ROBERTS SYNDROME : PHENOTYPIC VARIATION, CYTOGENETIC DEFINITION AND HETEROZYGOTE DETECTION

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MASERATI Emanuela, PASQUALI F., ZUFFARDI Orsetta, BUTTITTA Piera, CUOCO Cristina, DEFANT G., GIMELLI G., FRACCARO M. – Roberts syndrome : phenotypic variation, cytogenetic definition and heterozygote detection.

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SUMMARY : Five cases of Roberts syndrome (RS) in four nuclear families are reported and the wide range of phenotypic variation among them is described. This is in contrast with the remarkable uniformity of the cytogenetic findings. Indirect immunofluorescence with seric antibodies from patients with CREST, revealed that the centromeric structures are normal in RS thus confirming J. German's assumption that the chromatid repulsion is confined to the heterochromatin. The authors quantified the phenomenon of centromeric heterochromatin separation (as occasionally revealed by C-bands in normal subjects) in obligate heterozygotes and possible heterozygotes for RS. The results are indicative of the possibility to screen for heterozygotes. The nosology of RS and related syndromes is discussed in view of the cytogenetic findings and the natural history of the disease.

KEY-WORDS : Roberts syndrome. – Chromosomes. – Heterochromatin repulsion. – Heterozygote screening.

MASERATI Emanuela, PASQUALI F., ZUFFARDI Orsetta, BUTTITTA Piera, CUOCO Cristina, DEFANT G., GIMELLI G., FRACCARO M. – Le syndrome de Roberts : variation phénotypique, définition cytogénétique et détection des hétérozygotes. *(En Anglais).*

Ann Génét, 1991, **34,** n° 3-4, 239-246.

RÉSUMÉ : Cinq cas de syndrome de Roberts (RS) appartenant à quatre familles nucléaires sont rapportés et le spectre étendu des variations phénotypiques est décrit. Celui-ci contraste avec l'uniformité remarquable des observations cytogénétiques. L'immunofluorescence indirecte avec des anticorps sériques de malades atteints de CREST, a montré que les structures centromériques sont normales dans le RS, confirmant ainsi l'opinion de J. German selon laquelle la répulsion des chromatides est limitée à l'hétérochromatine. Les auteurs ont quantifié la séparation de l'hétérochromatine centromérique (telle qu'elle est occasionnellement observée en bandes C chez des sujets normaux) chez des hétérozygotes obligatoires et possibles pour le RS. Les résultats sont compatibles avec une détection des hétérozygotes. La nosologie du RS et de syndromes apparentés est discutée en fonction des observations cytogénétiques et de l'histoire naturelle de la maladie.

MOTS-CLÉS : Syndrome de Roberts. – Chromosomes. – Répulsion de l'hétérochromatine. – Détection des hétérozygotes.

INTRODUCTION

The nosology of Roberts syndrome (RS) and of the SC phocomelia syndrome (SCS) has been extensively discussed (e.g. Herrmann and Opitz, 1977) and recently Römke et al. (1987) concluded that the two conditions are likely to be one and the same genetic entity. In fact, both are inherited as recessive and characterized by pre- and postnatal growth retarda-

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tion, symmetrical limb defects of varying severity, and craniofacial abnormalities. The most striking common characteristic is that seen at the cytogenetic level, namely the typical centromeric appearance discovered by German (1979) and called RS effect, premature centromere separation (Parry et al., 1986) or chromatid repulsion (Krassikoff et al., 1986).

We report clinical and cytogenetic investigations on five cases of RS three of them familial and two sporadic, with variable phenotypic expression. The cytogenetic analysis was extended to the obligate heterozygotes.

CASE REPORTS

Case 1

M.M., a male, born after 39 weeks of uneventful pregnancy. He was the first son of unrelated parents aged 20 (mother) and 23 (father) years. Birth weight was 1400 g, length 35 cm, head circumference 26.5 cm. The following malformations and defects were ascertained. Sparse hair, microdolicocephaly, bilateral cleft lip and palate, hypertelorism, prominent eye bulbi, micrognathia, hypoplastic ears, capillary angioma of the forehead. Tetraphocomelia. Radiological examination of upper limbs showed at the right a single quadrangular rudimentary bone at the place of the humerus, ulna and radius, only three metacarpals, one of which was bifid and four fingers. On the left the humerus was normal. The ulna was severely hypoplastic; there were four metacarpals with partial fusion of the third and fourth, and four fingers. Both femora showed large exostoses at the distal third, while the tibia and the fibula were severely hypoplastic. The second metatarsal bone was longer than normal bilaterally, and a rudimentary sixth toe was present on the right. There was a slight dorsal kyphosis and dysplastic wings of ilia. An interventricular sept defect was diagnosed. The child suffered from frequent apnoea and died suddenly when 7 months old.

Cases 2 and 3

P.L. and P.D., two sisters born respectively in 1963 and 1971 from healthy second cousin parents, who had also five healthy daughters and sons (fig. 1).

P.L. was born with multiple limb deformities after an uneventful pregnancy. Birth weight was 2900 g. The radiological examination showed bilateral radius aplasia, hypoplastic ulnae and malformed hands with one supernumerary finger on the left. The lower limbs showed aplasia of the right fibula with bent tibia, knee stiffness and bilateral clubfoot. The head and trunk were normal. She was treated surgically for the clubfeet with good results.

P.D. was born after a normal pregnancy. Birth weight was 3000 g. The same deformities of the sister were present both on the upper and the lower limbs.

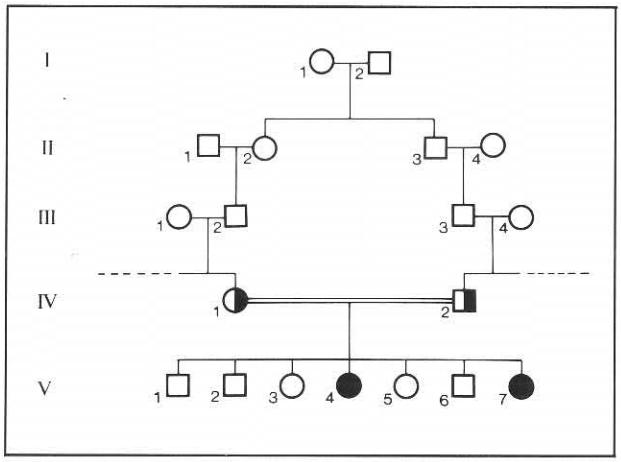

Fig. 1. – Pedigree of the family of cases 2 and 3.

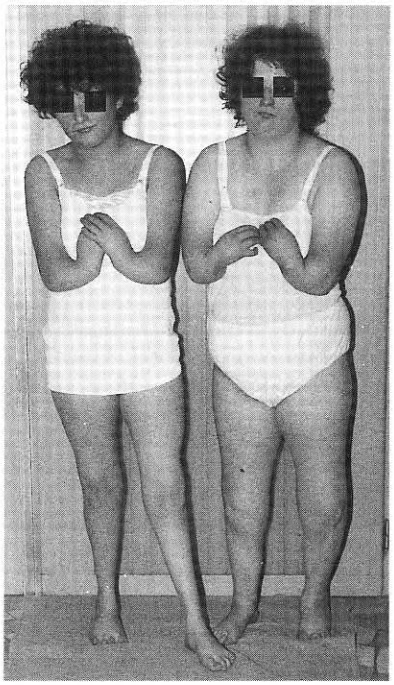

Fig. 2. – Patients 2 (right) and 3 at the age of 23 and 15 years, respectively.

Surgical treatment for the clubfeet was performed in the first year of life. The two sisters had a normal mental development and physical development resulted in short stature. The limb deformities are compatible with a nearly normal standard of activities. Figure 2 shows the patients aged 23 and 15 when their heights were 138 cm and 149 cm, respectively.

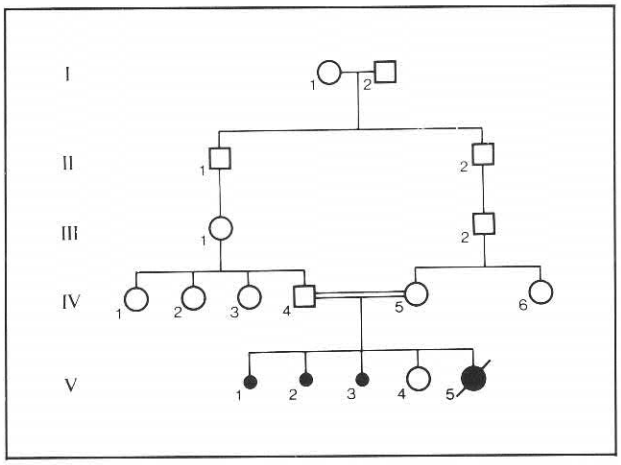

Fig. 3. – Pedigree of the family of case 4.

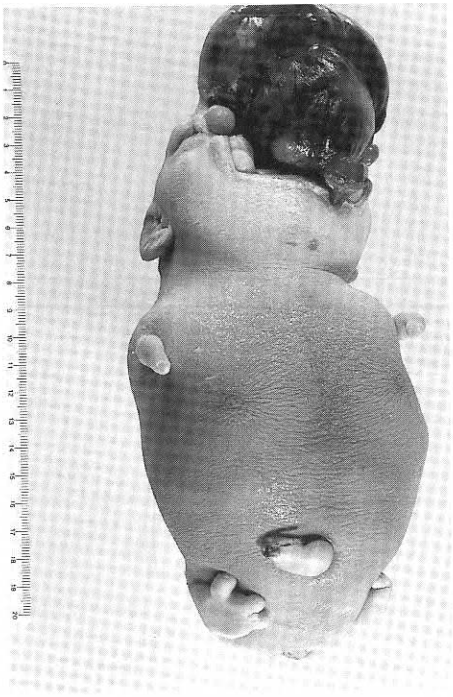

Fig. 4. – Patient 4 photographed at birth.

Case 4

A severely malformed female fetus. The mother, S.G., born in 1949 was admitted to hospital in 1985 because of polyhydramnios in her fifth pregnancy. She is a second cousin of her husband (fig. 3) and she had previously had three spontaneous abortions at the second month of pregnancy and one healthy daughter. The family history was uninformative except for a female first cousin of the father who was affected with hydrocephalus, lombosacral myelomeningocele and clubfoot. The pregnancy had progressed until the 38th week when echography confirmed the polyhydramnios and showed a severely malformed fetus. There was tetraphocomelia, with reduction of the number of fingers, the frontal and maxillary regions appeared abnormal due to the presence of a round mass due to an encephalocele. RS was suspected and confirmed at amniocentesis when a 46,XX karyotype with obvious RS-effect was found. Two weeks after amniocentesis a spontaneous delivery took place, and the newborn (fig. 4) died immediatly.

Autopsy findings

Female fetus of 920 g weight and 16.5 cm crown-rump length. Tetraphocomelia with 1.8 cm upper limb buds containing rudimentary humerus and only one finger ray and with 3 cm lower limb buds containing rudimentary femora and only two toe rays each. Broad medial facial and frontal cleft with involvement of the palatine, maxillar and zygomatic bones and with defects of frontal, ethmoidal, sphenoidal and nasal bones. Defect of the nasal soft tissue structure. Epidermal cysts at the medial margin of the non fused maxillary processes in the region of the former nasal pits. Hypoplasia and medial longitudinal fold of the tongue. Extreme hypertelorism. Bilateral ptosis and upward eye slant. Normal ears. Large partially coated fronto-ethmoido-nasomaxillary encephalocele completely filling up the facial cleft and protruding with the size of a tangerine containing almost the whole of the cerebrum. The cerebellum is in the normal intracranial position underneath the tentorium. Micro-and polygyria. Arhinencephalia with non recognizable olfactorian bulbs.

Cardiovascular malformations : segmental atresia of the left aortic arch between the descent of the left carotid artery and the junction with the ductus arteriosus Botalli (praeductal atresia). Supravalvular aorticopulmonary window of 3 x 4 mm diameter. Dilatation of the left and right atrium and of the right ventricle. Wide open foramen ovale due to increase of blood pressure in the left atrium, possibly persisting as small atrial septal defect. The ribs were long and slender ; the left 3 was shorter than the right one, the left 5 as well as the 11 and 12 were missing. High diaphragma. Hypoplasia of both lungs. Abnormal shape of both kidneys due to compression. Female external genitalia. Vagina, uterus, tubes and ovary were macroscopically normal. Microscopy revealed the persistence of primitive sex cords among the ovarian primordial follicles.

Case 5

D.D., a male born in 1986 from healthy second cousin parents. They had one spontaneous abortion at the 2nd month, a normal son and a normal daughter, and a son, born in 1981, who showed the same malfor-

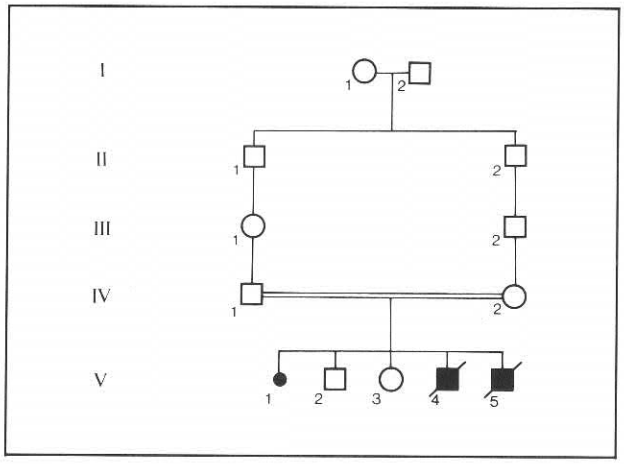

Fig. 5. – Pedigree of the family of case 5.

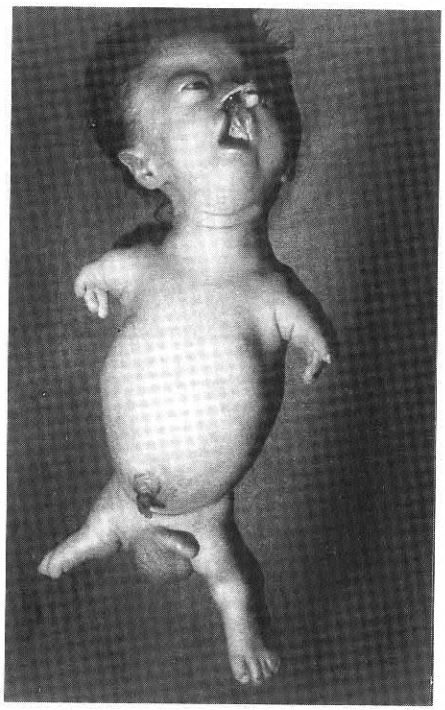

Fig. 6. – Patient 5 at birth.

mative picture of D.D. and died at 33 days age (fig. 5). During the 7th month of pregnancy an echo scan showed a development deficit, tetraphocomelia and cleft lip. The birth weight was 1300 g, length 32 cm, head circumference 27 cm.

The following malformations and defects were observed (fig. 6). Microcephaly. A symmetrical head and face. Hair sparse and low set on the forehead. The left ear was low-set with very thin helix. The orbital processes of the frontal bone were aplastic, so eyes and eyelids were prominent. There was hypertelorism and presence of corneal leukomas. The nasal bridge was flat and the tip of the nose was deviated to the left, the maxilla was abnormal. There were cleft lip and palate.

The upper limbs were rudimentary, the right 3 and the left 2.5 cm long, respectively.

A radiographic examination showed abnormal hypoplastic humeri, radii and ulnae. Each arm presented with three fingers, no spontaneous movement was possible. Simian creases were present. Lower limbs were rudimentary, 3 cm long the right, 6 cm the left. The femora were hypoplastic and the feet rudimentary, with four toes at the right and a syndactily between the fourth and fifth toe at the left. The thorax was short. At the radiological examination there was diastasis of vertebral bodies, schisis of the pubis and hypoplastic wings of ilium. Genitals were normal.

A C.T. scan of the head showed a partial aplasia of the frontal bone with encephalocele and a complex malformation of the basal skull bones, asymmetry of lateral ventricles, normal 3rd and 4th ventricles, normal parenchimal density.

The clinical course was characterized by marked psychomotor delay ; at 16 months of age his weight was 3,020 g, length 48 cm, head circumference 31.5 cm. Radiological examination showed craniosynostosis. He died at 2 years of age. Autopsy confirmed the clinical and radiological findings. The brain was asymmetrical, increased in volume, and weighed 530 g. Convolutions were thick with flat furrows. The thymus was atresic, the heart was macroscopically normal but histology showed a severe fibrocellular disarray with interstitial fibrosis.

MATERIAL AND METHODS

Cytogenetic investigations were performed on PHA-stimulated blood cultures in cases 1, 2 and 3, while in cases 4 and 5 they were performed on amniocytes and fibroblasts from a skin biopsy, respectively. All chromosome analyses were made with routine methods and the slides were stained with Giemsa and for Q- and C-banding. The Cd technique was applied following Maraschio et al. (1980) in patients 1, 2, 3 and 5. The Da-Dapi staining and the technique of indirect immunofluorescence with seric antibodies of patients with CREST-sklerodermia according to Peretti et al. (1986) were applied in patient No. 5.

In C-banded chromosome preparations from normal individuals centromeric heterochromatin separated in two distinct blocks on the two sister chromatids may be occasionally seen in any of the chromosomes, which on the other hand look normal with conventional Giemsa staining.

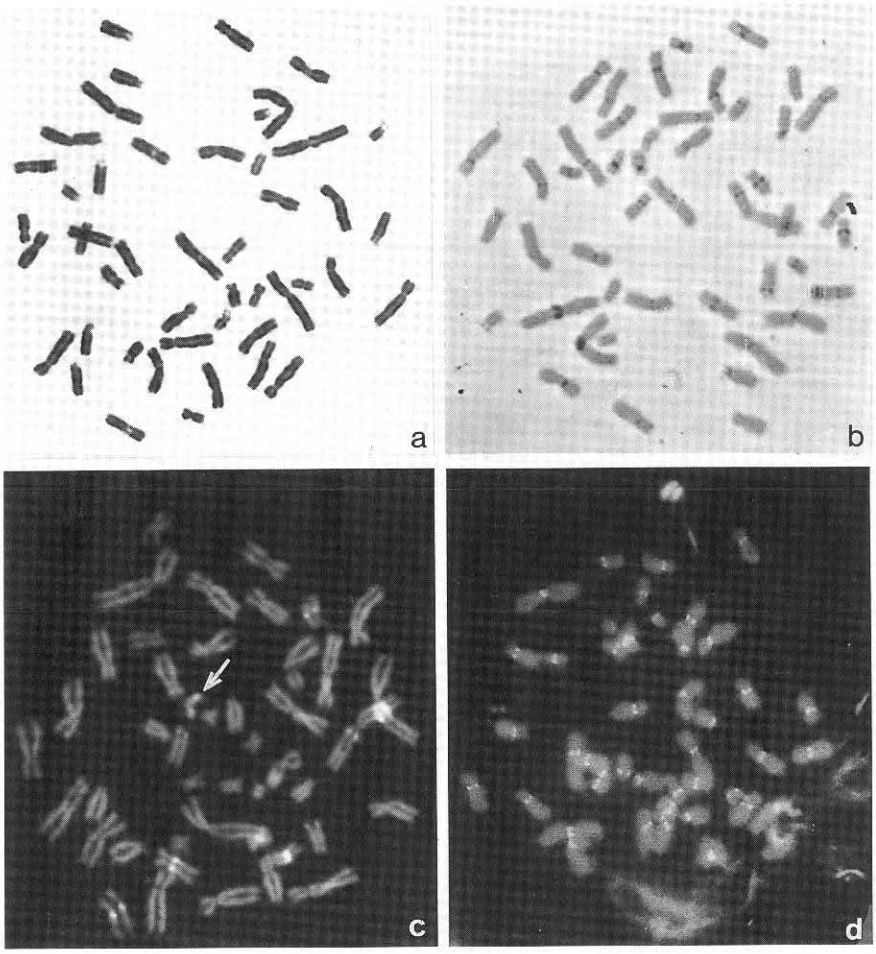

Fig. 7. — The same metaphase of patient 2 sequentially stained with Giemsa (a) and for G-bands (b). (c) Metaphase of patient 5 stained with Da-Dapi. The HR is evident in the chromosomes Nos 1, 9, 15, 16 and in the long arms of the Y chromosome (arrow). (d) Patient 5 after anticentromere immunofluorescence with CREST sera. The intensity of the fluorescence is within the normal range.

We attempted to evaluate quantitatively this phenomenon in RS obligate heterozygotes and to compare its frequency with normal controls and possible heterozygotes. We used 7 healthy subjects as controls, the parents of cases 2 and 3 as obligate heterozygotes and their 5 sibs as possible heterozygotes. We scored 40 C-banded metaphases in blood cultures from each of these subjects and scored the number of cells and the number of individual chromosomes in which it was present the « centromeric heterochromatin splitting » (CHS) phenomenon.

RESULTS

In conventional Giemsa stained preparations the phenomenon of chromatid repulsion was observed in all of the 5 patients, and C bands consistently revealed the separation in two blocks of the centromeric heterochromatin (fig. 7a, b). The technique for Cd demonstrated the normal presence of the proteic structure of the centromere. In patient No. 5 the indirect immunofluorescence with seric antibodies of patients affected with CREST was of the same intensity as that of the controls (fig. 7d). The Da-Dapi staining revealed clearly repulsion of the heterochromatic blocks specifically identified by this staining and the extreme repulsion of the Yq constitutional heterochromatin (fig. 7c) as first pointed out by Louie and German (1981). The phenomenon of CHS as seen with C-bands in some of the metaphase chromosomes is shown in figures 8 a, b and c. The frequencies of CHS in controls, obligate heterozygotes and possible heterozygotes are shown in table I. The number of chromosomes with CHS in the control group ranged from 20 to 65, with a mean of 41.3 ± 20.3. This mean is entered in figure 9 in which the values observed in the single subjects are also entered. The two obligate heterozygotes have values of 99 and 118, respectively. Among the possible heterozygotes three have values over 90 while the other two have values within the range of the controls. The scarce number of observations made impossible any refined statistical test but these results might as well be indicative of a significant trend.

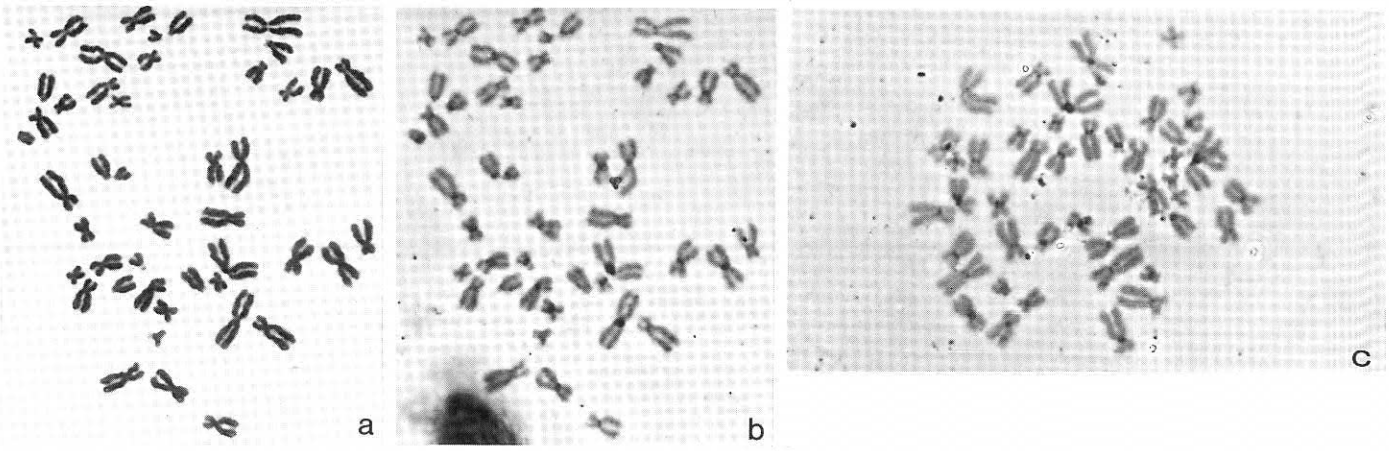

Fig. 8. — a and b : A metaphase of the father of patients 2 and 3 sequentially stained in Giemsa and for C-bands. There is no evidence of HR with Giemsa. With C-bands seven chromosomes present centromeric heterochromatin splitting. c : A metaphase of the mother of patients 2 and 3 stained for C-bands shows 12 chromosomes with centromeric heterochromatin splitting.

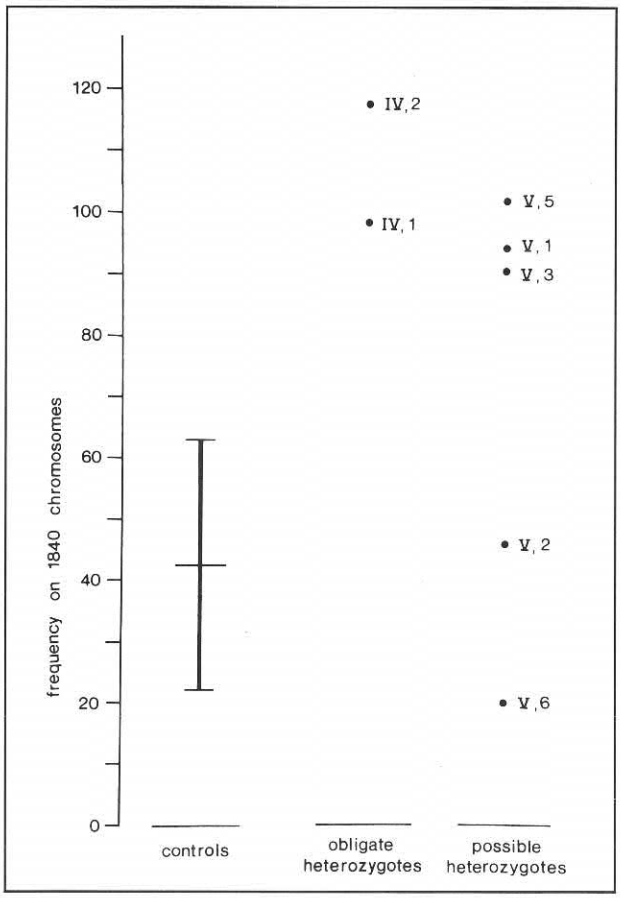

Fig. 9. — Quantitative scoring of CHS in the three groups of table I. From the left : the mean and standard deviation of the controls, the values of the obligate heterozygotes and those of the possible heterozygotes. Identification numbers refer to the pedigree of fig. 1.

TABLE I. — **Number of metaphases with at least one centromeric heterochromatin splitting (CHS) and number of chromosomes with CHS in controls, obligate heterozygotes and possible heterozygotes. For each subject 40 metaphases and 1840 chromosomes were scored. Identification figures are those of pedigree figure 1.**

Controls	N. of metaphases	N. of chromosomes
1	13	27
2	19	34
3	15	21
4	12	20
5	30	63
6	28	65
7	24	59
Obligate heterozygotes		
IV, 1	26	99
IV, 2	32	118
Possible heterozygotes		
V, 1	30	93
V, 2	22	46
V, 3	32	91
V, 5	33	102
V, 6	12	20

DISCUSSION

The cytogenetic phenomenon associated with RS is usually referred to as « premature centromere separation », but there seems to be a general consensus on the concept that the phenomenon involves specifically the heterochromatin (Tomkins et al., 1979 ; Petrinelli et al., 1984 ; Römke et al., 1987). All our patients showed the typical appearance of RS at the chromosome analysis, included also the Yq repulsion in the male patients Nos. 1 and 5 (fig. 7c).

In our patient we studied the centromeric regions with the Cd staining and with indirect immunofluorescence with centromeric antibodies and found that the proteic structures identified by these methods were normal. This findings indicate that only the heterochromatin blocks are involved in the repulsion and therefore we would suggest to call this phenomenon « Heterochromatin Repulsion » (HR).

A point which is worthy of discussion is the nosography of RS. The heterochromatin repulsion had the same appearance in all of our five patients and this indicates that they indeed had the same condition in spite of the wide phenotypic variability. Römke et al. (1987) postulated that RS and the so-called Appelt-Gerken-Lenz, SC-phocomelia and pseudothalidomide syndromes are identical and constitute a single genetic entity. Most cases, both sporadic and familial, show severe malformations, but a few RS patients with mild forms have been reported (Petrinelli et al., 1984 ; Parry et al., 1986 ; Stanley et al., 1988). In our sample the phenotypic variability is extreme, ranging from the mild form of the two sisters (cases 2 and 3) to the extreme severity of case 4.

In our familial cases the affected sibs seem to have the same degree of severity but relevant phenotypic variation between sibs was observed by Römke et al. (1987), Robins et al. (1989) and Holmes-Siedle et al. (1990). Our cases Nos. 2 and 3 show a similar mild form of RS while case No. 5 and his brother, although not directly observed, had similar, severe manifestations.

Our case 4 which is by far the most severe one, cannot be considered familial, but the mother had three spontaneous abortions.

Huson et al. (1990) suggested a possible overlap between RS and Baller-Gerold syndrome : the latter, mainly characterized by craniostenosis, could indeed represent a mild form of RS and not a distinct nosological entity. Our patients 2 and 3 have a considerable similarity with the case reported by Huson et al. (1990) in whom HR was noted at chromosome analysis.

What is the role of HR in the natural history of the disease ? German described in 1979 many aspects of the abnormal behaviour of chromosomes during mitosis and nuclei alteration in RS cells and postulated that these phenomena were independent of the microtubules function but rather due to a disturbance in heterochromatin separation. Tomkins and Sisken (1984) studied in detail the cell cycle of RS fibroblasts and found alterations in the duration of the various phases, with abnormally long metaphases. they suggested that the phenotype of RS could be a consequence of these disturbances. The gene product responsible of HR was demonstrated not to be diffusible in co-cultivation experiments (Petrinelli et al., 1984 ; Krassikoff et al., 1986), while HR was corrected in fusion hybrids between RS fibroblasts and an established chinese hamster cell line (Krassikoff et al., 1986).

Thus, if HR is a consequence of cell cycle alterations due to the mutation and these alterations in turn induce the phenotype, two alternative hypotheses are possible. The first is that in fact different mutations cause different syndromes (RS, SCS, Baller-Gerold) through pathogenetic mechanisms which all include cell cycle disturbances. These would lead to HR, but to explain the different phenotypes we should admit that the mutated gene is expressed also through other ways, which could be each specific for a given syndrome.

We favour a second possibility namely that a single RS mutation does exist, is expressed in the cell cycle disturbances, which result in the phenomenon HR but are channeled into a range of variable symptoms which in turn lead to different diagnoses. Huson et al. (1990) mention additional conditions which could be related to RS, such as the so-called Herrmann-Pallister-Opitz syndrome or cases of craniostenosis with bilateral fibular aplasia. In these latter syndromes no evidence of HR is demonstrated, while HR should remain a unifying feature for this group of syndromes.

There seems to be no obvious explanation for the wide phenotypic variability of RS. Holmes-Siedle et al. (1990) suggest the effect of what they call modifier genes as an explanation for the phenotypic variability among three RS sibs and this hypothesis could explain also the RS variability in general. Alternatively, the phenotypic variability may be due to different phases of embryogenesis in which the mutated RS gene is expressed, as suggested by Römke et al. (1987).

We attempted to arrive at a quantitative method which would permit the identification of the RS heterozygotes. Our preliminary results are suggestive of a trend that, if confirmed, would open up this possibility.

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