Abnormalities of craniofacial development: discussion report

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The following topics arose as major areas of discussion after individual papers and in the general discussion at the end of the session. Discussion from both sources has been merged here to facilitate easier reading.

(i) Retinoids and embryopathy
Comments focused primarily on the differential teratogenicity of the various retinoids used experimentally and on the dose levels concerned. Dr Johnston raised the issue of retinoid blood levels following application of retinol to embryos. Studies by Kochhar in the seventies using radiolabelled retinol showed that the blood levels went up to a high peak following administration and then came down a certain extent and levelled off because of retinol coming out of the fat in the liver where it had been stored (Kochhar, 1977). In more recent studies in Newcastle, UK, Vitamin A apparently seemed to concentrate within the cartilages.

Different retinoids have been used in the various experimental studies making comparisons difficult sometimes. For instance, Dr Poswillo had used retinol in his studies whereas Dr Sulik reported the effects of retinoic acid. The timing of exposure and pharmacokinetics can be rather important when using these different forms of vitamin A. Dr Johnston pointed out that retinol may act over a longer period than retinoic acid and possible differences in the results may be related to which retinoid is used. In response, Dr Sulik said that both she and Dr Poswillo saw analogues of the Treacher Collins syndrome. A more significant difference in their studies was that she analysed the treated embryos at an earlier stage during pathogenesis. However, as Dr Poswillo agreed, ultimately the same conclusions were reached. With regard to the effects of retinoic acid on the secondary palate, Dr Sulik reported that she had occasionally seen clefts and these were very large. It appears that the palatal shelves do not form and a portion of the maxillary prominence is wiped out.

The question was raised of how the doses of retinoic acid and Accutane® used by Dr Sulik and colleagues compared with the accumulative doses in humans. In reply, Dr Webster explained that the Accutane® levels in serum that are teratogenic in humans are well below the level achieved in the pregnant mouse. However, the whole embryo culture studies indicated that the 4-oxy-metabolite has roughly equal teratogenicity and that the combined human serum level of both compounds are teratogenic to the rat embryo in culture. Only trace amounts of the 4-oxy-metabolite are found in the mouse serum. With regard to excess intake of Vitamin A in humans the chief concern is when people are taking 'mega' doses between 50,000 and 100,000 international units per day.

(ii) Fetal alcohol syndrome
There was some discussion about the animal model for the fetal alcohol syndrome described by Dr Sulik in her paper. The brain defects observed fall into the holoprosencephaly series of syndromes. Just the anterior part of the brain is affected and there are single cerebral hemispheres. A puzzle was raised about the facial defects in the animal model. When ethanol is given at an early stage, a big (long) upper lip and some of the extravasations of blood mechanisms for the production of clots. There was no general consensus on this point. Several people work on early embryonic chicks and mice suggested that the clotting mechanism is not functional early in development. Human fetal blood samples at 17 weeks clots whereas chorionic blood taken at 9 weeks does not do so. In Dr Poswillo's thalidomide studies on monkeys, haemorrhagic blebs developed and remained as pooled blood, whereas in the human hemifacial microsomia, in the mouse model, the extravasated blood seemed to consist of aggregations of red cells. It was not clear whether the blood had clotted or merely been retained in tissue spaces.

Dr Brinkley offered one possible mechanism for the production of clots and some of the extravasations of blood observed by Dr Poswillo. She suggested that glycosaminoglycans (GAGs) could be involved. GAGs are important in vascular development and, according to their molecular weight, can either strengthen vascular walls or stimulate vascular proliferation and angiogenesis. The role of GAGs in strengthening vessel walls could be deduced from her own work on palatal shelves (Brinkley & Vickerman, 1982). If GAG production is interfered with, the vascular walls would be weaker and, even with normal blood pressure, the vascular walls could blow out leading to extravasation of blood.

Hemifacial microsomia is a term that may describe a wide variety of defects. The defect described by Dr Poswillo and which is associated with haemorrhaging, may arise rather late in development. In Goldenhaar's syndrome (the OAV spectrum), there are eye and cervical and thoracic vertebral defects in addition to facial changes. In this case, the syndrome may result from an early defect in development and involve not the formation of blood clots but a primary insult on neural crest cells.

(iii) Haemorrhagic disorder and craniofacial development
In his talk, Dr Poswillo had suggested that hemifacial microsomia may be due to the formation of blood clots and that delay in their absorption may disrupt development. This idea stimulated discussion about embryonic blood clots and the possible mechanisms that could lead to the formation of clots. There was no general consensus on this point. Several people work on early embryonic chicks and mice suggested that the clotting mechanism is not functional early in development. Human fetal blood samples at 17 weeks clots whereas chorionic blood taken at 9 weeks does not do so. In Dr Poswillo's thalidomide studies on monkeys, haemorrhagic blebs developed and remained as pooled blood, whereas in the human hemifacial microsomia, in the mouse model, the extravasated blood seemed to consist of aggregations of red cells. It was not clear whether the blood had clotted or merely been retained in tissue spaces.
(iv) Unilateral defects in craniofacial growth and development

A discussion on hemifacial microsomia led on to a consideration of the broader topic of asymmetries arising in craniofacial development. During discussion of Dr Poswillo's paper, it was explained that hemifacial microsomia can arise during adolescence by asymmetrical growth of the mandibular condyles. This is isolated from any general growth defect or indeed any alterations in the growth of any other cartilage. Dr Poswillo explained that 'overgrowth' of the secondary cartilage of a single condyle can be corrected by excision of that condyle after which an articulating surface will regenerate; the new condyle so formed will display normal growth and proliferative behaviour.

The cartilage of the mandibular condyle is secondary cartilage which, it is well known from a number of other systems, responds to shear and pressure by growth and proliferation (Hall, 1978). Hyperpropulsive devices fitted to the overlying masseter muscles can, in experimental situations, cause hypertrophy of the mandibular condyle (Petrovic, Oudet & Gasson, 1973). However, in clinical cases there is apparently no association between masseteric hypertrophy and hemifacial microsomia. Electromyographic recording reveals that there are no significant differences between the masseteric muscles on the two sides. The driving force therefore appears to be the mandibular condyle itself. Dr Poswillo reported that changes in the neck of the condyle can be seen which histology reveals to contain some trapped cartilage cells (within the bone), although the significance of this observation is far from clear. Clearly culture of condylar tissue to look for intrinsic differences in growth potential might be a sensible way to proceed but has not been possible to date. Thus, the factor or factors triggering this asymmetric growth of the mandibular condyles remain to be identified.

There are several well-known asymmetries occurring much earlier in embryonic/fetal development, in particular, unilateral clefting. The possibility of familial predisposition to right or left clefting was raised but there is insufficient data to answer this point. However, as Dr Poswillo pointed out, what is more important in terms of the type and severity of clefting is sex of the embryo in so far as males are more susceptible to cleft lip (with or without cleft palate) whereas females have a higher incidence of isolated cleft palate. Left-side clefting seems to predominate and, as Dr M. Johnston pointed out, the incidence may be twice that for right-side clefting. Comparable figures exist for a preponderance of left-side clefting in various animal models. A possible genetic basis for 'sidedness' of this defect was discussed but with no clear conclusions other than that the early and inherent asymmetry of the cardiovascular system was almost always implicated in asymmetrical abnormalities.

(v) Facial characteristics of certain craniofacial disorders

Professor Wolpert raised the question of whether Down's syndrome children look more alike than would be expected given the variation in the normal population. Dr Poswillo agreed that this could indeed be the case. In Down's syndrome, there are specific anatomical alterations such as the width of the skull, the prominence of the forehead and the flatness of the face in males, all of which characterize a face. Individuals with craniofacial malformation syndromes, including both Down's and Treacher-Colhns syndrome look much more like each other than their normal siblings.

(vi) The genetic basis of clefting (and related disorders)

A number of different aspects of this topic were raised for discussion; the principal ones concerned the role of growth (especially of the tongue), how many genes might be involved, what the gene products might be and speculation over the future application of molecular genetics in this area. There is considerable evidence that the tongue may be a major factor in some types of cleft palate. The palatal shelves grow down beside the tongue and the tongue itself has to be drawn down from between the shelves before they are able to swing up and contact each other. Dr Johnston reported studies on monozygotic twins that are discordant for facial clefts. The unaffected twin presumably only just missed getting the cleft and would grow like anyone else in the population in contrast to the affected twin whose growth would be affected by the presence of the cleft. Compared to controls that have no close relatives with clefts, the morphology in the lower facial region in non-cleft twins whose co-twin has cleft palate suggests an increase in tongue size by as much as 30% which puts them at the limits for their control groups. Such large tongues could interfere with shelf elevation and contact. Dr Johnston emphasized that these are the common types of cleft palate, not those associated with syndromes. These comments led to speculation about the possible role of cell death in normal tongue and palatal morphogenesis and whether an enlarged tongue and/or ankyloglossia reflected an absence of cell death.

There are known differences in the growth rates of male and female embryos detectable very early on. In the mouse, the order of magnitude during organogenesis is roughly two somite pairs, that is, a time difference of 4–5h. Given this difference, an error in timing of some developmental event, arising simply from an autosomal defect, would result in sex differences in phenotype. Clearly this is not so relevant in an X-linked disorder of the type reported by Dr Moore and his colleagues for cleft palate. Professor Williamson explained that this presents an unusual problem in that the female carrier, because of Lyonization, would be expected to have a partial defect but doesn't have. In fact, the females who are carriers are tongue-tied, which is underneath the tongue. The males who are affected have a cleft secondary palate and the penetrance is almost 100%. Professor Williamson felt that this had to be telling us something important about genetic control of palate and tongue morphogenesis but that it isn't yet clear what that might be.

Professor Ferguson, prompted by the observation that not only palate but tongue and teeth may also be involved in this disorder, speculated on whether a single gene or a cluster of genes, closely associated for cleft palate. Professor Williamson explained that this presents an unusual problem in that the female carrier, because of Lyonization, would be expected to have a partial defect but doesn't have. In fact, the females who are carriers are tongue-tied, which is underneath the tongue. The males who are affected have a cleft secondary palate and the penetrance is almost 100%. Professor Williamson felt that this had to be telling us something important about genetic control of palate and tongue morphogenesis but that it isn't yet clear what that might be.

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morphology) that is three megabases long and if you know that the great majority of cases are deletions, whether very small or somewhat larger, and if you know that the severity of dystrophy (that is, Duchenne or Becker) is related in many cases not only to the size of the deletion but whether the deletion creates an in-phase or out-of-phase space for the rest of the protein, then one begins to wonder about the precise number of genes involved. With such a good model on the other arm of the X chromosome, maybe what we are looking at is either one very large gene or a family of genes, or a family of genes plus a regulator. Most X-chromosome mutations are probably deletions. Where and how the deletion occurs may be central in determining the severity and the precise nature of the phenotype.

Can one determine the gene product and deduce its function having once isolated and sequenced the gene involved in the disorder? Dr Moore replied that this could be done by 'reverse genetics' as is currently being undertaken for the cystic fibrosis gene. One can speculate over a number of possible candidates for the gene product involved in the X-linked cleft palate disorder and, in the light of Professor Ferguson's results (see Ferguson - this volume) Dr Moore and colleagues are exploring the possibility that type IX collagen might be involved.

Current recombinant DNA techniques and DNA-sequencing strategies, coupled with enough manpower, mean that it is possible, theoretically at least, for these large segments of the chromosome consisting of several million base pairs, to be sequenced and for multiple cDNA probes to be produced. With these powerful approaches how can we be sure that a particular cDNA probe is precisely for that gene involved in the mutation and not simply for an associated gene? Professor Williamson replied that there are two answers.

"Firstly, from studies of sequence homology — for example, if it were a growth factor or growth factor receptor, that would be a pretty good candidate. The other way is to create animal models using transgenic experiments and this has now become an extremely powerful technique. One is not advocating models to replace human conditions but we now have an opportunity of applying reverse genetics in a fascinating way to create animal models, starting from human genes and clearly that’s going to be a very productive and interesting way to go."

References


