

SHORT REPORT

Lack of chromosomal imbalances in adamantinomatous and papillary craniopharyngiomas

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Craniopharyngiomas 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.

Craniopharyngiomas are benign epithelial, slow growing tumours of the sellar region thought to arise from embryonic remnants of Rathke's pouch.¹ They occur as either an adamantinomatous or a papillary subtype, corresponding histologically to WHO grade I, and they are among the most common childhood brain tumours.¹ It is not known whether there is a genetic susceptibility to these tumours, but—as some craniopharyngiomas have occurred within families or among siblings—there are clues that genetic events may be involved in the pathogenesis.^{2,3} However, as no investigations of genetic aberrations in biopsy tissue from craniopharyngiomas have so far been carried out, little is known about any possible chromosomal imbalances that could suggest the locations of tumour suppressor or proto-oncogenes. The paucity of data on the molecular basis of craniopharyngiomas prompted us to investigate 29 primary craniopharyngiomas by comparative genomic hybridisation (CGH).

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.⁴ 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).

DISCUSSION

Craniopharyngiomas are benign tumours that show a bimodal age distribution and arise in two distinct clinicopathological variants: the adamantinomatous and the papillary subtypes.¹ 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 siblings² as well as in a mother and daughter.³ To date, cytogenetic (that is, karyotypic) data on only 11 craniopharyngiomas have been published,⁵⁻⁸ and have shown multiple chromosomal abnormalities in two cases, both of which involved chromosomes 2 and 12,^{5,7} while the other nine cases presented with normal karyotypes^{6,8}; four additional craniopharyngiomas showed no mutations of the TP53 tumour suppressor gene.⁹ In view of the association of naevoid basal cell carcinoma or Gorlin syndrome with the occurrence of craniopharyngiomas, a recent study was carried out on 22 adamantinomatous craniopharyngiomas. This found no mutations in the Gorlin syndrome gene PTCH, localised on chromosome 9q22.3, while the putative proto-oncogenes encoding the α subunits of the stimulatory (G α) or the inhibitory (Gi2 α) GTP binding proteins on respective chromosome subunits 20q13.2 and 3p21 were also found not to be mutated.¹⁰ Interestingly, a subset of these adamantinomatous craniopharyngiomas turned out to be monoclonal in origin.¹⁰

While it has to be borne in mind that CGH is only sensitive for detecting deletions that are of the order of several megabases in size,¹¹ and that smaller deletions or balanced alterations may thus be missed, the lack of DNA copy number changes in any of our adamantinomatous and papillary craniopharyngiomas is also in accordance with previously published CGH data on low grade cerebral neoplasms: pineocytomas, subependymal giant cell astrocytomas, and pilocytic astrocytomas were reported to show 0,⁴ 0,¹² and 0.3¹³ imbalances per tumour, respectively. In view of our data, and most other molecular data on craniopharyngiomas, one has to assume that chromosomal aberrations do not play a major role in their tumorigenesis, and the only two cytogenetically abnormal cases may have represented tissue culture artefacts.^{5,7}

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

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