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IDENTIFIKASI MUTASI H63D GEN HFE PADA KELAINAN HBE

(Identification of H63d HFE Gene Mutation In HbE Disorder)

Yanuarita Tursinawati[1], Nyoman Suci Widyastiti[2], Moedrik Tamam[3]

ABSTRACT

The H63D HFE mutation has been reported to be responsible for primary haemochromatosis. The allele frequency in Indonesian population is about 2.8%. Co inheritance between H63D mutation and hemoglobin disorders such as Thalassemia may increase the severity of iron overload. Nevertheless, the coinheritance of this mutation with HbE disorder is the most common hemoglobin disorder in Indonesia and the gene frequency have not been reported especially in Javanese ethnic. To identify the presence and the frequency of H63D HFE mutation in HbE disorder among Javanese ethnic. A cross sectional study involved 24 Javanese individuals who consist of 21 HbE heterozygotes (HbAE) and 3 HbE homozygotes (HbEE) subjects. The subjects were screened for H63D mutation by digestion of PCR products with MbO I restriction endonuclease. The genotype frequency for wt/wt was 95.24% in HbAE, 100% in HbEE and for wt/H63D was 4.76% in HbAE. The allele frequency for H63D HFE mutation was 2.08% in total sample of HbE. The allele frequencies in HbAE and HbEE individual were 2.38% and 0%, respectively. H63D HFE mutation is found in 24 Javanese ethnic individual with HbE disorder. However, the allele frequency of H63D HFE mutation is low and almost similar to the allele frequency of H63D HFE mutation in Indonesian population.

Key words: *H63D mutation, HFE gene, HbE disorder*

ABSTRAK

Mutasi H63D gen HFE selama ini dilaporkan telah bertanggungjawab terhadap kejadian hemokromatosis primer dan kekerapan alelnya di populasi Indonesia sekitar 2,8%. Pewarisan bersama antara mutasi H63D dan kelainan hemoglobin seperti talasemia dapat meningkatkan derajat keparahan kelebihan beban zat besi. Namun demikian, pewarisan bersama antara mutasi tersebut dan HbE yang merupakan jenis kelainan hemoglobin yang paling banyak ditemui di Indonesia dan kekerapan gennya belum pernah dilaporkan khususnya bagi suku Jawa. Kajian ini bertujuan untuk mengetahui keberadaan dan kekerapan mutasi H63D gen HFE individu HbE bersuku Jawa secara mengidentifikasi. Kajian tertentu ini dilakukan secara potong lintang dengan melibatkan 24 individu suku Jawa yang terdiri dari 21 subjek heterozigot HbE (HbAE) dan tiga (3) dari subjek homozigot HbE (HbEE). Di subjek tersebut dilakukan penapisan mutasi H63D dengan memotong hasil PCR menggunakan enzim pembatas MbOI. Kekerapan genotip wt/wt sebesar 95,24% di HbAE, 100% di HbEE dan di kekerapan genotipe wt/H63D sebesar 4,76% di HbAE. Kekerapan alel mutasi H63D sebesar 2,08% di jumlah keseluruhan sampel HbE. Kekerapan alel di HbAE dan HbEE masing masing sebesar 2,38% dan 0%. Didasari telitian ini, dapat disimpulkan bahwa mutasi H63D gen HFE ditemukan di 24 individu HbE terkait suku Jawa. Kekerapan alel mutasi H63D gen HFE rendah dan hampir sama dengan kekerapan alel H63D gen HFE di populasi Indonesia.

Kata kunci: Mutasi H63D, gen HFE, HbE

INTRODUCTION

Hemoglobin disorder such as Thalassemia and hemoglobin E (HbE) are the major genetic burden in Asian population, include in the Indonesian population. HbE is the most common hemoglobin disorders in Indonesia. The highest frequency of HbE is found in East Sumba in the amount of 30 %.[1] Both of the HbE heterozygote (HbAE) and homozygote (HbEE) are asymptomatic or have a mild anemia with slightly abnormal laboratoric manifestations include the hypo chromic Microcytic one. Compound of hetero zygotes HbE/β^0 Thalassemia have variable phenotypes ranging

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from mild to severe, which are characterized with serious hemolytic anemia and require dependent tranfusions.[2]

Anemia leads to ineffective erythropoiesis in bone marrow which increases iron intestinal absorption to satisfy an augmented erythropoiesis activity. The increased iron absorption leads to the risk of hemochromatosis if the transfusion therapy is given. HbAEalso has some degrees of ineffective erythropoiesis and increased iron absorption with a lesser degree than HbEE or HbE/β^0 thalassemia.[3] Thiscondition may lead to the risk of iron accumulation which causes multi organ dysfunctions.

Mutations in HFEgene which is located on 6p21.3 have recently been reported to be responsible for the primary hemochromatosis.[4,5] The most common forms of HFEgene mutations associated with hemochromatosis are C282Y and H63D. The C282Y mutation carries a cysteine to tyrosine substitution at amino acid position 282 is more common in Caucasian people but extremely rare in South east Asian people. On the other hand, the H63D mutation carries histidine to Aspartic acid substitution at amino acid position 63 spreads worldwide include in Southeast Asia region.[6-8] A study by Merryweather et al[7] revealed that the allele frequency mean of H63D mutation in Thailand is 3%, while in Indonesian is 2.8%.

HFE gene encoded HFE protein which can bind to Transferrin Receptor (TfR) and inhibit the binding of Fe-transferrin to TfR.[9] Therefore, HFE can be expected to inhibit iron uptake by cells. The H63D HFE mutation may alter the normal function of HFE in inhibition iron uptake mechanism. The clinical appearance of HFE heterozygote is unclear; several studies indicate that co inheritance with hemoglobinopathies such as thalassemia may have a synergistic effect and leads to the increased iron uptake and storage.[5,10-12]

The co inheritance HFE with other type of hemoglobin disorders like HbE and frequency in Indonesian have not been reported especially in Javanese ethnic. This is the first study to identify the presence and frequency of H63D HFE gene mutation in HbE individuals which is the most common hemoglobin disorders in Indonesia. Results of this study will provide information during genetic counseling for prevention strategies of iron overload among HbE individuals or their offspring with HbE/β thalassemia.

METHODS

Twenty four samples from Javanese ethnic comprised of 21 subjects with HbAE and three (3) subjects with HbEE were included in this study. All of the samples are from Central Java region. The HbE individuals were obtained from family screening of thalassemia patient who had regular transfusion treatment in Indonesian Red Cross (PMI) of Semarang from January 2010 up to January 2011. The blood samples and secondary data derived from Complete Blood Count (CBC), the Hb electrophoresis was taken with permission from Dr.dr. Nyoman Suci Widyastiti, M.Kes, Sp.PK. A diagnosis of HbE was defined by low MCV (< 80 fL), low MCH (< 27 pg) in CBC results and increased of Hb A_2 (25-30% as Hb E heterozygote and 85% or more as Hb E homozygote) with no or mild elevated HbF in HbE electrophoresis. The subjects with previous history of liver diseases and impaired liver function which might increase ferritin levels were excluded.

Analysis mutation of HFE

The DNA extraction was performed in Molecular and Cito genetic laboratory of Center for Biomedical Research (CEBIOR), at the Medical Faculty of Diponegoro University of Semarang. DNA analysis of the H63D HFE mutation using PCR RFLP was performed in DNA laboratory of KK Hospital, Singapore.

The blood collection was obtained from peripheral blood and the DNA extraction using Wizard ® Genomic DNA. The purification kit used was from Promega (Cat #A1120). The PCR was performed in a final volume of 50 uL, containing 37.75 μL of sterile water, 5 μL 10x PCR Buffer $(NH_2)_4SO_4$,3 μL $MgCl_2$, 1 μL primers, 1 μL of each dNTP, 0.25 μl of DNA Taq polymerase (fermentase) and 1 μl DNA. The forward primer is 5' ACA TGG TTA AGG CCT GTT GC 3 'and reverse primer is 5' GCC ACA TCT GGC TTG AAA TT 3'. The mixture is amplified in a Gene Amp PCR system 2700 (applied biosystem) under the scheme of PCR by initial denaturation at 95°C for 5 minutes and will be completed after 35 cycles each comprising of: 95^0C for 30 second, 55°C for 45 second and 72°C in 30 second, then 10 minute extension at 72^0C. The PCR generated 208 bp DNA products which are visualized in 2% agarose gel.

The Amplified DNA were digested for four (4) hrs at 37^0C in 20 μL reaction mixtures containing 4,5 μL of sterile water, 2 μL of NE buffer 4, 12,5 μL of PCR product and 1 μL of enzyme then were visualized in 2% agarose gel with 100 bp DNA marker. In normal allele, the digested product are shown two bands at 138 and 70 bp, in heterozygote mutation is shown three bands at 208, 138 and 70 bp and in homozygote mutation is shown one bandat 208 bp.

The data of allele and genotype frequencies of H63D HFE gene mutation were analyzed using descriptive

methods and presented in tables. The comparison of mean value of hematological index between HbAE and HbEE was analyzed using independent two samples *t*-test, p value < 0.05 was considered has a significant differences.

RESULTS AND DISCUSSION

Data of characteristic and hematological index of subjects are presented in Table 1. All individuals were Javanese people who came from Central Java. There were 24 persons that comprised of 11 females (52.48%), 10 males (47.62%) were HbE heterozygotes and 1 female (33.3%), two (2) males (66.6%) were HbE homozygotes. The average age of HbE heterozygote was 33.67±12.89 year and for HbE homozygote group was 27.33±12.342 year.

The mean value of Hb in HbAE was 13.62±1.3 g/dL and in HbEE was 10.28±1.66 g/dL. In general, there are one (1) female subject with Hb< 12 g/dL and four (4) male subjects with Hb< 13 g/dL. The comparison between two groups, HbE homozygote group had significantly lower level of Hb (p=0.001), MCV (p=0.000), MCH (p=0.000) and MCH (p=0.045). Mean of HbA_2 plus HbE for HbE heterozygote was 30.88±2.27 and for homozygote was 92.97±13.42 (Table 1).

The genotype and allele frequency of H63D *HFE* gene mutation

The results of DNA analysis using PCR RFLP which were run into the 2% agarose gel can be seen as follows (see Figure 1).

The genotype frequency for wt/wt was 95.24 % in HbAE, 100% in HbEE and for wt/H63D was 4.76% in HbAE (see Table 2).

The H63D/H63D genotype was not found either in HbAE or in HbEE. The genotype frequency by sex was shown in Table 3. In the total sample, there were 12 females and 11 males sample with wt/wt genotype. Meanwhile, only one male sample carried H63D *HFE* mutation and no one female sample had this mutation.

The allele frequency of H63D mutation in total HbE was 2.08%. This study was divided HbE individuals into two (2) groups, HbE heterozygote with allele frequency of H63D mutation was 2.38% and HbE homozygote with allele frequency 0% (see Table 4).

The characteristic of research's subjects

The mean value of Hb in HbAE was 13.62±1.3 g/dL and in HbEE was 10.28±1.66 g/dL. There were two subjects in HbAE and three subjects in HbEE had anemia condition. Compared between the two groups, HbEE group had significantly lower level of Hb, MCV, MCH and MCHC than HbAE. This finding revealed that HbEE individuals in this study have mild anemia with hipochromic microcitic morphology of erythrocytes. This condition mimics thalassemia phenotype and may be confused with the iron deficiency anemia unless other laboratory studies are measured. Vichinsky et al[13] claims that the abnormality of HbE E's erythrocyte may be similar to the erythrocyte picture of thalassemia traits or mild thalassemia inter media. The abnormal β *globin* gene leads to reduced amounts of β^E m RNA and β^E globin chains may cause a mild β^+ thalassemia phenotype.[14,15]

The genotype and allele frequency of H63D HFE mutation

The H63D has a great variability of worldwide distribution. The highest H63D allele frequency in general Asian population is found in South Asia such

Table 1. Characteristic data and mean values of hematological indexes

	HbAE (n=21)	HbEE ((n=3)	p of t-test
Sex (n /%)			
Female	11 (52.38%)	1 (33.3%)	
Male	10 (47.62%)	2 (66.7%)	
Age	33.67±12.89	27.33±12.342	
Hb (g/dL)	13.62±1.3	10.28±1.66	0.001
Subject			
Female with Hb< 12 g/dL (n)	0	1	
Male with Hb< 13 g/dL (n)	2	2	
Mean corpuscular volume (fl)	73.93±2.99	61.60±1.76	0.000
Mean corpuscular Hb (pg)	25.33±1.5	19.23±1.44	0.000
Mean corpuscular Hb concentration (g/dl)	34.63±2.63	31.27±1.72	0.045
Red blood cell width (%)	14.28±1.3	19.30±6.99	0.339
HPLC			
HbA2 + HbE	30.88±2.27	92.97±13.42	0.015

HbAE :HbE Heterozygote

HbEE :HbE Homozygote

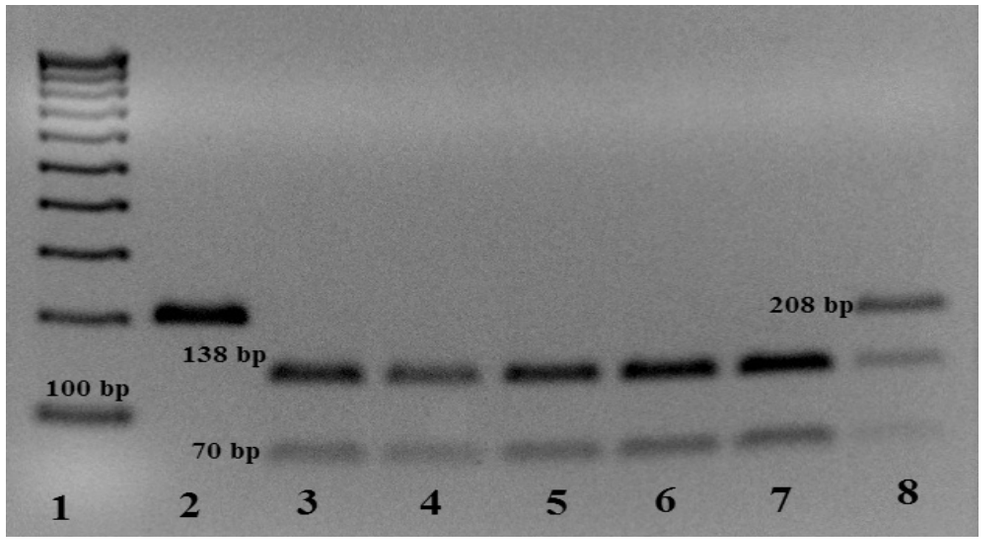

Figure 1. Detection of the H63D HFE mutation by RFLP. Lane 1 is a DNA marker for 100 bp, lane 2 is PCR product without MbO I digestion, lane 3 is (NORMAL) non HbE individual, lane 4 to 7 is HbE individual with wt/wt (138 and 70 bp), lane 8 is HbE individual with heterozygote H63D (208, 138 and 70 bp).

Table 2. Genotype frequency of HFE gene

	HbAE		HbEE	
	n	**%**	**n**	**%**
wt/wt	20	95.24	3	100
wt/H63D	1	4.76	0	0
Total	21	100	3	100

Table 3. HFE genotype frequency by sex in total sample

	Female		Male	
	n	**%**	**n**	**%**
wt/wt	12	100	11	92
wt/H63D	0	0	1	8
Total	12	100	12	100

as in India (9.2%).[6] The allele frequency of H63D mutation in South East countries such as Thailand is 3.2%, Vietnamese (4.9%) and none in Myanmar population.[8]

The co inheritance of β thalassemia that is the major public health problem in South East Asia with primary hemochromatosis may contribute in incidence of iron overload.[5,10-12] This study is aimed to know the presence of H63D HFEgene mutation in HbE which is extremely common β globin disorders in Indonesia by identifying. The study result giving overall genotype frequency for wt/wt was 95.24% in HbAE, 100% in HbEE and for wt/H63D was 4.76% in HbAE. H63D/H63D genotype was not found either in HbAE or in HbEE. These results seem to be higher compared to the genotype frequencies of wt/H63D among 36 Javanese people with β thalassemia trait in Central Java (2.78%) as found by Widyastiti.[16] This finding confirms that the H63D HFE gene mutation can be found in Indonesian population especially in HbE individuals.

The allele frequency was about 2.08% in the total sample and 2.3% in HbE heterozygote sample. This result appeared to be almost similar compared with the allele frequency of the H63D mutation among general population in Indonesia (2.8%) as reported by Merryweather.[7] It seems to be higher compared with its allele frequency among β thalassemia trait in Indonesia (1.4%).[16] This finding is supported by study which is carried out by Jazayeri et al that comparing the relative frequency of H63D HFE gene mutation between β thalassemia minor and healthy population

Table 4. Allele frequencies for H63D in total Hbe, Hbe heterozygote and homozygote

	Heterozygote	Homozygote	Total	Total frequency	
	H63D	**H63D mutation**	**allele**	**allele**	**(%)**
HbE heterozygote	1	0	1	42	2.38
HbE homozygote	0	0	0	6	0
Total HbE	1	0	1	48	2008

in Tehran. They claims that the occurrence of this mutation in thalassemia is not more frequent than in normal population.[17]

Our result also seems to be almost similar compared with the result of a study performed among 70 individuals with HbE homozygote in Thailand. Heterozygote H63D HFE mutation was the only mutation detected in that study giving allele frequency of 2.8%, which is similar to the average allele frequency of Thai population (3%).[3] Due to its relatively low prevalence and a rare mutation, the H63D associated iron overload does not seem become a common problem of hemochromatosis in Indonesia.

This study was performed among 24 Javanese peoples and discovered one hetero zygote H63D *HFE* mutation. Based on those facts, there is an interesting point in this study, the results were significantly different from the study by Culen et al[18] which examined 68 of healthy Javanese people. The Culen's study did not discover the presence of H63D mutation in Javanese people.[18] There is a question appeared from these new findings about the origin source of this mutation.

A study by Culen et al[18] which examined the ancestral origin of H63D mutation in nonCaucasian population, which revealed that in Australian Aborigin sample, this mutation was similar with HLA haplotypes common in Caucasian and possibly due to population admixture. Thus, it could be inferred that this mutation was from European origin. Meanwhile, the H63D mutation probably predates the C282Y mutation in South East Asia and Srilanka population, suggests that the H63D mutation may have formed once in the European and once in Asia as an independent mutation apart from Caucasian origin.[19] According to the presence of this mutation in Javanese people, thus further study needs to be conducted for haplotyping of H63D mutation to confirm the ancestral origin of this mutation in Indonesia.

The rarity of H63D HFE gene mutation in Asia especially in Indonesia may be due to the significantly differences of population characteristic, sample size and genotype variation compared to European population. Genotype variation in a population may be influenced by demographic effect and race diversity.[20] The people tends to form a cluster based on their geographic origin, ancestry or race. The smaller the population in a cluster the more vulnerable to genetic drift, resulting in reduced genetic variation. The allele frequency is more likely to be lost or changed from a small population if genetic drift occurs.

In addition, this study found that the sample who carried H63D mutation was an 18 year old male. The male sample carrying this mutation may have a tendency to be more at risk for iron overload because its co inheritance between HbE and heterozygosity of H63D HFE mutation as claimed by Madaniet al.[12] A study by Yan et al.[21] revealed that the symptoms in male rarely present before fourth or fifth decade of life. However, sometimes it does not result in clinically significant of iron overload. There is a high degree of variability in the phenotype expression due to additional factor such as: age, gender, alcoholism, viral hepatitis and genetic factors.[22] Thus, that additional factor must be appropriately observed in this male sample to prevent iron overload.

CONCLUSION AND SUGGESTION

In conclusion, H63D HFE mutation is found in 24 Javanese ethnic individual with HbE disorder. However, the allele frequency of H63D HFE mutation is low and almost similar to the allele frequency of H63D HFE mutation in Indonesian population (2.8%).

This study is not without limitations. As mentioned above, co inheritance of hemoglobinopathies and H63D mutation may result in iron accumulation, this study does not measure ferritin serum which represents the iron level. Furthermore, the participants were Javanese people who came from Central Java, it may not be generalized in to Indonesian populations in widely area. Therefore, further studies need to be developed in bigger sample size, wider sampling area and other tribes.

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REFERENCES

1. Lanni F, Sofro ASM, Ismadi M, Marzuki S. IVSI-5 (G>C): The most common β thalassemia mutation found in the island of Sumatera.Indones J Biotech 2004; 6: 571-7.

2. Wahidiyat PA, Djajadiman G, Tenny T, Harapan PR, Marzuki NS, Iswandari S *et al.* Phenotypic Diversity in beta-HbE thalassemia patients. Paediatr Indones 2006; 46(3): 82-6.
3. Viprakasit V, Prin V, Worrawut C, Kalaya T, Parichat PA, Victoria LCW *et al***.**Prevalence of HFE mutations among the Thai population and correlation with iron loading in hemoglobin E disorder**.** Eur J Haematol 2004; 73(1): 43-9.
4. Piperno A, Rappaela M, Cristina A, Anna V, Sandra B, Silvia F *et al.* Haemochromatosis in patients with -thalassemia trait**.** Br J Hematol 2000; 111(3): 908-14.
5. Argüelles RGJ, Garcés EJ, Reyes NV, Sánchez AJ, Ruiz DG, Jiménez C *et al*. Heterozygosity for the H63D mutation in the hereditary hemochromatosis (HFE) gene may lead into severe iron overload in beta-thalassemia minor: observations in a thalassemic kindred. Rev Invest Clin. 2001; 53 (2): 117-20.
6. Merryweather C, Pointon JJ, Shearman JD, Robson KJ. Global prevalence of putative haemochromatosis mutations. J Med Genet 1997; 34(4): 275–8.
7. Merryweather C, Pointon JJ, Jouanolle AM, Rochette J, Robson KJ.Geography of HFE C282Y and H63D mutations**.**Genet Test 2000*;* 4(2)*:* 183–98.
8. Pointon JJ, Viprakasit V, Miles KL, Livesey KJ, Steiner M, O'Riordan S *et al.* Haemochromatosis gene (HFE) mutations in South East Asia: a potential for iron overload. Blood Cells Mol Dis2003*;* 30(3): 302–6.
9. Feder JN, David MP, Alivelu I, Vince KL, José A. L, Nicole W *et al.* The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. Proc Natl Acad Sci U S A 1998; 95(4): 1472–7.
10. Jang MR, Patricia AG, Thad AH, Matthew RW, Chin SL, Russel EW. Identification of hemochromatosis gene polymorphisms in chronically transfused patients with sickle cell disease. Am J Hematol 2003; 74(4): 243-8.
11. Edward CQ, Skolnick MH, Kushner JP. Coincidental nontransfusional iron overload and thalassemia minor: association with HLA linked hemochromatosis. Blood 1981; 58(4): 844-8.
12. Madani HA, Afify, Abd El Aal, Salama, Ramy. Role of Hfe gene mutations on developing iron overload in β thalassemia carriers in Egypt. EMHJ, 2011; 17(6): 546-51.
13. Vichinsky E. Hemoglobin E Syndromes. Am Soc Hematol Educ Program 2007; 79-83.
14. Orkin SH, Kazazian H, Antonarakis SE. Abnormal RNA processing due to the exon mutation of the β^E globin gen. Nature 1982; 300(5894): 768-9.
15. Fucharoen S, David JW. Hemoglobin E disorder In : Steinberg MH, Bernard GF, Douglas RH, David JW, eds. Disorders of hemoglobin, genetics, pathophysiology and clinical management, 2nd Ed**.,** USA, Cambridge University, 2009; 442-8.
16. Widyastiti NS. Hubungan Polimorfime Gen *HAMP-P* c -582 A>G dan Gen *HFE* H63D Dengan Status Besi Carrier Talasemia Beta (dissertation). Surabaya, Airlangga University, 2012; 40-57.
17. Jazayeri M, Bakayev V, Zali MR, Mohaghegh HR, Esmaeili A, Haghighirad F *et al.* Comparison of relative frequency of *HFE* gene mutations in minor beta thalasssemia and healthy population in Tehran. J Hepatol 2003; 38(2): 211-2.
18. Cullen LM, Xiaojiang G,Simon E, Elizabeth CJ. The hemochromatosis 845 G → A and 187 C → G mutations : Prevalence in Non Caucasian Populations. Am J Hum 1998; 62(2): 1403-7.
19. Rochette J,Pointon JJ,Fisher CA,Perera G,Arambepola M,Arichichi DS *et al.* Multicentric origin of haemochromatosis gene (HFE) mutations. Am J Hum 1999; 64(6): 1056-62.
20. Jorde LB, Wooding SP. Genetic variation, classification and "race". Nat genet 2004; 36(11): S28-33.
21. Yen AW, Tonya LF, Christopher LB. Revisiting hereditary hemochromatosis: current concepts and progress. Am J Med 2006; 119(5): 391-9.
22. Rochette J, Gac GL, Lassoued K, Ferec C, Robson KJH. Factors influencing disease phenotype and penetrance in HFE haemochromatosis. Hum Genet 2010; 128(3): 233-48.