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Original scientific paper

Population Genetic Studies of the Pancreatic Amylase (AMY2, E.C. 3.2.1.1) in Bulgaria

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A B S T R A C T

*The pancreatic amylase (AMY2, E.C. 3.2.1.1) polymorphism has been studied in 2346 individuals from south-central and south-eastern Bulgaria. The allele frequencies have been determined as AMY2*1 = 0.9520 and AMY2*2 = 0.0480. The neighbor joining tree of seven subpopulations revealed only small genetic distances. Compared with other populations, the Bulgarian sample clustered with samples from Romania, Hungary, Germany and Switzerland, with larger distances to Albania, Greece and Macedonia.*

Key words: *AMY2, phenotypes, polymorphism, allele frequencies, population data, Bulgaria*

Introduction

Anthropological and historical evidence indicates that the main component of the present Bulgarian population arose from a tri-ethnic group – Thraki, Slavic and Proto-Bulgarians. There exist a number of more or less isolated ethnic groups such as Turks and Romanis. The population of South Bulgaria comprises 62% of the whole population in Bulgaria; and 90% of them are ethnic Bulgarians. There is a higher migration than in other parts of Bulgaria.

The genetic heterogeneity of the amylase isoenzymes (E.C. 3.2.1.1) in humans was first described in 1965[1]. There are only a few studies of the pancreatic amylase (AMY2) polymorphism (cf. for example[2–5]). Studies of serum protein polymorphisms in Bulgarians are relatively scarce and mostly restricted to the Sofia region[6,7]. As a contribution to the hemogenetic characteristics of Bulgarians AMY2 phenotype and allele frequencies are reported

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in the following for the south-central and south-eastern regions of Bulgaria.

Material and Methods

The serum samples studied came from 2346 individuals (mainly from paternity cases) of both sexes aged between 18 and 45 years from south-central and south-eastern regions of Bulgaria (Figure 1, Table 1). They were clinically healthy and were unrelated. The individuals belonged to the Bulgarian population, ethnic minorities were not included. Most of them (approx. 90%) came from the larger cities (Bourgas, Haskovo, Kurdzhali, Pazardzhik, Plovdiv, Smolyan, Sliven).

The serum samples were stored at –4 °C and analyzed within one week or frozen at –20 °C for later analysis. Horizontal electrophoresis on agar gel (1%) was performed at 9 V/cm and +4 °C for 17 hours. The bridge buffer consisted of 0.089 M Tris, 0.06 M Histidine x HCl, 2.3 mM Ca-lactate (pH 7.6), the gel buffer was a 1:2 dilution of the bridge buffer, containing 40% w/v sucrose. After electrophoresis the gel plate was incubated at 37 °C for 2 hours in a reactive mixture

Fig. 1. Geographical distribution of the Bulgarian population samples studied.

TABLE 1
PHENOTYPE FREQUENCIES OF PANCREATIC AMYLASE (AMY2) IN THE BULGARIAN SAMPLES STUDIED (EXPECTED VALUES IN PARENTHESES)

	AMY2 1	AMY2 2-1	AMY2 2	N	$H_{observed}$	$H_{expected}$
Bourgas	101 (101.19)	9 (8.63)	– (0.18)	110	0.0818	0.0785
Haskovo	167 (166.32)	13 (14.37)	1 (0.31)	181	0.0718	0.0794
Kurdzhali	88 (87.17)	6 (7.66)	1 (0.17)	95	0.0632	0.0807
Pazardzhik	79 (79.18)	8 (7.64)	– (0.18)	87	0.0920	0.0878
Plovdiv	1419 (1416.01)	142 (148.12)	7 (3.87)	1568	0.0906	0.0945
Sliven	86 (86.21)	9 (8.58)	– (0.21)	95	0.0947	0.0903
Smolyan	15 (15.06)	2 (1.88)	– (0.17)	17	0.1176	0.1107
Stara Zagora	121 (120.26)	10 (11.47)	1 (0.27)	132	0.0758	0.0869
Yambol	55 (55.15)	6 (5.70)	– (0.15)	61	0.0984	0.0936
Total	2131 (2126.19)	205 (214.41)	10 (5.40)	2346	0.0874	0.0914

consisting of barbital buffer pH 8,5 containing 1% starch, 0.5% NaCl, and 0.06% Ca-lactate. Staining was done by the iodine-starch reaction. The amylase isoenzymes migrated towards the cathode for a distance of 3–6 cm from the starting line as white zones on a blue background.

The neighbor joining trees have been created with the PHYLIP program[8], based on Reynolds genetic distances.

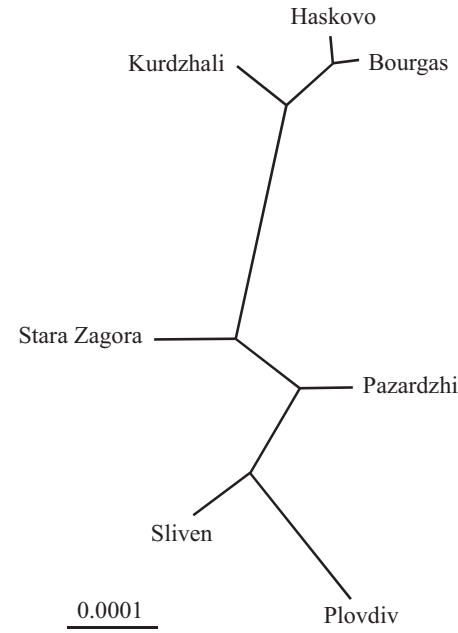

Fig. 2. AMY2 – Neighbor joining tree (based on Reynolds genetic distances) of the Bulgarian population samples.

Results and Discussion

Table 1 shows the observed and expected phenotype frequencies, Table 2 the allele frequencies of the samples studied. Due to missing degrees of freedom, the tests for Hardy-Weinberg equilibrium were not possible in the subpopulations. The expected values however correspond well to the observed frequencies. The combined data show significant differences between observed and expected values ($\chi^2 = 4.6162$; df = 1; $2.5 < p < 5$), excluding Hardy-Weinberg conditions. This may be due to the different subpopulations, although there are only small differences between the allele frequencies (Table 2). It is known that composed populations and populations of larger cities, such as the combined sample, often show no HW conditions, cf. e.g.[9,10]. This may be due to even minor differences in allele frequencies between the subpopulations and different sample sizes.

TABLE 2
PANCREATIC AMYLASE (AMY2) – ALLELE FREQUENCIES IN THE SAMPLES STUDIED

	AMY2*1	AMY2*2	N
Bourgas	0.9591	0.0409	110
Haskovo	0.9586	0.0414	181
Kurdzhali	0.9579	0.0421	95
Pazardzhik	0.9540	0.0460	87
Plovdiv	0.9503	0.0497	1568
Sliven	0.9526	0.0474	95
Smolyan	0.9412	0.0588	17
Stara Zagora	0.9545	0.0455	132
Yambol	0.9508	0.0492	61
Total	0.9520	0.0480	2346

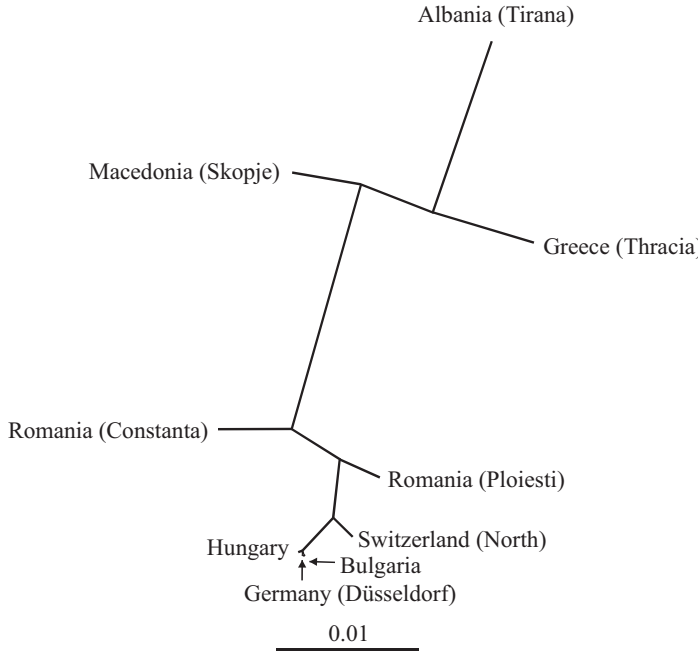

Fig. 3. AMY2 – Neighbor joining tree (based on Reynolds genetic distances) of the Bulgarian combined data, compared with population samples of the Balkans and with data from Germany and Switzerland as »outgroups«.

Figure 2 shows the neighbor joining tree, based on Reynolds distances, of seven subpopulations. Due to the small sample sizes, the data from Smolyan and Yambol have been omitted. The genetic distances are very small. There seems to be a little cluster composed of Kurdzhali, Haskovo and Bourgas. The coefficient of gene differentiation[11,12] G_{ST} is relatively small (0.0966), indicating only small differences between the subpopulations.

The non-Bulgarian data used in Figure 3 are taken from the literature[4,5,13–15]. The combined Bulgarian sample clustered with Romania, Hungary, Germany and Switzerland, with larger distances to Albania, Greece and Macedonia. Due to the geographical distances, one has to expect lower distances between Bulgaria, Romania, Greece and Macedonia and higher distances between Bulgaria, Switzerland and Germany. But it might be that the observed relationships must be traced back to the fact that there is only one marker studied with one more common and one more rare allele.

Further studies with additional genetic markers will clarify the position of Bulgaria in the context of Balkan populations.

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POPULACIJSKO-GENETIČKA STUDIJA PANKREATIČKE AMILAZE (AMY2, E.C. 3.2.1.1) U BUGARSKOJ

SAŽETAK

Istraživali smo polimorfizam pankreatičke amilaze (AMY2, E.C. 3.2.1.1) na uzorku od 2.346 ispitanika iz središnjo-južne i jugo-istočne Bugarske. Učestalost alela iznosio je za AMY2*1 = 0.9520 i AMY2*2 = 0.0480. »Neighbor joining tree« sedam ispitivanih pod-populacija pokazao je male međusobne genetske udaljenosti. U usporedbi s drugim populacijama, uzorak iz Bugarske pridružio se skupini u kojoj su uzorci iz Rumunjske, Mađarske, Njemačke, Švicarske dok je genetska udaljenost u odnosu na Albaniju, Grčku i Makedoniju bila veća.