Retinoblastoma: a possible link with low level radiation

A recent report of cases of retinoblastoma linked to the village of Seascale in West Cumbria is of particular interest because this village has already been shown to have an increased incidence of childhood cancer. A workshop was held in Lancaster on 21 September 1991 to discuss the possible link between these cases and low level radiation exposure.

**Background: childhood leukaemia in Seascale**

The possibility that childhood cancer is increased in the vicinity of nuclear installations was first suggested by the observation of an increased incidence of childhood acute lymphoblastic leukaemia (ALL) in the village of Seascale, close to the Sellafield nuclear plant. At first it seemed likely that the cause of the observed excess of ALL was environmental radionuclide contamination from Sellafield nuclear plant, but detailed physiological and mathematical models indicated that exposure experienced by Seascale residents from Sellafield discharges was less than natural background radiation. The population of Seascale is unusual in that it consists predominantly of higher socioeconomic groups, mobility is high, and the area is relatively isolated, all factors which have been associated with a higher incidence of childhood leukaemia. Initially these associations were suggested to be related to an altered response to infection but recently it has been shown that men who worked in the Sellafield plant were at increased risk of fathering a child with leukaemia and the risk was related to the degree of preconceptional radiation exposure. This observation has led to speculation that the increased incidence of leukaemia is the result of radiation damage to spermatocytes.

**Retinoblastoma linked to Seascale**

Dr Morris told the meeting that the original report was of three cases of retinoblastoma occurring in children whose mothers had spent part of their childhood in Seascale and whose maternal grandfathers had worked at Sellafield. One case was bilateral owing to a new germline mutation with a partial gene deletion. The other two cases were unilateral, but unusual in that they presented early, one at 6 months of age and the other at 12 months of age. All three cases were born outside Cumbria and in one case the birth occurred 26 years after the mother left Seascale. Retinoblastoma is extremely rare, being 10 times less common than ALL and occurring in approximately 1 in 20 000 live births. The three mothers were members of the group of 2614 children born in 1950 or later, who lived in Seascale between 1950 and 1983 and whose ages now range between 8 and 42 years. Although the exact number is not known, they are unlikely to have produced more than 5000 offspring and there would have been an expectation of retinoblastoma of 0.25 case: the probability of three cases would have been 0.002.

Following this report a number of other cases have been described, including a fourth case of unilateral retinoblastoma in a boy presenting at 11 months of age. The mother, who was born before 1950, had attended boarding school in Seascale in the 1950s. Her parents did not work at Sellafield or live in Cumbria. A fifth case occurred in a boy with unilateral retinoblastoma diagnosed at the age of 8 months whose parents had lived in Seascale but had moved from the village three months before his birth. There are an additional three cases of unilateral retinoblastoma since the 1950s in children born in Copeland (the district which includes both Seascale and Sellafield). One was a boy, diagnosed at 3 years 4 months of age, whose family had no connection with the nuclear industry. A second was a girl, diagnosed at 11 months of age, whose father worked at Sellafield, and a third was a girl, diagnosed at 14 months of age, whose paternal grandfather worked at Sellafield. Thus there is a total of eight cases of retinoblastoma of which seven are unilateral, but six of these presented before 14 months of age. That this was an unusually early age for sporadic disease was confirmed by comparing the age of presentation of the seven unilateral cases with the age at presentation of all the other 415 cases of non-hereditary unilateral retinoblastoma born in Britain during 1962 to 1980 and included in the National Registry of Childhood Tumours (Mann–Whitney U test, \( p = 0.036 \)). Other points of interest are that five cases linked to Seascale occurred in professional families (social class I), and in all cases the Copeland residential link was in the period 1950 to 1970. Because of the different ways in which the cases were ascertained, it is difficult to make an objective epidemiological assessment of these data. However, the con-

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sensus view at the meeting was that the observations were sufficiently interesting to warrant further study, and in particular to identify possible causal mechanisms.

Possible mechanisms
Dr Morris said that there are two related questions to answer: what is cause of the putative increased mutation rate in the retinoblastoma gene and at what stage in development does this aetiologic factor act? The increased mutation rate could occur in utero or in the germ cells of the parents or grandparents. It is unlikely that radiation damage to the germ cells of Sellafield workers is the common factor as only one case occurred in the child of a Sellafield employee. However, four cases occurred to mothers who had spent part of their childhood in Seascale and it might be speculated that there had been an increased mutation rate in the mothers' oocytes. This is unlikely as the incidence of new germline mutations in the retinoblastoma gene in the general population is about 1 in 100 000 live births and it is thought that the majority of these mutations arise during spermatogenesis,13 compared with an incidence of retinoblastoma in the offspring of Seascale children which approaches 1 in 2000 live births. This would imply a fifty-fold increase in the oocyte mutation rate and is not consistent with the apparent radiation exposure of Seascale children.17 An alternate and more plausible hypothesis involves radiation induced somatic mutation in utero. All cell generations would be exposed throughout intrauterine development and the expectation of childhood cancer would be proportional to the mutation rate raised to the power n, where n is the number of mutations for cancer.1415 This would only require a four-fold increase in the mutation rate to get a sixteen-fold increase in the expectation of retinoblastoma, and although much higher than the known exposure levels in the mother, there might be some mechanism by which radionuclides are transferred across the placenta and concentrated in the developing fetus.

Molecular genetic studies
Dr Cowell indicated that participants at the meeting agreed that study of the DNA from the subjects with retinoblastoma offered the best available method for solving the puzzle and molecular genetics might distinguish somatic mutations in utero from germline mutations. The DNA sequence of the retinoblastoma gene (RB1) is known and oligonucleotide primers are available to amplify by PCR each of the 27 exons of RB1, including the coding regions and the splice junction regions, although mutations of the promoter/enhancer regions upstream of the initiation codon might be missed by this procedure. Once each exon of the gene has been amplified using PCR it can be analysed by single strand conformation polymorphism (SSCP),16 and any of the 27 exons showing abnormal banding profiles on the SSCP gel can then be sequenced to show the specific mutation. Because constitutional mutations are usually heterozygous, a proportion of the mutations will be missed by this technique and it would be useful to analyse tumour DNA which must carry a mutation. (Unfortunately fresh tumour tissue is not available in any of the cases but it is possible to isolate DNA from formalin fixed paraffin embedded sections which are available.) If a tumour mutation could be found, this would give a lead to the search for constitutional mutations. If the same mutation is found in the constitutional cells of the patient, then he/she must carry a germline mutation. If germline mutations are found the type of mutation might shed light on causation as, for example, radiation, albeit in high doses, tends to cause deletions. Interestingly the single child who has bilateral retinoblastoma carries a large constitutional chromosome deletion and it will be possible to determine whether this was derived from the mother or father. Somatic mutation in utero will not be easy to prove if, as seems likely, most unilateral cases are not associated with germline mutations. The mutation rate of the retinoblastoma gene cannot be measured directly and only indirect measures are available, for example, by investigating the proportion of T lymphocytes from peripheral blood which have undergone mutation of the X linked gene hypoxanthine phosphoribosyl transferase (HPRT).17

Conclusion
There are similarities between the reported excess of retinoblastoma and the clustering of leukaemia in Seascale. However, in both cases there are difficulties inherent in the statistical interpretation of small numbers of rare cancers, particularly when the population from which they are derived is defined retrospectively. The observed incidence of cancer is also far in excess of what would be expected from the radiation exposure levels in the local population. These difficulties have not yet been resolved but new approaches involving the well characterised molecular genetics of retinoblastoma allow new studies of the relationship between low dose radiation and childhood cancer. The meeting concluded that in spite of methodological epidemiology the observations that had been made on retinoblastoma were remarkable and deserved further study.

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