Makara Journal of Science

Volume 18
Issue 2 *June*

Article 8

6-25-2014

Detection of Papua New Guinea Thalassemia Alpha Mutation in Gayo, Sumba, Ternate, and Timika Populations

Risya Nurfitriani
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok 16424, Indonesia

Abinawanto Abinawanto
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok 16424, Indonesia, abinawanto.ms@ui.ac.id

Rintis Noviyanti
Laboratory of Red Blood Cell Disorders, Lembaga Biologi Molekuler Eijkman, Jakarta 10430, Indonesia

Lely Trianti
Laboratory of Red Blood Cell Disorders, Lembaga Biologi Molekuler Eijkman, Jakarta 10430, Indonesia

Ita M. Nainggolan

Detection of Papua New Guinea Thalassemia Alpha Mutation in Gayo, Sumba, Ternate, and Timika Populations

Cover Page Footnote

The authors wish to thank the Laboratory of Red Blood Cell Disorders at Lembaga Biologi Molekuler Eijkman for funding this research. Thanks also to Dr. Iswari Setianingsih, Sp.Ak., and Dr. Alida Harahap, Sp.PK., Ph.D., for valuable comments and suggestions, and also to Muhammad Anindika for his correction of grammatical errors throughout the writing of this article.

Makara J. Sci. 18/2 (2014), 47-51
doi: 10.7454/mss.v18i2.3138

Detection of Papua New Guinea Thalassemia Alpha Mutation in Gayo, Sumba, Ternate, and Timika Populations

Risya Nurfitriani[1], Abinawanto[1*], Rintis Noviyanti[2], Lely Trianti[2], and Ita M. Nainggolan[2]

1. Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok 16424, Indonesia
2. Laboratory of Red Blood Cell Disorders, Lembaga Biologi Molekuler Eijkman, Jakarta 10430, Indonesia

**E-mail: abinawanto.ms@ui.ac.id*

Abstract

Papua New Guinea (PNG) mutation is a point mutation that occurs in noncoding region of alpha globin clusters. Polymorphism promotes an additional recognition site for transcription factor (GATA-1) which presumably downregulates alpha globin synthesis. The aim of this research is to detect PNG mutation in other populations in Indonesia, thus the results will be used for completing standard diagnoses in detecting alpha thalassemia mutation based on ethnic background. The method used in detecting PNG mutation was PCR-RFLP. Detection of 399 samples (MCH <80 fL) using the PCR-RFLP method showed positive results for the Timika population. However, negative results were found in the Gayo, Sumba, and Ternate populations. PNG mutation frequency in the Timika (Papuan ethnic) population is 18.1% (28 of 154 samples). High malaria prevalence in East Indonesia did not show a positive correlation with the absence of PNG mutation in the Sumba and Ternate populations. The results showed that PNG mutation is only found groups that are infected with *Plasmodium falciparum* malaria, but not in *Plasmodium vivax*-infected ones. However, PNG mutation is common in the eastern Indonesia population.

Abstrak

Deteksi Mutasi Papua Nugini (PNG) Thalassemia Alfa di Populasi Gayo, Sumba, Ternate, dan Timika. Mutasi PNG merupakan mutasi titik di luar gugus globin alfa. Polimorfisme menyebabkan terbentuknya promoter baru sebagai situs pengikatan faktor transkripsi GATA-1 yang diduga menurunkan laju transkripsi normal globin alfa. Tujuan dari penelitian ini adalah untuk mengetahui keberadaan mutasi PNG di populasi lain di Indonesia, sehingga hasil penelitian ini dapat digunakan untuk melengkapi standar diagnosis dalam mendeteksi mutasi penyebab thalassemia alfa berdasarkan latar belakang etnik. Teknik yang digunakan dalam mendeteksi mutasi PNG adalah PCR-RFLP. Hasil menunjukkan 18,1% (28 dari 154 sampel) positif pada populasi Timika, namun hasil negatif ditunjukkan pada semua sampel DNA populasi Gayo, Sumba, dan Ternate. Prevalensi malaria yang tinggi di wilayah Indonesia Timur tidak menunjukkan korelasi positif terhadap keberadaan mutasi PNG di populasi Sumba dan Ternate. Hasil penelitian menunjukkan bahwa mutasi PNG ditemukan hanya pada kelompok individu yang terinfeksi *Plasmodium falciparum* tetapi tidak pada kelompok individu yang terinfeksi *Plasmodium vivax* dan mutasi PNG juga ditemukan pada satu individu beretnik Ambon yang tinggal di Timika.

Keywords: ethnic, malaria, PNG mutation, polymorphism, population, Plasmodium

1. Introduction

Thalassemia is an inherited blood disorder caused by an abnormality of globin synthesis. Thalassemia is largely found in tropical and subtropical regions, such as the Mediterranean, the Middle East, and Southeast Asia, which are areas endemic to malaria [1]. These disorders probably occur as protection against *Plasmodium.* A mutation of the globin gene causes abnormality in globin synthesis, which forms abnormal erythrocyte that *Plasmodium* cannot utilize [2].

A major component of human adult hemoglobin is Hemoglobin A. Hemoglobin A is composed of two pairs of polypeptide chains: one pair of alpha globin chains and one pair of beta globin chains. A reduced rate of alpha globin synthesis results in alpha thalassemia. There are two kinds of mutations of alpha thalassemia: deletion and non-deletion mutation [3]. In general, non-deletion thalassemia may give rise to a more severe reduction in alpha chain synthesis than deletion mutation because non-deletion mutation affects mRNA processing, mRNA translation, and alpha globin stability [4]. The

large numbers of alpha thalassemia mutations illustrate a distinct variety of clinical patients, ranging from asymptomatic thalassemia to severe anemia [5]. Severe anemia leads to jaundice, bone changes, and hepatosplenomegaly. As a consequence of the more severe hematologic phenotype, these patients may need red blood cell transfusions more frequently [6]. Asymptomatic thalassemia is clinically normal with no symptoms like severe anemia, but shows low MCV and MCH levels. Moreover, a combination of deletion and non-deletion mutations causes the severe phenotype of alpha thalassemia [2,4].

Humans have two alpha globin genes located in the alpha globin gene cluster in chromosome 16 [5]. Alpha thalassemia mutation commonly occurs in the alpha globin gene, but the mutation can also occur outside the alpha globin gene [6-8]. In 2006, Gobbi discovered polymorphism at the 91st nucleotide of the interzeta-Hypervariable region (inter-ζHVR), located between the zeta gene and the pseudogen zeta alpha globin gene cluster. Polymorphism promotes an additional recognition site for the transcription factor (GATA-1). Preferential interaction of alpha major regulatory element to the new promoter causes disruption of the normal transcription activity of the alpha globin gene which downregulates alpha globin synthesis. Polymorphism was first discovered in a Hemoglobin H (Hb H) disease patient from Vanuatu, thus the mutation was named Vanuatu mutation but is also known as Papua New Guinea (PNG) mutation [9].

PNG mutation was first discovered in Indonesia in a patient of Bugis ethnicity. Interestingly, PNG mutation is a point mutation in the noncoding region of alpha globin gene clusters but causes severe thalassemia if combined with another point mutation. The patient was suffering from phenotypically severe thalassemia (Hb H disease) and the DNA analysis showed a combination of PNG mutation and Codon 59 mutation ($GGC^{Gly}>GAC^{Asp}$) [10-11]. Related to the finding of a PNG mutation in a patient of Bugis ethnicity, previous research has been done to detect PNG mutations in South Celebes populations of different ethnicities, such as Marang, Bugis, Mandar, Toraja, Kajang, and Makassar, but the results were negative. Several samples of the Timika population have also been detected and, surprisingly, positive samples of the PNG mutation were found (3/15 samples). These results underlied our research to detect PNG mutation within a larger population, especially among the Timika population. We hypothesized that there is a higher possibility of the presence of PNG mutations in regions with high malaria prevalence. This hypothesis is supported by research that was done by Elyazar *et al*. in 2011. East Indonesia, especially Maluku, Nusa Tenggara, and Papua, has the highest percentage (63%) of malaria parasites in Indonesia [12]. Thus, Ternate and Sumba populations were also detected for PNG mutations. The aim of this research was to detect PNG mutation using the PCR-RFLP method in Gayo, Sumba, Ternate, and Timika populations. Thus the result will be used for completing standard diagnoses in detecting alpha thalassemia mutations based on ethnic background.

2. Methods

Samples. A total of 399 DNA samples were used: 31 samples from the Gayo population, 92 samples from the Sumba popuation, 111 samples from the Ternate population, and 165 samples from the Timika population. Gayo, Sumba, Ternate and Timika populations respectively from west, central, and east region of Indonesia. All samples were from alpha thalassemia patients with MCH values below 80 fL and whose ethnical backgrounds were verified based on the lineage of three previous generations.

PCR amplification of genome DNA. Reaction mix is composed of NEB Buffer 10x, primer forward 0745F, primer reverse 149776R, taq pol NEB, and H_2O. DNA is amplified using the primer primer *forward* 0745F(5'-GGGAGCACCAGGACACAGATG-3' (bases 13--33), primer *reverse* 149776R (5'-CTTGCACCAACAGC-TTTTCA-3' bases 157--176), 30 cycles 15 sec at 94 ^{o}C, 15 sec at 57 ^{o}C, 45 sec at 72 ^{o}C; the size of the normal PCR product was 164 bp; the reaction mix composition, primers and PCR cycles used were the optimization results accomplished by the Laboratory of Red Blood Cell Disorders at Eijkman Insitute.

RFLP. Digestion of the PCR product was done with control and subject variables. The reaction mix is composed of NEB 4, *Hpy*188I, H_2O, and PCR product. The PCR product digested using *Hpy* 188I was incubated at 37 ^{o}C and 450 rpm for four hours. The size of a normal cell is 164 pb (not digested) and, if positive PNG mutation occurs, the size becomes 79 bp and 85 bp. The visualization of the PCR and RFLP results was done by using agarose gel 2% and marker ΦX174/*Hae*III.

3. Results and Discussion

The detection experiment of 399 samples (MCH <80 fL) using the PCR-RFLP method showed positive results for the Timika population with a frequency of 17.6% (Table 1). However, negative results were found in Gayo, Sumba, and Ternate populations. These results

Table 1. The Result of Detecting PNG Mutation in Indonesian Populations

Population	N	%
Gayo	0/31	0
Sumba	0/92	0
Ternate	0/111	0
Timika	29/165	17.6
Total	29/399	7.3

showed that there was no positive correlation between PNG mutation and malaria prevalence, referring to malaria distribution conducted in Eastern Indonesia (Mollucas, Lesser Sundas and Papua) [10].

The PNG mutation frequency was 7.3% (29 of 399 samples). The ethnical background and malaria status of 29 positive samples have been recognized. All positive samples are categorized as having either Papuan or non-Papuan ethnicity. Twenty-eight samples were Papuan and one sample was non-Papuan, being of Ambonese ethnicity. On the other hand, samples are categorized based on malaria status in three groups: not infected, *Plasmodium falciparum*-infected, and *Plasmodium vivax*-infected. All positive samples were from *P. falciparum*-infected patients. From these findings we may conclude that PNG mutation is a polymorphism of an alpha globin gene cluster in order to protect itself from *P. falciparum* invasion. *P. falciparum* initiate cerebral malaria, a disease with severe symptoms that causes death. Immune systems undergo self-resistance against high *P. falciparum* exposure that causes hemoglobinophaty. Hemoglobinophaty leads to red blood cell abnormalities. Abnormal red blood cells are less supportive of *Plasmodium* growth [13]. In fact, both thalassemic and normal red blood cells are susceptible to *Plasmodium* invasion, but thalassemic patients will not suffer severe symptoms of malaria [14]. The body has a mechanism that destroys abnormal red blood cells which accelerate hemolysis and thus impact the infected red blood cells. Moreover, this also decreases the percentage of parasitemia in blood [15].

Geographic conditions correlated with malaria vector. Timika population samples were classified into three groups according to their geographical background, such as Papuan highland, Papuan lowland, and Non-Papuan. The presentation of PNG mutations from Papuan lowlands and Papuan highlands were 34.61% (9/26) and 14.84% (19/128), respectively, shown in Figure 1. Data showed that PNG mutation is more frequent in Papuan lowlands than in Papuan highlands. Hence, geographic conditions should be considered for further research in detecting PNG mutation. Furthermore, Minakawa showed that altitude plays a significant role in the presence of a malaria vector. Warm temperatures in lower altitudes will support larvae growth, which increases the presence of malaria. In contrast, colder temperatures cause physiological pressure and shorten the life span of mosquitoes, which reduces the presence of malaria [16].

Table 2. Geographical Background of Timika Population

	Positive	Negative	Total
Papuan Highland	19	109	128
Papuan Lowland	9	17	26
Non-Papuan	1	10	11
	29	136	165

Fitness to malaria exposure. Single Nucleotide Polymorphism (SNP) in PNG mutation could be classified as malaria-selected polymorphism [17]. Referring to heterozygous advantage as a mechanism in balanced polymorphism, the high *P. falciparum* exposure in Papua shows that alleles with PNG mutation tend to be more fit, and eventually PNG mutation frequency will be significantly high in the Timika population. It has been shown that all positive samples were hetero-zygotes (Figure 2).

Previous studies at the Laboratory of Red Blood Cell Disorders showed that genetic variations from each ethnic group play significant roles in various mutations of alpha thalassemia (unpublished data). There are common and uncommon alpha thalassemia mutations in the population. By using the same samples, the studies revealed that mutation 3.7kb deletion was found in the Gayo, Sumba, and Ternate populations. Furthermore, SEA deletion was found only in the Gayo population, but not in the two other populations. According to these findings, we can assume that PNG mutations are more common in East Indonesia, in contrast to SEA deletion, which is found mostly in the Gayo population.

As seen in our findings, one sample of PNG mutation originated from an Ambonese ethnicity, whereas PNG mutation was not found in Ternate population even though they were from the same region (Mollucas). The separate population would tend to be genetically different compared to their new habitat, which affects

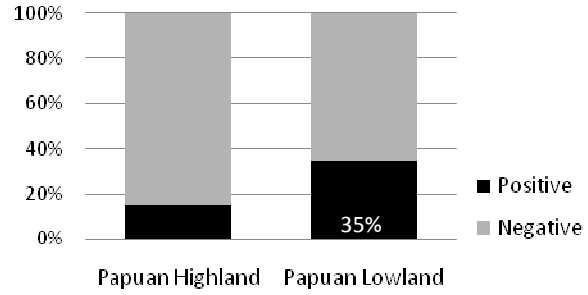

Figure 1. Ratio of Positive PNG Mutations in Papuan Lowlands and Papuan Highlands

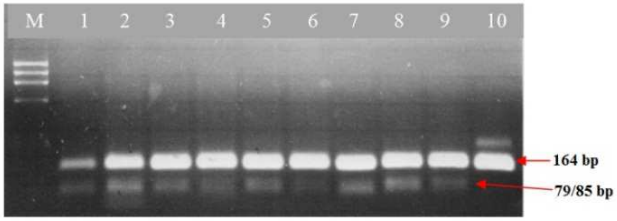

Figure 2. Result of PCR-RFLP. M: Φ X174/*Hae* III Marker. Lane 1: Positive Control. Lane 2-9: Positive PNG Mutation from Timika Samples. Lane 10: Uncut Sample

polymorphism. Single Nucleotide Polymorphism (SNP) in PNG mutation may increase fitness through malaria selection, therefore SNP will decline along with high malaria exposure and also increase mutation frequency within the population [18]. The presence of PNG mutation in Ambonese patients may occur because of outbreeding depression [19]. As depicted in this case, the patients were forced to adapt to their new habitat (Timika), which has the SNP been declined to increase fitness in a place with high malaria exposure.

Migration history correlated with the origin of PNG mutation. The origin of PNG mutation may be presumed using two assumptions. The first assumption is conducted with the Slow Boat hypothesis, which attempts to explain Pacific colonialization based on mitochondrial DNA and Y chromosomes. Austronesians as Polynesian ancestors moved eastward and mixed extensively with local Melanesians before colonizing the Pacific islands [20-21]. As depicted by the Austronesian migration path according to the Slow Boat hypothesis, we presumed that PNG mutation might be discovered in Maluku before the gene flow occurred to Papua (Timika). This assumption is supported by the finding of one positive sample from Ambon.

On the other hand, the second assumption states that PNG mutation originated among Melanesians, which accords with the research about the genetic backgrounds of North Maluku's population using mitochondria DNA [22]. North Maluku's population are non-Austronesian (Papuan Language) and Austronesian speakers. The research revealed that Papuans moved to North Maluku and mixed extensively with Austronesians before migrating westward. Therefore, the PNG mutation which was carried forth by Papuans would be introduced to the Austronesians and then brought to the western populations. These assumptions might also corroborate the findings of PNG mutations in Bugis and Ambonese patients.

4. Conclusions

The PNG mutation frequency is 0% in Gayo, Sumba, Ternate populations, but is quite high in the Timika population (18.1%), though the frequency in the total population is 7.3%. All positive samples are *P. falciparum*-infected and mostly found in the lowlands. This research revealed no positive correlation between malaria prevalence and the presence of PNG mutations in the overall population. Further research should be done to detect them among other populations in Indonesia, especially among other ethnic groups in Central Maluku since the one positive sample of PNG mutation came from the Timika population, which derived from the Ambonese. Detection of PNG mutations also should be done in *P. vivax*-infected patients to validate whether this mutation is a protection against *P. falciparum* or *P. vivax*.

Acknowledgements

The authors wish to thank the Laboratory of Red Blood Cell Disorders at Lembaga Biologi Molekuler Eijkman for funding this research. Thanks also to Dr. Iswari Setianingsih, Sp.Ak., and Dr. Alida Harahap, Sp.PK., Ph.D., for valuable comments and suggestions, and also to Muhammad Anindika for his correction of grammatical errors throughout the writing of this article.

References

[1] G. Stamatoyannopoulos, A.W. Nienhuis, P.W. Majerus, H. Varmus, The Molecular Basis of Blood Diseases, 3rd ed., W.B. Saunders Company, Philadelphia, 1994, p.331.
[2] I. Setianingsih, A. Harahap, I.M. Nainggolan, In: S. Marzuki, J. Verhoef, H. Snippe (Eds.), Advances in Experimental Medicine and Biology, Tropical Diseases, Kluwer Academic/Plenum Publishers, New York, 2003, p.531.
[3] S.B. McKenzie, J.L. Williams. Clinical Laboratory Hematology, 2nd ed., Prentice Hall, Upper Saddle River, New Jersey, 2010, p.1080.
[4] C.L. Harteveld, D.R. Higs, Orphanet J. Rare Dis. 5 **(**2010) 6.
[5] I. Wahidiyat, P.A. Wahidiyat, Pediatrica Indonesiana. 46 **(**2006) 190.
[6] P. Lio, N. Goldman, Genom Res. 8 (1998) 1233.
[7] R. Galanello, A. Cao, Gene Reviews. 25 (2008) 28.
[8] D. Provan, J.G. Gribben, Molecular Hematology, 3rd ed., John Willey & Sons, Ltd., London, 2010, 425.
[9] M. Gobbi, V. Viprakasit, J.R Hughes, C. Fisher, V.J Buckle, H. Ayyub, R.J. Gibbons, D. Vernimmen, Y. Yoshinaga, P. Jong, J.F. Cheng, E.M. Rubin, W.G. Wood, D. Browden, D.R. Higgs, Science Mag. 312 (2006) 1216.
[10] I. Nainggolan, A. Harahap, D.D. Ambarwati, R.V. Liliani, D. Megawati, M. Swastika, I. Setianingsih, Hemoglobin. 10 (2012) 2 [In Indonesia].
[11] N. Djunaidi, Deteksi Mutasi GATA-1 Papua Nugini Thalassemia Alfa Populasi Sulawesi Selatan, Universitas Katolik Indonesia Atmajaya, Indonesia, 2008 [In Indonesia].
[12] I.R.F. Elyazar, S.I. Hay, J.K. Baird, Adv. Parasitol. 74 (2011) 47.
[13] V.R.R. Mendonca, M.S. Goncalves, M.B. Netto. J. Trop. Med. 17 (2012) 3.
[14] B. Marcus, Malaria Deadly Diseases and Epidemics, 2nd ed., Infobase Publishing, New York, 2009, p.120.

[15] K. Pattanapanyasat, K. Yongvanitchit, P. Tongtawe, K. Tachavanich, W. Wanachiwanawin, S. Fucharoen, D.G. Walsh, Blood. 93 (1993) 3118.
[16] N. Minakawa, G. Sonye, M. Mogi, G. Yan, Med. Vet. Entomol. 18 (2004) 303.
[17] C.E. Tosta, Mem. Inst. Oswaldo Cruz. 102/3 (2007) 385.
[18] K.L. Heckman, C.L. Mariani, R. Rasoloarison, A.D. Yoder, Mol. Phylogenet Evol. 43 (2007) 353.
[19] R. Leimu, M. Fischer, Plos One. 5 (2010) 3.
[20] S. Oppenheimer, M. Richards, Sci. Progress. 84 (2011) 157.
[21] M. Kayser, S. Brauer, R. Cordaux, A. Casto, O. Lao, L.A. Zhivotosky, C.M. Faurie, R.B. Rutledge, W. Schiefenhoevel, D. Gil, A.A. Lin, P.A. Underhill, P.J. Oefner, R.J. Trent, M. Stoneking, Mol. Biol. Evol. 23 (2006) 2234.
[22] P. Kusuma, Thesis, Faculty of Medicine, Universitas Indonesia, Indonesia, 2013 [In Indonesia].