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HLA typing as a method of genetic counselling in congenital adrenal hyperplasia

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SUMMARY A Turkish Cypriot family with 3 consanguineous marriages is described. Segregation of the 21-hydroxylase deficiency gene was traced by HLA genotyping and this information used for genetic counselling.

Close linkage between the 21-hydroxylase deficiency gene and the HLA complex on chromosome 6 has now been established.^{1,2} Two genes are thought to be concerned in the genetic control of this condition, one between the HLA B and DR loci and a second between the DR and GLO loci.³ Before the recognition of the HLA linkage of this enzyme deficiency, detection of heterozygote carriers by measurement of the response of plasma 17OH-progesterone to adrenocorticotrophic hormone (ACTH) stimulation was described.⁴ Comparison of HLA genotyping and ACTH stimulation as methods for detection of heterozygotes has suggested that the former was more reliable because of overlap in plasma 17OH-progesterone levels between obligate heterozygotes and homozygous normal family members, making classification of some family members difficult.⁵ We describe a family with 3 consanguineous marriages in which segregation of the 21-hydroxylase deficiency gene was traced by HLA typing, and this information used for genetic counselling.

Patients and methods

The proband was the first child of Turkish Cypriot parents who were 1st cousins. Ambiguous external genitalia were present at birth and the infant was thought to be a boy with perineal hypospadias and undescended testes. Aged 3 weeks,

investigations for poor weight gain showed plasma sodium 124 mmol/l, potassium 9.5 mmol/l, and a karyotype of 46 XX. Plasma 17OH-progesterone measured by radioimmunoassay was greatly raised at 2370 nmol/l (normal range <10 nmol/l). These features are consistent with the salt-losing form of 21-hydroxylase deficiency. The infant was brought up as a girl.

The family history showed that the pedigree (Figure) contained two other 1st-cousin marriages and that several siblings of both parents had died unexpectedly in infancy in Cyprus. After explanation of the genetic nature of the condition to the parents, the family requested genetic counselling. Accordingly, HLA genotyping of the proband and 12 other family members who were clinically normal was carried out.

HLA genotyping. Typing for HLA A, B, and C loci was performed by a microlymphocytotoxicity test using a modified NIH technique.⁶ HLA-DR antigens were defined on unseparated peripheral blood lymphocytes using a double colour immunofluorescent technique.⁷ All A, B, C, and DR antigens which were officially recognised by the WHO nomenclature committee in 1980 were typed for.

Results

The results of HLA genotyping are shown in the Figure. As far as can be judged there had been no recombination in the major histocompatibility complex, the 5 haplotypes in this family being inherited unchanged.

One of the predictions for the proband born of 1st cousins was that she would probably be homozygous for an ancestral HLA-haplotype, which is

Figure Pedigree of family with 21-hydroxylase deficiency. The proband is indicated by an arrow. $\square$ shows children who died unexpectedly in infancy.

unlikely if the parents are unrelated. The proband is homozygous for the haplotype A3, BW35, (BW6), CW4, DR2, (MT1), and therefore we can conclude that the 21-hydroxylase deficiency gene is contained within the length of DNA which is identified by that parental haplotype. Family members, II₃, III₁, III₂, III₃, III₉, III₁₁, and III₁₄ are therefore heterozygous. GLO typing was performed by Dr Simon Welch, Department of Biochemistry, London Hospital Medical College in the proband, her parents, and 4 other relatives. All were homozygous GLO 2.2, which was therefore uninformative.

Genetic counselling. The 3 consanguineous couples each requested genetic counselling. Couple III₂/III₁₁, the parents of the proband, were told of the 1 in 4 risk of each subsequent child being affected. Couple III₃/III₁₂ had recently married and had waited for the HLA results before deciding whether to have children. The wife III₃ was a carrier. The husband III₁₂ was probably not a carrier unless paternal recombination of the second gene outside the DR locus had occurred. This is likely to be only a small

risk and cannot be checked because typing of the GLO locus was not informative. Consequently they were counselled that there was no appreciable risk of their children having 21-hydroxylase deficiency. Couple III₁/III₉ had 2 normal children and requested counselling about the risk in subsequent pregnancies. Both the wife III₉ and the husband III₁ were carriers. The risk in their case was felt to be effectively less than 1 in 4 because recombination may have removed either the intra or extra DR gene from the relevant haplotype. Neither of their children was a carrier.

Discussion

The linkage of the 21-hydroxylase deficiency gene to the major histocompatibility complex is now firmly established.⁸ HLA genotyping for genetic counselling however, is only reliable in the event of no recombination between the 21-hydroxylase deficiency gene and the HLA marker. The results in this family strongly suggest that recombination did not occur. Significant variation in the severity of the 21-hydroxylase deficiency may occur, even within a

single family, and this point was stressed during the counselling. HLA genotyping may therefore have a practical clinical application in ascertaining the distribution of the 21-hydroxylase deficiency gene as the basis for genetic counselling.

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Hepatic cellular injury during varicella

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SUMMARY Serological evidence for hepatic cellular injury may occur during uncomplicated varicella. The magnitude of abnormalities may be helpful as a guide in the management of children with progressive varicella or varicella-associated Reye's syndrome, but liver function tests may be in the normal range. Viraemia was not detected during the acute stage of varicella-associated Reye's syndrome.

Susceptible children who are immunologically compromised by disease or treatment are at risk of developing progressive varicella if exposed to varicella-zoster virus. Serological evidence of hepatic cellular injury has been used to corroborate the clinical impression of progressive infection in such patients. In addition, hepatic cellular injury is a diagnostic criterion for Reye's syndrome¹ which may complicate varicella. Because varicella is a self-limited childhood exanthem, evidence of hepatic cellular dysfunction is not generally sought in the

normal child undergoing a typical course of infection. However, recent reports suggest that chemical hepatitis may occur during apparently uncomplicated varicella.²⁻⁴ Therefore, normal children with varicella, children at risk of developing progressive varicella, and children with Reye's syndrome complicating varicella were evaluated for serological evidence of hepatic cellular dysfunction. Because progressive varicella has been associated with viraemia during exanthem,⁵ children with Reye's syndrome were also examined for the occurrence of viraemia during the early stages of encephalopathy.

Materials and methods

After informed consent had been obtained, 75 patients with varicella, confirmed by the recovery of varicella-zoster virus from vesicle fluid in human fibroblast tissue cultures or by the demonstration of a 4-fold rise in membrane fluorescence antibody titre,⁶ were evaluated for serological evidence of