Cranioopharyngiomas are among the most common paediatric tumours and are thought to arise from embryonic remnants of Rathke’s pouch. The molecular mechanisms involved in their formation remain elusive and little is known about chromosomal imbalances that could suggest the locations of tumour suppressor or proto-oncogenes involved in the pathogenesis. The paucity of published data on the molecular basis of such tumours prompted this investigation of 20 adamantinomatous and nine papillary craniopharyngiomas for genetic abnormalities by comparative genomic hybridisation (CGH). CGH revealed no DNA copy number changes in any of the 29 primary craniopharyngiomas, regardless of their histological subtype. These data suggest that chromosomal imbalances are a rare event in both adamantinomatous and papillary craniopharyngiomas.

METHODS

Patients and tumours

Twenty nine formalin fixed and paraffin wax embedded biopsy specimens of primary craniopharyngiomas (13 male patients, 16 female; mean age 39.8 years, range 2 to 77 years) were investigated by CGH. These consisted of 20 adamantinomatous craniopharyngiomas (from 10 male and 10 female patients; mean age 33.6 years, range 2 to 77 years) and nine papillary craniopharyngiomas (from three male and six female patients; mean age 53.7 years, range 34 to 75 years). Only tumour portions that had been shown histologically to contain more than 80% tumour cells were included. Where necessary, dissection and trimming of the tissue blocks was undertaken in order to optimise tumour tissue retrieval.

CGH analysis

DNA was isolated by phenol-chloroform extraction according to standard protocols. CGH analysis was undertaken as described previously. Briefly, tumour DNA was labelled with biotin-16-dUTP (Boehringer Mannheim, Mannheim, Germany) and reference DNA from a healthy male donor with digoxigenin-11-dUTP (Boehringer Mannheim) in a standard nick translation reaction. The DNase concentration in the labelling reaction was adjusted in order to reveal an average fragment size of 200–500 base pairs. The labelled DNA fragments were purified from remaining nucleotides by column chromatography.

For CGH, 500 ng of tumour DNA, 300 ng of reference DNA, and 30 µg of human Cot1 DNA (Gibco, Karlsruhe, Germany) were co-precipitated and redissolved in 10 µl of hybridisation buffer. Denaturation of DNA (75°C for five minutes) was followed by a preannealing time of 45 minutes at 37°C. Target metaphase spreads (46,XY), which had been prepared following standard procedures, were denatured separately in 70% formamide/2×SSC for two minutes at 72°C.

Hybridisation was allowed to proceed for three to four days. Post-hybridisation washes were carried out to a stringency of 50% formamide/2×SSC (NaCl/sodium citrate) at 45°C and 0.1×SSC at 60°C. Biotinylated and digoxigenated sequences were detected simultaneously using avidin-FITC (Boehringer Mannheim, 1:200) and anti-digoxigenin-rhodamine (Boehringer Mannheim, 1:40). The slides were counterstained with DAPI and mounted in an antifade solution (Vectashield, Vector Laboratories).

Microscopy and digital image analysis

Separate digitised grey level images of DAPI, FITC, and rhodamine fluorescence were taken with a CCD camera connected to a Leica DMRBE microscope. The image processing was carried out by use of Applied Imaging software. Average green-red ratios were calculated for each chromosome in at least 10 metaphases.

Chromosomal regions with CGH ratio profiles surpassing the 50% CGH thresholds (upper threshold 1.25, lower threshold 0.75) were defined as loci with copy number gains or losses. For the assignment of these gains to chromosomal bands, the signal intensities were compared to the DAPI banding on individual chromosomes. As tumour specimens and normal DNA were not sex matched, X and Y chromosomes were excluded.

RESULTS

CGH revealed no DNA copy number changes in any of the 29 primary craniopharyngiomas, regardless of their histological subtype. Successful completion and the quality of CGH investigation in each case was established by checking the narrowness of the 95% confidence interval as well as the loss of the Y and gain of the X chromosome in tumour material from female patients hybridised on metaphase spreads of a male donor (internal positive control).
Lack of chromosomal imbalance in craniopharyngiomas

DISCUSSION
Craniopharyngiomas are benign tumours that show a bimodal age distribution and arise in two distinct clinicopathological variants: the adamantinomatous and the papillary subtypes. The molecular mechanisms involved in craniopharyngiomas remain elusive. While a genetic susceptibility is not known, there are reports describing the occurrence of craniopharyngiomas in consanguineous siblings as well as in a mother and daughter. To date, cytogenetic (that is, karyotypic) data on only 11 craniopharyngiomas have been published, and have shown multiple chromosomal abnormalities in two cases, both of which involved chromosomes 2 and 12, while the other nine cases presented with normal karyotypes.

In conclusion, our CGH data suggest that chromosomal imbalances are a rare event in primary adamantinomatous and papillary craniopharyngiomas.

ACKNOWLEDGEMENTS
The invaluable help and skilful assistance of Ms Beate Schröder is gratefully appreciated.

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Competing interests: none declared

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Received 19 August 2002
Accepted 4 November 2002

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J Neurol Neurosurg Psychiatry 2003 74: 260-261
doi: 10.1136/jnnp.74.2.260

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