DETERMINATION OF GENOTYPE OF DIZYGOTIC CATTLE TWINS USING BLOOD AND HAIR ROOT MICROSATELLITE ANALYSIS

M. Ron, Y. Blank and M. Band
Institute of Animal Sciences, A.R.O., The Volcani Center, Bet Dagan, 50250, Israel

SUMMARY

Eighteen pairs of female twins were genotyped for 3 to 9 microsatellites using diplex PCR systems from blood and hair roots. Blood chimaerism was evident in 17 out of 18 sets of twins tested. Chimaerism was recognized by discrepancies between blood and hair analyses for microsatellite markers and by detection of more than 2 alleles per genotype. Chimaerism of 2, 3 and 4 alleles were shown in genotypes of twins from blood as compared to 1 and 2 alleles from hair. We found that genotyping of microsatellites from hair follicles is a highly sensitive method for discrimination between the inherited and acquired genotypes in dizygotic twins.

INTRODUCTION

Most multiple pregnancies in cattle result in anastomosis of placental blood vessels allowing interchange of blood cells between the twin fetuses (Lillie, 1916). The evidence of cell exchange between heterosexual cattle twins leading to a sterile female has been well documented (Dunn et al., 1979). Somatic cell chimaerism in twins was estimated between 3% to 100% (Dunn et al., 1979; Telpitz et al., 1967) and germ cell chimaerism between 5 to 10% (Telpitz et al., 1967). Leveziel et al. (1988) noticed discrepant results when genotyping for k-casein from milk protein and by RFLP of DNA from blood. Plante et al. (1992) showed extensive leucochimaerism using DNA fingerprinting between pairs of dizygotic twins in cattle which may hamper genotyping based solely on blood samples. Bowling et al. (1993) demonstrated blood chimaerism by different genotypes for microsatellites in blood and hair bulbs. In this paper we further explore chimaerism for microsatellite genetic markers in dizygotic cattle twins, and suggest a highly sensitive method for discrimination between the inherited and acquired genotypes in dizygotic twins.

MATERIALS AND METHODS

Eighteen pairs of female twins from six Israeli kibbutz herds were sampled for blood and hair roots. The age of cows varied from 6 mo to 4 yrs. In addition, a pair of male twin was sampled for semen. The PCR protocols for DNA isolated from semen, blood cells and 2-10 hair roots using GeneReleaser (Bioventures) were as described by Ron et al. (1994) using OmniGene thermocycler (Hybaid, Middlesex, UK). Genotypes were carried out for the following microsatellites using diplex PCR systems: ARO28, BRN, CYP21, D21S4, DU17S2, DU2S1, DU23S1, D5S1, HBB, ILST001, ILST002, UWCA1, UWCA4 and UWCA9 (Barendse et al., 1994) as described by Ron et al. (1994). To determine the sensitivity of our PCR system to detect different proportions of templates, DNA from two individuals who were homozygous for different alleles of a microsatellite were titrated together such that a range of proportions of each allele was obtained in the following ratios: 0:1, 1:0, 1:1, 1:1.5, 1:2, 1:10, 1:20, 1:50, 1:100, 1:1000 in a final concentration of 50 ng. PCR was performed separately for three microsatellites BRN, CYP21 and DU2S1 with independent replicates for each dilution ratio.

Autoradiograms were scanned and analyzed for intensity of bands by optical densitometry with Image 1.41 software on a Macintosh computer.

RESULTS

PCR from blood for 3-9 microsatellites per cow showed identical banding patterns between co-twins in 18 sets. In 17 twin sets the banding pattern showed the existence of 1 to 4 alleles per microsatellite genotype. PCR from hair roots showed only 1 and 2 alleles per genotype in all twin sets. Only one set of twins showed identical genotypes for both blood and hair root PCR for all 7 microsatellites tested indicating monozygosity. PCR amplification of microsatellites from hair and blood of female twins is shown in Figure 1. Chimaerism of 2, 3 and 4 alleles are shown in genotypes of twins from blood as compared to 1 and 2 alleles from hair: Twin set number 2 consists of homozygous and heterozygous cows for CYP21 from analysis of hair while both cows appear as heterozygous from analysis of blood. Both cows of twin set number 3 are heterozygous from hair analysis for D21S4 with one allele in common, however, three allele genotypes are evident for these cows when analyzed from blood. Twin set number 4 shows two different alleles for each cow from hair as compared to four alleles each from blood. Genotypes from blood were identical between co-twins for all cases. However, in a few cases there were predominant bands representing alleles of one of the cows. This is demonstrated in Figure 1 for twin set 4 where both alleles of the cow on the left were predominant in her co-twin's genotype. Nevertheless, this phenomenon was not consistent with genotypes for other microsatellites for the same twin. The set of male twins was genotyped for 12 microsatellites. Total number of alleles per genotype did not exceed two. Genotypes of 7 microsatellites were different for the twins. Thus, no sign of germ cell chimaerism was detected.

In Figure 2 the sensitivity of PCR to detect different amounts of templates is shown for microsatellite CYP21. A good estimate of the relative amounts of each allele was obtained by densitometry measures across the range of dilutions tested. The power of our PCR system to detect the presence of an allele representing 1% of the total amount of DNA (0.5 ng of template DNA) is demonstrated. Similar results were derived for two other microsatellites, BRN and DU2S1. The intensity of allele product increased with amount of DNA in template in the mixes. Significant linear regressions were found for allele product on dilution ratio for the three microsatellites tested. Correlation between band intensity of double loads of the same samples on the gel was 0.99 indicating the accuracy of both manual loading of samples and densitometry measures.

DISCUSSION

Blood chimaerism was recognized by discrepancies between blood and hair analyses for microsatellite markers and by detection of more than 2 alleles per genotype. Chimaerism was evident in 17 out of 18 sets of twins tested. The set with identical genotypes for blood and hair roots over all microsatellites can be considered monozygotic. Few microsatellites were needed to confirm chimaerism for each twin set. In a few cases there were predominant bands representing alleles of only one of the cows which may result in erroneous genotyping. This phenomenon was not repeated for other microsatellites for the same twin indicating that it is not due to a predominant blood system of one of the cows as suggested by Wilkes et al. (1981). Blood chimaerism was evident regardless of age of the cows which varied from 6 mo

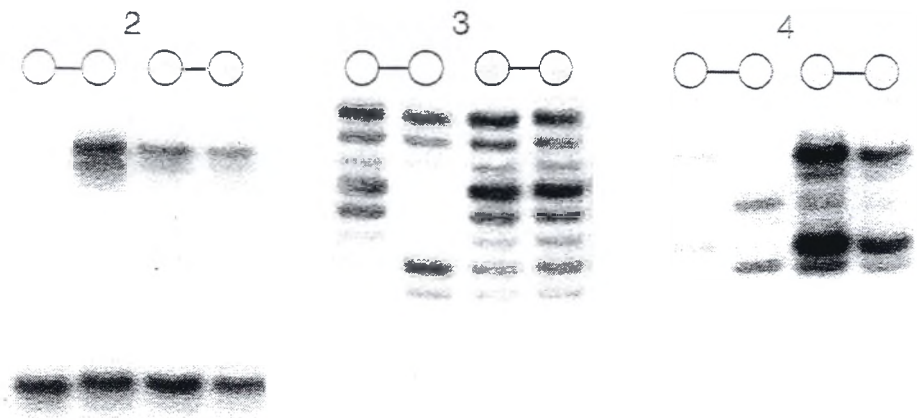

Figure 1. Chimaerism of 2, 3 and 4 alleles per genotype of female twins from blood (full circles) as compared to the genotype from hair (empty circles).

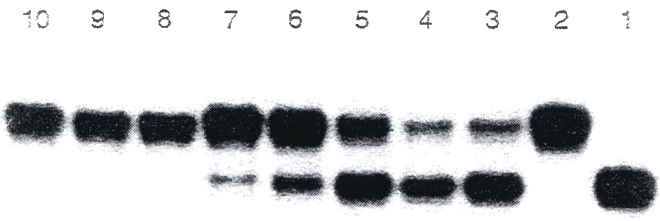

Figure 2. PCR products from proportional DNA mixes of two homozygous individuals for different alleles of microsatellite CYP21. Lanes 1 to 10 correspond to mixing ratios of 0:1, 1:0, 1:1, 1.5:1, 2:1, 10:1, 20:1, 50:1, 100:1 and 1000:1.

to 4 yr. Thus, it may be due to early anastomosis of the placental vessels of the two twins and the exchange of leukocytes as well as stem cells that become established in both embryos (Dunn et al., 1979; Plante et al., 1992). Although evidence for chimaerism of leukocytes in blood of twins is well documented there is little regarding the immunological aspects of the combined blood systems as to differences in disease resistance between dizygotic twins and non-twin sibs.

We found that genotyping of microsatellites from hair follicles can be used to discriminate between the inherited and acquired genotypes in all dizygotic twins. These results are concordant with those of Bowling et al. (1993). However, Lipkin et al. (1993) found chimaerism in female twins also in hair roots, based on presence of Y specific PCR product. This can be explained by analysis of DNA from large number of hair follicles (30-60) and amplification of a repetitive sequence. Telpitz et al. (1967) found 6-10 % XX-germ cells in newborn bull testis. Therefore, there is a possibility that a bull born with a twin might transmit to his progeny his twin's genotype. However, we were not able to demonstrate the existence of germ cells chimaerism in a male twin although our PCR system could detect cells mixes as small as a 1:100 ratio.

Genotyping for DNA microsatellites from blood of cattle twins can be used to diagnose chimaerism in both same-sex and heterosexual twins. Hair roots are a reliable source of DNA for detection of the inherited genotype of an individual twin. It can be applied in identification of monozygotic twins either for experiments with environmental effects or to avoid duplicate progeny testing of monozygotic male twins. Paternity and maternity testing is dependent on accurate identification of genotype of offspring and parental allele origin (Bowling et al., 1993). Unequivocal determination of sire allele origin for multiallelic microsatellites is essential for detection of quantitative trait loci within paternal half-sib designs (Ron et al., 1994) and for application in marker assisted selection (Weller and Fernando, 1991).

REFERENCES

Barendse W., Armitage S.M., Kossarek L., Kirkpatrick B.H., Ryaan A.M., Shalom A., Clayton D., Li L., Neibergs H., Nan Z., Grosse M., Creighton P., McCarthy F., Ron M., Soller M., Fries R., McGraw R.A., Moore S.S., Teale A., Georges M., Womack J.E., & Hetzel D.J.S. (1994) Nature Genetics (In press)

Bowling A. T., Stott M. L., & Bickel L. (1993) Anim. Genet. (24): 323-324.

Dunn H. O., McEntee K., Hall C. E., Johnson R. H., & Stone W.H. (1979) J. Reprod. Fert. 57:21-30.

Leveziel H., Metenier L., Mahe M. F., Choplain J., Furet J. P., Paboeuf G., Mercier J. C., & Grosclaude F. (1988) Genet. Sel. Evol. 20(2): 247-254.

Lillie F. R. (1916). Science 43: 611-613.

Lipkin E., Tikoschinsky Y., Arbel R., Sharoni D., Soller M., & Friedmann A. (1993) Anim. Biotech. 4(2): 195-201.

Plante Y., Schmutz S. M., Lang K. D. M., & Moker J. S. (1992) Anim. Genet. 23: 295 302.

Ron M., Band M., Yanai A., & Weller J.I. (1994) Anim. Genet. (In press)

Teplitz R. L., Moon Y. S., & Basrur P. K. (1967) Chromosoma. 22: 202-209.

Weller J.I., & Fernando R.L. (1991) In: Gene mapping: Startegies, Techniques and Applications. L. B. Schook, H. A. Lewin, & D.G. McLaren (Ed.) Marcel Dekker, Inc. New York. pp 305-328.

Wilkes P. R., Wijeratne W. V. S., & Munro I. B. (1981) The. Vet. Rec. 18: 349-353.