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Original Synthetic Report

Carrier frequencies of the common *GJB2* (connexin-26) *35delG* mutation in the Greek-Turkish area: predominance of the mutation in Crete

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Abstract – Mutation *35delG* in the connexin-26 gene is the main cause of recessive deafness in Europe. The aim of this study is to determine the percentage of carriers of this mutation in seven regions of the Greek-Turkish area; previous studies indicated that the prevalence of the mutation was relatively elevated in Greece. This study has been carried out on the genomic DNAs of a total of 1038 healthy subjects originating from Albania, Bulgaria, Athens (Greece) and Crete, and within Turkish origin from Ankara, Istanbul and Cyprus. Genotyping were performed using an allele-specific PCR protocol. The most elevated value of carrier frequency (5.36%) obtained concerns the Cretan population. Carrier frequencies are = 3.66% in Athens, = 1.34% in Istanbul and = 0.40% in Ankara. No carrier of the mutation was found in individuals with Turkish origin from Cyprus, Albania and Bulgaria. These results were compared to those already obtained in other published populations in the same area. A *35delG*-allelic map in this area was constructed, which confirms that Crete corresponds to the most elevated value obtained. We discuss the historico-geographical and genetic arguments explaining this *35delG* focus of elevated frequency in Crete.

Key words: Connexin-26; *GJB2* gene; *35delG* mutation; Greek-Turkish area; Crete.

Introduction

Hearing impairment is a frequent disorder that affects approximately 1 in 1000 children and more than 50% of the cases are of genetic origin. Genetic deafness is divided into syndromic (30%) and non-syndromic (70%) forms (Hereditary Hearing Loss Homepage-http://davinci.crg.es/deafness/), and non-syndromic autosomal recessive deafness accounts for the majority (70-80%) of the latter form.

Mutations in the *GJB2* gene coding for connexin-26 (*CX26*) are responsible for about half the cases of autosomal recessive deafness (Kelsell et al., 1997). A deletion of a guanosine (*G*) in a sequence of 6 *G*s, extending from position 30 to position 35 (the *35delG* mutation) accounts for the majority of the *CX26* mutant alleles (Zelante et al. 1997). The carrier incidence of this mutation alone is estimated at around 1 in 50 overall in Europe (Gasparini et al., 2000), but some important differences exist between various populations; notably a lower carrier frequency in northern European countries compared to southern Europeans (Gasparini et al., 2000; Lucotte and Mercier, 2001). In our more recent meta-analysis on the subject (Lucotte and Diéterlen, 2005), we showed that the relatively more elevated incidence value for the *35delG* mutation in Europe (1/31) concerns the Mediterranean region, with Greece as the focus.

In the present study, we have tested the *35delG* percentage heterozygosity in seven new populations (that we have not studied previously) of 1 038 healthy unrelated subjects, representing groups of individuals originating from various countries of the Greek-Turkish area and surrounding countries, and have compared the percentages heterozygosities we obtained with those already published for other populations of the same geographic regions.

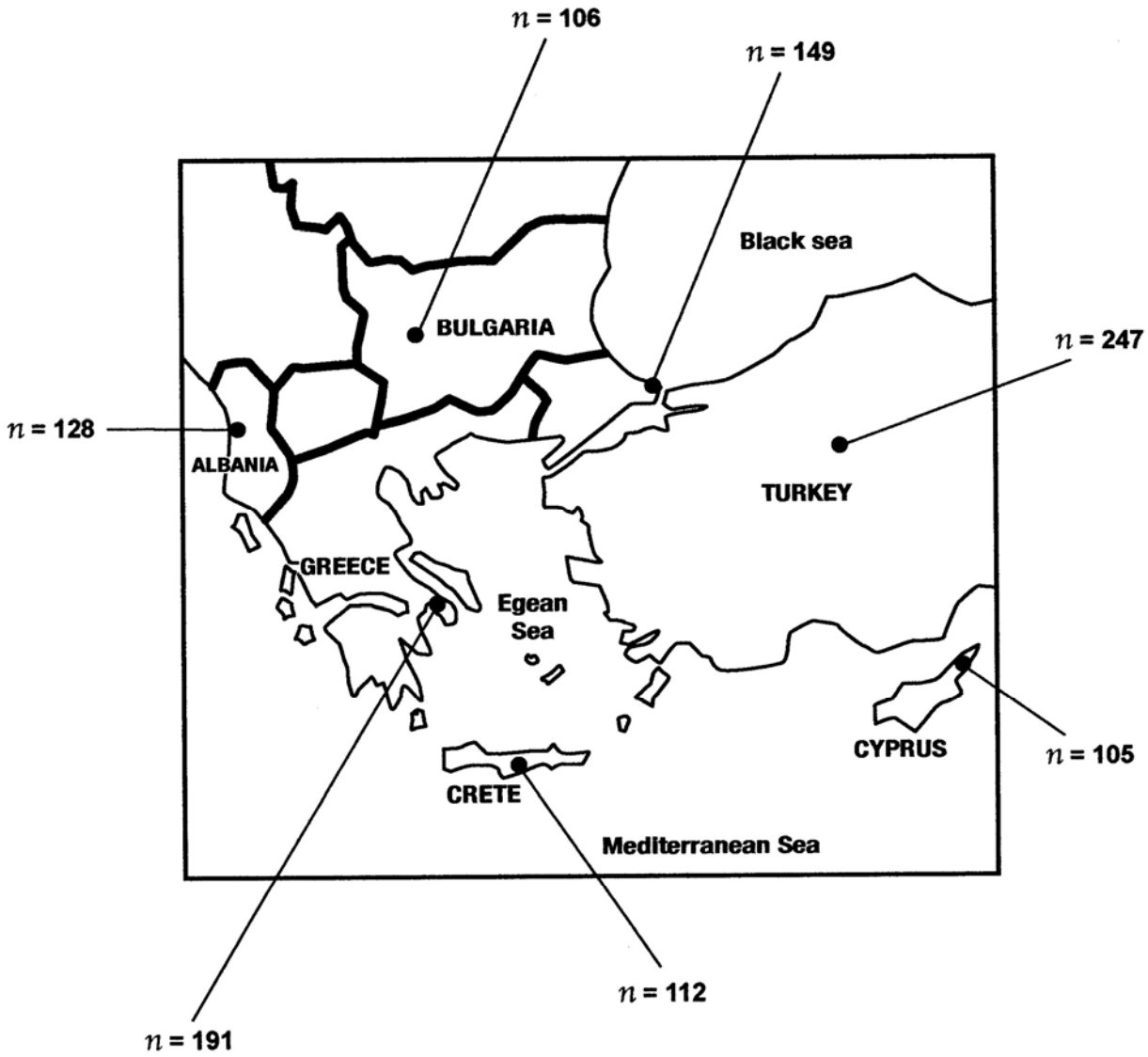
Subjects and methods

Subjects : A total number of 1 038 healthy adult unrelated subjects of both sex have been studied in the present study. All are immigrants in France since 1985, or have at least one generation of local ancestry. One hundred twenty eight of them originate from Albania and 106 from Bulgaria. Others originate from the Greek-Turkish area: 191 from Greece (Athens) and 112 from Crete; 247 from Ankara in Turkey, 149 from Istanbul and 105 of Turkish origin from the northern area of Cyprus.

The geographic location of all the seven populations is indicated on the map of Fig. 1. Some of the male subjects were previously incorporated in an anthropological study (Lucotte et al., 2006).

Figure. 1.

Map of the Greek-Turkish area. Approximate locations of subjects in countries (*n*: numbers of subjects in each of the 7 populations studied) are indicated.



Molecular methods : Blood of these subjects were collected, after informed consent. Genomic DNA was extracted from the whole blood, using a classical method (Gautreau et al., 1983) involving proteinase K treatment and successive phenol-chloroform extractions. *35delG* genotyping were performed using our established allele refractory mutation system (ARMS-PCR) protocol (Lucotte et al., 2001).

Statistical and Informatic methods : Comparisons of the carrier frequencies of the *35delG* mutation between the various populations were carried out using the Poisson probability distribution. For each population, the ratio of the frequency of *35delG* carriers (*i*) over the number of subjects tested (*n*) was modeled as a rate (lower confidence limit: $\frac{\chi^2_{2i,\,(1-\alpha)/2)}}{2n}$, upper confidence limit: $\frac{\chi^2_{2(i+1),\,\alpha/2)}}{2n}$, where $1-\alpha = 0.95$ and $\chi^2_{r,s}$ is the chi-square quantile on *r* degrees of freedom for upper tail probability *s)*.

The map of *35delG* carrier frequencies was drawn with the "Spatial Analyst" program (Arcview software), using the classical Kringing procedure (Lucotte and Diéterlen, 2005) We have used the inverse distance weighting (IDW) method, which is well adapted to scarce data (5 neighbors in each quadrant with a power of 2, so that influence is greater at large distances); the grid has 250 rows and 355 columns.

Results

Percentage carrier rates on these 1 038 subjects originating from the seven population we studied are displayed on Table 1. The most elevated percentage carrier rate we observe concerns the population of the Crete island (5.36%), where the frequency value of the *35delG* carriers = 1/18.7 in healthy subjects. The second value in order observed (3.66%) concerns the population of Athens (corresponding to an incidence = 1/27.3), a value comparable to that previously obtained (3.54%) in healthy subjects of the Greece mainland (Antoniadi et al., 1999).

Table 1. *35delG* heterozygosities, % carrier rates and confidence interval (CI) in the 7 populations studied.

Countries/towns /regions	Rates of *35delG* heterozygosities	% carrier rates	95% CI (Poisson distribution)
Albania	0/128	0	0- 2.88
Bulgaria	0/106	0	0- 3.48
Athens	7/191	3.66	1.47- 7.55
Crete	6/112	5.36	1.97-11.66
Turkey (Ankara)	1/247	0.40	0.01- 2.26
Istanbul	2/149	1.34	0.16- 4.85
Cyprus (northern geographical area)	0/105	0	0- 3.51

Table 2 permit comparisons between percentage carrier rates we observed to those previously published for the Greece main land (Antoniadi et al., 1999) and for two other populations of healthy subjects originating from the West main land of Turkey (Tekin et al., 2001) and or the Greek Cypriots (Neocleous et al., 2006). In this table, each population is represented by a geographic point of coordinates (*x*: degrees of longitude; *y*: degrees of latitude north), centered on main lands for Greece and for Turkey, on islands, and on Capital towns for other countries.

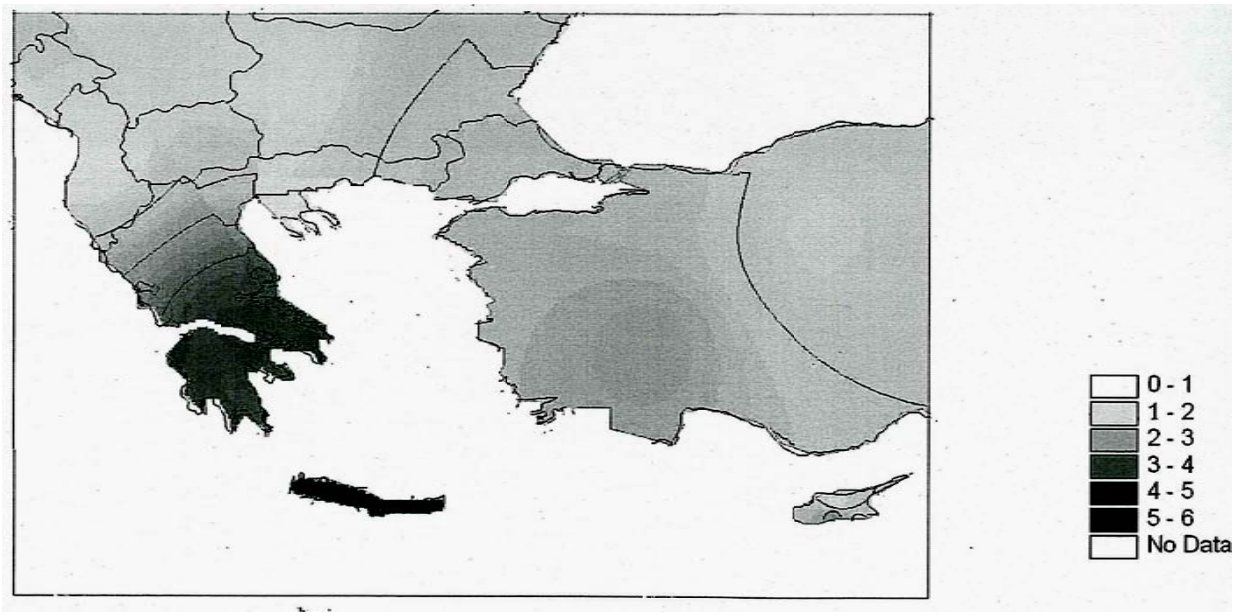
Table 2 . Percentages *35delG*-carrier rates in 10 populations.

Country	Island	Town/area	Geographic coordinates		Percentage carrier rates	References
			x	*y*		
Albania		Tirana	20	41.5	0	Present study
Bulgaria		Sophia	23	43	0	Present study
Greece			22.5	38.34	3.54	(Antoniadi et al., 1999)
Greece		Athens	23.9	38.1	3.66	Present study
Greece	Crete		25	35.1	5.36	Present study
Cyprus	Cyprus	(southern geographical area)	33.5	34.95	2.47	(Neocleous et al., 2006)
Turkey			30	38	1.78	(Tekin et al., 2001)
Turkey		Ankara	33	40	0.40	Present study
Turkey		Istanbul	28.4	41.2	1.34	Present study
Cyprus	Cyprus	(northern geographical area)	33.35	35.2	0	Present study

A *35delG*-allele map is represented on Fig. 2, on the basis of carrier data compiled in Table 2. Five artificial discontinuities in *35delG* frequency values are introduced on this map. The region of highest frequencies (>5%) corresponds to Crete; regions of relatively elevated frequencies (>3% <4%) correspond to continental Greece. The southern geographical area of Cyprus corresponds to moderate values (>2% <3%), and low values (0.4% to <2%) correspond to West continental Turkey, Istanbul and Ankara. Peripheral regions of Albania, Bulgaria and the northern geographical area of Cyprus correspond to a zero value in the populations studied.

Figure 2.

Distribution of the *35delG* carrier frequencies in the Greek-Turkish area. The various nuances of grey correspond to artificial discontinuities, with density percentages as indicated in the text. The independent country of Cyprus is in fact not a divided region.



Discussion

Because it was initially argued (Denoyelle et al., 1997) that the *35delG* deletion could arise through polymerase slippage, it was assumed that the high frequency of this mutation in various populations could reflect a mutational hot-spot within the *GJB2* gene.

In fact, focusing on a relatively short genomic region containing the *GJB2* gene and using six single nucleotide polymorphisms mapped to its immediate vicinity, it was established (van Laer et al., 2001) that the high frequency of *35delG* was the result of a founder effect. This alternative view was amply confirmed by subsequent studies (Tekin et al., 2001; Rothrock et al., 2003; Belguith et al., 2005].

In the van Laer et al. (2001) study were also furnished both an estimation of the age of the *35delG* mutation (approximately 10 000 years old) and the context in which it originated and then spread: somewhere in the Middle East, an then a spread throughout Europe along the classic Neolithic population movement routes. But (Lucotte and Diéterlen, 2005) *35delG* carrier frequencies are relatively low in Turkey (Tekin et al., 2001; Uyguner et al., 2001) and no carrier of the mutation was observed in series of Jordanian (Medley-Hashim et al., 2002) and Palestinian (Shahim et al., 2002) controls.

A general rule in population genetics is that the geographic center of a mutation corresponds to the area where it is the most frequent (Watterson and Guess, 1997). Among the published datas, actual Greece (Antoniadi et al., 1999) –with a heterozygosity = 3.54– probably represents the focus of the *35delG* mutation. We have previously suggested (Lucotte and Diéterlen, 2005) that gene flow of the *35delG* mutation from Greece to the south of Italy could be explained by the Ancient Greek colonizations in the so-called "Magna Grecia" in historical times (six century B.C.). I have shown more recently (Lucotte, 2007) that there are some correspondences between the relative prevalences for the *35delG* mutation and historico-geographic events concerning Ancient Greek Territories and successive colonizations.

To go further in the research of the Greek population of origin of the *35delG* mutation, we have studied 1 038 healthy subjects originating from the Greek-Turkish area and from surrounding countries. We found no *35delG*-carriers in samples from Albania, Bulgaria and in Turkish Cypriots. On the 699 remaining subjects originating from Greece and from Turkey tested for the presence of the mutation, 16 of them are carriers (mean percentage of heterozygosity = 2.28); the mean incidence in the corresponding area = 1/43.7.

In the present study, percentage carrier rates in Turkey = 0.40 in Ankara and = 1.34 in Istanbul (Table 1). This value attains a percentage = 3.66% in Athens (continental Greece). The most elevated value we obtain (=5.36%) concerns the Greek

population of Crete. The allele map pictured on Fig.2 designates effectively Crete as the Greek region representing the focus of the *35delG* mutation.

To our knowledge, the Albanian population was not studied previously concerning the *35delG* incidence. Table 3 summarizes statistical comparisons between already published studies of *35delG*-carrier rates in the various countries covered in our present study. The percentage of carriers previously obtained in Bulgaria (Gasparini et al., 2000] is statistically comparable to that we obtain here (test non-significant: N.S.). The percentage of carriers previously obtained in Turkey (Tekin et al., 2001) is statistically comparable to those we obtained, both in Istanbul and in Ankara. The percentage carrier previously obtained in Greece main land (Antoniadi et al., 1999) is also statistically comparable to that we obtained in the present study in Athens. The percentage of carrier = 2.47% obtained in Greek Cypriots (Neocleous et al., 2006) is significantly more elevated to that we obtained in Turkish Cypriots; but in fact it might be the difficult to predict whether there is a really significant difference, since the 0/105 rate we obtained for Turkish Cypriots concerns a relatively small sample of people and this can cause the width of the CI interval to be comparatively large.

Table 3. Statistical comparisons between previously and presently published populations for *35delG*-carrier rates, based on hypothesis tests with a 5% significance level (S.: significant, N.S.: non-significant).

Countries	References	Rates of 35delG heterozygosities	% Carrier rates	95% CI (Poisson distribution)	Statistical significances
Bulgaria					
(main land)	(Gasparini et al., 2000]	1/157	0.64	0.02 - 3.55	N.S.
(Sophia)	Present study	0/106	0	0 - 3.48	
Turkey					
(Istanbul)	Present study	2/149	1.34	0.16 - 4.85	N.S.
(West main land)	(Tekin et al., 2001)	12/674	1.78	0.92 - 3.11	
(Ankara)	Present study	1/247	0.40	0.01 - 2.26	N.S.
Greece					
(main land)	(Antoniadi et al., 1999)	14/395	3.54	1.94 - 5.95	N.S.
(Athens)	Present study	7/191	3.66	1.47 - 7.55	
Cyprus					
(southern geographical area)	(Neocleous et al., 2006)	10/405	2.47	1.18 - 4.54	S.
(northern geographical area)	Present study	0/105	0	0 - 3.51	

The observed predominance of the *35delG* in Crete needs some comments on this peculiar island. Crete is a southeastern and the largest island of Greece (located 25°E and 35°N) of about 650 000 inhabitants, who share the same genetic and cultural background. Referred to by some as the birthplace of western European civilization, the island of Crete is one of the most exclusively excavated locations in the world. Crete, which is situated approximately equidistant from main lands Greece and Turkey and linked to the two by archipelagos of stepping-stone islands, has been influenced during history by periodic waves of dispersing migrants.

The first inhabitants of the island are believed to have arrived around 7 000 B.C (Broodbank and Strasser, 1991) possibly from Anatolia. This founding group was composed of Neolithic farmers who established their first settlements in the fertile lowland regions of Crete. About 4 000 years later, this population formed the basis of what has since been termed the Minoan civilization, a pre-Hellenic Bronze-Age culture that prospered and has exchanged with other Mediterranean civilizations. During the later Bronze Age, Crete received an influx of Mycenian Creeks, who controlled the island from ca. 1 450 B.C. until the twelfth century (Fitton; 2002). After the Bronze Age, Crete came under the influence and control of various external cultures: in 69 B.C., Crete was annexed by the Romans, and was passed on to the Byzantines almost five centuries later; the Arabs invaded the island in the year 824 A.D.; it was followed by more than four centuries of Venetian rule, that started in the early 1 200 s. Then, after a two-decades siege by the Ottoman Empire, the Turks controlled the island from 1 669 A.D. until the early twentieth century, when it was unified with the main land Greece.

Recent analysis of Y-chromosome markers defining haplotypes and haplogroups revealed a high degree of heterogeneity within Crete (Malaspina et al. 2001; Di Giacomo et al., 2003; Lucotte, 2006). A remarkable finding obtained in these three studies consists on the preponderance, in most Cretan populations studied, of haplogroups belonging to the J2 lineage; these haplogroups are homologous to haplotype VII in the 49*a,f*/*Taq*I polymorphisms we referred (Lucotte and Mercier, 2003), a haplotype typical of Middle Eastern origin. In a more recently published study (Martinez et al., 2007) concerning the local population of the Lasithi Plateau in Crete, a mountain plains region that have played possible natural refuges for late Minoans,

lineages corresponding to haplogroups R1a1 and R1b predominate; these two haplogroups, typical of European populations, are homologous in our 49*a,f*/*Taq*I polymorphism of reference to haplotypes XI and XV (Lucotte and Mercier, 2003; Diéterlen and Lucotte, 2005), respectively.

So, the non-uniform distribution of Y-chromosome signatures reflects the complex history of colonization of Crete by different main land sources during the last 9 000 years. Cumulative data suggest that Cretans exhibit certain genetic differences in comparison with other populations (Stiakaki et al., 2005; Apostralis et al., 2005; Fragouli et al., 2008) and distribution of HLA alleles is peculiar (Arnaiz-Villena et al., 1999). At this stage of analysis, we can't establish if the highest frequency of the *35delG* mutation we observed in Crete could be the result of genetic drift owing to founder effect and/or to geographic isolation of the island. Further studies on the subject are needed to resolve this problem. Studying the *35delG* mutation in such a peculiar country has definitive advantages because may allow the detection of some population-specific genetic effect which otherwise might be undetectable in a larger continental population (Peltonen et al., 2000).

The elevated prevalence of the *35delG* in Crete, in Continental Greece and in other historically related Mediterranean countries, constitute a veritable problem of health in the corresponding populations.

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References

Antoniadi T., Rabionet R., Kroupis C., et al. 1999: High prevalence in the Greek population of the *35delG* mutation in the connexin-26 gene causing prelingual deafness, *Clin. Genet.* 55:381-382.

Apostralis S., Baritaki S., Krambovitis E., Spandidos D.A., 2005: Distribution of HIV/AIDS protective SDF1, CCR5 and CCR2 gene variants within Cretan population. *J. Clin. Virol.* 34:310-314.

Arnaiz-Villena A., Iliakis P., Gonzalez-Hevilla M. et al. 1999: The origin of Cretan populations as determined by characterization of HLA alleles, *Tissue Antigens* 53:213-226.

Belguith H., Hadjji S., Salem N., et al. 2005: Analysis of *GJB2* mutation: evidence for a Mediterranean ancestor for the *35delG* mutation. *Clin. Genet.* 68:188-189.

Broodbank C., Strasser S., 1991: Migrant farmers and the Neolithic colonization of Crete. *Antiquity* 65:233-245.

Denoyelle F., Weil D., Maw M.A., et al. 1997: Prelingual deafness: high prevalence of *30delG* mutation in the connexin 26 gene, *Hum. Mol. Genet.* 6:2173-2177.

Di Giacomo F., Luca F., Anagnou N., et al. 2003: Clinal patterns of human Y chromosomal diversity in continental Italy and Greece are dominated by drift and founder effects. *Mol. Phylogenet. Evol*. 28:387-395.

Diéterlen F., Lucotte G., 2005: Haplotype XV of the Y-chromosome is the main haplotype in West-Europe, *Biomed. Pharmacother.* 59:269-272.

Fitton F.L., 2002: Minoans. London, the British Museum Press.

Fragouli E., Eliopoulos E., Petraki E. et al. 2008: Familial Mediterranean fever in Crete: a genetic and structural biological approach in population of "intermediate risk", *Clin. Genet.* 73:152-159.

Gasparini P., Rabionet R., Barbujani, G. et al. 2000: High carrier frequency of the *35delG* deafness mutation in european populations. Genetic analysis consortium of *GJB2 35del*G, *Eur. J. Hum. Genet.* 8:19-23.

Gautreau C., Rahuel C., Cartron J.P., Lucotte G. 1983: Comparison of two methods of high-molecular-weight DNA isolation from human leucocytes. *Anal. Biochem.* 134:320-324.

Kelsell D.P., Dunlop J., Stevens H.P., et al. 1997: Connexin 26 mutations, in hereditary non-syndromic sensineuronal deafness, *Nature* 387:80-83.

Lucotte G., 2006: Frequencies of Y-chromosome DNA haplotypes in Europe show an early dispersion from the Levant: a synthesis, *Int. J. Anthropol.* 21:3-17.

Lucotte G., 2007: High prevalences of carriers of the *35delG* mutation of connexin-26 in the Mediterranean area, *Int. J. Pediatr. Otorhinolaryngol.* 71:741-746.

Lucotte G., Bathelier C., Champenois T. 2001: PCR test for diagnosis of the common *CJB2-35delG* mutation on dried blood spots and determination of the carrier frequency in France. *Mol. Cell. Probes* 15:57-59.

Lucotte G., Diéterlen F. 2005: The *35del*G mutation of the connexin-26 gene (*GJB2*) associated with congenital deafness: European carrier frequencies and evidence for its origin in Ancient Greece. *Genet. Testing* 9:0-25.

Lucotte G., Mercier G. 2001: Meta-analysis of *GJB2* mutation *35delG* frequencies in Europe, *Genet. Testing* 5:149-152.

Lucotte G., Mercier G., 2003: Y-chromosome DNA haplotypes in Jews: comparisons with Lebanese and Palestinians, *Genet. Testing* 7:67-71.

Lucotte G., Mercier G., Diéterlen F., 2003: Y-chromosome DNA haplotype XI in Western Europe, *Hum. Biol.* 75:405-410.

Lucotte G., Yanakakis N., Diéterlen F. 2006: Y-chromosome haplotypes in the Greek-Turkish area. *Int. J. Anthropol.* 21:123-129.

Malaspina P., Tsopanomichalou M., Duman T., et al. 2001: A multistep process for the dispersal of a Y chromosomal lineage in the Mediterranean area, *Ann. Hum. Genet*. 65:339-349.

Martinez L., Underhill P.A., Zhivotovsky L.A., et al. 2007: Paleolithic Y-haplogroup heritage predominates in a Cretan highland plateau, *Eur. J. Hum. Genet.* 15:485-493.

Medley-Hashim M., Mustapha M., Chovery M., et al. 2002: Non syndromic recessive deafness in Jordan: mapping of a new locus to chromosome 9q34.3 and prevalence of DFNB1 mutations. *Eur. J. Hum. Genet.* 1:391-394.

Neocleous V., Portides G., Anastasiadou V., Phylactou L.A. 2006: Determination of the carrier frequency of the common *GJB2* (connexin-26) *35delG* mutation in the Greek Cypriot population, *Int. J. Pediatr. Otorhinolaryngol.* 70:1473-1477.

Peltonen L., Palotie A., Lange K., 2000: Use of population isolates for mapping complex traits, *Nat. Rev. Genet.* 1:182-190.

Rothrock C.R., Murgia A., Sartorato E.L., et al. 2003: Connexin-26 *35del*G does not represent a mutational hotspot, *Hum. Genet.* 113:18-23.

Shahim H., Walsh T., Sobe T. et al. 2002: Genetics of congenital deafness in the Palestinian population: multiple connexin-26 alleles with shared origins in the Middle East, *Hum. Genet.* 110:284-289.

Stiakaki E., Germanakis I., Sfyridaki C., Katzilakis N., Danilatou V., Kalmanti M., 2005: Prevalence of Factor V Leiden and other thrombophilic traits among Cretan children with malignancy, *Pediatr. Blood Cancer*. 444:386-389.

Tekin M., Akar N., Cin S., et al. 2001: Connexin-26 (*GJB2*) mutations in Turkish population: implications for the origin and high frequency of the *35delG* mutation in Caucasians, *Hum. Genet.* 108:385-389.

Uyguner O., Emiroglu M., Uzumcu A., et al. 2001: Spectrum of conexin-26 gene (*GJB2*) mutations in Turkish families with inherited non syndromic deafness and determination of the *GJB2 35delG* mutation carrier frequency in Turkish populations, *Eur. J. Hum. Genet.* 9 (S1) 283.

van Laer L., Coucke P, Mueller R.F., et al. 2001: A common founder for the *35delG GJB2* mutation in connexin-26 hearing impairment, *J. Med. Genet*. 38:515-518.

Watterson G.A., Guess H.A., 1997: Is the most frequent allele the oldest? *Theor. Popul. Biol.* 11:141-160.

Zelante L., Gasparini P., Estivill X., et al. 1997: Connexin 26 mutation associated with the most common form of non-syndromic neurosensory autosomal deafness (DFBB1) in Mediterraneans, *Hum. Mol. Genet.* 6:1605-1609.