

Molecular Genetic Diagnostic Difficulties in Two Hungarian Gypsy Samples with Cystic Fibrosis

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ABSTRACT The frequency of $\Delta F508$ mutation in the CFTR gene was compared in Gypsy and European samples from 3 different geographical regions of Hungary. The frequency of $\Delta F508$ mutation in a total of 21 Gypsy patients was 43%, with 0.144 homozygosity index. This frequency was 50% with 0.127 homozygosity index in a total of 531 European Hungarian patients. Among the Gypsy patients 52 % had unknown mutations, but not the G542X, G551D, R553X and N1303K ones. However, there was a *geographical difference* in the distribution of homozygous $\Delta F508$ mutations. In the two Gypsy samples of 13 Gypsy patients from *North-East* Hungary, only one possessed $\Delta F508$ homozygote genotype, while all 7 Gypsy patients harboured this genotype from *South-West* Hungary. The difference in the occurrence of this mutation between the two geographically different Hungarian Gypsy samples can be explained by their different gene pools connected with their previous and present location, genetic drift and their isolation from each other. These findings need to be considered when planning any population screening programme for CF.

INTRODUCTION

Cystic fibrosis (CF) is the most common severe autosomal recessive genetic disorder in Hungary as in other European populations with the most frequent $\Delta F508$ mutation (worldwide frequency: 0.68) (The CF Genet. Anal. Consortium 1990). There is an obvious geographical trend in occurrence of $\Delta F508$ mutation in Europe and neighbouring regions of Asia from south-east (0.27 in Turkey, Istanbul) to north-west (0.87 in Denmark, Copenhagen) (Lucote et al. 1995). Hungarian data showed a 0.61-0.65 frequency for $\Delta F508$ mutation on CF chromosomes (Németh et al. 1996; Némethi et al. 1991; Endreffy et al. 1992).

Hungarians settled in the Carpathian Basin in 895-902 and they are more or less tight link with

linguistically related Finno-Ugrians population groups in the Ural region. The conquering Hungarians -after their westward migration from southern Ural region- met variety of groups, predominantly Slavic population groups, in their new homeland. Nowadays Gypsies form the largest ethnic group in the 12 Hungarian ethnic groups with some particular features (north Indian origin, inbreeding, high number of children). Their place of origin are different from the Hungarians.

The ancestors of the Gypsy population living in Hungary started to settle in great number from north India at the beginning of the 15th century (Hummel et al. 1991) afterwards the Western-European countries introduced regulations against the Gypsy "Kumpanias". However some data indicate even earlier Gypsy settlements. The process of their isolation started already in the 15-16th century with the preservation of a certain amount of autonomy. The 18th century was the period of restoration of state order, public services and resettlements of deserted areas in Hungary after the Turkish devastation. Gypsies migrated to the *south and south-eastern part* of the country. In the second half of the 19th century happened the most important events concerning the Gypsies: their migration in great number *to the north-eastern part of Hungary* (it was the exodus of Gypsies from Rumania after the emancipation of Gypsy slaves in 1856 (Habsburg 1894; Tomka 1983; Kemény 1974). The number of Gypsy population is about 800 000 (approx. ten percentage of the 10 million Gypsies resident in Europe) within the 10 million Hungarian citizens (Hummel et al. 1991) and out of the 19 counties their number is the highest in the north-eastern (Borsod-Abaúj-Zemplén, Szabolcs-Szatmár-Bereg counties) and south-western part (Baranya, Somogy counties) of Hungary. There-

fore we studied the frequency and geographical distribution of $\Delta F508$ mutation in three groups of Hungarian Gypsies and Hungarians.

SUBJECTS AND METHODS

Patients originated from hospitals in three geographic regions of Hungary: the central region including the capital (II. Pediatric Dept. Semmelweis Univ. Budapest and Pediatric Dept. Albert Szent-Györgyi Med. and Pharmaceutical Center of Szeged University), the north-east (Pediatric Unit, County Hospital Nyíregyháza, Postgraduate Medical University, Miskolc) and south-west (County Hospital for Chest and Heart Diseases, Mosdós, Department of Pediatrics, Medical University, Pécs) regions, respectively. Unrelated patients were differentiated in the European patients, but the Gypsies are well known endogamous population. 451 Europeans and 1 Gypsy patient form the Central region, 41 European and 13 Gypsy patients from the north-east region and 39 European and 7 Gypsy patients from the south-east region were involved in this study.

Detection of CF Mutations: The $\Delta F508$, G542X, G551D, R553X and N1303K mutations were detected from DNA, extracted from EDTA-anticoagulated venous blood as described (Woodhead et al. 1986). PCR analysis for $\Delta F508$ and other mutations was performed as published (Matthew et al. 1989; Ng et al. 1991). Homozygosity index (HI) for $\Delta F508$ mutation was defined as the squared allele frequency (Hummel et al. 1991).

RESULTS

189 of 531 European patients (36%) and 8 of 21 gypsy patients (38%) had homozygous ΔF

508 genotype (Table 1.). However, there was a *considerable difference* in the occurrence of this mutation among Gypsies *in the three regions* studied. The *central* region had only one Gypsy patient who carried non $\Delta F508$ mutations. Thus we can evaluate only two other regions.

In the north-east region 1 (8%) of 13 Gypsies was $\Delta F508$ homozygote (HI=0.006), 2 patients (15%) were $\Delta F508$ heterozygotes (compound heterozygotes, the second mutation remained unknown) and 10 children (77%) had other unknown mutations. All of the 7 Gypsy patients (100%) were $\Delta F508$ homozygotes in the *south-west region*. The difference was significant ($\chi^2 = 16.15$; $p < 0.001$).

From the analysed other CF mutations G542X, G551D, R553X and N1303 were detected, but only in the European population in 1.6%, 0%, 0.85%, and 0.94% of all CF chromosomes, respectively (this data are not separated on Table 1).

DISCUSSION

The Gypsies live in isolated communities with high inbreeding (the proportion of their first cousin marriages was about 2-20% in the 1980s compared with 0.3% figure in the Hungarian population). Analysing the genetic distances of North-Indians to 12 population of Hungary (Hummel et al. 1991) in 15 systems (haptoglobin, subtypes of group specific component, immunoglobulin: Gm1,2,b and Km1, glutamate pyruvate transaminase, acid phosphatase, subtypes of phosphoglucomutase₁, adenylate kinase, adenosine deaminase, 6-phosphogluconate dehydrogenase, third component of complement, subtypes of transferrin, esterase D, glyoxalase, amy-

Table 1: Distribution of CF genotypes in different regions of Hungary

Geographical region	Number of patients	Number and % of mutations			$\Delta F508$ on CF chrom (%)	Homozygosity index
		homozygotes	$\Delta F508$ homozygotes	Others		
Central region						
European	451	165 (37%)	138 (31%)	148 (33%)	52%	0.134
Gypsy	1	0	0	1 (100%)	0	0
North-east region						
European	41	6 (15%)	9 (22%)	26 (63%)	26%	0.021
Gypsy	13	1 (8%)	2 (15%)	10 (77%)	15%	0.006
South-west region						
European	39	18 (46%)	9 (23%)	12 (31%)	58%	0.213
Gypsy	7	7 (100%)	0	0	100%	1
Total						
European	531	189 (36%)	156 (29%)	186 (35%)	50%	0.127
Gypsy	21	8 (38%)	2 (10%)	11 (52%)	43%	0.144

lase₂) relatively low genetic distance was found between the two Hungarian Gypsy population and the North-Indians. All other genetic distances were larger, supported the view that the Gypsies originated from Northern India. This resulted in a high homozygosity index and some population genetic characteristics (e.g., they have higher proportion of B and Rh positive blood groups and their particular HLA haplotypes explains that multiple sclerosis does not occur among Hungarian Gypsies) (Van Loghem et al. 1985; Tauszik 1986). The Hungarian-German population genetic study showed an obvious difference in some genetic markers as ACP, PGM, 6-PGD, C3, GC between north-east and south-east Gypsies (Hummel et al. 1991). The result of our study confirms it. Though the proportion of the $\Delta F508$ mutation on 42 CF chromosomes was 43% in the total Gypsy sample and it was only slightly lower than the 50% figure of the European Hungarians, but it was 100% in south-west Gypsies and 15% in the north-east Gypsies. The number of subjects did not allow a good power, but the difference was significant. Population genetic data on four STR (short tandem repeat) loci in a Hungarian Gypsy population revealed also a relatively distant genetic relationship of the south-west Romanies with other Caucasian populations (Füredi et al. 1999).

Gypsies originated from North India and Pakistan (Tomka 1983). The high mutation rate for $\Delta F508$ mutation in the Hungarian Gypsies indicates a strong contradiction with the very rare occurrence of CF and of this common European mutation in Asian populations (Curtis et al. 1993). However, CF with $\Delta F508$ mutation was found in 53.8% of mutant chromosomes of Indian children (Kabra et al. 1996). The difference in the occurrence of this mutation between the two geographically different Hungarian Gypsy samples can be explained by their different gene pools connected with their previous and present location, genetic drift and their isolation from each other (Hummel et al. 1991; Gyódi et al. 1981). These findings need to be considered when planning any population screening programme for CF.

Angelicheva et al. (1997) presented their population genetic data of cystic fibrosis in Bulgaria. 262 CF alleles of the three main ethnic groups were analysed and $\Delta F508$ accounted for 100% of Gypsy CF alleles, which differed significantly from both Bulgarians and ethnic Turks.

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