Genetics and Molecular Biology, 23, 4, 725-727 (2000)

Short Communication

Multilocus DNA fingerprinting in paternity analysis: a Chilean experience

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Abstract

DNA polymorphism is very useful in paternity analysis. The present paper describes paternity studies done using DNA profiles obtained with the $(CAC)_5$ probe. All of the subjects studied were involved in nonjudicial cases of paternity. Genomic DNA digested with *Hae*III was run on agarose gels and hybridized in the gel with the $(CAC)_5$ probe labeled with ^{32}P. The mean number of bands larger than the 4.3 kb per individual was 16.1. The mean proportion of bands shared among unrelated individuals was 0.08 and the mean number of test bands was 7.1. This corresponded to an exclusion probability greater than 0.999999. Paternity was excluded in 34.5% of the cases. The mutation frequency estimated from non-excluded cases was 0.01143 bands per child. In these cases, the paternity was confirmed by a locus-specific analysis of eight independent PCR-based loci. The paternity index was computed in all non-excluded cases. It can be concluded that this method is a powerful and inexpensive alternative to solve paternity doubts.

INTRODUCTION

Genetic marker analysis is a powerful tool for paternity testing. The most polymorphic genetic markers in the human genome are the tandemly repeated mini- and micro-satellites (also known as VNTR or STR loci, respectively) (Pena *et al*., 1993). The simultaneous detection of several VNTR loci using a single DNA probe was first reported by Jeffreys *et al*. (1985a). The banding pattern obtained with this method is individual-specific and is referred to as a DNA fingerprint (Jeffreys *et al*., 1985b). Many multilocus probes have been described, and the specificity of the patterns obtained with them provides a reliable means of exclusion in paternity testing (Jeffreys *et al*., 1985b).

In this report, we describe the use of multilocus DNA fingerprinting to solve problems of paternity in Chile.

MATERIAL AND METHODS

The studied group consisted of all the individuals who requested for a paternity analysis at the University of Chile, Clinical Hospital, between June 1996 and July 1998. This hospital is a national center for paternity testing for cases in which no judicial process is involved. All of the mother and alleged father pairs studied were unrelated middle-class Chileans. The Chilean population is an admixture of Caucasians (mostly Spaniards) and Amerindians (Valenzuela, 1988).

Human genomic DNA was obtained from peripheral blood (Comey *et al*., 1994), digested with *Hae*III, and run on 7% agarose gels. After electrophoresis, and *in situ* hybridization with a $(CAC)_5$ probe (Jeffreys *et al*., 1991), the bands were labeled with ^{32}P (Armanet *et al*., 1995). The $(CAC)_5$ probe was developed by Nurnberg *et al*. (1989), who described the somatic and germline stability of the pattern obtained with this probe (Nurnberg *et al*., 1989). Only bands larger than the 4.3-kb *Hin*dIII fragment of lambda phage were studied. Paternity was excluded when more than two bands in the child's DNA profile were not present in the mother's or putative father's profile. If only one or two unassignable bands were present, they were interpreted as mutations. In those cases in which mutant bands were found, the paternity was corroborated with a locus-specific analysis of eight independent PCR-based loci (Jorquera and Budowle, 1998). The *a posteriori* probability of paternity was computed using the paternity index proposed by Pena and Chakraborty (1994) based on preliminary estimates of band sharing (0.1315) and the mutation rate (0.0015 bands/child) performed in our laboratory.

RESULTS

Eighty-four couples and their children requested a paternity investigation during the two-year period. Paternity was established in 55 cases (65.5%) and excluded in the remaining 29 (34.5%). The confirmation of paternity was based on an *a posteriori* probability greater than 0.999 in 44 cases (52.4%), using the paternity index proposed by Pena and Chakraborty (1994). In the remaining cases, those with one mutant band, the *a posteriori* probability of paternity ranged from 0.945 to 0.998. In one case with two mutant bands and nine test bands, the probability of paternity was 0.942.

The mean number of bands per individual was 16.1 ± 2.7 SD (range: 8-27). The number of bands shared among

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unrelated individuals, computed by comparing those of the mother and the alleged father in each case, was 1.4 ± 1.4 SD (range: 0-5), which yielded a mean proportion of shared bands of 0.09. The mean number of test bands was 7.1 ± 2.1 SD. These values resulted in an exclusion probability >0.999999.

The distribution of the bands shared between the a) mother and alleged father, b) child and excluded fathers, c) mother and child, and d) child and included alleged father is shown in Figure 1. The distribution in the latter two cases (c and d) was similar, but differed from the first two cases (a and b) (Figure 1). There was a small overlap between the distributions of the first two cases and the last two.

Of the 55 cases in which paternity was confirmed, in 14 an unassigned band in the child was detected, and it was interpreted as a mutation. In one case, there were two mutant bands. In all, 1400 child bands were detected which corresponded to a mutation frequency of 16/1400 on 0.01143 bands per child.

The distribution of the unassigned bands (as a percentage of the total number of test bands) is shown in Figure 2. There was a clear distinction between excluded and assigned paternity cases. This distinction was even more marked when the bands shared by the alleged father and child were included. Figure 3 shows that there was a negative correlation between band-sharing and the proportion of unassigned bands in the cases analyzed. In addition, the two paternity groups - those with confirmed paternity (upper left) and those excluded from paternity (lower right) were well separated.

DISCUSSION

Multilocus DNA fingerprinting is considered a powerful tool in paternity testing. In this study we validated the usefulness of the $(CAC)_5$ probe for DNA analysis in paternity cases in Chile. The technique distinguished fathers from non-fathers in all cases. The low proportion of bands shared among unrelated individuals (0.088) and the high number of test bands (mean of 7.1) provided a high probability of excluding falsely accused men. The best discrimination criterion was the ratio of unassignable bands relative to the total number of test bands. This ratio was less than 0.23 in fathers and greater than 0.68 in non-fathers, with no overlapping. An additional criterion was the number of bands shared by the alleged father and child. Compared to those shared with the true fathers, these groups showed only a small overlap in their numbers.

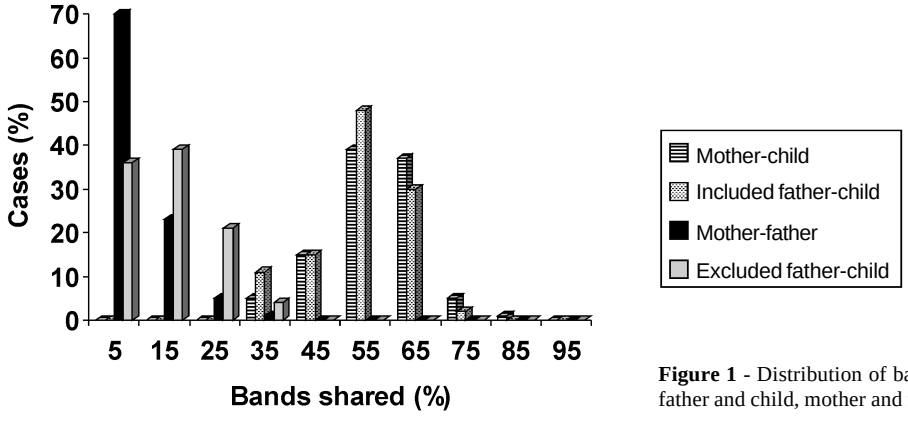

Figure 1 - Distribution of bands shared between mother and child, included father and child, mother and father and excluded father and child pairs.

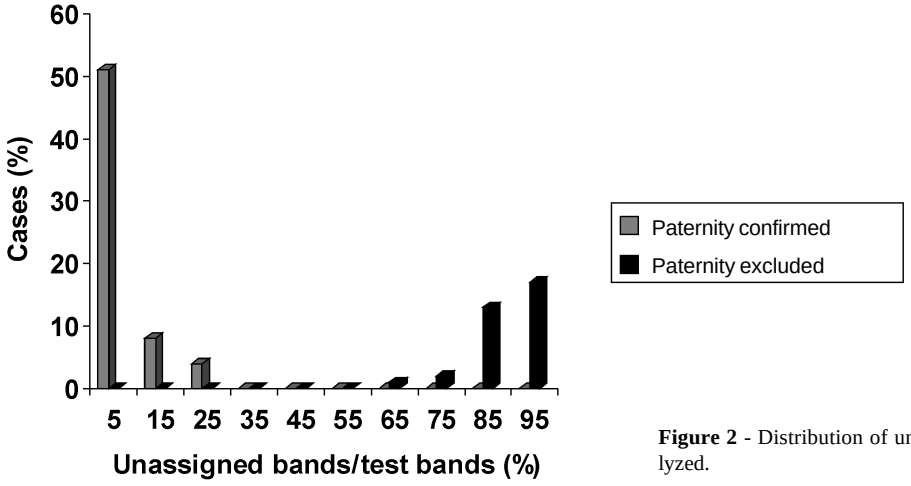

Figure 2 - Distribution of unassigned bands among the paternity cases analyzed.

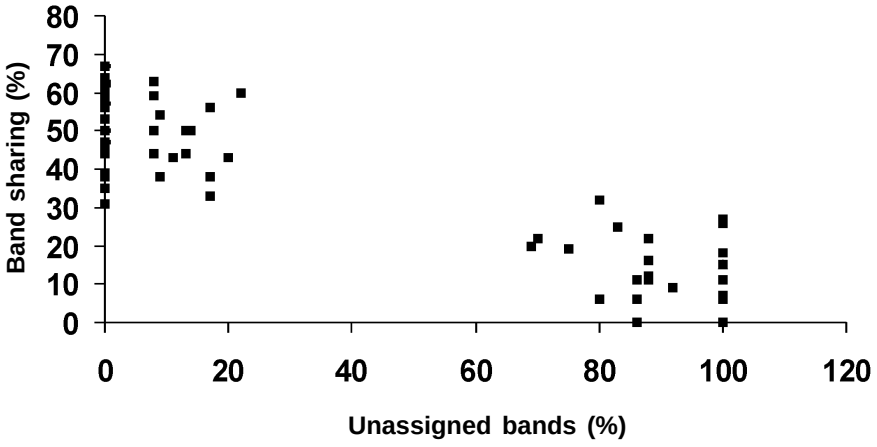

Figure 3 - The relationship between shared bands and unassigned bands in child-alleged father pairs. Each dot represents a single case. The cluster on the left upper of the graph indicates cases of confirmed paternity while that on the lower right indicates cases of no paternity.

The paternity index proposed by Pena and Chakraborty, is useful in DNA analysis, particularly since it includes mutation rates, which are not low in minisatellites. When only one or two unassignable bands exist, additional DNA testing is recommended in order to confirm the results of the paternity analysis; this confirmation is strongly recommended if there is more than one mutant band.

Exclusion from paternity was indicated in only 34.5% of the cases, despite the high exclusion probability offered by this technique. This finding suggests that, in most cases, those involved already knew who the father was.

ACKNOWLEDGMENTS

Research partially supported by grants from Fondecyt, Chile (No. 1960405).

RESUMO

O polimorfismo de DNA é muito útil em pesquisa de paternidade. O presente trabalho descreve estudos de paternidade usando perfis de DNA obtidos com a sonda $(CAC)_5$. Todos os indivíduos estudados estavam envolvidos em casos não judiciais de paternidade. DNA genômico digerido com *Hae*III foi colocado em gel de agarose e hibridizado no gel com a sonda $(CAC)_5$ marcada com ^{32}P. O número médio de bandas maiores que 4,3 kb por indivíduo foi 16,1. A proporção média de bandas compartilhadas entre indivíduos não aparentados foi 0,08 e o número médio de bandas de teste foi 7,1. Isto correspondeu a uma probabilidade de exclusão maior que 0,999999. A paternidade foi excluída em 34,5% dos casos. A freqüência de mutação estimada em casos não excluídos foi 0,01143 bandas por criança. Nestes casos, a paternidade foi confirmada por uma análise locus-específica de oito locos independentes obtidos com PCR. O índice de paternidade foi computado em todos os casos não excluídos. Pode-se concluir que este método é uma alternativa poderosa e econômica para resolver dúvidas de paternidade.

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(Received July 21, 2000)