Familial gonadal tumours

Following the letter from Huddart *et al.*,¹ we would like to report a further family with different gonadal tumours. The index case presented with stage 1 ovarian carcinoma at the age of 54. Histology showed a borderline serous tumour and there was no evidence of dissemination. She did not require chemotherapy and has remained well since surgery. Her mother died at the age of 78 having had a diagnosis of well differentiated papillary serous adenocarcinoma of the ovary made two years previously and her mother's cousin was also diagnosed as having ovarian carcinoma. Her son was diagnosed as having a malignant teratoma at the age of 19, which was successfully treated with an orchidectomy and chemotherapy. She has one sister aged 52 who is under surveillance and has so far not developed any malignancies. There is no history of breast cancer or other cancers in the family. The families reported by Huddart *et al.*¹ all had germ cell tumours whereas this family has a combination of germ cell and serous gonadal tumours. While this could be a chance association, the pedigree suggests an autosomal dominant pattern of inheritance. The index case has given permission for her DNA to be used by any interested research groups and we should be happy to hear from those involved with molecular studies in this field.

Although it is recognised that a small proportion of testicular teratomas are familial,² and there have been a handful of reports of families with both male and female germ cell tumours, we are unaware of any reports of familial predisposition to both germ cell and common epithelial gonadal tumours.

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1 Huddart RA, Thompson C, Houlston R, Nicholls EJ, Horwich A. Familial predisposition to both male and female germ cell tumours? *J Med Genet* 1996;33:86.

2 Forman D, Oliver RTD, Brett AR, *et al.* Familial testicular cancer: a report of the UK family register, estimation of risk and an HLA class I sib pair analysis. *Br J Cancer* 1992;65:255-62.

Cystic fibrosis in a Puerto Rican female homozygous for the R1066C mutation

Patients with cystic fibrosis show a wide variety of clinical symptoms, but the relationship between genotype and clinical phenotype is incompletely understood. The R1066C missense mutation is one of the four point mutations found at the CpG dinucleotide (3328-3329) mutation "hot spot".¹⁻³ The underlying nucleotide change is a C to T transition at nucleotide 3328 in the second half of exon 17, located in the region corresponding to the second transmembrane domain of the CFTR protein. The arginine residue at 1066 is conserved in humans and mice,⁴ suggesting the structural/functional importance of this amino acid. The substitution of the hydrophobic amino acid leucine for the positively charged arginine, R1066L, results in a pancreatic insufficient phenotype.⁵ The substitution of another positively charged histidine for arginine, R1066H, however, was

found in both pancreatic sufficient and insufficient patients.^{1,5} The R1066C mutation was found in a CF carrier with bronchiectasis.² All these reports referred to subjects who were heterozygous for the R1066 hot spot mutations. Thus, our patient with homozygous R1066C presents the opportunity to investigate the phenotype of this mutation.

The patient is the product of a consanguineous mating between first cousins. Her parents were from Arecibo in Puerto Rico. She was diagnosed at 1 year of age because of failure to thrive and recurrent pneumonia. Sweat chloride was 106 mEq/l. During her childhood, the patient claimed to take enzymes, and maintained low to normal levels of vitamins, cholesterol, and albumin. There was no significant change after withdrawal of enzyme therapy at the age of 16. Despite her bronchiectasis, she was extremely active in sports and noted little impairment in her lung function. At the age of 20, she gave birth to a healthy daughter, who continues to be in good health. During her late 20s, the patient began to have frequent episodes of bronchitis that required antibiotic treatment. At the age of 30, a positive culture was obtained for *Aspergillus fumigatus*. At the age of 31, she sustained a spinal injury in a serious car accident, was unable to breathe because of the back pain, and developed a severe pneumonia. Her lung function then deteriorated rapidly. At the age of 32, her pulmonary function was extremely poor. The peak flow, FEV₁, and FVC were 30%, 26%, and 32% of predicted, respectively. The difference in pulmonary function obtained before and after administration of the meter dose inhaler was not significant. The patient was colonised with *Pseudomonas aeruginosa* that were not multiply resistant. The patient died at the age of 36 from respiratory failure compounded by malnutrition. Before death she weighed 32 kg, was 147 cm tall, and had become diabetic. Since fewer than 5% of currently surviving CF patients exceed the age of 36, her clinical course can be characterised as moderate.

Mutations in the CFTR gene of this patient were originally studied by a commercial laboratory. None of the 34 point mutations analysed was detected. This patient's mutant CF alleles were identified by single strand conformational polymorphism (SSCP) analysis of the polymerase chain reaction product of exon 17b⁶ followed by direct DNA sequencing and confirmatory allele specific oligonucleotide (ASO) hybridisation. Neither of her parents was available for DNA testing. In order to eliminate the possibility that the apparent homozygosity is the result of amplifying only one allele, additional primers were used to show that the absence of the normal allele is not the result of mutations at the primer binding sites. No other mutations were detected by SSCP screening of 16 exons (exons 3, 4, 5, 6a, 7, 9, 10, 11, 12, 13a, 13b, 15, 17b, 19, 20, 21) in the CFTR gene. Since the R1066C is a rare mutation and the patient is the product of a consanguineous mating, we conclude that the patient is homozygous for the R1066C mutation.

The R1066C missense mutation is relatively uncommon in white populations, occurring with a frequency of 0.3% in the German population.⁷ It is more common in people of Spanish descent with a frequency of 0.72%,⁸ and has an unusually high frequency of 4.8% in CF patients from Portugal.⁹ Reviewing the point mutations that were

analysed by several well known commercial laboratories in the United States, we found that the R1066 mutation hot spot was not included in any of the mutation panels screened. We recommend that the R1066 mutation hot spot be analysed, especially if the patients are of Spanish, Portuguese, or Hispanic origin.

All reported R1066C cases were heterozygous, and most of the clinical courses were not described except one compound heterozygous carrier, with a rare DNA polymorphism, whose major clinical manifestation was disseminated bronchiectasis.² In this latter report, the results indicated that disseminated bronchiectasis of unknown origin is frequently associated with CFTR gene mutations or rare DNA polymorphisms. Our patient, homozygous for mutation R1066C, had a classical presentation of CF. During the course of screening 16 exons, including part of the flanking intron sequences, we discovered three polymorphisms. Two of them, 875+40 A→G in intron 6a and 3601-65 C→A in intron 18, have been previously reported.^{10,11} The other one, which has not been reported, is 622-58 T→G in intron 4. It is unclear at the present time if these polymorphisms have any clinical significance.

The substitution of a cysteine residue for arginine could potentially be a severe mutation since these two amino acids are structurally and electrochemically distinct. However, R1066 is located at the cytoplasmic loop between the fourth and fifth transmembrane segments of the second membrane spanning domain.¹² Replacement of the positively charged arginine with a neutral, slightly hydrophilic cysteine at the cytoplasmic loop may not completely knock out the protein function. This may explain the patient's overall moderate clinical features and borderline pancreatic insufficiency. Functional studies of the in vitro expressed R1066C mutant CFTR protein will be necessary in order to understand further the potential effect of this mutation on the biochemical and clinical outcome.

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