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1p36 Microdeletion Syndrome: Diagnostic Problems and the Use of Molecular Cytogenetic Technologies for the Solution

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ABSTRACT

Partial 1p36 monosomy is one of the most frequent microdeletion syndromes. However, the diagnosis appears to be hindered due to the exceptional clinical diversity. Here, we present a comparative analysis of cytogenetic and molecular cytogenetic methods employed for testing of this chromosomal aberration in children with mental retardation and congenital malformations. Application of cytogenetic techniques allowed us to reveal terminal 1p36 deletions in 0.1% of cases (2 of 1874). Molecular cytogenetic analysis performed by conventional comparative genomic hybridization (CGH) uncovered 1p36 deletions in 1.3% cases (2 of 150). Using high-resolution microarray CGH (array CGH) we found microdeletions in 2.4 % (3 of 125) cases. Detected chromosomal rearrangements were confirmed by a fluorescent in situ hybridization. Array CGH allowed us to characterize the loss of genetic material in microdeletions with 1-10 kbp resolution. It was concluded that molecular diagnosis of 1p36 microdeletion syndrome requires the application of such innovative molecular cytogenetic technologies as array CGH. Our study enabled to estimate for the first time the frequency of 1p36 microdeletion syndrome among children with mental retardation and congenital malformations in the Russian Federation, which appeared to be about 2%.

Keywords: 1p36 deletion, microdeletions, molecular cytogenetics, whole genome scan, comparative genomic hybridization.

INTRODUCTION

1p36 deletion syndrome is characterized by severe intellectual disability, developmental delay, microcephaly, facial dismorphisms (prominent forehead , deeply set eyes, midface retrusion, depressed nasal bridge, asymmetric ears, cleft lip and palate) and congenital heart disease. This chromosomal pathology is considered as one of the most common causes of mental retardation and congenital malformations associated with microdeletions with estimated incidence about 1:5000 in general population, and 0.5-0.7 % — among children with mental retardation [1-4, 6].

Despite the fact that 1p36 deletion syndrome represents one of the most common genetic diseases associated with an unbalanced structural genomic rearrangement, its molecular diagnosis is complicated. This problem is apparently associated with exceptional clinical diversity and size variability of DNA sequences affected by deletions [5]. The aim of this work is to compare diagnostic techniques used for 1p36 deletion detection, which are based on either conventional cytogenetic or molecular cytogenetic innovative methods towards detection of unbalanced chromosomal and genomic rearrangements.

MATERIALS AND METHODS

We analyzed samples of peripheral blood lymphocytes obtained from 1874 children with mental retardation and congenital malformations using conventional cytogenetic methods of G- and C-banding. One hundred fifty cases were studied by applying conventional comparative genomic hybridization (CGH) in according to previously described protocol [7]. Additionally, 125 cases of mental retardation and congenital malformations were investigated using a genome-scanning technology with a resolution from one to several thousand bp or array CGH [8]. Patients presented with loss of genetic material in the aforementioned region of chromosome 1 were confirmed by fluorescence in situ hybridization (FISH).

RESULTS AND DISCUSSION

Cytogenetic analysis revealed terminal deletions of the short arm of chromosome 1 in 2 cases out of 1874 (0.1%). Although both cases were confirmed by FISH, deletion size and genes involved in chromosomal rearrangements were not identified. Conventional CGH revealed deletions in 1p36 region in children with severe mental retardation and congenital malformations in 2 cases (1.3%). These aberrations were microdeletions within 1p36.1p36.3 and 1p36.13p36.21 chromosome regions. Deletion sizes were estimated as 12+/- 2Mb in the first case and 7+/- 1Mb in the second one. Nevertheless, the use of conventional CGH did not allow us to determine the gene imbalance caused by these microdeletions in the short arm of chromosome 1. High-resolution genome scan by array CGH (with 1 kbp resolution and more) revealed the presence of deletions in 1p36 region in three cases out of 125 (2.4%). Deletions sizes were determined as 4.44, 7.09 and 8.15 Mb. The whole-genome scan technology has made possible to characterize the genomic rearrangements up to 1 kbp and uncover genes which became homozygous due to the deletion.

These data suggest that the use of the high-resolution array CGH is the most effective method for detecting recurrent chromosomal rearrangements in children with mental retardation and congenital malformations. It is noteworthy that, despite of retrospective clinical

ascertainment showed the presence of 1p36 deletion syndrome phenotype, all patients were not clinically diagnosed until molecular testing was performed. It is also important to note that cytogenetic analysis revealed only terminal deletions, which size was more than 25 Mb. Conventional CGH revealed deletions in 1p36 but did not allow accurate description of DNA sequence losses. Whole genome scan or array CGH proved to be the most effective in terms of molecular diagnosis. This correlates with the previously obtained data on microdeletion syndromes investigated by a genome-wide scan [1, 2, 6]. In addition, our data demonstrates that the occurrence of 1p36 deletion syndrome among children with mental retardation and congenital malformations is not less than 2% in the Russian Federation.

CONCLUSION

Comparative analysis of cytogenetic and molecular cytogenetic methods used in genome analysis showed that for highly effective diagnosis of one of the most common syndrome associated with microdeletions, the use of high-resolution array CGH is essential, because it as increases the efficiency of molecular diagnostics as allows to define DNA sequences and genes involved in the rearrangement. Considering current achievements in molecular therapy, the information obtained can serve as a basis for the development of science-based tactics for treatment of this disease. It should also be noted that similar studies of 1p36 deletion syndrome in the Russian Federation has not been carried out. This is probably due to the fact that the methods of high-resolution molecular cytogenetic diagnostics were only recently introduced into genetic diagnosis. This study allowed us to solve this problem and showed that the occurrence of 1p36 deletion syndrome among children with mental retardation and congenital malformations in the Russian Federation is about 2%.

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