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EDITORIAL

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Intestinal neuronal dysplasia

Why does it only occur in parts of Europe?

Childhood constipation is a relatively common condition judged by the number of suction rectal biopsies received in paediatric pathology laboratories as part of the investigation of this condition. Once Hirschsprung's disease has been excluded, the question of intestinal neuronal dysplasia arises in the mind of the physician or surgeon. This condition is hardly ever diagnosed in the United Kingdom, and it is often asked why the incidence of intestinal neuronal dysplasia (usually erroneously described as neuronal intestinal dysplasia) varies so widely between Europe (excluding the United Kingdom) and the rest of the world. The current article by Dr Meier-Ruge again raises this issue, and the several possible explanations are addressed separately below.

Different clinical and surgical practices

In the United Kingdom the investigation of Hirschsprung's disease and of other causes of delayed passage of meconium and neonatal constipation is usually made early in the neonatal period when these problems first present. A suction rectal biopsy taken at that time will clearly identify those patients suffering from Hirschsprung's disease. In our practice at Great Ormond Street Hospital for Children, the mean age at diagnosis is 6.9±5.4 days. The methods of choice are H & E staining on 60 serial paraffin sections, and staining for acetylcholinesterase activity using a sensitive method on cryostat sections. An H & E stain appropriate for cryostat sections is also performed in parallel. With the frozen sections the diagnosis of Hirschsprung's disease can be accomplished with as few as 8 sections, and usually with only 2. Rectal biopsies at this age are taken no higher than 3–4 cm from the pectinate line to avoid the risk of perforation. Patients with Hirschsprung's disease have their aganglionic segment resected (mean age 5.0±3.0 months), and those in whom this diagnosis can be excluded are treated conservatively, or if necessary a colostomy is raised for management of the acute problem.

Dr Meier-Ruge, in his recent article (this Journal) and previously [13], has described patients from quite a different age range. Those with Hirschsprung's disease were not diagnosed until later (4±2 months), and those with intestinal neuronal dysplasia later still (18±12 months). However, he also states that in 95% of infants with intestinal neuronal dysplasia, gut motility normalizes in the first year of life. It would appear that the investigation and management of infant constipation is different in Germany and Switzerland from the remainder of Europe.

Different biopsy procedures

In the investigation of infant constipation, rectal mucosal biopsy samples are generally taken no higher than 3 cm above the pectinate line, to avoid the real possibility of perforation. In Dr Meier-Ruge's practice [13], biopsies are taken at various distances up to 12 cm, a procedure which United Kingdom paediatric surgeons regard as not only difficult but dangerous. Since the changes of intestinal neuronal dysplasia B do not appear to be present below 5 cm [3, 17] or 8–12 cm [13] and 6–7 cm (present article), only those surgeons who take very high biopsies are likely to find the changes considered to be diagnostic.

Different ways of handling the biopsy samples

The biopsy samples which Dr Meier-Ruge examines are 3–4 mm^3 and are serially sectioned at 12–15 μm. The characteristic hyperplastic ganglia with more than 7 ganglion cells, which occur only above 5–8 cm, represent only a small proportion of the total number of ganglia. Previously [13] he reported that giant ganglia comprised 20–30% of the total, but now he quotes a figure of only

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3–5%: hence the necessity to examine up to 160 serial sections stained with different techniques in order to find the pathological change. The normal procedures adopted by most histopathology laboratories require fewer, thinner sections, which could miss the features regarded as essential, even if they were present below 5 cm.

Different staining methods

The traditional baseline of all histopathological investigations is an H & E stain. This is usually poorly done on cryostat sections, but when performed properly [11] there is no difficulty in recognizing ganglia and ganglion cells. Dr Meier-Ruge and his colleagues do not use H & E staining but prefer a stain for lactate dehydrogenase activity to identify ganglia and ganglion cells, and also use a reaction for succinate dehydrogenase to identify "mature" ganglion cells. The use of an acetylcholinesterase (AChE) staining method, pioneered by Dr Meier-Ruge for the diagnosis of Hirschsprung's disease [12], is crucial to show the nerve bundles, fibres and ganglia. The Karnovsky & Roots method used by Dr Meier-Ruge for demonstration of the localization of AChE activity is a good general method, but its sensitivity can be improved [10] so that dubious manoeuvres such as increasing diffraction patterns by lowering the microscope condenser [4] are not necessary. It is surprising that in thick (15 μm) sections most ganglia contain fewer than 7 ganglion cells, since even in 5 μm sections ganglia of this size are often seen in biopsies taken at 1–3 cm in the neonatal period. Section thickness is an important factor in assessment of number of ganglion cells per ganglia, since ganglion cells measure up to 10 μm diameter in infants and values of up to three times that seen in 5 μm sections may be apparent in 15 μm sections.

Different ages of patients

In the neonatal period "giant" ganglia are frequently observed, and this is often accompanied by a prominent submucosal plexus. These features become much less prominent in rectal biopsies taken after 1 month of age and are clearly an age-related phenomenon [19]. Dr Meier-Ruge's patients tended to be older: the mean age at biopsy in his series of 773 patients was 15±9 months).

Different definitions

The criteria for the diagnosis of intestinal neuronal dysplasia keep changing. The current paper emphasizes the differences that occur with age, a feature not addressed by the Consensus of German Pathologists in 1991 [3], although introduced by Dr Meier-Ruge in 1994. He also now discriminates between age-dependent and age-independent findings in intestinal neuronal dysplasia. The increase in acetylcholinesterase-positive nerve fibres in the lamina propria, considered the most significant finding in 1981 [18], now becomes an optional feature. Hyperplasia of ganglia, regarded as an important finding in 1974 [12], was considered a less reliable indicator in 1981 but regained its importance as *the* diagnostic feature in 1991 [3]. The regular findings of increased AChE-positive nerves in the adventitia of arteries and in the circular muscle coat [3] no longer appear to be important. While most pathologists are under the impression that in intestinal neuronal dysplasia the myenteric plexus usually shows hyperganglionosis, it now appears that the myenteric plexus may be hypoganglionic, showing hypogenesis (?immaturity) or even heterotopic distribution of ganglion cells in the circular muscle layer.

Conclusions

While it is clear that the microscopical observations published by Dr Meier-Ruge are genuine, it is not clear that the findings relate to any real pathology. His concept of intestinal neuronal dysplasia as a problem of developmental immaturity of the enteric nervous system is supported by the resolution of the clinical symptoms in 95% of the patients by 1 year of age, but raises the question of whether there is any real pathology present or whether what is seen is within the normal range. Since there can be no ethically justifiable control biopsy samples, this question can only be resolved on post-mortem samples. This is something that should be addressed with some degree of urgency so that the apparent discrepancies of incidence can be resolved.

It is accepted, however, that there is a condition which has many of the features described by Dr Meier-Ruge in 1974, and this is intestinal neuronal dysplasia occurring proximal to the aganglionic segment in around 2–3% of all patients with Hirschsprung's disease. In these cases there is a definite quantitative two- to three-fold increase in the number of ganglion cells in the myenteric plexus. Such changes, together with giant submucosal ganglia and an increase in AChE-positive fibres in the lamina propria, have only been observed at Great Ormond Street Hospital for Children in the absence of Hirschsprung's disease in one patient in an experience spanning well over 20 years.

If intestinal neuronal dysplasia is a real disease having close association with Hirschsprung's disease, then molecular genetic studies of these patients with reference to the *ret* gene [1, 7, 8, 15], the endothelin B receptor [14], the endothelin 3 ligand (EDB3) [2] and possibly the *trk* gene [9] may also help in the resolution of the problem and clarify the position in relation to the multiple endocrine neoplasia syndromes with mutations in the *ret* gene [6, 8] in which giant submucosal ganglia and myenteric plexus hyperplasia are also observed.

The possibility that intestinal neuronal dysplasia might be a secondary phenomenon induced by congenital obstructive factors and/or inflammatory disease was raised by Sacher [16]. This possibility is further support-

ed by a recent report [5] of histologically characterized intestinal neuronal dysplasia occurring in 10 patients with colonic strictures following high-dose pancreatic enzyme supplements for cystic fibrosis.

The answer to the initial question is clear. The patients included in Dr Meier-Ruge's series and from whom he has derived the latest definition of microscopical features of intestinal neuronal dysplasia are of a different age range from that encountered by most paediatric surgeons and pathologists; the biopsy samples come from sites that many paediatric surgeons would consider hazardous; and the definition has changed yet again. It also appears that the clinical and surgical management of children with constipation is different in Germany and Switzerland, with later diagnosis and more invasive procedures. Whether the pathological changes described are induced or represent part of normal development or reflect a histological appearance without a defined clinical correlation, is something that is not yet clear and can only be resolved by careful observation. Proper control studies are lacking but are vital to the elucidation of a persistent problem.

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