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Characterization of Constitutive Heterochromatin, in Particular of Fluorescence Polymorphisms, in a Central European Population

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ABSTRACT Polymorphisms of constitutive hetrochomatin, in particular fluorescence polymorphisms after QFQ-staining, show variations in size and staining character with unequal intra- and interchromosomal distribution in the chromosomes 1, 3, 4, 9, 13, 14, 15, 16, 21, 22 and Y, revealing significant patterns. In some cases, significant differences are found in size as well as in fluorescence, according to the type of cell culture analysed (amniocytes or lymphocytes). Within the individual sizes, the findings for flurescence are almost equivalent in both groups. Complete pericentric inversions show a maximum in chromosomes 4. The majority of polymorphisms in the acrocentrics 13 and 22 for p11.2 and in 15 and 22 for p13. Fields of application show analyses of polymorphisms of constitutive heterochromatin in twins and triplets demonstrating discrepancies in dizygotics and trizygotics but identical polymorphisms in monozygotic twins. Case presentations deal with proof or exclusion of percentage on the basis of comparison of polymorphisms of the constitutive heterochromatin.

INTRODUCTION

Cytogenetic and molecular cytogenetic analyses of human chromosomes deal almost exclusively with the euchromatic regions, disregarding the constitutive heterochromatin. We performed a qualitative and quantitative study of the constitutive heterochromatin on a large scale. Polymorphisms of the human chromosomes were analysed according to different banding techniques, in particular following fluorescence banding (QFQ). Their frequency, localization, and the occurrence of mutations were determined for an investigation group consisting of Central Europeans. Fields of application were investigated.

CHARACTERIZATION OF POLYMORPHISMS

Polymorphisms of human chromosomes are regions of constitutive heterochromatin in preferential intrachromosomal positions. The term "constitutive heterochromatin" defines segments that do not decondense in the telophase of mitosis but stay condensed during the interphase and remain transcriptionally inactive on a permanent basis. They consist of different types of highly repetitive DNA, namely: satellite-DNA type I-IV and α- and β- DNA. Constitutive heterochromatin partially stains bright with QFQ, defined as i(5) according to ISCN 1995, but does not distinguish between different DNA-types.

Each centromer is surrounded by pericentromeric heterochromatin with a different structure and size in each single chromosome. Four human chromosomes (1, 9, 16, Y) show large blocks of heterochromatin of chromosome-specific tandem repeats of a pentanucleotide. They are located proximally in the long arm of the autosomes 1, 9, and 16 and distally in the Y-chromosome. These regions used to be considered to be without any function. They react less to DNAase-digestion as compared to euchromatin. Replicating late, constitutive heterochromatin prolongs the S-phase of the cell cycle and tends to cluster in the interphase. These chromosomes with a large block of heterochromatin are known to separate late in the anaphase together with the acrocentric chromosomes. In pachytene of meiosis I, heterochromatin maintains a special behaviour without any crossing-over. Recent investigations revealed the fact that constitutive heterochromatin is important for gene regulation, thereby modifying gene inactivity in various ontogenetic phases and specific tissues (Brown et al. 1997).

The individual blocks of heterochromatin show specific reactions to staining and have different frequencies of mutation (Verma 1988). Duplications, deletions, and inversions in these regions are caused by pairing abnormalities of homologous chromosomes in prophase of meiosis I.

Special heterochromatic structures are the short arms of the acrocentric chromosomes 13-15, 21, and 22, without any euchromatic regions and built of various DNA-types. The pericentromeric heterochromatin in p11.1 consisting of α-satellite-DNA is followed by the region p11.2 with repeats (68 base pairs) of β-satellite-DNA proximally and satellite-DNA type I – IV distally. P11.2 is characterized by a high variation in size and a specific staining pattern in each of the chromosomes. 13p11.2 for example shows a preference for bright fluorescence after QFQ-banding, 15p11.2 shows a specific staining after DA-DAPI. The satellite stalks (p12) contain the nucleolus organizing regions with multiple copies of 68 base pair sequences of ribosomal RNA-genes. These regions are narrower than p11.2; therefore they are easily identified and they can specifically be stained by silver nitrate. The satellites (p13) form the distal part of the short arms, which vary considerably in size and staining behaviour, just like p11.2. Proximally, they consist of β-satellite-DNA and distally of the telomeric sequences. Satellite stalks and satellites can be duplicated or deleted. A detailed description of the individual polymorphic regions follows:

1q12 is located between the pericentromeric heterochromatin q11.1 and the euchromatic GC-rich band q21.1. Partial and complete pericentric inversions occur. The band consists of two parts. Further sub-bands can be displayed using special methods. BrdU-incorporation demonstrates a characteristic lateral asymmetry that is usually inherited. The region consists of satellite-DNA I-IV, preferably type II and III. Homogenous staining (Orcein, Giemsa) will show a higher condensation of the region than the euchromatin. 1q12 does not stain with QFQ, is dark with CBG, and shows bright fluorescence with DA-DAPI.

3q11.2 is located between the pericentromeric heterochromatin in q11.1 and the euchromatic GC-rich band q12.1. Partial and complete pericentric inversions occur. The band consists of α-satellite-DNA and will resist any Alu I-digestion. The region does not stain with CBG or DA-DAPI. A bright fluorescence (i5) is possible with QFQ.

4q11.2 is located between the pericentromeric heterochromatin in q11.1 and the euchromatic GC-rich band q12. Partial and complete pericentric inversions are frequently observed. The band consists of α-satellite-DNA and will resist any Alu I-digestion. The region does not stain with CBG or DA-DAPI, and may show a bright fluorescence (i5) with QFQ.

9q12 is a block of heterochromatin located between the pericentromeric heterochromatin q11 and the euchromatic GC-rich band q13. Deletions of the band are less usual, duplications and partial or complete inversions are frequent. The band consists of satellite-DNA I-IV in equal amounts and possesses two parts, comparable to 1q12, with the proximal segment usually being smaller than the distal one. The size of both units can vary independently. Homogenous staining shows a more intense condensation of the band than of the adjacent euchromatin. The region is QFQ-negative and CBG-and DA-DAPI- positive. DAPI added to the cell culture will decondensate the band.

16q11.2 is located between the pericentromeric heterochromatin in q11.1 and the euchromatic GC-rich band q12.1. Deletions, duplications and partial or complete pericentric inversions occur. The region consists mainly of satellite-DNA II. Homogenous staining shows a more intense condensation of the band (see 1q12 and 9q12). It is QFQ-negative and CBG- and DA-DAPI-positive. Treatment of cell cultures with DAPI results in decondensation of the block of heterochromatin in the following mitosis.

Yq12 is located between the euchromatic GC-rich band q11.23 proximally and a PAR and the telomeric region distally. The length of this band can vary to the extremes either missing completely or amplifying to double the length of the Y-chromosome. Partial and complete pericentric inversions occur. Translocations of this region favour the short arms of the chromosomes 15 and 22, exchanging the whole block of heterochromatin, not parts thereof. The band q12 consists of two main subunits of different highly repetitive DNA-sequences (satellite-DNA type I-IV with a subunit of type I that is characteristic for the Y-chromosome), proximal and distal components of which vary in size independently. Up to 5 subunits can be differentiated in pro- and prometaphase. Pretreatment of the cell

cultures (BrdU, e.g.) reveals a lateral asymmetry. Homogenous staining results in a tighter pairing of the chromatids in Yq12 than in the euchromatin. The region is CBG-, QFQ (i5)-, and DA-DAPI-positive. The DA-DAPI-stained brightly fluorescent segment reaches further proximal than the QFQ-one, which might be explained by the various subunits of the region.

13: The short arm (p11.2, p12, p13) consists of heterochromatin in these three regions with a different DNA-structure in each of them, that is located between the pericentromeric heterochromatin (p11.1) proximally and the telomeric region distally. Duplications have been observed for all three of the segments as well as deletions for the entire short arm or individual regions, especially for p12 and p13. Translocations are frequent to the short arms of the chromosomes 15, 21, and 22. 13p11.2 consists of satellite-DNA type I-IV, p12 of rRNA-genes and p13 of β-satellite-DNA, as well as the telomeric region. P11.2 is CBG-positive, DA-DAPI-negative and brightly fluorescent (i5) with QFQ to some extent. P12 is silver nitrate-positive. P13 is CBG-positive, DA-DAPI-negative and QFQ-positive (i5) in a number of cases.

14: Morphology and structure of subunits of the short arm are comparable to chromosome 13. Duplications as well as deletions occur in all three of the regions. Translocations to the other acrocentrics show a somewhat different distribution than in chromosome 13. 14p11.2 consists of all four types of satellite-DNA. Their distribution is different, though, in comparison to chromosome 13. 14p11.2 is CBG-positive and DA-DAPI-negative. P12 is silver-nitrate-positive. In some cases, QFQ-staining can result in a bright fluorescence (i5) in p11.2 and p13.

15: Morphology and structure are comparable to the chromosomes 13 and 14. Different types of deletions, duplications and translocations occur. In particular, formation of derivative marker-chromosomes and translocations with the other acrocentric chromosomes and the Y-chromosome take place (see above). P11.2 consists mainly of satellite-DNA II and III. P12 and p13 match the other acrocentric chromosomes. P11.2 is positive for DA-DAPI and CBG. QFQ-staining can cause bright fluorescense (i5) in p11.2 and p13 in some cases. P12 is silver nitrate-positive.

21: Morphology, structure, and forms of mutation are comparable to the other acrocentric chromosomes. P11.2 is CBG-positive, DA-DAPI-negative and with QFQ bright fluorescence (i5) can occur in p11.2 and p13.

22: Morphology , structure, and staining properties match the other acrocentrics.

INVESTIGATION GROUP

600 unrelated persons of Central European origin were investigated, 310 of which had a male, 290 a female karyotype. 582 were shown to have a constitutive normal karyotype, and 18 carried various aneuploidies. 500 analyses derived from prenatal chromosome diagnostics (amniotic fluid cultures) of normal pregnancies. Any association to diseases and correlation to specific chromosome polymorphisms could be excluded. Furthermore, 100 chromosome investigations were performed postnatally using lymphocyte cultures.

During the course of the documentation, it became obvious that regions of size very small (vs, ISCN 1995) and brilliant fluorescence (i5) were discovered less frequently in lymphocytes than in amniocytes. This observation corresponds with findings of Verma (1988) stating that preparations of different cell systems may lead to dissimilar results. This fact was taken into consideration regarding the evaluation of these polymorhisms.

In addition to the main investigation group, 2 smaller groups were analysed for specific questions, one of which dealt with polymorphisms among 20 twins and 7 triplets and the other one with proof or exclusion of parentage in 3 cases.

METHODS OF INVESTIGATION

The analyses of polymorphisms were performed on metaphases between prometaphase and late metaphase. At least 10 mitoses per case were evaluated in a fluorescence microscope. In cases of problems of determining polymorphic regions, the amount of mitoses was increased to 20. All metaphases were primarily stained with QFQ, since the analysis of fluorescence polymorphisms (i5) was prevalent. In addition, this technique enabled the representation of eu- and heterochromatin while at the same time ensuring the safe classification of each single

polmorphism to the respective autosome or Y-chromosome. Photos were taken of the mitoses, and the final analyses were performed on negatives, using a special apparatus enlarging the negatives 10-fold with no loss of information through the prints. CBG- and DA-DAPI techniques were added as needed.

The polymorphic regions of 7 autosomes, the chromosomes 3, 4, 13, 14, 15, 21, and 22 were analysed, as well as the Y-chromosome. The polymorphic regions in the acrocentrics included the short arm in its entirety (p11.2, p12, and p13). The other chromosomes analysed each showed one polymorphic region only. The blocks of heterochromatin of the chromosomes 1, 9, and 16 were investigated for specific questioning. All together, 30 polymorphic regions were analysed, 24 (80 %) of which show fluorescence polymorphisms. Since all autosomes are present as homologous their polymorphisms per person were evaluated twice.

Documented for each region were fluorescence intensity i(5), size, and mutations such as amplifications or deletions as well as complete inversions. The polymorphic regions were classified vs, s, m, and l (very small, small, medium and large) according to size, using 17p12 as measure for vs, twice vs for s and so on. The heterochromatic regions of 1, 9, 16, and Y were defined in relation to the entire length of the specific chromosome. Duplications respectively amplifications of the polymorphic regions increasing as well as deletions reducing their length to more than 50% were considered mutations of the polymorphisms. In analysing the change of position of polymorphic regions in chromosomes 1, 3, 4, 9, 16, and Y, complete pericentric inversions only were taken into account since partial pericentric inversions could not be measured precisely. Mutations such as duplications occurred in the short arms regions of the acrocentrics distal of p11.2, including either one or both segments (p12 and p13). Deletions were not observed.

STATISTICS

The program SPSS 10.0 of SPSS Inc. was used to check the significance of differences in results favouring the Chi-square distribution in nonparametric tests.

RESULTS

Polymorphisms of the chromosomes 1, 3, 4, 9, 13, 14, 15, 16, 21, 22, and Y were characterized in a group of Central Europeans with emphasis on fluorescence intensity i(5) following QFQ-staining. 14400 regions in 600 persons were analysed separately according to the type of the cell culture (amniocytes and lymphocytes). Polymorphisms of size were found in 89.2 % of the investigation group A (amniocytes) and 87.0 % of the group Ly (lymphocytes), polymorphisms of fluorescence i(5) in group A in 25.8 % and in group Ly in 18.5 % (Table 1). Differences between groups A and Ly in size, as well as in fluorescence i(5) proved to be statistically significant, as was the comparison for vs (96.1 % in A and 96.3 % in Ly). The sizes s (3.6 and 3.5 %) and m (0.3 and 0.2 %) showed no significant differences in frequency, and the findings for fluorescence i(5) within the individual sizes were almost equivalent in both groups (A and Ly) with vs (89.3 and 89.4 %), s (9.9 and 9.7 %), and m (0.8 and 0.9 %) (Table 1).

Table1: Polymorphisms vs, s and m and fluorescence polymorphisms i(5) (analysis of 24 chromosome regions on 7 pairs of autosomes). A=amniocytes; Ly=lymphocytes; vs=very small; s=small; m=medium; i=intensity.

Polymorphisms		*Group*	
		A (N=500)	*Ly (N=100)*
Number of chromosomes analysed		12000	2400
Frequency of observed polymorphisms (%)		89.2	87.0
In size classes	vs	96.1	96.3
	s	3.6	3.5
	m	0.3	0.2
Frequency of fluorescence polymorphisms i(5) (%)			
Related to number of chromosomes analysed		25.8	18.5
Related to chromosomes with defined polymorphisms		28.9	21.2
In size classes	vs	89.3	89.4
	s	9.9	9.7
	m	0.8	0.9

The amount of fluorescence polymorphisms per case reached from 0 to 15, showing a maximum of 6 for group A and 4 for group Ly (Table 2).

The size of the average length of Yq12 showed a maximum at 30 % in both groups (Table 3).

Duplications of the size of the constitutive heterochromatin of the chromosomes 1, 9, and 16 were found in 10.5 % of chromosome 1, 20.6 % of 9, and 4.5 % of 16 in group A and in 4.5 % (1), 14.0 % (9), and 1.0 % (16) in group Ly. Duplications of the satellites (p13) of the acrocentric chromosomes had a frequency of 0.8 % in group A and of 0.3 % in group Ly (Kalz, 2003). Complete pericentric inversions occurred in group A in 0.1 % (chromosome 1), 1.8 % (3), 10.0 % (4), 1.1 % (9) and 0 % (16), in group Ly in 0 % (1), 0 % (3), 2.5 % (4), 1.5 % (9), and 0 % (16) (Table 4), determined in relation to the number of chromosomes analysed. The frequency of inversions becomes even more significant related to the number of polymorphisms per chromosome (in %), especially for the maximum at chromosome 4 (37,6 % for A and 38,5 % for Ly) (Table 4). Differing frequencies of polymorphisms in the individual chromosomes could be demonstrated for the short arm regions of the acrocentrics. The size vs showed a frequency between 90.6 and 100 % in group A and Ly. Duplications of satellites (ss) were observed in a frequency of 0 to 3.2 % (A, Ly; Table 5). The size s of polymorphisms was observed in 0-7.0 % (A, Ly), revealing the highest values in p11.2 in the chromosomes 13 and 22, and the lowest in 21 (A, Ly). In p13, maxima for s were found in the chromosomes 15 and 22 (A, Ly), and in 14 only in group A. The minimum was found in chromosome 13 (A, Ly). Size m showed frequencies between 0 and 1.0 %, with a maximum in 22p13 (A, Ly; Table 5).

The frequency of chromosomes with intense fluorescence i(5) ranged from 0.5 % (chromosomes 15p11.2 ; Ly and 21 p11.2; Ly) to 69.6 % (chromosome 13p11.2; A). Fluorescence polymorphisms i(5) and size vs dominated with a frequency of 68.0 to 100 % (A, Ly). Duplications of satellites (ss) were observed in a frequency of 0 to 0,5%. Size s i(5) was observed in 0-27.7 %. There existed significant differences between the

Table 2: Distribution of all fluorescence polymorphisms i(5) per case (analysis of 24 chromosome regions on 7 pairs of autosomes) (abbreviations Table 1).

Group	*Frequency of the occurrence of fluorescence polymorphisms/case (%)*															
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
A (N=500)	0.4	1.0	3.4	7.0	11.0	15.8	18.2	17.8	9.4	8.4	3.8	1.8	1.6	0.2	0	0.2
Ly (N=100)	3.0	1.0	5.0	22.0	25.0	18.0	12.0	10.0	3.0	0	1.0	0	0	0	0	0

Table 3: Frequency of the different sizes and structural aberrations of Yq12 with regard to the 2 cell types investigated. (inv.=inversion; del.=deletion; other abbreviations Table 1).

		Size of Yq12 compared to the total length of the Y-chromosome (%)							*Aberrations (%)*	
Group		<2	20	30	40	50	60	>60	inv.	del.
A (N=267)	**Frequency**	0	5.2	56.6	28.5	8.6	0.8	0	0.4	0
Ly (N=44)		4.6	2.3	50.0	25.0	9.1	2.3	4.6	0	2.3

Table 4: Amount of inversions among all chromosomes and polymorphisms diagnosed (inv.= inversions; polym.=polymorphisms; chr.=chomosome; other abbreviations Table 1).

Chromosome	*Group*	*Number of chromosome analysed*	*Number of polymorphisms/ cromosomes*	*Number of inversions/ chromosome*	*Frequency of inv. related to number of polym./chr. (%)*	*Frequency of inv. observed (%)*
1	A	1000	105	1	1.0	0.1
	Ly	200	9	0	0	0
3	A	1000	472	18	3.8	1.8
	Ly	200	79	0	0	0
4	A	1000	266	100	37.6	10.0
	Ly	200	13	5	38.5	2.5
9	A	1000	206	11	5.3	1.1
	Ly	200	28	1	3.6	0.5
16	A	1000	45	0	0	0
	Ly	200	2	0	0	0

Table 5: Frequency of the polymorphisms of size vs, s und m and of the polymorphisms of fluorescence i (5) of the chromosomes 3, 4, 13, 14, 15, 21, and 22 of the investigation groups A and Ly. (ss=duplicated satellites; other abbreviations Table 1). Statistically significant differences are marked cursive and bold.

Chromosome		*Group N(A)=500 N(Ly)=100*	*Frequency of the polymor phisms of size in regard to number of polymorpisms analysed (%)*				*Frequency of the polymorphisms of fluorescence i(5) in regard to number of chromosomes with polymorphisms (%)*				
			vs	*vs ss*	*s*	*m*	*in all*	*of those* *vs*	*vs ss*	*s*	*m*
3	q11.2	A	-	-	-	-	***47.2***	***97.2***	-	2.8	0
		Ly	-	-	-	-	***39.5***	***100***	-	0	0
4	q11.2	A	-	-	-	-	***26.6***	***100***	-	0	0
		Ly	-	-	-	-	***6.5***	***92.2***	-	7.8	0
13	p11.2	A	94.1	-	5.8	0.1	***69.6***	91.7	-	8.3	0
		Ly	95.0	-	5.0	0	***63.5***	93.7	-	6.3	0
	p13	A	97.6	0.1	2.1	0.2	23.2	90.5	0	***8.6***	0.9
		Ly	96.5	0.5	3.0	0	11.5	77.0	0	***23.0***	0
14	p11.2	A	97.9	-	2.1	0	1.0	90.0	-	10.0	0
		Ly	97.0	-	3.0	0	1.0	100	-	0	0
	p13	A	94.0	0.2	5.4	0.4	30.1	***81.1***	0	17.6	1.3
		Ly	98.0	0.5	1.5	0	21.0	***92.9***	0	7.1	0
15	p11.2	A	97.5	-	2.4	.1	1.2	91.7	-	8.3	0
		Ly	96.5	-	3.5	0	0.5	100	-	0	0
	p13	A	***90.6***	3.2	5.6	0.6	32.0	80.7	0.5	16.9	1.9
		Ly	***95.5***	0.5	3.5	0.5	23.5	83.0	0	14.9	2.1
21	p11.2	A	99.9	-	0.1	0	2.6	96.2	-	3.8	0
		Ly	100	-	0	0	0.5	100	-	0	0
	p13	A	95.4	0.2	4.1	0.3	30.5	85.4	0.1	13.5	1.0
		Ly	97.5	0	2.5	0	20.5	90.2	0	9.8	0
22	p11.2	A	95.5	-	4.5	0	8.7	86.2	-	13.8	0
		Ly	92.5	-	7.0	0.5	9.0	88.8	-	5.6	5.6
	p13	A	93.7	0.1	5.2	1.0	***36.8***	***83.2***	0	***14.1***	2.7
		Ly	92.0	0	7.0	1.0	***23.5***	***68.0***	0	***27.7***	4.3

two investigation groups (Table 5). Polymorphisms of the size s i(5) showed different frequencies in the two investigation groups for all the chromosomes, but maxima could be delineated for 14p13 (A), 15p13 (A, Ly) and 22p13 (A, Ly). Size m i(5) ranged from 0 to 5.6 % with maxima for 15 and 22 (Table 5).

In the chromosomes 3 and 4, only the polymorphic regions with a brilliant fluorescence could be detected, therefore only this group is included for the analysis of size variations. Size vs also dominated with a frequency of 92.2 to 100 %, followed by s in 0 to 7.8 %. Size m was not observed. The two investigation groups revealed significant differences, too, as to the frequencies of the size vs and s in both of them (Table 5).

CASE PRESENTATIONS

Case 1, exclusion of a gonosome mosaic respectively formation of a chimera: Prenatal chromosome analysis from amniocytes of a 35 year old woman showed cells with male and female karyotype in 4 cultures (46,XY:46,XX=1:1). There were 3 possibilities of explanation: first, a complex gonosome mosaic starting with a zygote with the karyotype 47,XXY and different postzygotic nondisjunction steps, second, a chimera and third, a contamination of the fetal cell cultures with maternal cells. A comparison of the female cells of the amniotic cell culture with a lymphocyte cell culture of the mother revealed identical polymorphisms (30/30) thus proving a maternal contamination.

Case 2, analysis of a pregnancy after a mixed homologous and heterologous insemination: Previous homologous insemination because of severe oligospermia of the husband´s had led to abortion twice. The first miscarriage revealed a trisomy 20. The second one revealed a trisomy 21. A combined homologous and heterologous insemination showed the structural aberration in 15p in the female fetus with brilliant fluorescence in the additional segment. A translocation Y/15 could be delineated by FISH. WCP Y excluded a transfer of euchromatin which would lead to

virilisation of the female fetus [Karyotype:46, XX,t(Y:15)(q12:p11.2)]. Additional analyses of the previous miscarriages revealed the trisomy 20 to be of paternal, whereas the trisomy 21 was of maternal origin. Comparisons of the polymorphisms of the trisomy 21 (21p11.2, p12, p13) demonstrated the non-disjunction originating from a meiosis I error, since both maternal differently structured chromosomes 21 were present. The blood of the semen-donor showed the translocation Y/15 and a length of Yq12 comprising 30 % of the entire length of the Y-chromosome, thus leading to the conclusion that the translocation did not occur de novo in the donor or his father but at the earliest in his maternal grandfather.

Case 3, questionable formation of a mosaic of a translocation de novo and determination of its genetic significance: Pregnancy was induced in a couple with reduced fertility after hormone stimulation. Ultrasound revealed dichorial female twins. Fetus I showed a completely normal female karyotype, and fetus II exhibited a normal female karyotype in one of two cell cultures and a reciprocal translocation 6/8 [karyotype: 46,XX,t (6:8)(q11:q12)] in more than 60 % of mitoses in the second one. The discrepancy of polymorphisms in 3 regions indicated dizygotic twins. The identity of 100 % of the polymorphisms in fetus II (normal and translocation cells) excluded a chimera. Because of the isolated presence of the translocation in one of the 2 cell cultures there was reason to believe the rearrangement to be a pseudo-mosaic originating in-vitro. 2 healthy girls were born at term.

POLYMORPHISMS IN TWINS AND TRIPLETS

20 sets of twins and 7 of triplets were analysed. All of them showed a normal male or female karyotype and resulted from prenatal analyses of amniocytes. 14 of the 22 sets of twins were of the same sex, and 8 were of a different one. Of those fetuses of equal sex, 8 were monozygotic, and 6 were dizygotic. Of the 5 sets of triplets, 3 were of different sex, and 2 were of equal sex and female. Of the 3 sets of triplets of different sex, 2 consisted of a set of monozygotic twins and the third child of different sex. The 3rd set was trizygotic. All sets of twins and triplets with different sexes (n = 11) showed differences in polymorphisms, in 4 features each on an average. Of the sets of twins and triplets with the same sex, 12 appeared to be monozygotic, whereby 8 of those were twins and 4 triplets. The mean number of identical polymorphisms was 7 in them. In all these cases, the results of polymorphism analysis coincided with findings of prenatal ultrasound-diagnostics. Thus, the polymorphisms were identical in 100 % of the monozygotic twins and triplets but showed discrepancies in all the dizygotics and trizygotics.

DISCUSSION

The discussion about the significance of the constitutive heterochromatin has been controversial for a long time. It was considered „junk DNA“ (Verma 1988).

The „selfish DNA hypothesis“ (Orgel and Crick 1980) stated that the frequently repeated DNA-sequences associated with heterochromatin were of no value to the organism. Hilliker and Sharp (1988) and Marchant (1986) investigated the centromeric heterochromatin of Drosophila melanogaster and discovered 18 genes within the heterochromatin of the second chromosome, 11 within the third, 1 within the fourth, 12 within the heterochromatin of Y-chromosome, and 3 in Xh.

The well known “position effect variation” (PEV) of the heterochromatin (Lewis 1950; Spofford 1976) states that the expression of a gene is strongly influenced by the neighbourhood to constitutive heterochromatin. Euchromatin-loci adjacent to heterochromatin do not function perfectly. Translocations of an euchromatin locus into the neighbourhood of constitutive heterochromatin in Drosophila melanogaster were observed to partially suppress the euchromatic gene.

Research on Drosophila and Sacharomyces pombe revealed the importance of pericentromeric heterochromatin for the function of the chromosome (Steiner and Clark 1994). Henikoff et al. (2001) tried to find the cause of the steady evolutionary increase of the pericentromeric areas in the asymmetry in the female meiosis with just one of possible 4 genomes to become the nucleus of the oocyte. This competition of the chromosomes in order to gain a favourable orientation in meiosis I can be won by a centromer with an advantage by an increased centromeric region

to which more microtubuli can be attached.

Increase and reduction of size in heterochromatic regions on a cytogenetic level can be explained by partial or complete deletions or duplications or by unequal crossing-over in meiosis being demonstrated by Jackson et al. (1999), who found out that a minimum of 50 Mb of chromosome 10 in pericentromeric regions are unstable and subject to an elevated level of rearrangements. Analyses of the human constitutive heterochromatin show a variety of methods in different studies, most of which were conducted for the chromosomes 1, 9, 16, and y, following CBG-banding (Craig-Holmes et al. 1973, 1975; Patil and Lubs 1977; Mikelsaar et al. 1978; Balicek et al. 1977; Erdtmann et al. 1981; Cavalli et al. 1985; Starke et al. 2002). The standards of comparison of the heterochromatic regions varied: Regions were compared with each other, with the length of the short arm of chromsome 16, with the area of fluorescence intensity of the distal part of chromosome 13 or measured densitrometrically. The Y-chromosome was related in length to chromosome 18 or a G-chromosome. Studies ranged from a small number of families (Craig-Holmes et al. 1975) to thousands of cases (Hsu et al. 1987; Rosser et al. 2000). Each of these publications took into account only a fraction of the polymorphisms following different staining methods and standards, thus complicating a comparison of the results.

A high variability of the constitutive heterochromatin of the chromosomes 1, 9, and 16 following C-banding of 20 blood donors was observed and compared in a qualitative evaluation by Craig-Holmes et al. (1973). Familial C-band patterns in five pedigrees revealed the stable inheritance of variants (Craig-Holmes et al. 1975). Duplications of the regions 1q12, 9q12, and 16q11.2 were found in chromosome-specific frequencies in our investigation group, with values for amniocytes being significantly higher than for lymphocytes: 1q12: 10.5 % (A) and 4.5 % (Ly); 9q12: 20.6 % (A) and 14.0 % (Ly); 16q11.2: 4.5 % (A) and 1.0 % (Ly). The same sequence of frequency was stated by Hsu et al. (1987) in a large series of prenatal cases. The findings of Hsu et al. and our study (Table 4) are comparable regarding the correlation of the inversions in the different chromosomes, but the percentage of inversions per chromosome in both studies shows different values.

Barker et al. (1977) investigated 108 Q-banded karyotypes derived from amniocytes in the chromosomes 3, 4, and 13, 14, 15, 21, and 22 (proximal arms and satellites). The bright fluorescence i(5) was compared to the distal region of the long arm of 13, but no attempt was made to document differences in size. The frequency of the fluorescence polymorphism i(5) per case lay in the range of 0 to 11, with the modal of 4 thus matching our results in group Ly (see Table 2) but being lower than the average value of 6 in group A in the present investigation. Their most frequent polymorphisms were the bright short arm of chomosome 13 (13p) followed by 3cen and 21p13. Our results for group A and Ly (amniocytes and lymphocytes) showed similar results for the first two regions with 69.6 (63.5) % for 13p11.2 and 47.2 (39.5) % for 3q11.2, but 22p13 took third place in the present investigation with 36.8 (23.5) %, followed by 21p13 (30.5 / 20.5 %) in fourth place. Barker et al. compared their results with those of a group of 221 persons from Geraedts and Pearson (1974) and found similar frequencies even though Geraedts and Pearson did not analyse the proximal short arms (p11.2, called short arms) and the satellites (p13) separately on the D and G chromosomes. An additional comparison to a study (N = 90) of Hauge et al. (1975) revealed significant discrepancies to the results of Barker et al. and of Geraedts and Pearson. The authors explained these differences with various techniques applied and differences in criteria for scoring the variants.

In 57 Caucasians, Olson et al. (1986) investigated QFQ-polymorphisms of the chromosomes 3, 4, 13, 14, 15, 21, 22, and Y. Of these, 39 were unrelated and findings were compared to 12 families (father, mother, child) with a paternal translocation. The authors delineated from their investigation that 2 persons can be distinguished best by the polymorphic regions of the chromosomes 15 and 22 and that the chromosomes 3, 4, and Y were the least informative. These results are in accordance with our observations. In the present investigation, the analyses of inter- and intrachromosomal distribution of the polymorphism sizes s and m revealed that for size s 1.8 % of the probands showed a bright fluorescence in the region 22p13 and 1.7 % for 15p13. Size m was found most often in chromosome 22p13 (0.3 %) followed by 15p13 (0.2 %) (Kalz 2003).

A pericentric inversion was observed in 33.3

% of metaphases in chromosome 4 and in 7,7 % in 3 in the study of Olson et al. (1986) these findings being in accordance with our results. Based on the observed fluorescence polymorphisms, 37.6 % (A) and 38.5 % (Ly) were found in chromosome 4 and 3.8 % (A) and 0 % (Ly) in 3. While the results of size and fluore-scence (Table 5) for the chromosome regions 3q11.2, 4q11.2, 13p11.2, 14p11.2, 15p11.2, 21p11.2 and 22p11.2 lay within the range of the other postnatal studies, the frequencies of size and fluorescence intensity of the regions 13p13, 14p13, 15p13 and 21p13 were higher than those of other studies. Cell type A showed a higher amount of fluorescence i(5) in the regions 13p11.2, 13p13, 14p13, 15p13, and 22p13. Differences in frequencies of polymorphisms were published for defined ethnic groups (Verma 1988; Kalz 2003).

The analyses presented here were gained from negatives instead of prints thus rendering higher accuracy in our research. Bright fluorescence polymorphisms i(5) were more frequently found in amniocytes than in lymphocytes. An aging effect could not be detected, but additional treatment resulting in a better comprehension of vs i(5) led to the conclusion that differences in frequency of the fluorescence polymorphism i(5) can be modified by exogen factors. A comparison of chromosome polymorphisms cannot be valid unless it is based on the same evaluation- and cell system.

In addition to the population genetic studies, it was our intention in this investigation to demonstrate the significance of the constitutive heterochromatin for diagnostic purposes. The analysis of the polymorphisms may reveal evidence of contamination of fetal cell cultures with maternal cells, and proof of parentage can be performed, as well as determination of zygocity in sets of twins and triplets.

Studies on the clinical importance of constitutive heterochromatin show controversial results. Mikelsaar et al. (1978) compared Q- and C-bands of 102 normal children with those of 45 metally retarded ones. In each of these cases, one of the parents had an inversion of the constitutive heterochromatin of chromosomes 3. The authors could not prove a relation between an inversion of chromosome 3 and mental retardation. Their results showed a frequency of 2-6 % for inv 3 (1,5 % in our study, Table 4), in mentally retarded children 11,1 %, judging this difference to be insignificant.

Reddy and Sulcova (1998) analysed 3 polymorphic regions in acrocentrics that had been translocated to different chromosomes. These mutations were explained with the existence of homologous sequences in the breakpoints. Two children showed malformations, and the third patient was sterile. In each of the cases, clinical importance was derived from the heterochromatin by the authors.

Genes are only a small part of the genome of the mammal. New epigenetic research is looking for correlations between coding and non-coding DNA and adjacent proteins. There is mounting evidence that histones and their covalent modifications can change chromatin structure. A model introduced by Jenuwein and Allis (2001) claims that the morphology of eu- or heterochromatin is subject to local concentra-tions and combinations of differently modified nucleic acids.

Recent investigations on the role of constitutive heterochromatin in the human genome are concentrating on function studies in different ontogenetic stages and might give us new perspectives to the possible importance of its mutations in clinical cytogenetics.

SUMMARY

Regions of constitutive heterochromatin were characterized as to qualitative and quantitative peculiarities in a group of 600 Central Europeans. Predominantly fluorescence polymorphisms i(5) after QFQ-banding were analysed. In 2 different cell systems, amniocytes and lymphocytes, 500 and 100 non-related persons were investigated. The most frequent class of polymorphisms was the size very small (vs) with or without brilliant fluorescence i(5) in 96.1 and 96.3 %. The size small (s) comprised 3.5-3.6 % and size medium (m) less than 1 %. The amount of fluorescence polymorphisms of size i(5) was significantly lower in lymphocytes than in amniocytes. This altered detection rate was influenced by exogenous factors like mode of preparation. In the acrocentrics there were significant chromosome- and region-specific differences in size of bands and frequency of fluorescence intensity i(5) with maxima in the chromosomes 13, 15, and 22.

The region Yq12 showed a length variation from less than 2 % to more than 60 % of the total

length of the Y-chromosome, with a maximum at 30 % in more than 50 % of the cases.

The number of fluorescence polymorphisms per proband reached from 0 to 15, with a maximum of 6 in amniocytes and 4 in lymphocytes.

Duplications of the heterochromatin blocks were most frequent in chromosomes 9 and least in 16.

Complete pericentric inversions occurred in 1, 3, 4, 9, and not in 16, with a maximum in 4, followed by 3, 9, and 1, thus stating new maxima of heterochromatin inversions in the human karyotype.

Examples of applying polymorphism analyses to clinical cytogenetics and to twin research demonstrated the relevance of this investigation system.

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