The *AQP1 del601G* mutation in different European Romani (Gypsy) populations

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Dear Sir,

The extremely rare Colton (Co) null phenotype enables iso-immunisation against the Co3 antigen. Co3 antibodies may induce haemolytic disease of the foetus or the newborn (HDFN) or haemolytic transfusion reactions that represent a challenge for blood centres to provide compatible blood for the patients. In most published cases, a frame shift or single amino acid change were causative for the Co-negative phenotype in single individuals[1]. In 2014, we published in this Journal an *AQP1 c.601delG* mutation (rs199474670) related to the Co-negative phenotype in 4 independent patients belonging to the Romani (Gypsy) ethnic group[2], adding to the single case report of this mutation already published[3]. Three of these 5 patients were of Spanish origin. Because there are an estimated 9 million Romani in Europe[4], a disproportional distribution of the *AQP1 601delG* mutation in this ethnic group implies an enhanced risk of incompatible blood transfusions for Romani patients. We, therefore, aimed to screen different European Romani populations for the mutation to estimate the risk of potential iso-immunisation.

We genotyped 748 DNA samples from European Romani from Bulgaria, Slovakia, Romania, Lithuania and Spain for the *AQP1 601delG* mutation after informed consent. A custom 5'-exonuclease allelic discrimination assay (Taqman SNP genotyping assay, Applied Biosystems, Foster City, CA, USA) was used to genotype 672 samples (Assay ID AH701NE) and the remaining samples were typed by PCR-RFLP (amplification primer CAACCTCTCCCTCCTCTCAC and CCAGAACAGGAAGGGACACT, restriction enzyme SacII; test conditions are available on request). Carrier status was confirmed in heterozygotes by Sanger sequencing (PCR primers CTCACTCTCTCTTCACCTATGAC and CAGAGCCTCCAGAACAGGAAG)[2].

Of the 748 Romani samples screened, the majority from Eastern Europe, only one of 137 Spanish individuals was a carrier of the *AQP1 601delG* mutation (Table I). Because this individual was heterozygous for the mutation, a Colton positive phenotype was predicted. The mutation is, therefore, rare in the European Romani, with an allele frequency of 0.0036 in the Spanish Romani. In an estimated population of 700,000 Romani in Spain, there could be some thousand carriers of the mutation. Due to a higher percentage of consanguinity in closed societies, an important number of homozygous carriers resulting in a Co-negative phenotype is very likely, and is confirmed by the 3 published cases of Spanish Romani origin.

The data presentation summarised according to migratory category highlights our hypothesis that the

Table I - Results of *AQP1 601delG* carrier screening in European Romani populations.

Country	Romani group	N. of samples	N. of *AQP1 601delG* carriers
Bulgaria	Kalderash	28	
	Rudari	52	
	Lom	72	
	Kalaidjii South	40	
	Darakchii	47	
	Koshnichari South Central	1	
	Koshnichari South West	4	
	Sievemakers	3	
	Blacksmiths	55	
	Kalaidjii North	81	
	Musicians	52	
Romania	Romanian Romani	36	
Lithuania	Lithuanian Romani	20	
Slovakia	Slovak Romani	120	
Spain	Spanish Romani	137	1
Total		748	1

AQP1 601delG mutation originated late in the Romani diaspora, especially in Spanish Romani whose ancestors immigrated to the Iberian Peninsula around 500 years ago. Screening of additional Western European Romani would provide additional information about the spread of the mutation in this region. The data collected from mainly Eastern European Romani do not indicate an enhanced risk for patients from these regions for iso-immunisation against the Colton blood group.

The Authors declare no conflicts of interest.

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