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CONGENITAL CHROMOSOME ANOMALIES

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Summary

The introduction of chromosome banding techniques has led to the detection of a greatly increased number of congenital chromosome anomalies, especially those involving duplication and/or deficiency of short segments of chromosomes. Of most widespread use are the general banding techniques: Q-, G- and R-banding. Special staining methods provide help in certain cases. These include C-banding, G11 staining of some C-bands, Ag-staining of active ribosomal RNA gene clusters, differential staining of sister chromatids and differential staining of early or late replicating bands. Every banding method that can be produced by a fluorescent or nonfluorescent dye can be duplicated by the use of antinucleoside antibodies, providing strong support for the idea that the organization of DNA of particular nucleotide base composition and sequence is responsible for chromosome banding.
As a result of the application of banding methods, a wealth of structural chromosome changes have been detected. In the balanced form, as translocations or inversions, these may have no effect on their carrier. However, such carriers have a markedly increased risk of having children with congenital chromosome anomalies. Most of these can be diagnosed prenatally using the generally available banding techniques.

Zusammenfassung

Die Entwicklung von Färbetechniken, die entlang der einzelnen Chromosomen ein reproduzierbares Bandenmuster zur Darstellung bringen und somit das Erkennen von Duplikationen und/oder Deletionen von kleinen Chromosomensegmenten ermöglichen, führte zu einer beachtlichen Zunahme der heute diagnostizierbaren angeborenen Chromosomenanomalien. Routinemässig werden weltweit die Q-, G- und R-Bändertechnik angewendet. In Einzelfällen sind die Ergebnisse, wie sie mit den nachgenannten zusätzlichen Färbemethoden erzielt werden können,

aufschlussreich: C-Bändertechnik: Giemsa 11-Technik zur Anfärbung bestimmter heterochromatischer Regionen; Silberfärbung zur Visualisierung der aktiven Gengruppen für ribosomale RNS; differentielle Anfärbung der Schwesterchromatiden und Banden mit früh- resp. spätreplizierender DNS. Die mit den verschiedenen Bändertechniken erzielten Bandenmuster können auch unter Verwendung von bestimmten Antinukleosid-Antikörpern gesehen werden. Diese Analogie stützt die Annahme, dass die Organisation der DNS in bezug auf Basensequenz und -zusammensetzung für die individuellen Bandenmuster der Chromosomen verantwortlich sein muss.
Die Bändertechniken eignen sich ganz besonders zur Identifikation von strukturellen Chromosomenaberrationen. In einem balancierten Zustand (Translokation, Inversion) brauchen diese keine Auswirkungen auf die Gesundheit ihres Trägers zu haben. Individuen mit einer balancierten Chromosomenanomalie haben aber ein deutlich erhöhtes Risiko, abnorme Kinder zu zeugen. Dank der Bändertechniken können diese Chromosomenanomalien bereits pränatal diagnostiziert werden.

CASPERSSON's discovery of metaphase chromosome banding (1) ushered in a new era in mammalian cytogenetics. Caspersson used an alkylating derivative of the fluorescent DNA-binding substance, quinacrine. Others have used different fluorescent dyes, or even Giemsa stain after various pretreatments, e.g. growing the cells in the presence of Actinomycin D or Ethidium bromide for the last few hours of culture, or treating the fixed, air-dried chromosome spreads with trypsin, oxidizing agents, solutions deficient in the divalent cations Ca^{2+} and Mg^{2+}, or solutions heated to nearly 90°C (2). Although the number of different banding methods continues to grow, there are basically only two or three general banding patterns (Table 1): Q- and G-banding, which are virtually identical except for the intensity of staining of polymorphic regions of highly repetitious sequence DNA, and R-banding, which is almost exactly reciprocal to Q- or G-banding i.e. a band that stains intensely by one method stains faintly by the other, and vice versa. The consistency and specificity of chromosome banding patterns, which makes them useful in chromosome identification, arises from the organization of the metaphase chromosomes, and reflects differences in base composition in the DNA along each chromosome. Thus the intensity of quinacrine fluorescence is enhanced in AT-rich sequences and quenched in GC-rich sequences in the DNA. In contrast, the antibiotic olivomycin shown GC-specific DNA binding and produces a fluorescent R-banding pattern (3). The importance of differences in nucleotide base sequence in chromosome banding is also shown by the ability to reproduce both forward (Q- and G-) and

Table 1. Methods for producing chromosome banding

General banding methods	
Quinacrine Fluorescence	Q-banding
Trypsin-Giemsa or variants	G-banding
87° heat-Giemsa or acridine orange	R-banding
Olivomycin Fluorescence	R-banding
Special banding methods	
Alkali-2X SSC-Giemsa	C-banding
In situ hybridization, satellite DNA	C-banding
Giemsa at pH 11	G11 banding
In situ hybridization, rRNA	rRNA gene sites
Ag+ reduction (silver staining)	NORs (active rRNA genes)
BrdU pulse-Hoechst 33258	DNA replication patterns
BrdU for 2 cycles-Hoechst 33258	Differential chromatid staining and SCEs

Table 2. Chromosome banding with immunochemical probes

Denaturing Agent	Antibody	Pattern
Formamide	Anti-adenosine	Q (except Y)-banding
UV-irradiation	Anti-adenosine	Q + C-banding
UV-irradiation	Anti-5 methylcytidine	C-banding
UV-irradiation	Anti-cytidine	G11-banding
Photooxidation	Anti-cytidine	R-banding
Heat (after BrdU)	Anti-BrdU	Differential chromatid staining

reverse (R-)-banding patterns by immunofluorescence or immunoperoxidase techniques for visualizing binding of antibodies to specific bases in DNA (Table 2 and ref. 4). These antibodies react only with the bases in single-stranded or denatured DNA. For example, after formamide denaturation, anti-adenosine binding produces a Q-banding pattern throughout the human complement (except for the Y chromosome). After UV-denaturation, antiadenosine binding also produces Q-banding, but with additional "hot spots" of very intense binding at the sites of AT rich satellite DNAs on chromosomes 1, 9, 16 and the distal part of the Y. After photooxidation of guanine residues in DNA, anti-cytidine produces an exact R-banding pattern.

In most clinical cytogenetics laboratories, G-banding is the standard procedure. Its use makes possible the identification of every chromosome and the detection of a large number of hitherto indeterminate structural changes such as translocations, inversions, deletions and duplications of specific chromosome segments, down to the level of a single band. This is important because deletions and to a lesser extent duplications can have a deleterious effect

on embryonic differentiation and development. Duplication of a single band from the long arm of chromosome 3 was associated with moderately severe mental retardation and mild physical malformations in two related individuals (5). In another family a brother and sister with duplication (partial trisomy) of the distal band from the long arm of chromosome 1 had similarly phenotypic abnormalities. Chromosome studies on the parents made it clear how this abnormality had come about. One parent had a translocation, with the distal band of the long arm of chromosome 1 now present on the tip of the short arm of chromosome 18. Only the latter chromosome was included in the gametes from which the two affected children arose (6). Of course, it is also possible for the reciprocal translocation chromosome alone to go to a gamete, and this can lead to the reciprocal unbalanced chromosome complement. That is, when the father or mother is a carrier of a reciprocal translocation, more than one type of chromosomal imbalance can occur among the progeny, leading to greater or lesser phenotypic effects. In one family where the father was a carrier of a t(5;14), one child had the 5p- (cri du chat) deletion syndrome while two children had the reciprocal 5p+ duplication (7). One had severe epilepsy and a behavior problem, while the other, who had febrile convulsions in early childhood, was severely disturbed emotionally, with a condition diagnosed as childhood schizophrenia. The other children in the family included a normal daughter, and a normal son who was a balanced carrier of the translocation. Such translocations clearly place their carriers in a high risk category in terms of producing children with congenital chromosomal aberrations. The risk may be as high as 50 %, if random segregation of the chromosome occurs during meiosis. The risk could be less if either (or both) type of imbalance leads to early abortion. On the other hand, the risk could be even higher, because the presence of a translocation tends to raise the frequency of nondisjunction, which could lead to still more types of aneuploid gametes.

Consider the case of a woman with an X-autosome translocation, t(X;14), in which virtually the entire long arm of the X was translocated to the distal end of the long arm of chromosome 14. This woman had a series of spontaneous abortions; none were karyotyped so it is uncertain whether any were secondary to chromosomal imbalance. However, consideration of the types of gametes she could produce indicates that she could have produced embryonic lethal karyotypes as well as karyotypes that should lead to Turner, Klinefelter and XXX syndromes. Her two children, both male, included one with Klinefelter's syndrome. His karyotype was noteworthy for the presence of two copies of the long translocation chromosome, one copy of the short one, and a Y chromosome. The two copies of the long arm of the X were sufficient to produce Klinefelter syndrome, i.e. the second short arm of the X

is not required. It is noteworthy that this patient had very few significant abnormalities other than those seen in Klinefelter syndrome despite being trisomic for virtually the entire chromosome 14. The explanation for this lack of effect appears to be that inactivation of the second long arm of the X occurred and spread from the X chromosome segment of the translocation chromosome into the chromosome 14-derived segment. The evidence for this is both the lack of phenotypic effects attributable to trisomy 14 and the late replication of all but the most proximal part of the long translocation chromosome (8).

Analysis of the timing of DNA replication in particular chromosomes is one of a number of special methods whose use has increased the accuracy of chromosome diagnosis. Markedly improved resolution has resulted from the replacement of ^{3}H-thymidine autoradiography by the use of a special staining method. Bromodeoxyuridine (BrdU) incorporation during a portion of the S phase of the cell cycle, followed by staining with the fluorescent bis-benzimidazole derivative, Hoechst 33258, permits analysis of the timing of DNA replication at the level of individual bands (9). BrdU incorporation throughout two cell cycles (specifically, two successive S periods) leads to differential staining of sister chromatids and permits demonstration of sister chromatid exchanges (10), which serve as a measure of DNA damage and repair.

Other special banding methods (Table 1) lead to delimitation of a limited part of the genome, e.g., C-banding reveals only constitutive heterochromatin, while G-11 differentially stains a restricted subset of the C-bands in the human, specifically the C-band of chromosome 9 and, to a variable extent, those of some of the acrocentric chromosomes. In contrast, in the mouse G-11 stains everything except the C-bands. In situ hybridization with radioactively labeled DNAs reveals the location of these DNAs (11), or with 18S and 28S ribosomal RNA (rRNA) reveals sites of reiterated rRNA genes (12). Silver (Ag)-staining also reveals the sites of rRNA genes (13) but only those that were actively transcribed in the preceding interphase (14).

Virtually every differential staining method, including differential chromatid staining, can be reproduced using antibodies that react with the nucleotide bases in denatured or single-stranded DNA (4). For example, after UV irradiation, anticytidine binds specifically mainly to the C-band of chromosome 9, although it sometimes stains the C-bands of some of the acrocentric chromosomes, a pattern resembling that seen after staining with Giemsa at pH 11 (G11). After UV-irradiation, anti-5-methylcytidine binding, visualized by immunofluorescence or immunoperoxidase staining, produces a C-banding pattern, with the most striking binding to the C-band region of human chromosomes 1, 9, 15, 16 and the Y. This provides a

relatively specific probe for the centromeric end of chromosome 15, usually distinguishing it very clearly from the other acrocentric chromosomes. This staining reaction has clinical application in the differential diagnosis of trisomy-22 and in determining the origin of bisatellited chromosomes, i.e. with secondary constrictions at both ends. Both ends of more than half the latter appear to have arisen from chromosome 15, based on antibody staining (15). In two individuals (first cousins), an extra acrocentric chromosome that closely resembled a number 22 by Q- and G-banding was shown to have the centromeric end of a number 15. Examination of the mother of one of the individuals and a sister of the other revealed a t(11;15) translocation. The centromeric end of the smaller translocation chromosome was derived from chromosome 15.

Immunological methods can also be used to enhance our understanding of abnormalities of sex differentiation (especially those commonly associated with a chromosome abnormality). Professor Wolf has referred to the use of antisera to H-Y antigen in cases of XX males with Klinefelter syndrome or true hermaphroditism, and XY females with pure gonadal dysgenesis. The latter sometime occur in familial patterns that suggest a role for an X-linked gene in the expression of male determining factors (16). Such a gene has been found in the wood lemming (17), and its presence in man would not be surprising in view of the evolutionary conservatism of the mammalian X. Some XY females with streak gonads occur as sporadic cases. Some may be the result of fresh mutations of X or Y linked genes for male sex determination, but others may be the result of a gross chromosomal change. In one such case (18), a young girl with short stature, cubitus valgus, lymphedema and streak gonads was found to have an apparent 46,XY karyotype, with a prominent Y body in interphase cells and a Y chromosome almost identical to that of her father. It differed only in having a deletion of the tiny short arm, thus removing the locus of the H-Y gene and blocking testicular differentiation. In another case, translocation of the short arm of the Y to a chromosome 22 was found in a male (with scrotal testes) whose karyotype was first thought to be 45,X. Demonstration of H-Y antigen on the surface of his cells has strengthened the cytogenetic interpretation of a translocation (19).

Recently, a silver staining technique has been introduced for studying nucleolus organizer regions (NORs), the sites of ribosomal RNA gene clusters on chromosomes. Only recently active genes are stained by this technique. The amount of staining generally parallels the number of copies of rRNA genes in the NOR (20, 21), but there are exceptions. For example, a 14p+ chromosome with a six-fold increase in the number or rRNA gene copies had no more than a twofold increase in silver staining, suggesting the operation of some kind of dosage

regulation. The variant chromosome was transmitted to six healthy people in three generations (22). Other variants, with a reduced number of rRNA gene copies, might have a deleterious effect if the total number of rRNA genes fell below a certain threshold number. If so, the methods now available should make it possible to diagnose prenatally those midtrimester fetuses at increased risk of a hyporibosomal state.

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