Available online at http://www.bretj.com

INTERNATIONAL JOURNAL OF CURRENT LIFE SCIENCES

RESEARCH ARTICLE

ISSN: 2249- 1465

International Journal of Current Life Sciences - Vol.4, Issue, 11, pp. 10958-10961, November, 2014

CYTOGENETIC STUDY OF MENTALLY RETARDED CHILDREN AND DETECTION OF 15 Q11-Q13 DELETION WITH FISH TECHNIQUE

Mina Niusha[1], Seyed Ali Rahmani[1] and Mohammad Barzegar*[2]

[1]Department of Molecular biology¸ Ahar Branch-Azad University¸Ahar ¸Iran
[2] Neurology, Pediatric Health Research Center, University of Medical Sciences, Tabriz, Iran

A R T I C L E I N F O

Article History

Received 7th, October, 2014
Received in revised form 17th, October, 2014
Accepted 12th, November, 2014
Published online 28th, November, 2014

Key words:

Mental retardation, Cytogenetic, FISH, Praderwilli syndromes, Angelman syndrome

A B S T R A C T

Background: Mental retardation is one of children's major problems in different communities. The most important way to solve this problem is the prevention of mental retardation. About 50% of mental retardation, results from genetic factors and a big part of genetic abnormalities that cause mental retardation are Chromosomal anomalies.

Material and methods: In this study, we analyzed chromosomes of 60 mentally retarded children with karyotype methods and among them the children who were suspicious to have praderwilli and angelman's syndromes were studied with FISH method for 15.q11.q13 deletion. The slides for study chromosomes of patient were prepared with GTG-banding technique and the 15 q11-q13 deletin was studied with FISH technique.Result: In this study, 18.33% of the cases had chromosome abnormality. 1 boy had 15.q11.q13 deletion, 5 girls had 47 XX+21 and 3 boys had 47 XY+21, 1 boy had Rob,t(14q:21q) and 1 girl had 47xx+18.

Conclusions: With diagnosing these abnormalities at an early stage through genetic counseling and PGD in families, we can prevent their recurrence. Therefore, we should develop the techniques for genetic abnormalities diagnosing.

INTRODUCTION

Mental retardation is one of the children's major problems in different communities whose care affect their families and society (Krishnan BR, Ramesh A, Papa KM, Gopinath PM, 1989).

Mental retardation is divided into 4 groups based on Intelligence Quotient'' (IQ): Mild (IQ level: (50- 70), Moderate (IQ level: 35- 50), Severe (IQ level: 20- 35) and Profound (IQ level: below 20) (Frumkin B, 2003).

Mental retardation is caused by environmental factors and genetic disorders. About 50% of them are caused by genetic factors. Some studies have shown that common genetic abnormalities associated with the occurrence of Mental Retardation are chromosomal abnormalities. Chromosomal abnormalities involve two groups of changes: Numerical abnormalities and Structural abnormalities (Feuk L, Carson AR, Scherer SW, 2006).

Numerical aberrations involve copy number changes such as aneuploidy. Structural rearrangements include translocations, insertions and inversions, deletions and duplications (Karnebeek CD, Jansweijer MC, Leenders AG, 2005). Several techniques are used for the diagnosis of chromosomal abnormalities, for example karyotype technique, fluorescent in situ hybridization (FISH) and comparative genomic hybridization array. Numerical aberrations and some of structural rearrangements can be found through karyotype technique. These chromosomal aberrations visible in the light microscope were detected in 10-16% of mental retardations cases (Rauch A, Hoyer J, Guth S, 2006).

All the chromosome abnormalities are not detected through routine cerotype method; submicroscopic rearrangements such as micro deletions are indistinguishable in the light microscope. Micro deletions have been identified in 2.5-6% of MR individuals. These micro deletions can be detected by the FISH (fluorescence in situ hybridization) technique or other cytogenetic molecular techniques (Rooms L, Reyniers E, Kooy RF 2005 & Ravnan JB, Tepperberg JH, Papenhausen P 2006). For instance, Prader-Willi /Angelman syndromes can occur because of 15 q11-q13 micro deletion. However, there are other known genetic defects causing Prader-Willi or Angelman that are uniparental disomy of chromosome 15, imprinting defects of chromosome region 15q11-q13 and other structural chromosomal rearrangements involving chromosome 15. Among these causes 15 q11-q13 micro deletion is common (Madon FP, Athalye SA, Sanghavi K, Parikh RF 2010, Kokkonen H 2003).

Corresponding author:* **Mohammad Barzegar
Neurology, Pediatric Health Research Center, University of Medical Sciences, Tabriz, Iran

Severe hypotonia, short stature, mental retardation, obesity with increasing age, hypogonadism, small hands and feet, fair hair and skin are clinical features of prader-willi syndrome. Angelman syndrome is known as Happy Puppet syndrome. In other words, happy predisposition with outbursts of laughter by frequent hand flapping, Mental retardation with microcephaly, jerky movements affecting the trunk and upper limbs, ataxia, unsteady and wide-based gait, wide mouth with constant dribbling and prominent chin are clinical features of Angelman syndrome (Madon FP, Athalye SA, Sanghavi K, Parikh RF ,2010).

Table 1 chromosomal anomalies that were found among the positive subjects in the present study

Case no.	Chromosomal abnormality	Sex	Age in years
1	15 q11-q13 deletion	Male	5
2	47xy+21	Male	2
3	47xy+21	Male	1
4	47xx+21	Female	1
5	47xx+21	Female	1
6	Rob,t(14q:21q)	Male	1
7	47xx+18	Female	1
8	47xx+21	Female	1
9	47xx+21	Female	2
10	47xy+21	Male	1
11	47xx+21	Female	8

In this study, we analyzed the chromosomes of 60 mental retarded children and among them, the symptoms of children with Prader-Willi or Angelman syndromes were tested for 15 q11-q13 microdeletion through FISH technique.

MATERIALS AND METHODS

In this cytogenetic study, we analyzed chromosomes of 60 mentally retarded children with karyotype method and among them the children who were suspicious of having praderwilli and angelman's syndromes were studied with FISH technique for 15.q11.q13 deletion.

Patients

The subjects include of 26 males and 34 females in the age group of 1-12 were referred to cytogenetic laboratory and analyzed with karyotype method and among them, the suspicious cases of praderwilli and angelman's syndromes were analyzed with FISH technique for 15.q11.q13 deletion.

Karyotyping procedure

Chromosomal samples were taken from peripheral lymphocytes. 10 ml of RPMI 1640 medium (plus phytohemagglutinin and penstripe and FBS) with 0.5 ml of heparinized whole blood were incubated at 37 $^{\circ}C$ for 72 hours. After 71 hours and 40 minutes 100µl of Colcemid solution were added to each culture tube then the tubes were incubated at 37 $^{\circ}C$ for an additional 20 minutes. They were spun at 2000 RPM for 10 minutes. The supernatant was removed and re-suspended the cells in 10 ml of hypotonic 0.075 M KCl prewarmed to 37 $^{\circ}C$.

The tubes were incubated at 37 $^{\circ}C$ for 20 minutes. They were spun at 2000 RPM for 10 minutes then the supernatant was removed. Next, 1ml of fresh ice cold fixative(1vol acetic acid and 3vol methanol) was added drop-by- drop (with vortexing). They were spun at 2000 RPM for 10 minutes. This stage was repeated until the supernatant got clear. The cell pellet was re-suspended with a 1ml of fixative.

We then dropped 4-5 drops onto a clean slide carefully on the drops to spread them on the slide from a height of 50 cm. The slides were kept at 55 $^{\circ}C$ overnight. After that the slides were put in Trypsin buffer for 1 minute. The slides were stained with Giemsa stain solution for 2 minutes. Finally, they were studied with microscope after they were washed with water.

In each case 50 metaphases were examined for numerical and 5 metaphase for structural abnormalities (Krishnan BR, Ramesh A, Papa KM, Gopinath PM, 1989).

FISH procedure

The Prader-Willi/Angelman (SNRPN) kit was provided by Cytocell Company. This probe is 170 Kb, labeled in red, and covers the whole SNRPN gene as well as the entire imprinting center. The 15qter subtelomere specific probe labeled in green allows identification of chromosome 15 and acts as a control probe (Madon FP, Athalye SA, Sanghavi K, Parikh RF, 2010).

FISH Protocol

The kit is designed to be used on cultured peripheral blood cells fixed in fixative and provides air dried samples according to standard cytogenetic procedures.

The slides were immersed in 2x SSC for 2 minutes at room temperature (RT) without agitation. Then they were each dehydrated in an ethanol series (70%, 85% and 100%) for 2 minutes at RT. After that the slides were allowed to dry. 10µl of probe were spotted onto the cell sample and carefully were applied a coverslip. The slides were on a hotplate at 75$^{\circ}C$ (+/- 1$^{\circ}C$) for 2 minutes. Next, the slides were placed in a humid, lightproof container at 37$^{\circ}C$ (+/- 1$^{\circ}C$) overnight. After removing the coverslip and all traces of glue carefully, the slides were immersed in 0.4x SSC (pH 7.0) at 72$^{\circ}C$ (+/- 1$^{\circ}C$) for 2 minutes and then were immersed in 2x SSC, 0.05% Tween-20 at RT (pH 7.0) for 30 seconds. Finally, the slides were drained and applied 10 µl of DAPI antifade each, were covered with a coverslip after 10 minutes and were studied with fluorescent microscope. In each case 100 interphases cells were examined (Madon FP, Athalye SA, Sanghavi K, Parikh RF, 2010).

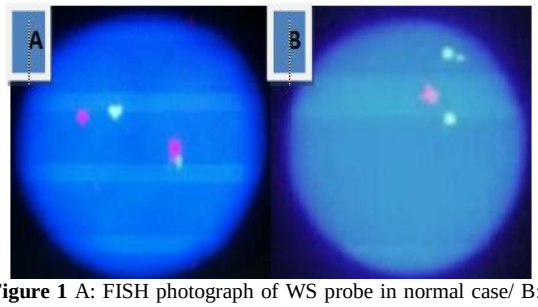

Figure 1 A: FISH photograph of WS probe in normal case/ B: FISH photograph of WS probe in WS patient

RESULTS

All 60 subjects were mentally retarded, Out of 60 subjects, 11 showed chromosomal aberrations (18.33%) included 2 (3.33%) structural variations, 9 (15%) numerical

anomalies. The results have shown in Table 1. Structural variations include deletions, translocations. The numerical variations included Down syndrome, and Trisomy 18. Surprisingly, no sex chromosomal abnormalities were noticed. Among subjects, 19.23% of the male and 17.65% of the female were suffering from chromosomal anomalies. As shown in Table 2, the patients studied were under 13 years of age.

The maximum cases of 5 (8.34%) females showed 47, XX, +21 karyotype and 3 (5%) males showed 47, XY, +21 karyotype. One family with a single child with Robertsonian translocation showed the karyotype 46, XY, +t (14; 21). Cytogenetic in another male showed 47, XX, + 18. Fifteen cases were tested with FISH technique and one boy showed microdeletion in 15 q11-q13. Out of 100 Interphases cells studied, 97 cells were positive for Mentioned microdeletion. In cells with microdeletion, 2 green signals and one red signal were obtained (Figure 1. A,B).

Table 1 Influence of age and sex

S. No.	Sex and age	Total cases	No. of abnormal cases	%
1	Female	34	6	17.65
2	Male	26	5	19.23
3	< 2 years	12	7	58.33
4	2-4 years	8	2	25
5	4-6 years	5	2	40
6	6-8 years	4	-	0
7	8-10 years	10	1	10
8	10-12 years	21	-	0

DISCUSSION

One important factor in the etiology of mental retardation is Cytogenetic investigation. Detection of new causes of mental retardation is a major challenge for human geneticists.

In this study, out of 60 subjects, 11 showed chromosomal aberrations (18.33%) including 2 (3.33%) structural variations, 9 cases (15%) showed numerical anomalies. Trisomy 21 is the most frequent genetic cause of mental retardation. In the present study, as shown in Table 1, 15% of the cases were referred as Down syndrome and 8 cases (13.33%) showed regular trisomy and 1 case (1.67%) showed translocation. In several study this abnormality is reported less than 11.3% and 12.09% and 64.33% (Kaur A, Singh JR , 2010, Rajasekhar M, Murugesan R, Shetty H, Gopinath PM, Satyamoorthy K. , 2010, Kaur A, Mahajan S, Singh JR 2003). Various studies were reported chromosomal abnormalities in MR patients. Chromosomal abnormalities in Taiwan were founded in 22.43% of MR children, with trisomy 21 occurring in 18.38%= (13). Another study of 341 MR children was done in Taiwan and chromosomal abnormalities were found in 20.3% including 10.7% of trisomy 21 (Li SY, Tsai CC, Chou MY, Lin JK, 1988). In a cytogenetic study of MR children in Argentina chromosomal abnormalities were reported in 21% (Coco R, Penchaszadeh VB, 1982).

Another study was performed in Amsterdam and chromosomal abnormalities were found in 22.1% of the MR patients. Of these cases, 14.3% were Down's syndrome patients, and 6.1% had other chromosomal abnormalities (Schreppers-Tijdink GA, Curfs LM. Wiegers A. Kleczkowska A. Fryns J P. A, 1988). Similar study was done in Amsterdam in 266 MR children, 7.5% had chromosome abnormalities in karyotyped (Karnebeek CD, Koevoets C, Sluijter S, Bijlsma EK, Smeets DF, Redeker EJ, Hennekam RC, Hoovers JM, 2002). The results that we were found in present study were according to the results of different studies that were done in different countries. However, in other studies, either lower or higher percentages of chromosomal aberrations were found. For example, in a study in Turkey, 457 MR Patients were karyotyped and 4.81% of them showed Chromosomal abnormalities (Celep F, Sonmez FM, Karaguzel A, 2006).

In the study was carried out on 324 MR patients, in Slovakia, a higher percentage of chromosome abnormalities were found, 53% of them showed chromosomal aberrations (Srsen S, Misovicova N, Srsnova K, Volna J, 1989).The criteria for patient selection could be regarded as the reason for the differences among the occurrences of chromosomal abnormalities in the literature. In this study, out of 60 patients, 15 cases were suspicious of having Prader-Willi and Angelman syndromes. They were tested for 15 q11-q13 deletion with FISH technique.

And one of them was positive for this deletion. 25% of mentally retarded children were suspicious of having Prader-Willi and Angelman syndromes and 6.7% had 15 q11-q13 deletions. In a study 73 cases were suspicious of having Prader-Willi and Angelman syndromes. They were tested for 15 q11-q13 deletion with FISH technique and 3.12% of them had this microdeletion (Madon FP, Athalye SA, Sanghavi K, Parikh RF, 2010). The results of this study are similar to the present study. In another study 118 patients were suspicious of having Prader-Willi and Angelman syndromes. They were tested for 15 q11-q13 deletion with FISH technique and 24.57% were positive for that microdeletion (Teshima L, Chadwick D, Chitayat D, Kobayashi J, Ray P, Shuman C, Siegel-Bartelt J, Strasberg P, Weksberg R., 1998). Another study was done and 72 patients who were suspicious of having Prader-Willi and Angelman syndromes were tested for 15 q11-q13 deletion with FISH technique and the result showed that 27.77% of them had the microdeletion (Varela MC, Fridman C, Koiffmann CP, 2002). The difference between the result of the above-mentioned and present study could be because of the criteria for patient selection.

CONCLUSION

By diagnosing cytogenetics abnormalities and microdeletion, we cannot treat genetic diseases but through genetic counseling and PGD in families we can detect these abnormalities at an early stage using karyotype and FISH techniques and prevent their recurrence.

Acknowledgement

We wish to thank Dr. rahmani's cytogenetic laboratory, Hematology laboratory of Tabriz University of Medical Science, and Pediatric Health Research Center of Tabriz University of Medical Science.

References

Krishnan BR, Ramesh A, Papa KM, Gopinath PM. Genetic analysis of a group of mentally retarded children. Indian J pediatr. 1989; 56: 249- 258.

Frumkin B. Challenging Expert Testimony on Intelligence and Mental Retardation. Journal of Psychiatry and Law. 2003; 34: 51- 71,

Feuk L, Carson AR, Scherer SW. Structural variation in the human genome. Nat Rev Genet. 2006; 7: 85- 97.

Karnebeek CD, Jansweijer MC, Leenders AG. Diagnostic investigations in individuals with mental retardation: a systematic literature review of their usefulness. Eur J Hum Genet. 2005; 13: 6- 25.

Rauch A, Hoyer J, Guth S. Diagnostic yield of various genetic approaches in patients with unexplained developmental delay or mental retardation. Am J Med Genet. 2006; 140: 2063- 2074.

Rooms L, Reyniers E, Kooy RF. Subtelomeric rearrangements in the mentally retarded: a comparison of detection methods. Hum Mutat. 2005; 25: 513- 524.

Ravnan JB, Tepperberg JH, Papenhausen P. Subtelomere FISH analysis of 688 cases: an evaluation of the frequency and pattern of subtelomere rearrangements in individuals with developmental disabilities. J Med Genet. 2006; 43: 478- 489.

Madon FP, Athalye SA, Sanghavi K, Parikh RF. Microdeletion Syndromes Detected by FISH –73 Positive from 374 Cases. Int J Hum Genet. 2010; 10: 15- 20.

Kokkonen H. Genetic changes of chromosome region 15q11-q13 in prader-willi and angelman syndromes in finland. PhD, Finland, University of Oulu, 2003.

Kaur A, Singh JR. Chromosomal abnormalities: Genetic disease burden in India. Int J Hum Genet. 2010; 10: 1- 14.

Rajasekhar M, Murugesan R, Shetty H, Gopinath PM, Satyamoorthy K. Cytogenetic analysis of 1400 referral cases: Manipal experience. Int J Hum Genet. 2010; 10: 49- 55.

Kaur A, Mahajan S, Singh JR. Cytogenetic investigations in mentally retarded individuals. Int J Hum Genet. 2003; 3: 13- 16.

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: 218- 224.

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: 292- 296.

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: 155- 173.

Schreppers-Tijdink GA, Curfs LM. Wiegers A. Kleczkowska A. Fryns J P. A systematic cytogenetic study of a population of 1170 mentally retarded and/or behaviourly disturbed patients including fragile X-screening. The Hondsberg experience. J Genet Hum. 1988; 36: 425- 446.

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: 546- 553.

Celep F, Sonmez FM, Karaguzel A. Chromosomal abnormalities in 457 Turkish patients with MCA/MR. Turk J Pediatric. 2006; 48: 130- 134.

Srsen S, Misovicova N, Srsnova K, Volna J. Chromosome aberrations in a group of mentally retarded persons. Cesk Psychiatr J. 1989; 85: 9- 16.

Teshima L, Chadwick D, Chitayat D, Kobayashi J, Ray P, Shuman C, Siegel-Bartelt J, Strasberg P, Weksberg R. FISH detection of chromosome 15 deletions in Prader-Willi and Angelman syndromes. Am. J. Hum. Genet. 1998; 62: 216- 232.

Varela MC, Fridman C, Koiffmann CP. Diagnosis of patients with Prader-Willi and Angelman Syndromes: the importance of an overall investigation. Genetics and Molecular Biology J. 2002; 25: 7- 12.

****