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Microcytogenetics 1984

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Introduction

A new trend in 'classical' cytogenetics (as contrasted with molecular cytogenetics) is undoubtedly the evolution towards microcytogenetics[21]. Due to the refinement of techniques, i.e. the development of high resolution techniques, it has become possible to identify some 850 discrete bands on the haploid genome[29]. This has allowed the discovery of small deletions or triplications which were formerly not detectable. Such microdeletions are obviously of great value in increasing the precision of cytogenetic diagnosis[22,24]. But probably their most remarkable feature is that they have been observed in clinical conditions known or supposed to be of genetic origin.

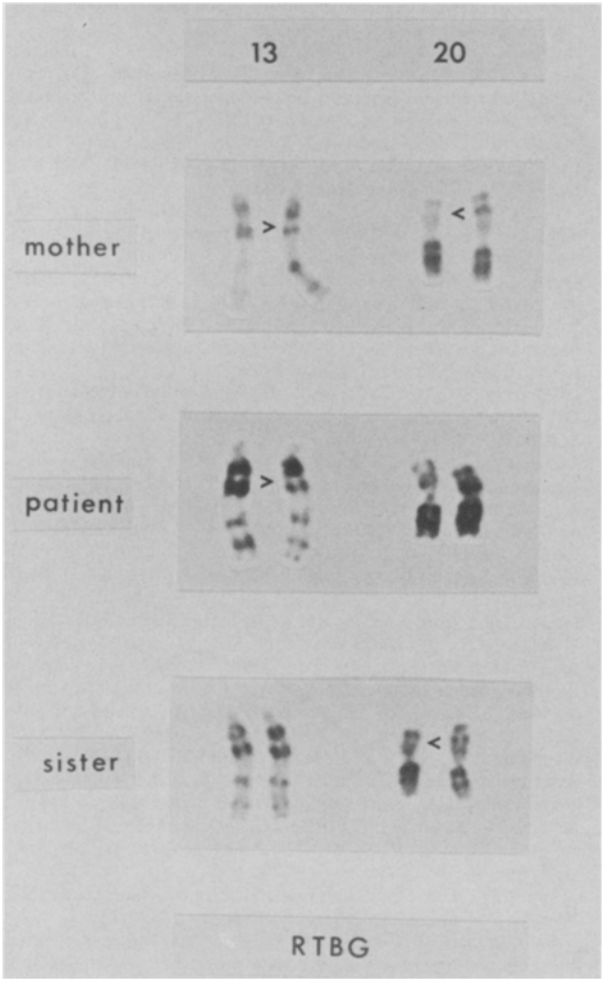

Figure 1. Familial rearrangement in a case of retinoblastoma. The mother is carrier of a balanced insertion: ins(20;13). The patient is monosomic for 13q14. His sister is trisomic for the same segment (from: Rivera et al.[53]).

The best example is that of retinoblastoma, in which regional mapping of the gene responsible for the dominant form of the tumor was made possible through a visible microdeletion of chromosome 13 and the coexistence of the closely linked genetic marker, esterase D. A comparable situation is illustrated by another childhood tumor, nephroblastoma. When associated with aniridia and other congenital malformations it is due to a specific microdeletion of 11p. Remarkably, nephroblastoma may also be part of the Beckwith-Wiedemann syndrome which in turn may be associated with triplication of the distal segment of 11p.

Further striking instances of microdeletions liable to be responsible for genetic conditions, other than childhood tumors, are: the deletion of chromosome 15 with Prader-Willi syndrome, that of 8q in Langer-Giedion syndrome, that of chromosome 22 in DiGeorge disease, and that of 17p in Miller-Diecker lissencephaly.

A word on the techniques

High resolution chromosome preparations are obtained by increasing the number of cells in prometaphase. This is done by synchronizing cell divisions; DNA synthesis is blocked by an inhibitor such as methotrexate, thymidine, or BrdU[9,67,73]. Blockage is released and the cells are harvested after a period previously determined, to obtain a maximum number of mitoses at the proper stage (usually 5-7 h).

Banding techniques may then be the same as for metaphases, for example heat denaturation (R-bands) or trypsin digestion (G-bands). They may also involve incorporation of BrdU followed by fluorescence – plus – Giemsa staining.

The del13q/retinoblastoma story

Retinoblastoma is a rare tumor that occurs in early childhood with an incidence of around 1 per 20,000. The majority of cases are not transmissible and are supposed to be due to somatic mutation(s). They are usually unilateral and unifocal. Other cases are due to germinal mutations. They occur as new mutations or are inherited as an autosomal dominant trait with about 90% penetrance. Lastly, a number of cases, which are considered exceptional but may well prove far more important in the future, are due to a chromosome rearrangement of chromosome 13[68].

Lele et al.[39] first reported in 1963 a partial deletion of a D group chromosome in a case of retinoblastoma. This chromosome was further recognized by banding techniques as a 13. The critical segment was progressively reduced to band 13q14[53,72]. A simultaneous and momentous discovery was the mapping of the esterase D gene locus to the same band[32,57]. This marker is polymorphic and linkage studies showed that the gene responsible for the dominant form of retinoblastoma and the locus involved in the deletion are the same[58].

From a clinical standpoint chromosomal forms of retinoblastoma, when due to a more or less conspicuous deletion, are often associated with congenital defects, mainly mental retardation, but no characteristic phenotype could be delineated.

The most frequently observed chromosome rearrangements are de novo interstitial deletions of which more than fifty have been recorded in the literature[28]. Their length is variable and their smallest region of overlapping is the proximal half of band 13q14. They account probably for 80–90% of all cytogenetic forms. Other observed rearrangements are de novo apparently balanced translocations with a breakpoint in or near 13q14. They involve another autosome or an X. Submicroscopic deletion, gene disruption, or position effect with X-inactivation spreading have been suggested to explain retinoblastoma in these events. Mosaicism has been reported in several instances (see for review refs 14, 63).

Of particular interest are parental balanced rearrangements which, by aneusomie de recombinaison or malsegregation, may result in monosomy 13q14. The most frequent mechanism is insertion (fig. 1). Five families are known in which retinoblastoma has been transmitted through unaffected parents who were carriers of such a balanced insertion. These families also include in some instances individuals trisomic for the inserted segment. Their phenotype is usually normal or only mildly affected. As in the case of de novo deletions, the breakpoints and the receptor chromosome are variable[52,53,60,62]. A recognized chromosome rearrangement does not, however, account for all families in which retinoblastoma is transmitted through unaffected carriers. A 'classical' hypothesis for explaining such families is delayed mutation or premutation. This notion remains somewhat hazy, however. Unrecognized insertions, at the submicroscopic level, offer a concrete explanation which will be amenable to proof as soon as molecular probes are at hand. Another convincing argument would be the demonstration of linkage disruption between retinoblastoma and esterase D or any other closely linked locus.

The del11p/aniridia complex

Aniridia is a genetic condition transmitted as an autosomal dominant trait with a frequency of 1.8×10^{-5} births[43]. This condition may, however, be occasionally part of a syndrome affecting psychomotor, renal, and genital development. The association of aniridia and nephroblastoma or Wilms tumor was first reported in 1953 by Brusa and Torricelli[2]. Miller et al.[45] found six cases of aniridia in a series of 440 patients with Wilms tumor; i.e. an incidence of 1/73. In 1977 François et al.[15] reviewed nearly 50 instances of the aniridia/Wilms tumor association. Francke et al.[12,13] and Riccardi et al.[51] demonstrated a short interstitial deletion of 11p in a patient with this association. The chromosomal etiology was confirmed by numerous reports and the critical segment common to all deletions was shown to be band 11p13. Wieacker et al.[70] mapped the catalase (CAT) locus to 11p by cellular interspecific hybridization. Junien et al.[31] regionally assigned the CAT gene to the same 11p13 band by gene dosage studies in three patients with various types of imbalance of 11p13.

Some forty patients with the del11p/aniridia complex are known[64]. Aniridia is present in all cases, except one[65]. The question remains open whether the situation is analogous to that of retinoblastoma, i.e. a single locus being responsible for cytogenetic and dominant forms, or whether at least two genes are responsible for aniridia. Indeed a dominant aniridia gene has been tentatively assigned to 2p by linkage with the acid phosphatase 1 locus (ACP1)[11]. Evidence for a dominant locus in 11p13 stems from the del1p13/aniridia association and from the observation of a family with an apparently balanced t(4;11)(q22;p13) transmitted through three generations and associated with isolated aniridia[55].

Tumors are an important but inconstant component of the complex. The type of malignancy is usually nephroblastoma and exceptionally gonadoblastoma. The overall risk of tumor formation is of the order of 1/3 although it is difficult to evaluate due to the mode of ascertainment. The tumor appears to be bilateral more often than in the isolated Wilms tumor. Its development is not related to the size of the deletion, as is proved by a couple of twins and a pair of brothers with identical deletions but different for the tumor[35,42]. The sex ratio is strongly in favor of males: 2 (XY)/1 (XX). Genital anomalies are practically constant in XY patients but of various degrees, from cryptorchidism to pseudohermaphroditism. Mental retardation is almost constant but highly variable, and so is growth retardation.

Like esterase D activity in the case of retinoblastoma, catalase activity is a useful complementary investigation to detect microdeletions. It is decreased in all instances, except two[33,46,50].

The chromosome rearrangement is variable in its type and in the size of the resulting monosomy. As with retinoblastoma, de novo deletions are by far the most frequent (fig. 2). A small proportion is due to complex de novo rearrangements. Three familial insertions are known[27,35,74]. An interesting point is that the deletion is

Figure 2. Interstitial deletion 11p13 in a case of aniridia/nephroblastoma complex (from Turleau et al.[64]).

associated in several cases with another independent de novo rearrangement[64].

The gene order within 11p13 is still a matter of controversy[46,65]. The vicinity on 11p to two loci, that of the oncogene HRAS and that of insulin, brings us to the next condition to be considered in this review.

Triplication 11p15 and Beckwith-Wiedemann syndrome

The Beckwith-Wiedemann syndrome was described, independently, in 1963 by Beckwith[1] in three infants and in 1964 by Wiedemann[71] in three siblings. It has also been called the EMG syndrome because of the three major signs: exomphalos, macroglossia, and gigantism. Other signs are: prematurity and hydramnios; neonatal hypoglycemia; microcephaly; facial naevus flammeus; fissures on the ear lobes; visceromegaly; gastro-intestinal anomalies, mainly malrotation; posterior diaphragmatic eventration; cardiac anomalies; genitourinary anomalies; mental retardation; and increased frequency of neoplasm[56] (fig. 3).

Trisomy 11p is a rare chromosomal disorder. Since the first report by Falk et al.[10], seven patients have been described, but with no reference to Beckwith-Wiedemann syndrome. Waziri et al.[60] were the first to relate specifically the clinical syndrome and trisomy for 11p15, by

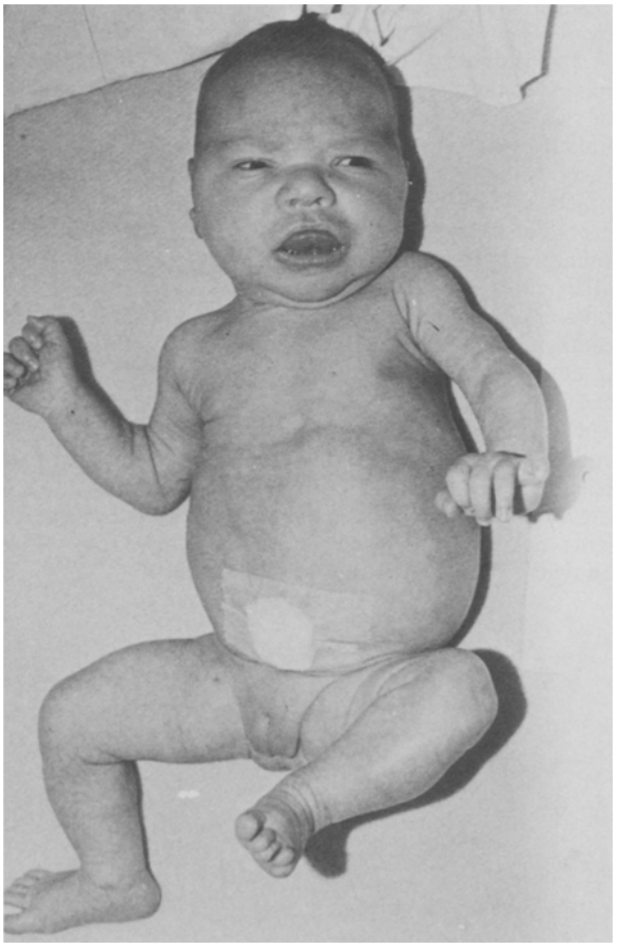

Figure 3. Patient with Beckwith-Wiedemann syndrome and trisomy 11p15 (from Turleau et al.[66]).

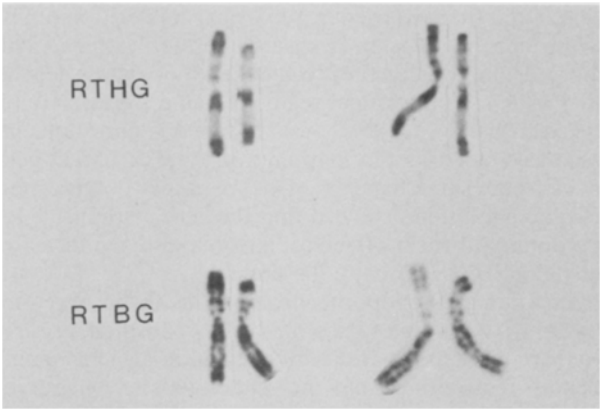

Figure 4. Trisomy 11p15 by tandem duplication in a girl with features of Beckwith-Wiedemann syndrome (from Turleau et al.[66]).

reporting two new patients with trisomy 11p15 and by emphasizing that these and the previously reported patients did indeed have features of the syndrome. This was confirmed by Turleau et al.[66] who also reported on two patients with the syndrome and trisomy 11p15 by de novo duplication in one (fig. 4), and a familial t(4; 11) in the other.

Using high resolution banding we have studied twelve other patients with Beckwith-Wiedemann syndrome, without mental retardation, including one patient with Wilms' tumor and a sibship of three affected children. We failed in each instance to demonstrate triplication of 11p15.

The question therefore arises whether Beckwith-Wiedemann syndrome is univocal or heterogeneous. As mentioned above, the genetic household of 11p affords some interesting clues in this respect. Firstly, the insulin gene is known to be located in 11p15 and trisomy for this gene could explain neonatal hypoglycemia and maybe gigantism, visceromegalia and its consequences.

The other inhabitant of 11p is the HRASI oncogene, the regional assignment of which is probably 11p15. This oncogene could be related to the occurrence of nephroblastoma in del11p13 as well as in some cases of Beckwith-Wiedemann syndrome. Tentatively one could hypothesize that a repressor gene acting on the oncogene in p15 is located in 11p13. In the case of 11p13 deletion only one repressor gene would be left for two oncogenes and in triplication of 11p15 (visible or submicroscopic) there would be three oncogenes for two repressors, both situations predisposing to Wilms' tumor.

Obviously, this hypothesis remains very tentative. In our experience the main difference between Beckwith-Wiedemann patients with and without a visible chromosome triplication remains mental deficiency. Before any knowledge of a chromosome rearrangement, the incidence of mental retardation was estimated to be some 12%. Although neonatal hypoglycemia may be considered responsible in some instances, the major component is perhaps the degree of chromosomal imbalance.

Del15q and Prader-Willi syndrome

In 1976 Hawkey and Smith[26] reported the first t(15q15q) in a patient with Prader-Willi syndrome. In their publica-

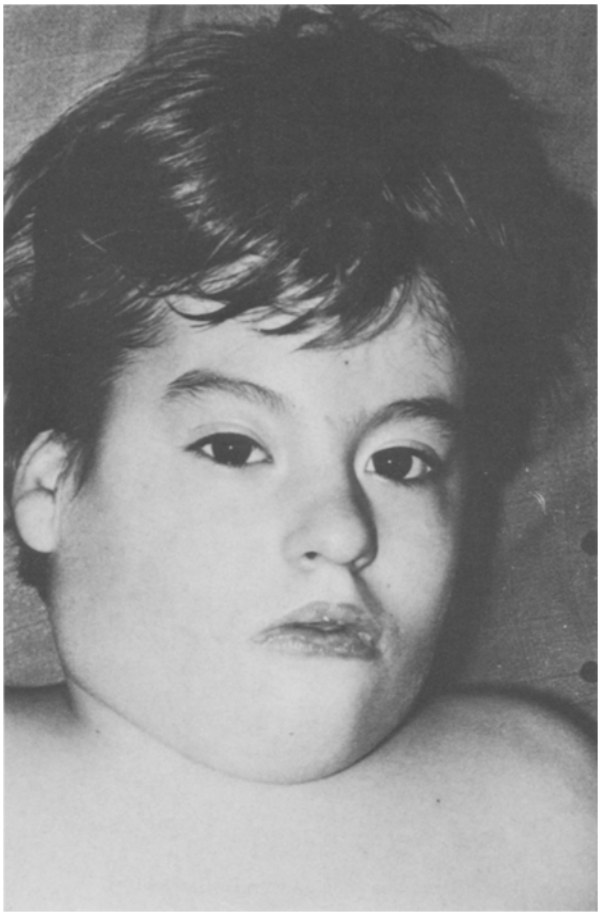

Figure 5. Patient with Prader-Willi syndrome and del 15q11 (Courtesy of M.6. Mattei, J.F. Mattei, and F. Giraud, personal collection).

tion they suggested that the dwarf Maribarbola in Velasquez's Las Meninas may have been a case of this syndrome rather than achondroplasia, as commonly assumed. Whatever the truth may be, the syndrome originally described in 1956 by Prader, Labhart, and Willi[49] in nine Swiss children is not a rare condition. The cardinal features are: neonatal hypotonia with feeding difficulties, respiratory distress, diminished spontaneous movements and weak or absent cry; hypogonadism and cryptorchidism; mental retardation; obesity with hyperphagia appearing around two years of age; and short stature. Gestation is characterized by diminished intrauterine movements and frequent breech presentation. The facies is distinctive with a narrow bifrontal diameter, almond shaped eyes with strabismus, a triangular mouth. The hands and feet are small (acromicria) (fig. 5).

A variety of structural rearrangements involving chromosome 15 have been reported since the first t(15q15q). All implicate the proximal region of the long arm of chromosome 15. They include reciprocal and Robertsonian translocations, small additional bisatellited chromosomes, pericentric inversions, and interstitial deletions (fig. 6). If visible deletions, unbalanced translocations, and a number of Robertsonian translocations involve loss of chromosome material, the other mechanisms may involve more complex reorganization of genetic sequences. In particular, the small supernumerary chromosomes represent apparent tetrasomy of the proximal 15q region[41]. Whatever the imbalance may be, microcytogenetics draws attention to a specific target band (15q11–12) and opens the way to the use of recombinant DNA technologies, which alone will answer the question of the uniqueness of the syndrome.

At present the observed frequencies of chromosome 15 rearrangements vary greatly but are generally more than 50%[38]. These estimates depend obviously on the use of adequate techniques and on the accuracy of clinical diagnosis. In the long run, the Prader-Willi syndrome may prove to be of chromosomal origin in nearly 100% of cases.

Langer-Giedion syndrome and the 'wandering' critical 8q segment

Giedion[18] reported in 1966 on the association of an abnormal facies, poor scalp hair growth, and specific radiological features. He named the syndrome 'tricho-rhino-phalangeal'. In 1969, he described a patient with this syndrome associated with multiple exostoses[19]. Independently, Langer[37] found the same association in another patient. The name 'Langer-Giedion' or 'tricho-rhino-phalangeal type II (TRP II)' was coined by Hall et al.[25] as opposed to type I, essentially without the multiple exostoses, as first delineated by Giedion[18].

Langer-Giedion syndrome is a very rare condition. The main clinical features are: a bulbous, pear shaped nose with tented alae, a prominent and long philtrum, a thin upper lip, micrognathia, and large protruding ears; sparse scalp hair; multiple exostoses; short stature; clinobrachydactyly; and cone shaped epiphyses. There is mild to moderate mental deficiency with or without microcephaly (fig. 7).

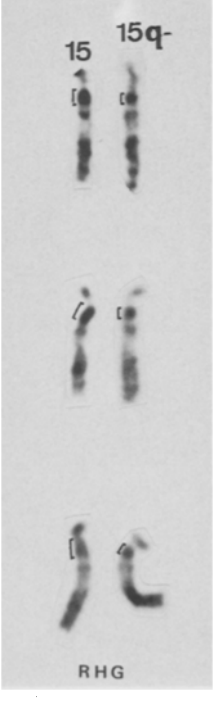

Figure 6. Interstitial deletion 15q11 in a patient with Prader-Willi syndrome (Courtesy of M.G. Mattei, J.F. Mattei, and F. Giraud, personal collection)

Bühler et al.[3] reported the first interstitial deletion of 8q. Pfeiffer[48] and Fryns et al.[16,17] reported on other deletions or rearrangements. These three reports indicated breakpoints varying greatly and showing no obvious overlapping. Zabel and Baumann[75] published the first description of prometaphase chromosomes from a Langer-Giedion patient and concluded that 'the deletion is interstitial, resulting form loss of material of the light G-band q22 and the main part of the prominent dark G-band q23'. Turleau et al.[61] presented two additional patients with typical Langer-Giedion syndrome, studied by high resolution banding. One had an intercalary deletion of 8q23: 'bands q22 and q24 seemed to have been preserved considering the degree of resolution obtained by the technique' (fig. 8). The other patient was considered as having a normal karyotype in spite of 'a very slight asymmetry of band q24.1'. Turleau et al.[61] suggested that in accordance with Zabel and Baumann[75], the critical segment is 8q23. A controversy thus arose, since the first reported case by Bühler et al.[3,4] showed no deletion of 8q23.

A total of 15 cases are now known. They are reviewed by Bühler and Malik[5]. After a reevaluation of the breakpoints in some of these cases, the authors conclude that the critical band is a tiny segment of 8q24.1 adjacent to 8q23. It seems reasonable in our view to admit that the matter remains open, considering the minuteness of the critical segment and the resolving power of the techniques at hand.

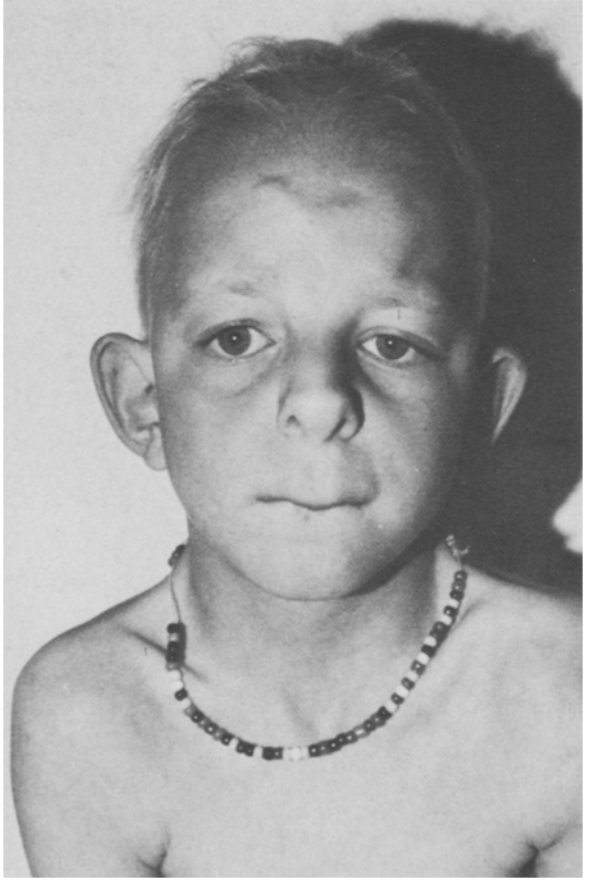

Figure 7. Patient with Langer-Giedion syndrome and del 18q23 (from Turleau et al.[61]).

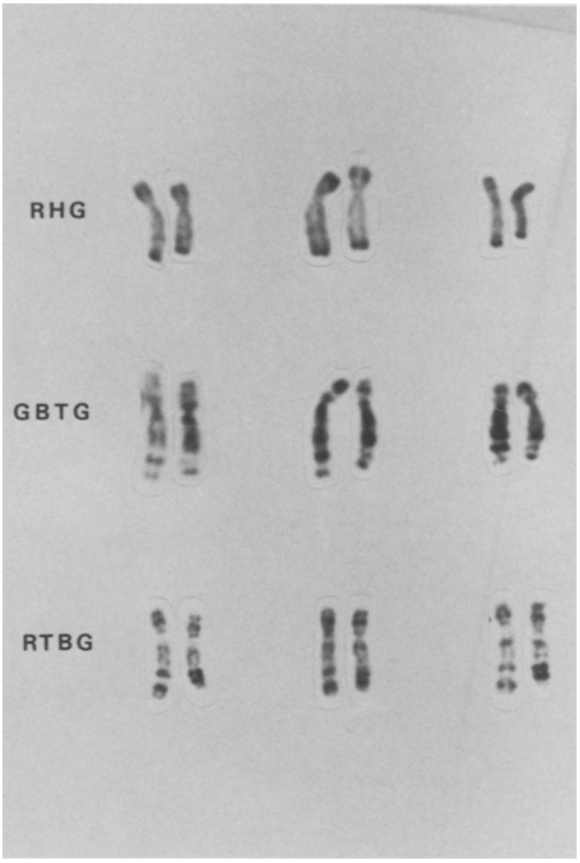

Figure 8. Interstitial deletion of 8q23 (from Turleau et al.[61]).

Another point of interest raised by Bühler and Malik[5] is the relationship between Langer-Giedion syndrome and tricho-rhino-phalangeal syndrome type I. One observation is known of a del 8q22.3→q23.2 (reinterpreted by Bühler and Malik as q24.1) in a type I patient. Further observations are likely to shed light on this matter in the near future. They should reveal whether the two conditions are really distinct[40] or whether they are closely related, differing essentially by the presence or absence of exostoses[5].

Partial monosomy of 22q and DiGeorge syndrome

The syndrome described in 1969 by DiGeorge[7] consists of: neonatal hypocalcemic tetany due to aplasia or hypoplasia of the parathyroids; increased susceptibility to infection due to aplasia or hypoplasia of the thymus; a characteristic facial dysmorphism with hyperterlorism, downward slanted palpebral fissures, short philtrum, low-set ears, and micrognathia; cardiovascular malformations, mainly conotruncal and aortic arch anomalies. These malformations are considered to be due to a failure of embryonic differentiation of the 3rd and 4th pharyngeal pouches.

La Chapelle et al.[36] were the first to report an association of partial monosomy 22 and DiGeorge syndrome. They studied a large family including four affected children in different sibships all of whom were carriers of an unbalanced t(20;22)(q11;q11). None of the six unaffected children had this translocation. Since none of the features

of DiGeorge syndrome is part of the trisomy 20p phenotype, the disease was attributed to monosomy 22q11. This hypothesis was supported by a previous report of child with complete monosomy 22 and several cardinal features of DiGeorge syndrome[54].

Kelley et al.[34] confirmed La Chapelle et al.[36] pioneer observation by publishing the observations of three unrelated patients with DiGeorge syndrome and carriers of a deletion of 22q11 due to balanced parental or unbalanced de novo translocations.

Lastly, Greenberg et al.[20] described another family (briefly referred to by Kelley et al.) in which two of three sibs and their mother had complete or partial DiGeorge syndrome associated with partial monosomy 22q due to an unbalanced t(4;22)(q35.2;q11.2).

A deletion of 22q11 is now well established in DiGeorge syndrome. Different points must however be emphasized. Firstly, the chromosomal rearrangement is observed only in a small number of cases. The above-mentioned authors have studied, in all, 24 patients with apparently normal karyotypes. Conversely, patients with monosomy 22pter→q11 are known without the cardinal signs of DiGeorge syndrome. Secondly, the only rearrangements to be observed were reciprocal translocations which produced proximal monosomy 22q by either adjacent- 2 or 3-1 type disjunction. Thirdly, no interstitial de novo deletion has been described. This may reflect a reality due for instance to close 'linkage' with the centromere. Deletions may on the other hand be overlooked due to the fact that banding of this particular region of 22q is not very discriminating. In other words, we might be in a situation similar to that when the Willi-Prader syndrome was being studied, and for which true deletions were observed only some time after translocations.

Partial monosomy 17p and Miller-Dieker lissencephaly syndrome

'Lissencephaly per se is an etiologically nonspecific brain malformation and may occur without other associated malformations and possibly, in more or less complete form, in other syndromes. Conversely, it is quite conceivable that lissencephaly is not an obligatory part of 'the' lissencephaly syndrome, but that other CNS defects may occur in that syndrome. Under such circumstances a diagnosis of 'the' lissencephaly syndrome can be made only if an affected sib had lissencephaly'[47].

Lissencephaly means 'smooth brain', without convolutions or gyri. In 1963 Miller[44] described this disorder in two sibs. Dieker et al.[6] reported in 1969 a family with three affected members and delineated a syndrome associating lissencephaly and malformations of the heart, kidneys, and other organs, as well as polydactyly and an unusual cranio-facial appearance. Microcephaly is constant. The forehead is high and may be narrow in temporal areas. The remarkable ability to fold the forehead skin into many wrinkles is a striking feature of the syndrome. The occiput is prominent. There is hypertelorism. The palpebral fissures slant slightly downward. Corneal clouding can be observed. The nostrils are anteverted. The lips may have a puckered appearance. The ears are slightly rotated posteriorly with anomalies of differentiation. Hirsutism may be extensive. The neurological picture is severe with initial hypotonia, seizures, and progressive decerebration. Death occurs invariably in infancy or childhood.

In 1980 Jones et al.[30] suggested the name Miller-Dieker syndrome to distinguish this entity from other types of lissencephaly of which at least five are known. It is a very rare disorder considered as an autosomal recessive trait. Dobyns et al.[8] were the first to report an association between the syndrome and 17p in two of three patients. One had a ring 17 and the other an unbalanced t(7p;17p). Additional support came from the literature where Dobyns et al.[8] found three patients with similar associations.

The same group further confirmed that Miller-Dieker lissencephaly is due to monosomy 17p13.3 by reporting one new patient with de novo 17p deletion and by reinvestigating four families already reported in the literature. In three of these they showed familial translocations involving 17p and a different autosome. In the fourth family the clinical history was reevaluated and diagnosis refuted[59].

Although the chromosomal origin of this rare syndrome has been demonstrated by a single group of investigators its reality appears well documented. The existence of a specific chromosomal imbalance emphasizes the individuality of Miller-Dieker lissencephaly syndrome. The necessity of a precise clinical diagnosis appears paramount, in particular for genetic counselling.

Concluding remarks

The 'morbid anatomy of the genome'[43] is the genetic disorders map. These disorders have mainly been assigned by assigning the gene locus of the enzyme whose deficiency is responsible for them[23]. Microcytogenetics have contributed to this map by assigning conditions not due to enzymatic deficiencies. These we have reviewed in this presentation. Another approach to this mapping has been the association of genic diseases and apparently balanced translocations. A remarkable example is the X/autosome translocations with a breakpoint in Xp21 observed in females with Duchenne muscular dystrophy. An important contribution of microdeletions is to pinpoint target bands on the genome and encourage linkage studies with known neighboring genetic markers of which DNA probes (bearing RFLP) are well-known examples, recently devised. Such technology will demonstrate whether genic and cytogenetic forms of a given disease are due to the same or different genes. The implications for genetic counselling and prenatal diagnosis are evident.

Another point, illustrated by this review, is the rapid evolution of cytogenetics. Hence it is difficult for a clinician to be knowledgeable about all new developments. This means that a very thorough cooperation between physician and cytogeneticist is needed. It becomes essential that the former gives all necessary biological, medical, and paramedical information when requesting a cytogenetic investigation, thus allowing the proper techniques to be applied, and the target band to be carefully investigated.

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First trimester fetal karyotyping using chorionic villi: technical development and diagnostic application

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Introduction

Transabdominal amniocentesis was introduced as a procedure suitable for fetal sex determination during the second trimester of pregnancy[5, 11, 19], but soon it was realized as offering a great potential for diagnostic purposes in cytogenetic and biochemical abnormalities because of the improvements in culture techniques for amniotic fluid cells[4, 12, 13]. However, the advanced time of gestation necessary for amniocentesis, and the cell culture time of 2–4 weeks, mean that selective abortion when an abnormal fetus is diagnosed may have to be performed at a late stage of pregnancy, 18–20 weeks. This frequently causes serious psychological stress to the couple, and the abortive procedures close to the fifth month of gestation are not completely free from medical complications.

In order to reduce the emotional stress and the possibility of clinical complications related to mid-trimester prenatal diagnosis, alternative techniques for fetal diagnosis before the 14th week of gestation have been evaluated in several studies. These techniques included transvaginal amniocentesis between 10 and 13 weeks of pregnancy[18] and analysis of fetal cells in the maternal circulation[1, 17], but at present these methods have no application in the diagnostic activity. Another technique for obtaining cells from the conceptus for cytogenetic, biochemical and