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High carrier frequency of the 35delG deafness mutation in European populations

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Congenital deafness accounts for about 1 in 1000 infants and approximately 80% of cases are inherited as an autosomal recessive trait. Recently, it has been demonstrated that connexin 26 (*GJB2*) gene is a major gene for congenital sensorineural deafness. A single mutation (named 35delG) was found in most recessive families and sporadic cases of congenital deafness, among Caucasoids, with relative frequencies ranging from 28% to 63%. We present here the analysis of the 35delG mutation in 3270 random controls from 17 European countries. We have detected a carrier frequency for 35delG of 1 in 35 in southern Europe and 1 in 79 in central and northern Europe. In addition, 35delG was detected in five out of 376 Jewish subjects of different origin, but was absent in other non-European populations. The study suggests either a single origin for 35delG somewhere in Europe or in the Middle East, and the possible presence of a carrier advantage together with a founder effect. The 35delG carrier frequency of 1 in 51 in the overall European population clearly indicates that this genetic alteration is a major mutation for autosomal recessive deafness in Caucasoids. This finding should facilitate diagnosis of congenital deafness and allow early treatment of the affected subjects. *European Journal of Human Genetics* (2000) 8, 19–23.

Keywords: *GJB2*; 35delG; carrier frequency; genetic deafness

Introduction

Congenital deafness accounts for about 1 in 1000 infants.¹ Approximately 80% of cases of congenital deafness are inherited in an autosomal recessive fashion or are apparently sporadic.² The *DFNB1* locus for non-syndromic sensorineural deafness on human chromosome 13q11^{3–5} was demonstrated to correspond to the connexin 26 (*GJB2*) gene in Pakistani⁶ and Mediterranean families,^{7,8} suggesting that *GJB2* is a

major gene for congenital sensorineural deafness. At least a total of 29 different mutations have been detected in the *GJB2* gene, mainly in autosomal recessive and in sporadic cases of congenital deafness^{6–12} (<http://www.iro.es/cx26deaf.html>). Among these, 35delG is the most common mutation in patients with autosomal recessive sensorineural deafness from Australia, France, Israel, Italy, Lebanon, Morocco, New Zealand, Spain, Tunisia, UK and USA, with relative frequencies ranging from 28% to 63%.^{8–12} Mutation 35delG has also been detected in apparently sporadic cases of deafness, accounting for 33% of deaf patients from Italy and Spain,⁸ and about 10% of those from Belgium and the UK.¹² The overall relative frequency of the 35delG mutation in patients with congenital deafness (familial and sporadic) has

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Received 23 March 1999; revised 27 August 1999; accepted 2 September 1999

been estimated at 40%,⁸ a high carrier frequency considering that genetic heterogeneity has been suggested¹³ for congenital deafness.

Since 35delG is found in a large proportion of patients with either recessive and/or sporadic deafness, and congenital deafness accounts for about 1 in 1000 live births, we expect a large number of asymptomatic 35delG carriers in the general population. Based on the frequencies of 35delG homozygotes among Italian and Spanish patients with congenital deafness, a carrier frequency of about 1 in 30 was estimated, remarkably close to the figure (1 in 31) observed in 280 unrelated normal subjects.⁶ Here, we report the data of a large population screening that confirm the high 35delG carrier frequency in Europe.

Materials and methods

Samples

A total of 3931 samples from 17 European countries, five different Jewish populations, plus Egyptians and North American Blacks was obtained for the analysis of the 35delG mutation. The samples were unrelated random controls with a normal male/female sex ratio. They were sent to either Barcelona or San Giovanni Rotondo for analysis, with the exception of the samples from Denmark, Estonia, and 200 samples from Greece.

Mutation analysis

DNA was extracted from peripheral blood according to standard protocols. Samples analysed in the Barcelona laboratory and those from Sardinia (Italy) were studied by allele specific oligonucleotide hybridisation using the oligonucleotide probes and hybridisation conditions previously described.¹⁴ Samples analysed in San Giovanni Rotondo and in Estonia were studied by GENSCAN analysis after electrophoretic separation on an ABI373 or 377 machine as previously described.⁷ Two hundred samples were analysed in Greece using a modification¹⁵ of an ARMS-PCR method. Ninety-five samples were studied in Denmark by dideoxy-fingerprinting with GTP as a single nucleotide.

Analysis of molecular variance

Chi-square test was used to estimate the difference of proportion, whilst Amova¹⁶ was used to partition the overall genetic diversity into three hierarchical components, representing differences between individuals within populations, among populations, and among groups, respectively.

Results

We have analysed the carrier frequency of 35delG in 3270 unrelated random subjects of 17 European populations, in an additional sample of 376 Jewish individuals of different origin, and in two other population groups (Egyptians and African-American) for an overall number of 3931 random subjects investigated. Although the highest 35delG carrier

frequency was found in Estonia (1 in 22), the Mediterranean countries showed significantly higher carrier frequencies than northern and central European countries (χ^2 10.54, $P = 0.002$). 35delG carrier frequencies in Mediterranean countries ranged between 1 in 29 and 1 in 45, with an overall carrier frequency of 1 in 35. Interestingly, the carrier frequencies of 35delG in the Mediterranean region are higher than in the common cystic fibrosis $\Delta F508$ mutation carrier frequencies in the *CFTR* gene in the same area.¹⁷

Conversely, the heterozygote frequency for 35delG in central and northern Europe varied between 1 in 22 (Estonia) to 1 in 200 (France) (Table 1). No carriers of 35delG were found in 119 individuals from the UK. The overall carrier frequency of mutation 35delG in these parts of Europe was 1 in 79, about half the figure estimated for the Mediterranean region.

Mutation 35delG was also detected in 1 of 29 Jews of Persian origin, 1 in 50 north African Jews and 1 in 115 Iraqi Jews, but was not found in Ashkenazi Jews, Egyptians and African-Americans (Table 2).

Although a lower carrier frequency for 35delG was detected in northern and central European countries compared with southern Europeans, the individual country frequencies are heterogeneously distributed. On a continental scale, the geographical pattern, described by spatial autocorrelation,¹⁸ does not depart significantly from randomness and does not indicate the presence of a gradient. This can be partly due to

Table 1 Carrier frequency of mutation 35delG in the *GJB2* gene in 17 European countries

Country	Detected/ studied	Carrier frequency	95% CI
<i>Northern and Central Europe</i>			
Denmark	2/95	1/47.5	
Norway	1/190	1/190	
Estonia	5/113	1/22.5	
United Kingdom	0/119	0/119	
Germany	4/200	1/50	
Belgium	1/190	1/190	
Holland	2/89	1/44.5	
France (Brittany)	1/96	1/96	
France	1/200	1/200	
Czech Republic	4/195	1/48.7	
Slovenia	1/182	1/182	
Bulgaria	1/157	1/157	
Total	23/1826	1/79.3	
	1/55–1/142		
<i>Southern Europe</i>			
Portugal	4/179	1/45	
Spain	5/200	1/40	
Italy	8/255	1/32	
Italy (Sardinia)	4/116	1/29.5	
Malta	4/144	1/36	
Greece	12/400	1/33	
Turkey	4/150	1/37.5	
Total	41/1444	1/35.2	1/32–1/39
Total	64/3270	1/51.1	1/41–1/64

the large standard errors associated with the very low allele frequency estimates, but also to the high carrier frequency among Estonians, an obvious exception to the low 35delG frequencies elsewhere in northern Europe. In synthesis, spatial autocorrelation shows that, given the carrier frequency observed in one country, no statistically robust prediction can be made of the carrier frequency in neighbouring countries.

However, a form of analysis of variance (Amova)¹⁶ was able to identify some degree of geographical structuring. The largest fraction of genetic diversity in humans is represented by individual differences within populations,¹⁹ here accounting for more than 99% of the total diversity. Less than 0.1% of the total is due to differences among populations, whereas the differences among groups of populations, albeit limited in extent, reach statistical significance in five independent runs of an analysis, based on five models of population structure (Table 3).

Under Model 1, seven groups were considered (northern, central, eastern and southern Europe, near East, Jewish populations, and non-European samples). The inter-group component of variance appeared to be significantly greater than 0, and it was further increased in Model 2, in which Estonians, in an evident outlier country (Figure 1), were removed from the analysis. In Model 3, the five Jewish samples were grouped with their geographical neighbours. This resulted in an increased fraction of variance explained

by inter-group differences as compared with Model 1, showing that the Jewish populations do not differ significantly from their non-Jewish neighbours. Model 4 showed a decrease in the fraction of diversity explained by differences among groups when three such groups, namely northern, southern and non-Europeans, were considered. However, when Estonians who, like Finns, speak an Uralic non-Indo-European language, are removed from the analysis (Model 5), the best discrimination among groups ($P < 0.001$) was obtained. Genetic diversity in the Finn population is known to reflect a probable founder effect.^{20,21} A similar demographic history, characterised by some kind of founder effect whose consequences may include the increase of 35delG allele frequency, can now also be suggested for Estonians. Once this population is excluded from the analysis, the distribution of 35delG allele reflects a rough subdivision of Europe into two areas, central-northern to southern, with frequencies in the latter being roughly twice those in the former.

Discussion

Our data clearly demonstrate a high carrier frequency and a different geographic distribution of 35delG in central northern and southern Europe. There are several possible explanations for this finding. The high frequency of 35delG carriers suggests either a founder effect or a selective advantage for heterozygotes or a combination of both. Since *GJB2* is expressed in a large number of tissues,^{22,23} it is possible that the putative carrier advantage is related to a function of *GJB2* in one of these tissues, but clearly not the cochlea. This carrier advantage could be related to specific functions of gap junctions and be involved in climate, food, toxic factors, infectious agents, or other factors, reflecting different geographic and cultural conditions that could influence the frequency of 35delG.

The high frequency of mutation 35delG in the Caucoid population has been attributed to the fact that the mutation occurs within a sequence of T(G)₆T, which may favour slippage and mispairing during DNA replication.⁷ The fact that mutation 35insG occurs in the same stretch of six guanines of 35delG,⁸ would support the view that this sequence is a hot spot for mutations and that there are several origins of the 35delG mutation. However, a mutational hot spot is expected to have two consequences, namely (i) little linkage disequilibrium between 35delG and the flanking markers, and (ii) the presence of comparable frequencies of 35delG carriers outside Europe. The latter does not seem to be the case. As for linkage disequilibrium, high levels thereof would suggest that the diffusion of the 35delG allele is due to some form of migrational process. In addition, preliminary data on haplotype analysis of Italian patients demonstrate that the majority of them carry the same haplotype, suggesting the presence of a common founder

Table 2 Carrier frequency of mutation 35delG in the *GJB2* gene in other populations

Country	Detected/ studied	Carrier frequency	95% CI
Persian Jews	2/59	1/29.5	
Iraqi Jews	1/115	1/115	–
Yemenite Jews	0/13	–	–
Askenazi Jews United	0/89	–	
North African Jews	2/100	1/50	
Total	5/376	1/75.2	1/23–1/310
Arabs	1/58	1/58	
Egyptians	0/95	–	–
North American Blacks	0/190	–	–

Table 3 Comparison of components of genetic variance (Amova) under five models describing the population distribution of the 35delG allele. (Estonian sample removed from the analysis)

Model	No of groups	Variance between groups	P of a more extreme value
1	7	0.42%	0.009
2	7 ^a	0.49%	0.0019
3	6	0.47%	0.001
4	3	0.37%	0.009
5	3 ^a	0.50%	<0.001

^aEstonia excluded.

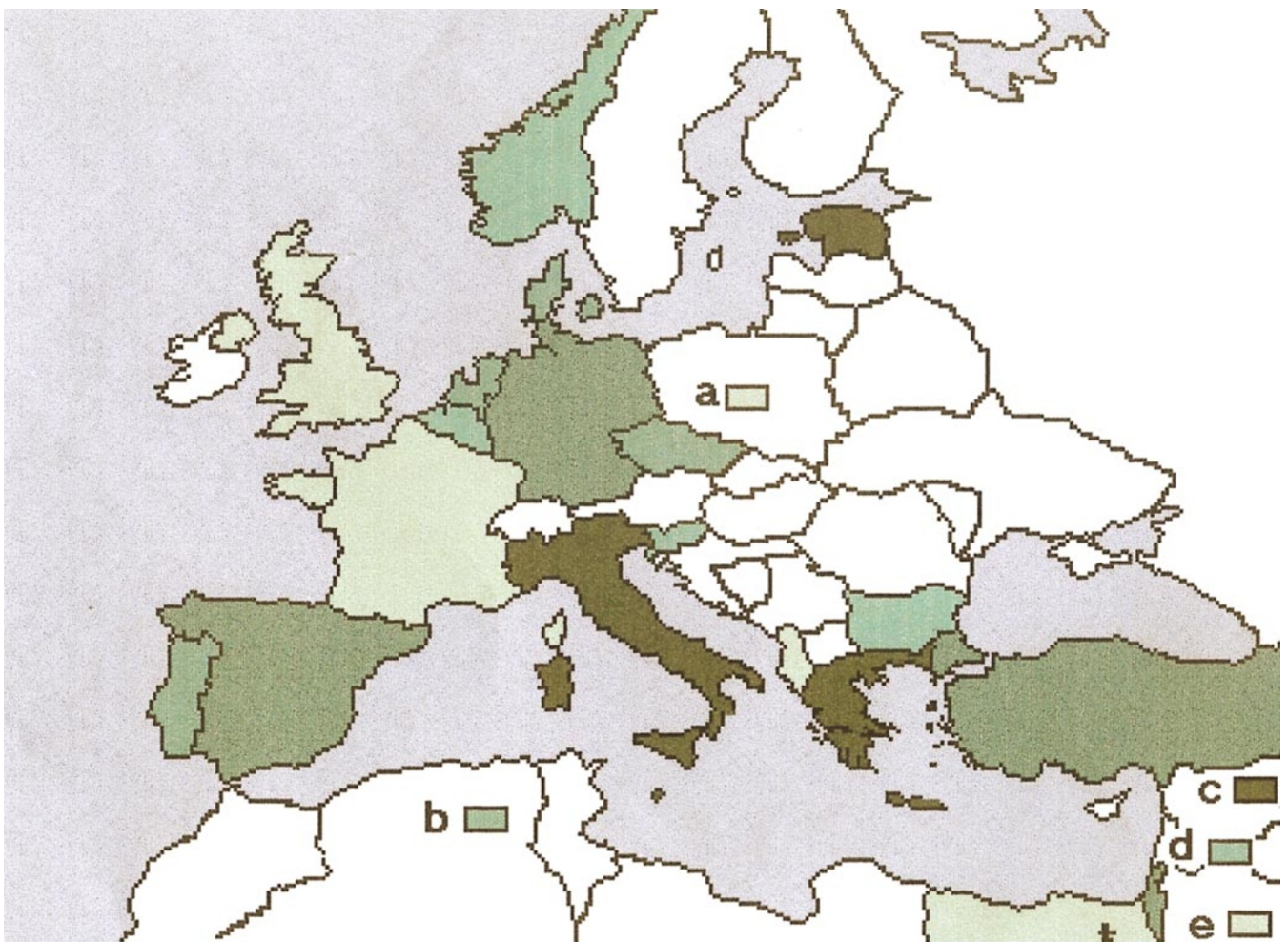


Figure 1 Estimated frequencies of the 35delG heterozygotes in several European and Mediterranean populations. Different countries have different shading corresponding to the different 35delG carrier frequencies; a darker shade corresponds to a higher carrier frequency. Rectangular insets represent Jewish populations (a: Ashkenazi; b: north African; c: Persian; d: Iraq; e: Yemenite); Arabs from Israel are mapped in Israel; independent frequencies have been estimated for France and Brittany. Populations that have not been tested are in white

(data not shown). The patterns of cancer mortality in Europe, for example, correlate with the patterns of gene flow between populations, as inferred from the historical record of population contacts.²⁴ In any case, the absence of the 35delG mutation outside Europe can be explained as the consequence of a single origin, somewhere in Europe or in the Middle East. The fact that the Jewish populations do not differ much from non-Jewish neighbours suggests that, after the occurrence of 35delG, sufficient gene flow has occurred to make the two communities similar. However, the sample sizes from these communities are small. Also, it would be pointless to estimate admixture rates, but the relationship observed here between the two communities seems closer than previously described.²⁵ In a recent paper, a high carrier frequency of another *GJB2* mutation has been described in

Ashkenazi Jews.²⁶ This finding is in agreement both with the negative results we obtained for 35delG carrier frequency in this selected Jewish population and with the major role played by *GJB2* in determining deafness in Jewish populations, also here described.

In conclusion, this study shows (i) a high carrier frequency of 35delG in most European countries, (ii) a higher frequency of 35delG in southern, as opposed to northern and central, Europe, and (iii) a likely single origin for 35delG, somewhere in Europe or the Middle East. The study also suggests that a carrier advantage together with a founder effect could explain the extremely high frequency of this common frameshift mutation in the *GJB2* gene. This high frequency of 35delG should facilitate diagnosis of congenital deafness and allow early treatment of the affected subjects.

Acknowledgements

This work was supported by a grant from the Fundació La Marató de TV3 (1998). RR is supported by a grant from the Spanish Ministry of Health and Social Security (BEF198/9207) and XE by a grant EU BIOMED BMH4-CT96-1364. PG is supported by a Telethon grant and by the Italian Ministry of Health. PG and RR contribute equally to this work. The Genetic Analysis Consortium of GJB2 35delG included: Gert Matthijs (Leuven, Belgium), Ivo Kremensk (Sofia, Bulgaria), Milan Macek Jr, Milan Macek Sr, Alice Krebsova (Prague, Czech Republic), Cecile Cazeneuve (Paris, France), Claude Ferec (Brest, France), Manfred Stuhmann (Hannover, Germany), Edwin Mariman (Nijmegen, Holland), Motti Shoat, Mordechai Shohat, Nurit Magal (Petah Tikva, Israel), Andrea Novelletto (Rome, Italy), Cristina Patrosso (Milan, Italy), Dragana Josifova, AE Felice (La Valletta, Malta), Kristin Eiklid (Oslo, Norway), Paula Pachecho (Lisboa, Portugal), Damjan Glavac, Metka Glavac (Ljubljana, Slovenia), Radovan Komel, Lana Strmecki (Ljubljana, Slovenia), Asli Tolun (Istanbul, Turkey) and Martin Schwarz (Manchester, UK).

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