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Hammersmith Staff Rounds

An independent diagnosis

A treatable metabolic disorder is diagnosed by molecular analysis of human genes

A young man referred himself after reading an article in a national newspaper that described two patients with an inherited disorder whose symptoms he had recognised. He had a 10 year history of grand mal seizures. Investigations had previously shown nothing, and he had been obliged to surrender his driving licence and modify his work. After self referral hereditary fructose intolerance was diagnosed by analysis of epithelial cell DNA recovered from mouth-wash samples. Since following a strict exclusion diet free from fructose and related sugars he has reported an improvement in wellbeing and has been free from seizures. This case history illustrates the usefulness of genetic analysis in diagnosing this treatable genetic disorder and detecting symptomless carriers of the disease.

Case history

The patient was an environmental health officer who had been well until the age of 20 in 1979, when he had had an episode of generalised convulsions and loss of consciousness. He was examined by a neurologist shortly after the attack and sodium valproate was prescribed. By 1984 he had had no further seizures while taking regular anticonvulsant. An electroencephalogram was found to be normal and the valproate was stopped.

In February 1988, when he was 29, he had a second generalised seizure attack. This occurred at 11 am after a late breakfast of cereal. The attack was preceded by a distant sensation and impaired awareness of the surroundings; witnesses reported that the patient sank to the ground and then showed generalised clonic movements rapidly thereafter. He had no incontinence. The night before the attack he had consumed a large quantity of home brewed beer, which he described as "unfinished." He had also drunk half a bottle of wine. Investigations immediately after the episode included repeat encephalography, blood count, measurement of blood glucose concentrations, and an automated multiple biochemical analysis of serum. All these tests gave normal results. He was thought to have had an alcohol induced fit and for a time thereafter consumed non-alcoholic beers and lagers. These, however, provoked attacks of sweating that were relieved only by eating starchy foods such as bread.

Later in 1988 he applied for a year's leave of absence to travel abroad with his wife. In August they visited Pakistan, where ambient temperatures exceeded 28°C. After a day's sightseeing he purchased and drank a bottle (0.3 litres) of a local brand of carbonated sweet drink but immediately became nauseous. After 20 minutes he noticed epigastric discomfort, dizziness, and profuse sweating. These sensations worsened over the next hour and his speech became incoherent. Finally, he lost consciousness and had generalised convulsions. On this occasion he bruised his back and was incontinent of urine. After a series of seizures that were witnessed by his wife he remained unconscious for about an hour, after which he remained disoriented and drowsy for several hours. The symptoms improved in the evening after he had eaten a light meal. He returned home immediately and was further investigated. An electroencephalogram was normal, as were the results of a two hour glucose tolerance test. Blood sugar determinations after a prolonged fast remained normal and nothing was clearly diagnosed, although attacks of hypoglycaemia were considered to be possible. He was advised to discontinue his travels abroad, and he surrendered his driving licence.

The patient has three siblings, who are all healthy, as are his parents, who are not known to be related by blood. He had been healthy throughout childhood, but unlike all other members of his family he had avoided fruit, sugar, and confectionary for as long as he could remember because he did not enjoy the taste. On his return from abroad the patient noted that when he drank drinks containing sugar he became lightheaded, especially when the drinks were followed by exercise. He recalled that this sensation resembled closely the premonitory symptoms of his last two seizures.

In December 1988 he read an article in a national newspaper that described two young doctors who had hereditary fructose intolerance and had diagnosed their disorder correctly. The genetic defect responsible for this disorder had been characterised, and on noting the resemblance to his complaint and with his general practitioner's consent the patient sought further advice on the nature of his dietary preferences and their relation to his seizure attacks. Later, on review at this hospital, he was found to have no other important past illness or injury and was not taking drugs regularly. Physical examination showed no abnormality.

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He was investigated and hereditary fructose intolerance was diagnosed from his history and the results of genetic tests that were carried out on samples of genomic DNA. These were tested before he had attended hospital. A generally applicable method that uses the polymerase chain reaction to amplify the specific region of the aldolase B gene, in which mutations responsible for this disease occur, was used in combination with synthetic allele specific oligonucleotides to search for the presence of the molecular lesions responsible for hereditary fructose intolerance. Sufficient DNA for the selective amplification of the target sequence may be obtained from buccal epithelial cells recovered from mouthwashes and so we were sent saline mouthwashes from the patient and his two parents. After recovery of cells and extraction of DNA the target sequences were amplified by the polymerase chain reaction, applied to filters, and then probed with radiolabelled wild type and mutation specific oligonucleotide; under appropriate conditions these hybridise selectively to their respective complementary sequences in the amplified DNA. Samples from the patient hybridised to only the A149P mutation specific probe at the discriminatory temperature. Amplified DNA from both his parents hybridised to the corresponding wild type probe (A149⁺) as well as the A149P allele specific probe. It was thus concluded that the patient was homozygous for the A149P allele and that his parents, as expected, were symptomless heterozygotes.

In view of the importance of establishing beyond doubt that this patient suffered from hereditary fructose intolerance and that the disorder was responsible for his symptoms the effects of challenge with fructose were formally investigated. He was admitted briefly to hospital for an intravenous challenge with fructose at a dosage of 0.25 g/kg body weight, which was administered over one minute. As shown in figure 1 administration of fructose induced a prompt fall in serum phosphate concentrations which was accompanied by pronounced hypoglycaemia. The hypoglycaemia was noted during the conduct of the test to be accompanied by irritability, loss of concentration, and poor memory.

We concluded that he had hereditary fructose intolerance and that inadvertent consumption of sucrose and fructose had led to episodic hypoglycaemia. Since the institution of a strict exclusion diet, in which fructose, sucrose, and sorbitol consumption was reduced to less than 40 mg/kg body weight/day, he has had no further episodes of seizures and has noted a distinct improvement in his general sense of wellbeing.

Comment

Hereditary fructose intolerance is an autosomal recessive disorder in which abdominal pain, vomiting, and metabolic disturbances including hypoglycaemia are induced by fructose and related sugars. Many patients develop an aversion to sweet tasting foods, as well as to fruit and vegetables that provoke their symptoms, and this aversion usually becomes established during infancy. The disorder is due to a deficiency of aldolase B, which is responsible for the assimilation of fructose from the diet in the liver, intestine, and kidney. These organs develop functional and structural damage should large amounts of the noxious sugars continue to be ingested. The mechanism by which challenge with fructose causes the metabolic disturbance in this disorder has been much studied and is too complex for discussion here, except to say that absorption of fructose generates a milieu in which both glycogenolysis and gluconeogenesis are appreciably depressed in the liver.³ Exclusion of fructose and sucrose from the diet is accompanied by a dramatic improvement, but if this is not achieved early and if the

FIG 1—Intravenous fructose tolerance test in patient. Dotted line represents lower limit of normal range. Reproduced by permission of Oxford University Press from the *Quarterly Journal of Medicine*²

patient does not restrict his or her intake because of an aversion to the offending sugars growth is retarded and progressive renal and hepatic disease develop, which may lead to death. Thus early recognition is critical to its effective management.

Hereditary fructose intolerance has a frequency of one in 20 000 live births, and the diagnosis may be confirmed by the intravenous fructose test. The predicted carrier frequency for the disorder based on the Hardy-Weinberg equation is about one in 70 of the population but, as yet, no simple method for detection of symptomless heterozygotes has been devised.

Recently Dr Nicholas Cross studied in our laboratory the molecular defect in the gene for aldolase B in this disorder.⁴ We previously showed that the deficiency of aldolase B was most frequently attributable to the synthesis of a catalytically inactive variant in the tissues. The first aldolase B gene from the patient with such a catalytic defect was cloned and sequenced and found to contain a single point mutation.⁵ This mutation, termed A149P, results from a transversion of guanine to cytosine, which is responsible for the replacement of an aldolase B specific residue—namely, alanine—at position 149 of the protein by a proline residue. This lesion was detected in all British patients with the disorder so far studied and is the most prevalent mutation responsible for this disease in the European population.²

Published work on the management of the first seizure in adult life is somewhat controversial, as is the management of a single seizure attack. Hopkins *et al* studied 408 adults over three to four years and found that just over half of them (52%) had a second convulsion within three years after the first attack.⁶ They examined the predictive features that were associated with the second attack and found that the following were somewhat predictive: attack in the early hours of the morning, an age of more than 50, a history of febrile convulsions, and a family history of convulsions. They looked at the diagnostic yield of a range of frequently performed investigations and found that computed tomography carried out in 370 of the 408 adults detected only 13 tumours, two of which had been missed originally and were discovered by other means. The electroencephalogram was abnormal in only four of these patients. They concluded from this that results of electroencephalography, skull radiography, and routine blood testing, including measurement of serum calcium and blood glucose concentration, did not generally contribute to the eventual diagnosis and were not predictive variables for a second seizure. They recommended that the presence of

FIG 2—Crystal structure of aldolase subunit I (inferred from x ray diffraction data obtained at 0.27 nm from rabbit muscle aldolase). Cylindrical structures depict α helical regions and arrowed strips represent β strands. Reproduced from the Proceedings of the National Academy of Sciences of the United States with the permission of the authors

appropriate clinical features and selective use of computed tomography could be used to predict the natural course of first seizures occurring in adult life. That may be a good generalisation, but I think that there is little doubt that blood tests at the time of a seizure attack would have been highly informative in the investigation of this patient.

Discussion

SRB: The proline substitution at position 149 is obviously by far the commonest genetic abnormality. Do you think that this is an original error that has been carried through as a single mutation, in which case do you speculate that there is some evolutionary advantage, or do you think that there is a local instability that gives rise to the same mutation on different occasions?

TMC: Although this mutation is widespread in European peoples, it is particularly prevalent in France and Britain and there is a strong belief from this distribution that it has arisen as a single point mutation and spread by so called genetic drift from a single person or founding group over millennia. The other factor that supports the concept of genetic drift is that another point mutation, A174D, accounting for about 15% of mutant alleles, is again widespread, although it does have a completely different geographical distribution within the regions of Europe. The occurrence of two point mutations—incidentally, each in the same exon of aldolase B and each with a different distribution—would support the concept of genetic drift.

The selection against aldolase B mutations was almost certainly minimal before the Industrial Revolution—that is, before the refining of raw sugar on the

present large scale—and there is some historical evidence to support this. Before the colonisation of the West Indies the amount of sugar consumed, at least in northern Europe, was minimal when added to the diet. As to heterozygote advantage, there is no information about this—although homozygotes who display a strikingly reduced incidence of dental caries as a result of their profound aversion to sweet tasting food and drinks may possess a selective advantage. The prevalence of dental abscesses in the Egyptian mummies suggests that exposure to harmful sugars in honey, for example, has occurred from time to time in the course of human history.

RWEW: The replacement of alanine by proline is particularly interesting as proline is a substituent amino acid that introduces a hinge into the tertiary structure of the protein. Have you had an opportunity to examine the effects of this mutation on the tertiary structure of the protein?

TMC: Thank you for that perceptive question. Figure 2 is a representation of the crystal structure of one subunit of the tetramer of the closely related enzyme rabbit muscle aldolase. The A149 mutation occurs at the substrate binding residue, that would be drastically altered by the substitution of alanine by the amino acid proline. The mutation would alter the spatial relation of critical residues that are known to be essential for the binding of fructose phosphoesters in the enzyme molecule. To answer the question whether this mutation is responsible for the absence of catalytic activity we have performed experiments to examine the effects of this substitution. The enzyme has been expressed in the normal wild type form in *Escherichia coli* and by introduction of this mutation we have also expressed a mutant form of the protein abundantly in *E. coli*. The mutant enzyme has less than 1% of the activity of the wild type. We therefore believe that the altered tertiary structure at the substrate binding site as a result of this amino acid substitution is the likely explanation of the altered catalytic activity.

SW: To return to the origin of the mutation, have you examined haplotypes?

TMC: There are no easily defined polymorphisms in this gene, although there has been a single report of a polymorphism in a non-coding region that occurs in about 30% of Italian subjects. To answer your question definitively we would clearly like to see linkage of a specific allele at a polymorphic site with a given mutant allele in all patients affected by the disease. We have not found any informative polymorphisms, including the one described from Naples, in patients we have studied from British and European populations and so we have not been able to decide about this interesting point that you raise.

This patient was described briefly in an article on the molecular genetics of fructose intolerance in the *Quarterly Journal of Medicine*.¹

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