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ORIGINAL ARTICLE

Screening for subtle chromosomal rearrangements in an Egyptian sample of children with unexplained mental retardation

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Abstract Mental retardation is present in about 1–3% of individuals in the general population, but it can be explained in about half of the cases. A descriptive study was carried out to screen for subtle chromosomal rearrangements in a group of Egyptian children with idiopathic mental retardation (IMR) to estimate its frequency if detected. The study enrolled 30 patients with IMR, with the perquisite criteria of being <18 years at referral, their IQ <70, and manifesting at least one of the criteria for selection of patients with subtelomeric abnormalities. Males were 63.3% and females were 36.7%, with a mean age of 7.08 ± 4.22 years. Full history taking, thorough clinical examination, IQ, visual, and audiological assessment, brain CT scan, plasma aminogram, pelvi-abdominal ultrasonography, echocardiography, and cytogenetic evaluation using routine conventional karyotyping, high resolution banding (HRB), and fluorescent in situ hybridization (FISH) technique with appropriate probes were carried out for all studied patients.

All enrolled patients had apparently normal karyotypes within 450 bands resolution, except for one patient who had 46, XY, [del (18) (p11.2)]. HRB and FISH showed subtle chromosomal rearrangement in 10% of cases that have been proven to be subtelomeric in 2 cases, i.e., 6.8%: 46, XY, dup (17) (p13.3), 46, XY, del (2) (q36.1–36.3), and non-subtelomeric in one case, 5.5%, 46, XX, ins

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(7;?) (q22;?). To conclude, in children with IMR and clinical phenotype indicative of a suspected chromosomal anomaly, once recognizable syndromes have been excluded, abnormalities that include the ends of chromosomes must be searched for using HRB and subtelomeric FISH even when conventional karyotyping fails to demonstrate any abnormality.

1. Introduction

A significant diagnostic challenge exists to identify the new causes of mental retardation. Mental retardation is present in about 1–3% of individuals in the general population, but it can only be explained in about half of the cases, despite thorough clinical and laboratory investigations. Several lines of evidence indicate that genetic factors are involved in many of the idiopathic cases, as they often show prenatal and postnatal signs such as dysmorphic features, growth retardation, and malformations or have a family history of mental retardation [1].

The subtelomeric regions are interesting from a genomic perspective, as they are gene rich and often involved in chromosomal rearrangements [2]. Most telomeres stain lightly with G-banding, and small rearrangements are therefore difficult to detect but with the advent of high resolution banding (HRB) and fluorescent in situ hybridization (FISH), it is now possible to identify submicroscopic rearrangements of the chromosomes that may otherwise go undetected using conventional cytogenetic studies [3].

The current study was carried out to screen for subtle chromosomal rearrangements in a group of Egyptian children with unexplained (idiopathic) mental retardation (IMR) and estimate its frequency if detected.

2. Subjects and methods

A descriptive study was conducted, enrolling 30 patients with IMR (i.e., no etiological diagnosis has been reached after complete examination and detailed investigations), after being approved by the Faculty Ethical Committee. Patients were recruited consecutively from the Genetics Clinic, Children's Hospital, Ain Shams University, Egypt with the perquisite criteria of being <18 years at referral, their IQ <70, and manifesting at least one of de Vries et al. [4] criteria for selection of patients with subtelomeric abnormalities. On the other hand, patients with known genetic etiology including well defined syndromes due to single gene anomalies, chromosomal aberrations, and metabolic causes were excluded from the study. Males were 63.3% (19 cases) and females were 36.7% (11 cases), their ages ranged between 1.5 and 17 years with a mean age of 7.08 $\pm$ 4.22 years. An informed written consent was taken from legal caregivers of included cases. Full history taking, thorough clinical examination, IQ, visual (fundus and slit lamp examination), and audiological assessment (Brain Stem Evoked Visual and Auditory Responses and or audiometry), brain CT scan, plasma aminogram (to exclude aminoacidopathies), and pelvi-abdominal sonar and echocardiography for detection of any concomitant congenital malformations were carried out for all studied patients. Cytogenetic evaluation of enrolled cases included routine conventional karyotyping, HRB, and FISH technique with appropriate probes. de Vries et al. criteria [4] for selection of patients with subtelomeric abnormalities include: +ve FH of affected individuals, prenatal or postnatal growth retardation, facial dysmorphic features and/or congenital anomalies, and behavioral problems (hyperactivity, aggression, self mutilation).

IQ assessment was carried out using Vineland Social Maturity Scale for enrolled infants (age <2 years) [5] and Stanford-Binet Intelligence Scale for children (age >2 years) [6]. The degree of mental retardation was subsequently categorized according to the *DSM IV TR* criteria [7] into profound (IQ <20), severe (IQ 21–35), moderate (IQ 36–50), mild (IQ 51–70).

Cytogenetic evaluation included the following techniques:

- Routine conventional karyotyping using G-banding [8].
- High resolution banding study by synchronization using MTX, FUDR and thymidine release [9].
- Molecular cytogenetics using FISH technique (fluorescence in situ hybridization) with appropriate probes as indicated for individual cases [10,11].

3. Results

Table 1 shows the frequency of different non-parametric clinical variables of the studied sample with unexplained MR. Fundus and slit lamp examination, audiometry, auditory and visual evoked brain stem responses, and pelvi-abdominal sonar were free in all enrolled patients while echocardiography was normal in all but one who had congenital heart disease (ASD, VSD). On the other hand, mild brain atrophy was revealed using brain CT in three cases (10%). IQ assessment showed that 19 patients had mild degree of MR (63%), 9 patients had moderate degree of MR (30%), and 2 patients had severe degree of MR (7%) (Fig. 1).

Table 1 Frequency of different non-parametric clinical variables of the studied sample with unexplained MR.

	No.	%
Studied non-parametric variable		
Females	11	36.7
Males	19	63.3
Consanguinity	18	60
Significant family history of similar or related conditions	16	53
Exposure to hazardous perinatal event	15	50
Recorded clinical manifestations		
Craniofacial dysmorphic features	21	70
Short stature	7	23
Microcephaly	10	33.3
Hand anomaly	3	10
CHD (ASD + VSD)	1	3.3
Seizures	3	10
Hyperactivity	3	10
Speech disorders	5	16

All patients had apparently normal conventional karyotype within 450 bands resolution, except for one patient whose karyotype showed evident deletion of the short arm of chromosome 18: 46, XY, [del (18) (p11.2)], accordingly he has been excluded from FISH analysis. Molecular cytogenetic study using FISH with specific unique sequence probes confirmed the high resolution findings that were suspected in the 550 bands resolution in three cases: one of them had mild MR (IQ = 60) and duplication of band p13.3 on the terminal end of the short arm of chromosome 17: 46, XY, [dup (17) (p13.3)]; the origin of this recorded chromosomal rearrangement could not be traced because of the parental refusal to be examined (Fig. 2). The second case was severely mentally retarded (IQ = 25) and shown to have interstitial deletion of the sub-terminal band of the long arm of chromosome 2 (q36.1): 46, XY, [del (2) (q36.1–36.3)] denovo. It was suspected by high resolution G-banding and confirmed by FISH using LSI spectrum orange and DAPI counter stain (Fig. 3), his parental karyotypes were normal. The cytogenetic studies of the third case who had mild degree of MR (IQ = 55), and normal facial features showed 46, XX, [ins (7;?) (q22;?)] denovo on using HRB while FISH using WCP 7 spectrum green and DAPI counterstain revealed non-painted band on the long arm of chromosome 7 (Fig. 4); her parental karyotypes were normal. Frequency of the different cytogenetic findings encountered among enrolled cases is shown in Fig. 5.

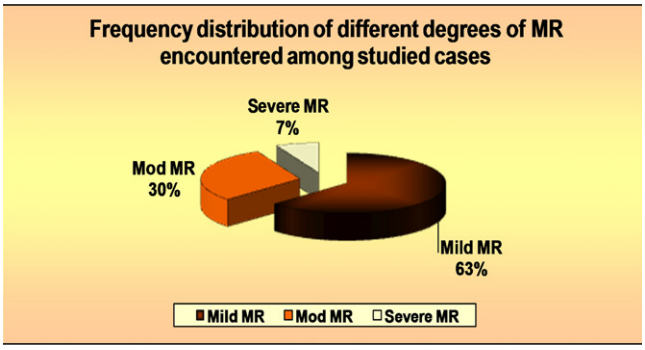

Figure 1 Frequency distribution of different degrees of MR encountered among studied cases.

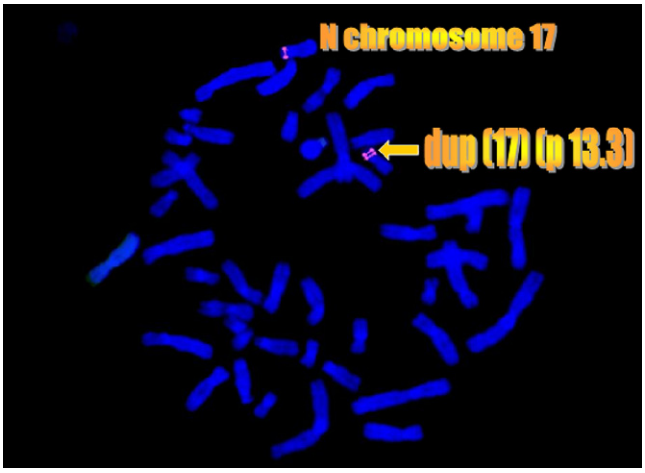

Figure 2 A metaphase spread with an abnormal chromosome 17 short arm (duplication 17p13.3) as shown by FISH using LSI LIS1 spectrum orange probe, and DAPI counter stain. The arrow indicates the two copies of the probe (N = normal).

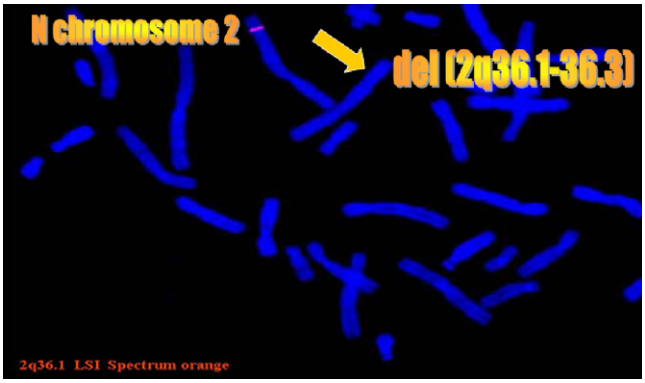

Figure 3 A metaphase spread with an abnormal deleted long arm of chromosome 2; del (2q36.1–36.3) as shown by FISH using LSI spectrum orange probe, and DAPI counter stain. The arrow indicates the deleted part (N = normal).

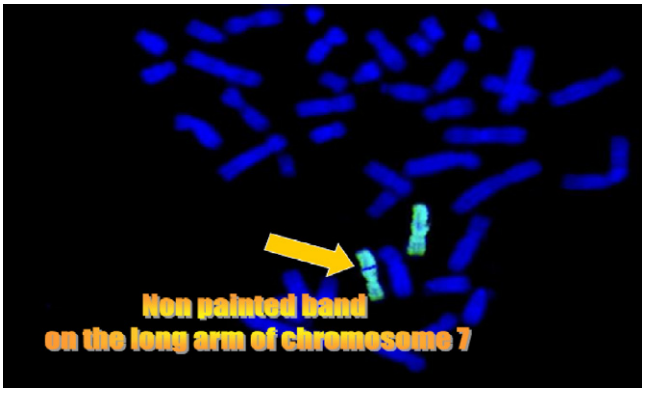

Figure 4 A metaphase spread for the third patient, with painted chromosome 7 by FISH using WCP spectrum green and DAPI counter stain. The arrow indicates the non-painted band on the long arm of chromosome 7.

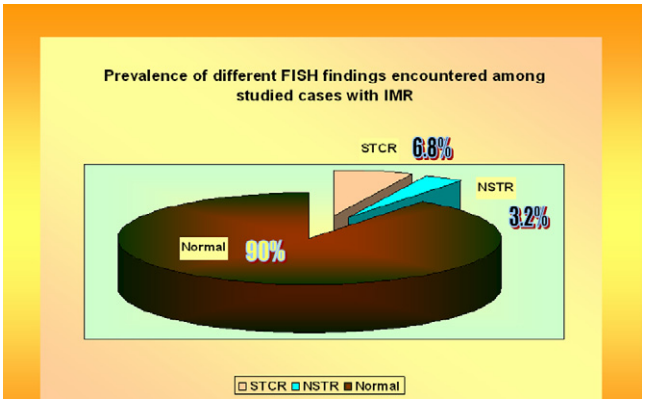

Figure 5 Prevalence of different FISH findings encountered among studied cases with IMR (STCR = sub-telomeric chromosomal rearrangement, NSTCR = non-subtelomeric chromosomal rearrangement).

4. Discussion

Chromosomal analysis by classic cytogenetic studies relies on interpretation of banding patterns resulting from dye uptake in individual metaphase chromosomes. Cytogenetic analysis at 400–500 band resolution is the standard method for investigating syndromes suspected to have a chromosomal etiology. Although large aberrations are detectable with standard chromosome analysis, this technique cannot easily detect structural abnormalities that are small (< 4 megabases) or within negative G-bands and/or abnormalities involving exchanges of segments with similar G-banding patterns. So, conventional cytogenetic studies can overlook many subtle chromosome aberrations, especially those close to the telomeres within G-negative bands. With the advent of HRB and molecular cytogenetic techniques including FISH, it is possible to identify submicroscopic chromosomal rearrangements that may otherwise go undetected using conventional cytogenetic studies [12]. FISH as a molecular cytogenetic tool using specific DNA probes solves this problem because FISH staining is based on the DNA sequence of the target and can be optimized for particular applications [13].

In the current study, FISH findings confirmed the diagnosis of three cases with chromosomal abnormalities which were missed by conventional karyotypes and suspected by HRB. Accordingly, the detection rate of subtle or cryptic chromosomal abnormalities was 10% (3/29 FISH studied cases). These cases were two patients with mild MR (IQ 50–70) and one patient with severe MR (IQ = 25). The frequency of subtelomeric abnormalities was 6.8% (2/29), 5.5% in cases with mild MR (1/18), 9% (1/11) in cases with moderate to severe MR, 3.5% (1/27) in cases with mild to moderate MR and 50% in cases with severe MR (1/2). Two cases were denovo while the origin of the third case could not be identified because of parental refusal to be examined. The foregoing recorded rates support the view that subtle chromosomal rearrangements represent a considerable cause of unexplained MR. Similarly, Bocian et al. [14] studied 84 families with IMR and unspecific clinical features (including 59 patients with moderate to severe MR and 24 with mild MR) but they found subtle chromosomal rearrangements with a slightly higher frequency of 11.9%. Subtelomeric abnormalities were recognized in six of their cases with moderate to severe MR (i.e., 10%; 6/59) and in three cases with mild MR (i.e., 12.5%; 3/24). Retrospective G-banding analysis in the foregoing study [14] showed that six of their recorded nine rearrangements could be suspected at the 450–550 band levels, in contrast to the current study, in which all patients were suspected in the 550 band resolution.

On the other hand, a lower frequency has been recorded by Rong and Zhao [15] as they had discovered two submicroscopic chromosomal abnormalities detected by subtelomeric FISH out of their studied 46 children with idiopathic MR or developmental delay with normal G-banded karyotypes. Those two patients were confirmed to carry microdeletions of 2qter, and 6qter, respectively. Their detection rate of subtle chromosomal abnormalities; 4% included one in 33 patients with mild MR (3%) and one in 13 patients with moderate to severe MR (7.6%).

In contrast to the findings of the current as well as other previously discussed studies, Joyce et al. [16] reported that true cryptic telomeric rearrangements were not a significant cause of IMR as they studied two groups of patients with unexplained MR (selected and unselected) compared to control individuals to determine the frequency of submicroscopic telomeric rearrangements associated with IMR compared to normal population. Unexpectedly, they found two cryptic telomeric abnormalities among their controls. Accordingly, they have suggested that submicroscopic telomeric abnormalities are not uncommon findings in the general population. van Karnebeek et al. [17] agreed with Joyce et al. [16] and showed that subtelomeric rearrangements do not represent a common cause of IMR, since they have identified only one rearrangement (0.5%) in a non-familial MR female, (de novo del 12q24.33-qter) out of 184 patients with unexplained MR studied using subtelomeric FISH probes. This finding might be explained by the low number of their studied cases with moderate to severe MR (44%); it is remarkable that the degree of MR in the single patient recorded in their series was mild. The difference in yield between the foregoing study and other studies may be also explained by the fact that van Karnebeek et al., did not search for subtelomeric microdeletions in patients with a cytogenetic anomaly visible by light microscopy, thus possibly missing concurrent micro deletions. Generally, there were different rates of subtelomeric rearrangements reported by different investigators. Knight et al. [18] detected a subtelomeric anomalies frequency rate of 5–7.4% in patients with moderate to severe MR and only 0.5% in mild cases of MR in their study which included 284 children with moderate to severe MR, and 182 children with mild MR and half of their cases (nearly 466) were familial. Congenital anomalies, behavioral problems, and post natal growth retardation were the most frequent associated features in their series of MR children while intrauterine growth retardation (IUGR) and family history (FH) of MR were less frequent.

Koolen et al. [19] also screened 210 patients with unexplained MR, for subtelomeric rearrangements using molecular cytogenetic study by MLPA and confirmed by FISH. They have identified subtelomeric aberration in 14 patients (6.7%) including ten deletions and four duplications. Abnormalities occurred in 6.3%, 5.1%, and 1.7% of mildly, moderately, and severely retarded patients, respectively. They indicated that testing for subtelomeric aberrations among mildly retarded individuals is necessary. This might be explained by the increased detection of smaller aberrations and by the identification of submicroscopic duplications that cause less severe phenotypes in general. Also, Rooms et al. [20,21] had detected a frequency rate of 5.3% of subtelomeric rearrangements in their studied 70 patients with mild to severe unexplained MR with dysmorphic features and/or familial history of MR. Their recorded abnormalities were: two terminal 1p deletions, a terminal 1q deletion, and a terminal 3p deletion. Fluorescent in situ hybridization (FISH) was performed in 76 patients referred to the Department of Genetics, Institute of Psychiatry and Neurology, Warsaw, Poland because of intellectual disability and dysmorphic features that can be related to subtelomeric microaberrations. In all those patients, conventional cytogenetic methods revealed normal karyotype. Four (5.3%) subtelomeric rearrangements were detected by FISH: 2 subtelomeric 1p36 deletions, an unbalanced translocation involving chromosomes 1 and 12 with 1p36 deletion, and a de novo balanced translocation involving chromosomes 19

and 22. Thus, three cases of 1p36 subtelomeric deletion were found (3.95%) [22].

Because of the cost of FISH technique, it is advisable to use clinical criteria to select patients who are at a much higher risk of small deletions as a high rate of inherited anomalies has two important implications. First, because of the divergence of the phenotypes due to the presence of contrasting chromosomal rearrangements, it will be possible to establish a genetic diagnosis of an idiopathic disorder. Second, the high frequency of familial translocation alters the balance in favor of screening of all children with moderate to severe unexplained MR for the possibility of subtle chromosomal rearrangements.

In their survey of 29 patients with known subtelomeric rearrangements, de Vries et al. [4] documented that 83% had two or more facial dysmorphic features, 50% had a similar family history, and 37% had prenatal growth retardation. The structural malformations that have been reported in association with subtle subtelomeric rearrangements include cardiac defects, fascial clefts, brain abnormalities and skeletal defects [23]. It was difficult in the present study to determine the most frequent features among enrolled patients who had subtelomeric abnormality owing to the low number of documented cases (2/29, i.e., 6.8%) in comparison with the previously discussed studies conducted on larger samples with a relatively higher number of different subtelomeric rearrangements [24]. However, our first patient had broad forehead, low set ears and micrognathia, while our second patient had microcephaly, low set ears, high arched palate and squint. In our study we did not search for the frequency of subtelomeric abnormalities in the normal population, in contrast to Knight et al. [18] who revealed zero percent in the normal population. However, the frequency of these subtelomeric rearrangements in the normal population and the clinical relevance of every subtelomeric rearrangement that may be detected needs a study on larger number of population, although many investigators would not support a population screening study because of ethical and practical considerations [23].

5. Conclusion

In children with IMR and clinical phenotype indicative of a chromosomal anomaly, once recognizable syndromes have been excluded, abnormalities that include the ends of chromosomes must be searched for using HRB and subtelomeric FISH studies even when conventional karyotyping fails to demonstrate any abnormality. Proper diagnosis ascertained by HRB and sub-telomeric FISH studies in such cases is crucial for proper genetic counseling, recurrence risk estimate, and prenatal diagnosis.

Conflict of interest

The authors declare no conflict of interest.

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