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Cytogenetic analysis: Is there a need to discover greater detail in the karyotype?

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The University of Arizona, 1991

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CYTOGENETIC ANALYSIS: IS THERE A NEED TO DISCOVER GREATER DETAIL IN THE KARYOTYPE?

by

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STATEMENT BY AUTHOR

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APPROVAL BY THESIS DIRECTOR

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Finally, I owe special thanks to my family: my husband, my parents, my brother and my son, whose love and expectations have been the major motivation for me to work toward a Master's Degree. They have always been very supportive and understanding throughout my life.

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ABSTRACT

Chromosome studies are mandatory to confirm the diagnosis in even "typical" cases of genetic or malformation syndromes, since the phenotype does not unambiguously define the genotype. With the inception of banding methods to facilitate the unequivocal identification of chromosomes, major interest has been devoted to obtaining prometaphase chromosomes, Since they allow for greater detail with a larger number of bands. The purpose of this study is to define in which circumstances prometaphase chromosome analysis is most useful and the minimum band level required for such high resolution detail in the karyotype.

Karyotypes of 4 amniotic fluid samples and 14 prepheral blood specimens were prepared and studied at a range of band levels. The 18 cases were selected because of the presence of a variety of chromosomal anomalies. The results revealed that in most cases, a structural anomaly of a single chromosome could be surmised at banding level of 400-500. However, banding levels of 650-850 allowed for more clear delineation of the exact anomaly and accurate assignment of break points.

INTRODUCTION

Each chromosome in the human somatic cell complement can be unequivocally identified by following a number of different banding procedures. The banding patterns are highly characteristic. Trypsin-Giemsa (G-banding) is commonly used. It is necessary to define the optimal chromosome band level that is required to perform an adequate chromosome analysis. For the study of aberrant chromosomes and, in particular, for the assignment of breakpoints, more detail can usually be seen with the finer bands present at prometaphase which condense and form thicker bands in late metaphase. This forms the basis of high resolution banding techniques. This study attempts to discover what chromosome band level is required in a karyotype to correlate with the given clinical data.

An International System for Human Cytogenetic Nomenclature (ISCN)(1985) provides the schematic representation and nomenclature of chromosomes corresponding to approximately 400, 550 and 850 bands per haploid set. The number of bands seen along the length of a chromosome is a function of the stage of the cell cycle. Each band visible

during metaphase is composed of 2 to 4 bands in prometaphase, an earlier stage of the cell cycle. During mid to late metaphase, there are 300 to 400 bands in a hapoid set of 23 chromosomes. During prometaphase, approximately 550 to 850 bands can be visualized. Because a much larger number of bands is present at prometaphase, detection of smaller deletions is possible. Through cell synchronization techniques, it is possible to enrich the percentage of cells in prometaphase.

The phenotypic effects of an autosomal chromosome abnormality are usually related to disturbances of gene dosage and regulating functions, rather than to structural defects in the individual genes. The fundamental concept is imbalance. Any deviation from the normal diploid karyotype is potentially hazardous to normal development. Autosomal imbalance usually has very serious consequences. Growth retardation, dysmorphic features, multiple malformations and mental deficiency are the most common findings caused by a chromosome aberration. Using the banding studies of the rearranged chromosomes along with human gene mapping, relationships have been sought between the abnormal chromosomes and the present clinical manifestations.

There have been several recent modifications to the standard lymphocyte culture method. These are aimed at allowing analysis of human chromosomes at earlier stages of cell division in order to gain additional information from more elongated chromosomes which permit high resolution banding. The technique commonly used for high resolution chromosome analysis introduced by Yunis is based principally on cell synchronization using methotrexate and thymidine to increase the number of cells in early mitotic stages and obtain prometaphase chromosomes.

Methotrexate (MTX) is an analogue of folic acid (FA) with a higher affinity for dihydrofolate reductase than FA, so that the synthesis of folinic acid is potently inhibited. Folinic acid is required for the production of thymidine which in turn is required for DNA synthesis. The cells are therefore blocked prior to DNA synthesis at the G1/S interphase. The MTX block can be released by washing and adding thymidine. Minimum exposure of colcemid is important for the inhibition of spindle formation while having a negligible effect on chromosome condensation.

In the present study, ethidium bromide (EB), has been evaluated for its usefulness in obtaining large numbers of early mitoses from the culture of human lymphocytes. EB has an inhibitory effect on mitotic chromosome condensation. Thus its application to high resolution banding analysis can be found in its ability to produce a high proportion of mitotic cells having elongated chromosomes.

MATERIALS AND METHODS

Materials used for the chromosomal analysis consisted of 4 aminotic fluid samples and 14 peripheral blood samples.

Methotrexate treatment of peripheral blood cultures to yield prometaphase chromosome for high resolution banding

Sterile sodium heparinized peripheral blood was mixed by gently inverting the vacutainer tube several times, then 7-8 drops were added to 5 ml of RPMI 1640 culture medium (GIBCO) supplemented with 20% fetal bovine serum (FBS)(GIBCO), L-glutamine penicillin streptomycin (GPS) and phytohemagglutinin (PHA). The cultures were incubated at 37°C with the tube slightly inclined for approximately 72 hours (48 hour cultures will usually yield enough metaphases for analysis if there is a rush for the evaluation). Culture incubation was started at 4:00-5:00P.M. to make subsequent processing convenient. At the end of the 72 (or 48) hour incubation, 0.05ml of 10^{-5}M methotrexate was added per 5ml culture to synchronize the mitoses. The tube was then inverted several times until no pellet was left. The cap was loosened and the tube returned to the incubator. 17 hours

later, 0.1ml, 10^{-3}M thymidine was added per 5ml culture. This removed the methotrexate block. The tubes were inverted until well mixed, the time was noted, and the tubes were retured to the incubator and incubated for 4.5-5.5 hours. 15 minutes before harvesting, 0.05ml (per 5ml culture) of colcemid (10^{-5}M) was added, measured with a 1ml in 1/100 pipet. The tubes were inverted to mix it well and returned to the incubator. At end of incubation, the cells were resuspended and centrifuged for 7 minutes at approximately 1000-1200rpm. The tubes were checked to be sure that the cells were well pelleted. The supernatant was pipetted off and 5ml of 0.075M KCL solution added (prewarmed to 37°C). The tubes were mixed again if necessary and incubated at 37°C for 17 minutes. (The chemical addition times may be adjusted for future cultures according to how satisfactorily the metaphases spread. Metaphases spread more in higher humidity, less in very low humidity.) After hypotonic incubation, the tube were centrifuged for 7 minutes at 1000-1200rpm. Almost all of the supernatant was pipetted off, the tubes recapped, the cells resuspended with a mixer, making sure that there was no pellet left in the bottom of the tube. Slowly 5ml of fresh cold fixative was added to the tube (prepared with 3 parts absolute methanol to 1 part

glacial acetic acid). The tube was chilled in the freezer at least 15 minutes. The cell suspension was then mixed and centrifuged and washed two more times with changes of fixative. After the last wash with fixative, the slides were made using the air-dried method and then stained by the standard GTG (G-bands by trypsin using Giemsa) technique. (In our laboratory, viocase (pancreatin solution) is used instead of trypsin). Stock viocase was diluted to 1:20 with Hanks' BSS (balanced salt solution)(1.0ml viocase plus 19.0ml Hanks in a plastic slide mailer) and used at room temperature. The slide was quickly vertically dipped into the viocase, and immediately rinsed with tap water. Next, the slide was stained in freshly prepared Giemsa working solution (1ml Giemsa stock solution in 50ml Gurr's buffer 6.8) Various tissues and types of slides required different exposures: peripheral blood - 2 min 45 sec, amniotic cells - 3 min 30 sec. The slides were checked under the microscope after staining and coverslipping or stained for a longer period of time if the stain intensity needs adjusting. The slide was then rinsed in tap water, air dried, cleared in xylene and mounted with coverbond using a coverslip. Adequate time for the slides to dry was allowed before analysis with an oil objective lens.

Ethidium bromide for high-resolution banding analysis of chromosomes

Ethidium bromide (EB) has been found to inhibit chromosome condensation without disadvantages (except that it is carcinogenic) and can be useful for high-resolution chromosome banding of human lymphocytes (Ikeuchi and Sasaki 1979, Ikeuchi 1984). Cultivation of EB in our laboratory is 1 hour and 50 minutes before harvest. 1 hour before adding colcemid, 0.05ml EB (10^{-5}M) per 5ml culture is added. 50 minutes before harvesting, 0.05ml of colcemid is added. The culture medium, cell fixative, harvest and slide preparation as well as staining techniques were the same in this study as those mentioned above.

Amniotic fluid cell chromosome analysis

Amniotic fluid was usually collected by trans-abdominal amniocentesis at 14 - 17 weeks of fetal gestation. Sterile tissue culture reagents and aseptic technique were utilized until the processing was complete. Each specimen was set up separately. The specimen was transferred into the

specified conical tube and centrifuged at 1200rpm for 7 minutes. After centrifuging, about 1 - 5ml of supernatant was poured into a plastic tube for alpha fetoprotein testing and a 1 - 5ml portion was poured into another tube to reserve in the laboratory freezer until the karyotypic analysis was finished. Approximately 0.5ml fluid was left on each pellet. 22mm square glass coverslips were dipped into methanol, flame dried, and placed into 35mm (Nunc) culture dishes. Alpha MEM medium (GIBCO or IRVINE) supplemented with 20% FBS and 1 x GPS solution (IRVINE) was added to the cell pellets using a separate bottle of medium for each tube, making sure that the cell pellets were well mixed into the medium. Then 0.5ml of medium was added to each coverslip and 3ml to the flask. (The flask is intended as a "back up" culture and is usually not needed for the analysis). The tray of dishes was then carefully placed in a humidified incubator at 37°C with 5% CO_2, 95% air atmosphere. The next day, the coverslip cultures were flooded by carefully adding 2.5ml of culture medium to each dish. The dishes were left undisturbed for 3 or 4 days. After 3 to 4 days the medium was changed by swirling each dish separately and then dumping the medium into a waste bottle. The cultures were reflooded with 2.5ml medium / dish. The coverslips were

evaluated every day on the inverted microscope after the initial 3 to 4 days to determine if the coverslips were ready for harvest. (Coverslips are ready to harvest when there are several colonies of 50 - 200 cells present). The average harvest time was at 7 - 8 days. To the cultures ready to process, 2 drops (from 1ml pipet) of colchicine, 10^{-5}M (GIBCO) were added, then incubated 30 minutes at 37°C. The medium was carefully removed with a capillary pipet, and replaced with 2ml of 0.8% fresh sodium citrate (hypotonic solution) by gently adding it down the side of the dish and allowing the dishes to sit at room temperature for 20 minutes. Then, dropwise down the side of the dish , 2ml of fresh fixative (3 parts methanol : 1 part glacial acid) were added and let stand for 2 minutes. All fluid was removed and replaced with 2ml of fixative and let stand 20 minutes and repeated once again. The coverslips were then steamed, heated and air dried. After aging at 85°C for 1/2 hour or overnight at 60°C on a slide warmer. The coverslips were stained by the standard GTG technique and mounted on slides.

Chromosome banding level analysis method

I studied specimens from 16 abnormal and 2 normal cases. These included patient karyotypes with constitutional deletions, inversions,translocations, and 2 normal cases for control. For each case, I examined a range of chromosomes from prometaphase to metaphase. Using photomicroscopic techniques, I took pictures of each chromosome or chromosomes at several different band levels and prepared a partial karyotype of each. I counted the number of bands found in each chromosome and analyzed the chromosome band levels for each of 22 pairs of homologs and each of the sex chromosomes.

RESULTS

Among the 18 selected cases, 16 had structurally abnormal chromosomes. These included 4 deletions, 7 translocations, and 5 inversions. Two normal chromosome karyotypes were used as a control for high resolution banding analysis. Using methotrexate techniques, these two cases were greater than the 850 band level (Fig.1 and Fig.2). In many cases, particularly with respect to subtle deletions, karyotypes with band levels less than 550 failed to reveal the structural chromosomal anomalies.

The new banding techniques have allowed the characterization of even more subtle deletions of chromosomes which have been found to be associated with clinical syndromes. For example, three cases contained interstitial deletions of chromosome 15 (q11-q12). Two of them had a clinical history of Prader Willi syndrome; one had Angelman syndrome. A range of chromosomes from prometaphase to metaphase was examined and abnormal partial karyotypes were prepared at stages that correspond to the 400, 550 and 850 band levels (Fig.3, Fig.4, Fig.5). Note

that the deletions were more readily recognized at the 850 band level.

Sometimes a partial deletion 5p results in a phenotype with some features similar to Down syndrome. A newborn girl had short digits and upslanting eyes. The clinical information provided indicated Down syndrome. Chromosome analysis using EB technique showed del(5)(pter->p15.1) (Fig.6). Thus, the cytogenetic diagnosis confirmed one of the most frequently occurring, but subtle, autosomal deletion syndromes, the cri du chat syndrome.

In another case cytogenetic investigation of the cultured amniocytes showed a reciprocal balanced 3/6 translocation. A pericentric inversion 9 was also present. Investigation of the parental karyotypes showed that a balanced reciprocal translocation between chromosomes 3 and 6 had been inherited from the phenotypically normal father, and a pericentric inversion 9 from the phenotypically normal mother. Both the fetus and father had the same breakpoints: t(3;6)(p21.3;q16.2) (Fig.7).

Two cases of a Robertsonian translocation 14/21 were observed with an 850 band level. One patient was a 7 year old boy who had clinically dysmorphic features and developmental delay. The high resolution band study showed a balanced 14/21 Robertsonian translocation with no deletion identified(Fig.8a,Fig.8b). Another newborn boy was hypotonic, and had a simian crease and excessive skin folds on the neck. Chromosome analysis revealed a Robertsonian 14/21 centric fusion translocation in addition to two other copies of chromosome 21. This was a Down syndrome karyotype (Fig.9).

In another case, a family study was instigated because an amniotic fluid analysis showed a paracentric inversion 7 (q11.22q22.3) (Fig.10). The chromosome analysis of the parental karyotypes revealed that this paracentric inversion 7 had been genetically inherited from the father who had a normal phenotype. Band levels had been observed from 400-750.

A reciprocal translocation 14/17 was observed in a 6 year old boy who had a clinical history of omphalomesenteric duct cyst (now repaired), deformed ears, flattened facies,

open fontanelle, smooth philtrum, micrognathia, redundant neck skin, simian creases and a single umbilical artery. The reciprocal translocation first noted in a 1984 study was confirmed. The breakpoint on 14 was q32.3 and the breakpoint on the 17 was q21.3. The modal count was 46 (Fig.11a and Fig.11b). No obvious net excess or deficiency was noted in the rearrangement at the 715 band level. Parents of the patient had normal karyotypes. It is possible that this de novo translocation involves a submicroscopic deletion leading to the abnormal phenotype. Future studies with molecular markers of 14q and 17q could shed more light on this possibility.

Cytogenetic study of a cultured peripheral blood showed a reciprocal translocation 8/17 (Fig.12). This child had multiple congenital abnormalities, and two previous pregnancies of the mother had ended in spontaneous abortions. The breakpoint on chromosome 8 was p21, while on 17 it was q11. The significance of this apparently balanced translocation is unclear. Cytogenetic studies of the parents will be necessary to determine whether one of them carries this translocation, or if it is de novo.

A 2 year old boy was referred for chromosome analysis because of developmental delay. EB high resolution techniques were used, and a 2/9 reciprocal translocation with breakpoints of q31; q22.3 was observed. Banding studies demonstrated that there was no apparent imbalance (Fig.13). As above, parental studies are indicated to infer clinical significance.

Cytogenetic analysis of a cultured amniotic fluid showed a pericentric inversion 4 (p14q34.2) (Fig.14). The same inversion was demonstrated in the paternal karyotype (Fig.16). No imbalance was present from the 600 to 850 band level.

In a chromosomal study of amniocytes, a pericentric inversion 9(p13q21) was observed (Fig.15). No chromosomal imbalances were noted, and phenotype was normal.

DISCUSSION

Cytogenetic analysis using new banding techniques has allowed the characterization of even more subtle deletions of chromosomes which have been found to be associated with clinical syndromes. For example, deletion 15 (q11 or q12) had been demonstrated in both Prader-Willi syndrome (PWS) and Angelman syndrome (AS). The number of bands seen along the length of a chromosome is a function of the stage of the cell cycle. Each band visible during metaphase is composed of 2-4 bands in prometaphase. High- resolution methods applied to patients with PWS and AS reveal an identical small interstitial deletion in the proximal portion of the long arm of chromosome 15 (q11-q12) (Fig.3,Fig.4,Fig.5). During late metaphase, there are 400 or less bands in a haploid set of 23 chromosomes; during prometaphase, approximately 550 to 850 bands can be visualized. Because a much larger number of bands is present in prometaphase, detection of smaller deletions is possible. Through cell synchronization techniques, it is possible to obtain a higher percentage of cells in prometaphase. These Prader-Willi syndrome and Angelman syndrome patients have the same breakpoints and share a common chromosome 15 deletion, but they have very different clinical phenotypes and differ in parental origin of the deletion. With the present

cytogenetic level of resolution, the deletion found in these two syndromes does not appear to differ. The partial chromosome deletion of 15(q11-q12) has been demonstrated (Fig.3,Fig.5); this study confirms that a higher band level clearly reveals a chromosome deletion. At the 400 band stage (mid to late metaphase) one only can see the shortened region of the deleted band, but the q11-q12 bands are not discernible, and it is difficult to tell that the specific band is missing. At approximately the 750-850 band level (prometaphase), the deleted region of q11-q12 can be easily seen. The diagnosis of PWS and AS depends on characteristic clinical features and natural history. With the addition of prometaphase chromosome analysis, the diagnosis of PWS and AS diseases may be confirmed. These data suggest that high resolution techniques at band level > 650 should be routine in peripheral blood karyotypes. This is especially true for patients having unusual clinical manifestations and/or a positive family and natural history.

The least rigorous criteria for banding resolution have been applied to prenatal diagnosis specimens and routine bloods. Longer chromosomes are required for the diagnosis of a child with multiple congenital anomalies and/or mental retardation since this is the population in which small deletions are most likely to be found. The

phenotypic expression in deletion syndrome will vary depending on the region of the deletion. If euchromatin is involved in the deletion, the more serious the consequence may be. This is due to the fact that euchromatin contains the normally functioning genes, while heterochromatin is, for the most part, genetically inert.

Translocations occur when two chromosomes break and then rejoin in mispaired combinations. This involves breaks in two chromosomes with rejoining of mismatched segments. If no genes are lost, the translocation is balanced and the phenotype is usually normal. A Robertsonian translocation is the most common form of translocation. It is composed of a centric fusion involving two acrocentric chromosomes. These may be monocentric or dicentric. The corresponding small marker chromosomes formed by the fusion of the satellited short arms of these chromosomes is usually lost, but it can sometimes be seen as an accessory marker chromosome. Unbalanced gametes of Robertsonian translocations produce trisomy or monosomy for a complete chromosome. The translocation involving an extra number 21, or at least extra material corresponding to band 21q22 is clinically important. Also, Robertsonian translocations involving 13/14, 13/13, 13/21 will produce trisomy 13, 13, 13(or21), respectively. One of the patients in this study had a

Robertsonian translocation producing trisomy 21 with Down syndrome features (Fig.9). The significance of a high-resolution banding study in the instance of a Robertsonian translocation is that there is only one active centromere, but it can not be shown which centromere belongs to which acrocentric chromosome. One centromere or partial centromere was lost. Some phenotypes can be mapped to specific chromosomal segments since not all chromosomal syndromes involve a whole chromosome. If we can find a specific partial trisomy or monosomy chromosome on a high-resolution banding study, this will help to associate it with a clinical syndrome. For example, Down Syndrome can be diagnosed by finding extra material corresponding to band 21q22.

In some cases, patients have a very clear abnormal phenotype, but have an apparently balanced chromosomal rearrangement. For instance, in the case of a 6 year old boy with a balanced reciprocal translocation (14/17), the breakpoints are 14(q32.3) and 17(q21.3) (Fig.11a,Fig.11b). His clinical symptoms were omphalomesenteric duct cyst, deformed ears, flattened facies, open fontanelle, smooth philtrum, micrognathia, redundant neck skin, simian creases, and a single umbilical artery. Chromosome studies on his parents were normal, suggesting a de novo translocation in

the child. Despite the rearrangement looking perfectly balanced, the breakage and reunion event may have disrupted the function of the genes at the breakpoints. The breakpoint might have damaged an essential gene function by splitting the gene or by altering position effects, which caused the gene to be lost or mutated. Alternatively a very small deletion not visualized with present techniques may be present. Molecular techniques may be helpful in delineating the presence or absence of such submicroscopic deletions.

If two breaks occur in the same chromosome, the interstitial segment may become inverted prior to repair of the breaks. Such an inversion is paracentric if the centromere is not included; it is pericentric if the centromere is included in the inverted segment. A chromosome containing an inversion theoretically still has a full complement of genes and therefore is genetically balanced. Problems arise only during meiosis, when normal pairing cannot occur. In order for homologous regions to pair in a cell that is heterozygous for an inversion, the chromosomes must form an inversion loop. The larger the inversion, the larger the loop and, thus, more crossing over is possible within it. Crossing over within the loop may lead to unbalanced complements giving rise to gametes with duplications and deficiencies. The pericentric inversion is

more commonly observed than the paracentric inversion. The general rule of interpretation of pericentric inversions is that the closer both breakpoints are to the telomeres, the greater the risk that an unbalanced offspring will survive to birth. When the breakpoints are close to the centromere on a large chromosome, there will usually be no risk of abnormal offspring. In the case of the inverted chromosome 4 (Fig.14), in this study the fetus and his father had the same chromosomal rearrangement. The breakpoints were p14 and q34.2. The anaphase chromosomes had a balanced complement of genes with phenotypically normal effects. Specific phenotypic abnormalities found in the offspring of patients with inversions depend on the chromosome segments involved in the duplication/deficiency. Very small deletions may be difficult to observe without high-resolution banding techniques. The number of bands involved has been used to estimated the risk to the unbalanced progeny. Therefore, there is a pressing need to discover greater detail in the karyotype.

CONCLUSION

The results of the study herein reported reveal that high resolution cytogenetic techniques are essential to the current practice of pediatric and obstetric clinical genetics. The finding of a chromosome abnormality does not always imply multiple defects in morphogenesis, growth disturbances and mental retardation in the patient. Some anomalies cause no harm whatsoever. Their effects on the phenotype obviously depend on both the quality and the quantity of genetic material involved. In general, If more deleted euchromatin is involved, the more serious the consequences may be. Some plan for investigating whether any particular chromosomal findings are relevant or not must be applied. Using high-resolution banding techniques, one can observe small deletions and, indeed, there must be many deletions below the limits of resolution of any present microscopic technique. Any missing genetic material (bands) should be recorded and evaluated in relation to the clinical findings since missing genes are generally more deleterious.

Apparently balanced rearrangements in patients with clinical findings that might suggest unbalanced chromosome anomalies are occasionally found. Some cases have very clearly significant clinical manifestations, but the

structurally rearranged chromosomes appear balanced. Alterations of structure may involve only a few base pairs. Many so-called single gene mutations have been proved with a more detailed molecular analysis to involve deletions of small segments of chromosomes containing more than one gene. However, these are still too small to cause visible chromosome changes. Even if a rearrangement appears balanced, an essential gene could be missing, mutated or damaged within the breakpoints when the chromosome structure was rearranged. A change could also occur at the molecular level. More detail will hopefully be confirmed in the future with molecular DNA investigations.

This study has demonstrated that minimal banding level karyotypes (400 bands) will easily show whole chromosome abnormalities. However, a higher chromosome band level > 650 will give more detail regarding which specific bands were missing since these finer bands can be made visible. If the deleted band is contained in a wide region, it does not matter whether short bands or high-resolution band levels are used; the deletion can be seen. A clinical service laboratory, however, should find greater detail in the karyotype in order to correlate with the clinical data.

High-resolution studies can become valuable tools in human cytogenetics. Selecting elongated chromosomes at the 700 - 750 band level should be required for the karyotype, especially in amniotic fluid cell analysis or peripheral blood analysis of the dysmorphic child. In amniocyte karyotypes, it is very important to discover greater detail to assist the doctor to make a prenatal diagnosis of a genetic disease. If a chromosome abnormality is confirmed, the parents may choose to terminate the pregnancy. The relatively high chance of finding a chromosomal anomaly in the dysmorphic child likewise makes high resolution analysis essential in that case.

This study also confirmed that although the high-resolution banding technique has been applied routinely in blood samples for precise chromosome analysis in every patient with a structural chromosomal aberration, we cannot always obtain elongated chromosome. This occurs since some specimens do not react well to the high-resolution technique. Most of the specimens in this study where the MTX technique was used revealed a better band level than the EB procedure. Some high-resolution studies, however, have a tendency to artifactually simulate homologue discordance in banding patterns. These situations must be distinguished from real variation, which is sometions very difficult.

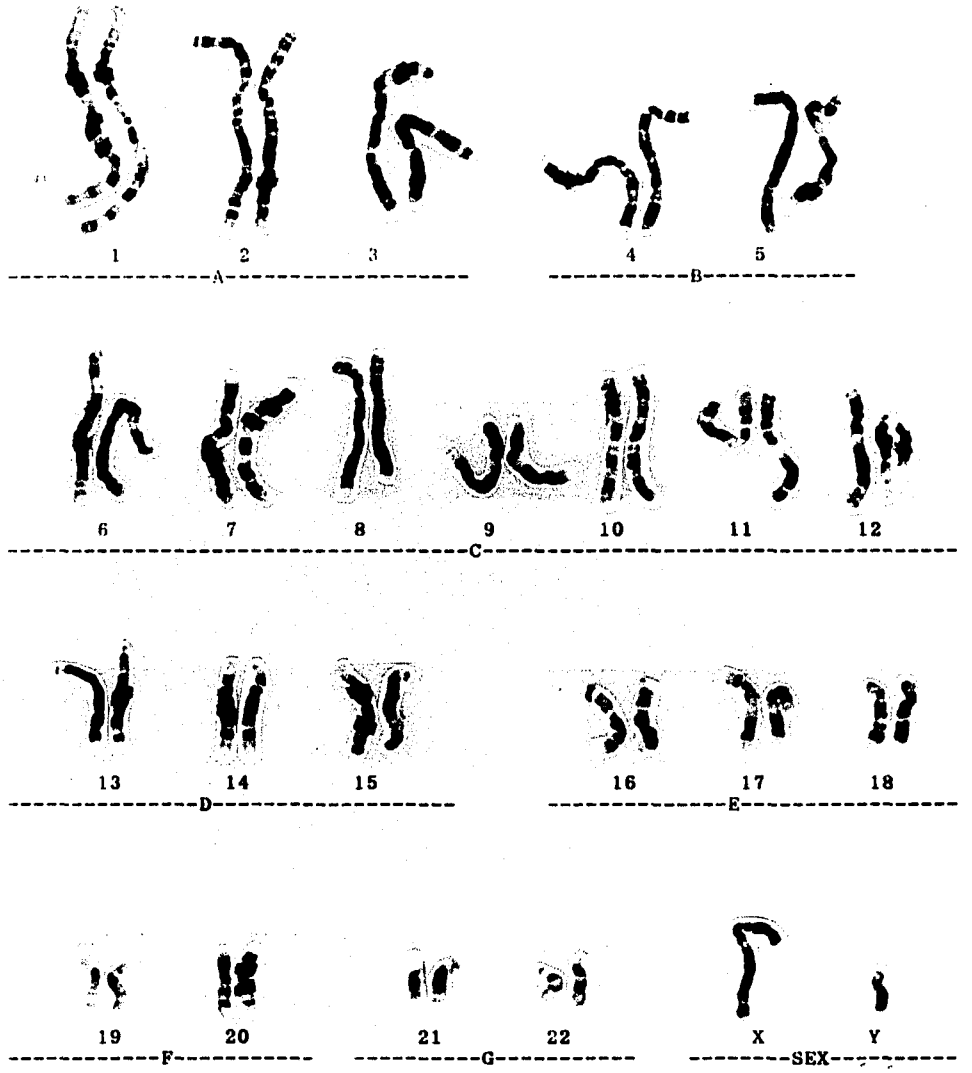

Fig.1 G-banded chromosome with 850 bands per haploid set from a single mitotic cell with 46 chromosomes.

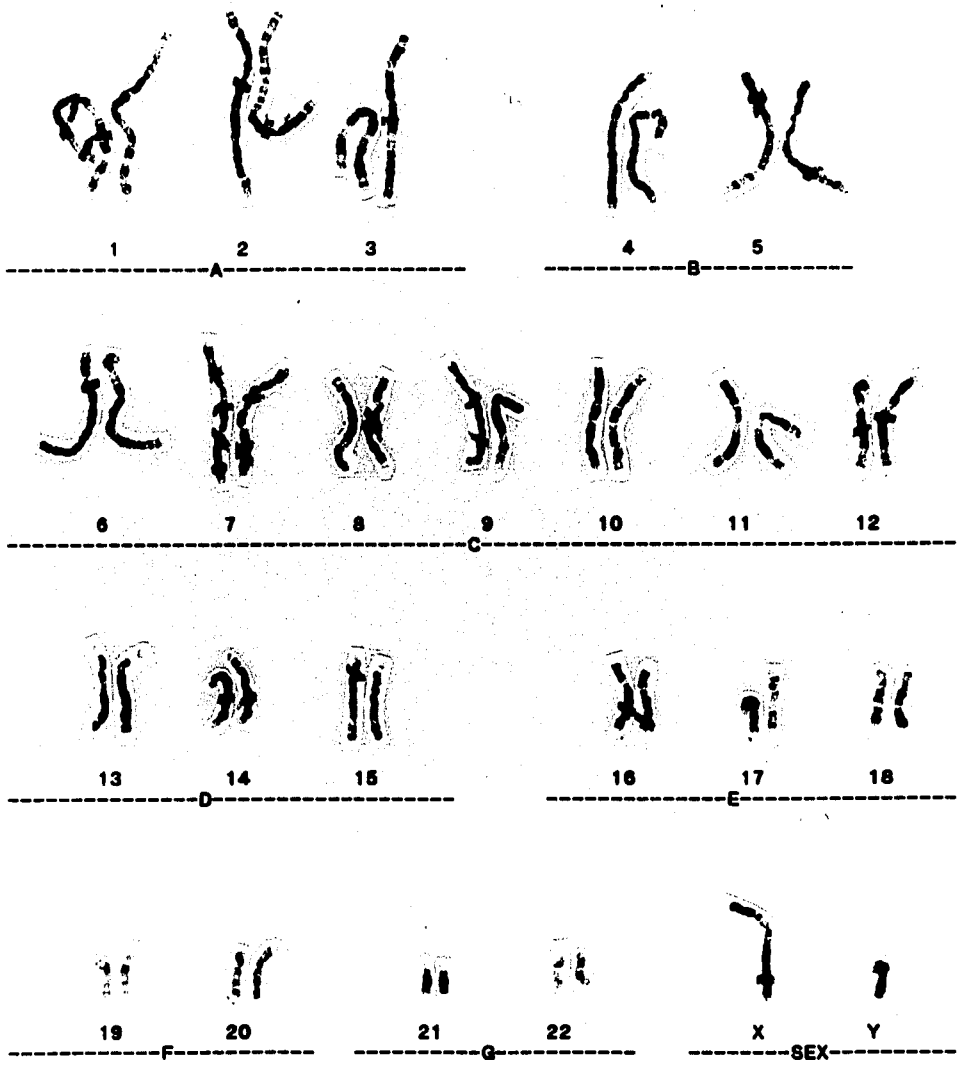

Fig.2 High-resolution G-banded human prometaphase chromosomes above 850-band level.

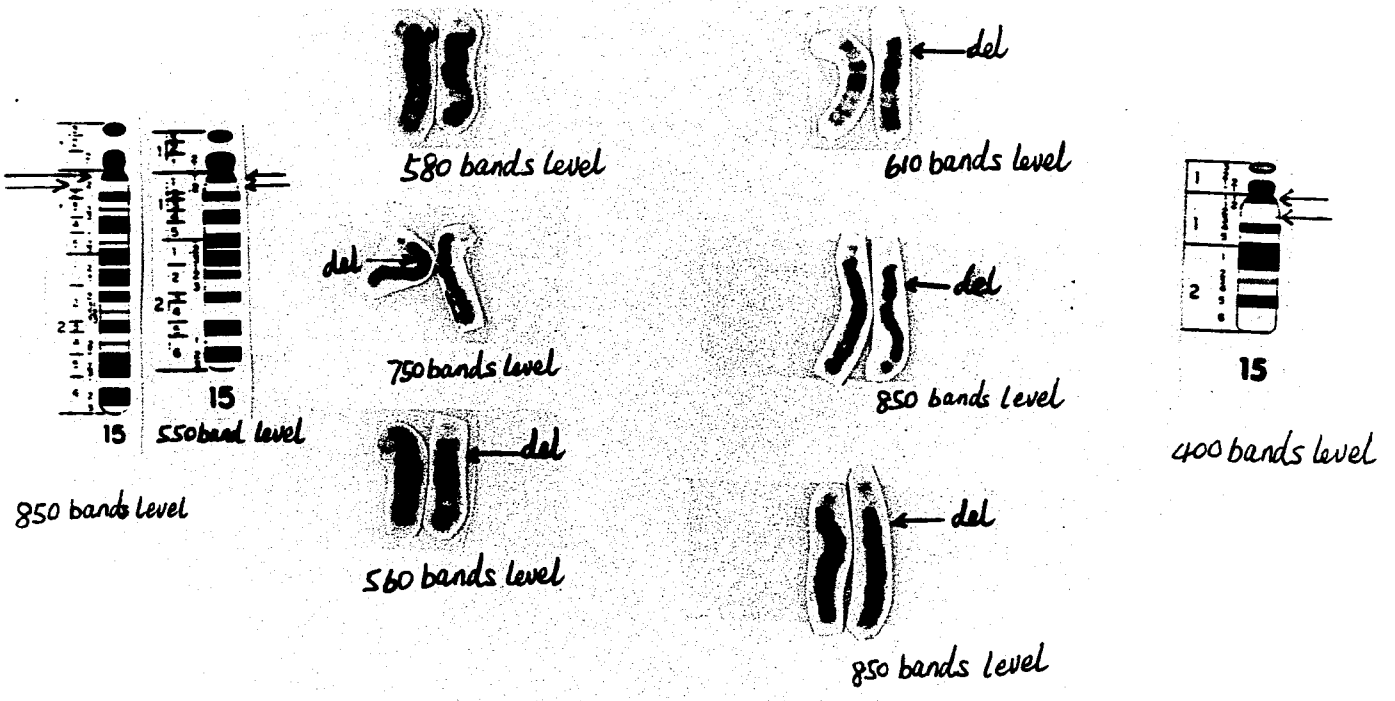

Fig.3 PWS associated with the chromosome 15q deletion(q11-q12). Arrows show the breakpoints and the deleted chromosomes.

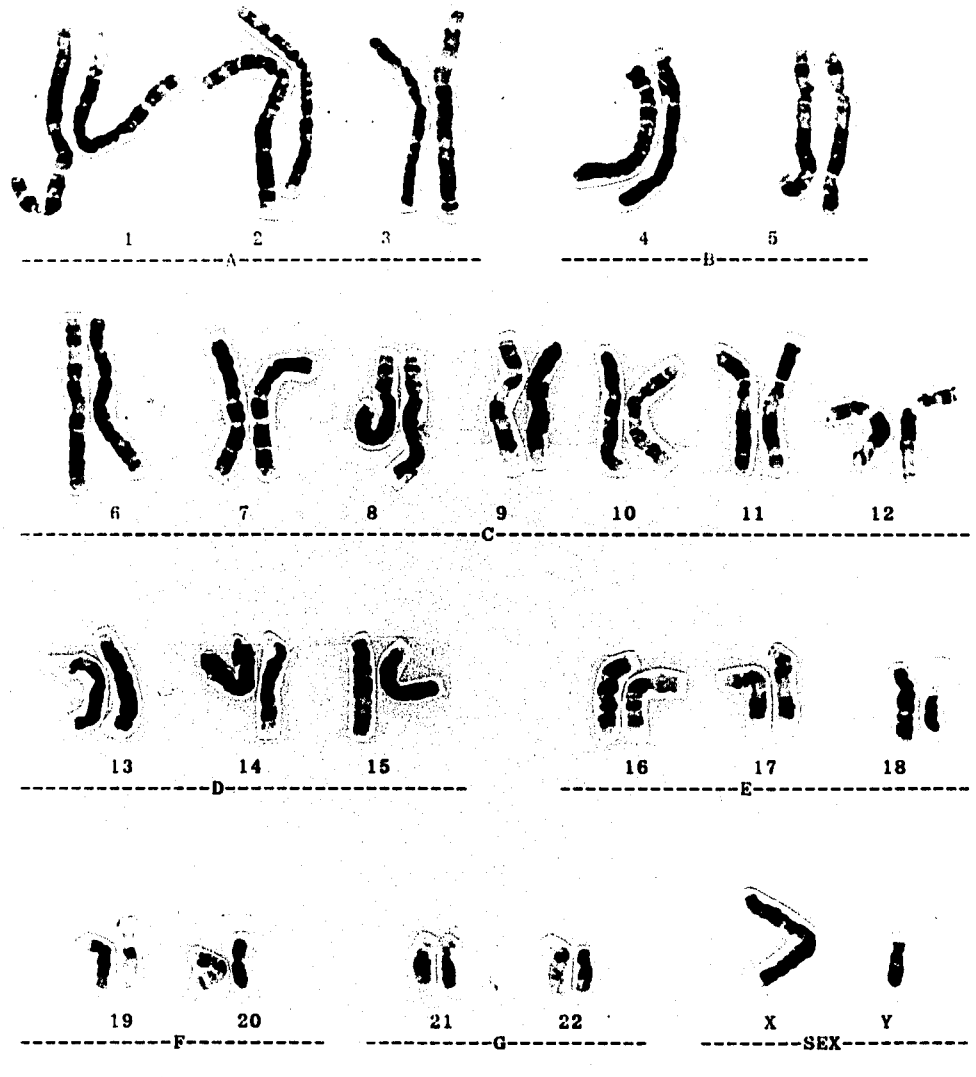

Fig.4 High resolution G-banded chromosome karyotype with a 46,XY,del(15)(q11) in PWS.

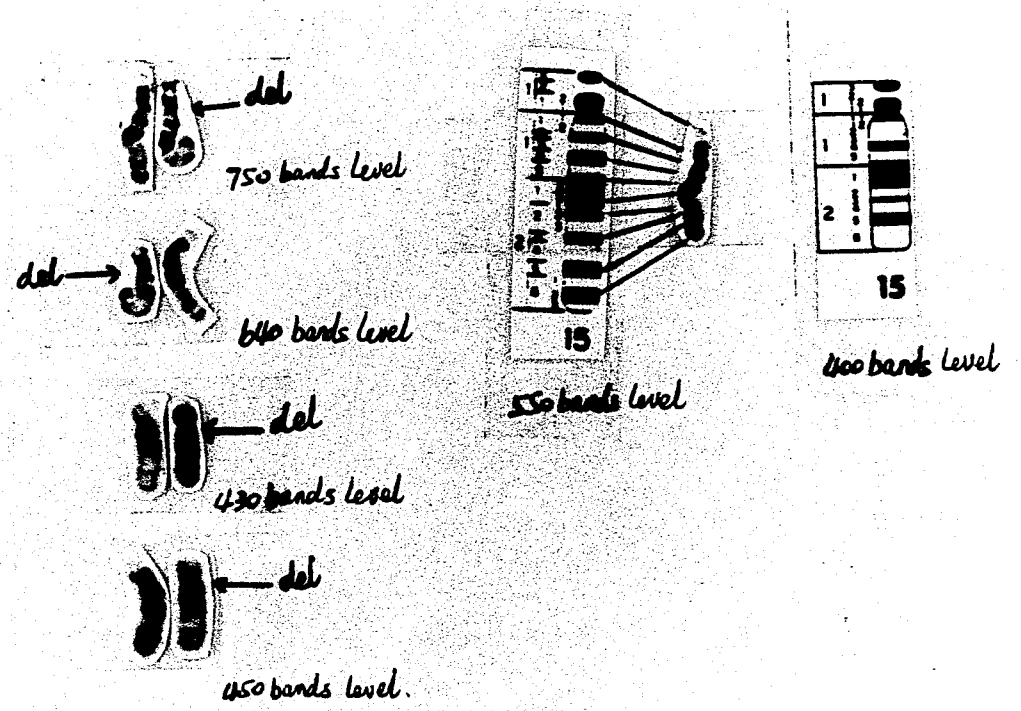

Fig.5 Photographs of G-banded chromosome 15 pair from a patient with AS at different band levels.

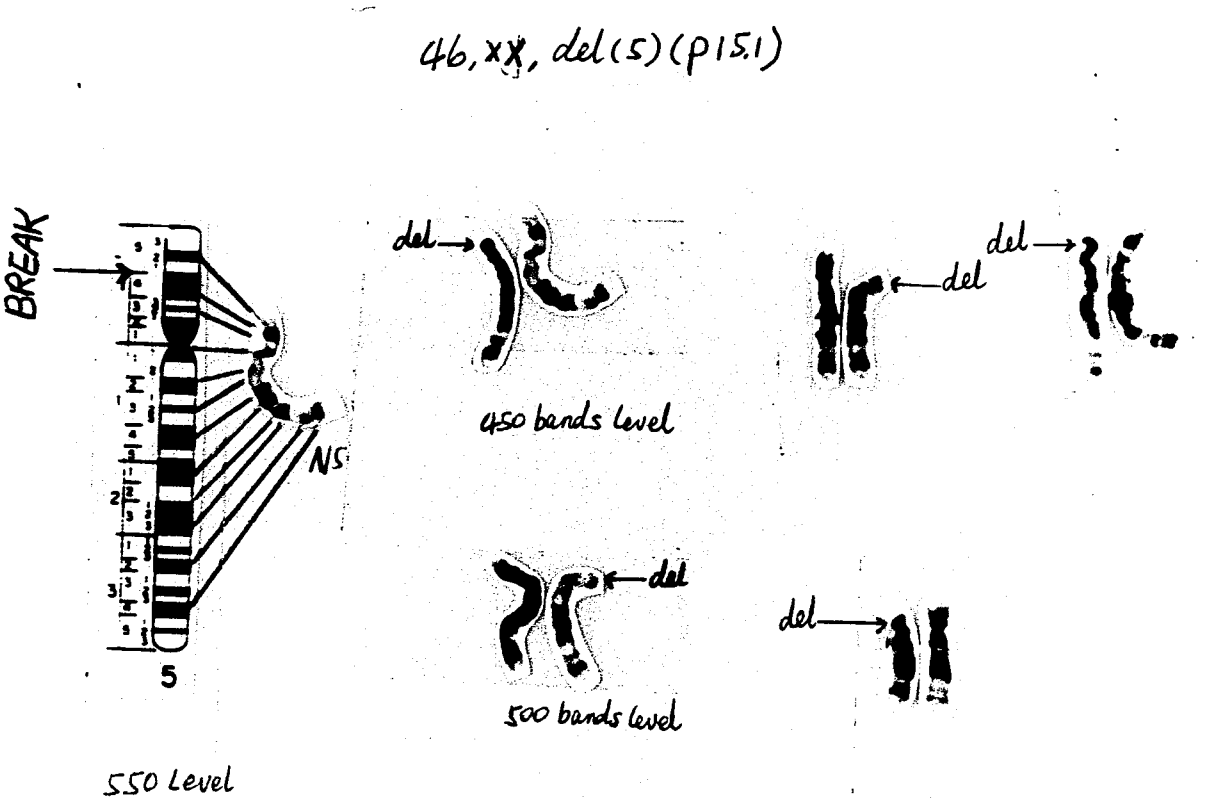

Fig.6 G-banded chromosomes with a 46,XX,del(5)(pter->p15.1).

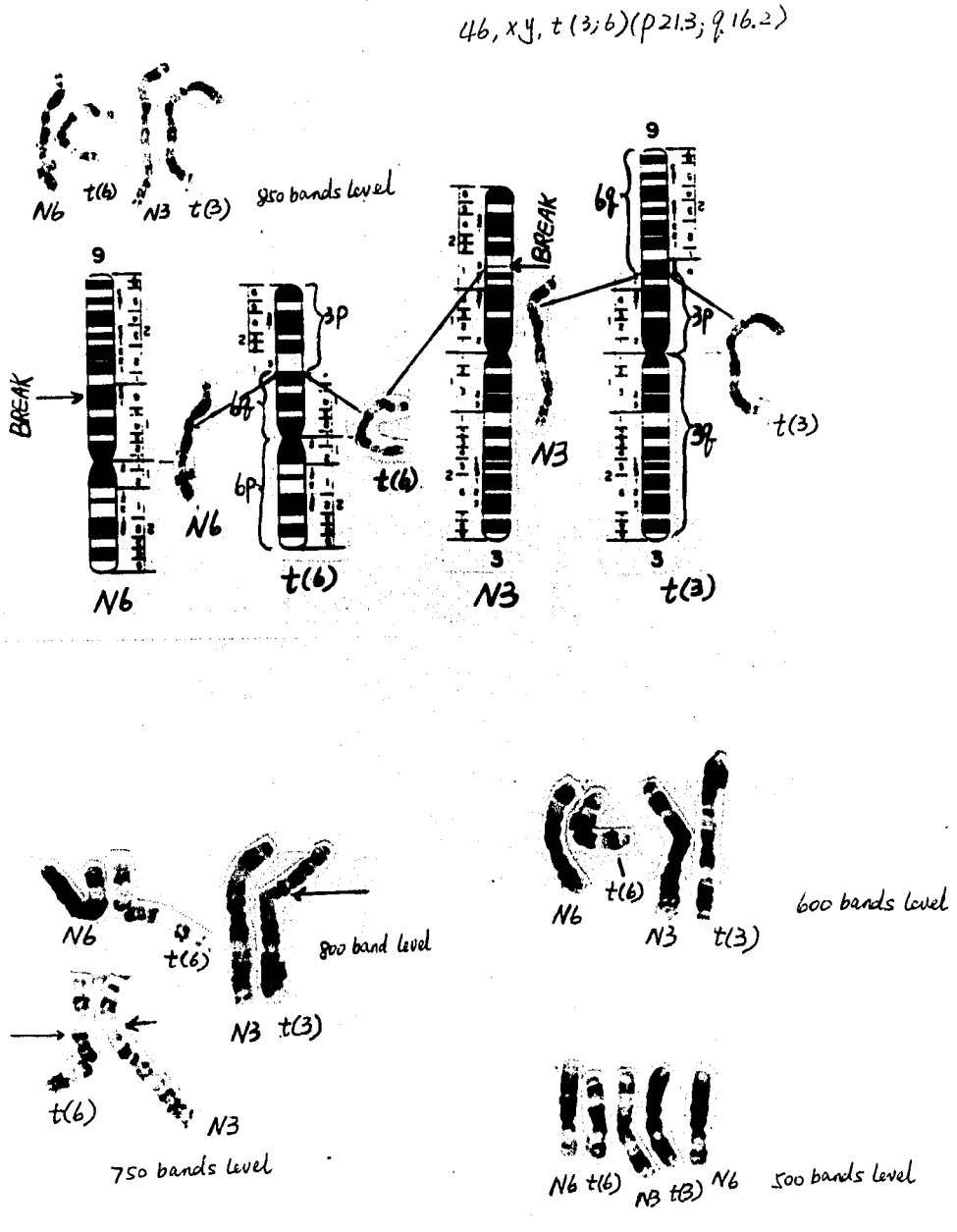

Fig.7 Selected chromosomes from a karyotype of a fetus with a 46,XY,t(3;6)(p21.3;q16.2).

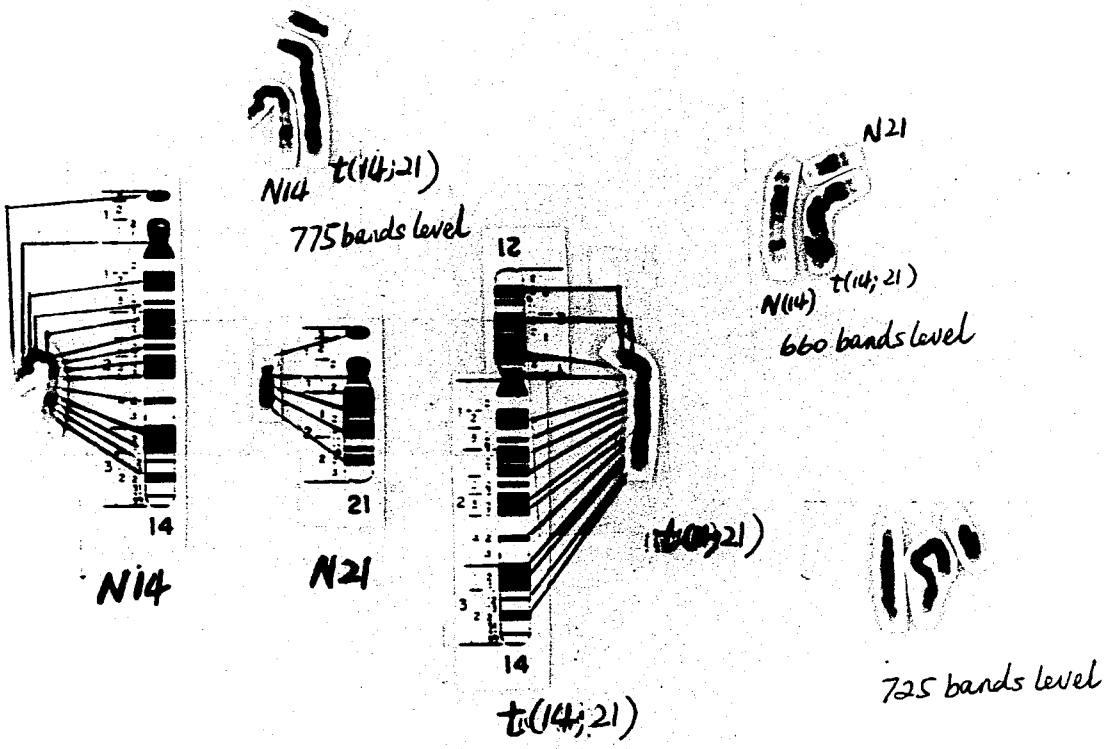

Fig.8a Partial karyotype prepared with a 45,XY,robertsonian translocation(14;21)(14qter->cen->21qter).

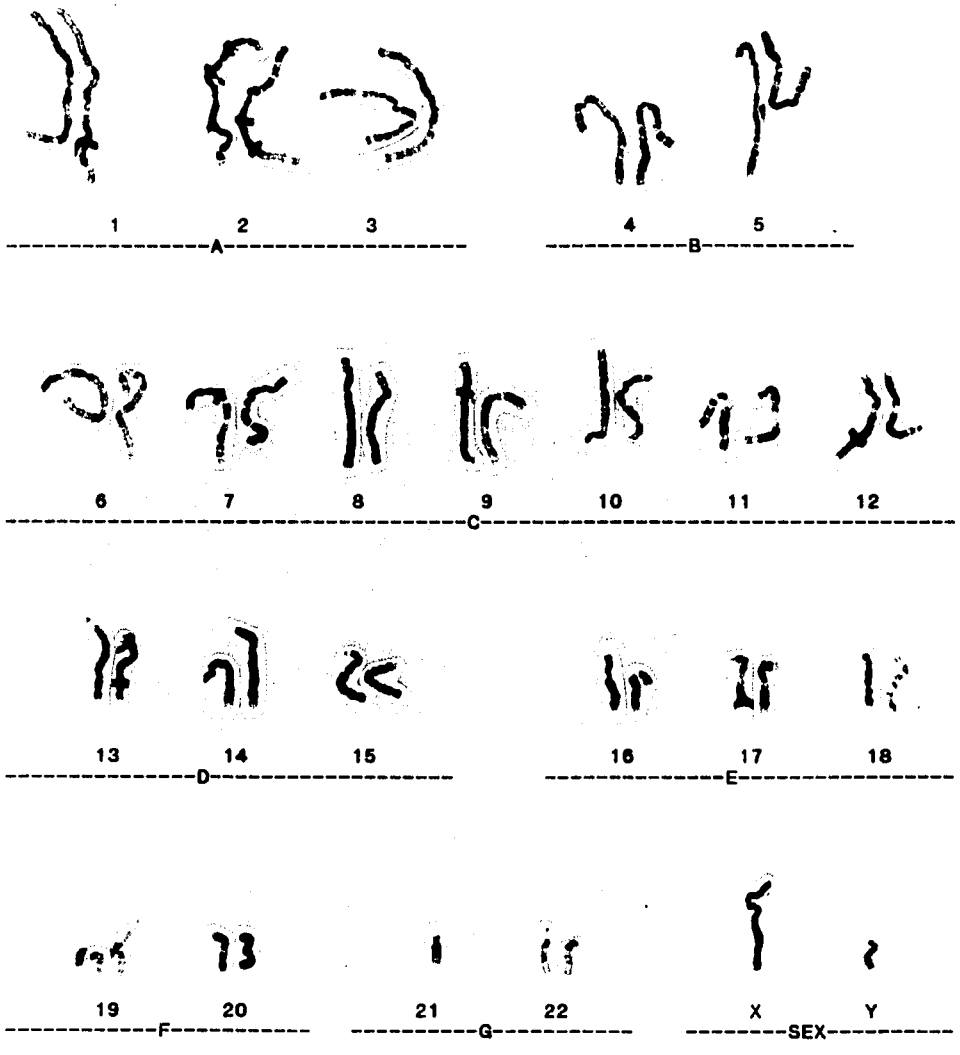

Fig.8b High resolution G-banded study with a 45,XY,robertsonian translocation(14;21)(14qter->cen->21qter) at 850-band level.

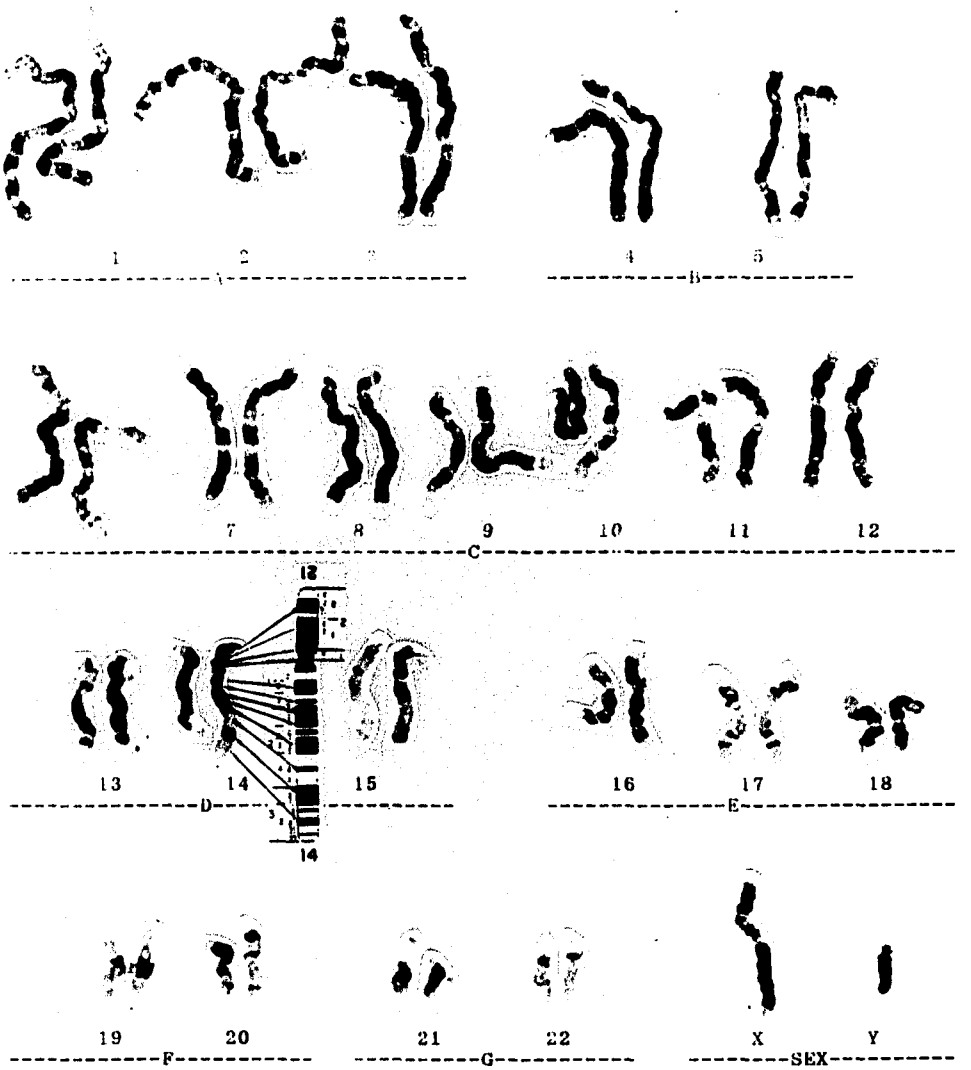

Fig.9 A cultured peripheral blood shows 46,XY,robertsonian translocation(14;21)(14qter->cen->21qter), a Down Syndrome karyotype.

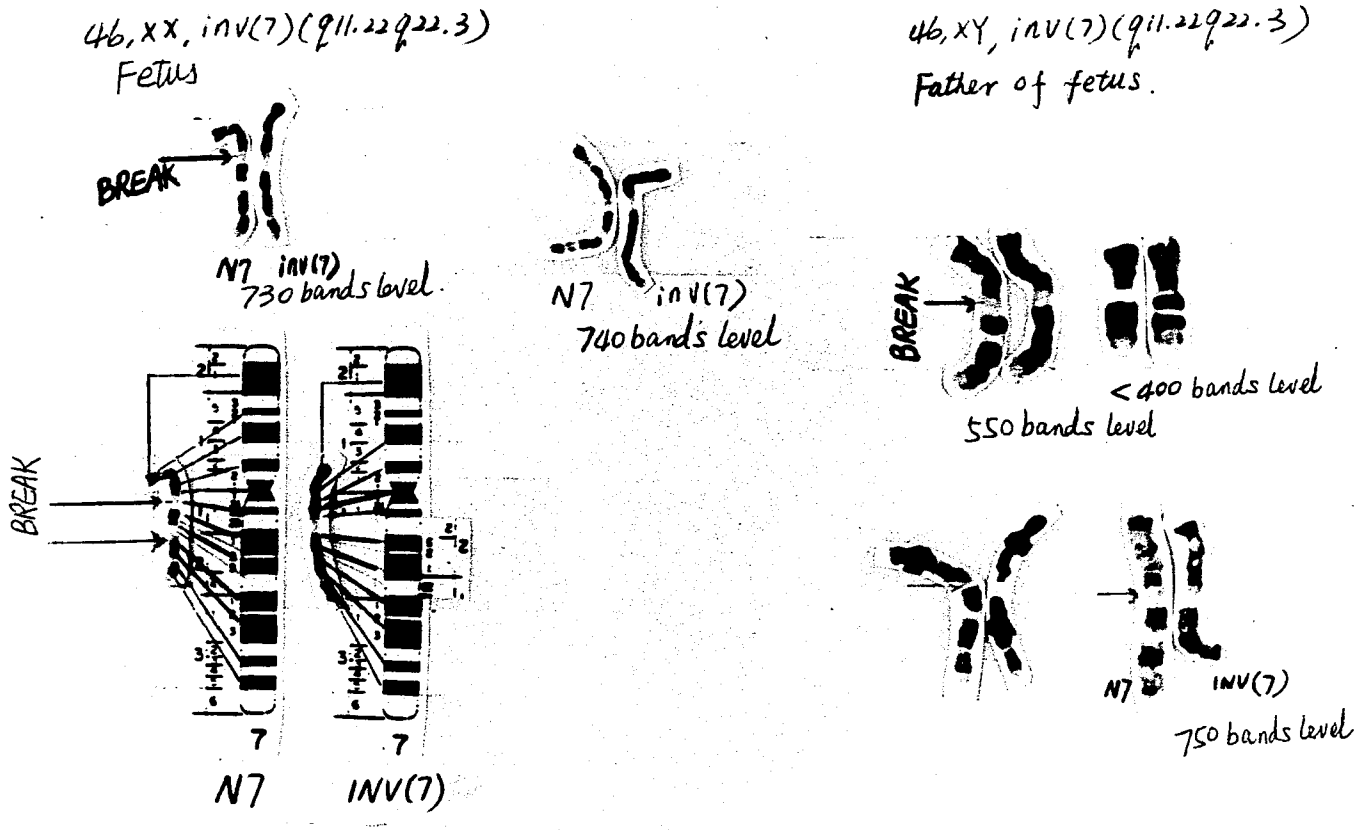

Fig.10 Chromosome analysis of amniocytes and father of fetus show same paracentric inversion(7)(q11.22q22.3).

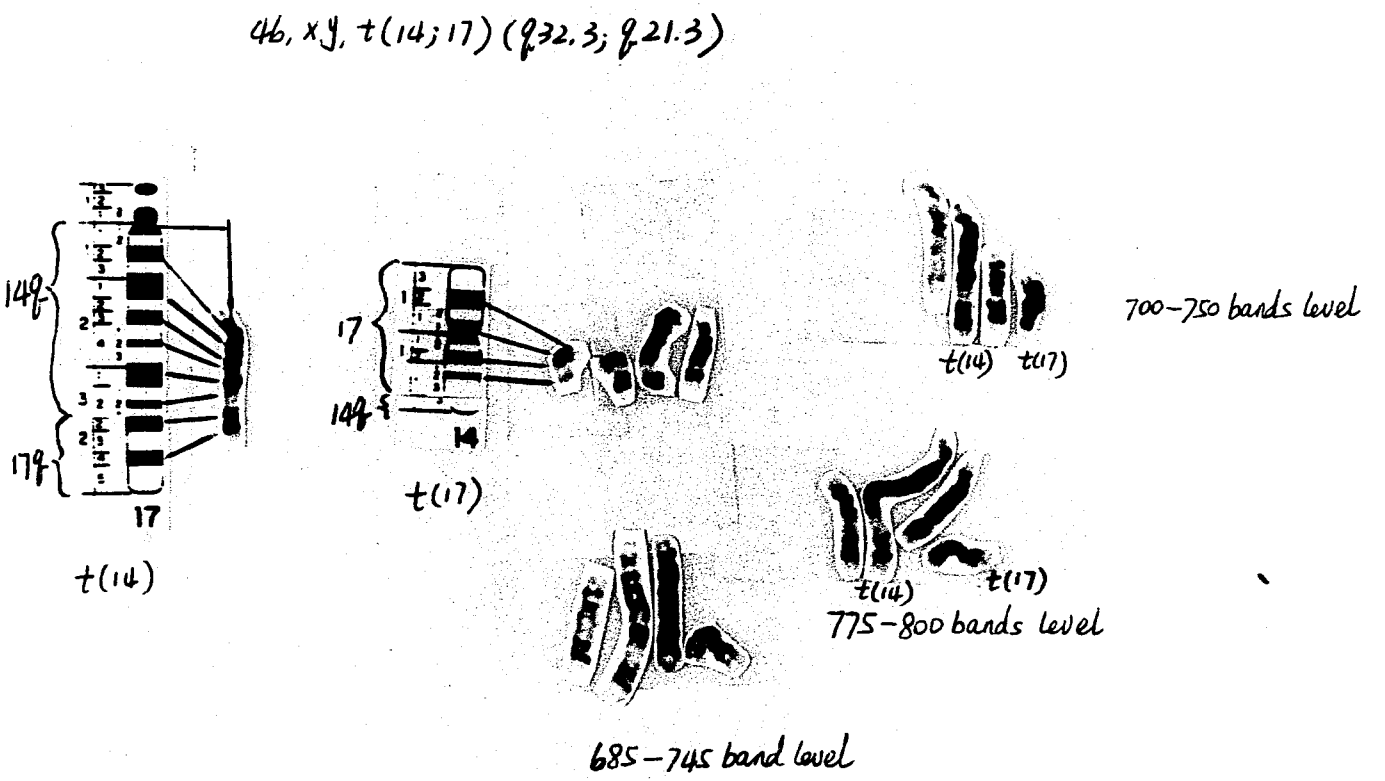

Fig.11a Partial G-banded karyotype prepared with a 46,XY,reciprocal translocation(14;17)(q32.3;q21.3).

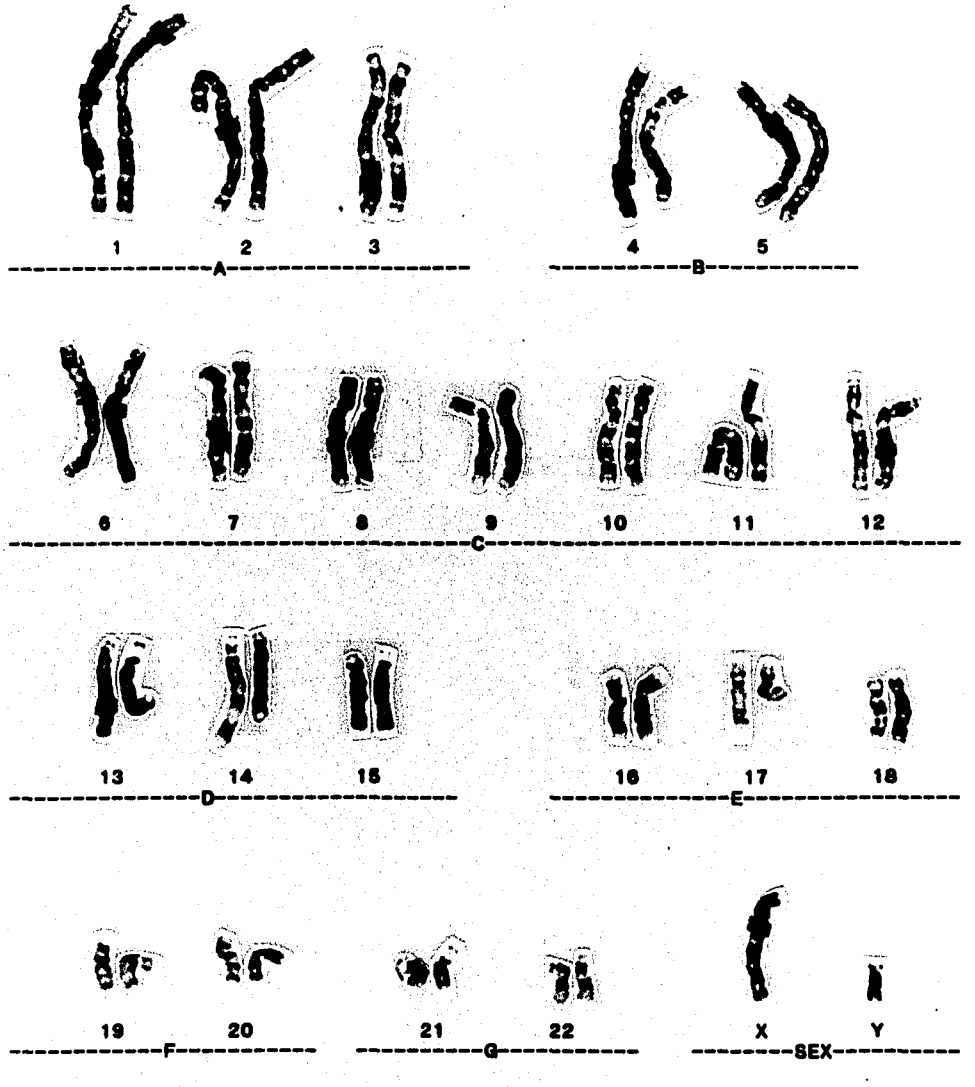

Fig.11b G-banded chromosomes from a 46,XY,reciprocal translocation(14;17)(q32.3;q21.3) karyotype at 715-band level.

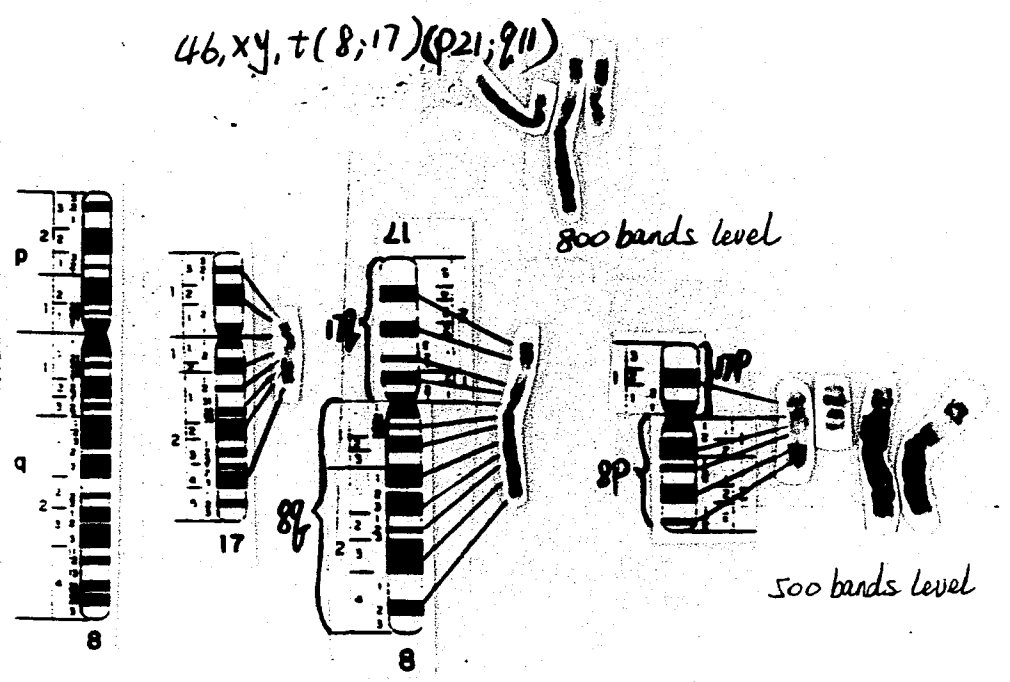

Fig.12 G-banded chromosome partial karyotype with a 46,XY,reciprocal translocation(8;17)(p21;q11).

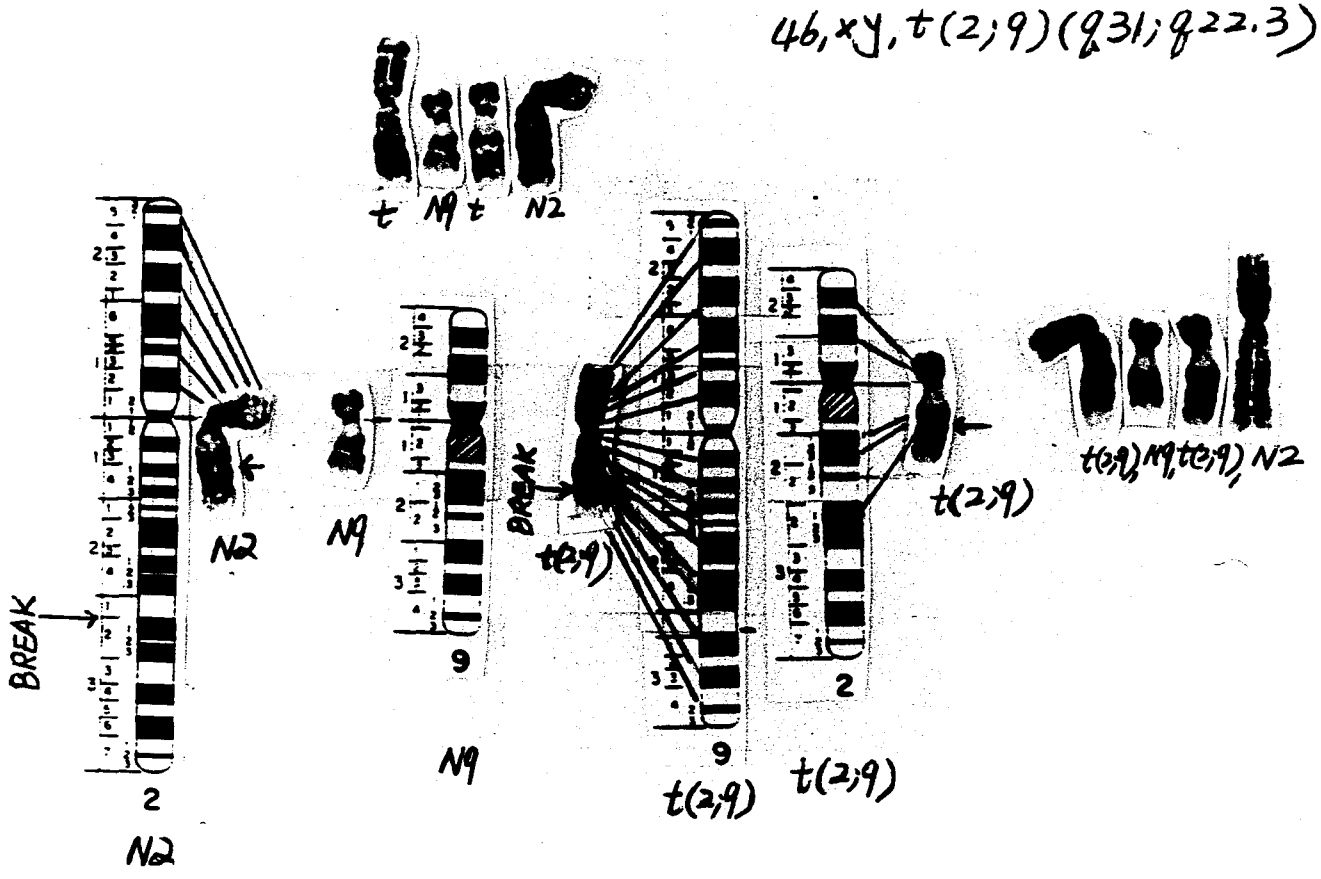

Fig.13 A 2 years old boy had developmental delay with a 46,XY,reciprocal translocation(2;9)(q31;q22.3).

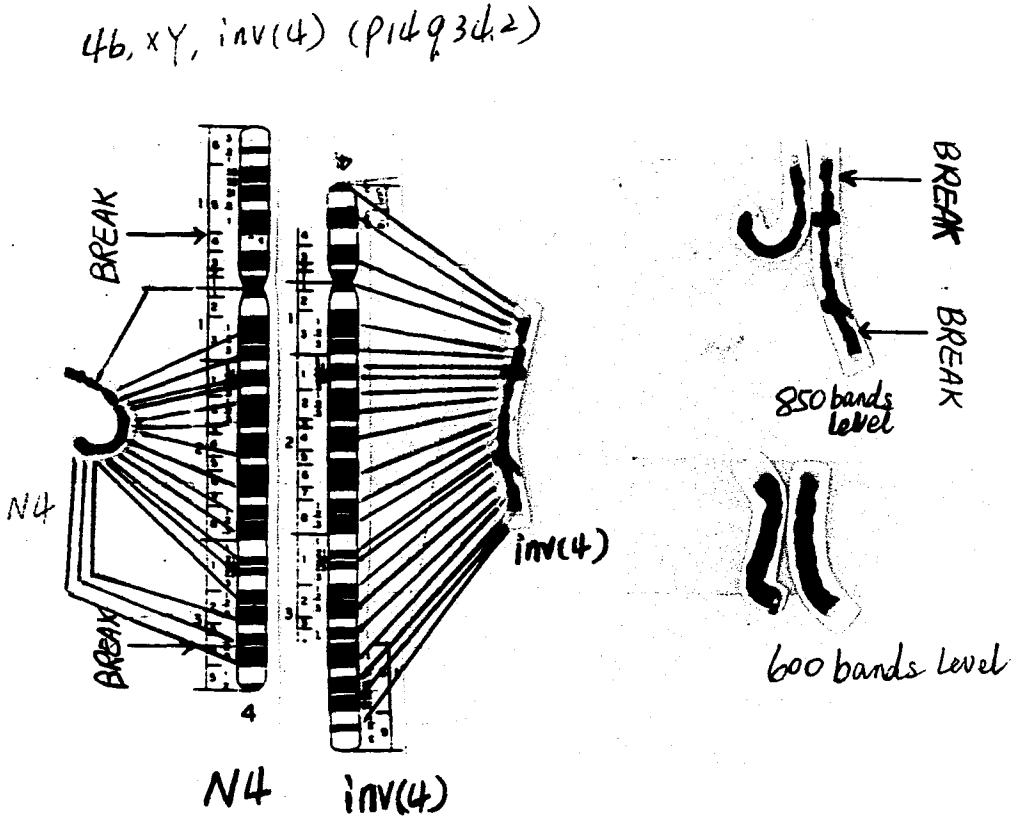

Fig.14 A pericentric inversion(4)(p14q34.2) in cultured amnio fluid at different band level.

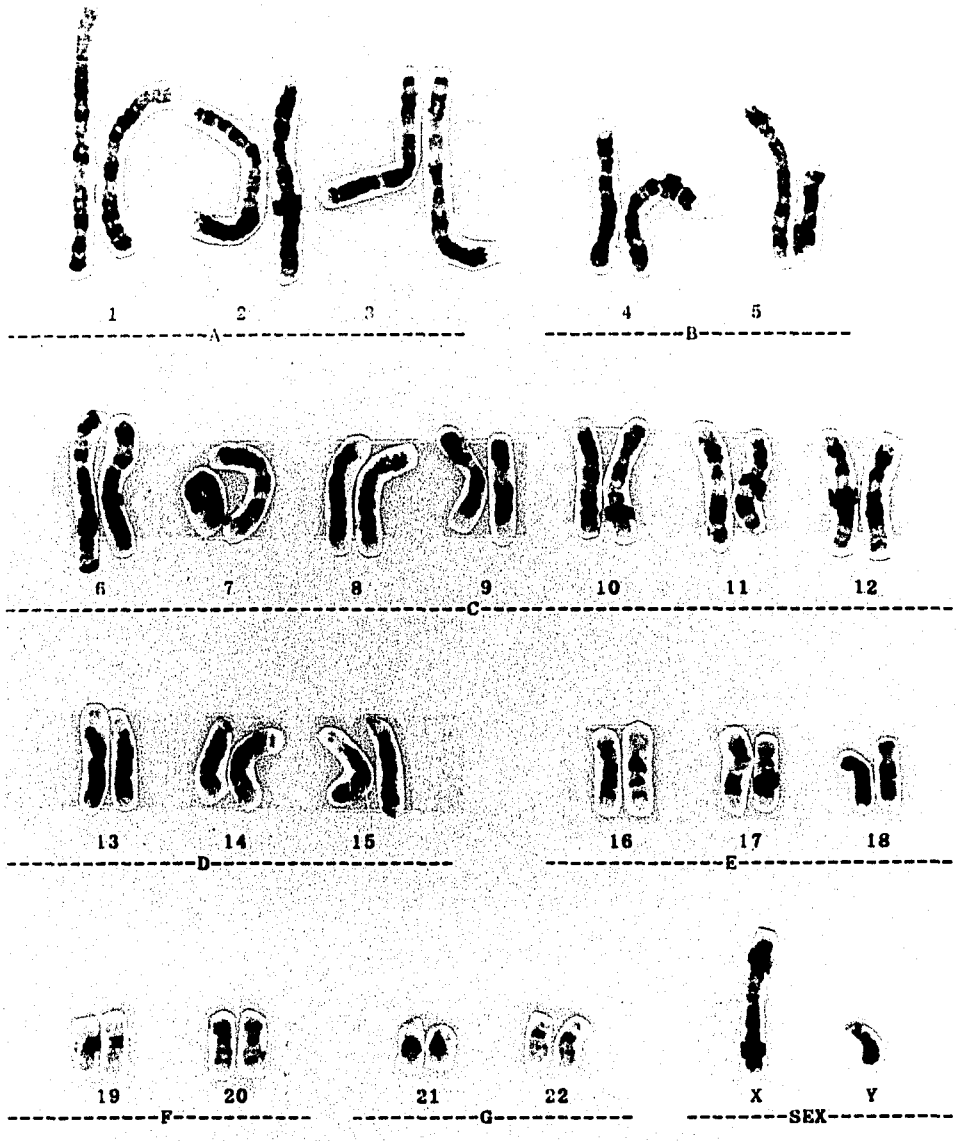

Fig.15 High resolution G-banded karyotype with a 46,XY,inv(9) at 850-band level.

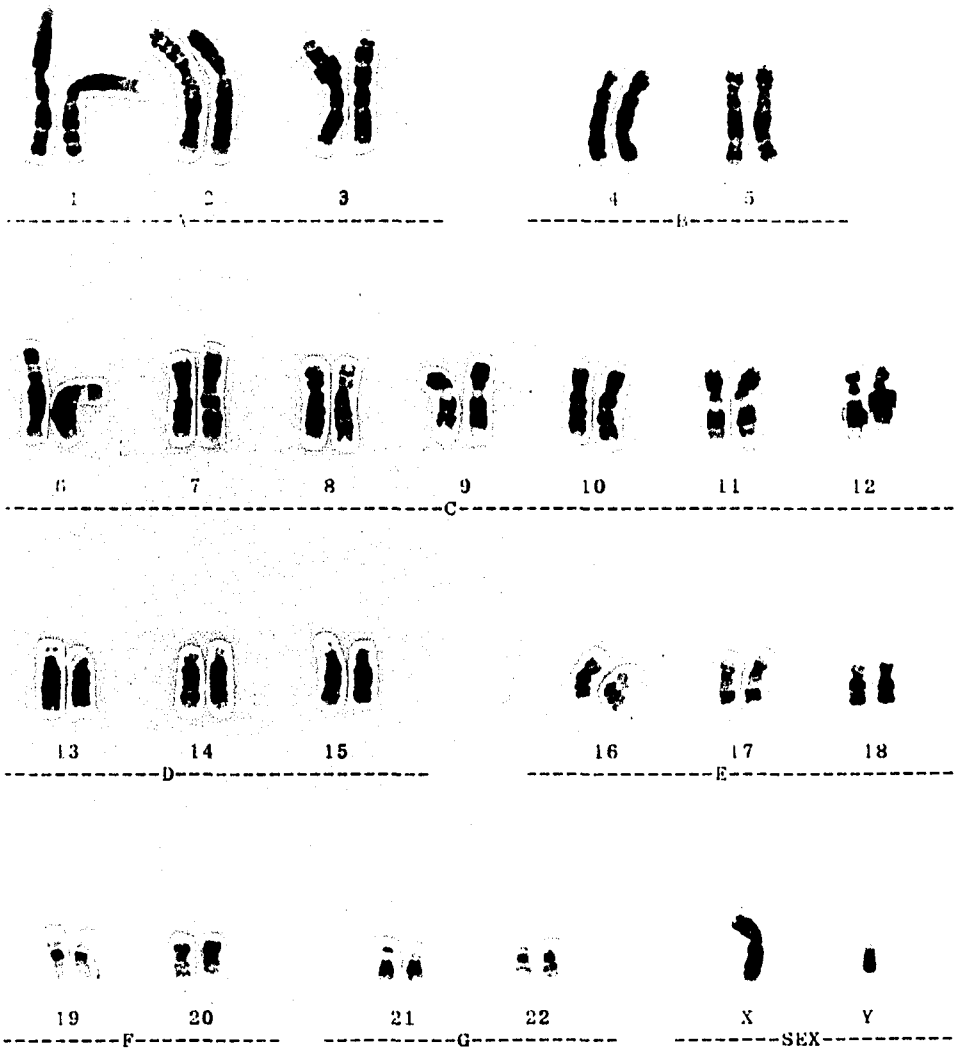

Fig.16 Chromosome analysis of amniocytes shows pericentric inversion(4)(p14q34.2).

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