

# 23\_Cd60\_GTG\_GAGHb\_Cagliari \_mutation\_was\_found\_in\_scan.p df *by*

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**Submission date:** 14-Sep-2020 02:25PM (UTC+0700)

**Submission ID:** 1386598430

**File name:** 23\_Cd60\_GTG\_GAGHb\_Cagliari\_mutation\_was\_found\_in\_scan.pdf (544.14K)

**Word count:** 2446

**Character count:** 12962



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### Short Communication

## 3 Cd60 (GTG > GAG)/Hb Cagliari mutation was found in scanning of $\beta$ -thalassemia alleles from patients of East Kalimantan, Indonesia

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### ARTICLE INFO

#### Keywords:

$\beta$ -thalassemia  
Mutation  
 $\beta$ -Globin gene  
Samarinda-East Kalimantan

### ABSTRACT

**Background and purpose:** Thalassemia is a genetic disorder with a fairly high prevalence worldwide. Three to 10% of Indonesian people are estimated to be carriers for thalassemia. This study was intended to figure out the spectrum of genetic mutations of patients with thalassemia in Samarinda City, East Kalimantan.

**Methods:** The research subjects consisted of 31  $\beta$ -thalassemia patients registered with the Association of Thalassemia Patients' Parents (POPTI) of Samarinda. DNAs were extracted from the patients' blood samples then amplified by the direct sequencing technique with polymerase chain reaction to analyze  $\beta$ -globin gene mutations.

**23** **It:** The study results show that the male/female ratio was 51.6%:48.4%, the patients' ages ranged from 4 years to 56 years with an average age of 14 years, and the dominant ethnic group was Javanese (64.5%). The DNA analysis yielded 7 types of mutant alleles, namely Cd26/HbE (GAG > AAG) at 48.4%, IVS-1-5 (G > C) at 14.5%, IVS-1-2 (T > C) at 12.9%, Cd35 (-C) at 8.1%, IVS-1-1 (G > T) at 6.5%, and, the least frequently encountered mutant alleles, Cd30 (AGG > ACG) and Cd60 (GTG > GAG) each at 3.2%.

**Conclusion:** This study discovered unreported mutant in Indonesia, namely Cd60 (GTG > GAG).

### 1. Introduction

Thalassemia is a genetic disorder fairly common across the world-wide areas belonging to the thalassemia belt [1]. The WHO (World Health Organization) estimated 7–8% of world population to be carriers for  $\beta$ -thalassemia, resulting in 60,000 children born with severe thalassemia disease every year, most of whom have origins in developing countries, including Indonesia. Results of previous surveys state that 3–10% of the Indonesian people of varied ethnicities across the archipelago are carriers for  $\beta$ -thalassemia. Some ethnic <sup>22</sup> carry specific mutations: Javanese-Sundanese (HbE (GAG > AAG), IVS-1-5 (G > C), IVS-1-1 (G > T), Cd35 (-C)); Malay (Cd19 (AAC > AGC), HbE, IVS-1-5); Makassar (HbE, Fil <sup>25</sup>  $\beta$ -thalassemia deletion, Hb Lapore Boston); and <sup>24</sup> <sup>24</sup> (Cd42-42 (-TCTT), IVS-2-654 (T > C) [2].

Mutations of the  $\beta$ -globin gene are highly variable with over 300 types of mutations reported [3]. Thalassemia is a disease which up until now is devoid of definitive therapies, making prevention the most appropriate and WHO-recommended disease management. It has been reported that  $\beta$ -globin gene mutations are determined by ethnic variants. Ethnic-specific types of mutations that may differ by frequency

pattern can be used for preparing prevention-stage thalassemia screening frameworks that are focused on ethnic-based mutation determinants. This step can minimize the costs the authorities must incur because of the elimination of total gene screening.

The present study was carried out in the multiethnic-community-dwelled East Kalimantan <sup>21</sup> city of Samarinda. This study was deemed necessary to find out the distribution of  $\beta$ -globin gene mutations in Samarinda City, the data of which can later be added to the national database and used by local authorities in conducting thalassemia screening based on local data.

### 2. Methods

Ethical clearance for the study was granted by Ethical Committee, Faculty of Medicine Universitas Jenderal Soedirman with reference number: Ref.0574/KEPK/II/2019. This research involved 31 patients registered with the Association of Thalassemia Patients' Parents (POPTI) of Samarinda City, consisting of males and females 4–56 years of age. All respondent candidates were given informed consent forms and detailed explained, thus participation was voluntary without any

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<sup>2</sup> <https://doi.org/10.1016/j.ymgmr.2019.100550>

Received 16 October 2019; Received in revised form 4 December 2019; Accepted 13 December 2019

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coercion. Patients' blood samples were extracted by professionals at RSU Abdul Moelis Samarinda and taken to the research laboratory of the Faculty of Medicine of Jenderal Soedirman University, Purwokerto, Indonesia. DNAs were isolated from the blood samples at 3 mL using PureLink® Genomic DNA Kits, Invitrogen (Life Technologies, Carlsbad, CA 92008 USA). The DNAs extracted were amplified with a PCR (Polymerase Chain Reaction) kit (Applied Biosystem Veriti 96 well thermal cycler). The  $\beta$ -globin gene mutation analysis was performed by a DNA sequencing technique using the forward and reverse primers flanking the  $\beta$ -globin gene as employed in earlier research [4]. The sequencing technique was applied with BigDye® Terminator v3.1 cycle sequencing kit ABI PRISM® 3730XL Genetic Analyzer by Applied Biosystem, Foster City, CA, USA at the First BASE Laboratory Singapore.

### 3. Results

From the sequencing of the 31 samples, the following genotypes were obtained: Cd26 (GAG > AAG)/IVS-1-2 (T > C) as the most abundant (25,8%), Cd26 (GAG > AAG)/Cd26 (GAG > AAG) in the second (22,6%), and Cd60 (GTG > GAG)/Cd60 (GTG > GAG) as the least abundant (3,2%). Sixty-two mutant alleles were identified, including Cd26 (GAG > AAG) as the most common (48,4%), followed by IVS-1-5 (G > C) (14,5%), and Cd30 (AGG > ACG) and Cd60 (GTG > GAG) as the least frequently encountered (3,2% each). The results are outlined in Tables 1 and 2.

### 4. Discussion

Samarinda, the capital city of East Kalimantan Province, houses 10 districts: Samarinda Utara, Sungai Pinang, Samarinda Ulu, Samarinda Kota, Samarinda Ilir, Sambutan, Sungai Kunjang, Samarinda Seberang, Loa Janan Ilir, and Palaran. The thalassemia patients enrolled in this study were spread in all districts in Samarinda except for Sambutan District. There were patients from outside Samarinda but registered with YTI/POP/IT Samarinda. The mapping of the thalassemia patients' locations in Samarinda was performed to figure out the thalassemia patients' distribution. The Samarinda thalassemia patients' distribution map can be seen in Fig. 1.

There were 31 patients with  $\beta$ -thalassemia enrolled as this research's subjects in total, 16 (51.6%) males and 15 (48.4%) females. The subjects' ages ranged from 4 years through 56 years, and the age average was 14 years. This wide  $\beta$ -thalassemia patient age range (4–56 years of age) is indicative of improvement in thalassemia patient handling compared to preceding years. Curative programs and clinical management of thalassemia patients in Indonesia developed in a right direction after the start of the era of particular health insurance for assemia (Jampertal) in 2011; then followed by BPJS which adopted the national health insurance in 2014. The Ministry of Health has also issued a special regulation regarding the clinical management of thalassemia patients which contains guidelines for doctors or thalassemia centers in providing transfusion services, handling complications, and

**Table 1**  
Genotype distribution of  $\beta$ -thalassemia patients in Samarinda, East Kalimantan.

Mutation	n	(%)
Cd26 (HbE)/IVS-1-2 (T > C)	8	25.8
Cd26 (HbE)/Cd26 (HbE)	7	22.6
Cd26 (HbE)/Cd35 (-C)	5	16.1
Cd26 (HbE)/IVS-1-1 (G > T)	3	9.7
IVS-1-5 (G > C)/IVS-1-5 (G > C)	3	9.7
(HbE)/IVS-1-5 (G > C)	2	6.5
IVS-1-5 (G > C)/IVS-1-1 (G > T)	1	3.2
Cd30(AGG > ACG)/Cd30(AGG > ACG)	1	3.2
IVS-1-5(G > C)/Cd60(GTG > GAG)	1	3.2
	31	100

**Table 2**  
Prevalence of mutant alleles of the  $\beta$ -globin gene in patients with  $\beta$ -thalassemia in Samarinda, East Kalimantan.

Allel Mutan		n	%
Allel	Nomenklatur HGVS		
Cd26 (GAG > AAG)	HBB: c79 G > A	30	48.4
IVS-1-5 (G > C)	HBB: c92 + 5 G > C	10	16.1
IVS-1-2 (T > C)	HBB: c92 + 2 T > C	8	12.9
Cd35 (-C)	HBB: c110delC	5	8.1
IVS-1-1 (G > T)	HBB: c92 + 1 G > T	4	6.5
Cd30 (AGG > ACG)	HBB: c92 G > C	2	3.2
Cd60 (GTG > GAG)	HBB: c182T > A	1	1.6



Fig. 1. Distribution of thalassemia patients in Samarinda City, East Kalimantan.

improving the quality of life of patients [5].

In terms of ethnicity, Javanese was the dominant ethnicity in this research (64.5%), followed by Banjarese (12.9%), Buginese (9.7%), Kutai Malay (6.5%), and Toraja and Sundanese (3.2% each). The determination of the patients' ethnicities was conducted by asking questions regarding the ethnicities of previous three generations. Javanese ethnic occupies the largest number due to transmigration in the past, Kalimantan's native ethnicities such as Banjar, Bugis, Kutai, and Toraja ranks second. Ethnic distribution that spreads between regions has consequences in prevention programs, because the determination of mutation panels must be adjusted [1].

Beta-globin gene mutation screening was performed on the 31 blood samples from the research subjects. From the blood samples DNAs were extracted, which were later subjected to PCR for  $\beta$ -globin-gene-specific DNA amplification. Afterwards, gene mutation analysis



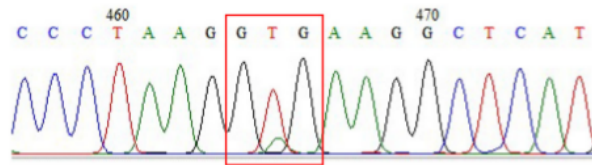


Fig. 2. Cd60 (GTG > GAG) mutation in heterozygote state.

was conducted by the direct sequencing technique. The results of the subjects'  $\beta$ -globin gene sequencing were compared with normal  $\beta$ -globin gene sequences retrieved from the database at [www.ncbi.nlm.nih.gov/genbank](http://www.ncbi.nlm.nih.gov/genbank) (reference sequence accession: NC\_000011.10).

Some mutations in Indonesia have been reported by earlier studies. A study by Lie-Injo reported that, in the case of Javanese ethnicity, the mutations most frequently encountered was IVS-1-5 (G > C) at 54%, followed by Cd26/HbE (GAG > AAG) 18%, IVS-II-654 (C > T) at 9.7%, and Cd41/42 (-TTCT) and Cd35 (-C) each at 1.4% [6]. A separate work reported IVS-1-5 (G > C) at 21% and, the most common, Cd26/HbE (GAG > AAG) at 37% [7]. Another research study reported HbE/IVS1-nt5 as the most prevalent mutation at 55.3%, followed by HbE/Cd35 and IVS1-nt5/IVS1-nt5 each at 13.2%, HbE/IVS1-nt1 at 7.9%, and, the least, IVS1-nt5/IVS1-nt1, IVS1-nt1/IVS1-nt1, and HbE/Cd41/42 each at 2.6% [8]. Yet a different work reported IVS1-5 (G > C) as the most pervasive at 43.5%, trailed behind by Cd26 (GAG > AAG) at 28%, and, the least, IVS1-1 (G > A) at 5% and Cd15 (TGG > TAG) at 3.8% [4]. A study in Riau found 4 mutant alleles in the HBB gene, namely IVS1-nt5 (G > C), Cd26/HbE (GAG > AAG), IVS1-nt1 (G > T), and IVS1-nt2 (T > C), with IVS1-nt5/Cd26 being the most ubiquitous (41.1%).

This study identified a number of mutations reported by foregoing studies, namely Cd26/HbE (GAG > AAG) with the highest prevalence at 48.4%, followed by IVS-1-5 (G > C) at 14.5%, IVS-1-2 (T > C) each at 12.9%, Cd35 (-C) at 8.1%, and IVS-1-1 (G > T) at 6.5%, while Cd30 (AGG > ACG) and Cd60 (GTG > GAG) were the last at 3.2% (Fig. 2).

Aside from the abovementioned, this research also found new mutant allele unreported previously in Indonesia, namely Cd60 (GTG > GAG). This mutation causes changes in the protein Valin to Glutamine and is known by the name of Hb Cagliari which is of Italian ethnic origin. This Hb variant is synthesized at a normal rate but is rapidly broken down (highly unstable) resulting in markedly ineffective erythropoiesis [9]. Combination of Hb Cagliari with other types of mutations in beta gene can result in moderate to severe clinical appearances. A very rare mutant allele in Indonesia, Cd30 (AGG > ACG) or Hb Monroe, was also discovered in this research. It plays a role in pathogenesis of thalassemia by reducing the splicing genes. This allele was previously reported in Jakarta, Tunisia, and India [6,10,11]. The

discovery of new mutants in the thalassemia patients in East Kalimantan presumably results from spontaneous mutations given that there was no descendants or regions of origin from the first mutation finding. These independent mutant allele also may explain the distribution of some mutation types in different geographical areas.

## 5. Conclusion

It can be concluded that the most commonly encountered mutations in the subjects were Cd26/HbE (GAG > AAG), Cd2 (CAT > CAC), IVS-1-5 (G > C), IVS-1-2 (T > C), Cd35 (-C), and IVS-1-1 (G > T). New mutants unreported in previous studies were also found in this study, namely Cd 60 (GTG > GAG).

## Acknowledgments

Authors express the gratitude to Sinta and Ely Yulieta for helping laboratory preparation, and Ministry of Research Technology and Higher Education for funding support.

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