TECHNICAL NOTE

Mohammad I. Kamboh,[1] *Ph.D. and Robert E. Ferrell,*[1] *Ph.D.*

An Improved Method for Complement Subcomponent C1R Typing

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ABSTRACT: An improved method has been developed for the reliable classification of different C1R genetic variant forms from human serum or plasma. The method combines the use of neuraminidase-digested samples followed by monodimensional isoelectric focusing in the pH range 5 to 8 followed by immunoblotting. The method yields a simple pattern, with one major band in homozygote and two major bands in heterozygote cases.

KEYWORDS: forensic science, genetic typing, isoelectric focusing, immunoblotting, C1R, genetic variants, forensic serology, neuraminidase, blood samples

The C1R, along with C1S and C1Q subcomponents, constitutes the first component of the classical complement system [*1*]. Using isoelectric focusing (IEF) and immunoblotting, genetic polymorphisms for C1R and C1S have been described recently in native serum or plasma samples [*2–3*]. The products of two common alleles, *C1R*1*, *C1R*2* [*2*], and one rare allele, *C1R*3* [*4*], have been described. In its native form C1R consists of a complex multibanded pattern on IEF gels. This complex pattern may cause problems in some cases for their unequivocal classification. This paper describes a simple, reliable, and reproducible method to score different C1R phenotypes without any difficulties.

Materials and Methods

Blood Samples

Twenty-microlitre plasma or serum samples were simultaneously dialyzed and desialylated overnight at room temperature by the addition of 10 μL of neuraminidase solution (1 U/28 μL in 0.2*M* disodium ethylenediaminetetra-acetate, pH 7.2) in a continuous flow microdialysis system (Model 1200, Bethesda Research Laboratories) against 0.03*M* phosphate buffer, pH 6.8.

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[1]Assistant professor and professor, respectively, Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA.

Isoelectric Focusing and Immunoblotting

Isoelectric focusing was carried out in 0.5-mm thin-layer polyacrylamide gels (T = 5%; C = 3%) containing 6*M* urea and 2% pharmalyte pH range 5 to 8. Polymerization was achieved by adding 20 μL of 0.1% riboflavin solution and exposing the gel overnight to fluorescent light. Catholyte and anolyte solutions were 1*M* sodium hydroxide and 1*M* phosphoric acid, respectively. The gels were prefocused for 30 min at 1000 V, 30 W, and 50 mA before focusing at 2000 V for 180 min. The neuraminidase-treated samples were applied 1 cm from the catholyte strip using 5- by 7-mm paper wicks (Whatman 3 MM), and the wicks were removed after 60-min focusing time. IEF on native samples was performed in pH range 4 to 6.5 gels as described previously [2]. After electrofocusing, proteins were allowed to transfer passively for 60 min from the gel onto a nitrocellulose membrane, followed by sequential exposing of the membrane to the following: 5% nonfat dry milk for 45 min; goat anti-human C1R antiserum (Atlantic antibodies) at 1:750 dilution for 60 min; and rabbit anti-goat IgG conjugated with alkaline phosphatase at 1:5000 dilution for 60 min. In between the primary and secondary antibody incubations the membrane was washed extensively with Tris-buffered saline, pH 8.0. Finally, the membrane was stained histochemically with 25 mg of β-naphthyl phosphate, 25 mg of Fast Blue BB salt, and 60 mg of magnesium sulphate dissolved in 50 mL of staining buffer (1.8 g of sodium hydroxide, 3.7 g of boric acid/L).

Results and Discussion

Figure 1 shows IEF-immunoblotting banding patterns of different C1R phenotypes obtained from neuraminidase-treated samples. The asialo pattern is very simple, and C1R phenotypes can be easily scored. Three phenotypes designated C1R 1-1, 2-1, and 2-2 are controlled by two codominantly expressed alleles, *C1R*1*, *C1R*2*, at the C1R structural locus. The homozygote phenotypes (1-1, 2-2) are illustrated by a single major band, each with a characteristic isoelectric point. The heterozygote phenotype (2-1) represents the exact combination of two homozygote patterns.

The C1R banding pattern obtained using native plasma or serum samples is shown in Fig. 2. Each C1R phenotype consists of multiple major and minor bands in its native form. Comparison of Figs. 1 and 2 reveals that desialylated patterns of each phenotype are relatively simple and easily interpretable. Using both native and neuraminidase-treated samples, we have screened 140 U.S. whites and 136 U.S. blacks for C1R polymorphism. Using native samples, several cases of 2-1 phenotype were misread as 1-1 and

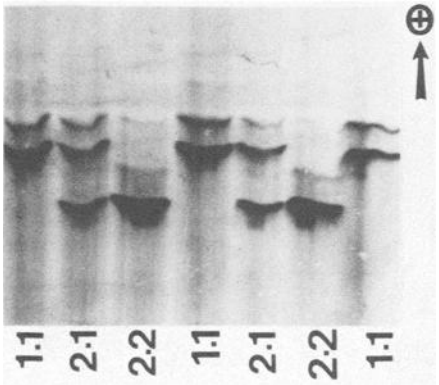

FIG. 1—*C1R phenotypic patterns obtained on neuraminidase-treated samples followed by IEF in pH range 5 to 8.*

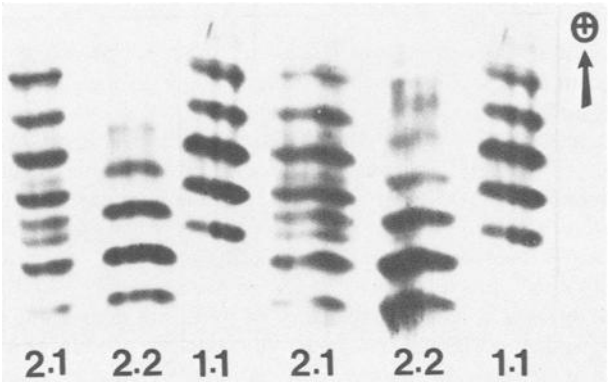

FIG. 2—*C1R phenotypic patterns obtained on native samples using IEF in pH range 4 to 6.5.*

the products of several new variants were found to be indistinguishable from the 1-1 phenotype. In U.S. whites, the frequencies of the *C1R*1* and *C1R*2* alleles were 0.93 and 0.07, respectively, using native samples, and these values changed to 0.89 and 0.11, respectively, using neuraminidase-treated samples. This improvement in correcting typings in U.S. whites increased the average heterozygosity from 0.12 to 0.19. Similarly, the products of the *C1R*1* and *C1R*2* alleles were observed at frequencies 0.90 and 0.10, respectively, in U.S. blacks using native samples. The use of neuraminidase treated samples in U.S. blacks not only changed these values to 0.83 and 0.16, respectively; the product of a third allele was observed at 0.01 frequency. Thus, in U.S. blacks, the average heterozygosity increased significantly from 0.18 using native samples to 0.29 using desialylated samples. The C1R polymorphism is already proving to be useful in the field of human genetics [*2,4–6*]. The significant improvement in exact C1R typing and potentially detecting new C1R variants makes this method a useful tool to apply routinely in disputed paternity cases. The method also has potential applicability in forensic serology, although we have not tested its application to bloodstains.

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Address requests for reprints or additional information to
Dr. M. I. Kamboh
Department of Human Genetics
Graduate School of Public Health
University of Pittsburgh
Pittsburgh, PA 15261