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# Quinacrine Fluorescence and Giemsa Banding in Trisomy 22[1,2]

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**Summary.** Using quinacrine fluorescence and Giemsa banding techniques we have identified an extra chromosome 22 in three non-mongoloid children with similar phenotypes and 47 chromosomes. In one of the children, the long arm of the extra 22 was shorter than usual. This 22q−chromosome was observed in 4 normal family members with 46 chromosomes. In a fourth child, with similar physical findings, the extra G chromosome was shown to be neither a normal 21 nor 22. It must have arisen from a rearrangement in a parental gamete since it was not present in either parent's karyotype.

No constellation of clinical findings, in association with an extra G chromosome, is sufficient evidence for the diagnosis of trisomy 22. The positive identification of the extra chromosome must be made using fluorescence and banding.

## Introduction

Lejeune's (1959) initial finding of 47 chromosomes (trisomy 21) in children with Down's syndrome was followed by sporadic reports of children with non-mongoloid features and an extra G group chromosome. The phenotype of these children varied widely (see Nielsen et al. 1969 and Hsu et al. 1971 for partial reviews) but for each the possibility was raised that he represented trisomy 22, a potential clinical entity.

Until recently, chromosomes 21 and 22 could not be distinguished from each other on the usual morphologic grounds. Autoradiographic studies of DNA replication failed to distinguish between the two pairs. Caspersson and his colleagues (1970a) have shown however, that each chromosome in the human complement has a unique pattern of fluorescence when stained with DNA binding fluorochrome, quinacrine mustard hydrochloride. These banding patterns are sufficiently distinctive to permit visual identification of each chromosome with fluorescent micrography. Quinacrine dihydrochloride is equally effective for the demonstration of fluorescent banding.

A second technique for differentiating human chromosomes is based on the observation by Pardue and Gall (1970) that the centromeric heterochromatin on NaOH treated chromosomes stains more densely than the rest of the chromosome. Modifications of this technique have been used to produce banding patterns which also allow for identification of each chromosome, and provide a complementary method to that of fluorescence.

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## Family and Case Histories

*Case 1.* S.C. (SCHC 65-00-720) was the male product of a gestation complicated by bleeding during the first trimester. He was born at full term by breech delivery, weighing 3312 g. His 31 year old mother had 2 older living children and had had a miscarriage. His father was 38 years old. The patient had aspiration pneumonia as a newborn and at 3 months of age was referred to St. Christopher's Hospital for Children because of severe respiratory distress. At this time, micrognathia, high arched palate, and very small uvula were noted. The tongue retracted into the pharynx. Laryngoscopic examination revealed an infantile type epiglottis and flabby, elongate arytenoids. Ears were low set, with bilateral preauricular pits (Fig. 1a, b). Thumbs were hypermobile and extensible. The phallus was embedded within a hypoplastic scrotal sac but was of near normal length. Testes were undescended. A grade II/VI short systolic murmur was heard. Electrocardiogram was normal. Radiographic examination revealed an abnormal cardiac shape, suggesting absence of the pericardium. Upon exploratory thoracotomy, only a small portion of pericardium was found at the inferior vena cava. There were also an atrial septal defect and an aberrant right subclavian artery. No renal abnormality was seen on intravenous pyelogram. No unusual appendages of granulocytes were observed. He was reported as a possible case of trisomy 22 (Punnett and Vaughan 1966).

The patient's subsequent course has been marked by a slow mental and physical development and frequent episodes of serous otitis media. At 5 years, his weight was 13.4 kg., height was 103.4 cm. At 7 years of age, he was severely retarded. He could not sit or walk without support; he had no speech or purposeful activity.

*Case 2.* M.R. (SCHC 72-00-050) was a female infant born at full term by elective caesarean section to a 32 year old mother and 35 year old father. Pregnancy was uncomplicated. There had been one previous pregnancy. Birth weight was 2340 g., head circumference 32 cm, and length 40.6 cm. The patient was a small-for-date infant with wrinkled dry skin, and rapid respirations. A preauricular skin tab was present on the right, an ear pit on left, and redundant skin was noted at the back of the neck. At one week of age, she was transferred to St. Christopher's Hospital for Children for evaluation of respiratory distress and cardiac failure (Fig. 1c).

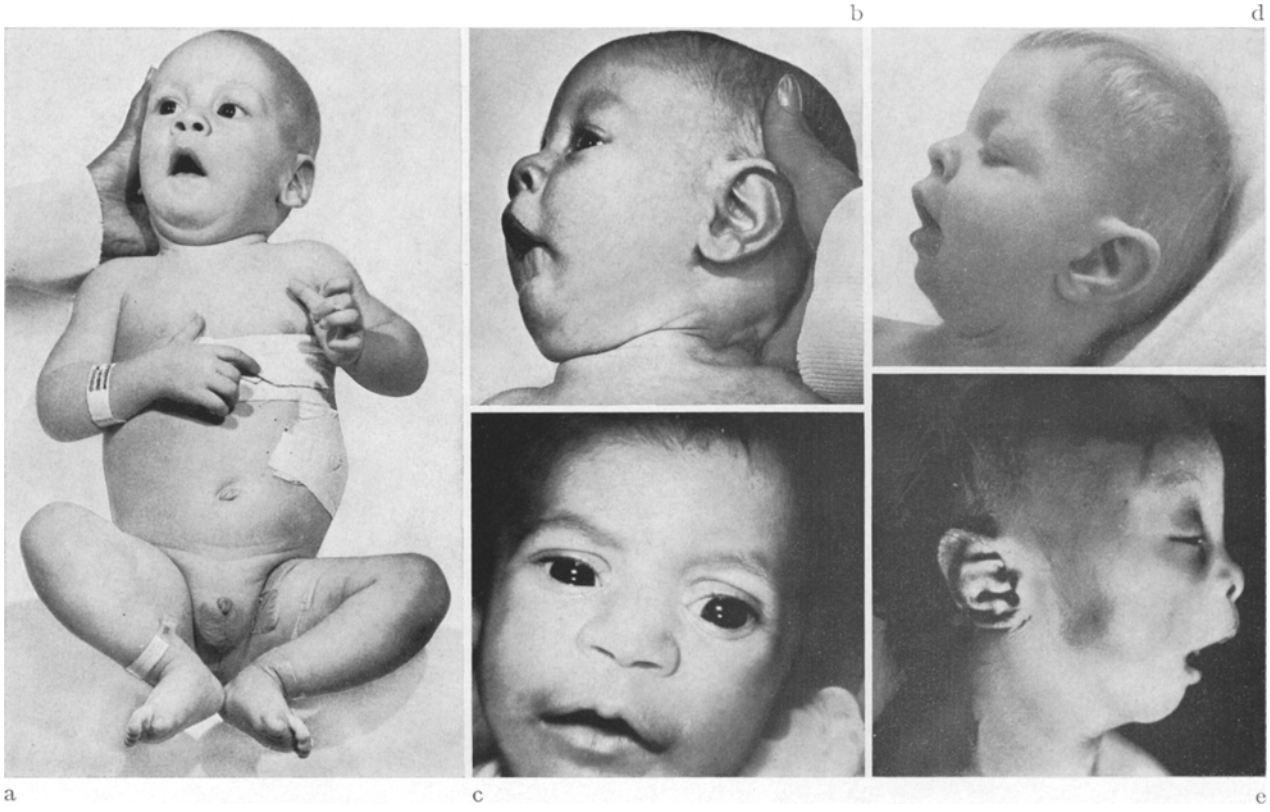


Fig. 1. a, b: Case 1 at 3 months of age; note micrognathia, hyperextensible thumbs, small phallus and ear pit, c: Case 2 at 3 months of age, d: Case 3 at 3 months of age showing ear pit and micrognathia, e: Case 4 at 6 weeks of age, post-mortem photograph

Radiographic examination suggested the presence of a fused crossed ectopic kidney. Granulocytes on peripheral bloodsmear resembled those seen in trisomy 13. There were many drumstick and sessile appendages on the nuclei which had a coarse, rope-like appearance.

Cardiac catherization demonstrated a patent ductus arteriosus, atrial septal defect and persistant left superior vena cava. At two weeks of age, the patent ductus was ligated.

At 6 months of age, the patient weighed 3676 g, was 60 cm long and had a head circumference of 36 cm, all measurements below the third percentile for age. Development was extremely retarded; she did not smile or have good head control.

*Case 3.* T.O. (CHOP 70 7335) was a male infant born after 44 weeks gestation to a 29 year old mother and a 28 year old father. The mother had taken Librium and Bonadoxin during the otherwise uneventful pregnancy. There were two older children. Birth weight was 3569 g, length 52 cm, and head circumference 35 cms. The patient was transferred to Children's Hospital of Philadelphia at one day of age for repair of imperforate anus with fistula.

Physical examination revealed multiple congenital anomalies: anti-mongoloid slant of his eyes, large ears with bilateral preauricular pits, cleft palate, micrognathia, redundant skin at back of neck, low set nipples, underdeveloped scrotum, undescended testes, a very small phallus with mild hypospadias, imperforate anus with fistula, bilateral dislocated hips and marked hypotonia (Fig. 1d). The right fronto-parietal area transilluminated excessively. Additional anomalies noted were a long preductal coarctation of the aorta, an extra rib bilaterally, and an absent left kidney. At 5 days of age, the patient had a generalized seizure in association with a low calcium level in blood. Appropriate treatment was given. Subsequent calcium levels were normal.

The patient has had many hospital admissions for recurrent infections. Following a left focal seizure and respiratory arrest at 15 months of age, he developed a permanent left hemiparesis.

At 17 months of age, his weight was 7,820 g, height 76 cm and his head circumference 42 cm (all below the third percentile). He had a residual Moro response and tonic neck reflex. He had poor head control, did not hold his bottle or sit, but he could roll over, follow moving objects with his gaze, smile, and say "mama" and "dada".

*Case 4.* L.M. (HMCP 55991) a male infant, was born at 41 weeks of gestation to a 25 year old, unwed mother. The father's age was unknown. There was an older sibling. The patient was delivered by caesarean section because fetal distress and bradycardia had occurred 10 minutes prior to the section. Birth weight was 2,750 g, length 45.7 cm and head circumference 35.4 cm. The patient was lethargic and flaccid at birth. There were a brachycephalic head, prominent occiput, low set square ears, and a pit anterior to the right ear. The eyes had a slightly mongoloid slant and the lips were puffy. There were micrognathia, a high arched palate, and thickened alveolar ridges. Loose, redundant skin was present on both sides of the neck (Fig. 1e). The chest was narrow and triangular, with flaring of the lower ribs. No heart murmur was heard. The testes were undescended. The fingers were long; the thumbs low set. There was a deep crease between the first and second toes bilaterally, and hammer toe of the fourth toe.

The infant failed to gain weight and at four weeks of age was hospitalized with diarrhea and vomiting. At this time, opacities were noted in the cornea of the left eye.

Despite vigorous treatment, the patient died at six weeks of age with an overwhelming *Pseudomonas* infection. At autopsy, in addition to the previously mentioned anomalies, enteritis and incomplete rotation of the cecum were noted. No gonadal tissue could be found.

## Methods

Peripheral lymphocytes from all four patients and their parents and a skin biopsy from case 1 were cultured by standard methods. For fluorescent examination, slides were washed in McIlvaine's buffer at pH 4.4 for 3 minutes, stained for 20 minutes in quinacrine mustard (50 ug/ml), washed, and mounted in buffer. Slides were examined with Zeiss photoscope, exciting filter BG 3, barrier filters 44 and 53, and were photographed with a vertically mounted camera using either High Speed Ektachrome or Tri X. Differential staining was carried out using a modification of Seabright's method (1971).

## Genetic Studies

The karyotypes of the peripheral lymphocyte cultures of the four patients and the fibroblast culture from case 1 all indicated 47 chromosomes, with an extra member of the G group. By fluorescent examination and differential staining, cases 1 and 2 were each demonstrated to have an extra chromosome 22 (Fig. 2). Their parents had normal karyotypes. By fluorescence and differential staining Case 3 had two chromosomes 21, two 22, and an unusual short G group chromosome (Gq-) (Fig. 3). The patient's mother, maternal grandmother, aunt, and sister all had 46 chromosomes including a normal 22 and the short 22 (Fig. 4).

*Case 4* was found to have two chromosomes 21, two 22 and a fifth acrocentric which by fluorescence was neither 21 nor 22, having a very short bright band close to the centromere and fluorescences of intensity between 21 and 22 for the rest of the long arm (Fig. 2). It was possible to determine the parental origin of each 21 and 22 in this case (Fig. 5) taking advantage of satellite size and fluorescence, and an unusual band in a paternal 21. The extra chromosome was present in neither parent, each of whom had two normal 21, two normal 22 and no suggestion of a translocation involving one of the G group.

Dermatoglyphic studies were unremarkable and blood group analyses of case 1, his siblings, and parents, were unrevealing.

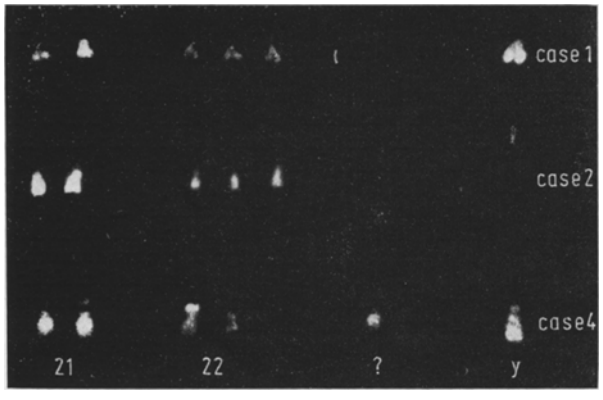


Fig. 2. Quinacrine fluorescence pattern of G group and Y chromosomes in Cases 1, 2, 4. Note the atypical chromosome, indicated by ?

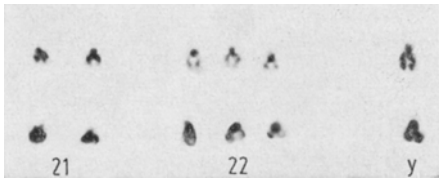


Fig. 3. Differential banding of chromosomes 21 and 22 from 2 cells of Case 3, demonstrating the short 22

## Discussion

This study of the extra G group chromosome in phenotypically similar, non-mongoloid children with 47 chromosomes demonstrates the necessity for precise identification of the extra chromosome with fluorescence and differential staining. An extra G chromosome and the physical findings listed in Table 1 are not sufficient to establish the diagnosis of trisomy 22. Case 4 was similar physically to the other 3 cases herein presented, yet the fluorescence pattern of the extra G was clearly different from that of 21 and 22.

Moreover, it was possible to demonstrate that neither parent carried this chromosome so that case 4 did not receive an aneuploid product of segregation from a carrier. Each of the parents' small acrocentric chromosomes was identifiable by the relative size and fluorescence of satellites and length of the satellite

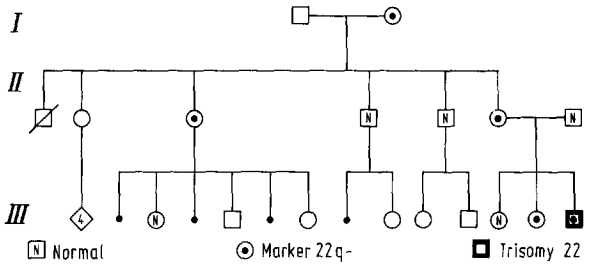


Fig. 4. Pedigree of Case 3

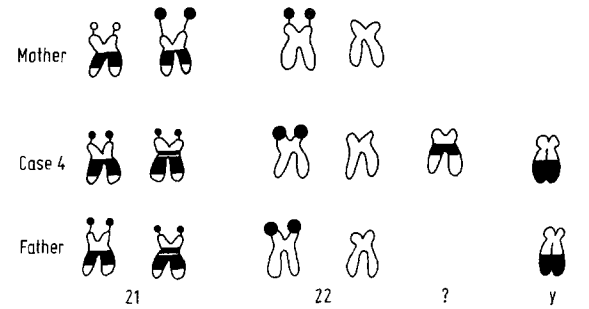


Fig. 5. Diagrammatic representation of fluorescence patterns (indicated by dark bands) of G group and Y chromosomes of case 4 and his parents. Note that the origin of the expectional G, indicated by ?, cannot be determined. There was no detectable difference in the fluorescence of the satellites of the left most 21 in mother, father and child

stalk; this made it possible to determine from which parent each 21 and 22 in the child had come. We conclude that the patient with presumed trisomy 22 and his parents must be studied with fluorescence and banding to be certain that the extra chromosome is a normal member of the complement of one of the parents. Had his parents not been studied, the atypical G of case 4 might have been accepted as a 22 variant. Its origin is not known. It cannot be a deleted D, owing to its fluorescent pattern. Its short arm is seen in satellite association frequently, which suggests its derivation, in part, from a G or D chromosome.

Hsu et al. (1971), in their discussion of trisomy 22, accept equal size as evidence for the fact that the extra chromosome is a G and genetically the same in all of their cases. In view of the fact that the extra G in our case 4 was morphologically indistinguishable by the usual examination, it is evident that identical size is not enough. Chromosomes of apparently identical size may arise from a variety of translocations and deletions, and morphologic similarity with conventional staining is insufficient to establish a diagnosis of trisomy 22 in a non-mongoloid child with an extra acrocentric chromosome. Fluorescence and differential staining are required to make the diagnosis. All cases reported as trisomy 22 merit reinvestigation using these new techniques.

Two previous instances reported as G trisomy have been shown to have neither an extra 21 nor 22 by fluorescence. Gustavson, Hitrec, and Santesson (1972) restudied a previously reported child whom Hsu et al. (1971) included in their series as a case of presumed trisomy 22. The fluorescence pattern of the extra G differed from both 21 and 22, with fluorescence intermediate between the two. Unfortunately, the parents were not studied so the possibility of aneuploidy resulting from unbalanced segregation due to a parental translocation was not ruled out.

Table 1

| | case 1 S.C. | case 2 M.R. | case 3 T.O. | case 4 L.M. |
|---|---|---|---|---|
| Mental retardation | + | + | + | +(?) |
| Physical retardation | + | + | + | + |
| Microcephaly | + | + | + | − |
| Low set, abnormal ears | + | + | + | + |
| Preauricular tab and/or pit | + | + | + | + |
| Micrognathia | + | + | + | + |
| High arched or cleft palate | + | + | + | + |
| Abnormal insertion of thumb | + | + | + | + |
| Abnormal genitalia | + | − | + | + |
| Congenital heart defect | + | + | + | − |
| Kidney anomaly | − | + | + | − |
| Sex | M | F | M | M |

Caspersson et al. (1970b) described an extra G, morphologically indistinguishable from 21 and 22 by conventional staining in a non-mongoloid child. The fluorescent pattern differed from both 21 and 22. The parents were not reported. In the same paper, Caspersson et al. report another patient with an extra G chromosome whose phenotype is compatible with trisomy 22. Both the patient (47, XY, G+) and his normal mother (46, XX) had a short G (Gq−) chromosome which by fluorescence studied could not be distinguished from a chromosome 22 with shorter than normal arms, and closely resembles our case 3 and his mother.

Neither in Caspersson's case or in ours could irregularities be detected in the other chromosomes of the karyotype. Considering the small amount of material involved, and its lack of definitive fluorescent banding, it would be difficult to detect the distal third of the long arm of 22 in a translocation. The amount of chromatin missing is roughly equivalent to that deleted in some cases of 5p−(cri-du-chat) or 18p− or 18q−. In the families in which a clinically normal parent also has a number 5 or 18 deleted chromosome and no other detectable chromosome abnormality, a balanced translocation is assumed despite the failure to identify the second involved chromosome.

Two other families in which a deleted G was found to be segregating were reported before the advent of the fluorescent and banding techniques. In one family, the deleted chromosome G was found in four members in three generations. The propositus had spina bifida and mild mental retardation. The other three family members were normal (Ricci et al. 1970).

A more complex situation, analogous to that of our case 3 but involving chromosome 21, was described by Day and Miles (1965). Three siblings with Down's syndrome were born to a normal mother. The mother had 46 chromosomes including one deleted G which was assumed to be one member of a pair involved in a reciprocal translocation. Although the second chromosome could not be detected, the 21 translocation was presumed because of the presence of Down's syndrome in 3 children. Two had apparently normal karyotypes which must have included the undetected translocation, the third child had 47 chromosomes, including the deleted G 47, XX t (?; 21q−) 21+, Adjacent −1 segregation in the maternal meiosis would account for the karyotype of the first two affected children; unequal (3:1) segregation would explain the third.

If the mother of our case 3 carried an undetected exchange involving chromosome 22 and another autosome, the child's complement 47, XY t (?; 22q−) 22+ could have resulted from an unequal segregation. The small size of these deleted G chromo-

somes probably leads to reduction in synapsis and/or crossing over which in turn produces discordant orientation. A 3:1 segregation is not uncommon in non-Robertsonian translocations involving chromosome 21. We have recently observed an example: a child with Down's syndrome 47, XY t (Bq−; 21q+) 21+ whose mother was a balanced carrier 46, XX, t (Bq-; 21q+).

German et al. (1972) suggest that trisomy 22 is a cytogenetic entity because of the phenotypic variability which they encountered in three non-mongoloid, G+ patients studied with quinacrine fluorescence and Giemsa banding. It must be stressed that the age at which the patient is first seen is an important factor in determining phenotypic similarities. Our case 1, at age 7, is remarkable only for the profound retardation, both physical and mental. His micrognathia has disappeared and his facies is unremarkable. His phallus is now of normal length and appearance and the only other anomalies are his unusual cardiac lesions, cryptorchidism, preauricular pits and hyperextensible thumb. At 3 months of age the similarity between case 1 and case 3 was remarkable. In our experience, trisomy 22 is a recognizable syndrome. Case 2 was diagnosed clinically and then confirmed by karyotyping and we know of another unreported case similarly diagnosed. However, we have also seen children with all of the findings listed in table 1 with apparently normal chromosomes. Because of its comparative rarity the full spectrum of the trisomy 22 phenotype will not be known until more cases, proven by fluorescence and differential staining, have been reported.

It is difficult to estimate the incidence of trisomy 22. In analyses of 2,400 karyotypes of children with congenital anomalies studied at St. Christopher's Hospital for Children between 1964 and 1972, trisomy 22 has been observed with the same frequency as the cri-du-chat (5p−) and the 18q− syndromes. In this same time interval, we have seen 40 children with trisomy 18 and 17 with trisomy 13, suggesting that trisomy 22 occurs once in every 30,000−50,000 births.

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