

## The quantitative PCR technique resolves ambiguities concerning a small rearrangement of human chromosome 6q12-13

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**Abstract** Karyotype analysis, performed on the basis of chromosome banding pattern, is a standard method used for identification of chromosomal aberrations, both numerical and structural. The application of classic cytogenetic techniques fails, however, to solve all diagnostic problems in certain types of chromosome aberrations. In this study, quantitative polymerase chain reaction technique (Q-PCR) application was applied to verify a cytogenetic diagnosis, which assumed that a difference observed in the banding pattern of homologous chromosome 6q12-13 region of a foetus had resulted from an inversion and/or duplication of the region in question. The obtained results indicate a possibility to use the Q-PCR method in the diagnostics of unbalanced chromosomal aberrations.

**Key words:** chromosome 6, duplication, human, inversion, Q-PCR.

### Introduction

The resolution potential of classic banding cytogenetic techniques sometimes seems to be rather insufficient for an accurate description of chromosomal breaking points in cases of chromosomal aberrations. This problem reveals not only theoretical significance but it also exerts a fairly practical impact on the prognoses of clinical effects resulting from chromosome aberrations. A need then emerges to design a method of quicker and more accurate evaluation of the regions involved in chromosomal aberrations, especially in cases of prenatal diagnostics. The quan-

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titative polymerase chain reaction (Q-PCR) technique is an example of such an evaluation. The Q-PCR has been used in the diagnostics of the most frequent aneuploidies of chromosomes 21, 18, 13, X and Y, as well as of other unbalanced chromosomal aberrations, such as deletions or duplications (VON EGGELING et al. 1993, ADINOLFI et al. 1997, LEE et al. 1997, DEL RIO et al. 1998). Being a very sensitive method, with a much quicker availability of results, the Q-PCR technique enables an effective dose effect determination.

In our study, this technique was applied to verify a cytogenetic diagnosis of a female foetus, assuming that a difference observed in the banding pattern of homologous chromosome 6q12-13 region had been caused by an inversion and/or duplication of the region in question (Figure 1).

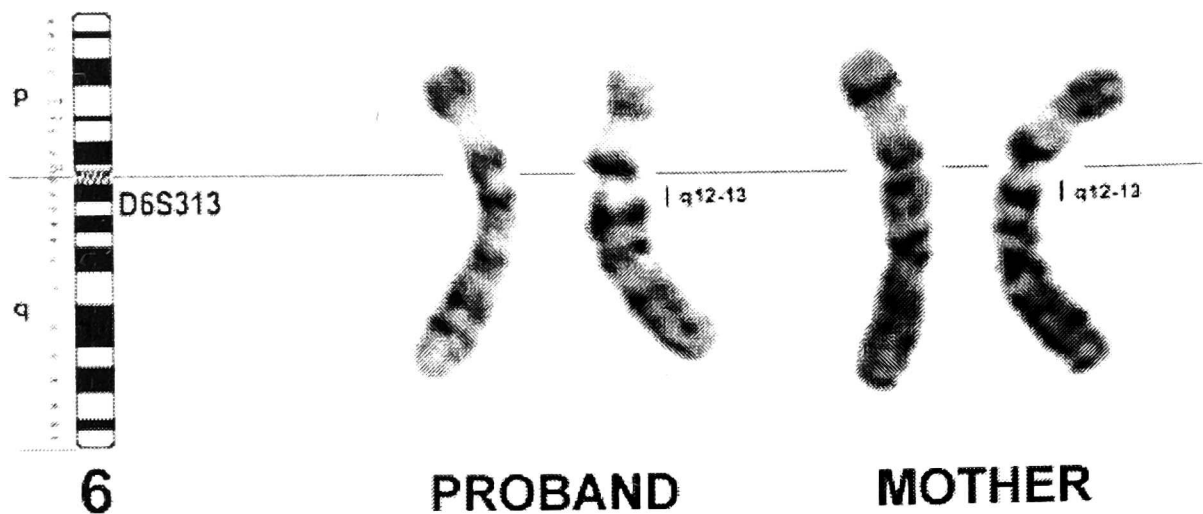


Figure 1. Ideogram of the human chromosome 6 and partial karyotypes of the foetus and mother showing chromosome pair 6. Abnormal region indicated by lanes. Karyotypes are 46,XX,dup(6) (pter→q13::q13→qter) and/or inv(6) (pter→q12::q13→q12::q13→qter).

## Material and methods

### Material

The study was a part of prenatal diagnostics, performed because of the mother's age (41 years). Karyotypes of the foetus, of both parents and of controls were evaluated by means of the GTG technique (G-bands by Trypsin using Giemsa) of high resolution, aided by the Multiscan-karyotype computer image analysis. Genomic DNA was isolated from foetal amniocytes by ERLICH'S method (1989) and from peripheral blood lymphocytes from both parents, as well as from the controls (n = 20), according to MILLER et al. (1988).

## Polymerase chain reaction (PCR)

DNA samples were subjected to amplification, using primers specific for the D6S313 sequence, located in 6q12-13 region, and for the D21S16 sequence from chromosome 21q11.1, the latter standing for internal control. The PCR primers were selected with the help of the primer analysis software VECTOR NTI, version 5.0. Primer sequences included: D6S313A 5'-AGG TAG GGA AAT ACA GTT AGT GCA G-3', D6S313B 5'-TTC CAT GTG CCA TAC ACT ATG C-3', D21S16A 5'-CCA TAA ACA CAA TCT TCA AGG CC-3', and D21S16B 5'-CCC AAG GAC AAT AGT ACA CAT TCT C-3'. The reaction mixture (50  $\mu$ l) contained 1  $\mu$ g genomic DNA, 5 nmol dNTPs, 5  $\mu$ Ci  $\alpha^{32}$ PdCTP (Amersham), 40 pmol of each primer and 1  $\times$  *Taq* polymerase buffer, and 2U *Taq* DNA polymerase. After denaturation at 95°C for 150 seconds, 30 cycles at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds were performed in an UNO II thermocycler (Biometra). Final extension lasted 10 minutes.

## Electrophoresis and the quantitative analysis of PCR products

PCR products were separated by electrophoresis in 8% polyacrylamide gel (Figure 2). After ethidium bromide staining, samples corresponding to the amplified DNA fragments (D6S313, D21S16) were densitometrically analysed by

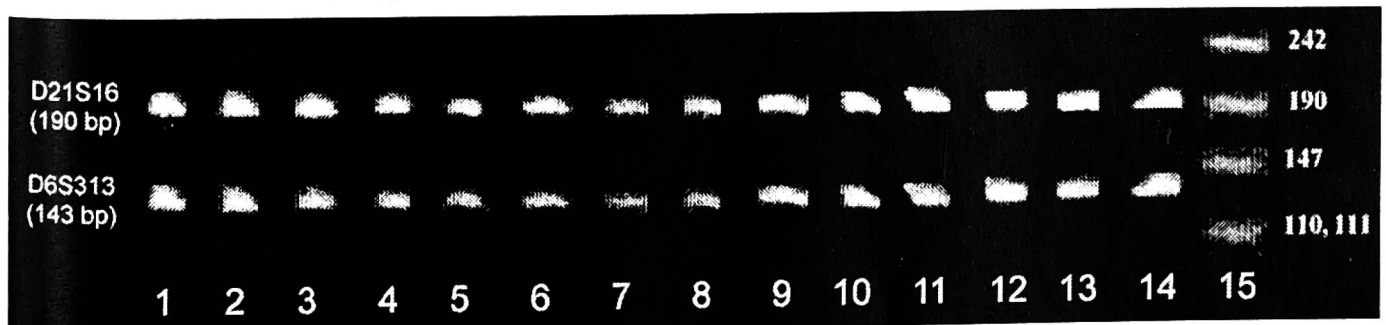


Figure 2. Acrylamide gel electrophoresis of PCR products of D6S313 (143 bp) and D21S16 (190 bp). Lanes 1-8: patients with inv/dup (?) 6q12-13; lanes 9-14: the control group; lane 15: molecular weight standard (pUC19/MspI). Densitometric analysis did not show any increase in quantity of PCR products, as compared with the control group.

the Multiscan system and then were cut and subjected to isotopic analysis (Beckman Scintillator). We assessed the ratio of PCR product quantity in the studied sequence to the quantity of the product in internal control. The obtained results were compared with those of the control group. The statistical analysis of results was performed by Student's *t* test with the level of significance at  $p \leq 0.05$ .

## Results

The evaluation enabled foetal karyotype recognition 46,XX,dup(6)(pter→q13::q13→qter) and/or inv(6)(pter→q12::q13→q12::q13→qter). A similar aberration was found in the mother. Following the results of the performed densitometric and isotopic evaluations, no statistically significant increase was found in the PCR product quantity of the studied D6S313 sequence in both the foetus and its mother, when compared with the control group, consisting of 20 persons with normal karyotypes (Table 1). The obtained results indicate that the observed aberration in the location of bands in 6q12-13 region was not caused by duplication of that region and the observed aberration is probably an inversion only.

**Table 1.** Quantitative analysis of PCR product of D6S313 in a case with inv/dup 6q12-13 in comparison to the control group

No.	Subject	Densitometric analysis [%]	Isotopic analysis
1	mother	100.79 ± 3.71	97.68 ± 6.25
2	father	100.61 ± 5.13	97.59 ± 6.73
3	foetus	100.65 ± 6.97	97.72 ± 7.88

## Discussion

Reports from the recent years confirm the usefulness of the quantitative PCR technique for the assessment of chromosomal abnormalities (TÓTH et al. 1998). In the presented study, a fragment of studied DNA was amplified, together with another fragment from another site of the same matrix (internal control) on which the studied DNA fragment is replicated. The applied method indicates certain technical requirements (RAEYMAEKERS 1998). The two used pairs of primers should be similar with respect to their length and melting point ( $T_m$ ), while they should not bind with each other. In contrast, the studied and control sequences should differ in length, so that both PCR products could be easily identifiable.

The presented case confirms the potential of the Q-PCR technique for the verification of structural aberrations. The lack of PCR product increase in the analysed DNA fragments indicates that the difference in the banding pattern of the homologous chromosome 6q12-13 region is not caused by duplication of this region, being rather dependent on paracentric inversion. The confirmation of results obtained in such cases is possible through further molecular analysis, employing, for examples, the chromosome orientation and direction fluorescent *in situ* hybridisation (COD-FISH) which is a modification of the standard fluorescent *in situ* hybridisation (BAILEY et al. 1996). Presumably, that it is the only

method which, in cases of inversion, approached from the practical point of view, can bring up conclusive information. The carriers of inversions do not present with any clinical symptoms but they bear the risk of procreation of chromosomally unbalanced offspring (DJALALI et al. 1986). The risk to deliver an abnormal child depends on a number of factors, e.g., on the size of the aberrant chromosome fragment.

The performed study confirmed the usefulness of the quantitative PCR technique as a valuable diagnostic tool, enabling a quantitative evaluation of genetic material from the region involved in chromosomal aberration. This method can be used with equal effectiveness for the analysis of numerical aberrations, as well as for the evaluation of unbalanced structural aberrations.

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