Inversion Analysis in Cytogenetics Researches

Sitogenetik Araştırmalarda Inversiyon Analizi

Hakkı TAŞTAN, MD,[a]
Ayla TÜZÜN, MD,[a]
Serdar YÜKSEL[b]

[a]Department of Biology,
Ankara University Faculty of Science
[b]Department of Genetic,
Dr. Zekai Tahir Burak
Women's Hospital's, Ankara

Geliş Tarihi/*Received:* 25.04.2008
Kabul Tarihi/*Accepted:* 18.09.2008

Yazışma Adresi/*Correspondence:*
Serdar YÜKSEL
Dr. Zekai Tahir Burak Women's Hospital's, Department of Genetic, Ankara,
TÜRKİYE/TURKEY
serdarykl@yahoo.com

ABSTRACT Objective: There have been great improvements in chromosome analysis software paralleling the developments in computer technology since 1980s. There are mainly three stages in generating karyotypes in chromosome analysis systems. These are 1. Capturing photography of the metaphase region by microscope using a video camera and saving it on a computer, 2. Eliminating the background noise by reducing the threshold value of the saved photography and differentiating the chromosomes, 3. Grouping each chromosome in the karyotype table according to their length and band structure. The purpose of using this method was to differentiate the normal and abnormal chromosomes with using the numerical data sequence and chart which obtain chromosomes photography. **Metarial and Methods:** In this study, chromosomes with inversions and normal chromosomes were compared with software. The software used in this study transforms the banded chromosome photograph to numeric data sequence. The numeric data sequence compared with Pearson Correlation analysis. **Results:** The software used in this study transforms the banded chromosome photograph to numeric data sequence. The resulting numeric data revealed the differences of chromosomes with normal and pathological structures using statistical analysis. **Conclusion:** Numeric data sequencing using a software program identified chromosomes without taking into account chromosome morphology. We suggest that the method described is beneficial in determining chromosome inversion and the classification of chromosomes.

Key Words: Cytogenetic analysis; inversion; chromosome

ÖZET Amaç: 1980'lerden bu yana kromozom analizi yazılım ve sistemlerinde büyük ilerlemeler olmuştur. Bu sistemlerin çalışma şekli; 1- mikroskoba bağlı dijital kamera ile metafaz bölgesinin resminin çekilmesi, 2- resmin arka planının temizlenmesi ve kromozomların belirginleştirilmesi ve 3- kromozomların gruplandırılması şeklinde özetlenebilir. Kullandığımız metodun amacı normal ve anormal yapıdaki kromozomların ayrımını kromozom fotoğrafından elde edilen grafikler ve sayısal veri dizilerini kullanarak sağlamaktır. **Gereç ve Yöntemler:** Bu çalışmada normal yapıya sahip kromozomlar ile inversiyonlu kromozomlar yazılımla karşılaştırılmıştır. Bu çalışmada kullanılan yazılım, bantlanmış kromozom fotoğrafını sayısal veri dizisine dönüştürmektedir. Sayısal veri dizileri Pearson korelasyon analizi ile karşılaştırılmıştır. **Bulgular:** Bu çalışma için geliştirilen yazılımla anormal bant yapısına sahip kromozomların, normal yapıya sahip olan kromozomlar ile karşılaştırılarak inversiyonların, grafiksel ve sayısal olarak tespiti yapılmıştır. Sayısal veriler kromozomun normal ve patolojik yapı farkını istatistiksel olarak ortaya koymuştur. **Sonuç:** Elde ettiğimiz sonuç kromozomların morfolojisine bakılmaksızın sayısal veri dizileri ile kromozomların identifiye edilebileceğini göstermiştir. Tarif edilen bu metodun kromozom inversiyonunun belirlenmesinde ve kromozomların sınıflandırılmasında yararlı olduğunu düşünmekteyiz.

Anahtar Kelimeler: Sitogenetik analiz; inversiyon kromozom

Turkiye Klinikleri J Med Sci 2008;28(6):811-21

Cytogenetic analysis is an important tool in the diagnosis of genetic disorders. It involves cell culture, metaphase arrest, hypotonic treatment, cell fixation, slide preparation and use of banding methods.

Following the banding, analysis is done by capturing photomicrographs of the metaphase spreads, and developing and printing the film in the dark room. Individual chromosomes are then manually cut, paired, pasted and a karyotype is made. This whole procedure is time consuming, labour intensive and expensive. With the increasing workload of the cytogenetic laboratories and the availability of improved computer capabilities for image processing, the Image Analysing System with appropriate software, have become the choice for cytogenetic analysis. The system includes a light microscope with or without an automatic metaphase scanning stage, charged couple device (CCD) camera and computer with special software for image capturing, chromosome counting, automatic karyotyping etc.[1]

Since 1980s, computers have been utilized in karyotype analysis in cytogenetic studies. The first utilized systems were composed of computers with LSI/11 microprocessor and Fortran and Macro programming language designed for them.[2] Currently, karyotype analysis systems suitable for PC and Mac are used.[3] Except for some karyotype analysis software used today, properties that are able to detect the structural anomalies of chromosomes do not exist. In the software developed for this study, normal and abnormal (having an inversion) chromosomes were compared and their difference were presented numerically as well as graphically.

A chromosomal aberration in which a segment of a chromosome is reversed in orientation but not relocated is called an inversion. There are two types of inversion. Paracentric inversions involve only one arm of a chromosome, whereas pericentric inversions involve both arms of a chromosome and therefore, include the centromere. The type of inversion does not have to be specified, as this will be evident from the breakpoints. Thus, generally, large inversions are associated with a greater risk of producing abnormal liveborn offspring, because the recombinant chromosomes associated with them carry small duplications and deficiencies that have a greater probability of being compatible with survival. Furthermore, the larger the inversion, the greater the likelihood that a recombination event within the inversion loop will occur and form recombinant chromosomes. The opposite is true of small inversions with large distal segments, which are usually associated with a very low risk of liveborn abnormal offspring.[4]

In previous studies indicated inversions were observed in recurrent pregnancy losses 46,XX,inv (9)(p11q12), 46,XY,inv(9)(p24q13) male infertility, 46,XY,inv(9)(p24q34.1) FTT and congenital anomalies in offspring, 46,XY,inv(9)(p11q13) acute leukemia, 46,XY,inv(9)(p11q21), familial bipolar disorder, 46, XY, inv(9)(p11q13) Asperger Syndrome Pia, 46,XY,inv(9)(p11q13) Goldenhar Syndrome or oculo-auriculo-vertebral spectrum, 46,XX, inv(9) (p11q13) schizophrenia-like psychosis, 46,XY, inv(9)(p11q13) schizophrenia, 46,XX,inv (9)(p13q24) neuroblastoma, 46,XX,inv (9)(p13q21) ectodermal dysplasia, 46,XX,inv(9) (p33.2q34.1) Blepharophimosis Syndrome, 46,XX, inv (9)(p11q11) recurrent spontaneous first trimester abortions, 46,XY, inv(9)(q31.2q34.3) schizoaffective disorder, short stature, depressed nasal bridge, hypertelorism and slender shoulders, 46,XX,inv(11)(q13q23) pediatric osteosarcoma, 46,XX,inv(14)(q11q32) T-Cell leukemia, 46,XY, inv(18)(q21.1q22.1) Gilles de la Tourette syndrome, 46,XY,inv(X)(q11q28) FG syndrome, 46,XY, inv(9)(p21q21) primary infertility, moreover, inversion chromosome 9 induced mental retardation, familial schizophrenia, immotile/ultrastructural sperm defect, double aortic arch (CHD), hydronephrosis, encephalocele, neurofibromatosis, prune belly syndrome, habitual abortions, spontaneous abortions, and amenorrhea.[5-33]

MATERIAL AND METHOD

The software uses the chromosome images saved on the computer or the image from the video camera source. Analysis is started by turning on the images of normal chromosomes and chromosomes with inversion respectively. The size of the images of normal chromosomes and chromosomes with inversion equaled to 400 pixels by the software. Moreover, images taken from the video source and chromosome photos taken directly from the microscope that do not have karyotype analysis systems are fixed with this software due to its options

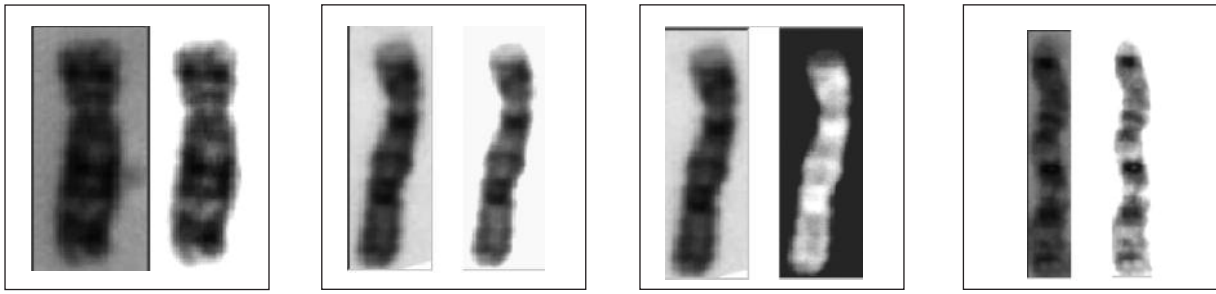

FIGURE 1: The original images of some chromosomes (a-d, left) and their modified profiles by software (a-d, right).

such as Color Balance 4(R-G-B), Brightness/Contrast, Ligthness (B), Darkness (W), Color, R/G and Negative image (Figure 1).

Software is packaged in two different modes. The first software Band Analysis 10 divided the chromosome into 10 equal parts and assessed the chromosome in 200 lines. The first software was suitable for the low-resolution chromosomes whose band level was 400. The second software Band Analysis 20 divided the chromosome into 20 equal parts and assessed the chromosome in 400 lines. The second software was suitable for chromosomes with a high band level (800 and above). The software scanned the image of the chromosome starting from the first line and attributed a numerical value to the parts with a black band in line with the darkness of the color. The color values of all pixels on the line were recorded and their averages were calculated. Chromosome's numerical nature is determined both from the average band concentration and numerical data file. Correlation analysis of normal and abnormal chromosomes was done by comparing the data files created simultaneously with the Band analysis. In Band Analysis 10 software data files were divided into 10 pieces and 10 different correlation analyses were made. On the other hand, in Band Analysis 20 software the data files were divided into 20 pieces. The reason of dividing chromosome into pieces for statistical examination was to find out the chromosome regions that show low or negative correlation.

In this study, chromosomes with inversions and normal chromosomes were compared. In addition, two normal chromosomes were compared to test the software.

Statistical Analysis

Pearson correlation analysis is used for the classification and identification of chromosomes in this study. For inversion parts of inverted chromosomes,only the correlation values either zero or the values near zero are considered.

EXPERIMENTAL RESULTS

PERICENTRIC INVERSION OF CHROMOSOME 8 46,XX, inv(8)(p21q11.2)

Chromosome 8 that shows a pericentric inversion was compared with the same individual's normal chromosome 8. The lengths of both chromosomes were equaled to 155 pixels (Figure 2). The starting point of inversion p21 is the third region in the graphic generated by the software (Figure 3). Inversion ends in the seventh region in the graphic (q11.2 region) (Table1).

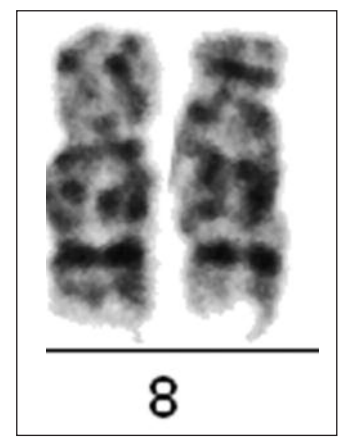

FIGURE 2: Chromosome 8 (left), pericentric inversion of chromosome 8 (rigth).

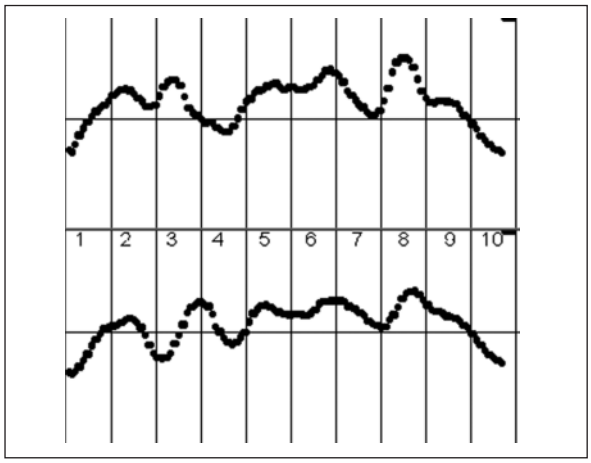

FIGURE 3: Software generated graphic from normal (top) compared to inverted (bottom).

PERICENTRIC INVERSION OF CHROMOSOME 2 46,XX, inv(2)(p11.2 q13)

In p11.2 q13 inversion observed in chromosome 2, the lengths of both chromosomes were equaled to 160 pixels (Figure 4). Chromosome 2 that has a normal band structure was shown on the left and the chromosome that shows inversion was shown on the right. Inversion started in the fourth region and ended in the eighth region in the graphic. An asymmetrical structure was observed in the graphic in the fourth and eighth regions (Figure 5). Compatible with the graphic and chromosome images, values given in the fourth and eighth regions in the correlation table formed by the software had a lower correlation compared to the other regions. Correlation table itself can even give information about the inversion regions (Table 2). In Figure 6 chromosome picture was examined elaborately in 20 regions.

COMPARING CHROMOSOME 11 WITH A NORMAL BAND STRUCTURE

In this part of the study, the software was tested by using only normal chromosomes. Graphics designed for both chromosomes matched one-to-one. Euchromatin regions were unstained whereas heterochromatin regions were stained (taken a band) (Figure 7). In both graphics chromosome 11 centromer corresponded to 10 regions. Chromosome p arm was located on the left side of the graphic and chromosome q arm was on the right side of it (Figure 8).

The chromosome photos used in this study were taken by Zeiss Axioplain microscope. Chromosomes were attained from peripheric blood culture on a Pb Max ready medium. Microscope slides were prepared with Giemsa-Trypsin staining method.

RESULTS

Numeric data is obtained from giemsa –trypsin staining method photographs. Numeric data sequences separate the normal and inverted chromosomes.

TABLE 1: Software measured and calculated result from normal chromosomes 8 and inverted chromosomes 8. (Pearson correlation is significant at the 0.01 level).

Chromosome regions from arm p to arm q	Average band intensity from normal chromosome 8	Average band intensity from inverted chromosome 8	The correlation between normal and inverted chromosome band intensities	Sig.(0.01 level) p value
1	10504.63	11954.81	0.993601	0.000
2	8368	10364.44	0.8518	0.000
3	8221.813	10975.75	-0.73149	0.053
4	11254	10281.75	0.218205	0.328
5	7650.188	8623.25	0.714115	0.013
6	6794.375	8294.375	0.95316	0.277
7	8600	8602.75	0.94482	0.020
8	6169.875	7923.188	0.623171	0.241
9	9246.875	9010.563	0.862544	0.042
10	9741.063	9492.063	0.999945	0.000

FIGURE 4: Chromosome 2 (left), inverted chromosome 2 (rigth).

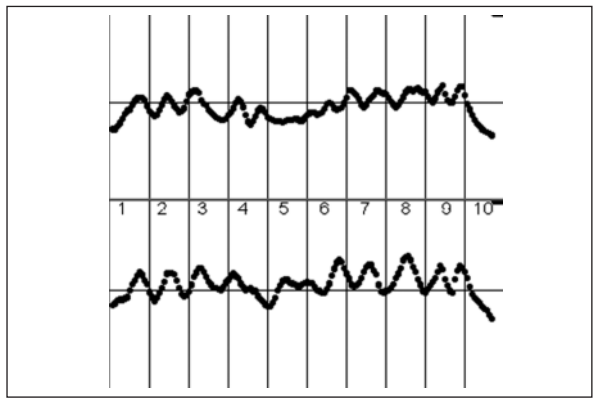

FIGURE 5: Software generated graphic from normal chromosomes 2 (top), compared to inverted chromosomes 2 (bottom).

Normal and inverted chorosome numeric sequences were included in the correlation analysis. Morever, inverted regions can be determined statistically without using chromosome morphology), In addition, numeric data sequences of normal and inverted chromosomes were compared with correlation analysis. Numeric data sequence is divided into 10 equal parts. Then, the correlation value of each part is calculated . In Table 1 and 2, the correlation value of 10 equal parts from p to q is calculated. Normal and inverted chromosomes are separated with this method. In Table 3, the chromosome 11 of two different individuals with normal structure is analyzed. Since both have normal structure, no statistical difference is observed. In Table 4, chromosome 11 is compared with an individual's chromosomes (22 autosomal and 1 sex chromosome). The highest correlation value is observed between the chromosomes 11. These results showed that numeric data sequences using software program identified chromosomes without taking into account of chromosome morphology.

DISCUSSION

The subject matter software complements other chromosome analysis software used in clinical cytogenetic laboratories in terms of the below stated properties.

I- It transforms chromosome photography to a chart on an X, Y two dimensional platform.

II- It makes chromosome photography numerical and statistically analyzes chromosome photos that were transformed into numerical data (Supplemental 1).

By this way, the software will be helpful in differentiating the normal band structured chro-

TABLE 2: Software measured and calculated result from normal chromosomes 2 and inverted chromosomes 2. (Pearson correlation is significant at the 0.01 level)

Chromosome regions from arm p to arm q	Average band intensity from normal chromosome 2	Average band intensity from inverted chromosome 2	The correlation between normal and abnormal chromosome band intensities	Sig.(0.01 level) p value
1	11062	10026.31	0.989735	0.000
2	11294.13	10419.5	0.76146	0.000
3	11227.44	9670.438	0.563199	0.053
4	11860.63	10466.75	0.285693	0.328
5	12887.31	10569.44	-0.4389	0.013
6	11602.63	9460.375	0.367009	0.277
7	10008.13	9311.75	-0.5427	0.020
8	9939.813	8914.063	0.256672	0.241
9	9803.875	9412.625	0.771236	0.042
10	9783.25	8888	0.99803	0.000

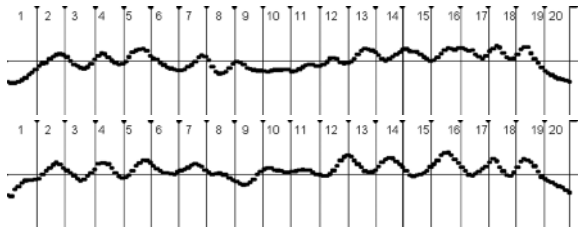

FIGURE 6: Software generated large scaled graphic from normal chromosomes 2 (top), compared to inverted chromosomes 2 (bottom).

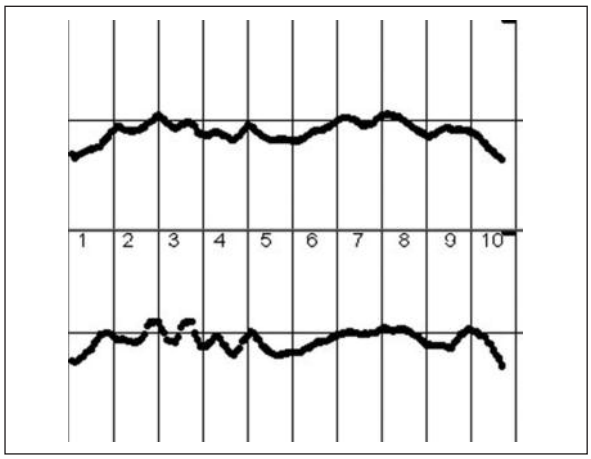

FIGURE 7: Software generated graphic from two normal chromosomes 11.

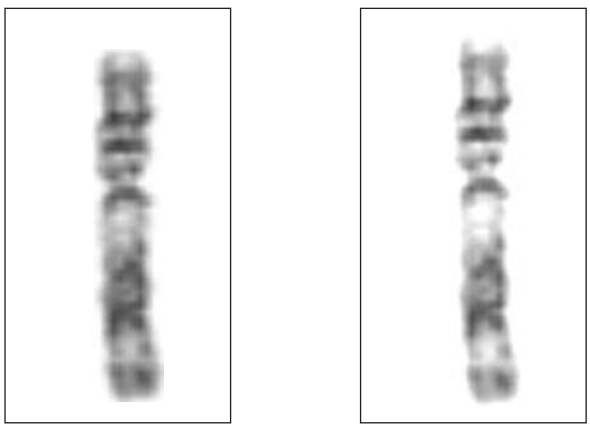

FIGURE 8: Chromosomes 11 (left and right).

mosomes from abnormal ones. Moreover, color, contrast, threshold and negative image properties make the chromosome photos suitable for analysis. Another utilization area of the software is that it can be used for ultramicrospectrophotometry that is based on a principle of defining which chromosome band takes what amount of stain during the preparation of chromosome slide phase.

According to Ahmed et. al the classic method of chromosome identification is as follows; 1. Determination of Chromosome centromere 2. Describing banding patterns 3. A context-sensitive classification procedure based upon relative length, centromeric index and banding description of the chromosomes.

In contrast, only numeric data sequences are used in this study. Our software separates numeric data sequences from normal and inverted chromosomes without using chromosome morphology.

Hiller B et al showed that for statistical analysis in cancer cytogenetics, the genomic changes encoded by the karyotype should be translated into numerical codes. We developed a program, which extracts chromosomal gains and losses as well as breakpoints from the karyotype. The changes are compiled in tables according to the chromosome bands involved and/or depicted in projection to the respective chromosome ideogram.[34]

According to Hiller, genomic changes in karyotype should be converted to numeric codes. With the software that we used in our study, banded chromosomes are converted to numeric codes.

Martin et al demonstrated that the Image Analysis Systems had varying amounts of decision-making ability. The metaphase chromosomes are manipulated (cut) and arranged (pasted) in pairs on the karyotype card (projected onto the computer monitor). Chromosomes are classified based on chromosome dimension (e.g., relative length of chromosomes, centromeric index, i.e. ratio of short arm to long arm) and banding pattern profile. Assuming absorption imagery, bands are considered the dark regions of the chromosomes by the system. An optical density based threshold selects the dark parts as regions potentially bearing a band. In this way, the vague connections between clearly separated bands are avoided. From this and some more information, a subset of the bands is extracted and used for classification. Some of the systems use the central position of the following bands to get an initial karyotype.[35]

TABLE 3: Software measured and calculated result from normal chromosomes 11 (Pearson correlation is significant at the 0.01 level).

Chromosome regions from arm p to arm q	Average band intensity from normal chromosome 2	Average band intensity from inverted chromosome 2	The correlation between normal and abnormal chromosome band intensities	Sig.(0.01 level) p value
1	12853.38	11517.19	0.9819434	0.000
2	11480.69	11061.94	0.9289895	0.000
3	11214.06	10755.25	0.6795448	0.008
4	12330.75	12070.56	0.8956381	0.000
5	12434.19	12277.94	0.9710914	0.000
6	12095.13	12112.31	0.9942735	0.000
7	10823.06	10809.06	0.4571935	0.171
8	10919.06	10690.75	0.9102486	0.000
9	11914.25	11743.63	0.4213372	0.262
10	10089.88	8910.125	0.9973502	0.000

TABLE 4: The table shows correlations between the numeric data sequence of chromosome 11 and all other chromosomes. (Pearson correlation is significant at the 0.01 level)

Chromosomes name	Correlation value	p value	Chromosomes name	Correlation value	p value
1	0.196719887	.014	15	0.213333689	0.008
2	0.510773597	.000	16	0.372612688	0.000
3	0.299917282	.000	17	0.695293285	0.000
4	0.600123669	.000	18	0.173412437	0.031
5	0.283456899	.000	19	0.024312984	0.764
6	-0.013101545	.871	20	0.517839413	0.000
7	0.197831778	.014	21	-0.11046621	0.171
8	0.372335067	.000	22	0.243541392	0.002
9	0.523482713	.000	X	0.660654771	0.000
10	0.320474647	.000	8	0.605791967	0.000
11	**0.846952535**	.000	inverted 8	0.448876838	0.000
12	0.697742216	.000	2	0.603784655	0.000
13	0.6134873	.000	inverted 2	0.452368429	0.000
14	0.173143415	.031	11	**0.789103493**	0.000

In contrast, Martins study similarly numeric data is used regardless of relative length of chromosomes, centromeric index i.e. ratio of short arm to long arm.

Attempts for an accurate automated chromosome classification using a neural network have led to partial results.[36]

Although the image analysis system makes the process of cytogenetic analysis rapid, cost effective and eliminates labor involved in the conventional method, the system cannot itself detect any abnormality (structural or numerical) present in a metaphase. All its decision-making features are just to help in improving the resolution of the band and making the cytogenetic analysis correct and faster. Therefore, a qualified, skilled and well-trained cytogeneticist is essential for cytogenetic analysis.

SUPPLEMENTAL 1: Chromosomes numerical datas (155 pixel density).

Normal chromosome 2	Inverted chromosome 2 (inv(2)(p11.2 q13))	Normal chromosome 8 8 (inv(8)(p21;q11.2))	Inverted chromosomes 11 (46, XX)	Normal chromosome 11 (46, XX)	Normal chromosome
14001	12607	14039	15037	14398	13740
14111	12365	14305	15304	14788	13953
13626	11932	13436	14957	14568	13787
13268	11896	12514	14443	14384	13458
12723	11897	12514	14443	14244	13308
12095	11760	11735	13789	14040	12914
11759	11598	11119	13079	13969	12721
11596	10770	11119	13079	13865	12520
10995	10059	10479	12213	13792	11909
10556	9531	9937	11494	13647	11541
10251	8946	9937	11494	13590	11003
10143	8570	9610	10889	13114	10944
10198	8897	9244	10398	12795	10769
10496	9549	9244	10398	12487	10788
11174	10044	8842	10260	11973	10920
11823	11009	8227	10112	11780	11258
12080	11596	8227	10112	11377	11451
12347	12034	7963	10012	11425	11476
12274	11720	7631	9772	11668	11474
11678	11071	7631	9772	11780	11484
11007	10508	7441	9472	11855	11644
10328	9774	7719	9317	11921	11722
9953	8761	7719	9317	11907	11831
10130	8657	8065	9390	11846	11763
10602	8701	8476	9692	11775	11675
11161	8855	8476	10227	11625	11412
11554	9547	8919	10227	11474	10944
11879	10523	9378	11048	11124	10039
11806	11177	9378	12175	10953	9588
11457	11450	9376	12175	10781	9642
10627	11329	9262	13011	10400	9588
9805	10967	9262	13512	10246	9646
9498	10106	8284	13512	10513	10402
9350	9112	7339	13530	10690	10819
9228	8601	7339	13501	11046	11491
9577	8085	6788	13501	11211	11642
10498	8085	6612	12977	11512	11718
10916	8508	6612	11963	11612	11801
11083	9112	6564	11963	11493	11247
11654	9686	7130	11051	11228	10184
11929	10094	7130	9949	11126	9744
12303	10470	8365	9949	10987	9665
12541	10439	9589	8695	10922	9571
12736	10620	9589	8091	11147	9543
12846	10735	10100	8091	11413	10851
12919	10304	10423	7783	12071	11536
12756	9803	10423	7544	12208	12224
12276	9485	10787	7544	12274	12312
11967	9065	11162	7778	12369	12198

11428	8690	11162	7998	12369	11919
10746	9030	11027	7998	12156	11692
10370	9317	11170	8866	12061	11206
10627	9957	11637	10138	12052	11124
11289	10364	11637	10708	12173	11360
12285	10783	11927	10708	12352	11943
13115	10618	12169	11358	12463	12210
13433	10554	12169	11851	12611	12719
13052	10843	12174	11851	12882	12999
12451	10841	11490	12043	12850	13202
11742	11428	11490	11810	12573	12724
11428	11852	10691	11810	12447	12376
11576	12122	9686	11312	11959	11716
11985	12519	9686	10735	11701	11429
12574	12740	8911	10735	11290	11060
12722	12680	8592	9666	11408	10610
12872	12276	8592	8727	11611	10783
12992	11700	7962	8727	11935	11290
13007	10735	7705	8292	12099	11557
13038	10132	7705	7913	12438	12102
13129	9651	7644	7913	12586	12367
13007	9441	7255	7777	12755	12629
12901	9498	7255	8029	12850	12857
12903	9878	6996	8029	12914	12989
12842	9957	6937	8348	12878	13140
12753	10002	6937	8590	12824	13127
12784	10240	7337	8590	12797	13082
13051	10284	7600	8852	12802	13001
12961	10048	7600	8871	12866	12956
12661	9849	7375	8913	12894	12897
12314	9814	7344	8913	12946	12863
12075	9909	7344	8875	12954	12852
11987	9891	7654	8829	12934	12880
11971	10362	7650	8829	12788	12866
12194	10768	7650	8914	12686	12596
12164	10830	7541	8946	12447	12475
11952	11057	7337	8946	12307	12328
11548	11038	7337	8765	11971	12092
10916	10646	7158	8361	11883	11986
10779	9971	6610	8361	11834	11772
10960	9019	6610	7709	11794	11768
11442	8125	6102	7465	11741	11701
11594	7420	5585	7465	11545	11638
11534	7120	5585	7522	11445	11538
11338	7336	5419	7405	11199	11278
10874	8060	5784	7405	11048	11164
10130	8750	5784	7351	10728	11022
9226	9350	6138	7389	10626	10942
9319	10121	6805	7389	10522	10847
9544	10300	6805	7507	10452	10776
9832	10222	7744	8009	10461	10716
10357	9850	8213	8009	10582	10712
10987	9308	8213	8182	10650	10757
11311	8478	8742	8319	10863	10811

10993	7906	9192	8722	11041	10890
10496	7649	9522	8722	11341	10889
10163	7678	9522	9003	11315	10868
9836	8387	10063	9525	11245	10882
9365	8867	10298	9525	11218	10811
9304	10106	10298	9874	11057	10788
9619	10903	10361	10059	10626	10740
9648	11113	9900	10059	10442	10494
9774	10872	9900	10250	10246	10349
10134	10735	8792	10179	10191	10245
10627	10499	7511	10179	10091	10324
11022	10030	7511	9462	10134	10440
11327	9293	5726	8924	10259	10551
11099	8745	4708	8924	10309	10496
10658	8098	4708	8035	10409	10342
10119	7093	4268	7142	10653	10302
9485	6684	4274	7142	10768	10390
9182	6642	4274	6592	11064	10542
9213	7122	4509	6401	11247	10631
9410	7892	5225	6401	11541	10899
9098	8571	5225	6317	11657	11041
9065	9414	6517	6763	11909	11173
9337	10105	7785	6763	12040	11573
9487	10830	7785	7297	12187	11754
9427	11084	8513	7830	12445	12076
10055	10604	8978	7830	12508	12075
10509	10214	8978	8407	12314	12123
10777	9717	9167	8514	12241	12106
10244	9277	8815	8530	11969	12113
9520	8491	8815	8530	11808	12113
9081	7815	8778	8772	11626	12111
8626	8270	8877	9053	11507	12248
9774	9414	8877	9053	11597	12352
10470	10150	8977	9115	11744	12371
10728	10870	9150	9279	11808	11869
10695	11053	9150	9279	11768	11545
10033	9456	9676	9530	11760	10975
9257	8328	10279	10037	11798	10832
8842	7787	10279	10037	11844	10634
8824	8072	10641	10373	11891	10355
9907	8495	11283	10906	12045	10452
10903	9244	11283	10906	12158	10608
11581	10297	11841	11622	12362	10707
12305	11225	12558	12175	12579	11019
12917	11656	12558	12175	13041	11098
13310	12016	13015	12716	13262	11254
13628	12250	13469	13137	13713	11787
14001	12701	13469	13137	13955	11997
14239	13029	13912	13491	14175	12674
14407	13219	14054	13788	14535	13141
14569	13806	14054	13788	14687	13543
14765	14270	14361	14032	14926	14282

REFERENCES

1. Ahmad ME, Kucheria K. Image analysis system and its use in cytogenetic analysis. Indian J Hum Genet 2002;8:15-9.
2. Walter RJ, Berns MW. Computer-enhanced video microscopy: digitally processed microscope images can be produced in real time. Proc Natl Acad Sci U S A 1981;78:6927-31.
3. Hiller B, Bradtke. J. Balz. H, Reider H. Cydas: A Cytogenetic Data Analysis System. Bioinformatic Advance Acces . Oxford Unv. Pres. 2004
4. Gersen SL, Keagle MB (eds): The principles of clinical cytogenetics, 2nd. ed. Humana Press, 2005 p:185
5. Sasiadek M, Haus O, Lukasik-Majchrowska M, Slezak R, Paprocka-Borowicz M, Busza H, et al. [Cytogenetic analysis in couples with spontaneous abortions] Ginekol Pol 1997;68: 248-52.
6. Dávalos IP, Rivas F, Ramos AL, Galaviz C, Sandoval L, Rivera H. Inv(9)(p24q13) in three sterile brothers. Ann Genet 2000;43:51-4.
7. Shapira SK, Orr-Urtreger A, Gagos S, Shaffer LG. Constitutional mosaicism for a chromosome 9 inversion resulting in recombinant aneusomy in an offspring. Am J Med Genet 1997;69:360-4.
8. Keung YK, Knovich MA, Powell BL, Buss DH, Pettenati M. Constitutional pericentric inversion of chromosome 9 and acute leukemia. Cancer Genet Cytogenet 2003;145:82-5.
9. McCandless F, Jones I, Harper K, Craddock N. Intrafamilial association of pericentric inversion of chromosome 9, inv (9)(p11-q21), and rapid cycling bipolar disorder. Psychiatr Genet 1998;8:259-62.
10. Pia Verri A, Cimbro C. Observation of an Asperger Syndrome's case with a diagnosis in adulthood and a pericentric inversion chromosome 9. Minerva Psichiatr 2002:43:38.
11. Stanojević M, Stipoljev F, Koprcina B, Kurjak A. Oculo-auriculo-vertebral (Goldenhar) spectrum associated with pericentric inversion 9: coincidental findings or etiologic factor? J Craniofac Genet Dev Biol 2000;20:150-4.
12. Miyaoka T, Seno H, Itoga M, Ishino H. A case of small cerebral cyst and pericentric inversion of chromosome 9 that developed schizophrenia-like psychosis. Psychiatry Clin Neurosci 1999;53:599-602.
13. Demirhan O, Taştemir D. Chromosome aberrations in a schizophrenia population. Schizophr Res 2003;65:1-7.
14. Satgé D, Moore SW, Stiller CA, Niggli FK, Pritchard-Jones K, Bown N, et al. Abnormal constitutional karyotypes in patients with neuroblastoma: a report of four new cases and review of 47 others in the literature. Cancer Genet Cytogenet 2003;147:89-98.
15. Fuenmayor HM, Roldan-París L, Bermúdez H. Ectodermal dysplasia in females and inversion of chromosome 9. J Med Genet 1981;18: 214-7.
16. Mhanni AA, Dawson AJ, Chudley AE. Vertical transmission of the Ohdo blepharophimosis syndrome. Am J Med Genet 1998;77:144-8.
17. Parmar RC, Sira P. Prenatal diagnosis of partial trisomy 21 associated with maternal balanced translocation 46xx der 21 t(21q;22q) with pericentric inversion of chromosome 9. J Postgrad Med 2003;49:154-6.
18. Inayama Y, Yoneda H, Fukushima K, Sakai J, Asaba H, Sakai T. Paracentric inversion of chromosome 9 with schizoaffective disorder. Clin Genet 1997;51:69-70.
19. Batanian JR, Cavalli LR, Aldosari NM, Ma E, Sotelo-Avila C, Ramos MB, et al. Evaluation of paediatric osteosarcomas by classic cytogenetic and CGH analyses. Mol Pathol 2002;55:389-93.
20. Baer R, Heppell A, Taylor AM, Rabbitts PH, Boullier B, Rabbitts TH. The breakpoint of an inversion of chromosome 14 in a T-cell leukemia: sequences downstream of the immunoglobulin heavy chain locus are implicated in tumorigenesis. Proc Natl Acad Sci U S A 1987;84:9069-73.
21. State MW, Greally JM, Cuker A, Bowers PN, Henegariu O, Morgan TM, et al. Epigenetic abnormalities associated with a chromosome 18(q21-q22) inversion and a Gilles de la Tourette syndrome phenotype. Proc Natl Acad Sci U S A 2003;100:4684-9.
22. Briault S, Villard L, Rogner U, Coy J, Odent S, Lucas J, et al. Mapping of X chromosome inversion breakpoints [inv(X)(q11q28)] associated with FG syndrome: a second FG locus [FGS2]? Am J Med Genet 2000;95: 178-81.
23. Sasagawa I, Ishigooka M, Kubota Y, Tomaru M, Hashimoto T, Nakada T. Pericentric inversion of chromosome 9 in infertile men. Int Urol Nephrol 1998;30:203-7.
24. Cora T, Demirel S, Acar A. Chromosomal abnormalities in mentally retarded children in the Konya region--Turkey. Genet Couns 2000;11: 53-5.
25. Kunugi H, Lee KB, Nanko S. Cytogenetic findings in 250 schizophrenics: evidence confirming an excess of the X chromosome aneuploidies and pericentric inversion of chromosome 9. Schizophr Res 1999;40:43-7.
26. Lee KB, Kunugi H, Nanko S. Familial schizophrenia with pericentric inversion of chromosome 9: a case report. Schizophr Res 1998;32:123-6.
27. Baccetti B, Collodel G, Crisà D, Moretti E, Piomboni P. Notulae seminologicae. 8.Ultrastructural sperm defects in two men, carriers of autosomal inversion. Andrologia 1997;29:277-82.
28. Brzezinska-Kolarz B, Sanak M, Musial J, Szczeklik A. Double aortic arch associated with pericentric inversion of chromosome 9. Kardiol Pol 1997:47:39.
29. Flego V, Radojcić Badovinac A, Plese V, Kapović M, Beg-Zec Z, Zaputović L. Malignancy risk in patient with neurofibromatosis and autosomal dominant polycystic kidney disease. Croat Med J 2003;44:485-8.
30. Salihu HM, Boos R, Tchuinguem G, Schmidt W. Prenatal diagnosis of translocation and a single pericentric inversion 9: the value of fetal ultrasound. J Obstet Gynaecol 2001;21: 474-7.
31. Tural Ş, Güneş S, Kara N, Koçak İ, Ökten G. [A case of habitual abortus karyotyped 46, XX,inv (9) (P11Q13) X2 with inv 9 (P11Q13) in both of homolog chromosome pairs] Turkiye Klinikleri J Gynecol Obst 2007;17:331-3.
32. Yılmaz A, Yakut T, Gülten T, Karahasan A, Avcı İ, Develioğlu O. [The Type And Frequency Of Genetiic Abnormalitiesin Spontaneous Abortions] Turkiye Klinikleri J Gynecol Obst 2005;15:63-5.
33. Saatçi Ç, Özkul Y, Müderris İİ, Kiraz A, Taşdemir Ş, Çağlayan AO, et al. Cytogenetic analysis of patients with amenorrhea. Turkiye Klinikleri J Gynecol Obst 2008;18:83-7.
34. Hiller B, Bradtke J, Balz H, Rieder H. CyDAS: a cytogenetic data analysis system. Bioinformatics 2005;21:1282-3.
35. Martin AO, Isenstein BS, Thomas CW, Rzeszotarski MS, Johnson WE. Stylized chromosome images. Cytometry 1990;11:40-50.
36. Malet P, Benkhalifa M, Perissel B, Geneix A, Le Corvaisier B. Chromosome analysis by image processing in a computerized environment. Clinical applications. J Radiat Res (Tokyo) 1992;33 Suppl:171-88.