Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medical Genetics 51 (2008) 93–105
EUROPEAN JOURNAL OF MEDICAL GENETICS
http://www.elsevier.com/locate/ejmg

Original article

Prospective screening of patients with unexplained mental retardation using subtelomeric MLPA strongly increases the detection rate of cryptic unbalanced chromosomal rearrangements

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Received 1 August 2007; accepted 8 October 2007
Available online 18 October 2007

Abstract

This study was designed to increase the diagnostic detection rate for subtelomeric unbalanced chromosomal rearrangements (UCRs) that are believed to cause 3–5% of all cases of mental retardation (MR), but often remain undetected by routine karyotyping because of limited resolution in light microscopy. Increased detection of such cryptic UCRs may be achieved by CGH- or SNP-array technology adapted for genome wide screening but these techniques are labor-intensive and expensive. We have implemented subtelomeric Multiplex Ligation-dependant Probe Amplification (MLPA), a relatively low cost and technically uncomplicated molecular approach, as a high throughput prospective screening tool for UCRs in MR patients. We prospectively studied a cohort of 466 MR patients and detected 53 aberrant MLPA signals. After exclusion of false-positives, potential familial polymorphisms and of non-cryptic UCRs also found in routine chromosome analysis, 18 cases or 3.9% of total could be confirmed as true cryptic subtelomeric UCRs. These were 6 terminal deletions, 8 unbalanced translocations, 3 Prader-Willi deletions and 1 subtelomeric interstitial deletion. This result increases our laboratory's detection rate in this

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1769-7212/$ - see front matter
doi:10.1016/j.ejmg.2007.10.003

patient cohort from 8.3% (without MLPA) to 12.2% (with MLPA), representing a 47% improvement. This study demonstrates that when applying MLPA in a routine cytogenetic diagnostic setting, a major increase of the diagnostic yield can be achieved.

Keywords: Molecular karyotyping; Mental retardation; Cryptic unbalanced chromosomal rearrangements; MLPA

1. Introduction

Mental retardation (MR) has a prevalence ranging from <1% to 3% in the general population varying with the definition of inclusion criteria of the populations described in the available publications [21]. In over half of the cases the cause of the MR remains unknown, either because patients have never been genetically evaluated or because a causative genetic defect was not found. Unbalanced chromosomal rearrangements (UCRs) are identified in a substantial proportion of patients as the cause of the observed MR with detection rates again varying widely from 5 to 30% as reported in the literature [21,26]. In particular, UCRs involving the distal ends or (sub-)telomeric regions of chromosomes have been shown to be a significant cause of MR [2,6,7,12,19,27] putatively based on the structural characteristics of chromosome ends and their role in the segregation process [15]. Detecting these subtelomeric UCRs by classic karyotyping is hampered by the limited resolution of light microscopy, which in optimally stretched and G-banded metaphase preparations typically is in the range of 5–10 Mb. Resolution in the subtelomeres is even further limited by the fact that chromosome endings most frequently appear as white unbanded stretches under the microscope. Small subtelomeric UCRs therefore are commonly referred to as 'cryptic' and are believed to represent a substantial proportion of undetected UCRs in every cytogenetic laboratory.

In order to increase the power of resolution and thus facilitating increased detection rates for cryptic UCRs a wide range of techniques have been developed and successfully implemented that either target the entire genome or are specifically aimed at the telomeres. In particular genome wide array based screening approaches, such as CGH or SNP arrays, are now available that effectively target a large number of loci in one assay, ranging from several thousands in classical BAC arrays, to as much as 500,000 in automated SNP arrays [9,11,17,18,28]. The downside to this technology is that is not readily available for many diagnostic cytogenetic laboratories and requires a substantial investment in equipment and resources. Of the currently available techniques specifically targeting the telomeres, multiprobe fluorescence *in situ* hybridization assays (often referred to as total telomere FISH), have been successfully employed and are now common in most cytogenetic labs [7,8,16,19]. However, even total telomere FISH is still an expensive and labor intensive assay, requiring lengthy analysis for every patient.

In a recent review the efficacy of various molecular subtelomeric screening approaches, allowing rapid screening of multiple patients in a single assay, was evaluated [23]. From this analysis and from several other studies MLPA has emerged as the most promising candidate technique with adequately robust characteristics and relative ease-of-use. The actual detection rate for UCRs is above average for MLPA compared to other screening techniques, although it is difficult to compare between published studies since success rates seem to be determined mostly by the widely diverging patient inclusion criteria. Our aim in this study was to perform a high throughput prospective screen using MLPA and establish if and by what margin we