serum ferritin concentration (243 μg/L). However, this was in a 78-year old *C282Y/H63D* female and in an earlier measurement at 72 years, the transferrin saturation had been found to be in the normal range (32%). This is the first phenotypic analysis in individuals with digenic inheritance of *HJV* and *HFE* mutations and suggests that at least in these individuals there is no increased risk of iron loading.

In conclusion, we report three cases of JH in Australia caused by *HJV* mutations. In two of the families digenic inheritance of *HJV* and *HFE* mutations did not cause iron loading. Although this is a small study, given the rarity of *HJV* and *HAMP* mutations in the general population it may be difficult to identify sufficient individuals to study the effect of these genes as modifiers of *HFE*. The analysis of family members of JH patients may yield more information on the effect of digenic inheritance of *HFE* and either *HJV* or *HAMP* on iron loading.

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*Red Cell Disorders*

## HbO-Arab mutation originated in the Pomak population of Greek Thrace

**HbO-Arab emerged about 2,000 years on a rare haplotype, characteristic of the Greek Pomaks. Its frequency increased as a consequence of high genetic drift within this population, and it was dispersed throughout the Mediterranean basin and Middle East with minor variations of its haplotypic pattern.**

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The non-randomness of some restriction fragment length polymorphisms (RFLP) across the *HBB* cluster, was recognized two decades ago.[1] These RFLP segregates, known as *HBB* cluster haplotypes, are associated with certain mutations and populations, thus constituting a link between a mutation and its possible origin.[2] Moreover, a pattern of five polymorphic sites within the *HBB* gene itself, known as the *HBB* gene framework, has been proved to represent archaic gene patterns.[3]

HbO-Arab (*HBB, E121K*) is most frequent worldwide in Greek Pomaks, in whom the frequency of this mutant allele has been calculated to be 0.027.[4] To investigate its origin, we determined 20 single nucleotide polymorphisms (SNP) flanking *HBB* in 52 Pomak (21 HbO-Arab carrying and 31 normal) and 7 non-Pomak (2 HbO-Arab carrying and 5 normal) chromosomes. Fifteen of them alter restriction sites, are dispersed across the *HBB* cluster and constitute the *HBB* cluster haplotype (Figure 1) and 5 are not linked with restriction sites, segregate within *HBB* and form the *HBB* framework (Table 1). All polymorphisms and the mutation site were confirmed by DNA sequencing. DNAsp 3.99 software was used for recombination analysis.[5] Genetree software was used for *time to most recent common ancestor* (TMRCA) analysis.[4]

A summary of the results is presented in Table 1. Almost half of the normal Pomak chromosomes (13/31) are connected with Greek haplotype VI and Pomak haplotype I; these two haplotypes are most unusual (in the case of Greek haplotype VI) or even absent (in the case of Pomak haplotype I) from the normal Greek population. The remaining 18 normal chromosomes revealed the universal haplotypes I, II and III, which are common among the normal Greek population.[6-8] All but three (18/21) HbO-Arab genes in the Pomaks and both HbO-Arab genes of non-Pomak origin are carried over the haplotype Greek VIa. The remaining three HbO-Arab carrying Pomak chromosomes display a variety of the universal haplotype I (Id). It is notable that the 3' subhaplotype is the same [(-) + (+ - - +) +] in all Pomak and non-Pomak chromosomes carrying the HbO-Arab mutation.

Of the 12 normal chromosomes of Pomak origin examined, 8 displayed the CCGCT framework pattern and 4 the CCTCT pattern. In contrast, all the HbO-Arab carrying chromosomes examined (12 of Pomak and 2 of non-Pomak origin) displayed the CCTCT framework pattern. Possible recombination sites in the Pomak population have been detected between (i) *Hind*III/HBG1 and *Hinc*II/HBBP1 and (ii) between 3' and 5' subhaplotypes. These data explain both Greek VI and Pomak I haplotypes. Figure 1 depicts the mechanism of recombination

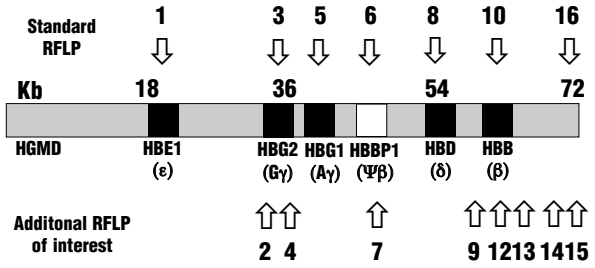


**Figure 1.** A schematic representation of the *HBB* cluster for better interpretation of the mechanisms and chronology of the events that prevailed in the HbO-Arab mutation history. Numbers in the upper and lower rows refer to standard[2] and additional[8] RFLP, respectively. 5 *subhaplotype*: 1. *Hinc*II/HBE1, 2. *Xmn*I/5´HBG2, 3. *Hind*III/HBG2, 4. *Taq*I/3´HBG2, 5. *Hind*III/HBG1, 6. *Hinc*II/HBBP1, 7. *Ava*II/HBBP1, 8. *Hinc*II/3HBBP1, 3 *subhaplotype*: 9. *Rsa*I/HBB, 10. *Ava*II/HBB, 12. *Hinf*I/HBB, 13. *Hpa*I/3HBB(i); 14. *Hpa*I/3HBB(ii); 15. *Hpa*I/3HBB(iii); 16. *Bam*HI/3'HBB. Number 11 is omitted as it refers to the HbO-Arab mutation position lying very close to the *Ava*II/HBB polymorphism (see Table 1).

**Appendix to Figure 1.**

| *Time elapsed in coalescent units* | *Time elapsed in years* | *Event* |
|---|---|---|
| Before 0.252±0.216 | Before the period between 1,740 B.C. and 1,716 A.D. | Formation of Greek haplotype VI via recombination between sites 5 and 6. |
| 0.252±0.216 | Between 1,740 B.C. and 1,716 A.D. | HbO-Arab evolution via *de novo* mutation |
| Between 0.252±0.216 and 0.032±0.049 | After 1,740 B.C. | HbO-Arab survival and distribution due to genetic drift |
| 0.032±0.049 | After 1,356 A.D. | Formation of Universal haplotype I carrying HbO-Arab via recombination between sites 8 and 9. |

**Table 1.** Incidence of studied universal and non-universal *HBB* haplotypes studied in the normal and HbO-Arab carrying Pomak and non-Pomak chromosomes. The *HBB* cluster haplotypes in the 428 chromosomes of Greek origin were based on the studies by Athanassiadou *et al.*,[6] Boussiou *et al.*,[7] and Kollia *et al.*[8] Classical polymorphisms are contained in the white columns; [2]additional polymorphisms are shown in gray columns.[8] Numbers in columns indicate polymorphisms as shown in Figure 1.

| | *5 subhaplotype* | | | | | | | | | *3 subhaplotype* | | | | | *Non-Pomak Greek chromosomes* | | *Pomak Greek chromosomes* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Polymorphisms** | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* | *9* | *10* | *11* | *12* | *13* | *14* | *15* | *16* | *17* | *18* | *19* |
| Universal haplotypes (Nomenclature from Antonarakis)[2] | | | | | | | | | | | | | | | | | | | |
| Universal Ia | + | – | – | – | – | – | + | – | – | + | + | – | – | + | + | 169 | 0 | 8 | 3 |
| Universal Ib | + | – | – | – | – | – | + | – | + | + | – | – | – | + | + | | 0 | 5 | 0 |
| Universal Ic | + | – | – | – | – | – | + | – | – | + | – | – | – | + | + | | 0 | 1 | 0 |
| Universal Id | + | – | – | – | – | – | + | – | + | + | + | – | – | + | + | | 0 | 1 | 0 |
| Universal IIa | – | – | + | – | + | – | + | + | – | + | + | – | – | + | + | 62 | 0 | 1 | 0 |
| Universal IIb | – | + | + | – | + | – | + | + | – | + | + | – | – | + | + | | 0 | 1 | 0 |
| Universal III | – | + | + | – | – | + | + | + | + | + | + | – | – | + | – | 16 | 0 | 1 | 0 |
| Universal IV | – | ? | + | ? | – | + | ? | + | ? | – | ? | ? | ? | ? | + | 11 | 0 | 0 | 0 |
| Universal V | + | ? | – | ? | – | – | ? | – | ? | + | ? | ? | ? | ? | – | 54 | 0 | 0 | 0 |
| Universal VI | – | ? | + | ? | + | – | ? | – | ? | – | ? | ? | ? | ? | + | 10 | 0 | 0 | 0 |
| Universal VII | + | ? | – | ? | – | – | ? | – | ? | – | ? | ? | ? | ? | + | 19 | 0 | 0 | 0 |
| Universal VIII | – | ? | + | ? | – | + | ? | – | ? | + | ? | ? | ? | ? | – | 0 | 0 | 0 | 0 |
| Universal IX | – | ? | + | ? | – | + | ? | + | ? | + | ? | ? | ? | ? | + | 77 | 0 | 0 | 0 |
| Universal X | – | ? | + | ? | – | – | ? | – | ? | – | ? | ? | ? | ? | + | 2 | 0 | 0 | 0 |
| Total | | | | | | | | | | | | | | | | 428 | 0 | 18 | 3 |
| Non-universal haplotypes (Nomenclature from Kollia)[8] | | | | | | | | | | | | | | | | | | | |
| Chinese | – | ? | + | ? | + | – | ? | + | ? | – | ? | ? | ? | ? | + | 4 | 0 | 0 | 0 |
| Greek I | – | ? | + | ? | + | – | ? | – | ? | + | ? | ? | ? | ? | + | 2 | 0 | 0 | 0 |
| Greek V | – | ? | – | ? | – | + | ? | + | ? | + | ? | ? | ? | ? | + | 1 | 0 | 0 | 0 |
| Greek VIa | + | – | – | – | – | – | + | + | – | + | + | – | – | + | + | 1 | 2 | 1 | 18 |
| Greek VIb | + | – | – | – | – | – | + | + | + | + | + | – | – | + | + | | 0 | 1 | 0 |
| Greek VIc | + | – | – | – | – | – | + | + | – | + | – | – | – | + | + | | 0 | 2 | 0 |
| Pomak Ia | – | + | + | – | + | + | + | + | + | + | + | – | – | + | + | 0 | 0 | 5 | 0 |
| Pomak Ib | – | + | + | – | + | + | + | + | – | + | + | – | – | + | + | | 0 | 4 | 0 |
| Total | | | | | | | | | | | | | | | | 8 | 2 | 13 | 18 |

*1: HincII/HBE1; 2: XmnI/5´HBG2; 3: HindIII/HBG2; 4: TaqI/3´HBG2; 5: HindIII/HBG1; 6:HincII/HBBP1; 7: AvaII/HBBP1; 8: HincII/3HBBP; 9: RsaI/HBB; 10: AvaII/HBB; 11: HinfI/HBB; 12: HpaI/3HBB(i); 13: HpaI/3HBB(ii); 14: HpaI/3HBB(iii); 15: BamHI/3HBB; 16: normal (n=436); 17: Hb O-Arab (n=2); 18: normal (n=31); 19: Hb O-Arab (n=21). *Nomenclature from Antonarakis.[2]*

between the former *hotspot* which might explain the formation of Pomak Ia/b haplotypes. In chronological order, the most ancient event seems to have been a recombination between the universal haplotypes or VI and III or πX at the 6.1 kb long sequence between *Hind*III/HBG1 and *Hinc*II/HBBP1 polymorphisms, to which the formation of

the initial haplotypic pattern on which HbO-Arab evolved (haplotype Greek VI) can be attributed. A *de novo* mutation event leading to the formation of HbO-Arab was superimposed on that certain haplotype, whose frequency in the meanwhile increased due to genetic drift. Using the coalescent method to compute TMRCA it appears that this happened 0.252±0.216 coalescent units ago. A second recombination event, between the 3' and 5' subhaplotypes of the Greek haplotype VIc and the universal haplotype I followed before 0.032±0.049 coalescent units and accounts for the minority of HbO-Arab-carrying Pomak chromosomes that display a variety of the universal haplotype I (Id). As a coalescent unit (measured in years) represents the sum $2*N*t_g$ (where N is the population size and $t_g$ is the mean years passed per generation), the approximate time elapsed since the two above-mentioned events is 2000 and 250 years, respectively (for N=200, which is a reasonable estimate for isolated populations, and $t_g$=20) (Figure 1).

Summing up, we found that 42% of the normal Pomak chromosomes display two unusual *HBB* cluster haplotypes, one of which is also strongly associated with the HbO-Arab mutation, and that 33% of normal Pomak chromosomes carry the CCTCT *HBB* framework, with which all HbO-Arab chromosomes are linked. These results suggest that Pomaks constitute a population group characterized by a high genetic drift that can be attributed to historically confirmed physical isolation and intense inbreeding. This environment might have given rise to novel haplotypes, as products of intra-*HBB* cluster recombination events, in one of which the HbO-Arab mutation emerged and increased in frequency. As the HbO-Arab related haplotype and framework is widely dispersed among normal Pomak chromosomes and not in any other known population,[2] the HbO-Arab mutation might have emerged among Pomaks. Heterozygosity for HbO-Arab may be beneficial, which would further ensure the survival of the mutation. Pomaks might have spread HbO-Arab throughout the Mediterranean basin. Minor HbO-Arab related haplotype variations reported[9,10] could be attributed to local recombination events. Finally, the uniformity of the presence of the CCTCT framework and the close association with the Greek VIa haplotype in all our HbO-Arab cases as well as all others already reported in the literature – with only minor variations – indicate a single origin of the mutation.

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*Key words: HbO-Arab, hemoglobinopathies, Pomaks.*

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*Red Cell Disorders*

## Phosphoglycerate mutase BB isoenzyme deficiency in a patient with non-spherocytic anemia: familial and metabolic studies

**We previously reported the first case of red blood cell phosphoglycerate mutase (PGAM) isozyme BB deficiency due to the homozygous point mutation cDNA 690G→A, which causes a substitution of methionine 230 by isoleucine. In the present work we analyzed the changes in glycolytic intermediates caused by this mutation. With the exception of hexose phosphates, all other intermediates were decreased. In contrast, lactate levels were increased. The methionine 230 isoleucine change did not alter the mutated PGAM levels.**

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Phosphoglycerate mutase (PGAM, EC 5.4.2.1) is a glycolytic enzyme that catalyses the interconversion of 2-phosphoglycerate (2PGA) and 3-phosphoglycerate (3PGA), with 2,3-bisphosphoglycerate (2,3-BPG) being required, in mammals, as a co-factor. In addition to acting as a PGAM cofactor, 2,3-BPG in red blood cells (RBC) acts as an allosteric modulator of the affinity of hemoglobin for oxygen and is present at much higher concentrations than in other cell types. In mammals, the enzyme is present in three isozymes which result from the homodimeric and heterodimeric combinations of two different subunits, M and B, coded by two different genes, although the gene coding subunit B is unknown. Only