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SCIENCE DIRECT
Clinical Biochemistry 39 (2006) 196–202
CLINICAL BIOCHEMISTRY

High rate of detection of subtelomeric aberration by using combined MLPA and subtelomeric FISH approach in patients with moderate to severe mental retardation

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Received 28 July 2005; received in revised form 17 November 2005; accepted 2 January 2006
Available online 23 February 2006

Abstract

Objectives: (1) To evaluate the prevalence of subtelomeric deletion in moderate to severe mental retardation population, (2) to assess the feasibility and cost-effectiveness of combined methodology in routine workup of this sub-population.

Method: Twenty unrelated patients using strict selection criteria were recruited for the study from the Clinical Genetic Service. Patients were initially screened by Multiplex Ligation-dependent Probe Amplification (MLPA) for subtelomeric imbalance followed by FISH analysis for anatomical integrity. This is then followed by parental subtelomeric FISH analysis.

Results: Three subtelomeric deletions were identified. They were Deletion 1p36, Deletion 1q44 and Deletion 10q26; these were previously unidentified by conventional technique.

Conclusions: The prevalence of subtelomeric deletion in our cohort of moderate to severe mental retardation patients is consistent with published findings of around 10%. The figure is on the higher side if more stringent criteria is used. The combination of strict clinical criteria, MLPA and selective subtelomeric FISH was shown to be feasible and cost-effective.

Keywords: Moderate to severe mental retardation; Subtelomeric deletions; MLPA; Subtelomeric FISH; MR, mental retardation; FISH, fluorescence in situ hybridization

Introduction

Mental retardation is common and occurs in approximately 2% of the population. 5–30% of moderate to severe mental retardation can be accounted for by chromosomal disorders; and the majority have associated malformations, growth retardation, dysmorphism and family history of similar occurrence.

Many studies have shown that subtelomeric aberrations are a significant cause of idiopathic moderate to severe mental retardation with figures varying from 5 to 10% [1,8,12,15,17] depending on sample selection criteria, as compared to less than 1% in mild mental retardation.

Subtelomeric regions are gene-rich. A small deletion in these regions would involve many genes, thus deleterious consequences result. Many of these subtelomeric deletions are now recognized as clinically recognizable phenotypes [3]. These terminal regions stain lightly on karyotyping and deletion sizes are variable, which are properties that often are difficult to detect on routine conventional examination. Although in retrospect, with awareness of the deletion, repeat culture with higher banding can often detect such deletions.

Multiplex Ligation-dependent Probe Amplification (MLPA) is a new, sensitive, economical and simple method for relative quantification of multiple nucleic acid sequences in a single reaction. Introduced by MRC-Holland in January 2002, the principle is a relatively simple one, in which denatured genomic DNA after standard extraction is hybridized with a mixture of standardized probes. Each MLPA probe consists of two oligonucleotides, the two parts of each probe hybridized to adjacent target sequences and are ligated by a thermostable ligase. All probe ligation products are amplified by PCR using

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doi:10.1016/j.clinbiochem.2006.01.003

Table 1
Subtelomeric patient selection criteria

Major criteria
1) Normal karyotype at 550 band level (46XY, 46XX)
2) Idiopathic moderate to severe MR (IQ < 50)

Plus minor criteria 3 out of 6 of the following
a) Facial dysmorphism (at least 2 areas from eyes, nose and ears that markedly differ from parents)
b) Non-facial dysmorphism or congenital anomalies (hands, brain, internal organ anomaly or abnormal genitalia)
c) Abnormal growth (pre or postnatal onset of growth retardation/or overgrowth)
d) Behavioural disorder (autistic symptom, hyperactivity, sleep disturbance, aggression, self-mutilations, etc.)
e) Family history of mental retardation
f) Family history of miscarriages or perinatal death

only one primer pair. Since the amplification product of each probe has a unique length, they can therefore be separated by electrophoresis. The relative amounts of probe amplification products reflect the relative quantity of target sequences.

Subtelomeric FISH probe is the most commonly used technique in detecting subtelomeric rearrangement [7]. Subtelomeric FISH probe sets are commercially available and are fully tested by their manufacturer.

Due to the laborious, time-consuming and costly procedures of using a complete set of subtelomeric FISH to analyze these selected patients, we developed a strategy of selecting the high risk group, screening with MLPA and then confirming with selective subtelomeric FISH probe(s), hoping to be of cost-effectiveness for future routine clinical use.

Methods

Subtelomeric testing selection criteria

The Clinical Genetic Service is a tertiary referral center and is also the only genetic center in Hong Kong. Twenty patients were selected consecutively from the genetic clinic from our service in year 2003 who satisfied major criteria of having a normal karyotype at 550 band level (46XY, 46XX) and with a diagnosis of idiopathic moderate to severe mental retardation (IQ < 50). They were all examined by a clinical geneticist. In addition, they all had 3 out of 6 of the followings: (a) facial dysmorphism (at least 2 areas from eyes, nose and ears, etc., that markedly differ from their parents), (b) non-facial dysmorphism or congenital anomalies (hands, brain, internal organ anomaly or abnormal genitalia, etc.), (c) abnormal growth (pre- or postnatal onset of growth retardation/or overgrowth), (d) behavioral disorder (autistic symptom, hyperactivity, sleep disturbance, aggression, self-injurious, etc.), (e) family history of mental retardation, (f) family history of miscarriages or perinatal death. When positive cases were detected in probands, karyotype (minimum 550 bands), MLPA and subtelomeric FISH analyses were performed in proband's parents and their mentally retarded relatives, where indicated (Table 1).

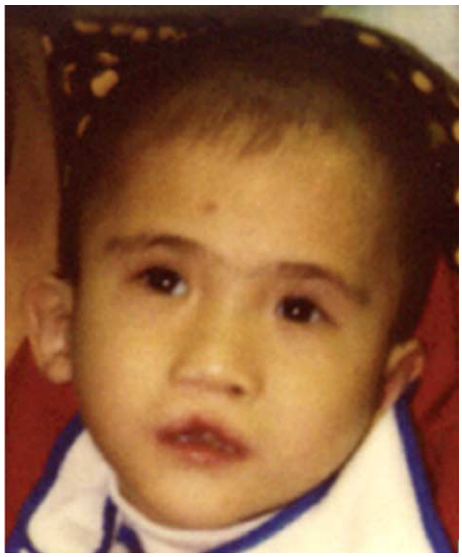

Fig. 2. Case 1 patient.

Subtelomeric FISH probes

Subtelomeric FISH studies were performed using Vysis ToTel Vysion selective probe(s) according to the manufacturer's procedure. It is comprised of a different combination of probes with different colors. The probe size ranged from 70 kb to 191 kb, and each contained a locus estimated to be within 300 kb of the end of the chromosome. We followed the standard hybridization method which consisted of 2× SSC aging of

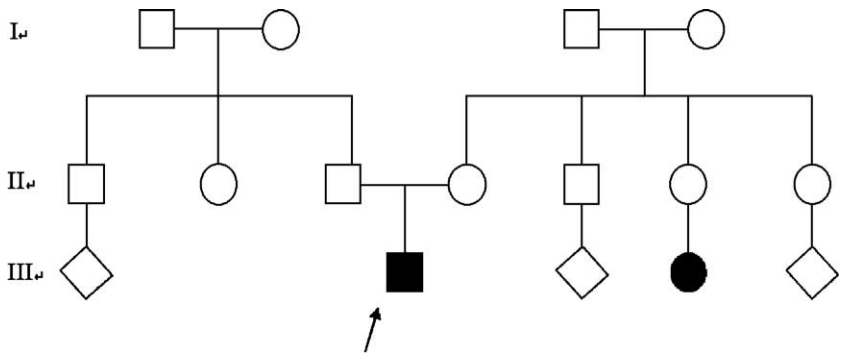

Fig. 1. Case 1 family pedigree.