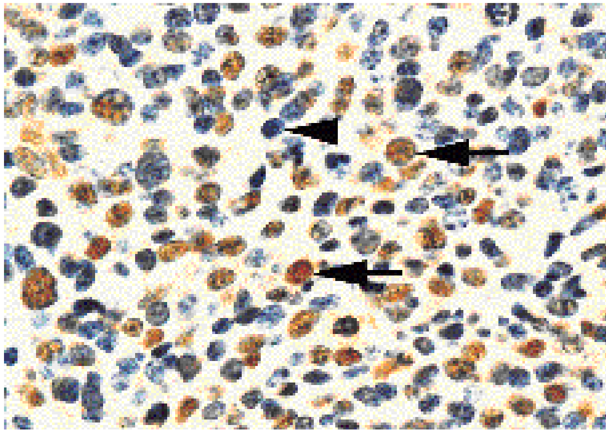

Detection of JC virus early in human medulloblastomas
Immunohistochemical staining with antibody detecting JC virus T-antigen shows nuclear localisation in the majority of tumour cells (arrows). Some tumour cells do not show nuclear staining (arrowhead). (Haematoxylin counterstain. Original magnification, ×400.)

neuronal marker proteins, and anatomical location. JC virus is a polyomavirus infecting more than 80% of the human population early in life. Replication of this virus in brain results in a fatal demyelinating disease, progressive multifocal leucoencephalopathy (PML) seen in immunocompromised individuals.[4] The observations from transgenic mice prompted us to examine paediatric medulloblastomas for a JC virus sequence in tumour tissue and expression of the viral early protein, T-antigen, in cells.

11 formalin-fixed, paraffin-embedded tumour samples from immunocompetent patients were collected from St Christopher's Hospital for Children and Hahnemann University Hospital in Philadelphia; Rhode Island Hospital, Providence; and the University of Virginia Hospitals, Charlottesville. Six of the tumours were histologically classified as desmoplastic and the rest as classical medulloblastomas. Patients' ages ranged from 10 days to 18 years, with the majority being less than 10 years old. Genomic DNA was isolated from paraffin sections and samples were examined by PCR with a pair of primers which amplify the viral genome corresponding to the C-terminal of T-antigen and a region from the viral late gene, VP1. After PCR, samples were analysed by southern blot hybridisation with JC virus-specific radiolabelled oligonucleotide probes.

72% of the samples contained DNA sequences corresponding to the JC virus T-antigen, whereas 90% had DNA sequences from VP1 in the tumour tissue. Control DNA from the cerebellum of five age-matched normal brain necropsy samples were negative for JC virus sequences.

Immunohistochemistry was done on paraffin-embedded tissue sections for JC virus T-antigen[5] in these neoplasms. JC virus T-antigen was detected in the nuclei of tumour cells (figure). About 5–20% of the tumour cells per high-power (400×) microscope field were immunopositive for anti-T-antigen antibody. Immunohistochemical examination of the tumour tissue for the viral late gene product showed no evidence of VP1 gene expression in the tumour cells. None of the control cerebellar specimens showed positive immunostaining for T-antigen.

The high frequency of detection of JC virus DNA sequences within human medulloblastomas, and demonstration of the viral oncoprotein, T-antigen, by immunohistochemistry suggests that JC virus may play a part in tumorigenesis of human medulloblastomas.

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Methanol/acetone treatment helps the amplification of FMR1 CGG repeat fragment in dried blood spots from Guthrie cards

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The fragile X syndrome is a cause of intellectual disability resulting from an absence of the protein FMR1. It has a prevalence in white populations of 1/4000 in men and 1/8000 in women.[1] The associated cytogenetic finding is almost always the demonstration of a fragile site at Xq27.3[2]—the associated molecular finding is usually an expanded CGG repeat identifiable by Southern blot or PCR techniques.[3] To facilitate the identification of fragile X syndrome, we developed a method to treat Guthrie blood-spot samples and improve the PCR amplification of CGG repeat fragment in *FMR1* gene.

Two adjacent 3 mm diameter discs near the centre of each Guthrie blood spot were punched and put in individual tubes. A 10 μL mixture of methanol and acetone (1:1) was then dropped into the tubes of the pretreatment group and dried at 55°C for 60 min. The discs were then incubated at room temperature with 25 μL PCR reaction mixture (0·4 μmol primers each,[4] 20 mmol tris-hydrochloric acid pH 8·75, 10 mmol potassium chloride, 10 mmol ammonium sulphate, 2 mmol magnesium sulphate, 0·1% triton X-100, 0·1 mg/mL bovine serum albumin, 0·2 μM deoxyribonucleoside triphosphate, 10% dimethyl sulfoxide for 24 h. The discs of the non-pretreated group were directly incubated with PCR reaction mixture at room temperature for 24 h. The reaction was initiated after the addition of 0·75 unit exo(-) *pfu* polymerase (Stratagene) with a programme of 40 cycles of 45 s at 98°C, 45 s at 65°C, and 45 s at 75°C. The PCR products were electrophoresed on 3% ethidium bromide stained agarose gel, and photographed under ultraviolet with a Coupled Charged Digital camera (figure). 500 randomly selected neonatal male Guthrie blood-spot

Comparison of CGG fragment with FMR-1 PCR efficiency in dried blood spots from Guthrie cards from eight people with (b) or without (a) methanol/acetone pretreatment
The length of PCR products is 158 bp (equivalent to 30 CGG repeats).

samples collected from Taiwan were screened using this method. All of the samples were successfully amplified with varying sizes of bands. One case having a size larger than 55 CGG repeats was identified. These results demonstrated the feasibility of screening for male fragile X syndrome using Guthrie dried blood-spot samples.

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Sham continuous positive airway pressure for placebo-controlled studies in sleep apnoea

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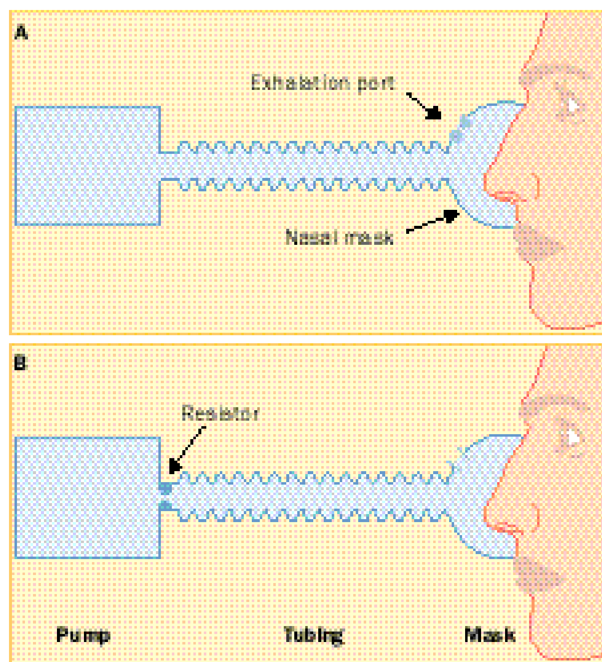
The sleep apnoea/hypopnoea syndrome is commonly treated with nasal continuous positive airway pressure (CPAP).[1] The efficacy of CPAP has been assessed in comparison with procedures which do not meet the requirements of a placebo because the patient is not subjected to the same instrumental constraints.[2,3] Given the lack of placebo-controlled studies together with the costs of CPAP, the usefulness of CPAP has been called into question.[4,5] We designed a method of sham CPAP for placebo-controlled studies in sleep apnoea.

CPAP equipment includes a pump, tubing, and a nasal mask with an exhalation port (figure, A). CPAP is generated by the pressure drop across the resistance of the exhalation port. To implement sham CPAP (figure, B), airflow resistance of the exhalation port is almost eliminated by increasing its area, thereby virtually cancelling nasal pressure, and an orifice resistor identical to the original exhalation port is connected between the CPAP unit and the tubing in order to load the blower with the same airflow resistance as in true CPAP. The ventilator operating noise and the airflow through the exhalation port remain unchanged, which are crucial to a CPAP placebo.

We tested a CPAP device (PV100, Breas Medica, Sweden). The exhalation port diameter was increased from 4 to 10 mm and a 4 mm diameter orfice-type resistor was placed between the pump and the tubing. Flow through the exhalation port and operating noise were the same (within 10% and 1 dBA, respectively) for the true CPAP and the sham CPAP systems. A preliminary study in eight awake patients with sleep apnoea showed that actual nasal sham CPAP was 0·4 (SD 0·1) cm H_2O and that rebreathing was avoided (oxygen saturation [SaO_2] and end-tidal CO_2, mean temperature, and humidity measured at the mask were the same with CPAP at 8 cm H_2O and with sham CPAP). An all-night polysomnographic study was carried out to verify whether or not the sham CPAP system modified the patient's sleep (efficiency; arousals/microarousals; percent night time in stages I, II III-IV, and REM) and the number of sleep and respiratory events (apnoea-hypopnea index (AHI) and sleep time with SaO_2<90%). Ten men with sleep apnoea (55 [8] yr, body-mass-index: 33·4 [7·4] kg/m^2, AHI=62 [25] events/h) seen consecutively at the outpatient sleep clinic and naive in the use of a nasal mask and CPAP treatment were studied for two consecutive nights. The study design consisted in a single blind two-way crossover trial with the treatment periods randomly assigned without a washout period between in order to compare sham CPAP with no-treatment. Patients and the polysomnography technician were not informed about the purpose of the study. When comparing sham CPAP and no-treatment, no significant differences (paired *t*-test, p>0·05) were found in sleep efficiency (79 [10]% *vs* 83 [11]%), arousals (57 [17] *vs* 55 [19] events/h), and AHI (66 [21] *vs* 62 [25] events/h), nor in the other sleep variables studied.



A: CPAP. B: sham CPAP

As the method we describe can be easily implemented on any conventional CPAP equipment, it may be a useful tool for future controlled clinical studies to ascertain the efficacy of CPAP in patients with sleep apnoea.

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