Disponible en ligne sur ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com

TRANSFUSION CLINIQUE ET BIOLOGIQUE

Transfusion Clinique et Biologique 22 (2015) 76–79

Original article

The Duffy blood group system in the Tunisian population

Le groupe sanguin Duffy dans la population tunisienne

M. Ouchari , H. Romdhane , T. Chakroun , S. Abdelkefi , I. Jarrey , B. Houissa , S. Jemni Yacoub *

Centre régional de transfusion sanguine, hôpital Farhat Hached, Sousse, Tunisia

Available online 27 April 2015

Abstract

Background. – Tunisia was described to as genetically heterogenous. Besides the 1% native Berber, the genetically influence of the Europeans seems much larger than that of sub-Saharan populations. Due to their ethnic variability, blood group variants have the potential to support population analyses. The aim of this study was to estimate the Duffy blood group system in this mixed population with enhanced characterization of samples with aberrant expression.
Materials and methods. – Standard serological testing for the Duffy antigen was done for 105 Tunisian blood donors. Samples with altered Fy expression underwent DNA sequencing of the *DARC*, *RHD* and *RHCE* genes.
Results. – The Fy(a-b+) was the most common phenotype identified in the Tunisian population (38.1%). Five samples with Fy(a-b-) phenotype were determined as *FY*02N.01/FY*02N.01* by a homozygous occurrence of the *FY*B-67C>T* alteration. Another three individuals exhibited a Fy(b+^{w})Fy^{x} expression, confirmed by a *FY*A/FY*02M.01 (n = 1)* and a *FY*02M.01/FY*02M.01 (n = 2)* genotype. *RHD* and *RHCE* sequencing ($n = 8$) revealed altered alleles observed in black populations in 5 samples. One individual with *FY*02M.01/FY*02M.01* have the silent 165C>T nucleotide substitution each in the *RHD* and *RHCE* gene.
Discussion. – The composition of blood group variants determined in this study confirms the genetically proximity of Tunisia to Europe. The small sub-Saharan genetic influence was approved by a limited number of variant samples associated with the black population.

Keywords: Duffy; Aberrant RH alleles; Tunisia

Résumé

Contexte. – La Tunisie est considérée comme génétiquement hétérogène. À côté d'une population Berbère initiale évaluée à 1 %, l'influence génétique issue des Européens semble supérieure à celle issue des populations sub-sahariennes. Du fait de leur variabilité ethnique, les groupes sanguins sont utiles dans les analyses de population. Cette étude a pour but de caractériser le système de groupe sanguin Duffy dans cette population métissée, en s'attachant aux cas des allèles aberrants.
Matériel et méthodes. – Cent cinq donneurs de sang tunisiens pris au hasard ont été testés en sérologie classique pour le groupe sanguin Duffy. Les cas présentant une expression altérée ont subi un séquençage ADN des gènes *DARC*, *RHD* et *RHCE*.
Résultats. – Le phénotype Fy(a-b+) est le plus fréquent dans la population tunisienne (38,1 %). Cinq échantillons Fy(a-b-) ont été trouvés *FY*02N.01/FY*02N.01* du fait d'une altération homozygote *FY*B-67C>T*. Trois autres cas avec une expression Fyb+w/Fyx avaient un génotype *FY*A/FY*02M.01* (1 cas) et *FY*02M.01/FY*02M.01* (2 cas). Le séquençage *RHD* et *RHCE* fait sur 8 échantillons a montré des allèles altérés analogues à ceux des populations négroïdes dans 5 cas. Une substitution muette 165C>T a été trouvée dans les deux gènes *RHD* et *RHCE* d'un individu *FY*02M.01/FY*02M.01*.
Discussion. – Les variants de groupes sanguins retrouvés dans cette étude confirment la proximité génétique de la Tunisie avec l'Europe. Une influence sub-saharienne plus faible est retrouvée avec certains variants associés à la population négroïde.

Mots clés : Duffy ; Allèle RH aberrant ; Tunisie

* Corresponding author.
E-mail address: saloua.jemni@rns.tn (S. Jemni Yacoub).

http://dx.doi.org/10.1016/j.tracli.2015.03.002

1. Introduction

Tunisia, located in North Africa, is believed to be inhabited by Berbers since from at least 10,000 B.C. Seafaring peoples like the Phoenicians, Vandals, Greeks, French and Romans facilitated the migration across the Mediterranean. Lastly, the Islamic influence of the "Moors" from Southern Spain and several Arab occupations have shaped Tunisia. Genetically, about 1% of the global Tunisian population are native Berber [1–3]. X-chromosomal based analysis demonstrated a smaller sub-Saharan genetic influence and elucidated a relative genetically heterogeneity within current Tunisian populations [4]. Due to their ethnic variability, blood group variants may support human variation studies. The Duffy (FY) blood group system encodes the DARC glycoprotein, which acts as an erythroid receptor for the malaria parasites *Plasmodium vivax* and *P. knowlesi* [5] and a chemokine receptor of both C-X-C and C-C classes [6]. Two major antigens, Fy^a and Fy^b, are encoded by the *FY*A syn. FY*01* and *FY*B syn. FY*02* allele, differing by a single amino acid change in the *DARC* exon 2 [7,8]. While the Fy^a and Fy^b phenotypes are distributed equally among Caucasians, Blacks predominantly express the Fy^b antigen [9]. Homozygosity or compound heterozygosity of the *FY*02N.01* allele results in a Fy(a-b-) phenotype frequently observed in the black population. Its molecular basis is a -67 T>C substitution in the *GATA-1* binding motif of the *DARC* promoter causing gene-silencing [8]. First discovered in persons of African descent associated with the *FY*B* allele and therefore called *FY*B* ^{ES}, and subsequently the same mutation was later determined to be linked to the *FY*A* haplotype in individuals living in a *Plasmodium vivax*-endemic region of Papua New Guinea and therefore annotated *FY*A* ^{ES} [10]. In addition, rare cases of Fy(a-b-) among the Caucasian population were described with a different molecular background [11].

Carriers with diminished expression of the Fy^b antigen annotated Fy $b+^w$ Fy^x were shown to harbour an altered *FY*B* genotype with the specific Arg89Cys and Ala100Thr amino acid substitutions [12,13]. An additional alteration, Ala49Ser, was described for some individuals with the Fy^X phenotype, annotated *FY*02M.02* [12,14].

The present study attempts to elucidate the Duffy phenotype composition among a Tunisian multiethnic donor cohort and to further characterize those samples with aberrant Fy expression with regard to the RH blood group system.

2. Materials and methods

2.1. Blood samples

EDTA blood samples of 105 random blood donors from the Sahel were collected at the Regional Blood Transfusion Center of Sousse, Tunisia.

2.2. Serologic testing

Duffy phenotyping of fresh blood samples was done by agglutination tests with anti-Fy(a)FY1 IgG (lot FYA226AX) and anti-Fy(b)FY2 IgG (lot FYB075BX) human antibodies (Ortho Clinical Diagnostics, Neckargemund, Germany) using ID coombs cards (anti-IgG/C3d) (Bio-Rad Laboratories GmbH, Vienna, Austria).

2.3. Molecular analysis

Genomic DNA of 9 samples exhibiting an altered Fy phenotype was automatically isolated from 200 μL of EDTA blood using the MagNA Pure Compact DNA Isolation Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. For amplification of a *DARC* gene segment encompassing exons 1 and 2, primers 5′-GTTCAAGGGGATGGAGGAGC-3′ and 5′-TCATCATTCACCTTCTTCCCAA-3′ [15] were introduced. The PCR was carried out in a LightCycler 2.0 instrument (Roche Diagnostics GmbH, Manheim, Germany) and consisted of an initial denaturation of 2 minutes at 95 °C, followed by 35 cycles of 30 seconds at 95 °C, 50 seconds at 60 °C, 2 minutes at 72 °C and a final elongation of 7 minutes at 72 °C. A 25 μL PCR mixture contained 1 × High Fidelity Enyzme buffer, 0.25 μmol/L each primer, 0.2 mM dNTPs, 1U High Fidelity Expand enzyme (Roche Diagnostics GmbH, Manheim, Germany) and 10 ng of DNA. *RHD* and *RHCE* exons 1 to 10 including intron/exon boundaries were amplified as published before [16,17]. PCR products were treated with alkaline phosphatase and exonuclease I (ExoSAP-IT, High Wycombe, UK) before analyzing by an automated ABI 3130 sequencer (Life Technologies, Vienna, Austria) using BigDye Chemistry (BigDye Terminator v1.1 Cycle Sequencing Kit, Life Technologies). Data analysis was performed with SeqScape v. 2.7 (Life Technologies).

3. Results

In this study, we verified six Fy phenotypes among 105 samples, comprising Fy(a-b+) ($n = 40$, 38.1%), Fy(a+b-) ($n = 24$, 22.9%), Fy(a+b+) ($n = 32$, 30.5%), Fy(a-b-) ($n = 5$, 4.8%), Fy(a-$b+^w$) Fy^x ($n = 3$, 2.9%), Fy(a+$b+^w$) Fy^x ($n = 1$, 1%). Standard serological Fy typing revealed a diminished expression of the Fy^bantigen in 4 samples and a Fy(a-b-) phenotype in 5 individuals (Table 1). Those samples underwent molecular characterization by nucleotide sequencing of the *DARC* gene. The *FY*02M.01* gene formation was determined by *DARC(Arg89Cys,Ala100Thr)* in a homozygous ($n = 2$) and heterozygous occurrence ($n = 1$) (Table 2). In one sample with diminished Fy^b phenotype, no alteration of the *DARC* gene

Table 1
Results of serological Duffy typing.

Duffy phenotype	*n*	%
Fy(a+b+)	32	30.5
Fy(a+b-)	24	22.9
Fy(a-b+)	40	38.1
Fy(a-b-)	5	4.8
Fy(a-$b+^w$)Fy^x	3	2.9
Fy(a+$b+^w$)Fy^x	1	1.0