

Study on the Frequent Expression of Rodgers and Chido, Red Blood Cell Antigens, in Iranian Healthy Population

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Abstract

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Rodgers (Rg) and Chido (Ch) are blood-group antigens and they determine the fourth-component of human complement C4. Rodgers and Chido are associated with two C4 isotypes (C4A and C4B). In addition to genotype determination, study on expression of Rg and Chido could be useful in disease studies.

DNA was extracted from the whole blood of 60 normal individuals. Then, PCR amplification of C4d gene fragment was followed by restriction digestion. This study demonstrated that the frequency of Ch and Rg in Iranian healthy population was 98.3 and 93.4 percent, respectively. Additionally, 6.6 percent of the studied population showed Chido-positive, Rodger-negative and 1.7 percent showed Rodger-positive, Chido-negative genotype. It may be concluded that upon receiving blood transfusion, 6.6 and 1.7 percent of individuals could produce anti-Rg and anti-Ch antibodies, respectively.

Keywords: Blood Group Antigens, DNA Restriction Enzyme, Compliment C4

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Rodger (Rg) and Chido (Ch) are not true blood group antigens because they are not located on intrinsic erythrocyte structures, but become bound as covalently attached to C4d fragments from plasma. The genes encoding the two C4 isotypes (C4A, C4B) lie 10 kb apart in class III region of the human major histocompatibility complex. The two isotypes exhibit extensive structural polymorphism (1). The region of C4d contains Rg and Ch blood group antigens. Usually, C4A expresses Rg and C4B expresses Ch. It has been revealed that an increased frequency of null alleles (lacking the protein) for C4A or C4B have been associated with severe infections and some autoimmune diseases such as systemic lupus erythematosus (2, 3). Rg and Ch allotypes are identified by specific antisera, anti-Rg and anti-Ch, in an immunofixation; immunoblot; and inhibition analysis (4-6) and in PCR with sequence-specific primers (PCR-SSP) or are recognized by the restriction fragment length polymorphism or real time PCR method (7-8). Ch/Rg antibodies are not considered clinically significant from red cell transfusion aspect. However, anti-Ch and -Rg have been implicated in severe anaphylactic reactions following infusion of fresh frozen plasma, plasma fraction, or platelet concentrates containing plasma (9). Because of these important events, we investigated the frequency of Chido/Rodger antigens in a healthy population of Iran. This information may help to predict the

possibility of alloimmunization to Ch/Rg upon blood transfusion.

Sixty blood samples from 60 healthy (donor candidates for Bone Marrow Transplantation, BMT) were referred from the Hematology, Oncology, and BMT Research Center of Shariati Hospital and Tehran University of Medical Sciences to Iranian Blood Transfusion Organization between years 2006 and 2007. You have to be more specific about your studied group, like: The donors were born and raised in different regions of Iran. They were from all different age group, race and gender.

DNA was extracted and purified from the whole blood collected in 5% EDTA. By using High Pure PCR Template Preparation Kit (Roche Diagnostics), Rg and Ch typing was carried out with polymerase chain reaction. The concentration, purity and integrity of DNA were assessed by biophotometer (Eppendorf) and electrophoresis on 0.8% agarose gel. Rg and Ch typing was carried out with polymerase chain reaction followed by restriction fragment length polymorphism.

PCR was carried out with 500ng of genomic DNA in a total volume of 25µL of the reaction mixture containing the following items: 0.4 µM of the primers C4-inF (5'- tgg gac aga gct ggt atg at- 3') and C4-27 inR (5' - gtt cct gag gaa aaa ggg ag- 3'), 1.1 x reaction buffer (Roche), 4.5 mM MgCl₂, 250 µM dNTP, and 2.5 U of Taq DNA polymerase (Roche).

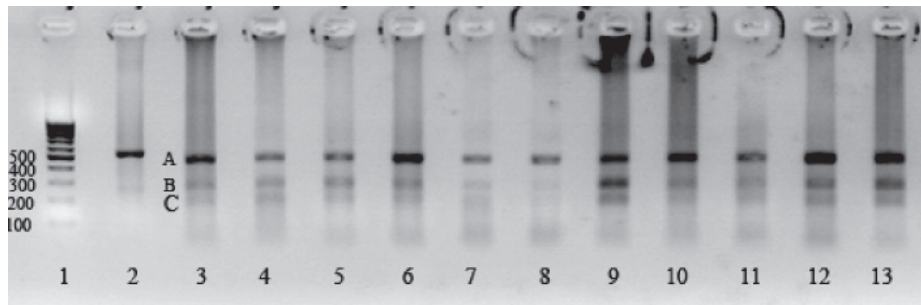


Fig 1: PCR-RFLP analysis of the Chido and Rodger in the studied healthy individuals. PCR products were digested overnight by restriction enzyme BspL-1 : Lane 1; 100 bp ladder; Lane 2; a C4d gene fragment before restriction digestion (537 bp); Lane 3-13 represent the fragments after restriction digestion; fragment A (467 bp) corresponds with Ch, and fragments B,C (276 and 191 bp) correspond with Rg.

Following PCR steps were taken: initial temperature for denaturation started at 94°C for 2min, followed by 10 cycles at 94°C for 15sec, 65.4°C for 30sec, and 68°C for 1min and another 29 cycles at 94°C for 15sec, 54.7°C for 30sec, and 68°C for 1min. The program was followed by a final extension step at 72° C for 8 min. The restriction fragment length polymorphism was carried out by overnight digestion with 1U of BspL-1 enzyme (Fermentas). The restriction-digested section was analyzed by using electrophoresis on a 2% agarose gel and stained with ethidium bromide.

This study showed that the frequencies of Chido and Rodger blood group antigens were 98.3 and 93.4 percent, respectively. Approximately, 6.6 percent of the studied population showed Chido-positive, Rodger-negative genotype whereas 1.7 percent of the studied population showed Chido-negative, Rodger-positive genotype (Fig 1, Table 1).

Table 1: The frequency of Chido and Rodger blood group antigens in a healthy Iranian population

Ch/Rg antigens (n=60)	Ch+	Rg+	Rg+ Ch+	Rg+ Ch-	Rg- Ch+	Rg- Ch-
Frequency	98.3	93.4	93.4	1.7	6.6	0

Middleton and Crookston used inhibition techniques and found that about 97% of white donors are Ch⁺ (10). Longster and Giles found that anti-Rg antibody reacted with red cells of about 97% of white people, strength of red cell Rg expression was variable and the reaction with Rg⁺ red cells was specifically inhibited by Rg⁺ plasma (11). The results were obtained in this study for the frequent expression of Ch and Rg in the Iranian healthy subjects were in accordance with the mentioned studies. Regarding to the existence of >41 allotypes in the two classes of C4, we can use the results of this research as a preliminary study for further elucidation of Ch/Rg genetic variation in Iranian people.

In summary, the frequent expression of Rodger and Chido antigens in the studied subjects of Iran could suggest that 6.6 and 1.7 percent of the population could produce anti-Rg and -Ch antibodies respectively upon

receiving blood transfusion.

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