ORIGINAL ARTICLE

DETECTING SEX-BIASED GENE FLOW IN AFRICAN-AMERICANS THROUGH THE ANALYSIS OF INTRA- AND INTER-POPULATION VARIATION AT MITOCHONDRIAL DNA AND Y- CHROMOSOME MICROSATELLITES

Battaggia C¹, Anagnostou P^{1,3}, Bosch I⁴, Brisighelli F⁴, Destro-Bisol G^{1,3}, Capocasa M^{2,3,*}

***Corresponding Author:** Dr. Marco Capocasa, Dipartimento di Biologia e Biotecnologie “Charles Darwin”, Università “La Sapienza”, Roma, Italy, P.le A. Moro 5, 00185 Roma, Italia; Tel.: +39-6-4991-2725; Fax: ++39-6-4991-2771; E-mail: marco.capocasa@uniroma1.it

ABSTRACT

This study reports on variations at the mitochondrial DNA (mtDNA) hypervariable region 1 (HVR-1) and at seven Y-chromosome microsatellites in an African-American population sample from Chicago, IL, USA. Our results support the hypothesis that the population studied had undergone a European male-biased gene flow. We show that comparisons of intra- and inter-population diversity parameters between African-Americans, Europeans and Africans may help detect sex-biased gene flow, providing a complement to quantitative methods to estimate genetic admixture.

Keywords: Admixture, African-Americans, Mitochondrial DNA (mtDNA), Sex-biased gene flow, Y-chromosome

INTRODUCTION

The European contribution to the gene pool of African populations deported to the United States

of America in the course of the Atlantic slave trade may be regarded to as a paradigmatic case of gene flow in human populations [1,2]. Coherently, studies of genetic structure of present day African-Americans have attracted a particular interest in molecular anthropology [3]. The earliest investigations based on protein loci estimated a 4.0-30.0% proportion of European genes in African-Americans, pointing to a higher admixture in northern rather than in southern US regions [6]. More recent studies, based on autosomal DNA poly-morphisms, highlighted the level of admixture for northern US populations to be lower than previously thought, and the lack of a close relationship between latitude and extent of European admixtures [7]. The introduction of unilinear DNA polymorphisms of mitochondrial DNA (mtDNA) and non recombining portions of Y-chromosome, has made it possible to separately study the male and female European contribution to the African-American gene pool [7-9]. Finally, further refinements have been obtained with the introduction of genome wide approaches [10,11].

In a previous study, we used 10 autosomal microsatellites and an Alu polymorphism to explore the genetic structure of an African-American population from Chicago, IL, USA [12]. In this study, we analyzed the variation at the mtDNA hypervariable region 1 (HVR-1) and at seven microsatellites of the Y-chromosome in the same population sample. Using a broad population dataset, we showed that comparisons of intra- and inter-population diversity

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¹ Dipartimento di Biologia Ambientale, Università “La Sapienza”, Roma, Italia

² Dipartimento di Biologia e Biotecnologie “Charles Darwin”, Università “La Sapienza”, Roma, Italia

³ Istituto Italiano di Antropologia, Roma, Italia

⁴ Istituto di Medicina Legale, Università Cattolica, Roma, Italia

parameters between African-Americans, Europeans and Africans may help detect sex-biased gene flow, providing a complement to quantitative methods to estimate genetic admixtures.

MATERIALS AND METHODS

A total of 50 individuals (23 males and 27 females) was available for this study. Appropriate informed consent was obtained from all individuals participating in the study. Sequencing of the HVR-1 of mtDNA from nucleotide (nt) positions 16024 to 16383, and determination of size variations at loci DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392 and DYS393, was performed as previously described [13,14]. The amplified products were analyzed in polyacrylamide denaturing gels using a semi-automated DNA sequencer (A.L.F. express; Pharmacia Biotech, Uppsala, Sweden). Allelic and internal standards were used for microsatellite typing.

The dataset for comparison was built selecting data from the mtDNA and Y-chromosomal literature for European and 10 African populations. We have preferentially used widely dispersed populations which could have contributed to the gene pool of present day African-Americans and/or have not undergone drift events in their evolutionary history (Supplementary Table 1). Moreover, data for four African-American samples were considered [15-17].

Parameters of intra-population diversity [haplotype diversity (HD) and mean number of pairwise differences (MNP)] and genetic distances between populations were calculated using the Arlequin software ver. 3.5 [18]. Given the relatively high differentiation expected between African and European haplotypes, molecular methods were used in addition to the haplotypic ones to detect further signatures of admixture.

RESULTS AND DISCUSSION

Mitochondrial DNA and Y-Chromosomal Variation. DNA sequences of the HVR-1 were determined in 50 unrelated individuals (Table 1). We found nucleotide differences at 63 out of 360 positions with respect to the 'consensus' sequence [19], five of which were transversions [16114 (A>C); 16183 (A>C); 16188 (C>G); 16258 (T>A)]. In total,

we were able to define 40 different lineages, with frequencies ranging from 0.002 to 0.008. We obtained a HD value of 0.987 ± 0.008 and a MNP value of 7.673 ± 3.637 .

Y-Chromosomal haplotypes, built using seven microsatellite loci, were determined in 23 individuals (Table 2). Nineteen different haplotypes were observed, with frequencies ranging from 0.042 to 0.125. We obtained a HD value of 0.996 ± 0.014 and a MNP value of 4.383 ± 2.246 .

Intra- and Inter-population Diversity in African-American and African Populations. Since the seminal work by [20], the approach based on the simultaneous use of mtDNA and Y-chromosomal polymorphisms has been used in numerous studies of human genetic diversity [21]. This approach may also turn out to be useful in the case of African-American populations. In fact, the availability of unilocal DNA polymorphisms offers the opportunity to detect possible signatures of a sex-biased gene flow from Europeans to African-Americans. Intriguingly, whereas a male-biased gene flow was expected on the basis of historical knowledge, demographic data indicate that since 1960, most of the mixed marriages involved African-American males to females of European origin [22].

In a study carried out among African-American students at the Texas University at Austin, Austin, TX, USA, Hsieh and Sutton [23] compared admixture estimates based on mtDNA and protein loci, concluding that there is no evidence of a European male-biased gene flow. On the other hand, the latter case was supported by more recent studies carried out in a number of African-American populations from different areas and based on population specific mtDNA and Y-chromosome alleles [7-10].

Estimates of sex-specific admixture suffer from two different orders of limitations. Obtaining reliable African parental populations is difficult for three main reasons. First, present day African-Americans originate from populations scattered in a wide area from the central and western parts of the continent. Second, it is tricky to identify the populations in continuity with those from which slaves were taken to the US, as their demography was reshaped by slave capture and forced migrations [24]. Third, there is also substantial variation among sub-Saharan groups for mtDNA and Y-chromosome polymorphisms [25,26], so that results may vary substantially depending on

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Table 2. Seven loci Y chromosome haplotype frequencies in 23 African-American subjects.

Sample	Number of Haplotypes	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	Frequency (%)	Standard Deviations
AA01	2	17	14	31	21	10	11	15	0.0870	0.0601
AA02	3	16	13	30	21	10	11	14	0.1304	0.0718
AA03	1	14	13	29	24	10	13	13	0.0435	0.0435
AA04	1	15	14	33	21	10	11	13	0.0435	0.0435
AA05	1	16	14	31	25	10	11	13	0.0435	0.0435
AA06	1	14	12	28	25	10	11	13	0.0435	0.0435
AA07	1	15	14	32	21	10	11	13	0.0435	0.0435
AA10	1	14	12	28	24	11	13	13	0.0435	0.0435
AA11	1	15	14	31	21	10	10	13	0.0435	0.0435
AA12	1	17	13	30	21	11	11	15	0.0435	0.0435
AA13	1	15	13	30	22	10	13	13	0.0435	0.0435
AA14	1	14	13	29	24	11	13	14	0.0435	0.0435
AA16	1	14	12	28	22	10	11	13	0.0435	0.0435
AA17	1	17	14	31	20	10	11	14	0.0435	0.0435
AA18	1	15	12	27	23	10	14	13	0.0435	0.0435
AA19	2	15	13	31	21	10	11	13	0.0870	0.0601
AA22	1	14	14	30	24	11	13	13	0.0435	0.0435
AA223	1	14	13	30	24	11	13	13	0.0435	0.0435
AA24	1	14	12	28	23	10	11	13	0.0435	0.0435

Table 3. Haplotype and molecular intra-population diversity measurement in African-Americans and Africans. Mitochondrial DNA estimates refer to nucleotide positions 16090 to 16365. Y-chromosome estimates refer to the five loci haplotypes (DYS389I, DYS3910, DYS391, DYS392 and DYS393).

		mtDNA				Y-Chromosome			
		<i>n</i>	HD (SE)	MNPD (SE)	MNPD/HD	<i>n</i>	HD (SE)	MNPD (SE)	MNPD/HD
African-Americans 1	AA1	50	0.984 (0.009)	7.291 (3.471)	7.410	23	0.976 (0.0201)	2.818 (1.543)	2.887
African-Americans 2	AA2	230	0.993 (0.002)	7.667 (3.587)	7.721	106	0.978 (0.0045)	2.839 (1.508)	2.902
African-Americans 3	AA3	78	0.987 (0.006)	6.651 (3.173)	6.739	426	0.984 (0.0022)	3.034 (1.584)	3.082
Bakaka	BAK	50	0.983 (0.008)	9.457 (4.414)	9.620	49	0.796 (0.0270)	1.232 (0.796)	1.548
Bamileke	BAM	48	0.988 (0.007)	7.869 (3.725)	7.965	50	0.850 (0.0375)	1.875 (1.091)	2.207
Bassa	BAS	46	0.991 (0.007)	9.194 (4.306)	9.277	49	0.843 (0.0355)	1.764 (1.040)	2.093
Beti	BET	48	0.965 (0.012)	8.395 (3.955)	8.699	36	0.916 (0.0232)	1.738 (1.035)	1.898
Cabinda	CAB	110	0.988 (0.003)	8.960 (4.159)	9.069	72	0.900 (0.0168)	1.993 (1.138)	2.215
Ewondo	EWO	53	0.983 (0.008)	9.666 (4.500)	9.833	39	0.804 (0.0449)	1.505 (0.926)	1.871
Fulbe	FUL	34	0.975 (0.016)	6.460 (3.134)	6.626	27	0.946 (0.0253)	2.644 (1.456)	2.795
Guinea Bissau	GUB	372	0.985 (0.002)	7.466 (3.496)	7.580	162	0.937 (0.0110)	2.183 (1.215)	2.330
Mozambique	MOZ	107	0.967 (0.007)	8.216 (3.839)	8.496	112	0.907 (0.0152)	1.975 (1.125)	2.177
Ngoumba	NGO	44	0.984 (0.007)	8.798 (4.137)	8.941	36	0.929 (0.0209)	2.324 (1.301)	2.502

HD: haplotype diversity; SE: standard error; MNPD: mean number of pairwise differences.

Figure 1. Scatterplots of MNP and HD values for mtDNA and Y-chromosomes in the populations under study. Abbreviations are given in Table 3. Dotted lines indicate the 95.0% confidence interval for the regression line obtained for African populations.

netic systems generally produce values with extended confidence intervals that substantially overlap (*e.g.*, see estimates for Maryland, Texas and Virginia of table 5 in [9]).

A possible approach is represented by the search for possible effects of gene flow on intra- and inter-population genetic variation. Since the two genetic systems cannot be directly compared due to substantial difference in types and rate of mutations and demographic dynamics, we have compiled a paired mtDNA and Y-chromosomal population database including both European and African populations [25,28]. This makes it possible to compare the extent of mtDNA and Y-chromosome variation within a given population, by contrasting it with data from other populations included in the database.

An incoming European male-biased gene flow predicts a greater ratio of Y-chromosome to mtDNA diversity for African-Americans than for African populations. This expectation is met by our African-American sample. In fact, their mtDNA HD is equal or lower than in five African populations, whereas their Y-chromosomal value is higher (Table 3). The greater diversity of African-Americans is even more evident comparing MNP, a measure which weighs molecular differences. In this case, the diversity between Europeans and Africans may have a greater impact than happens with HD, in which the extent of inter-haplotypic differentiation is not taken into account. In fact, there are eight African populations with a mtDNA MNP higher than our

African-Americans, and only one for the Y-chromosomal polymorphisms. It is noteworthy that the latter case involves the Fulbe, a population which has been shown to have undergone a male-biased gene flow due to African migrations [29]. Although no inference on asymmetric gene flow can be made for other African-American populations, it is remarkable that the ratio between molecular and haplotypic measures of intra-population diversity are the lowest for mtDNA and the highest for Y-chromosome polymorphisms (Figure 1).

A male-biased gene flow in African-Americans from Chicago, IL, USA is also supported by the analysis of genetic distances (Table 4). They show the lowest genetic distance (both molecular and haplotypic from Europeans for Y-chromosomal polymorphisms but not for the mtDNA ones. Furthermore, the ratio of mtDNA to Y-chromosomal genetic distances from Europeans is markedly higher (18.3-38.3% for molecular and haplotypic distances, respectively) than all African groups. The introgression of Y-chromosomes into other African-American populations is consistent with their comparatively low genetic distance from Europeans.

To sum up, our results coherently support the hypothesis that the African-American sample under study has undergone a European male-biased gene flow. On a more general note, our research showed that comparative analysis of intra- and inter-population variation for Y-chromosome and mtDNA polymorphisms in a broad dataset including African and

Table 4. Average genetic distances of Africans and African-Americans from Europeans.

		Molecular Distances			Haplotype Distances		
		mtDNA	Y Chrom.	mt/Y	mtDNA	Y Chrom.	mt/Y
African-Americans 1	AA1	0.219	0.281	0.779	0.027	0.024	0.151
Bakaka	BAK	0.240	0.624	0.385	0.028	0.120	0.232
Bamileke	BAM	0.267	0.456	0.585	0.025	0.095	0.270
Bassa	BAS	0.266	0.598	0.445	0.024	0.098	0.241
Beti	BET	0.312	0.477	0.653	0.036	0.058	0.624
Cabinda	CAB	0.250	0.435	0.575	0.025	0.066	0.374
Ewondo	EWO	0.278	0.528	0.527	0.028	0.114	0.244
Fulbe	FUL	0.198	0.399	0.496	0.032	0.038	0.832
Guinea Bissau	GUB	0.184	0.560	0.328	0.026	0.052	0.501
Mozambique	MOZ	0.254	0.415	0.612	0.035	0.067	0.528
Ngoumba	NGO	0.248	0.377	0.659	0.025	0.058	0.427

Europeans groups, may help detect admixture signatures, providing a useful complement to methods for admixture estimates.

REFERENCES

1. Curtain PD. The Atlantic Slave Trade: a Census. Madison: University of Wisconsin Press, 1969.
2. Conniff ML, Davis TJ.; Africans in the Americas: a History of the Black Diaspora. New York: St. Martin's Press, 1994.
3. Salas A, Richards M, Lareu MV, Scozzari R, Coppa A, Torroni A, *et al.* The African diaspora: mitochondrial DNA and the Atlantic slave trade. *Am J Hum Genet.* 2004; 74(3): 454-465.
4. Salas A, Carracedo A, Richards M, Macaulay V. Charting the ancestry of African-Americans. *Am J Hum Genet.* 2005; 77(4): 676-680.
5. Reed FA, Tishkoff SA. African human diversity, origins and migrations. *Curr Opin Genet Develop.* 2006; 16(6): 597-605.
6. Reed TE. Caucasian genes in American Negroes. *Science.* 1969; 165(3895): 762-768.
7. Parra EJ, Marcini A, Akey J, Martinson J, Batzer MA, Cooper R, *et al.* Estimating African-American admixture proportions by use of population-specific alleles. *Am J Hum Genet.* 1998; 63(6): 1839-1851.
8. Parra EJ, Kittles RA, Argyropoulos G, Pfaff CL, Hiester K, Bonilla C, *et al.* Ancestral proportions and admixture dynamics in geographically defined African-Americans living in South Carolina. *Am J Phys Anthropol.* 2001; 114(1): 18-29.
9. Kayser M, Brauer S, Schädlich H, Prinz M, Batzer MA, Zimmerman PA, *et al.* Y Chromosome STR haplo-types and the genetic structure of US populations of African, European, and Hispanic ancestry. *Genome Res.* 2003; 13(4): 624-634.
10. Lind JM, Hutcheson-Dilks HB, Williams SM, Moore JH, Essex M, Ruiz-Pesini E, *et al.* Elevated male European and female African contributions to the genomes of African-American individuals. *Hum Genet.* 2007; 120 (5): 713-722.
11. Tishkoff SA, Reed FA, Friedlaender FR, Ehret C, Ranciaro A, Froment A, *et al.* The genetic structure and history of Africans and African-Americans. *Science.* 2009; 324(5930): 1035-1044.
12. Destro-Bisol G, Maviglia R, Caglià A, Boschi I, Spedini G, Pascali V, *et al.* Estimating European admixture in African-Americans by using microsatellites and a micro-satellite haplotype (CD4/Alu). *Hum Genet.* 1999; 104(2): 149-157.
13. Kayser M, de Knijff P, Dieltjes P, Krawczak M, Nagy M, Zerjal T, *et al.* Applications of microsatellite-based Y-chromosome haplotyping. *Electrophoresis.* 1997; 18(9): 1602-1607.
14. Coia V, Boschi I, Trombetta F, Cavulli F, Montinaro F, Destro-Bisol G, *et al.* Evidence of high genetic variation among linguistically diverse populations on a micro-geographic scale: a case

- study of the Italian Alps. *J Hum Genet.* 2012; 57(4): 254-260.
15. Torres JB, Doura MB, Keita SOY, Kittles RA. Y Chromosome lineages in men of West African descent. *PLoS One.* 2012; 7(1): e29687.
 16. Kline MC, Vallone PM, Redman JW, Duewer DL, Calloway CD, Butler JM. Mitochondrial DNA typing screens with control region and coding region SNPs. *J Forensic Sci.* 2005; 50(2): 377-385.
 17. Ely B, Wilson JL, Jackson F, Jackson BA. African-American mitochondrial DNAs often match mtDNAs found in multiple African ethnic groups. *BMC Biol.* 2006; 4: 34.
 18. Excoffier L, Lischer HE. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour.* 2010; 10(3): 564-567.
 19. Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat Genet.* 1999; 23(2): 147.
 20. Seielstad MT, Minch E, Cavalli-Sforza LL. Genetic evidence for a higher female migration rate in humans. *Nat Genet.* 1998;20(3): 278-280.
 21. Destro-Bisol G, Coia V, Boschi I, Verginelli F, Caglià A, Pascali V, *et al.* The analysis of variation of mtDNA hypervariable region 1 suggests that Eastern and Western Pygmies diverged before the Bantu expansion. *Am Nat* 2004; 163(2);212-226.
 22. United States Census Bureau 2000. url: <http://www.census.gov/main/www/cen2000.html>.
 23. Hsieh CL, Sutton HE. Mitochondrial and nuclear variants in a US black population: origins of a hybrid population. *Ann Hum Genet.* 1992;56(Pt 2): 105-112.
 24. Whatley W, Gillezeau R. The impact of the transatlantic slave trade on ethnic stratification in Africa. *Am Econ Rev.*2011; 101(3): 571-576.
 25. Rosa A, Brehm A. African human mtDNA phylo-geography at-a-glance. *J Anthropol Sci.* 2011; 89: 25-58.
 26. Montano V, Ferri G, Marcari V, Batini C, Anyaele O, Destro-Bisol G, *et al.* The Bantu expansion revisited: a new analysis of Y chromosome variation in Central Western Africa. *Mol Ecol.* 2011; 20(13): 2693-2708.
 27. Mitchell P. Genetics and southern African pre-history: an archaeological view. *J Anthropol Sci.* 2010; 88: 73-92.
 28. Francalacci P, Morelli L, Useli A, Sanna D. The history and geography of the Y chromosome SNPs in Europe: an update. *J Anthropol Sci.* 2010; 88: 207-214.
 29. Cruciani F, Santolamazza P, Shen P, Macaulay V, Moral P, Olckers A, *et al.* A back migration from Asia to sub-Saharan Africa is supported by high-resolution analysis of human Y-chromosome haplotypes. *Am J Hum Genet.* 2002; 70(5): 1197-1214.

CLINICAL IMPACT OF PROXIMAL AUTOSOMAL IMBALANCES

Hamid AB, Weise A, Voigt M, Bucksch M, Kosyakova N, Liehr T,* Klein E

***Corresponding author:** Dr. Thomas Liehr, Universitätsklinikum Jena, Institut für Humangenetik, Kollegiengasse 10, D-07743 Jena, Germany; Tel.: +49-3641-935533; Fax. ++49-3641-935582; E-mail: i8lith@mti.uni-jena.de

ABSTRACT

Centromere-near gain of copy number can be induced by intra- or inter-chromosomal rearrangements or by the presence of a small supernumerary marker chromosome (sSMC). Interestingly, partial trisomy to hexasomy of euchromatic material may be present in clinically healthy or affected individuals, depending on origin and size of chromosomal material involved. Here we report the known minimal sizes of all centromere-near, *i.e.*, proximal auto-somal regions in humans, which are tolerated; over 100 Mb of coding DNA are comprised in these regions. Additionally, we have summarized the typical symptoms for nine proximal autosomal regions including genes obviously sensitive to copy numbers. Overall, studying the carriers of specific chromosomal imbalances using genomics-based medicine, combined with single cell analysis can provide the genotype-phenotype correlations and can also give hints where copy-number-sensitive genes are located in the human genome.

INTRODUCTION

Autosomal Proximal Chromosome Imbalances. The finding of unbalanced chromosomal abnormalities (UBCA) was recently reviewed and

summarized from a total of 200 families. The UBCA usually involve several megabases of DNA. Carriers of such UBCA are ascertained due to adverse reproductive effects or dysmorphic and/or mentally retarded offspring; the carriers themselves have an otherwise normal phenotype. Unbalanced chromosomal abnormalities have been reported for more than 50 euchromatic regions of almost all human autosomes [1,2].

Unbalanced chromosomal abnormalities leading to gain of genetic relevant material within the autosomal centromere-near region were not comprehensively followed in the above mentioned studies [1,2]. Such centromere-near, *i.e.*, proximal chromosomal imbalances (C-UBCA), can be induced by small supernumerary marker chromosomes (sSMCs) [3,4] and also by intrachromosomal duplications [4]. While the latter are rare events and no reliable data on their frequency is available, sSMCs are present in 0.043% of human beings [5]. With a given population size of 7×10^9 individuals, 3×10^6 sSMC carriers are presently alive. As $\sim 2/3$ of these do not show any symptoms, $\sim 2 \times 10^6$ do not even know of their condition. Euchromatin is present in $\sim 36.0\%$ of those sSMC cases that do not lead to any clinical symptoms (Table 1) [6]. *N.B.:* sSMC, irrespective of origin and genetic constitution may cause fertility problems, especially in males [7]. Thus, infertility was not considered as an 'abnormal phenotype' in this study.

Even though partial trisomy is the most frequent imbalance induced by sSMC, tetra- or even hexasomy

Table 1. Summarized here are 478 autosomal derived sSMC cases, which are characterized in detail for their size and genetic content; all of them can be found on the sSMC homepage [6]. All these cases are not associated with any clinical abnormalities. In 174, *i.e.*, 36.4%, proximal euchromatic material was present. As can be seen, the rates of cases with and without euchromatin vary from chromosome to chromosome. In general, in acrocentric derived sSMC, cases without euchromatin are in the majority, while it is the other way round in most non acrocentric derived sSMC.

Chromosomes	Cases With Euchromatin	Cases Without Euchromatin
1	4	11
2	9	2
3	10	2
4	1	0
5	8	5
6	1	1
7	1	0
8	9	2
9	18	1
10	6	1
11	2	1
12	6	2
13	1	0
14	6	53
15	35	136
16	11	8
17	2	0
18	9	2
19	5	1
20	6	6
21	8	1
22	16	69
Overall	174	304

of proximal euchromatin may be present in clinically healthy individuals [6]. Here we present the latest known proximal, centromere-near regions and their minimal molecular borders. The corresponding index cases were previously published and are summarized on the sSMC homepage [6]. This study intends to give a review on the clinical impact of proximal autosomal imbalances. A special focus is hereby laid on gain of copy numbers. For this, the following steps were necessary: *i)* define the pericentric regions that can be present as additional copy(ies) without causing any clinical phenotype. *ii)* After the definition of such copy number insensitive regions, in a second step, proximal autosomal regions including genes potentially sensitive to copy numbers can be defined. *iii)* Such copy number sensitive regions can be correlated

with specific, typical symptoms; the latter already being possible for nine centromere-near regions in this study and there will be more in the future.

What Can be Learned From Cases With Chromosome Imbalances? It was nicely summarized back in 1993 [8] that structural autosomal imbalances may lead in ‘typical cases’ to syndromes with a complex of minor anomalies and/or congenital malformations. The latter ‘suggests the importance of gene interaction in determining the phenotypic picture of autosomal imbalance syndromes’ [8]. Duplication-related syndromes are much more frequent than deletion-related ones, and thus, it is common sense that in general, duplications of several Mb in size are better tolerated by the human genome than deletions of the same size. This has also recently been confirmed on the level of micro-duplications and -deletions [9]. Overall, chromosomal imbalances can point towards dosage sensitive genes being responsible for specific syndromes or clinical features. A good example is the dosage sensitive peripheral myelin protein 22 (PMP22) gene in 17p11.2: a duplication of 1.4 Mb including PMP22 leads to the hereditary motor and sensory neuropathy type 1A and the reciprocal deletion to the hereditary neuropathy with liability to pressure palsies. However, also specific mutations in PMP22 itself can cause the identical syndromes [10].

Moreover, UBCA of several Mb in size have been reported, which surprisingly, do not have any clinical consequences [1,2]. At the same time, they are not pure copy number variants (CNV) such as those recently found for a cytogenetically visible amplified region in 8q21.2 [11]. In summary, there are genetically relevant regions which can be tolerated if ‘amplified’ as three or more copies; the reason for that is most likely that they do not comprise dosage sensitive genes. In summary, studying carriers of specific chromosomal imbalances can provide genotype-phenotype-correlations, and also give hints as to where copy-number-(in)sensitive genes are located in our genome.

Where to Find Proximal Chromosome Imbalances in Humans. Centromere-near imbalances may principally appear as deletions or duplications. However, practically no reports of proximal deletions are available in the literature. The only exceptions are offspring of carriers with an sSMC formed by the McClintock mechanism [12], *e.g.*, as reported for a child

having the karyotype 47,XY,del(2) (p12p11.1) due to a maternal cytogenetic condition (47,XX,del(2) (p12p11.1),+r(2)(:p12→p11.1::) [13]. Only eight corresponding cases are available in the literature [6] and all these patients were severely affected.

The best suited patients to study proximal duplications would be those with proximal intrachromosomal rearrangements, as direct or inverted duplications or unbalanced insertions, because these cases would be non mosaic [4]. However, such cases are scarce (summarized in Table 2). Most of these ~200 cases were only studied cytogenetically and no information on the molecular size of their duplicated region is available [6].

In contrast, the largest and best characterized group where to find proximal duplications are patients with sSMC [3-7,14-16]. Besides their cytogenetic characterization, more and more cases were characterized at the molecular level by array-comparative genomic hybridization (aCGH) studies [4,17,18]. However, when analyzing this group of patients one has to consider the following drawbacks: *i)* sSMC carriers may be mosaic with normal cell lines and/or may have different levels of mosaicism in different tissues; thus, harmful sSMC sizes may be rated as harmless [19], and *ii)* also harmless sSMC may be considered to be harmful if they appear together with a uniparental disomy (UPD) [20], or a mutation in a monogenic disorder gene [21]. Thus, results for regions including or excluding most likely dosage-sensitive genes, *i.e.*, C-UBCA, have to be handled carefully. Nevertheless, sSMC carriers are much more frequent and better characterized on the molecular level than intrachromosomal duplications, and are thus used here as a model system for proximal duplications.

MATERIALS AND METHODS

This study is based on the data summarized on the sSMC-homepage [6]. All raw data is freely available and can be followed down to each individual case. The data used for the present study is summarized in Tables 1 through 4.

Proximal Chromosomal Imbalances Without Clinical Consequences. The available in detail characterized sSMC cases [6] were studied by various approaches. In the majority of cases, the sSMC were characterized exclusively by molecular cytogenetics and the breakpoints are given as cytobands without

Table 2. The ~200 case reports of proximal intra-autosomal duplications are summarized per autosome and distinguished in clinically normal and abnormal cases [6].

Chromosomes	Clinically Normal	Clinically Abnormal
1	1	8
2	0	5
3	0	1
4	0	2
5	0	4
6	0	3
7	0	2
8	0	2
9	4	2
10	1	4
11	3	3
12	0	11
13	0	2
14	0	1
15	32	>50
16	2	16
17	0	5
18	22	3
19	0	0
20	0	4
21	0	3
22	3	3
Overall	68	>134

molecular assessment of the exact breakpoint. In addition, there are already numerous sSMC cases characterized by well-defined locus-specific probes used in fluorescence *in situ* hybridization (FISH) and/or by aCGH [6]. In Table 3, the presently characterized C-UBCA are summarized. Overall, it could be shown that at least 96.8 Mb of the proximal chromosomal regions are tolerated as triplicates or more (Table 3). While for proximal 6q there is neither molecular nor cytogenetic hint for any dosage independent C-UBCA, in all other proximal autosomal parts at least cytogenetic evidence for C-UBCA in healthy individuals is there.

Except for proximal parts of 1q, 6p, 6q and 13q, there are molecular hints on C-UBCA for every chromosome arm, being at least between 0.07 and 10.23 Mb in size. According to cytogenetics, no less than 16 of the 39 autosomal proximal non dosage sensitive regions (= C-UBCA) are larger than already proven by aCGH, *i.e.*, 2p, 3p, 3q, 6p, 8p, 8q, 9p, 9q, 10p, 10q, 11p, 12p, 19p, 19q, 20p and 22q (Table 3).

Table 3. All 39 proximal autosomal regions containing no copy number-sensitive genes are summarized. According to the sSMC-homepage [6], the positions and sizes of duplications are given in columns 2 and 3. Column 4 summarizes if the C-UBCA may be larger according to non molecular cytogenetic results. Additionally, in the last two columns it is indicated if the C-UBCA is based on mosaic or non mosaic sSMC cases, and if more than three copies were present in the corresponding index cases. (UCSC: University of California Santa Cruz genome browser; <http://genome.ucsc.edu>).

Chromosomes	Molecular Bands (UCSC hg18, 2006)	Size (Mb)	Region Expected to be Larger According to Molecular Cytogenetic Results	Mosaic	>Three Copies
1p	118.33-121.10	2.80	[+/-]	(-)	-
1q	142.40-??.??	n.a	[++]	+	-
2p	89.60-91.00	1.40	[++]	+	{+;4}
2q	95.70-101.58	5.88	[+/-]	+	{+;4}
3p	87.60-89.40	1.80	[++]	-	{+;4}
3q	93.20-96.01	2.81	[++]	+	{+;4}
4p	44.03-48.70	4.67	[+/-]	+	-
4q	52.40-62.63	10.23	[+/-]	+	-
5p	37.21-45.80	1.41	[+/-]	-	{+;4}
5q	50.50-55.27	4.77	[+/-]	+	{+;4}
6p	??.??-58.40	n.a.	[++]	+	-
6q	63.40-??.??	n.a	[+/-]	n.a.	n.a.
7p	56.45-57.40	0.95	[+/-]	+	-
7q	61.10-67.00	5.90	[+/-]	+	-
8p	42.50-43.20	0.70	[++]	+	{+;4}
8q	48.10-48.30	0.20	[++]	+	-
9p	42.96-46.70	3.74	[++]	+	{+;4}
9q	70.00-70.50	0.50	[++]	+	-
10p	34.75-38.80	4.05	[++]	(-)	-
10q	42.10-43.82	1.72	[++]	-	-
11p	50.95-51.40	0.45	[++]	-	-
11q	56.40-60.23	3.83	[+/-]	+	{+;4}
12p	28.47-33.20	4.73	[++]	+	-
12q	36.50-39.90	3.40	[+/-]	+	-
13q	18.40-??.??	n.a.	[++]	-	+;4
14q	19.10-19.88	0.78	[+/-]	-	+;4
15q	18.40-21.05	2.65	[+/-]	-	+;4 +;6
16p	28.86-34.40	5.54	[+/-]	-	-
16q	45.50-46.02	0.52	[+/-]	-	{+;4}
17p	18.68-22.10	3.42	[+/-]	+	-
17q	23.20-23.32	0.12	[+/-]	+	-
18p	12.80-15.40	2.60	[+/-]	(-)	{+;4}
18q	17.30-18.12	0.82	[+/-]	+	-
19p	22.98-26.70	3.72	[++]	+	{+;4}
19q	30.20-36.90	6.70	[++]	+	-
20p	24.96-25.70	0.74	[++]	+	{+;4}
20q	28.40-29.93	1.53	[+/-]	+	{+;4} {+;6}
21q	13.20-14.85	1.65	[+/-]	-	{+;4}
22q	16.30-16.37	0.07	[++]	-	-

+/-: no larger C-UBCA expected; (-): in part mosaic index cases; n.a.: not available; ++: larger according to molecular cytogenetic results; {}: mosaic; -: no mosaic; +: mosaic; +;4: four copies; +;6: six copies.

Table 4. Clinical consequences of larger proximal autosomal imbalances for nine corresponding regions are summarized [6]. Common specific clinical symptoms were observed in crucial parts of the 5-12 cases, each; for 4q only two cases were available, both showing overgrowth. Unspecific symptoms such as mental retardation developmental delay or dysmorphic face were neglected but normally also present in these cases. For 22q cases with cat eye syndrome were excluded.

Symptom – Chromosome Region	1p	1q	4q	5q	7q	10p	17p	18q	22q
Autism	+	–	–	–	–	–	–	–	–
Finger/toe/foot malformations	+	–	–	–	+	–	–	–	–
Growth retardation	–	+	–	–	–	+	–	–	–
Heart defects	–	+	–	–	–	–	–	–	–
Hernia	–	–	–	+	–	–	+	–	–
Hypotonia	+	–	–	+	–	–	–	–	+
Macrocephaly	–	–	–	+	–	–	–	–	–
Overgrowth	–	–	+	–	+	–	–	–	–
Seizures	–	–	–	–	–	–	–	–	–
Urethral problems	–	–	–	–	–	–	–	+	+

Twenty-four of the 38 informative proximal autosomal regions are based on mosaic sSMC cases. Thus, the data summarized in Table 3 is still to be considered as preliminary in those cases, even though in >99.0% of sSMC cases, mosaicism detected in peripheral blood plays a minor role for the clinical outcome [22]. Mosaicism may play a role for the phenotype if its rates are variant in different tissues of the body [23]. The C-UBCA regions 1p, 3p, 5p, 10p, 10q, 11p, 13q, 14q, 15q, 16p, 16q, 18p, 21q and 22q were reported in non mosaic cases. The remaining regions await such proof.

Another issue to be reflected is the copy number of a C-UBCA tolerated by the human genome. At least, for 15 C-UBCA low mosaics (maximum 20.0%) of cells having four (or in one case of 20q up to six) copies of the corresponding regions are tolerated. The C-UBCA of chromosomes 13q, 14q and 15q can be present in four copies in normal carriers in 100.0% of the studied cells. For 15q, even six copies are possible (Table 3).

Autosomal Proximal Imbalances Leading to Clinical Consequences. In case an sSMC or an intrachromosomal duplication is larger than the critical region for harmless sSMC, as summarized Table 3, a variety of clinical problems can be the consequence for the sSMC carrier. Besides well-known syndromes such as isochromosome-12p (Pallister-Killian syndrome) [24], -15q [25], -18p [26] or -22q (cat-eye-syndrome) [27], a variety of symptoms can be associated with an sSMC-induced imbalance [3,6]. In most cases the correlated symptoms are rather non

specific. However, first potentially specific symptom combinations for nine corresponding imbalances are summarized in Table 4. In future, it should be possible for at least some of these proximal autosomal imbalances to define new, possibly even clinically recognizable, syndromes [3].

CONCLUSIONS

The sSMC are a long time underestimated source for the understanding of proximal chromosomal imbalances in humans. New information on regions of the human genome, possibly inert to copy number changes, can be acquired from this group of patients. Moreover, effects such as heterochromatinization [3] or feedback-loops in gene regulation [28] might also be considered for the understanding of the effects of such imbalances. Comprehensive studies of more aberrant cases will also lead to new genotype-phenotype correlations and to the possibility of a clinical sub-differentiation of more sSMC cases. All these goals can only be achieved by a sophisticated balance of single cell analysis (such as in mosaic cases) and genomics-based medicine (such as for array-based approaches).

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REFERENCES

- Barber JC. Directly transmitted unbalanced chromosome abnormalities and euchromatic variants. *J Med Genet.* 2005; 42(8): 609-629.
- The chromosome anomaly collection: http://www.ngri.org.uk/Wessex/collection/ubca_chart.htm. (Accessed June 01 2011).
- Liehr T, Mrasek K, Weise A, Duke A, Rodríguez L, Martínez Guardia N, *et al.* Small supernumerary marker chromosomes – progress towards a genotype-phenotype correlation. *Cytogenet Genome Res.* 2006; 112(1-2): 23-34.
- Liehr T, Stumm M, Wegner RD, Bhatt S, Hickmann P, Patsalis PC, *et al.* 10p11.2 to 10q11.2 is a yet unreported region leading to unbalanced chromosomal abnormalities without phenotypic consequences. *Cytogenet Genome Res.* 2009; 124(1): 102-105.
- Liehr T, Weise A. Frequency of small supernumerary marker chromosomes in prenatal, newborn, developmentally retarded and infertility diagnostics. *Int J Mol Med.* 2007; 19(5): 719-731.
- sSMC homepage: <http://www.fish.uniklinikum-jena.de/sSMC.html>. (Accessed December 12, 2011).
- Manvelyan M, Riegel M, Santos M, Fuster C, Pellestor F, Mazaurik ML, *et al.* Thirty-two new cases with small supernumerary marker chromosomes detected in connection with fertility problems: detailed molecular cytogenetic characterization and review of the literature. *Int J Mol Med.* 2008; 21(6): 705-714.
- Lurie IW. Autosomal imbalance syndromes: genetic interactions and the origin of congenital malformations in aneuploidy syndromes. *Am J Med Genet.* 1993; 47(3): 410-416.
- Berg JS, Potocki L, Bacino CA. Common recurrent microduplication syndromes: diagnosis and management in clinical practice. *Am J Med Genet. A.* 2010; 152A(5): 1066-1078.
- Roa BB, Lupski JR. Molecular genetics of Charcot-Marie-Tooth neuropathy. *Adv Hum Genet.* 1994; 22(1): 117-152.
- Manvelyan M, Cremer FW, Lancé J, Kläs R, Kelbova C, Ramel C, *et al.* New cytogenetically visible copy number variant in region 8q21.2. *Mol Cytogenet.* 2011; 4: 1 (available from: <http://www.molecularcytogenetics.org/content/pdf/1755-8166-4-1.pdf>).
- Baldwin EL, May LF, Justice AN, Martin CL, Ledbetter DH. Mechanisms and consequences of small supernumerary marker chromosomes: from Barbara McClintock to modern genetic-counseling issues. *Am J Hum Genet.* 2008; 82(2): 398-410.
- Lasan Trcic R, Hitrec V, Letica L, Cuk M, Begovic D. Small supernumerary marker chromosome derived from proximal p-arm of chromosome 2: identification by fluorescent in situ hybridization. *Croat Med J.* 2003; 44(4): 477-479.
- Sheth, F, Ewers, E, Kosyakova, N, Weise, A, Sheth, J, Desai, M, *et al.* A small supernumerary marker chromosome present in a Turner syndrome patient not derived from X- or Y-chromosome: a case report. *Mol Cytogenet.* 2009; 2: 22 (available from: <http://www.molecularcytogenetics.org/content/pdf/1755-8166-2-22.pdf>).
- Kitsiou-Tzeli S, Manolakos E, Lagou M, Kontodiu M, Kosyakova N, Ewers E, *et al.* Characterization of a prenatally assessed de novo supernumerary minute ring chromosome 20 in a phenotypically normal male. *Mol Cytogenet.* 2009; 2: 1 (available from: <http://www.molecularcytogenetics.org/content/pdf/1755-8166-2-1.pdf>).
- Rodríguez L, Liehr T, Martínez-Fernández ML, Lara A, Torres A, Martínez-Frías ML. A new small supernumerary marker chromosome, generating mosaic pure trisomy 16q11.1-q12.1 in a healthy man. *Mol Cytogenet.* 2008; 1: 4 (available from: <http://www.molecularcytogenetics.org/content/pdf/1755-8166-1-4.pdf>).
- Sheth F, Andrieux J, Ewers E, Kosyakova N, Weise A, Sheth H, *et al.* Characterization of sSMC by FISH and molecular techniques. *Eur J Med Genet.* 2011; 54(3): 247-255.
- Backx L, Van Esch H, Melotte C, Kosyakova N, Starke H, Frijns JP, *et al.* Array painting using microdissected chromosomes to map chromosomal breakpoints. *Cytogenet Genome Res.* 2007; 116(3): 158-166.
- Loitzsch A, Bartsch O. Healthy 12-year-old boy with mosaic inv dup(15)(q13). *Am J Med Genet. A.* 2006; 140(6): 640-643.

20. Liehr T, Brude E, Gillessen-Kaesbach G, König R, Mrasek K, von Eggeling F, *et al.* Prader-Willi syndrome with a karyotype 47,XY,+min(15)(pter→q11.1:) and maternal UPD 15 – case report plus review of similar cases. *Eur J Med Genet.* 2005; 48(2): 175-181.
21. Nelle H, Schreyer I, Ewers E, Mrasek K, Kosyakova N, Merkas M, *et al.* Presence of harmless small supernumerary marker chromosomes hampers molecular genetic diagnosis: a case report. *Mol Med Report.* 2010; 3(4): 571-574.
22. Liehr T, Karamysheva T, Merkas M, Brecevic L, Hamid AB, Ewers E, *et al.* Somatic mosaicism in cases with small supernumerary marker chromosomes. *Curr Genomics.* 2010; 11(6): 432-439.
23. Fickelscher I, Starke H, Schulze E, Ernst G, Kosyakova N, Mkrtchyan H, *et al.* A further case with a small supernumerary marker chromosome (sSMC) derived from chromosome 1 – evidence for high variability in mosaicism in different tissues of sSMC carriers. *Prenat Diagn.* 2007; 27(8): 783-785.
24. Liehr T, Wegner R-D, Stumm M, Joksic G, Polityko A, Kosyakova N, *et al.* Pallister-Killian syndrome. Rare phenotypic features and variable karyotypes. *Balkan J Med Genet.* 2008; 11(2): 65-67.
25. Battaglia A, Parrini B, Tancredi R. The behavioral phenotype of the idic(15) syndrome. *Am J Med Genet. C. Semin Med Genet.* 2010; 154C(4): 448-455.
26. Sebold C, Roeder E, Zimmerman M, Soileau B, Heard P, Carter E, *et al.* Tetrasomy 18p: report of the molecular and clinical findings of 43 individuals. *Am J Med Genet. A.* 2010; 152A(9): 2164-2172.
27. McDermid HE, Morrow BE. Genomic disorders on 22q11. *Am J Hum Genet.* 2002; 70(5): 1077-1088.
28. DeBeauchamp JL, Moses A, Noffsinger VJ, Ulrich DL, Job G, Kosinski AM, *et al.* Chp1-Tas3 interaction is required to recruit RITS to fission yeast centromeres and for maintenance of centromeric heterochromatin. *Mol Cell Biol.* 2008; 28(7): 2154-2166.

CHROMOSOMAL POLYMORPHISMS INVOLVED IN REPRODUCTIVE FAILURE IN THE ROMANIAN POPULATION

Mierla D^{1, 2*}, Stoian V²

***Corresponding Author:** Dana Mierla MD, Department of Genetics, Faculty of Biology, University of Bucharest, Bucharest, Romania; Tel.: +40721479083; E-mail: dana_mierla@yahoo.com

ABSTRACT

Cytogenetic heteromorphisms are described as variations at specific chromosomal regions with no impact on phenotype. The purpose of this study was to investigate the effects of these chromosomal polymorphisms involved in reproductive failure in the Romanian population.

One thousand eight hundred and nine infertile patients, who were referred to Life Memorial Hospital, Bucharest, Romania, between January 2008 and April 2011, were investigated in this retrospective study. The frequency of chromosomal polymorphic variations was calculated for these patients. The control group is represented by 1116 fetuses investigated by amniocentesis between January 2009 and April 2011.

In this study 122 (6.74%) infertile patients and 63 fetuses (5.65%) showed chromosomal polymorphic variations. The differences between the two groups was not statistically significant ($p < 0.242$) but there was statistical significance for some specific chromosomal polymorphisms [inv(9), 1qh+, 9qh+, fra(17)].

Some chromosomal polymorphic variations appear to be associated with reproductive failure. The statistically significantly higher incidence of hetero-

chromatic variations found in infertile individuals emphasizes the need to assess their role in infertility and subfertility.

Keywords: Chromosomal abnormalities; Heterochromatic variations; Infertility; Inversion; Subfertility.

INTRODUCTION

Infertility is a significant marital problem, affecting up to 15.0% of couples of reproductive age [1,2]. Infertility can be caused by defects in the development of the urogenital system or defects in function of the endocrine system, including the hypothalamic-pituitary-gonadal axis, or by defects in gametogenesis, sexual function, fertilization or early embryonic development [3]. Secondary or acquired infertility, such as after tubal diseases, vasectomy or exposure to gonadotoxins may also occur [4].

Genetic pathology is an increasingly important part of general human pathology as the number of described genetic diseases and their frequency increases. Study of human chromosomes play a key role in diagnosis, prognosis, treatment and monitoring of chromosomal abnormalities. In order to provide genetical counseling for affected families, cytogenetic analysis is the crucial investigation.

Several studies have been published regarding chromosome analysis in couples with reproductive failure who are referred for IVF (*in vitro* fertilization)

¹ Life Memorial Hospital, Bucharest, Romania

² Department of Genetics, Faculty of Biology, University of Bucharest, Bucharest, Romania

or other treatments [5]. The incidence of chromosomal abnormalities in people with infertility appears to be greater than the overall incidence of chromosomal abnormalities in the general population [6]. It is unclear whether chromosomal abnormalities are one of the main causes of infertility in the human population. Many researchers believe that there is an association between genetic abnormalities and infertility in both men and women [7,8]. Approximately 40.0% of infertility cases are due to male pathology, 40.0% to female pathology, and the remaining 20.0% is a combination of the two [8]. About 5.0% of infertile men have chromosomal abnormalities, most of which involve sex chromosomes.

Chromosomal abnormalities are a major cause of male and female infertility and can be defined as an alteration of function and structure of chromosomes [9]. Cytogenetic abnormalities, both acquired and inherited, are one of the most common genetic causes of miscarriages early in pregnancies [10]. Most chromosomal abnormalities may cause a genetic imbalance that causes various phenotypic abnormalities (delayed growth and development, multiple congenital anomalies, disorders of sexuality and reproduction, *etc.*) due to partial trisomy or monosomy of the regions involved. Cytogenetic studies have been reported to determine the contribution of chromosomal abnormalities in patients with reproductive failure [11].

The purpose of the present study was to investigate the effects of chromosomal polymorphic variations involved in reproductive failure. Polymorphism variations mainly refer to the variants in the chromosomal heterochromatin region. To be classified as variants, chromosomal polymorphisms needed to be at least twice the size of the corresponding region on the second homologous chromosome [2]. Polymorphic variants on non acrocentric chromosomes usually occur in the paracentric heterochromatin on the long arms of chromosomes 1, 9 and 16, the short-arm regions of the D and G group chromosomes, and the distal heterochromatin of the Y chromosome. Increased lengths of the heterochromatic regions on the long arms of these chromosomes are designated as 1qh+, 9qh+, 16qh+ and Yqh+. The heterochromatin can be reduced in these chromosomes, such as in the case of 1qh-, 9qh- and 16qh-. Increased lengths of the short-arm satellites of the acrocentric D and G group chromosomes (13, 14, 15, 21 and 22)

are designated as 14ps+ and 13ps+, while increased lengths of the short arms themselves are designated as p+ (*e.g.*, 15p+) [12]. Because the heterochromatic region consists of highly repeated sequences of satellite DNA that does not encode proteins, the chromosomal polymorphism variations are considered normal karyotypic variations [13]. However, many recent studies indicate that chromosomal polymorphisms may cause certain clinical effects, such as infertility and spontaneous miscarriage [12,14,15].

MATERIALS AND METHODS

During the period from January 2008 to April 2011, 1809 infertile patients (969 men and 840 women) were referred to the Department of Reproductive Medicine, Life Memorial Hospital, Bucharest, Romania. Informed consent was obtained from the patients and donors prior to collection of heparinized blood samples. These patients were investigated for the frequency of chromosomal polymorphic variations. All the patients were evaluated by a skilled medical specialist and tested for antiphospholipid antibodies and relevant hormones. Ultrasonography was performed to rule out other causes of infertility. All cases were Caucasians.

The control group, considered to be a sample of the fertile population, consisted of 1116 fetuses (originating from spontaneous pregnancies). This group was investigated by amniocentesis in the period between January 2009 and April 2011. None of the pregnancies was obtained by an assisted reproductive technique (ART) and the reasons for referral were standard indications for amniocentesis such as abnormal serum screening levels or advanced maternal age.

Amniotic fluid samples were cultured in Amnioxmax complete medium (Gibco, Grand Island, NY, USA) and peripheral blood samples in PBmax and Chromosome B medium (Gibco); G-banded chromosomes were analyzed after harvesting [16]. At least 15 metaphases were analyzed for each case and 10 metaphases were karyotyped using light microscopy. The banding resolution was 400-550 bands per haploid set (BPHS). The results of the two groups were then compared.

Heteromorphism variations were reported according to the recommendations of the International System for Chromosome Nomenclature 2009 [17,18].

Statistical Analyses. The results for the two groups were compared using the two-tailed Fisher's exact test and calculated on line at the GraphPad Software website (<http://www.graphpad.com/quickcalcs/contingency1.cfm>).

RESULTS

Cytogenetic analysis revealed a number of numerical and structural abnormalities, but in this study only chromosomal polymorphisms involved in infertility are reported (Table 1). Chromosomal polymorphism were found in 122/1809 (6.74%) infertile patients in the study group and 63/1116 (5.65%) fetuses in the control group; this was not statistically significant ($p = 0.24$) (Figure 1). The difference between the patients and controls for some specific chromosomal polymorphisms is statistically significant [e.g., inv(9), 1qh+, 9qh+, fra(17)]. This shows that there was a noteworthy relation at risk of infertility and polymorphic variants. The frequencies, according to our study, of the chromosomal polymorphisms for patients and controls are shown in Table 2. The most common variant observed in infertile couples was inv(9) (2.27%). Other chromosomal variants with a high incidence were 1qh+ (1.22%) and 9qh+ (1.11%).

Figure 1. Comparison of the frequency of heterochromatic variations in the two studied groups. The distribution of the chromosomal polymorphisms in the two study groups (fetuses and infertile patients) is presented in this image.

The least common polymorphic variations in infertile couples were usually observed in the para-centric heterochromatin on the long arms of chromosomes 16, 16qh+ (0.28%), the short-arm of the D and G groups of chromosomes 15ps+ (0.22), 21ps+ (0.33), 22ps+ (0.44%), and the distal heterochromatin of the

Table 1. Numerical and structural abnormalities.

Chromosomal Abnormalities	Karyotype	
Numerical abnormalities	Trisomy X Trisomy XXY Trisomy XYY Monosomy 45,X	47,XXX 47,XXY 47,XYY 45,X
Structural abnormalities	Inversion	46,XY,inv(1)(p12q23) 46,XX,inv(1)(p31q13) 46,XX,inv(3)(p11q11.2) 46,XY,inv(3)(p11q11.2),inv(9)(p11q13) 46,XX,inv(5)(pterq13) 46,XX,inv(8)(p22q13) 46,XY,inv(10)(p11.2q21)
	Translocation	46,XY,t(1;4)(q43;q13) 46,XX,t(1;9)(q11;p13) 46,XX,t(1;19)(p13;p13.3) 46,XY,t(3;9)(q28;q32) 46,XX,t(3;13)(p21;p11.2) 46,XX,t(4;13)(p11;q11) 46,XX,t(9;22)(q34;q11) 46,XX,t(10;19)(q22;q13) 45,XY,t(13;14)(q10;q10) 45,XX,t(14;15)(q10;q10)

Table 2. Frequency of chromosomal polymorphisms in the studied groups.

Chromosomal Polymorphic Variations		Control Group Frequency (%)	<i>n</i>	Study Group Frequency (%)	<i>n</i>	<i>p</i> Values
Inversion	inv(9)(p11q12)	3.76	42	2.27	41	0.025
Heteromorphisms qh+	1qh+	0.36	4	1.22	22	0.016
	9qh+	0.18	2	1.11	20	0.002
	16qh+	0.36	4	0.28	5	0.711
	Yqh+	–	–	0.16	3	0.171
Fragile sites	fra(17)	–	–	0.66	12	0.006
	fra(16)	–	–	0.05	1	0.429
Pseudo satellites	14ps+	0.18	2	–	–	0.073
	15ps+	0.18	2	0.22	4	0.796
	21ps+	0.18	2	0.33	6	0.435
	22ps+	0.45	5	0.44	8	0.998
Total		5.65	63	6.74	122	

Y chromosome, Yqh+ (0.16%). The frequency of heteromorphisms in females was 2.76% and 3.98% in males. Twenty males who had heteromorphisms were oligozoospermic or azoospermic. The seven women with chromosome heteromorphisms had normospermic partners. As for the 1116 amniocentesis samples studied, we detected female karyotypes in 533 and male karyotypes in 583 fetuses. We observed polymorphisms in 63 fetuses (5.65%), 30 (1.65%) female and 33 (1.87%) male fetuses. The most frequent types of heteromorphisms in the control group were inv(9) at 3.76%, followed by 1qh+, 9qh+ and 16qh+ variants (0.36, 0.18 and 0.36%, respectively), followed by D and G group variants.

DISCUSSION

Reproductive disorders are closely associated with chromosomal polymorphisms that were considered normal for a long period of time [19]. In recent years, more and more studies have shown an increased incidence of chromosomal polymorphism variation in infertile couples [20,21]. Some studies have demonstrated that 2.0-14.0% of infertile men have constitutional chromosomal abnormalities [20,21]. Chromosomal polymorphism between the two sexes in our study group showed some differences: 72 out of 969 men (7.43%) and 50 out of 840 women (5.95%). In both male and female groups, the inversion of chromosome 9 was more frequent. The most common types of chromosomal polymorphism in human infertility include inversion of chromosome 9. The frequency of inversions

in the studied group was compared with rates in the population and estimated at 1.0-2.0% [22,23]. Involvement of chromosome 9 polymorphisms in reproductive failure has been reported previously [24]. There are multiple chromosome 9 heteromorphisms that cannot be detected by GTG-banding or C-banding [25].

In our study, inversion of chromosome 9 was found in 1.32% men and 0.95% women compared with literature data; 1.52% in men [26] and 0.66% in women [22]. Despite being categorized as a minor chromosomal rearrangement that does not correlate with abnormal phenotypes, many reports in the literature raised conflicting views regarding the association with sterility and subfertility [27,28].

In our study, morphological variations of constitutive heterochromatin were frequently detected during cyto-genetic analysis. Most often, chromosomes vary in size and position of heterochromatin in the 1qh, 9qh, and 16qh regions. Although inherited variants have been reported not to be associated with any risk for phenotypic abnormalities, chromosomal heteromorphisms have been found to have a higher frequency relative to the normal population and have been regarded as abnormalities in some studies [11,17,20,21]. Recent studies suggest that classical euchromatic variants of 9qh+/12qh+ and heteromorphism on chromosome 6q may be responsible for recurrent abortions [29,30]. However, in this study we found a statistical association between some chromosomal polymorphisms, namely, inv(9), 1qh+, 9qh+, fra(17) and infertility. In our study, the frequency of 1qh+ and 9qh+ was statistically signifi-

cantly increased in women with primary infertility and in men with azoospermia which was confirmed by other studies [31,32]. Earlier studies had not investigated polymorphism and chromosomal aberrations as a determining factor in infertility in Romania, therefore, this study could be important in this regard.

CONCLUSIONS

Chromosomal abnormalities and even heteromorphisms are significant etiological factors leading to fertility problems. The statistically significantly higher incidence of heterochromatic variations found in infertile individuals in this study emphasizes the need to evaluate their role in infertility and subfertility.

The overall high prevalence of chromosomal polymorphisms in infertile couples, compared to the normal population, needs to be confirmed with further investigations and larger study populations to delineate the role of “harmless” chromosomal aberrations in the etiology of infertility.

REFERENCES

1. de Kretser DM. Male infertility. *Lancet*. 1997; 349 (9054): 787-790.
2. Gersen SL, Keagle MB. *The Principles of Clinical Cytogenetics*. New Jersey: Humana Press Inc., 2005.
3. Kamel RM. Management of the infertile couple: an evidence-based protocol. *Reprod Biol Endocrinol*. 2010; 8(1): 8-21.
4. Shah K, Sivapalan G, Gibbons N, Tempest H, Griffin DK. The genetic basis of infertility. *Reproduction*. 2003; 126(1): 13-25.
5. Raziel A, Friedler S, Schachter M, Kasterstein E, Strassburger D, Ron-El R. Increased frequency of female partner chromosomal abnormalities in patients with high-order implantation failure after in vitro fertilization. *Fertil Steril*. 2002; 78(3): 515-519.
6. Babu A, Agarwal AK, Verma S. A new approach in recognition of heterochromatic regions of human chromosomes by means of restriction endonucleases. *Am J Hum Genet*. 1988; 42(1): 60-65.
7. Speroff L. Women's healthcare in the 21st century. *Maturitas*. 1999; 32(1): 1-9.
8. Wang Y, Han W, Guan C, Wang G, Zhu X, Jiang M, *et al*. Cytogenetic study on couples with a history of re-productive failure in China. *J Reprod Contraception*. 2009; 20(4): 237-246.
9. Cortés-Gutiérrez EI, Cerda-Flores RM, Dávila-Rodríguez MI, Hernández-Herrera R, Vargas-Villarreal J, Leal-Garza CH. Chromosomal abnormalities and polymorphisms in Mexican infertile men. *Arch Androl*. 2004; 50 (4):261-265.
10. Fritz MA, Speroff L, Eds. *Recurrent early pregnancy loss. Clinical Gynecologic Endocrinology and Infertility*, 8th ed. Philadelphia: Lippincott Williams & Wilkins. 2010: 1191-1215.
11. Madon PF, Anthalye AS, Parikh FR. Polymorphic variants on chromosomes probably play a significant role in infertility. *Reprod Biomed Online*. 2005; 11(6): 726-732.
12. Bhasin MK. Human population cytogenetics: a review. *Int J Hum Genet*. 2005; 5(2): 83-152.
13. Yuce H, Tekedereli I, Elyas H. Cytogenetic results of recurrent spontaneous miscarriages in Turkey. *Med Sci Monit*. 2007; 13(6): CR286-CR289.
14. Sahin FI, Yilmaz Z, Yuregir OO, Bulakbasi T, Ozer O, Zeyneloglu HB. Chromosome heteromorphisms: an impact on infertility. *J Assist Reprod Genet*. 2008; 25(5): 191-195. doi:10.1007/s10815-008-9216-3.
15. Daya S. Issues in the etiology of recurrent spontaneous abortion. *Curr Opin Obstet Gynecol*. 1994; 6(2): 153-159.
16. Shaffer LG, Slovak M.L, Campbell. *ISCN 2009. An International System for Human Cytogenetic Nomenclature*, 2009.
17. Babu A, Verma RS. Characterization of human chromosomal constitutive heterochromatin. *Can J Genet Cytol*. 1986; 28(5): 631-644.
18. Nakamura Y, Kitamura M, Nishimura K, Koga M, Kondoh N, Takeyama M, *et al*. Chromosomal variants among 1790 infertile men. *Int J Urol*. 2001; 8(2): 49-52.
19. Hong Y, Zhou W, Tao J, Wang S, Zhao X. Do polymorphic variants of chromosomes affect the outcome of in vitro fertilization and embryo transfer treatment? *Hum Reprod*. 2011; 26(4): 933-940.

20. Caglayan AO, Ozyazgan I, Demiryilmaz F, Ozgun MT. Are heterochromatin polymorphisms associated with recurrent miscarriage? *J Obstet Gynaecol Res.* 2010; 36(4): 774-776.
21. Yakin K, Balaban B, Urman B. Is there a possible correlation between chromosomal variants and spermatogenesis? *Int J Urol.* 2005; 12(11): 984-989.
22. Goud TM, Mohammed Al Harassi S, Khalfan Al Salmani K, Mohammed Al Busaidy SM, Rajab A. Cyto-genetic studies in couples with recurrent miscarriage in the Sultanate of Oman. *Reprod Biomed Online.* 2009; 18(3): 424-429.
23. Khaled R, Gaber, Hala T, El-Bassyouni HT, El-Gerzawy A. Pericentric inversion of chromosome 1 and 9 in a case with recurrent miscarriage in Egypt. *J Am Sci.* 2010; 6(8): 154-156.
24. Lissitsina J, Mikelsaar R, Punab M. Cytogenetic analyses in infertile men. 2006; 52(2): 91-95.
25. Yun JS, Jang SK, Choi OH. Cytogenetic abnormalities in patients with reproductive dysfunction. *Korean J Obstet Gynecol.* 2007; 50(5):760-768.
26. Starke H, Seidel J, Hen W, Reichardt S, Volleth M, Stumm M, *et al.* Homologous sequences at human chromosome 9 bands p12 and q13-21.1 are involved in different patterns of pericentric rearrangements *Eur J Hum Genet.* 2002; 10(12): 790-800.
27. Davalos IP, Rivas F, Ramos AL, Galaviz C, Sandoval L, Rivera H. Inv (9) (p24q13) in three sterile brothers. *Ann Genet.* 2000; 43(1): 51-54.
28. Teo SH, Tan M, Knight L, Yeo SH, Ng I. Peri-centric inversion 9-Incidence and clinical significance. *Ann Acad Med Singapore.* 1995; 24(2): 302-304.
29. Dundar M, Caglayan AO, Saatci C, Batukan C, Basbug M, Ozkul Y. Can the classical euchromatic variants of 9q12/qh+ cause recurrent abortions? *Genet Couns.* 2008; 19(3): 281-286.
30. Caglayan AO, Ozgun MT, Demiryilmaz F, Ozyazgan I. Can heterochromatin polymorphism of chromosome 6 affect fertility? *Genet Couns.* 2009; 20(2): 203-206.
31. Minocherhomji S, Athalye AS, Madon PF, Kulkarni D, Uttamchandani SA, Parikh FR. A case-control study identifying chromosomal polymorphic variations as forms of epigenetic alterations associated with the infertility phenotype. 2009; 92(1): 88-95.
32. Eiben B, Leipoldt M, Rammelsberg O, Krause W, Engel W. High incidence of minor chromosomal variants in teratozoospermic males. *Andrologia.* 1987; 19(6): 684-687.

ORIGINAL ARTICLE

CYTOGENETIC FINDINGS IN MENTALLY RETARDED IRANIAN PATIENTS

Nasiri F¹, Mahjoubi F^{1,2,*}, Manouchehry F¹, Razazian F¹, Morteza pour F¹, Rahnama M¹

***Corresponding Author:** Dr. Frouzandeh Mahjoubi, Genetics Department, Iran Blood Transfusion Organization Research Centre, High Institute for Research and Education in Transfusion Medicine, Hemmat Express Way, Next to the Milad Tower, Tehran, Iran; Tel.: +9821-44580389; Fax: +9821-44580399; E-mail: Frouz@nigeb.ac.ir

ABSTRACT

We conducted a cytogenetic study on 865 individuals with idiopathic mental retardation (MR) who were admitted to the Cytogenetics Department of the Iran Blood Transfusion Organisation (IBTO) Research Centre, Tehran, Iran; these were performed on blood samples using conventional staining methods. Chromosome anomalies were identified in 205 of the patients (23.6%). The majority were Down's syndrome cases ($n = 138$). In 33 males, a positive fragile X anomaly was found. The remainder ($n = 34$) had other chromosomal abnormalities including structural chromosome aberrations ($n = 23$), marker chromosomes with an unknown origin ($n = 3$), sex chromosome aneuploidy ($n = 6$) and trisomy 18 ($n = 2$). The contribution of chromosome aberrations to the cause of MR in this group of patients is discussed.

Keywords: Chromosome abnormality, Idiopathic mental retardation (MR), Iranian patients.

INTRODUCTION

Mental retardation (MR) is characterized by destruction in intellectual abilities, and by an inability to adapt to the environment and the social situation. Mental retardation is found in individuals either as an

isolated finding, or as part of an underlying disorder [1]. The worldwide prevalence of MR is about 2.3% [2]. Despite large studies being conducted to discover the etiology of MR, in less than 50% of MR cases is the cause identified and the genetic defect known to be responsible for 17-47% of MR cases [3].

It is known that numerical and structural chromosomal anomalies are one of the most common causes of MR seen in these patients [4-12]. Identification of the causes of MR in a patient is of great importance because of the consequences it has for the prognosis, risk of occurrence in other family members, and prevention. Mental retardation is the reason for a substantial portion of referrals of patients and families to the genetic counseling unit. Here we summarize the result of a cytogenetic study performed on 865 mentally retarded Iranian patients consecutively referred to the Cytogenetics Department of the Iran Blood Transfusion Organisation (IBTO) Research Centre, Tehran, Iran.

MATERIALS AND METHODS

Blood samples were collected from 865 idiopathic MR patients who were referred to IBTO for cytogenetic study. There were 287 females and 578 males. The median age of the patients was 9.5 years. The patients enrolled in this study had unexplained MR. In addition, some of them showed stigmata of dysmorphism, malformations, growth retardation, family history of MR, developmental delay, miscarriages, infertility/subinfertility suggestive of a familial chromosomal translocation/inversion.

¹ Genetics Department, Iran Blood Transfusion Organisation (IBTO) Research Centre, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran

² Clinical Genetics Department, National Research Institute of Genetic Engineering and Biotechnology, Tehran, Iran

Chromosomal analysis was performed on phytohemagglutinin (PHA)-stimulated peripheral lymphocyte cultures of the patients using standard cytogenetic methods [13,14]. A cytogenetic test for fragile X was performed upon request.

Briefly, peripheral blood lymphocytes were cultured in 5 mL RPMI 1640 (Gibco®; Invitrogen, Paisley, Scotland, UK), supplemented with 20% (v/v) fetal bovine serum (GIBCO®; Invitrogen) and 10 µL/mL phytohemagglutinin (PHA) (GIBCO®; Invitrogen) at 37°C. After 72 hours of incubation, 40 µL colcemid (10 µg/mL) (GIBCO®; Invitrogen) was added to the cells. The cells were incubated at 37°C for about 10 mins. The suspension was centrifuged, and the pellet was resuspended in 5-10 mL KCL (0.075 M) for about 20 mins. at 37°C. After centrifugation, the cells resuspended in fixative (3v methanol:1v acetic acid) (Merk, Frankfurt, Germany). The fixative was changed at least three times. Using a Pasteur pipette, a drop was dropped onto the slide. The chromosomes were viewed under phase contrast to assess quality of the metaphases and nuclei. The chromosomes were treated with trypsin, then stained with Giemsa (GTG-banded) after aging.

Twenty to 30 metaphases were analyzed per individual and in cases of suspected mosaicism, the

numbers of metaphases were increased to a total of 100 for analysis. A resolution of 450-band stage was considered as a minimum; for a more detailed structural analysis, 550-700-band stage was preferred. The routine analysis was based on GTG-banded staining. For patients with structural chromosome abnormalities or marker chromosomes, a chromosome study of the parents was recommended and performed if the parents were alive and available (some of the patients lived in orphanages) or cooperated.

RESULTS

In our study of 865 screened subjects (287 females, 578 males), anomalies were identified in 205 of the patients (23.6%). The majority were Down's syndrome cases ($n = 138$, 15.9% of all the screened MR cases, and 67.3% of the cases with chromosome abnormalities). In 33 males, a positive fragile X anomaly was found (3.8% of all the screened MR cases, and 16% of the cases with chromosome abnormalities).

The remainder ($n = 34$, 3.9% of all the screened MR cases, and 16.5% of the cases with chromosome abnormalities) had other chromosomal abnormalities (Table 1), mainly structural chromosome aberrations

Table 1. Structural and numerical chromosome anomalies in patients with mental retardation.

#	Sex-Age	Clinical Data (in addition to MR)	Karyotype (structural chromosomal anomalies)	Familial/ De Novo
1	F-9	developmental delay; history of three sepsis cases in family (probability of autosomal recessive immunodeficiency); epicanthic fold	47,XX,del(2)(q22q32.2)+r(2)(q22;q32.2)[81]/46,XX,del(2)(q22;q32.2)[9]	<i>de novo</i>
2	F-?	further clinical data not available	46,XX,del(6)(p25)[7]/46,XX[46]	<i>de novo</i>
3	M-13 mths	developmental delay	46,XY,del(18)(q23)	<i>de novo</i>
4	F-2	developmental delay; external ear duct stenosis; depressed nasal bridge; stoned peripheral fitness	46,XX,del(18)(q21.3)[18]/46,XX[33]	<i>de novo</i>
5	F-9	respiratory problems at birth; cardiovascular problems; protrusion of back of head and forehead bossing; unable to hold neck erect; autism	46,XY,del(4)(p15.31)	<i>de novo</i>
6	F-?	further clinical data not available	46,XX,del(11)(q23.2)	<i>de novo</i>
7	M-7	open mouth; micrognathia; high arched palate; recurrent respiratory infections	46,XY,del(22)(q11.2)	<i>de novo</i>
8	F-3.5	developmental delay; epicanthic fold; moon face	46,XX,del(5)(p15.3)[8]/46,XX	<i>de novo</i>
9	F-?	developmental delay; asphyxy; restlessness	46,XX,del(5)(p15.2)	not known

Continue

Table 1. Continued

10 11	M-15 M-9	low-set ears; epicanthal folds; speech problems low-set ears; epicanthal folds; micrognathia	46,XY,del(5)(p15.2) brother: 46,XY,del(5)(p15.2) parental chromosome study not done	paternal/ maternal
12	M-17 mths	developmental delay; minor dysmorphic features	46,XY,t(16;17)(q22;p13) father: 46,XY,t(16;17)(q22;p13)	paternal
13	F-14 mths F-20	severe developmental delay; minor dysmorphic features	45,XX,der(7)t(7;22)(q36.2;q11.1~11.21), -22 mother: 46,XX,t(7;22)(q36.2;q11.1~11.21)	maternal
14	F-47	depression; history of self injury and suicide	46,XX,t(2;3)(q23;p25)	not known
15	F-8	apparent dysmorphic features; developmental delay; abnormal EEG	46,XX,t(1;4)(q21;p16),add(22)(p13)	<i>de novo</i>
16	F-10 mths F-21	developmental delay; no apparent dysmorphic features	47,XX,t(11;22)(q23;q11.2),+der(22)t(11;22)(q23;q11.2) mother: 46,XX,t(11;22)(q23;q11.2)	maternal
17	F-8 F-?	learning disability	46,XX,inv(6)(p23p21) mother: 46,XX,inv(6)(p23q21)	maternal
18	M-11 M-35	epilepsy; right hemiplegia due to head trauma at 4 years old; strabismus	46,XY,inv(6)(q22.1q25.1) father: 46,XY,inv(6)(q22.1q25.1)	paternal
19	M-3 F-27	bilateral talipes equinovaiuse	46,XY,inv dup(10)(p11.2q26.3) mother: 46,XY,inv(10)(p11.2q26.3)	maternal
20	M-8	developmental dela; prominent nose; speech and behavior problems	46,XY,add(15)(pter)	<i>de novo</i>
21	F-?	further clinical data not available	46,XX,add(15)(p13)	not known
22	M-14 mths	frontal bossing; small jaw; low-set ears; deepset eyes; strabismus; drooping upper eyelid (left side); widely-spaced eyes; short nose; long philtrum; downcurved upper lip; camptodactyly; hypotonia	46,XY,dup(7)(q21.2q32)	<i>de novo</i>
23	F-?	developmental delay; hypotonia in hand and leg (power: 2/5); lack of eye contact; dysmorphic features; gastrointestinal reflex; cardiovascular defects (oval fromen); small head circumference; retarded growth; edema in one foot; third toe of right foot longer than the others; low breast line; fever of unknown origin; spcial nose feature	46,XX,r(18)(q21.2qter)	<i>de novo</i>
Karyotype (numerical chromosomal anomalies)				
1	M-19	developmental delay; minor dysmorphic features	47,XY+mar[8]/46,XY[12]	<i>de novo</i>
2	F-15	primary amenorrhea	47,XXX[22]/46,X+mar[8]/48,XXX,+mar[2]/45,X[2]	<i>de novo</i>
3	F-8	further clinical data not available	47,XX,+mar	<i>de novo</i>
4	F-13 F-?	further clinical data not available	45,X(n=2)	<i>de novo</i>
5	M-7 M-21	further clinical data not available	48,XXYY(n=2)	<i>de novo</i>
6	M-19	further clinical data not available	47,XXY	<i>de novo</i>
7	M-3	further clinical data not available	48,XXXX/49,XXXXY	<i>de novo</i>
8	M-3 mths M-20 days	some of the typical clinical symptoms of trisomy 18	47,XY,+18(n=2)	<i>de novo</i>

($n = 23$). The chromosomal anomalies in these patients were mostly of *de novo* origin except in six cases (patients #12, #13, #16, #17, #18 and #19). In five cases parental chromosome study could not be performed (patients #9, #10, #11, #14 and #21). Marker chromosomes with an unknown origin found in three *de novo* cases. Sex chromosome aneuploidy was detected in six patients. Twelve cases had inversion 9q which is believed to be a normal variant.

DISCUSSION

There is great variation in the frequency of the reported chromosomal abnormalities found in MR patients. A cytogenetic study of 419 MR school children in southern Taiwan, by Shiue *et al* [2], found chromosomal abnormalities in 22.43% of the cases, with trisomy 21 occurring in 77 cases (18.38%). Sex chromosome aneuploidies were found in three cases (0.72%). Structural abnormalities of autosomes were found in 13 cases (3.10%) (2). Another study of 341 MR children in Taiwan found chromosomal abnormalities in 89 cases (20.3%) including 63 of trisomy 21 (10.7%) and 13 of fragile X (3.8%) [4].

Coco and Penchaszadeh [5] reported on a cytogenetic study in 200 MR children in Argentina. They found chromosomal abnormalities in 42 (21%) with 26 cases having structural chromosome defects [5].

Two studies were performed in The Netherlands. One study done in Amsterdam (in the south of The Netherlands) indicated that a chromosomal base in 22.1% of the patients was responsible for their MR. Of these, 14.3% were Down's syndrome patients, and 6.1% had other chromosomal abnormalities [6]. Another study done in Amsterdam indicated that 20 patients had chromosomal anomalies (7.5%) in 266 karyotyped MR children. Interestingly, these were mainly structural chromosome aberrations [7].

A study performed in Poland showed that the incidence of abnormal karyotypes in MR patients was 10.1% [8]. However, the percentage of chromosome aberrations found in patients with non specific mental retardation was 2.2% [8]. A study done by Butler and Singh [9] in America showed that 39 out of 201 (6.6%) institutionalized MR patients had abnormal chromosome with Down's syndrome noted in 31 of the patients.

While the overall frequency of chromosomal abnormalities in these reports was similar, there are reports of either low or high percentages of chromosomal aberrations in other studies. For example,

Celep *et al.* [10] reported the percentages of chromosomal abnormalities in 457 Turkish MR Patients to be only 4.81%. Chromosomal abnormalities and polymorphisms were detected in 65 (14.21%) (structural and numerical chromosomal abnormalities in 22 patients and polymorphisms in 43) of 457 MR and/or multiple congenital anomaly (MCA) patients. On the other hand, a study done in Slovakia revealed a very high percentage of chromosome abnormalities in MR patients. Of 324 MR patients, 104 (53.0%) had chromosomal aberrations [11].

The differences between the incidences of chromosomal abnormalities in the literature could be caused by the criteria for patient selection, and the techniques applied [cytogenetics only or in combination with molecular cytogenetics such as fluorescent *in situ* hybridization (FISH) and comparative genomic hybridization (CGH)]. In our study of 865 screened subjects, chromosomal anomalies were identified in 205 of the patients (23.6%). The majority were Down's syndrome cases ($n = 138$, 15.9%). Interestingly, we found three cases with marker chromosomes (0.34%). Liehr and Weise [15] found that the incidence of marker chromosomes is about 0.288% in MR patients.

In general, van Karnebeek *et al.* [3] showed that the mean yield of chromosome aberrations in classical cytogenetics is about 9.5% (variation: 5.4% in school populations to 13.3% in institute populations; 4.1% in borderline-mild MR to 13.3% in moderate-profound MR; more frequent structural anomalies in females). They also indicated that for fragile X anomalies, yields were 5.4% (cytogenetic studies) and 2.0% (molecular studies) [3].

The incidence of fragile X positive cases in our study is slightly higher than some other reports although we only employed cytogenetic tests for fragile X. For example, Butler and Singh [9] reported 2.0% fragile X positive in his cases, while in our study it was 3.8%. Nevertheless, our results indicate that the diagnostic contribution of the fragile X screening could be considered equally important as conventional chromosome banding techniques for the detection of structural chromosome abnormalities.

Some of the chromosome aberrations were detected in more than one case. For example: in two cases, chromosome 2 was involved with a very close breakpoint of q22 and q23 (Table 1; patients #1 and #14); in two cases, chromosome 4 with breakpoints p16 and p15.3 (Table 1; patients #5 and #15); and in

another two cases, chromosome 6 with breakpoints p25 and p23 (Table 1; patients #2 and #17). Even more interesting, chromosome 11 in two cases and chromosome 18 in another two cases had the same breakpoints (Table 1; patients #6 and #16, and #4 and #23, respectively). This could be very interesting because some of the genes responsible for MR may be located in these breakpoints. For example, the chromosomal breakpoints for the two MR patients (patients #1 and #14) were at 2q22 and 2q23, respectively. Heterozygous mutations or deletions of the ZEB2 gene, which is located near to 2q22, is known to be responsible for Mowat-Wilson syndrome, with MR being one of the main features of this syndrome (16). Furthermore, heterozygous mutations/deletions of the DPAGT1 gene located at 11q 23 (the breakpoint for patients #6 and #16) may reduce up to 88.0% of the mature mRNA and cause clinical features including MR [17].

CONCLUSIONS

In conclusion, the results of this study illustrate the contribution of chromosomal abnormalities to the pathogenesis of MR in this group of mentally retarded Iranian patients. Therefore, we recommend cytogenetic analysis for every individual with idiopathic MR.

This can help the management of the MR patient much better. In addition, by discovering the cause of MR, *e.g.*, deletion or duplication/trisomy of a chromosomal segment resulting from a paternal/maternal balanced translocation, prenatal diagnosis could be applied for future pregnancies, thus preventing the birth of another MR infant(s) through therapeutic abortion, which is allowed in Iran. Furthermore, since a routine cytogenetic analysis gives a minimum resolution of only 4-10 Mb, other advanced molecular cytogenetic techniques would be helpful for the diagnosis of the MR patients with normal karyotype, as mentioned by some researchers [12,18].

REFERENCES

1. Bradinova I, Shopova S, Simeonov E. Mental retardation in childhood: clinical and diagnostic profile in 100 children. *Genet Couns.* 2005; 16(3): 239-248.
2. Shiue CN, Lin YH, Kuan LC, Lii LM, Tsai WH, Chen YJ, Lin SJ, Kuo PL. Cytogenetic surveillance of mentally-retarded school children in southern Taiwan. *J Formos Med Assoc.* 2004; 103(3): 218-224.
3. van Karnebeek CD, Jansweijer MC, Leenders AG, Offringa M, Hennekam RC. Diagnostic investigations in individuals with mental retardation: a systematic literature review of their usefulness. *Eur J Hum Genet.* 2005; 13(1): 6-25.
4. Li SY, Tsai CC, Chou MY, Lin JK. A cytogenetic study of mentally retarded school children in Taiwan with special reference to the fragile X chromosome. *Hum Genet.* 1988; 79(4): 292-296.
5. Coco R, Penchaszadeh VB. Cytogenetic findings in 200 children with mental retardation and multiple congenital anomalies of unknown cause. *Am J Med Genet.* 1982; 12(2): 155-173.
6. Schreppers-Tijdink GA, Curfs LM, Wiegers A, Kleczkowska A, Fryns JP. A systematic cytogenetic study of a population of 1170 mentally retarded and/or behaviourally disturbed patients including fragile X-screening. The Hondsberg experience. *J Genet Hum.* 1988; 36(5): 425-446.
7. van Karnebeek CD, Koevoets C, Sluijter S, Bijlsma EK, Smeets DF, Redeker EJ, Hennekam RC, Hoovers JM. Prospective screening for subtelomeric rearrangements in children with mental retardation of unknown aetiology: the Amsterdam experience. *J Med Genet.* 2002; 39(8): 546-553.
8. Bocian E, Mazurczak T, Stańczak H. Cytogenetic studies in clinical diagnosis analysis of 1611 examination results. *Przegl Lek.* 1995; 52(1): 5-9.
9. Butler MG, Singh DN. Clinical and cytogenetic survey of institutionalized mentally retarded patients with emphasis on the fragile-X syndrome. *J Intellect Disabil Res.* 1993; 37(Pt 2): 131-142.
10. Celep F, Sönmez FM, Karagüzel A. Chromosomal abnormalities in 457 Turkish patients with MCA/MR. *Turk J Pediatr.* 2006; 48(2): 130-134.
11. Srsen S, Misovicová N, Srsnová K, Volna J. Chromosome aberrations in a group of mentally retarded persons. *Cesk Psychiatr.* 1989; 85(1): 9-16.
12. Xu J, Chen Z. Advances in molecular cytogenetics for the evaluation of mental retardation. *Am J Med Genet C Semin Med Genet.* 2003; 117C: 15-24.

13. Gosden CM, Davidson C, Robertson M. Lymphocyte culture. In: Rooney DE, Czepulkowski BH, Eds. *Human Cytogenetics: A Practical Approach*, Vol. I: Constitutional Analysis. Oxford: IRL Press Ltd. 1992: 31-54.
14. Benn PA, Perle MA. Chromosome staining and banding techniques. In: Rooney DE, Czepulkowski BH, Eds. *Human Cytogenetics: A Practical Approach*, Vol. I: Constitutional Analysis. Oxford: IRL Press Ltd. 1992: 91-118.
15. Liehr T, Weise A. Frequency of small supernumerary marker chromosomes in prenatal, newborn, developmentally retarded and infertility diagnostics. *Int J Mol Med*. 2007; 19(5): 719-731.
16. Mowat DR, Croaker GDH, Cass DT, Kerr BA, Chaitow J, Ades LC, Chia NL, Wilson MJ. Hirschsprung disease, microcephaly, mental retardation, and characteristic facial features: delineation of a new syndrome and identification of a locus at chromosome 2q22-q23. *J Med Genet*. 1998; 35(8): 617-623.
17. Wu X, Rush JS, Karaoglu D, Krasnewich D, Lubinsky MS, Waechter CJ, Gilmore R, Freeze HH. Deficiency of UDP-GlcNAc:dolichol phosphate N-acetylgluco-samine-1 phosphate transferase (DPAGT1) causes a novel congenital disorder of glycosylation type II. *Hum Mutat*. 2003; 22(2): 144-150.
18. Kriek M, White SJ, Bouma MC, Dauwerse HG, Hansson KB, Nijhuis JV, Bakker B, van Ommen GJ, den Dunnen JT, Breuning MH. Genomic imbalances in mental retardation. *J Med Genet*. 2004; 43(4): 362-370.