prolactin receptor expression is heterogeneous in breast carcinomas and has been shown to be associated with highly differentiated tumours of low malignancy [33]. Although prolactin may be involved in class II induction in breast epithelium, its overall influence on the development and progression of breast carcinomas remains controversial [34].

A prognostic significance of class II expression may reflect a role for the immune system in the development of breast cancer. Studies with transplantable murine tumours have shown that the expression of individual MHC products can play a direct role in tumour growth and metastasis, apparently by controlling immune recognition of tumour antigens [35]. Although a role for the HLA-D region products in cell proliferation or cell–cell adhesion cannot be ruled out [36], the immune recognition of class II restricted tumour antigens may also be important in the control of the growth of human tumours. T cells which support or suppress development of an immune response recognise distinct combinations of class II molecules and antigen epitopes [37, 38] and this could account for the disparate prognostic significance of HLA-class II expression observed in different types of tumours (e.g. melanomas vs. breast carcinomas) as they presumably express different antigens.

The striking correlation observed between HLA-DQ expression and small tumour size may indicate a special role for this molecule in tumour–host interactions, or it may simply reflect an overall high level of class II expression, a situation which has also been shown to be associated with altered functional capacity [39]. Evidence is accumulating that the expression of the HLA-DQ and DR products can be independently regulated [40, 41] suggesting that the expression of DQ by breast carcinomas may reflect a particular differentiation stage of the tumour. The fact that only 50% of the tumours smaller than 2 cm expressed HLA-DQ raises the question of whether its expression will provide an additional prognostic marker in this group of patients.

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Inhibition of the Growth of Cultured Human Meningioma Cells by Recombinant Interferon-α

Jan W. Koper, Ellen C. Zwarthoff, Anne Hagemeijer, Reinder Braakman, Cees J.J. Avezaat, Mats Bergström and Steven W.J. Lamberts

In this paper the results of investigations on the effect of interferon-α (IFN-α) on the growth of meningioma cells in culture is reported. A consecutive series of six meningiomas and one meningioma/neurofibroma derived from a patient with neurofibromatosis type 2 was investigated and it was found that the growth of all seven tumours in response to mitotic stimuli (fetal bovine serum or epidermal growth factor) is strongly inhibited by IFN-α. Maximal response varied between 100% and 70% inhibition of the incorporation of tritiated thymidine. In some cases an inhibitory response was obtained already at very low doses (≤10 U of IFN-α per ml). These results indicate that further clinical investigation of the application of IFN-α to the treatment of meningioma is warranted.
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INTRODUCTION

MENINGIOMAS, tumours arising from the arachnoidial cell-layer surrounding the brain [1] are usually slowly growing, benign tumours. There are strong indications that the cause of meningiomas is the inactivation or loss of both copies of a putative tumour suppressor gene located on the long arm of chromosome 22 [2, 3]. This aetiology is probably shared with one of the inheritable forms of neurofibromatosis (bilateral acoustic neurofibromatosis or neurofibromatosis type 2) [3, 4] and possibly with some glial tumours [3].

The common treatment for meningiomas is surgical removal; however, the recurrence rate is considerable [5] and not all meningiomas are easily accessible to surgery. For these reasons other possibilities for treatment are investigated. The presence of receptors for progesterone in many meningioma tissues [6] has led to a number of research papers dealing with the possibilities of therapy using antiprogestins such as mifepristone [7–9].

Similarly, the discovery of the presence of high concentrations of specific receptors for somatostatin in meningioma membranes [10] has resulted in a number of reports about the effects of somatostatin (analogues) on the growth of meningiomas or cultured meningioma cells [11–13]. Contrary to one report in the literature [11], we have not been able to show inhibitory effects of somatostatin (analogues) on the growth of meningiomas (refs 12 and 13, and J.W.K. *et al.*).

Correspondence to J.W. Koper.
J.W. Koper and S.W.J. Lamberts are at the Department of Medicine, Room Bd 281; E.C. Zwarthoff is at the Department of Pathology; A. Hagemeijer is at the Department of Genetics and R. Braakman and C.J.J. Avezaat are at the Department of Neurosurgery, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands; and M. Bergström is at the Department of Neurology, Uppsala University Hospital, Uppsala, Sweden.
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