American Journal of Medical Genetics 34:237–245 (1989)

Invited Editorial

Clinical, Cytogenetic, and Molecular Approaches to the Genetic Heterogeneity of Holoprosencephaly

Maximilian Münke

Howard Hughes Medical Institute and Department of Human Genetics, University of Pennsylvania School of Medicine and Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, Philadelphia

INTRODUCTION

> Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of Nature by careful investigation of cases of rarer forms of disease. For it has been found, in almost all things, that what they contain of useful or applicable is hardly perceived unless we are deprived of them, or they become deranged in some way.
>
> —William Harvey [1657, cited by Garrod, 1928].

The abnormal development of brain and face in the holoprosencephaly sequence is one example of such "workings apart from the beaten path." Brain malformation in holoprosencephaly may include an abnormally small forebrain vesicle with complete failure of formation of the hemispheres as seen in alobar holoprosencephaly, a single ventricle with rudimentary lobes as seen in semilobar holoprosencephaly, or well-formed lobes with communication of the lateral ventricles caused by absence of the septum pellucidum as seen in lobar holoprosencephaly. These brain findings have been correlated with facial anomalies that range from the most severe types with anophthalmia, cyclopia, ethmocephaly, and cebocephaly to less severe forms with hypotelorism, flat nasal bridge, maxillary agenesis, median cleft lip, or single upper midline incisor [DeMeyer et al., 1964].

Embryologically, the craniofacial malformations of holoprosencephaly can be traced back to the gastrulation stage, which involves a conversion of the two-layered into a three-layered embryo during the third week after conception [Webster et al., 1988]. There is evidence that interference with gastrulation by teratogens or genetic factors results in detectable deficiencies in the neural plate which may then lead to facial abnormalities and CNS defects [Sulik and Johnston, 1982; Sulik et al., 1988].

WHAT THEN CAUSES HOLOPROSENCEPHALY?

There are numerous reports of holoprosencephaly associated with either teratogenic agents or genetic factors (Figs. 1, 2; Table I). Whether these factors are causally related to holoprosencephaly or are merely coincidental is not always certain. Among the embryopathies, maternal diabetes is a well-known association. Infants of diabetic mothers have a 1% risk and a 200-fold increase for holoprosencephaly [Barr et al., 1983; Dekaban, 1959; Farquhar, 1969; Probst, 1979, case 21]. Prenatal cytomegalovirus infection has been described in infants with congenital eye abnormalities, including cyclopia [McCarthy et al., 1980; Frenkel et al., 1980; Byrne et al., 1987]. Other prenatal infections that have been associated with holoprosencephaly are rubella [Castel et al., 1976; Probst, 1979] and toxoplasma [Lison et al., 1967]. Holoprosencephaly has been produced in mice by alcohol [Sulik and Johnston, 1982]. In the literature on humans, there are reports of four infants with holoprosencephaly whose mothers consumed alcohol heavily early in pregnancy [Jellinger et al., 1981; Ronen and Andrews, 1988]. Other causal agents that have been implicated in holoprosencephaly are maternal ingestion of salicylates [Khudr and Olding, 1973; Benawra et al., 1980; Agapitos et al., 1986], high doses of contraceptives [Stabile et al., 1985], quinine [Probst, 1979, case 10; Jellinger et al., 1981], retinoic acid [Lammer et al., 1985], cortisone [Khudr and Olding, 1973], and irradiation [Castel et al., 1976; Jellinger et al., 1981]. In animals, holoprosencephaly has been induced experimentally by a variety of agents [Binns et al., 1962; Rogers, 1963; Keeler and Binns, 1968; Keeler, 1970]. In addition to sporadic holoprosencephaly, e.g., without any identifiable cause, there is clear-cut evidence for a genetic cause of holoprosencephaly. This evidence comes from 1) observations of defined and undefined genetic syndromes associated with holoprosencephaly, 2) family studies with several affected relatives, and 3) nonrandom chromosome anomalies.

First, holoprosencephaly has been described in genetic syndromes with Mendelian inheritance and in as-

Received for publication March 29, 1989; revision accepted April 24, 1989.

Address reprint requests to Dr. M. Münke, Department of Human Genetics, University of Pennsylvania School of Medicine, 37th and Hamilton Walk, Philadelphia, PA 19104-6072.

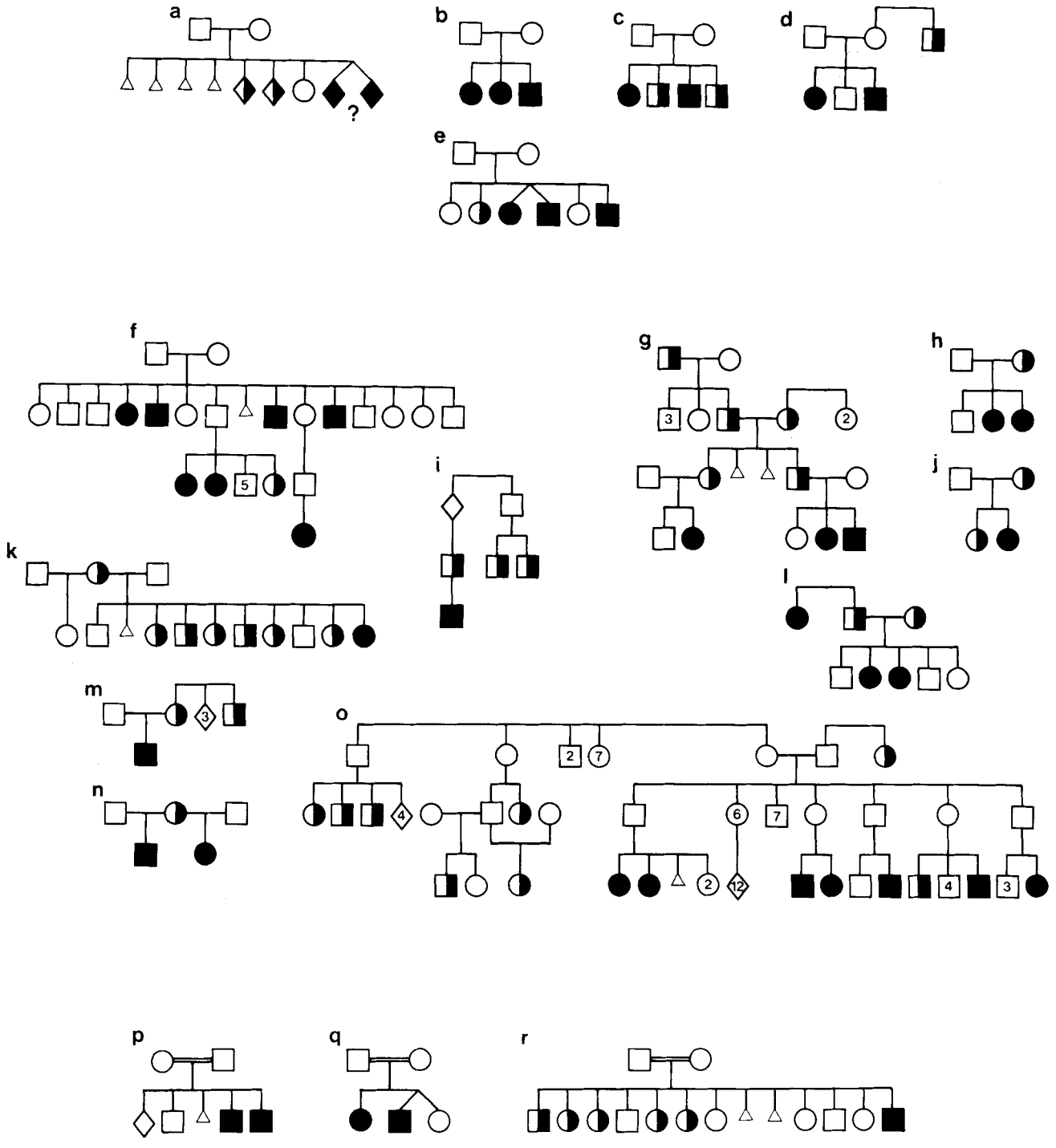


Fig. 1. Familial occurrence of holoprosencephaly (*a–e*) with autosomal dominant (*f–o*) and autosomal recessive (*p–r*) inheritance. Pedigrees are modified from the following reports: a, van Duyse, cited by Klopstock [1921]; b, Dominok and Kirchmair [1961]; c, Ardouin et al. [1968]; d, Nivelon-Chevallier and Nivelon [1975]; e, Khan et al. [1970]; f, Benke and Cohen [1983]; g, Berry et al. [1984], updated by Johnson [1989]; h, DeMeyer et al. [1963]; i, Fryns and Van den Berghe [1988]; j, Thomas [1988]; k, Cantú et al. [1978]; l, Roach et al. [1975]; m, Gilbert and Opitz [1976], same as Petterson [1976]; n, McDonald et al. [1988]; o, Dallaire et al. [1971]; p, Klopstock [1921]; q, McDonald et al. [1988]; r, Cohen and Gorlin [1969]. Full squares and circles represent individuals with holoprosencephaly, whereas half-filled squares and circles depict individuals with minor forms of the holoprosencephaly sequence.

TABLE I. Genetic Syndromes and Conditions With Holoprosencephaly

Váradi syndrome
Meckel syndrome
Sphrintzen syndrome
Majewski syndrome
Pallister-Hall syndrome
Rubinstein-Taybi syndrome
Zellweger syndrome
Kallmann syndrome
Walker-Warburg syndrome
CHARGE association and syndrome
Agnathia-holoprosencephaly association
DiGeorge sequence

sociations (Table I) [for a review, see Cohen, 1989a–c]. However, the frequency of holoprosencephaly within a clinical entity may be rare, e.g., 2 of 21 in Váradi or orofacial-digital syndrome [Váradi et al., 1980; reviewed by Münke et al., 1989], 3 of 25 in the DiGeorge sequence [Conley et al., 1979], and only one or two reports each in the velo-cardiofacial syndrome [Wraith et al., 1985], Majewski syndrome, Pallister-Hall and Smith-Lemli-Opitz syndromes [Donnai et al., 1987], Rubinstein-Taybi syndrome [DeMeyer, 1977], Walker-Warburg syndrome [Dobyns et al., 1989], Meckel syndrome [Opitz and Howe, 1969; Hsia et al., 1971], Zellweger syndrome [Opitz et al., 1969], and the CHARGE [Toriello, 1986] and the agnathia-holoprosencephaly associations [Pauli et al., 1983; Leech et al., 1988; Hersh et al., 1989; Robinson and Lenke, 1989].

Krassikoff and Sekhon [1989] describe a balanced t(6;18) translocation in a father and duplication of 6p and monosomy of 18p in a stillborn fetus of a previously reported family including two offspring with agnathia-holoprosencephaly [Pauli et al., 1983]. With this first report of a chromosome anomaly in familial agnathia-holoprosencephaly, previously thought to be inherited as an autosomal recessive trait, it seems worthwhile to reanalyze prometaphase chromosomes of parents who had a child with idiopathic syndromal holoprosencephaly. As with a number of other genetic syndromes (e.g., Miller-Dieker, Langer-Giedion, Wiedemann-Beckwith, Prader-Willi, and cat-eye syndrome and aniridia-Wilms tumor association), some of the above-mentioned syndromes associated with holoprosencephaly may turn out to be chromosomal in origin on high resolution banding analysis.

Second, although holoprosencephaly is most often sporadic, familial occurrence (Fig. 1a–e) has been observed [Dominok and Kirchmair, 1961; DeMeyer et al., 1963; Ardouin et al., 1968; Hintz et al., 1968; James and van Leeuwen, 1970; Khan et al., 1970; Nivelon-Chevallier and Nivelon, 1975; Seidlitz et al., 1983]. Roach et al. [1975] reported familial occurrence in 2 of 30 families of an otherwise unselected study group. In Figure 1, I have selected only those pedigrees with more than two affected individuals. From this figure it becomes clear that some of the familial cases may well represent autosomal dominant or recessive inheritance.

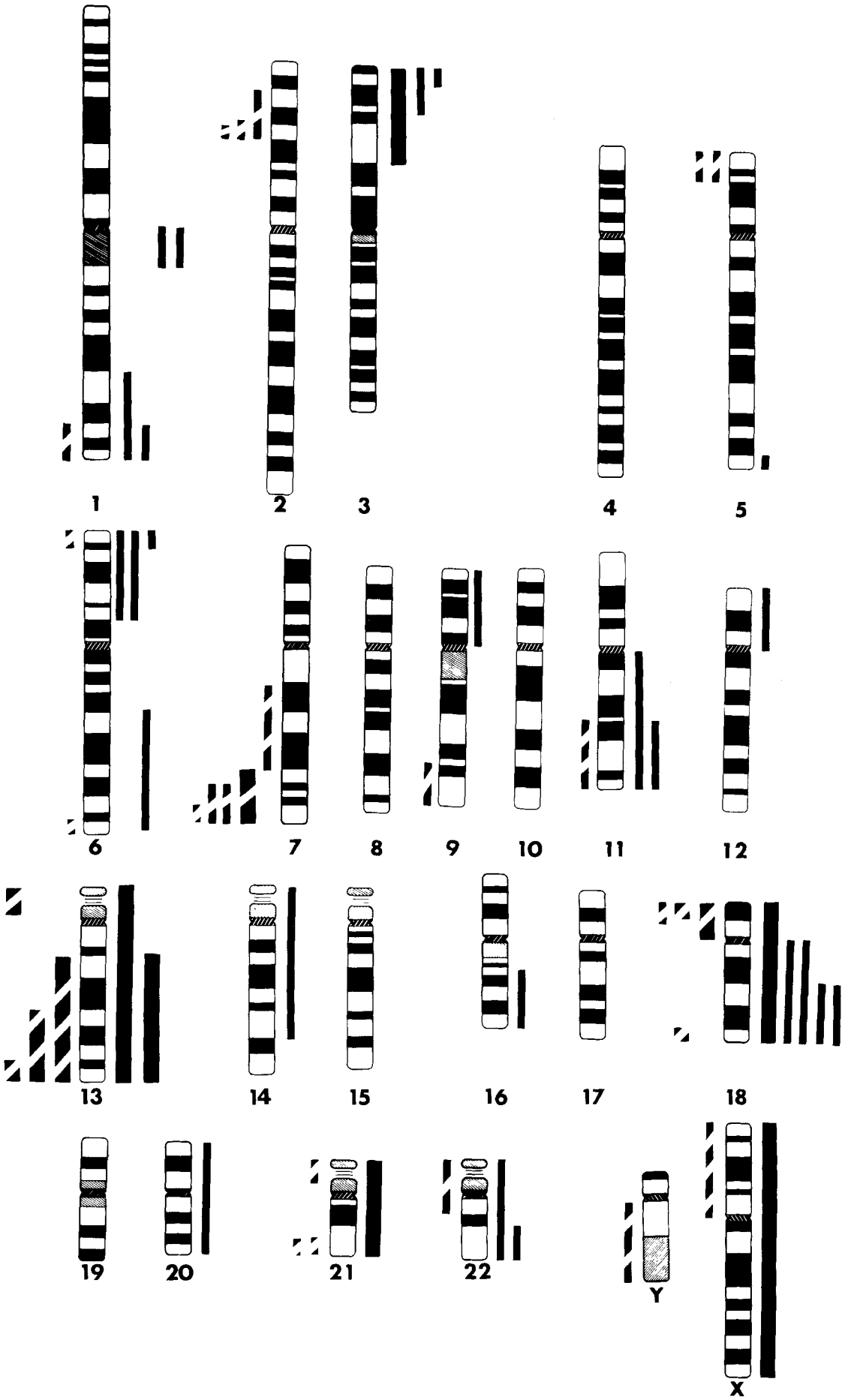
Autosomal dominant of holoprosencephaly has been well documented in the two families reported by Johnson [1989] as well as in other families (Fig. 1f–o) [Dallaire et al., 1971; Patel et al., 1972; Lowry, 1974; Roach et al., 1975, pedigree 20; Gilbert and Opitz, 1976, same as Petterson, 1976; Cantú et al., 1978; Benke and Cohen, 1983; Berry et al., 1984; Hattori et al., 1987; McDonald et al., 1988; Thomas, 1988; Fryns and Van den Berghe, 1988; Jaramillo et al., 1988; Ardinger and Bartley, 1988]. Extensive pedigree analysis, recognition of the wide variability in the holoprosencephaly sequence, and careful clinical examination of parents and other relatives is most important to confirm autosomal dominant inheritance. Normal or borderline intelligence with normo- or microcephaly has been described in relatives of holoprosencephalic patients. Other anomalies illustrating the variable expressivity include premaxillary agenesis or hypoplastic intermaxillary segment, single upper central incisor, mostly midline but also unilateral or bilateral clefts, bifid uvula, hypotelorism and hypertelorism, anosmia, pituitary deficiency (e.g., short stature), and iris coloboma [Johnson, 1989; Benke, 1989].

Affected sibs in a consanguineous family and clinically normal parents are evidence for autosomal recessive inheritance (Fig. 1p–r) [Klopstock, 1921; Grebe, 1954; Cohen and Gorlin, 1969; McDonald et al., 1988]. Until recently, there was not sufficient evidence for X-linked inheritance. The two families reported by Begleiter and Harris [1980] and Falk et al. [1982] with suggested X-linkage could well be explained by autosomal recessive inheritance. However, two reports of a new syndrome with holoprosencephaly, microcephaly, hypokinesia, and contractures clearly document X-linked inheritance [Morse et al., 1987; Hockey et al., 1988].

Third, chromosome anomalies in holoprosencephaly have been repeatedly described since the first report of this CNS defect in association with trisomy of a D group chromosome [Miller et al., 1963] and a deletion of the short arm of an E group chromosome [de Grouchy et al., 1963]. To date, both structural and numerical anomalies of chromosomes 13 and 18, especially trisomy 13 and del(18p), are the most common cytogenetic findings for two reasons. Trisomy 13 has a high incidence in newborn infants (1 in 5,000), and the frequency of holoprosencephaly (mostly hypoplastic or absent olfactory nerves and bulbs) within this entity is 70% [Warkany et al., 1966; Taylor, 1968; Gullotta et al., 1981]. Craniofacial anomalies occur in 16% of the cases with del(18p)[de Grouchy and Turleau, 1984], although accurate incidence figures for newborn infants are not available. Furthermore, there are no prospective or retrospective studies available on the proportion with or without chromosome anomalies in patients with holoprosencephaly.

In addition to chromosome 13 and 18 anomalies, duplications of the distal short arm of chromosome 3 and deletions of the distal long arm of chromosome 7 are more frequently involved than expected [Lurie et al., 1986; Münke et al., 1988a]. To my knowledge 8 of 50 patients with dup(3p) [Lurie et al, 1986] and 5 of 20 with del(7q) have manifestations of the holoprosencephaly sequence [Young et al., 1984; Tiller et al., 1988].

The following reports of phenotype/karyotype correlations have been added to Figure 2 since the previous review: del(1)(q42→qter) [Hill et al., 1982; Dewald,


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
Y
X

1988, personal communication]; del(2)(p2101→p2109) [Shanks et al., 1988, same as Wilson et al., 1989]; del(2)(p21→p23) [Grundy et al., 1989]; dup(3)(p25→pter) and del(6) (p25→pter) [Lurie et al., 1986]; dup(3)(p23→pter and del(7)(q36→qter) [Pfitzer et al., 1982]; possibly dup(3)(p21→pter) and del(6)(q26→qter) [Gottschall et al., 1983]; dup(6)(p24.1 or 24.2→pter) and del(18)(p11.21→pter) [Krassikoff and Sekhon, 1989]; del(7)(q36→qter) and dup(18)(q21→qter) [Borovik et al., 1987]; del(9)(q32→qter) [Kargas et al., 1987]; dup(9p) and dup(11q) [Dinno et al., 1974]; del(11)(q21→qter) [Helmuth et al., 1989]; r(18) [Neu et al., 1971]; del(21)(q22.3→qter) [Estabrooks et al., 1987]; trisomy 21 [Epstein et al., 1988; Urioste et al., 1988]; del(X)(p11→pter) [Saunders et al., 1984, case 4]; 47,XXY [Probst, 1979, case 17]; a "balanced" t(5;12)(p14.1;q24.4) translocation [Cousin et al., 1988]; and triploidy (69,XXX) [Filly et al., 1984].

These additions confirm the notion that dup(3p) and del(7q) are nonrandomly associated with holoprosencephaly. In contrast, four patients with this CNS defect and trisomy 21 [Pi et al., 1980; Rehder, 1981; Urioste et al., 1988; Epstein et al., 1988] do not constitute a causal relationship as discussed in detail by Epstein et al. [1988]. Similar arguments can be made against an involvement of the sex chromosomes in the etiology of holoprosencephaly. The association of Ullrich-Turner del(X)(p11→pter) [Saunders et al., 1984, case 4] and Klinefelter syndromes (47,XXY [Probst, 1979, case 17] and 49,XXXXY [Pallister, 1982]) and this craniofacial malformation may be coincidental. Holoprosencephaly in a patient with a Yq deletion and t(Yp;18) translocation [Münke et al., 1988b] and an individual with Klinefelter syndrome (47,XXY, 18p−) [Schnabel and Hansen, 1983] was due to loss of chromosome 18 short arm material. To date, cytogenetic analyses do not provide evidence that sex chromosome anomalies predispose to holoprosencephaly.

Three articles in this issue of the journal are fascinating from a cytogenetic standpoint: Krassikoff and Sekhon's report [1989] on a dup(6p) and del(18p) as cause for familial agnathia-holoprosencephaly and two reports on overlapping interstitial deletions of chromosome 2p: del(2)(p2101→p2109) [Wilson et al., 1989] and del(2)(p21→23) [Grundy et al., 1989]. We have previously published a similar overlapping deletion del(2)(p21→p22.2) [Münke et al., 1988a] and stated: "In the absence of other case reports with comparable chromosome deletions and brain anomalies, it is not possible to determine whether holoprosencephaly in our patient is due to the interstitial 2p deletion." Clinically these three patients are similar, as all of their findings are related to the holoprosencephaly sequence. However, one is less severely affected with premaxillary agenesis, midline cleft, and semilobar holoprosencephaly by CT scan [Wilson et al., 1989], whereas the two others have cyclopia and alobar holoprosencephaly on autopsy (Münke et al., 1988a; Grundy et al., 1989]. The smallest region of overlap of the three deletions is the one described by Wilson et al. [1989]. This deletion del(2)(p2101→p2109) encompasses most of the GTG-light staining band 2p21, which does not split into subbands even at the 850 band stage [Francke, 1981]. I am only aware of one other deletion that overlaps in part the one described by Wilson et al. [1989]. Fryns et al. [1979] report a 14-year-old moderately retarded boy without gross abnormalities and a *de novo* interstitial deletion del(2)(p11→21). In addition, he had a small acentric supernumerary fragment without any identifiable banding pattern, which was assumed to represent at least part of the deleted short arm material. Thus, to date, three of three patients with a deletion including most of band 2p21 have had manifestations of holoprosencephaly. Although the total number of patients with this deletion is small, a causal relationship between holoprosencephaly and del(2)(p21) is very suggestive.

WHAT IS THE MOLECULAR BASIS FOR THE DIFFERENT GENETIC FORMS OF HOLOPROSENCEPHALY?

Currently, we are unable to answer this question. In the following I will try to outline several molecular approaches that may eventually increase our understanding of normal and abnormal brain development. The aim of the different approaches is the cloning of human genes that are involved in the formation of the early embryonic brain either directly or indirectly by regulation of other genes. From both the complexity of the developing nervous system and the genetic heterogeneity of the holoprosencephaly sequence (Figs. 1, 2; Table I) we can hypothesize that a number of genes are involved in CNS formation. These genes may be expressed for a short period of time during embryogenesis only and/or have a different function in postnatal life, if any at all.

Animal Models of Holoprosencephaly

Although this specific brain malformation has been described in a large number of different animals, only a few reports may help to elucidate the underlying molecular mechanisms: 1) In *Drosophila,* mutations in the *single-minded (sim)* gene locus result in loss of neuronal and nonneuronal precursor cells, whereas the normal *sim* gene is expressed in cells along the midline of the developing CNS [Crews et al., 1988]. 2) Insertional mutagenesis by retrovirus integration into the mouse germline has yielded homozygous mice with holoprosencephaly and limb anomalies [McNeish et al., 1988]. It is suggestive that the gene that is interrupted by retroviral DNA plays a crucial role in the formation of early embryonic brain and limbs. Thus, human sequences homologous to the *Drosophila single-minded* gene or to flanking sequences in the transgenic mouse may be involved in the development of CNS midline structures.

Fig. 2. Correlation between chromosome abnormalities and the holoprosencephaly sequence (modified from Münke et al. [1988a] and updated). Hatched bars on the left side of chromosomes represent deletions, full bars on the right duplications. Thin Bars indicate individual case reports, whereas thick bars represent three or more case reports. Ring chromosomes are represented by hatched bars on the terminal ends. For details and references, see text and Münke et al. [1988a].

Deletion Cloning

As discussed above, there is cytogenetic evidence that at least five regions of the human genome are causally related to holoprosencephaly and may contain brain-specific genes. These chromosome regions include 2p21, 3(p21→pter), 7(q32→qter), 18p, and one or several regions on chromosome 13. With the exception of 2p21, all other regions are too large to be studied efficiently on a molecular level. In contrast, del(2)(p2101→p2109) [Wilson et al., 1989] comprises less than 0.2% of the haploid genome [Francke, 1981], which may approximate to 6,000 kb of DNA or less. A more accurate estimate of the deletion size could be obtained by flow cytometry [van den Engh et al., 1988].

Recently, microdissection of banded chromosomes has become a reproducible method to isolate DNA sequences from chromosome regions, e.g., 8q24.1 for Langer-Giedion syndrome, 11p13 (Wilms tumor-aniridia association), and 15q11-12 (Prader-Willi syndrome) [Lüdecke et al., 1989]. Similarly, DNA from chromosome band 2p21 can be dissected and cloned.

Isolation of DNA sequences from within the 2p21 deletion can also be achieved by the PERT method (phenol-enhanced reassociation technique) as has been successfully applied for sequences from the locus for Duchenne muscular dystrophy in Xp21 [Kunkel et al., 1985] or from near the locus for choroideremia in Xq21 [Nussbaum et al., 1987]. To use this technique for sequences from 2p, both the normal and the deleted chromosome 2 have to be separated by microcell transfer into different human X rodent somatic cell hybrids with identical rodent background.

A different approach is to identify available chromosome 2-specific sequences, e.g., from a human chromosome-specific genomic library [Fuscoe et al., 1986] located within the deletion. DNA sequences near the 2p deletion include those of a gene, CAD, encoding a multifunctional protein that carries out the first three steps of the pyrimidine biosynthesis. CAD has been mapped to chromosome 2 by Southern analysis of somatic cell hybrid DNA and more specifically to the chromosome region 2p21→22 by *in situ* hybridization [Chen et al., 1989]. As a first attempt to map more precisely probes within band 2p21, we have hybridized DNA from the deletion patient with holoprosencephaly [Wilson et al., 1989] and normal male and female control individuals with a CAD-specific probe and subsequently with an X chromosome-specific probe (DXS42). Dosage blot analysis and densitometry of the autoradiograms clearly excludes CAD from the deletion del(2)(p2101→p2109) (Münke et al., unpublished results). Presence of the CAD gene on both the normal and the deleted chromosome 2 allows for the selective retention of either chromosome 2 in somatic cell hybrids prepared with Chinese hamster ovary cell mutants of the Urd-A complementation group [Patterson and Carnright, 1977].

DNA sequences from within chromosome band 2p21 generated by microdissection of banded chromosomes, PERT cloning, or mapping of probes from a human chromosome 2-specific library to this region can then be used to isolate more sequences by chromosome jumping. Hybridization of 2p21-specific DNA fragments to a cDNA library prepared from the developing mouse brain of different stages of early embryogenesis could identify clones that contain homologous sequences to a gene expressed during or prior to brain formation. Analysis of this gene or its gene product and function will shed light on normal as well as abnormal development of the central nervous system, as seen in the holoprosencephaly sequence.

Linkage Analysis

Family studies by restriction fragment length polymorphisms (RFLPs) have allowed the mapping of the gene locus for Huntington disease to chromosome 4p, cystic fibrosis to 7q, neurofibromatosis to 17q, and so forth. This approach to map and eventually clone a disease-related gene has been undertaken when the basic defect, e.g, an abnormal gene product, is unknown. Since in a heterogeneous condition like the holoprosencephaly sequence the genetic defect may be different from one family to another, linkage to a DNA marker can be tested for individual families only with multiple affected and unaffected relatives. If we assume complete penetrance in a family with autosomal dominant holoprosencephaly and no recombination between marker and gene locus, 10 fully informative meioses are necessary to establish a likelihood ratio of 1,000:1 (lod score of 3). At least 14 meioses would be required to establish linkage for a marker that is 5 cM from a holoprosencephaly sequence locus.

DNA markers and/or candidate genes first tested for linkage to holoprosencephaly will be probes that map to chromosome regions associated with this brain defect (Fig. 2). The retinoic acid receptor gene is a possible candidate gene for several reasons. 1) In animals, prenatal exposure to retinoic acid has been demonstrated to cause craniofacial anomalies [Pratt et al., 1987; Abbott and Pratt, 1987]. 2) There is at least one report in the literature on humans of retinoic acid embryopathy [Lammer et al., 1985]. 3) The receptor for retinoic acid has been mapped to human chromosome 3p24 [Mattei et al., 1988], a region nonrandomly associated with holoprosencephaly. Large families with autosomal dominant holoprosencephaly are rare (Fig. 1), and the number of affected and unaffected individuals is usually small, since those with the more severe forms died in infancy. Thus linkage studies with DNA markers may only yield results in a few families [Dallaire et al., 1971; Benke and Cohen, 1983; Cantú et al., 1978].

Conclusion

To gain insight into the molecular basis of normal brain development and of abnormal formation as in holoprosencephaly, we need a "careful investigation of cases of rarer forms of the disease" [Harvey, 1657, cited by Garrod, 1928]. These careful investigations include a genetic physical examination of the affected newborn infant or prenatally of the fetus for other anomalies to exclude any genetic syndrome, tests for prenatal infection (TORCH titers) in mother and infant, a prenatal history to rule out teratogen exposure, a family history and examination of parents and other relatives for clefts

and other midline defects (see above), and storage of cells (amniocytes, fibroblasts, and/or transformed lymphoblasts) for later genetic work-up in patients with a rare cytogenetic anomaly or a positive family history.

ACKNOWLEDGMENTS

M.M. is an Associate in the Howard Hughes Medical Institute, University of Pennsylvania.

REFERENCES

Abbott BD, Pratt RM (1987): Retinoids and epidermal growth factor alter embryonic mouse palatal epithelial and mesenchymal cell differentiation in organ culture. J Craniofac Genet Dev Biol 7:219–240.

Agapitos M, Georgiou-Theodoropoulou M, Koutselinis A, Papacharalampus N (1986): Cyclopia and maternal ingestion of salicylates. Pediatr Pathol 6:309–310.

Ardinger HH, Bartley JA (1988): Microcephaly in familial holoprosencephaly. J Craniofac Genet Dev Biol 8:53–61.

Ardouin M, Lanchou G, Faivre J, Garnier J-P (1968): Un cas de cyclopie dans une fratrie présentant en outre plusieurs malformations médianes de la face. Rev Otoneuroophthalmol 11:259–270.

Barr M Jr, Hanson JW, Currey K, Sharp S, Toriello H, Schmickel RD, Wilson GN (1983): Holoprosencephaly in infants of diabetic mothers. J Pediatr 102:565–568.

Begleiter ML, Harris DJ (1980): Holoprosencephaly and endocrine dysgenesis in brothers. Am J Med Genet 7:315–318.

Benawra R, Mangurten HH, Duffell DR (1980): Cyclopia and other anomalies following maternal ingestion of salicylates. J Pediatr 96:1069–1071.

Benke PJ (1989): XK aprosencephaly may be a new mutation or a dominant genetic defect. Am J Med Genet 34:250.

Benke PJ, Cohen MM Jr (1983): Recurrence of holoprosencephaly in families with a positive history. Clin Genet 24:324–328.

Berry SA, Pierpont ME, Gorlin RJ (1984): Single central incisor in familial holoprosencephaly. J Pediatr 104:877–880.

Binns W, James LF, Shupe JL, Thacker EJ (1962): Cyclopian-type malformation in lambs. Arch Environ Health 5:20–22.

Borovik CL, Brunoni D, Sato AE, Stávale JN, Cardoso SH, Montezzo LC, Perez ABA (1987): Holoprosencephaly as a result of an unbalanced familial translocation rcp(7;18)(q36;q21). Rev Brasil Genet 10:269–276.

Byrne PJ, Silver MM, Gilbert JM, Cadera W, Tanswell AK (1987): Cyclopia and congenital cytomegalovirus infection. Am J Med Genet 28:61–65.

Cantú J-M, Fragoso R, García-Cruz D, Sánchez-Corona J (1978): Dominant inheritance of holoprosencephaly. In Summitt RL, Bergsma D (eds): "Annual Review of Birth Defects, 1977: Recent Advances and New Syndromes." New York: Alan R. Liss, Inc., for the National Foundation—March of Dimes. BD:OAS XIV(6B):215–220.

Castel Y, Riviere D, Toudic L, Nouaille Y, L'Henoret J, Duparcmeur H, Loroy JP, Palouet G (1976): Deux cas de cyclopie. Ann Pédiatr, 23:647–651.

Chen K-C, Vannais DB, Jones C, Patterson D, Davidson JN (1989): Mapping the gene encoding the multifunctional protein carrying out the first three steps of pyrimidine biosynethesis to human chromosome 2. Hum Genet 82:40–44.

Cohen MM Jr (1989a): Perspectives on holoprosencephaly. Part I. Epidemiology, genetics and syndromology. Teratology (in press).

Cohen MM Jr (1989b): Perspectives on holoprosencephaly. Part III. Spectra, distinctions, continuities, and discontinuities. Am J Med Genet 34:271–288.

Cohen MM Jr (1989c): Holoprosencephaly and cytogentic findings: Further information. Am J Med Genet 34:265.

Cohen MM Jr, Gorlin RJ (1969): Genetic consideration in sibship of cyclopia and clefts. In Bergsma D, McKusick VA, Hall JG, Scott CJ (eds): "The Clinical Delineation of Birth Defects: Part II, Malformation Syndromes." New York: Alan R. Liss, Inc., for the National Foundation—March of Dimes. BD:OAS V(2):113–118.

Conley ME, Beckwith JB, Mancer JFK, Tenckhoff L (1979): The spectrum of the DiGeorge syndrome. J Pediatr 94:883–890.

Cousin J, Creusy C, Croquette MF, Vittu G (1988): Syndrome malformatif majeur et anomalie chromosomique apparemment équilibreé: Holoprosencéphalie et translocation 5p;12q. J Génét Hum 36:83–88.

Crews ST, Thomas JB, Goodman CS (1988): The *Drosophila single-minded* gene encodes a nuclear protein with sequence similarity to the *per* gene product. Cell 52:143–151.

Dallaire L, Fraser FC, Wiglesworth FW (1971): Familial holoprosencephaly. In Bergsma (ed): "The Clinical Delineation of Birth Defects. Orofacial Structures." Baltimore: Williams & Wilkins Co., for The National Foundation—March of Dimes. BD:OAS VII(7):136–142.

de Grouchy J, Lamy M, Thieffry S, Arthuis M, Salmon C (1963): Dysmorphie complexe avec oligophrénie: Délétion des bras courts d'un chromosome 17–18. C R Acad Sci 256:1028–1029.

de Grouchy J, Turleau C (1984): 18p monosomy or 18p− syndrome. In de Grouchy J, Turleau C (eds): "Clinical Atlas of Human Chromosomes" New York: John Wiley & Sons, pp 308–313.

Dekaban AS (1959): Arhincephaly in an infant born to a diabetic mother. J Neuropathol Exp Neurol 18:620–626.

DeMeyer W (1977): Holoprosencephaly (cyclopia-arhinencephaly). In Vinken PJ, Bruyn GW (eds): "Handbook of Clinical Neurology." Amsterdam: North Holland Publishing Co., pp 431–478.

DeMeyer W, Zeman W, Palmer CG (1963): Familial holoprosencephaly (arhinencephaly) with median cleft lip and palate. Neurology 13:913–918.

DeMeyer W, Zeman W, Palmer CG (1964): The face predicts the brain: Diagnostic significance of median facial anomalies for holoprosencephaly (arhinencephaly). Pediatrics 34:256–263.

Dinno ND, Silvey GL, Weisskopf B (1974): 47,XY,t(9p+;11q+) in male infant with multiple malformations. Clin Genet 6:125–131.

Dobyns WB, Pagon RA, Armstrong D, Curry CJR, Greenberg F, Grix A, Holmes LB, Laxova R, Michels VV, Robinow M, Zimmerman RL (1989): Diagnostic criteria for Walker-Warburg syndrome. Am J Med Genet 32:195–210.

Dominok GW, Kirchmair H (1961): Familiäre Häufung von Fehlbildungen der Arhinencephaliegruppe. Z Kinderheilkd 85:19–30.

Donnai D, Burn J, Hughes H (1987): Smith-Lemli-Opitz syndromes: Do they include the Pallister-Hall syndrome? Am J Med Genet 28:741–743.

Epstein CJ, Seto S, Golabi M (1988): Chance vs. causality in the association of Down syndrome and holoprosencephaly. Am J Med Genet 30:939–942.

Estabrooks LL, Rao KW, Seeds JW, Donahue RP, Aylsworth AS (1987): Nonsyndromic holoprosencephaly in an infant with a minute deletion of chromosome 21(q22.3). Am J Hum Genet 41:A118.

Falk RE, Frohlich GS, Crandall BF (1982): Familial holoprosencephaly: Possible X-linkage with variable expression. Am J Hum Genet 34:87A.

Farquhar JW (1969): Prognosis for babies born to diabetic mothers in Edinburgh. Arch Dis Child 44:36–47.

Filly RA, Chinn DH, Callen PW (1984): Alobar holoprosencephaly: Ultrasonographic prenatal diagnosis. Radiology 151:455–459.

Francke U (1981): High-resolution ideograms of trypsin-Giemsa banded human chromosomes. Cytogenet Cell Genet 31:24–32.

Frenkel LD, Keys MP, Hefferen SJ, Rola-Pleszczynski M, Bellanti JA (1980): Unusual eye abnormalities associated with congenital cytomegalovirus infection. Pediatrics 66:763–766.

Fryns JP, Van den Berghe H (1988): Single central maxillary incisor and holoprosencephaly. Am J Med Genet 30:943–944.

Fryns JP, de Waele P, Van den Berghe H (1979): Interstitial deletion of the short arm of chromosome 2 in a moderately mentally retarded boy without gross clinical stigmata. Hum Genet 51:123–125.

Fuscoe JC, Clark LM, Van Dilla MA (1986): Construction of fifteen human chromosome-specific DNA libraries from flow-purified chromosomes. Cytogenet Cell Genet 43:79–86.

Garrod A (1928): The lessons of rare maladies. Lancet 1:1055–1060.

Gilbert EF, Opitz JM (1976): The pathology of some malformations and hereditary diseases of the respiratory tract. In Bergsma D, Schimke

RN (eds): "Growth Problems and Clinical Advances." New York: Alan R. Liss, Inc., for the National Foundation—March of Dimes. BD:OAS XII(6):239–270.

Gottschall A, Losan F, Pelz L, Wiedersberg H (1983): A new type of familial chromosome translocation involving 3p and 6q in two unrelated families. Hum Genet 64:304.

Grebe H (1954): Familienbefunde bei letalen Anomalien der Körperform. Acta Genet Med Gemellol 3:93–111.

Grundy HO, Niemeyer P, Rupani MK, Ward VF, Wassman ER (1989): Prenatal detection of cyclopia associated with interstitial deletion of 2p. Am J Med Genet 34:268–270.

Gullotta F, Rehder H, Gropp A (1981): Descriptive neuropathology of chromosomal disorders in man. Hum Genet 57:337–344.

Hattori H, Okuno T, Momoi T, Kataoka K, Mikawa H, Shiota K (1987): Single central maxillary incisor and holoprosencephaly. Am J Med Genet 28:483–487.

Helmuth RA, Weaver DD, Wills ER (1989): Holoprosencephaly, ear abnormalities, congenital heart defect, and microcephallus in a patient with 11q− mosaicism. Am J Med Genet 32:178–181.

Hersh JH, McChane RH, Rosenberg EM, Powers WH, Corrigan C, Pancratz L (1989): Otocephaly midline malformation association. Am J Med Genet 34:246–249.

Hill LM, Breckle R, Bonebrake CR (1982): Ultrasound findings with holoprosencephaly. J Reprod Med 27:172–175.

Hintz RL, Menking M, Sotos JF (1968): Familial holoprosencephaly with endocrine dysgenesis. J Pediatr 72:81–87.

Hockey A, Crowhurst J, Cullity G (1988): Microcephaly, holoprosencephaly, hypokinesia—Second report of a new syndrome. Prenat Diagn 8:683–686.

Hsia YE, Bratu M, Herbordt A (1971): Genetics of the Meckel syndrome (dysencephalia splanchnocystica). Pediatrics 48:237–247.

James E, van Leeuwen G (1970): Familial cebocephaly. Clin Pediatr 9:491–493.

Jaramillo C, Brandt SK, Jorgenson RN (1988): Autosomal dominant inheritance of the DeMeyer sequence. J Craniofac Genet Dev Biol 8:199–204.

Jellinger K, Gross H, Kaltenbäck E, Grisold W (1981): Holoprosencephaly and agenesis of the corpus callosum: Frequency of associated malformations. Acta Neuropathol 55:1–10.

Johnson VP (1989): Holoprosencephaly: A developmental field defect. Am J Med Genet 34:258–264.

Kargas SA, Kargas GA, Chandra S, Wu S, Meisner LF (1987): Newborn infant with del(9)(pter→q32:) and multiple congenital anomalies including arrhinencephaly, cardiac malformations, and rudimentary ears. Am J Med Genet Suppl 3:161–166.

Keeler RF (1970): Teratogenic compounds of *Veratum californicum* (Durand) X. Cyclopia in rabbits produced by cyclopamine. Teratology 3:175–180.

Keeler RF, Binns W (1968): Teratogenic compounds of *Veratum californicum* (Durand) V. Comparison of cyclopian effects of steroidal alkaloids from the plant and structurally related compounds from other sources. Teratology 1:5–10.

Khan M, Rozdilsky B, Gerrard JW (1970): Familial holoprosencephaly. Dev Med Child Neurol 12:71–76.

Khudr G, Olding L (1973): Cyclopia. Am J Dis Child 125:120–125.

Klopstock A (1921): Familiäres Vorkommen von Cyklopie und Arrhinencephalie. Mschr Geburtsh Gyn 56:59–71.

Krassikoff N, Sekhon GS (1989): Familial agnathia-holoprosencephaly due to an inherited unbalanced translocation and not autosomal recessive inheritance. Am J Med Genet 34:255–257.

Kunkel LM, Monaco AP, Middlesworth W, Ochs HD, Latt SA (1985): Specific cloning of DNA fragments absent from the DNA of a male patient with an X chromosome deletion. Proc Natl Acad Sci USA 82:4778–4782.

Lammer EJ, Chen DT, Hoar RM, Agnish ND, Benke PJ, Braun JT, Curry CJ, Fernhoff PM, Grix AW, Lott IT, Richards JM, Sun SC (1985): Retinoic acid embryopathy. N Engl J Med 313:837–841.

Leech RW, Bowlby LS, Brumback RA, Schaefer GB Jr (1988): Agnathia, holoprosencephaly, and situs inversus: Report of a case. Am J Med Genet 29:483–490.

Lison MP, Armbrust-Figueiredo J, Mega D (1967): Arhinencéphalie: Considérations a propos d'un cas diagnostiqué pendant la vie. Acta Neurol Belg 67:25–36.

Lowry RB (1974): Holoprosencephaly. Am J Dis Child 128:887.

Lüdecke H-J, Senger G, Claussen U, Horsthemke B (1989), Cloning defined regions of the human genome by microdissection of banded chromosomes and enzymatic amplification. Nature 338:348–350.

Lurie IW, Korotkova IA, Zaletajev DV, Smirnova LI, Podlechshuk LV, Gurevich DB (1986): Trisomy for the distal part of the short arm of chromosome 3. Helv Paediatr Acta 41:509–513.

Mattei M-G, de Thé H, Mattei J-F, Marchio A, Tiollais P, Dejean A (1988): Assignment of the *hap* retinoic acid receptor RAR β gene to the p24 band of chromosome 3. Hum Genet 80:189–190.

McCarthy RW, Frenkel LD, Kollarits CR, Keys MP (1980): Clinical anophthalmia associated with congenital cytomegalovirus infection. Am J Ophthalmol 90:558–561.

McDonald DM, Mennuti MT, Goodwin DC, Kistenmacher ML, Zackai EH (1988): Holoprosencephaly, a heterogeneous disorder: I. Monogenic etiology. Am J Med Genet [Suppl 4].

McNeish JD, Scott WJ Jr, Potter SS (1988): Legless, a novel mutation found in PHT1-1 transgenic mice. Science 241:837–839.

Miller JQ, Picard EH, Alkan MK, Warner S, Gerald PS (1963): A specific brain defect (archinecephaly) in 13-15 trisomy. N Engl J Med 268:120–124.

Morse RP, Rawnsley E, Sargent SK, Graham Jr JM (1987): Prenatal diagnosis of a new syndrome: Holoprosencephaly with hypokinesia. Prenat Diagn 7:631–638.

Münke M, Emanuel BS, Zackai EH (1988a): Holoprosencephaly: Association with interstitial deletion of 2p and review of the cytogenetic literature. Am J Med Genet 30:929–938.

Münke M, McDonald DM, Cronister A, Stuart JM, Gorlin RJ, Zackai EH (1989): Oral-facial-digital syndrome type VI (Váradi syndrome): Further clinical delineation. Am J Med Genet (in press).

Münke M, Page DC, Brown LG, Armson BA, Zackai EH, Mennuti MT, Emanuel BS (1988b): Molecular detection of a Yp/18 translocation in 45,X holoprosencephalic male. Hum Genet 80:219–223.

Neu RL, Watanabe N, Gardner LI, Galvis AG (1971): A single nasal orifice and severe intrauterine growth retardation in a case of 46,XX,18r. Ann Genet 14:139–142.

Nivelon-Chevallier A, Nivelon JL (1975): Forme familiale d'holoprosencéphalie. J Genet Hum 23:215–223.

Nussbaum RL, Lesko JG, Lewis RA, Ledbetter SA, Ledbetter DH (1987): Isolation of anonymous DNA sequences from within a submicroscopic X chromosomal deletion in a patient with choroideremia, deafness, and mental retardation. Proc Natl Acad Sci USA 84:6521–6525.

Opitz JM, Howe JJ (1969): The Meckel syndrome (dysencephalia splanchnocystica, the Gruber syndrome). In Bergsma D (ed): "The Clinical Delineation of Birth Defects. Malformation Syndromes." Baltimore: The Johns Hopkins Medical Institutions for the National Foundation —March of Dimes. BD:OAS V(2):167–179.

Opitz JM, ZuRhein GM, Vitale L, Shahidi NT, Howe JJ, Chou SM, Shanklin DR, Sybers HD, Dood RD, Gerritsen T (1969): The Zellweger syndrome (cerebro-hepato-renal syndrome). In Bergsma D (ed): "The Clinical Delineation of Birth Defects. Malformation Syndromes." Baltimore: The Johns Hopkins Medical Institutions for the National Foundation—March of Dimes. BD:OAS V(2):144–158.

Pallister PD (1982): 49,XXXXY syndrome. Am J Med Genet 13:337–339.

Patel H, Dolman CL, Byrne MA (1972): Holoprosencephaly with median cleft lip. Am J Dis Child 124:217–221.

Patterson D, Carnright DV (1977): Biochemical genetic analysis of pyrimidine biosynthesis in mammalian cells: I. Isolation of a mutant defective in the early steps of *de novo* pyrimidine synthesis. Somatic Cell Genet 3:483–495.

Pauli RM, Pettersen JC, Arya S, Gilbert EF (1983): Familial agnathia-holoprosencephaly. Am J Med Genet 14:677–698.

Petterson JC (1976): An anatomical study of two cases of cebocephaly. In Bosma JF (ed): "Development of the Basicranium." Bethesda, MD: U.S. Department of Health, Education, and Welfare. DHEW No. (NIH) 76-989:240–265.

Pfitzer P, Splitt M, Müntefering H, Friesenecker JE (1982): Familiäre Häufung von Cyklopie über mehrere Generationen. Verh Dtsch Ges Pathol 66:169–172.

Pi S-Y, Fineman RM, Wing SD, Grunnet M, Chan G (1980): Holoprosencephaly in a Down syndrome child. Am J Med Genet 5:201–206.

Pratt RM, Goulding EH, Abbott BD (1987): Retinoic acid inhibits migration of cranial neural crest cells in the cultured mouse embryo. J Craniofac Genet Dev Biol 7:205–217.

Probst FP (1979): "The Prosencephalies. Morphology, Neuroradiological Appearances and Differential Diagnosis." Springer-Verlag, pp 1–25.

Rehder H (1981): The pathology of trisomy 21—With particular reference to persistent common atrioventricular canal of the heart. Hum Genet [Suppl] 2:57–73.

Roach E, DeMeyer W, Conneally PM, Palmer C, Merritt AD (1975): Holoprosencephaly: Birth data, genetic and demographic analyses of 30 families. In Bergsma D (ed): "Malformation Syndromes." New York: American Elsevier Publishing Co. Inc., for the National Foundation-March of Dimes. BD:OAS XI(2):294–313.

Robinson HB, Lenke R (1989): Letter to the editor. Am J Med Genet 34:266–267.

Rogers KT (1963): Experimental production of perfect cyclopia in the chick by means of LiCl, with a survey of the literature on cyclopia produced experimentally be various means. Dev Biol 8:129–150.

Ronen GM, Andrews WL (1988): Holoprosencephaly as a possible embryonic alcohol effect. Pediatr Res 23:270A.

Saunders ES, Shortland D, Dunn PM (1984): What is the incidence of holoprosencephaly? J Med Genet 21:21–26.

Schnabel R, Hansen S (1983): Karyotype 47,XXY,18p− in a newborn child with holoprosencephaly. Clin Genet 23:186–190.

Seidlitz G, Kadow I, Theel L, Pietsch P, Rudel J, Schneider K, Schroeter C (1983): Genetische Aspekte und humangenetische Beratung der Holoprosencephalie. Dtsch Gesundheitsw 38:665–669.

Shanks DE, Wilson WG, Sudduth KW, Couper KA, McIlhenny J (1988): Holoprosencephaly with del(2)(p2101p2109). Am J Hum Genet 43:A122.

Stabile M, Bianco A, Iannuzzi S, Buonocore MC, Ventruto V (1985): A case of suspected teratogenic holoprosencephaly. J Med Genet 22:147–149.

Sulik KK, Cook CS, Webster WS (1988): Teratogens and craniofacial malformations: Relationship to cell death. Development [Suppl] 103:213–232.

Sulik KK, Johnston MC (1982): Embryonic origin of holoprosencephaly: Interrelationship of the developing brain and face. Scan Electron Microsc 1:309–322.

Taylor AI (1968): Autosomal trisomy syndromes: A detailed study of 27 cases of Edwards' syndrome and 27 cases of Patau's syndrome. J Med Genet 5:227–252.

Thomas IT (1988): Familial holoprosencephaly and intra-familial phenotypic variability. Am J Hum Genet 43:A73.

Tiller GE, Watson MS, Duncan, LM, Dowton SB (1988): Congenital heart defect in a patient with deletion of chromosome 7q. Am J Med Genet 29:283–287.

Toriello HV (1986): The arhinencephaly field defect. Am J Med Genet [Suppl] 2:73–76.

Urioste M, Valcarcel E, Gomez MA, Pinel I, de León G, de Bustamante AD, Tebar R, Martinez-Frias ML (1988): Holoprosencephaly and trisomy 21 in a child born to a nondiabetic mother. Am J Med Genet 30:925–928.

van den Engh GJ, Trask BJ, Lansdorp P, Gray JW (1988): Improved resolution of flow cytometric measurements of Hoechst- and chromomycin-A3-stained human chromosomes after addition of citrate and sulfite. Cytometry 9:266–270.

Váradi V, Szabó L, Papp Z (1980): Syndrome of polydactyly, cleft lip/palate or lingual lump, and psychomotor retardation in endogamic gypsies. J Med Genet 17:119–122.

Warkany J, Passarge E, Smith LB (1966): Congenital malformations in autosomal trisomy syndromes. Am J Dis Child 112:502–517.

Webster WS, Lipson AH, Sulik KK (1988): Interference with gastrulation during the third week of pregnancy as a cause of some facial abnormalities and CNS defects. Am J Med Genet 31:505–512.

Wilson WG, Shanks DE, Sudduth KW, Couper KA, McIlhenny J (1989): Holoprosencephaly and interstitial deletion of 2(p2101p2109). Am J Med Genet 34:252–254.

Wraith JE, Super M, Watson GH, Phillips M (1985): Velo-cardio-facial syndrome presenting as holoprosencephaly. Clin Genet 27:408–410.

Young RS, Weaver DD, Kukolich MK, Heerema NA, Palmer CG, Kawira EL, Bender HA (1984): Terminal and interstitial deletions of the long arm of chromosome 7: A review with five new cases. Am J Med Genet 17:437–450.