Editorial

GENETICS IN PEDIATRIC PRACTICE: AN INDIAN PERSPECTIVE

Recent advances in molecular technology have revolutionised the practice of medical genetics. The genes for an increasing number of human diseases are being mapped, cloned and sequenced. The human genome consists of some 3 billion base pairs. Possibly only 5% of this DNA codes for specific proteins, while the rest is made up of repetitive sequences, introns and flanking regions(1). McKusick's catalogue lists nearly 5,000 loci with phenotypes that are inherited in Mendelian fashion, *i.e.,* as single gene disorders(2). These would represent only about 5-10% of man's structural genes. Many of these loci have been mapped and about 500 genes have been cloned(3).

Classical Approach in Genetics

The classical approach in genetics has been one of moving from abnormal clinical features, through the pathology of organs and tissues and biochemical defects to an identified defective protein product and finally to the gene. This approach has been successful in delineating the molecular pathology of the hemoglobinopathies; the beta globin gene locus on chromosome 11 has been extensively studied and a variety of mutations have been identified which all produce abnormal/reduced hemoglobin resulting disease(4). However, genes for Duchenne muscular dystrophy and cystic fibrosis were mapped and cloned even though no gene product was known(5,6). Here the approach was to locate the gene using all available information, often large rearrangements of DNA or chromosomal deletions, closing in on the gene by using linkage to available DNA markers, cloning this region and characterising the gene and finally building up a complementary protein(1). By this process of "reverse genetics" the defective proteins in these diseases have been identified and sequenced(7,8). Recently, the gene defect causing Marfan syndrome has been successfully localised to chromosome15(9). This combined with the demonstration that fibrillin immunofluorescence is consistently decreased in the skin of patients of Marfan syndrome(10) may help to resolve the pathology of this disease. Attempts are under way to determine the function of proteins identified in this way. The hope is that this may eventually lead to rational therapy.

While treatment of most genetic disorders still remains a distant prospect, the direct benefit of localising disease genes and establishing closely linked DNA markers to these genes, if not actually direct study of the gene, lies in offering prenatal diagnosis to affected families. This would avoid the birth of another affected child. For example, this has been successfully carried out for conditions such as Werdnig Hoffman's disease(11), Freidreich's ataxia(12), neurofibromatosis 1(13) and retinoblastoma(14).

Genetics and Environment

Also, more effort is now being put in to

studying common disorders which have a genetic component interacting with the environment. These include several childhood disorders like asthma, insulin dependent diabetes mellitus (IDDM), epilepsy and some congenital malformations. Using linked markers, it is now possible that their complexities may be resolved. For IDDM it has been established that the presence of the aminoacid aspartic acid in position 57 of the beta chain of the DQ HLA antigen is protective; while its substitution with a non-charged aminoacid (alanine or serine) alters the configuration of the molecule in such a way that antigen presentation is enhanced, allowing for antigen-antibody reaction to occur that will produce beta cell damage(15). Ardinger *et al.*(16) have demonstrated an association of cleft lip and palate with two markers (RFLPs) at the transforming growth factor alpha locus. The role of homebox genes in limb development is being understood(17). All this information is expected to provide new insights into birth defects.

Cytogenetics

In the field of cytogenetics, advances have been chiefly in improving the resolution of chromosomal preparations to the level of identifying deletions and rearrangements involving tiny fragments. Such studies have led to the recognition of chromosome microdeletions as being the cause of dysmorphic syndromes such as the Prader-Willi syndrome, Angelman syndrome and, Langer Giedion syndrome(2).

The discovery of a fragile site on the long arm of the X-chromosome linked with mental retardation and particular phenotypic features has led to the elucidation of the fragile X-syndrome as the second commonest cause of mental retardation after Down syndrome, and the commonest inherited cause of mental retardation(18). Studies carried out at AIIMS have revealed a prevalence of 18 per 1,000 among patients with mental retardation, and 2.8% among boys with mental handicap(19). These figures probably are an underestimate, as only selected cases were screened cytogenetically. Most recently the gene for the disorder has been identified(20). More studies in India in this area would be justified, as this condition causes a considerable burden and can be diagnosed prenatally to reduce the risk for future generations.

Molecular cytogenetics is a newer field where hybridisation with specially treated DNA fragments permits their chromosomal localisation, both in metaphase and interphase cells. Even with high resolution banding it is difficult to detect deficiencies which measure less than 0.2% of the total haploid length. With FISH (fluorescent in situ hybridisation) and other cytogenetic techniques applied to interphase cells, the current limits of resolution lie around a genomic distance of 50 kilobases, which is greater than the distance of 5-20 cM (centiMorgans) achieved by DNA linkage studies(21). Hence these techniques have great potential in accurate gene mapping.

Indian Perspective

In a developing country, such as ours, committed to achieving the goals of the Alma Ata declaration, control of infectious diseases has been a major preoccupation within the pediatric health care system. A decline in common infections appears to be occurring and an increase in disorders of genetic origin among urban hospital births, out patients and admissions is becoming obvious. Increasing economic pressures on families particularly in urban areas forces a

limiting of family size. As a result many prospective parents now actively seek measures by which birth defects and genetic disorders can be prevented. A pregnancy is no longer undertaken lightly but is carefully planned. Though individually rare, within the particular family setting, and because of their generally incurable and crippling course, these disorders are responsible for considerable mental trauma and economic burden. We have argued earlier that genetic disorders deserve attention even in developing countries(22). Due to large population, a high birth rate and consanguineous marriages, the prevalence of genetic diseases is as high as elsewhere, while rehabilitative and support facilities are grossly inadequate(23). There is already great demand for genetic services in urban areas. At the AIIMS where specialist genetics services are provided for almost all of North India, statistics reflect this growing demand for diagnosis, counselling and prenatal diagnosis. Patients come from a wide variety of backgrounds, and are referred from many specialities. The types of disorders managed are shown in *Table I*. Unfortunately,

TABLE I—*Disorders Managed at the Genetics Clinic, AIIMS, (Jan, 1989 to Dec, 1990)*

Disorder		%	No.	%
Multiple congenital anomalies			374	24.7
Chromosomal			302	19.9
Down syndrome	187	(12.3)		
Turner syndrome	63	(4.2)		
Klinefelter syndrome	10	(0.6)		
Others	42	(2.8)		
Prenatal diagnosis			239	15.8
Neural tube defects	120	(10.5)		
Previous Down syndrome	41	(2.6)		
Thalassemia	26	(1.6)		
Others	52	(3.2)		
Obstetric problems			237	15.6
Recurrent miscarriages	151	(10.0)		
Bad obstetric history	86	(5.7)		
Metabolic disorders			112	7.4
Identifiable syndromes			45	3.0
Environmental disorders			25	1.6
Skeletal dysplasias			23	1.5
Miscellaneous			159	10.5
Total new cases			1516	100.0

(% of total.)

such specialist services are only available in a few large cities. Also, clinicians are often not aware of the existence and role of such a service. Hence, many families who could otherwise be helped either come too late or never realise that prenatal diagnosis could be relevant to them. Cost of tests unfortunately can be a limiting consideration particularly with molecular techniques where chemicals are expensive.

Most genetic or multifactorial disorders with a genetic component present in childhood, and the disease comes to light only after the birth of an affected child in the family. As a result the pediatrician is a key figure, often the first to be consulted. *The cornerstone of clinical genetics is accurate diagnosis and determination of the mode of inheritance*(24). The pediatrician should be able, therefore, to recognize a condition as genetic and counsel the family. Counselling should be non-directive; the family should be given necessary information and left to make their decisions on issues like prenatal diagnosis, future child bearing, *etc.* For illiterate patients with no knowledge of genetics, a more directive approach may be permissible(25). For common disorders like Down syndrome, or neural tube defects, further action will depend on the training of the counsellor, and the availability of specialist services for prenatal diagnosis. He should be familiar with various patterns of inheritance, principles of dysmorphology, diagnosis of chromosomal disorders and approach to a child with mental handicap.

A pediatrician must know how to deal with a child having *congenital malformations*, as these are common occurring with a frequency of 19.4 per 1,000 births, based on an analysis of 301,897 births from 25 hospitals(26). As these data are collected from different hospitals in India at different times by investigators with differing skills in dysmorphology and epidemiology, we have initiated a multi-centric study to collect this information, on a voluntary basis. Preliminary analysis of this data reveal a frequency of 16.8 per 1000 live births for congenital malformations, based on the data obtained from 34,650 births in 18 centres in India(27). We hope to secure funds to continue this project as it is likely to yield important etiology information, as well as help us to establish a Birth Defect Registry in India.

Another common problem encountered in genetic clinics is mental retardation. Expertise in its diagnosis and management is highly desirable. Recently, the results of an ICMR-sponsored multicentric study to determine the genetic cause of mental retardation has been published(28). This report, along with others(29), shows that the largest group of cases had undifferentiated mental retardation, *i.e.,* where the diagnosis was nonspecific mental retardation (59.7%). Chromosomal disorders accounted for 23.7% of cases, identifiable syndromes 11.5%, and metabolic disorders 4.9%.

For those in the field of neonatology, it may be necessary to occasionally investigate and manage a baby with a suspected metabolic defect or one with ambiguous genitalia. These situations may call for "crisis" counselling. Detection and management of congenital malformations at birth with appropriate counselling is a situation every pediatrician will face. Ascertaining the cause of a stillbirth is best initiated by the well informed neonatologist who is called to resuscitate the baby(30). It is useful to obtain a full body radiograph in every still born, especially if there is dwarfism. It must be

realised that once the baby is dead without adequate recording of malformations and appropriate investigations, counselling is impossible. Therefore, every pediatrician dealing with neonates in particular must be familiar with postmortem protocols for diagnosis of suspected genetic disease (*Table II*). Using this approach in two neonates dying of suspected metabolic disease, the authors' laboratory has recently diagnosed maple syrup urine disease and citrullinemia. A definite and precise diagnosis will be invaluable in counselling the family.

Clinical genetic subjects are covered in many pediatric textbooks and are already part of most undergraduate and postgraduate teaching. However, it would appear that this instruction should be consolidated into a clinical genetics module, at least in postgraduate courses. More-over in today's medical practice no clinician can do without knowledge of the basics of molecular genetics, in view of revolutionary advances which have immediate applications in clinical practice. Teaching in these fields, must, therefore, be carried out by those constantly in touch with recent developments.

Genetic Counselling

Genetic counselling must be followed up by action to be meaningful. Prenatal

TABLE II—*Postmortem Investigation of Suspected Genetic Disease*

1.	*Full body radiograph:*	In stillbirths/suspected skeletal dysplasias.
2.	*Chromosomal analysis:* 2-5 ml of heparinised blood, intracardiac (sterile), Skin biopsy in normal saline or special media (clean with alcohol or spirit, not iodine), keep at normal room temperature, transport to lab within 6 hours. *No freezing or formalin.	In multiple malformations.
3.	*Biochemical autopsy:* 5-10 ml of blood, separate plasma, freeze in 1 ml aliquots at–20°C. If urine/plasma are unavailable, collect CSF/ vitreous homor. Skin biopsy collected and stored as above. Biopsies of liver, muscle, kidney, brain frozen in liquid nitrogen, or in special fixative for electron microscopy (if facilities for these tests are available).	In suspected metabolic disease.
4.	*DNA studies:* 15-10 ml of blood in EDTA (sterile). Skin biopsy for fibroblast cultures.	In single gene disorders.

diagnosis is one such option. In this field efforts have been made to obtain fetal tissue at earlier gestations than conventional amniocentesis so that earlier and less traumatic termination of pregnancy can be offered. Chorionic villus sampling at 9-12 weeks of pregnancy by the transvaginal route has been demonstrated to be safe with a low risk of abortion. In later pregnancy the transabdominal route can be used depending upon the expertise of the obstetrician. Tissue thus obtained can be used for chromosomal analysis, DNA analysis or enzyme assay. Special mention must be made of the polymerase chain reaction (PCR), which amplifies small amounts of DNA a million fold or more, and makes it possible to carry out DNA studies even from filter paper blood specimens(31). This technique avoids the use of radioactive probes and requires very small amounts of blood. In our laboratory we apply it for prenatal diagnosis of disorders like thalassemia and Duchenne muscular dystrophy.

Amniotic fluid is commonly used for chromosomal analysis or enzyme assay, for those women reporting later in midtrimester(32). Ultrasound remains a noninvasive tool for the detection of structural abnormalities. High or low maternal serum alphafetoprotein is a test that is used for screening for neural tube defects and chromosome abnormalities and is often a predictor of complications in pregnancy. The alpha fetoprotein value should be expressed as multiples of the median value for a particular gestation. Such standards for the Indian population are available at the Genetics Unit, AIIMS and may be requested from the authors(33).

Needless to say *timing is critical for initiating prenatal diagnosis* and patients must be referred as soon as pregnancy is confirmed for optimal management. It is always preferable for the couple to be referred to a specialist geneticist *before pregnancy* for a discussion of risks, tests available and for appointments etc. Similarly, it is always of more value if an affected child is seen with the parents, rather than examining retrospectively records of a deceased child.

Ethical and moral considerations are inseparably linked to the field of genetics and must not be abandoned in our search for perfection. Fetal testing, rights of handicapped persons, confidentiality, embryo research, *etc.* are some of the issues involved. Sex determination remains controversial, linked to socio-economic and cultural values in our country(34). In general prenatal diagnosis is undertaken for high risk conditions which are potentially crippling or incurable. It must be remembered, that for a particular couple who have had one such affected child, any risk, however small, may be unacceptable.

To conclude, the facilities required for a basic genetics service are genetic counselling, chromosome analysis and aminoacid chromatography. These facilities are within the reach of any pediatric division of a teaching hospital. The role of an organisation like the Indian Academy of Pediatrics should lie in developing curricula for teaching of genetics at undergraduate and postgraduate level, organising updates for pediatricians in different parts of the country, helping to establish small genetic units in university hospitals, and developing guildelines for ethical considerations in genetic practice. The Academy should also publish simple instructional leaflets providing guidance on common

genetic problems to patients, parents and doctors to promote better and wider use of genetic services.

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REFERENCES

1. Gardiner R. The human genome: a prospect for pediatrics. Arch Dis Child 1990; 65: 457-461.
2. McKusick VA. Mendelian Inheritance in Man, 10th edn. Baltimore, The Johns Hopkins University Press, 1990.
3. Human Gene Mapping 10.5. Oxford Conference (1990). Update to the 10th International Workshop on Human Gene Mapping. Cytogenetics and Cell Genetics 1990; 55: Nos 1-4.
4. Steinberg MH, Adams JG III. Thalassemia: recent insights into molecular mechanisms. Am J Hemat 1982; 12: 81-92.
5. Kunkel LM, Monaco AP, Middlesworth W, *et al*. Specific cloning of DNA fragments absent from the DNA of a male patient with an *X*-chromosome deletion. Proc Nat Acad Sci 1985, *82*: 4778-4782.
6. Wainwright BJ, Scambler PJ, Schmidtke J, *et al*. Localisation of the cystic fibrosis locus to human chromosome 7 cen-p22. Nature 1985, 318: 384-385.
7. Hoffman EP, Brown RH Jr, Kunkel LM. The protein product of the DMD locus, Cell 1987, 51: 919-928.
8. Riordan JR, Rommens JM, Kerem B, *et al*. Identification of the cystic fibrosis gene: cloning and characterisation of complementary DNA. Science 1989, 245: 1066-1072.
9. Kainuleinen K, Pulkinnen L, Savolainen A, *et al*. Location on chromosome 15 of the gene defect causing Marfan syndrome. New Engl J Med 1990, 323: 935-939.
10. Godfrey M, Menashe V, Weleber RG, *et al*. Cosegregation of elastin associated microfibrillar abnormalities with the Marfan phenotype. Am J Hum Genet 1900, 46: 652-660.
11. Melki J, Sheth P, Abdelha KS, *et al*. Mapping of acute (type 1) spinal muscular atrophy to chromosome 5q12-q14. Lancet 1990, ii: 271-272.
12. Chamberlain S, Shaw J, Rowland A, *et al*. Mapping of the mutation causing Freidreich's ataxia to human chromosome 9. Nature 1988, 334: 248-250.
13. Collins FS, Ponder BA, Seizinger BR, *et al*. Editorial: The von Recklinghausen neurofibromatosis region on chromosome 17-genetic and physical maps come into focus. Am J Hum Genet 1989, 44: 1-5.
14. Dryja TP, Rapaport JM, Joyce JM, Petersen RA. Molecular detection of deletions involving band q14 of chromosome 13 in retinoblastoma. Proc Nat Acad Sci 1986, 83: 7391-7394.
15. Todd JA, Bell JI, McDevitt HO. HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature 1987, 329: 599-604.
16. Ardinger HH, Buetow KH, Bell GI, *et al*. Association of genetic variation of the transforming growth factor alpha gene with cleft lip and palate. Am J Hum Genet 1989, 45: 348-353.
17. Dolle P, Izpisua-Behmonte JC, Falkenstain H, *et al*. Coordinate expression of the marine. Hox-5 complex homebox—containing genes during limb pattern formation. Nature 1989, 342: 767-772.

18. Webb TP, Bundey S, Thake A, Todd J. The frequency of the fragile X-chromosome among school children in Coventry. J Med Genet 1986, 23: 396-399.

19. Elango R, Verma IC. Fragile X-syrdrome in Delhi. I. Prevalence among the mentally retarded (In press).

20. Ververk AJMH, Pieretti M, Sutcliffe JS, *et al.* Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 1991; 65: 905-914.

21. Ferguson-Smith MA. Invited editorial: Putting genetics back into cytogenetics. Am J Hum Genet 1991, 48: 179-182.

22. Verma IC. Genetic disorders need more attention in developing countries. World Hlth Forum 1986, 7: 69-70.

23. Verma IC. Genetic disorders in India. *In:* Medical Genetics in India, Vol 1. Ed Verma IC. Pondicherry, Auroma Enterprises 1978, pp 5-18.

24. Verma IC. Genetic counselling and control of genetic disease in India. In: Genetic Research in India. Ed Verma IC Delhi, Sagar Printers and Publishers. 1986, pp 21-37.

25. Verma IC. Ethical issues arising in molecular genetics in developing countries. *In:* Ethical Issues of Molecular Genetics in Psychiatry. Eds Sram RJ, Bulyzhenkov V, Prilipko L, Christen Y. Berlin, Springer-Verlag 1981: 134-148.

26. Verma IC, Elango R, Mehta L. Monitoring reproductive and developmental effect of environmental factors in India-A review. Program and Abstracts Indo-US Symposium–Effect of Environmental and Genetic Factors on Pregnancy Outcome. New Delhi, All India Institute of Medical Sciences, 1990, pp 63-73.

27. Mehta L, Verma IC. Congenital malformations in India–a multicentric study. Indian J Pediatr (In press).

28. ICMR Collaboratory Centres and Coordinating Unit. Multicentric study on genetic causes of mental retardation in India. Indian J Med Res 1991, 94: 161-169

29. Verma IC. Genetic causes of mental retardation in India. *In:* The Genetics of Mental Retardation. Eds Hicks EK, and Berg, Dardrecht, Kluver Academic Publishers, 1988, pp 99-106.

30. Mehta L, Verma IC. Diagnostic approach to stillbirths. Indian J Pediatr 1990, 57: 601-605.

31. Saiki RK, Gelfand DH, Stoffel S, *et al.* Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 1988, 239: 487-491.

32. Verma IC, Mathew S, Elango R, *et al.* Prenatal diagnosis of chromosomal disorders in Delhi. Indian Pediatr 1990, 27: 459-462.

33. Kaur M, Verma IC. Biochemical markers in maternal serum for prenatal diagnosis. *In:* Proceedings Indo-US Symposium Effect of Environmental and Genetic factors on Pregnancy Outcome, 1991 (In Press).

34. Verma IC, Singh M. Ethics and medical genetics in India. *In:* Ethics and Human Genetics–A Cross-cultural Perspective. Eds Fletcher JC, Wertz DE Hiedelberg, Springer-Verlag 1989, pp 250-270.