Original articles

Disialotransferrin developmental deficiency syndrome

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SUMMARY Seven mentally deficient children and adolescents (three pairs of siblings and one singleton) were studied. A peculiar external appearance, a characteristic neurohepatosubcutaneous tissue impairment syndrome and, as a biological marker, an abnormal sialic acid transferrin pattern were characteristic features. All seven seemed odd from birth and prone to acute cerebral dysfunction during catabolic states. Abnormal lower neurone, cerebellar, and retinal functions dominated from later childhood. The disialotransferrin pattern found in serum and cerebrospinal fluid is thought to be the biological marker of a newly discovered inborn error of glycoprotein metabolism with autosomal recessive inheritance.

Children who have unexplained developmental deficiency and similar, peculiar appearance and behaviour are diagnostic challenges. The ultimate aim is to establish diagnoses from the cause of the deficiency. When this is not feasible, eponyms, acronyms, or descriptive names may help in setting diagnoses, which is important for genetic counselling and prognosis and information, and sometimes for treatment.

A common biochemical marker may help in establishing a diagnosis and in tracing the basic defect, and may be useful for prenatal diagnosis. We recently succeeded in finding such a marker for three pairs of siblings and one single child in four unrelated families. The marker, an abnormal sialic acid transferrin pattern detectable in both serum and cerebrospinal fluid was found by Jaeken *et al* in twin sisters with psychomotor retardation, delayed nerve conduction velocity, increased protein in the cerebrospinal fluid, and numerous abnormalities in the serum.^{1,2} Quite recently Jaeken *et al*³ found additional information and reported two more cases. It now seems most likely that their four patients and our seven represent the same—or a closely allied—condition about which we have collected a considerable amount of clinical and laboratory data over the years while searching for the diagnosis and origin.

The obvious common clinical manifestations are so characteristic that two of our families, unaware of each others' existence, immediately recognised the

almost identical appearance and motor behaviour of their children when they met by chance in the street on holiday in a small Swedish town.

The aim of this paper is to present the disialotransferrin developmental deficiency syndrome, its clinical characteristics, and its common biochemical marker and to summarise the main diagnostic investigations carried out that so far have not discovered the aetiology.

Methods

Isoelectric focusing of serum and cerebrospinal fluid was carried out in flat beds of polyacrylamide gel and silver staining according to previously described methods.⁴ The transferrin content of each sample was determined by electroimmunodiffusion. About 0.8 µg of transferrin was applied to the isoelectric focusing gel.⁵ Crossed immunoelectrophoresis was carried out in a 1% agarose gel. The gel was prepared in 0.07 M trometamol (TRIS)/0.02 M barbitone buffer, pH 8.2, containing 3.4 mM calcium lactate and 1% sodium azide. The gel was cast on a Gelbond (FMC corporation) polyester film. Twenty microlitres of rabbit antihuman transferrin antibodies (Dakopatts) were added to 30 ml of agarose. After the first dimension electrophoresis, the part of the isoelectric focusing gel containing transferrin was transferred on a piece of Gelbond to an antibody free zone on the agarose gel. Electrophoresis in the second dimension was run at 15–20 V/cm for six hours.

Patients

Seven children were studied (table). Once the two index cases (cases 1 and 2) were found to carry the characteristic changes of the transferrin, five other children (cases 3–7) were investigated because of clinical, developmental, and behavioural similarities. The series comprised three boys and four girls, three pairs of siblings and one singleton in four unrelated families. No consanguinity was traced, and no child with a similar disease had been born before according to grandparents, uncles, aunts, or cousins. All seven children have been followed up by us from infancy. One couple of parents had samples of their serum and cerebrospinal fluid investigated.

For comparison serum samples from seven children with abnormal liver function were investigated to find out whether the transferrin changes reflected impaired liver function.

Eight thousand routine isoelectric focusing examinations of cerebrospinal fluid and serum carried

out at the Sahlgrenska Hospital cerebrospinal fluid laboratory during the years 1983–1987 were retrospectively reviewed for similar aberrations in the transferrin pattern.

Results

THE MARKER

All seven children had a predominating band of transferrin containing two molecules of sialic acid (disialotransferrin) in both cerebrospinal fluid and serum, sometimes stronger than the band of tetrasialotransferrin, which is usually the predominant band (fig 1).

In the two parents of one pair of siblings the transferrin patterns in serum and cerebrospinal fluid pattern were normal. In the seven children with abnormal liver function, the transferrin patterns in serum were normal. The described transferrin pattern was not detected in the 8000 samples of cerebrospinal fluid and serum except for two patients who had been investigated for neurological symptoms caused by severe alcohol abuse.

Table Age, sex, perinatal data, and clinical characteristics

Family	A		B		C		D	
Case No	1	2	3	4	5	6	7	
Sex	Boy	Girl	Boy	Girl	Boy	Girl	Girl	
Age (years)	3	5	11	13	16	18	21	
Birth weight (g)	3600	3500	2200	2800	3500	3200	2900	
Gestational age (weeks)	40	40	36	41	40	40	38	
Clinical picture								
General characteristics:								
Early floppiness	+	+	+	+	+	+	+	
Gluteal fat pads	–	+	+	+	–	+	+	
Failure to thrive	+	+	+	+	+	+	+	
Inverted nipples	+	+	–	+	–	+	+	
Joint restrictions	–	–	+	+	+	+	+	
Facial characteristics:								
High bridge of the nose	+	+	+	+	+	+	+	
Prominent jaw	–	+	+	+	+	+	+	
Large external ears	+	+	+	+	+	+	+	
Ocular abnormalities:								
Squint	+	+	+	+	+	+	+	
Retinitis pigmentosa	–	–	–	+	+	+	+	
Dystrophic maculas	+	+	+	+	+	–	–	
Neurological characteristics:								
Lower neurone weakness	+	+	+	+	+	+	+	
Mental retardation	+	+	+	+	+	+	+	
Microcephaly	–	+	+	+	+	–	–	
Epilepsy	–	+	+	+	–	+	+	
Stroke like episodes	–	+	+	+	–	+	+	
Neurogenic hearing impairment	–	–	–	–	+	+	+	
Skeletal abnormalities:								
Osteopenia	–	–	+	–	+	+	+	
Kyphoscoliosis	–	–	+	+	+	+	+	
Delayed skeletal maturation	+	+	+	+	–	+	+	

+ = Present.

– = Absent.

Fig 1 Isoelectric focusing of samples of serum (S) and cerebrospinal fluid (CSF) from two affected children (left and middle) and one healthy control (right). Numbers to the right indicate bands corresponding to transferrin with 0–5 molecules of sialic acid. Arrows indicate the characteristic marker.

SIGNS AND SYMPTOMS (table)

During the neonatal period unspecific features of ill health like respiratory distress syndrome, polycythaemia, hyperbilirubinaemia, and suspected septicaemia were noticed in five of the children, and only two (cases 1 and 2) had entirely uneventful neonatal periods. All seven affected children were extremely floppy infants from birth or from the early postnatal period. Feeding difficulties and diarrhoea were obvious problems from around 6 months of age, at which time signs of liver disease were noticed. The gastrointestinal symptoms were accompanied by a slowing of growth at the age of 6–8 months and a subsequent 'catch-up' in growth between 12 and 18 months of age when the intestinal symptoms subsided. Mild non-progressive liver disease persisted during the first years, with slightly increased liver transferase activities, mild hepatomegaly, and morphological changes.

During early infancy, remarkable fat pads (which later disappeared) were noticed over the buttocks in five of the seven children (fig 2). Particularly on the lower parts of the body, the skin tended to be thick and adherent (peau d'orange), or like patchy sclerema. The following additional characteristics were recorded: a dysmorphic facial appearance (figs 2 b and d) with a high bridge to the nose and eyes close together; an asymmetric, small, or even microcephalic skull; a convergent squint and large external ears; a prominent jaw with a short neck; and inverted nipples and restricted lower limb joints contrasting with an otherwise extreme floppiness.

Early psychomotor development was delayed in all children, particularly from the age of 4–5 months,

when gross motor abilities lagged behind. The developmental retardation was subsequently found to be generalised, affecting principally gross motor development but also fine motor, social, and mental performances. The children's ability to sit, crawl, stand up, and walk with support was severely delayed. They learnt to move by rolling over or crawl-shuffling, and (when older) wheel chairing. Hand skill development seemed to be less affected than gross motor performance. Some of them learned to make simple drawings but not to read or write. They managed to eat by themselves and to use a knife and fork, and they could be toilet trained. Their intellectual capacity progressed through the first decade of life. Their mental ability permits them to understand spoken words but their own active speech is limited. The children have developed a simplified language comprehensible to siblings and parents and useful for family social life.

The neurological manifestations successively developed towards those of a severe weakness of a lower neurone type affecting both legs with additional traits of cerebellar ataxia and coordination difficulties. Tendon reflexes became increasingly weak and were impossible to elicit after 1 year of age. All the children have ocular abnormalities; severe alternating squint ($n=7$), retinal pigmentation ($n=4$), and dystrophic maculas ($n=3$).

Five have presented with 'stroke like episodes' during or after acute infections. Three children have had recurrent transient hemiparesis. One girl developed a Reye like syndrome after varicella (no liver biopsy was carried out). Another girl has twice (during a common cold on each occasion) become comatose for one to four days without any obvious cause; intracranial pressure, glucose homeostasis, acid base balance, liver function (including the urea cycle), and mitochondrial and peroxisomal function have all been normal during these periods. After the first incident the girl was blind for three months; her vision then reappeared. One child (case 2) had recurrent severe pericardial effusions during infancy and pericardectomy was carried out because of cardiac tamponade.

The two eldest sisters (family D) still have no signs of pubertal maturation, and extensive investigations show that they have primary hypogonadism. In contrast, case 5 has matured normally and passed puberty and his sister is at pubertal stage 2 at the age of 12 years.

LABORATORY INVESTIGATIONS

As well as studies on the marker, extensive additional investigations have been carried out over the years. Tests for intrauterine infections, metabolic disorders (amino acid abnormalities, organic aci-

Fig2 Characteristic features of the disialotransferrin developmental deficiency syndrome. (a) Fat pads over the upper, outer parts of the buttocks in a 6 month old infant. (b) Facial dysmorphic appearance: a convergent squint, large external ears, a prominent jaw, high bridge of the nose in a 1 year old boy. (c) A 5 year old boy with the characteristic features, including the weakness of lower motor neurone type affecting both legs. (d) A 14 year old boy with the characteristic appearance, with microcephaly, large external ear, high bridge of the nose, prominent jaw and short neck. (e) The same child in standing posture with deformed thorax and the weakness of a lower motor neurone type affecting both legs.

daemias, organic acidurias, glycogenoses, and mitochondrial and peroxisomal disorders), and malabsorptive disorders of the pancreas and intestines have all been unhelpful. All seven children have excreted an excess of oligosaccharides and sialic acid in their urine, although highly variable in amounts and with no consistent change of the oligosaccharide pattern. Excess amounts of mucopolysaccharides were found in three of the children, always with an increase of chondroitin sulphate. Nevertheless, primary defects in mucopolysaccharide, oligosaccharide, and glycogen metabolism could be ruled out, as could the diseases of

glucoprotein degradation like aspartylglucosaminuria and sialidosis.

In two of the children (case 6 and 7) low concentrations of pre β lipoproteins and β lipoproteins were found during infancy, but later both cholesterol and triglyceride concentrations were normal in serum, as in the other five children.

In one child (case 3) suspected of having familial mucopolipidosis, several acid hydrolases (α -fucosidase, β -hexosaminidase, β -glucuronidase, iduronosulphate sulphatase) were clearly increased in serum. In four children α_1 -antitrypsin activity

was abnormal, probably reflecting an abnormal sialic acid pattern similar to that of transferrin.

Protein was increased in the cerebrospinal fluid during infancy (0.6–1 g/l) in all cases, with signs of damage to the blood brain barrier, which later reverted to normal.

Nerve conduction velocity was reduced in all children after the age of 6 to 8 months, distal parts being more affected than proximal ones. Electromyography was normal in all. Computed tomography showed cerebellar hypotrophy in three of the six children investigated. It was normal in two, and the sixth child had diffuse calcification in the white matter and a low density area in the parieto-occipital region.

Biopsy specimens of the sural nerve were obtained from five children: at 6 months of age that from one boy was normal on both light and electron microscopy. The other four specimens were taken between 1 and 10 years of age and showed mild forms of neuronal-axonal neuropathy. Biopsy specimens of muscle were obtained from all seven children; these were either normal or only slightly abnormal on histological examination, reflecting the neuropathy. Biopsy specimens of liver were taken from three of the children between the ages of 6–24 months. All displayed steatosis with fat vacuoles and fibrosis. In one child a cirrhotic pattern was discerned. Glycogen seemed to be increased. After the age of 2 years, however, the liver seemed to be less affected, and activities of liver transferases, α -fetoprotein, and γ -glutamyltranspeptidases returned nearly to normal. At the age of 12 and 14 years, two had liver biopsy specimens that were normal.

Discussion

In this study a biological marker—abnormal transferrin with a characteristic isoelectrophoretic pattern—was found in both serum and cerebrospinal fluid of seven mentally deficient children all of whom had a remarkable combination of peculiar clinical signs. These children had seemed clinically odd from birth or early infancy with anomalous appearance, floppiness, respiratory problems, liver dysfunction, unusual 'peau d'orange' skin, and fat pads on the buttocks, as well as deviating psychomotor development. Later in childhood they developed a neurological disorder with severe weakness of the lower neurone type affecting both legs, combined with cerebellar signs and atrophy, epilepsy, impaired vision with macular dystrophy and retinal pigmentation, neurogenic hearing defects in some, pronounced secondary kyphoscoliosis with a hump, peculiar fibrous restricted movements in the hip and

knee joints, and unexplained osteopenia. There was a high risk of acute cerebral dysfunction during or after infectious diseases or other catabolic states. Episodes similar to Reye's syndrome with stupor or coma then tended to develop, but without hyperammonaemia, hypoglycaemia, or acute liver dysfunction.

To judge from our older patients, the long term course seems to be stationary as regards mental performance. Both gross and fine motor performance improved during the years of observation, but a slow, progressive downhill tendency was seen in both the lower neurone and the cerebellar functions. Differential diagnostic considerations were mainly lysosomal disorders, but the combination of dysmorphic symptoms and slowly progressive neurological disabilities have suggested associations with the group of peroxisomal disorders. Laboratory tests, however, have so far excluded traditional groups of intermediary metabolic disturbance, as well as oligosaccharidoses, mucopolysaccharidoses, glycogenoses, mitochondrial, and peroxisomal diseases. The laboratory findings were all unspecific and of no diagnostic help except for the characteristic changes of the transferrin.

The actual abnormality in transferrin was described by Jaeken *et al*^{1–3} and associated with a set of clinical signs that might well tally with those of our cases. The disialotransferrin band in some children is even stronger than the tetrasialotransferrin band, which is usually the predominating one. A similar sialic acid-transferrin pattern is however, also recognised in adult alcoholics during periods of abuse.^{6,7} In our experience, the pattern caused by alcohol is often much less pronounced than in the children described here. It is known to be dose dependent, however, and among the 8000 samples that we re-examined there were two with patterns indistinguishable from that of the disialotransferrin developmental deficiency syndrome. The well known neuronal cell and tract impairments caused by alcohol are of certain interest in this connection.⁸ The reason for the changes in the sialic acid transferrin pattern in alcoholics is unknown but there is some evidence that ethanol may inhibit glycosylation of glycoproteins with derangement of the carbohydrate composition of the glycoproteins, transferrin in particular.⁹ It would not be unreasonable to suspect that the disialotransferrin-developmental deficiency syndrome described in this study might as regards clinical manifestations be the effect of similar mechanisms, perhaps representing an inborn error of metabolism.

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