
**UNIVERSITI PUTRA MALAYSIA**

***HAEMOGLOBIN ADANA IN ALPHA THALASSAEMIA INTERMEDIA IN THE MALAYSIAN POPULATION***

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# HAEMOGLOBIN ADANA IN ALPHA THALASSAEMIA INTERMEDIA IN THE MALAYSIAN POPULATION

**By**

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy**

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**HAEMOGLOBIN ADANA IN ALPHA THALASSAEMIA INTERMEDIA IN THE MALAYSIAN POPULATION**

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**June 2017**

**Chairman : Lai Mei I, PhD**
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Alpha thalassaemia is an autosomal blood disorder due to a quantitative reduction or total absence of alpha globin chain synthesis caused by alpha globin gene mutations. Alpha thalassaemia intermedia or more commonly known as HbH disease is a form of thalassaemia with intermediate severity. A total of 3 out of 4 alpha globin genes are non-functional. There are two main types of HbH disease, the deletional and non-deletional HbH disease. There are limited studies in Malaysia on alpha thalassaemia intermedia particularly the severe form of non-deletional alpha thalassaemia like Hb Adana. Hence, it is imperative to identify alpha thalassaemia intermedia with its spectrum, origin of mutations and genotype-phenotype correlation. We hypothesized that Hb Adana is an important severe alpha thalassaemia and its molecular basis needs to be further elucidated for better understanding. Genotype-phenotype correlation was done on the 320 alpha thalassaemia intermedia cases collected. Three main groups from the pool of alpha thalassaemia cases were categorized into deletional HbH, non-deletional HbH, non-deletional alpha thalassaemia. The most common alpha thalassaemia genotypes found in this study is the $-\alpha^{3.7}/--^{SEA}$ which forms more than 50% (55.3%) of the cases. The non deletional HbH cases have a lower Hb, RBC, MCHC and $HbA_2$ mean value than deletional HbH. However, the RDW, MCV and MCH of the non-deletional HbH cases are higher than the deletional HbH. A rapid detection method to screen α-thalassaemia variants was also evaluated by using the new technology of digital droplet PCR (ddPCR). The design of this assay encompasses the detection of triplications, common and rare deletional alpha thalassaemia by ultilising this new technology. This quantitative method was found to be able to measure and determine the copy number changes therefore could simultaneously detect deletions, duplications and triplications of the alpha globin gene cluster. Hence, ddPCR is an alternative method for rapid detection of alpha thalassaemia variants in Malaysia. The ddPCR was also able to detect Hb Adana (Cd59) which is a SNP mutation. Both the wild type and heterozygous Hb Adana mutation was successfully characterised using the ddPCR method. All Hb Adana cases were then subjected to direct sequencing and PCR RFLP to determine the position of Cd59 mutation on the alpha globin gene. All the 36 cases of Hb Adana have the Cd59 position on α2 globin gene. Majority of the Hb Adana samples found were of the Malay ethnicity

in the alpha thalassaemia intermedia pool. Further investigations on the haplotype patterns revealed that most of the Hb Adana cases belongs to the Haplotype I pattern (53.8%) in Malaysia. This suggests that the Hb Adana pool in Malaysia has a distinctive pattern and there is a high possibility that they might well be from the same genetic pool and quite similar to the one from Indonesia. In conclusion, Hb Adana with mutation at α2 globin gene and its distinctive haplotype pattern is found predominantly in the Malay population in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**HEMOGLOBIN ADANA DALAM ALPHA TALASEMIA INTERMEDIA DALAM POPULASI MALAYSIA**

Oleh

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Alpha talasemia adalah penyakit darah autosomal yang disebabkan oleh pengurangan kuantitatif atau tidak adanya sintesis rantai alpha globin disebabkan oleh mutasi gen alpha globin. Alpha talasemia intermedia atau lebih dikenali sebagai penyakit HbH merupakan sejenis talassemia yang mempunyai keparahan yang sederhana. Sebanyak 3 daripada 4 alpha globin gen untuk penyakit ini tidak berfungsi. Terdapat dua jenis penyakit HbH iaitu penyakit HbH delesi dan bukan delesi. Kajian tentang alpha talasemia di Malaysia adalah terhad terutamanya yang merupakan alpha talasemia bukan delesi yang berbentuk parah. Oleh itu, adalah penting untuk mengenalpasti alpha talasemia intermedia dengan spektrum, sumber mutasi dan korelasi genotip fenotip. Kami membuat hipotesis bahawa Hb Adana merupakan sejenis alpha talasemia parah dan asas molekularnya perlu dikaji secara mendalam untuk pemahaman selanjutnya. Korelasi genotip dan fenotip telah dilakukan ke atas 320 kes alpha talasemia intermedia yang terkumpul. Tiga golongan utama daripada kumpulan kes alpha talasemia telah terbahagi kepada HbH delesi, HbH bukan delesi dan alpha talasemia bukan delesi. Genotip alpha tal assemia yang paling biasa dijumpai adalah jenis $-\alpha^{3.7}/--_{SEA}$ yang merangkumi lebih daripada 50% (55.3%) kes. Kes-kes HbH bukan delesi mempunyai nilai min Hb, RBC,MCHC dan $HbA_2$ yang lebih rendah berbanding daripada HbH delesi. Namun begitu, nilai RDW, MCV dan MCH untuk kes HbH bukan delesi adalah lebih tinggi daripada HbH delesi. Satu kaedah cepat pengesanan variasi alpha talasemia telah dikaji dengan menggunakan teknologi terkini "digital droplet PCR (ddPCR)". Reka-bentuk kaedah ini merangkumi pengesanan alpha talasemia delesi yang berbentuk triplikasi, biasa dan yang jarang berlaku dengan penggunaan teknologi baharu ini. Kaedah kuantitatif ini juga didapati dapat mengukur dan menentukan perubahan "copy number" yang sekaligus dapat mengesan delesi, dulplikasi dan triplikasi untuk kluster gen alpha globin. Oleh itu, ddPCR merupakan kaedah alternatif untuk mengesan dengan pantas variasi alpha talasemia di Malaysia. ddPCR juga mampu mengesan Hb Adana (Cd59) yang merupakan sejenis mutasi titik. Kedua-dua jenis liar dan mutasi heterozigot telah berjaya dicirikan melalui kaedah ddPCR ini. Kesemua kes Hb Adana kemudian telah dilakukan teknik jujukan dan PCR RFLP untuk menentukan posisi mutasi Cd59 di gen alpha globin. Kesemua 36 kes Hb Adana mempunyai posisi

mutasi Cd59 di gen globin α2. Terdapat 32 daripada 36 kes Hb Adana merupakan kaum Melayu yang mencadangkan bahawa kumpulan gen kes Hb Adana di Malaysia hampir sama dengan kes yang terdapat di Indonesia. Kajian susulan ke atas corak haplotip mendedahkan kebanyakan kes Hb Adana mempunyai corak Haplotip I (53.8%) di Malaysia. Ini mencadangkan bahawa kumpulan Hb Adana yang terdapat di Malaysia mempunyai corak yang tersendiri and besar kemungkinannya kumpulan ini tergolong dalam kumpulan genetik yang sama. Konklusinya, Hb Adana berinteraksi dengan mutasi-mutasi alpha talasemia yang lain untuk membentuk alpha thalassemia intermedia di Malaysia.

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I certify that a Thesis Examination Committee has met on 15 June 2017 to conduct the final examination of Lee Tze Yan on his thesis entitled "Haemoglobin Adana in Alpha Thalassaemia Intermedia in the Malaysian Population" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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**TABLE OF CONTENTS**

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVATIONS

| | |
|---|---|
| ARMS | Amplification refractory mutations system |
| AS-PCR | Allele-specific PCR |
| ATMDS | Alpha thalassaemia and myelodysplastic syndrome |
| ATRX | Alpha thalassaemia mental retardation-X linked syndrome |
| Cd | Codon |
| CN | Copy number |
| CNV | Copy number variation |
| ddPCR | Digital droplet Polymerase Chain Reaction |
| DHPLC | Denaturing high-performance liquid chromatography |
| DNA | Deoxyribonucleic acid |
| DQ | Dosage Quotient |
| FAM | 6-carboxyfluorescein |
| HbH | Haemoglobin H |
| HRM | High-resolution melting |
| IVS | Intervening sequence |
| LCR | Locus control region |
| MCH | Mean corpuscular haemoglobin |
| MCHC | Mean corpuscular haemoglobin concentration |
| MCV | Mean corpuscular volume |
| MLPA | Multiplex Ligation-dependent Probe Amplification |
| MSC-R2 | Multispecies conserved sequences region 2 |
| NGS | Next-Generation sequencing |
| OH | Hydroxyl group |
| PCR-RDB | Polymerase chain reaction reverse dot blot |
| RE | Restriction enzyme |
| RBC | Red blood cell |
| RDW | Red cell distribution width |
| RFLP | Restriction length polymorphism |
| SNP | Single nucleotide polymorphism |
| VIC | 2'-chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein |

# CHAPTER 1

# INTRODUCTION

## 1.1 Structure of Haemoglobin

Haemoglobin is a tetrametric protein formed by 2 pairs of globin chains with an iron-containing haem molecule in each globin chain to transport oxygen in the human body (Lewis *et al*., 2012; Bain *et al*., 2010). HbA, a major haemoglobin in normal adults constitutes about 97% of the total haemoglobin and another component, $HbA_2$ accounts for a mere 2-3% of the total. Foetal haemoglobin (HbF) is the major haemoglobin in foetus and in most of the normal adults, however, a minute amount of HbF can still be present (Weatherall & Clegg, 2001).

The globin genes which encode the globin chains can be found in two clusters (Figure 1.1). They are located in chromosome 16 (alpha globin gene cluster) and chromosome 11 (beta globin gene cluster). Alpha globin gene cluster is made up of a few pseudogenes, a zeta (ζ) gene and two alpha (α) genes. On the other hand, beta globin gene cluster consists of an epsilon (ε) gene, two gamma (γ) genes, a delta (δ) gene and a beta (β) gene (Bain *et al*., 2010). Both alpha and beta globin gene cluster are arranged according to their developmental expression (Thein, 2004).

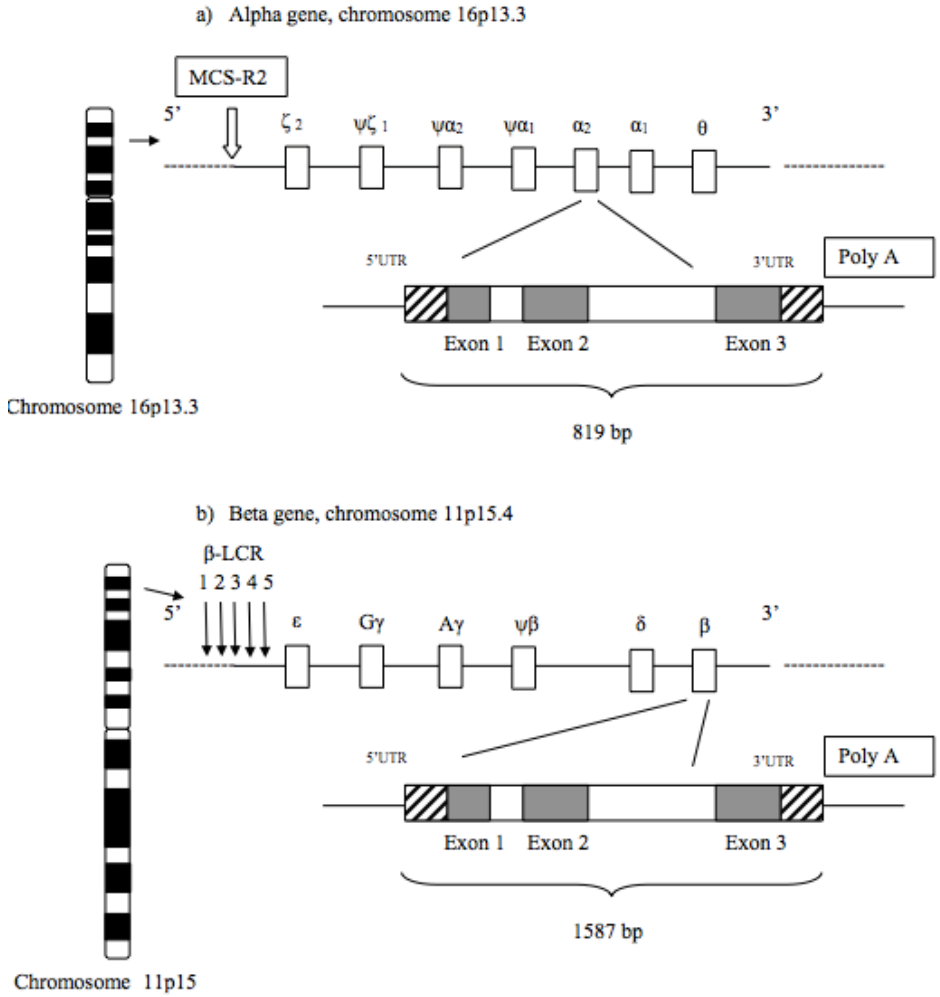


**Figure 1.1**: **Maps of the α-like and β-like globin gene clusters.** (a) Alpha gene locus on chromosome 16p13.3. Down-point vertical arrow represents the important gene expression locus, MSC-R2 (upstream hypersensitive site). (b) Beta locus on chromosome 11p15.4. Five hypersensitive sites are indicated by 5 vertical down point arrows, representing the gene regulatory sequences in the β-globin cluster, also known as locus control region (LCR) (Modified from Clark and Thein, 2004).

## 1.2 Haemoglobinopathies

Normal adult human haemoglobin is a paired complex of two α-globin and two β-globin chains (Bain *et al*., 2010). The other two minor haemoglobins, Hb $A_2$ and Hb F are made up of 2 α and 2 δ chains and 2 α and 2 γ chains respectively. In embryo, pre-alpha chains (ζ globin chains) contribute to embryonic haemoglobin and for fetal development, β-like globin chains (ε and γ) are involved (Nemati *et al*., 2010). There are basically two types of haemoglobin abnormalities (Figure 1.2). One is due to the synthesis of an abnormal haemoglobin (variant haemoglobin) while the other is caused

by the reduction or absence of normal alpha (α) and/or beta (β) chains production (thalassaemia syndromes) (Hoffbrand *et al*., 2006). Variant haemoglobin or structural haemoglobinopathies is the term used to describe structurally abnormal haemoglobins which includes haemoglobin E, haemoglobin C and haemoglobin S (Bain *et al*., 2006; Hoffbrand *et al*., 2006). In Malaysia, thalassaemia is a public health problem among the ethnic Malay and Chinese populations whereas ethnic Indians form a minority percentage (Weatherall & Clegg, 2001).

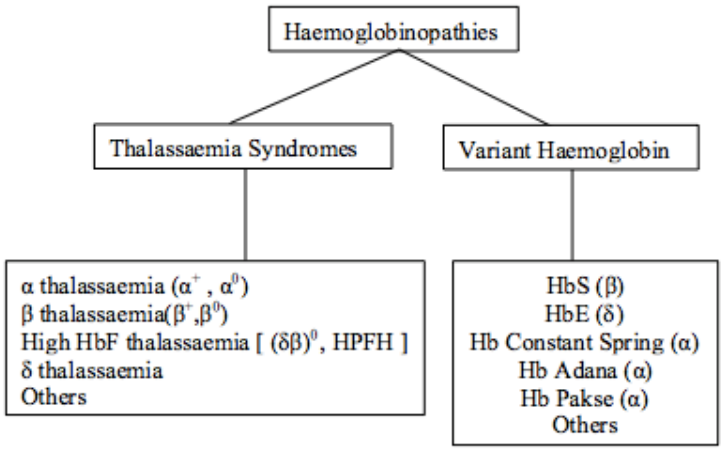


**Figure 1.2: The 2 different types of haemoglobinopathies.** Thalassaemia syndromes involve the reduction or absence of alpha or beta globin chains production while variant haemoglobin is the synthesis of an abnormal haemoglobin.

## 1.3 Alpha thalassaemia

Thalassaemia is the commonest monogenic disorder in human beings worldwide (Weatherall & Clegg, 2001). There is a double copy of the α-globin gene per haploid genome which gives rise to the annotation αα/αα. $\alpha^0$-thalassaemia with deletion of both α-globin genes will result in the absence of α-globin chain production whereas a reduction of α-globin chain production is caused by deletion of a single gene (-α/αα) resulting in $\alpha^+$ thalassaemia. Minor or trait (carriers) for $\alpha^0$-thalassaemia (--/αα), homozygous alpha thalassaemia (-α/-α) or $\alpha^+$-thalassaemia (-α/αα) are normally asymptomatic or have mild anaemia. Compound heterozygous $\alpha^0$ and $\alpha^+$-thalassaemia (--/-α) will cause HbH disease or α-thalassaemia intermedia. The major form, homozygous $\alpha^0$- thalassaemia (--/--), results in intrauterine death and hydrops foetalis in the absence of intrauterine blood transfusions (Weatherall, 1997; Weatherall, 1999).

## 1.4 Haemoglobin Adana (Hb Adana)

Hb Adana [HBA2: c179G>A (or HBA1); p.Gly60Asp] is a common non-deletional α-thalassaemia variant found among the Malays (Ahmad et al., 2013). This highly unstable variant is located at Cd 59 position either on α1 globin gene or α2 globin gene. Haemoglobin subtyping is unable to detect this highly unstable α-haemoglobin variant

since Hb Adana carriers have normal red blood cell indices and are therefore generally asymptomatic. (Azma et. al., 2014). Cases of Hb Adana were reported in the Malaysian Malays, Chinese and indigenous populations (Ahmad et al., 2013). Furthermore, the first reported case of Hb Adana in Malaysia was in a 52-year-old patient with α-thalassaemia intermedia (George et al, 2009). Confirmation of Hb Adana is a diagnostic predicament as this highly unstable haemoglobin variant has no product when observed during routine haematology tests. Hence, patients may remain undiagnosed until DNA studies are carried out. In Malaysia, the reliance on routine haematology studies and Hb subtype analysis to detect Hb Adana may not be sufficiently accurate in populations with different globin gene mutations (Tan J.A.M.A, et al., 2016). The interaction between Haemoglobin (Hb) Adana (HBA2:c.179>A) with deletional and nondeletional α-thalassaemia mutations will produce HbH disorders with varying clinical manifestations from asymptomatic to severe anaemia with significant hepatosplenomegaly.

### 1.5 Main challenges in Hb Adana

Similar to beta thalassaemia, screening, diagnosis and the understanding of the genotype-phenotype interactions of alpha thalassaemia often come with many challenges. For alpha thalassaemia intermedia, there are limited studies in Malaysia which focus on this category as most of the research are concentrated on the management and prevention of beta thalassaemia major. Besides that, the molecular basis of severe forms of alpha thalassaemia is not well-studied and a good example will be Hb Adana. Therefore, it is imperative to identify alpha thalassaemia intermedia at an early age with its spectrum of mutations, origin of mutations and genotype-phenotype correlation. It would be better if we could apply and evaluate rapid and newer techniques to detect some of the alpha thalassaemia mutations so that screening of alpha thalassaemia can be done faster and will then lead to accurate definitive diagnosis. Our hypothesis of this study is that Hb Adana is an important severe alpha thalassaemia when interacting with the other alpha mutations and its molecular basis needs to be further elucidated for better understanding.

### 1.6 Objectives

The general objective of this study is:

1. To determine the genotypes and its ethnic distribution of Hb Adana of alpha thalassaemia intermedia in Malaysia

The specific objectives of this study are:

1) To correlate the genotypes with phenotypes of alpha thalassaemia intermedia
2) To apply a rapid and newer technique to identify deletional alpha thalassaemia mutations and to screen for Hb Adana
3) To identify the position of Hb Adana mutation on the alpha globin gene in Malaysia
4) To determine the haplotypes associated with Hb Adana

## REFERENCES


Ahmad, R., Saleem, M., Aloysious, N.S., Yelumalai, P., Mohamed, N. and Hassan, S., 2013. Distribution of alpha thalassaemia gene variants in diverse ethnic populations in Malaysia: data from the institute for medical research. *International Journal of Molecular Sciences* 14, 18599-18614.

Aksu, T., Yarali, N., Bayram, C., Fettah, A., Avcı, Z. and Tunç, B., 2014. Homozygosity for HBA1: c. 179G> A: Hb Adana in an Infant. *Hemoglobin* 38, 449-450.

Alauddin, H., Jaapar, N.A., Azma, R.Z., Ithnin, A., Razak, N.F.A., Loh, C.K., Alias, H.,Abdul-Latiff, Z. and Othman, A., 2014. A case series of α-thalassemia intermedia due to compound heterozygosity for Hb Adana [HBA2: c179G> A (or HBA1); p. Gly60Asp] with other α-thalassemias in Malay families. *Hemoglobin* 38, 277-281.

Albano, F., Zagaria, A., Anelli, L., Coccaro, N., Tota, G., Brunetti, C., Minervini, C., Impera, L., Minervini, A., Cellamare, A., Orsini, P., 2015. Absolute quantification of the pretreatment PML-RARA transcript defines the relapse risk in acute promyelocytic leukemia. *Oncotarget* 6,13269.

Arena, S., Bellosillo, B., Siravegna, G., Martínez, A., Cañadas, I., Lazzari, L., Ferruz, N., Russo, M., Misale, S., González, I., Iglesias, M., 2015. Emergence of multiple EGFR extracellular mutations during cetuximab treatment in colorectal cancer. *Clinical Cancer Research* 21, 2157-2166.

Azma, R. Z., Ainoon, O., Hafiza, A., and Azlin, I., 2014. Molecular characteristic of alpha thalassaemia among patients diagnosed in UKM Medical Centre. *Malaysian Journal of Pathology* 36, 27–32.

Bain, B.J., Lewis, S.M. and Bates, I., 2006. Basic haematological techniques. Dacie and Lewis Practical Haematology 4, 19-46.

Bain, B.J., Barbara J.W., Adrian D. S., Lorraine A. P., 2010. Variant Haemoglobins : A guide to identification. Wiley-Blackwell.

Bhardwaj, U., Zhang, Y,H., Lorey, F.,MacCabe.L.L., MacCabe,E.R.B, 2005. Molecular Genetic Confirmatory Testing from Newborn Screeening Samples for the Common African-American, Asian Indian, Southeast Asian and Chinese β-Thalassaemia mutations. *American Journal of Haematology*. 78:249-255.

Bozdogan, S.T., Yuregir, O.O., Buyukkurt, N., Aslan, H., Ozdemir, Z.C. and Gambin, T., 2015. Alpha-thalassemia mutations in Adana Province, southern Turkey: genotype-phenotype correlation. *Indian Journal of Hematology and Blood Transfusion* 31, 223-228.

Cao, A.and Galanello, R. 2003. Beta-Thalassaemia. *Gene Reviews*, 1-20.

Carreira, S., Romanel, A., Goodall, J., Grist, E., Ferraldeschi, R., Miranda, S., Prandi, D., Lorente, D., Frenel, J.S., Pezaro, C., Omlin, A., 2014. Tumor clone dynamics in lethal prostate cancer. *Science Translational Medicine* 6, 254.

Chan, V., W-Y Chan, V., Tang, M., Lau, K., Todd, D. and Chan, T.K., 1997. Molecular defects in Hb H hydrops fetalis. *British Journal of Haematology* 96, 224-228.

Chanarin, I., 1989. Laboratory haematology: an account of laboratory techniques. Churchill Livingstone.

Chong, S.S., Boehm, C.D., Higgs, D.R. and Cutting, G.R., 2000. Single-tube multiplex-PCR screen for common deletional determinants of α-thalassemia. *Blood* 95, 360-362.

Chui, D.H. and Waye, J.S., 1998. Hydrops fetalis caused by α-thalassemia: an emerging health care problem. *Blood* 91, 2213-2222.

Chui, D.H., Fucharoen, S. and Chan, V., 2003. Hemoglobin H disease: not necessarily a benign disorder. *Blood* 101, 791-800.

Clark, B.E. and Thein, S.L., 2004. Molecular diagnosis of haemoglobin disorders. *Clinical & Laboratory Haematology* 26, 159-176.

Çürük, M.A., Dimovski, A.J., Baysal, E., Gu, L.H., Kutlar, F., Molchanova, T.P., Webber, B.B., Altay, C., Gürgey, A. and Huisman, T.H.J., 1993. Hb adana or α259 (E8) Gly→ Aspβ2, A severely unstable α1-globin variant, observed in combination with the - (α) 20.5 KB α - thal - 1 deletion in two Turkish patients. *American Journal of Hematology* 44, 270-275.

Criscione, F., Qi, Y., Saunders, R., Hall, B., Tu, Z., 2013. A unique Y gene in the Asian malaria mosquito Anopheles stephensi encodes a small lysine-rich protein and is transcribed at the onset of embryonic development. *Insect Molecular Biology*. 22,433-441.

de Silva, S., Fisher, C.A., Premawardhena, A., Lamabadusuriya, S.P., Peto, T.E.A., Perera, G., Old, J.M., Clegg, J.B., Olivieri, N.F. and Weatherall, D.J., 2000. Thalassaemia in Sri Lanka: implications for the future health burden of Asian populations. *The Lancet* 355, 786-791.

Devonshire, A.S., Honeyborne, I., Gutteridge, A., Whale, A.S., Nixon, G., Wilson, P,, Jones, G., McHugh, T,D., Foy, C.A., Huggett, J.F., 2015. Highly reproducible absolute quantification of Mycobacterium tuberculosis complex by digital PCR. *Analytical Chemistry* 87, 3706-13.

DNA Direct. Guidance & Decision Support for Genomic Medicine. Retrieved 3 June 2016 from http://services.dnadirect.com/grc/patient-site/alpha-thalassemia-carrier-screening/genetics-of-alpha-thalassemia.html

Douna, V., Papassotiriou, I., Garoufi, A., Georgouli, E., Ladis, V., Stamoulakatou, A., Metaxotou-Mavrommati, A., Kanavakis, E. and Traeger-Synodinos, J., 2008. A rare thalassemic syndrome caused by interaction of Hb Adana [α59 (E8) Gly→ Asp] with an $\alpha^{+}$-thalassemia deletion: clinical aspects in two cases. *Hemoglobin* 32, 361-369.

Durmaz, A.A., Akin, H., Ekmekci, A.Y., Onay, H., Durmaz, B., Cogulu, O., Aydinok, Y. and Ozkinay, F., 2009. A severe α thalassemia case compound heterozygous for Hb Adana in α1 gene and 20.5 kb double gene deletion. *Journal of Pediatric Hematology/Oncology* 31, 592-594.

Ekawa, K., Nishi, M., Okamura, T., Nomura, Y., Sanke, T. and Nanjo, K., 1996. β-Thalassemia (β90 GAG→ TAG): Genetic Diagnosis by Allele-Specific Polymerase Chain Reaction and Estimation of Mutant mRNA Expression by Reverse Transcription Polymerase Chain Reaction. *Internal Medicine* 35, 463-467.

Embury, S.H., Miller, J.A., Dozy, A.M., Kan, Y.M., Chan, V., Todd, D. 1980. Two different molecular organizations account for the single alpha-globin gene of the alpha thalassaemia-2 genotype. *Journal of Clinical Investigation* 66,1319-1325.

Eng, B., Patterson, M., Walker, L., Chui, D.H.K. and Waye, J.S., 2001. Detection of severe nondeletional α-thalassemia mutations using a single-tube multiplex ARMS assay. *Genetic Testing* 5, 327-329.

Farashi, S., Bayat, N., Faramarzi Garous, N., Ashki, M., Montajabi Niat, M., Vakili, S., Imanian, H., Zeinali, S., Najmabadi, H. and Azarkeivan, A., 2015. Interaction of an α-globin gene triplication with β-globin gene mutations in Iranian patients with β-thalassemia intermedia. *Hemoglobin* 39, 201-206.

Fisher, C.A., Premawardhena, A., De Silva, S., Perera, G., Rajapaksa, S., Olivieri, N.A., Old, J.M. and Weatherall, D.J., 2003. The molecular basis for the thalassaemias in Sri Lanka. *British Journal of Haematology 121*,662-671.

Forrester, W.C., Takegawa, S., Papayannopoulou, T., Stamatoyannopoulos, G. and Groudine, M., 1987. Evidence for a locus activation region: the formation of developmentally stable hypersensitive sites in globin-expressing hybrids. *Nucleic Acids Research* 15, 10159-10177.

Fucharoen, S., Winichagoon, P., Pootrakul, P., Piankijagum, A. and Wasi, P., 1986. Differences between two types of Hb H disease, alpha-thalassemia 1/alpha-thalassemia 2 and alpha-thalassemia 1/Hb constant spring. *Birth Defects Original Article Series* 23, 309-315.

Fucharoen, S. and Viprakasit, V., 2009. Hb H disease: clinical course and disease modifiers. ASH Education Program Book 2009, 26-34.

Gao, L., Liu, Y., Sun, M., Zhao, Y., Xie, R., He, Y., Xu, W., Liu, J., Lin, Y. and Lou, J., 2015. Rapid detection of α-thalassaemia alleles of--SEA/,-α 3.7/and-α 4.2/using a dual labelling, self-quenching hybridization probe/melting curve analysis. *Molecular and Cellular Probes* 29, 438-441.

Galanello, R., Aru, B., Dessi, C., Addis, M., Paglietti, E., Melis, M.A., Cocco, S., Massa, P., Giagu, N., Barella, S. and Turco, M.P., 1992. HbH disease in Sardinia: molecular, hematological and clinical aspects. *Acta Haematologica* 88, 1-6.

Galanello, R. and Origa, R., 2010. Beta-thalassemia. *Orphanet Journal of Rare Diseases* 5, 11.

Galanello, R. and Cao, A., 2011. Alpha-thalassemia. *Genetics in Medicine* 13, 83-88.

George, E., 2007. Practical algorithm to screen for thalassaemia: BHES+ F protocol. *Malaysian Journal of Medicine and Health Sciences* 3, 1.

George, E., Tan, J. A. M. A., Azian, A. S., Ahmad, R. and Zubaidah, Z., 2009. A rare case of alpha-thalassaemia intermedia in a Malay patient double heterozygous for $\alpha^{+}$-thalassaemia and a mutation in α1 globin gene CD59 (GGC→ GAC). *Medical Journal of Malaysia* 64, 321–322.

George, E., 2013. HbE β-Thalassaemia in Malaysia: Revisited. *Journal of Hematology & Thromboembolic Diseases*.

George, E., 2014. Screening of Thalassaemia Carriers and Its Limitations. *Journal of Hematology & Thromboembolic Disease*. 2:e109. doi:10.4172/2329-8790.1000e109.

Gibbons, R.J., Picketts, D.J., Villard, L., and Higgs, D.R., 1995. Mutations in a putative global transcriptional regulator cause X-linked mental retardation with α-thalassemia (ATR-X syndrome). *Cell* 80, 837-845.

Gibbons, R.J., Pellagatti, A., Garrick, D., Wood, W.G., Malik, N., Ayyub, H., Langford, C., Boultwood, J., Wainscoat, J.S. and Higgs, D.R., 2003. Identification of acquired somatic mutations in the gene encoding chromatin-remodeling factor ATRX in the α-thalassemia myelodysplasia syndrome (ATMDS). *Nature Genetics* 34, 446-449.

Grimholt, R.M., Urdal, P., Klingenberg, O. and Piehler, A.P., 2014. Rapid and reliable detection of α-globin copy number variations by quantitative real-time PCR. *BMC Hematology* 14, 4.

Grosveld, F., van Assendelft, G.B., Greaves, D.R. and Kollias, G., 1987. Position-independent, high-level expression of the human β-globin gene in transgenic mice. *Cell* 51, 975-985.

Harteveld, C.L. and Higgs, D.R., 2010. α-Thalassaemia. *Orphanet Journal of Rare Diseases* 5, 13.

Heid, C.A., Stevens, J., Livak, K.J. and Williams, P.M., 1996. Real time quantitative PCR. *Genome Research* 6, 986-994.

Henderson, S., Pitman, M., McCarthy, J., Molyneux, A. and Old, J., 2008. Molecular prenatal diagnosis of Hb H Hydrops Fetalis caused by haemoglobin Adana and the implications to antenatal screening for α - thalassaemia.*Prenatal Diagnosis* 28, 859-861.

Higgs, D.R., Vicker, M.A., Wilkie, A.O., Pretorius, I. M., Jarman A.P., Weatherall D.J. 1989. A review of the molecular genetics of the human α-globin gene cluster. *Blood* 73, 1081-1104.

Higgs, D.R. and Weatherall, D. eds., 1993. The haemoglobinopathies. Baillière Tindall.

Higgs, D.R. and Bowden, D.K., 2001. Clinical and Laboratory Features of the c v-Thalassemia Syndromes. *Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management* 431.

Higgs, D.R. and Wood, W.G., 2008. Long-range regulation of α globin gene expression during erythropoiesis. *Current Opinion in Hematology* 15, 176-183.

Higgs, D.R., Wood, W.G., Jarman, A.P., Sharpe, J., Lida, J., Pretorius, I.M. and Ayyub, H., 1990. A major positive regulatory region located far upstream of the human alpha-globin gene locus. *Genes & Development* 4, 1588-1601.

Higgs, D.R., Sharpe, J.A. and Wood, W.G., 1998, April. Understanding alpha globin gene expression: a step towards effective gene therapy. *In Seminars in Hematology* 35, 93-104).

Higgs, D.R., 2013. The molecular basis of α-thalassemia. *Cold Spring Harbor Perspectives in Medicine* 3, 011718.

Higgs, D.R. and Weatherall, D.J., 2009. The alpha thalassaemias. *Cellular and Molecular Life Sciences* 66, 1154-1162.

Hoffbrand, A.V. and Herbert, V., 1999. Nutritional anemias. *Seminars in Hematology* 36, 13-23.

Hoffbrand, A.V., Moss, P.A.H. and Pettit, J.E., 2006. Essential Haematology. Blackwell Publishing 5, 249.

Kanavakis, E., Papassotiriou, I., Karagiorga, M., Vrettou, C., Metaxotou-Mavrommati, A., Stamoulakatou, A., Kattamis, C. and Traeger - Synodinos, J., 2000. Phenotypic and molecular diversity of haemoglobin H disease: a Greek experience. *British Journal of Haematology* 111, 915-923.

Kleanthous, M., Kyriacou, K., Kyrri, A., Kalogerou, E., Vassiliades, P.H., Drousiotou, A., Kallikas, I., Ioannou, P. and Angastiniotis, M., 2001. Alpha-thalassaemia prenatal diagnosis by two PCR-based methods. *Prenatal Diagnosis* 21, 413-417.

Lal, A., Goldrich, M.L., Haines, D.A., Azimi, M., Singer, S.T. and Vichinsky, E.P., 2011. Heterogeneity of hemoglobin H disease in childhood. *New England Journal of Medicine* 364, 710-718.

Lewis, S. M., Bain, B. J., and Bates, I., 2012. Dacie and Lewis Practical Haematology, Eleventh edition, Churchill Livingstone.

Lee, T.Y., Lai, M.I., Ismail, P., Ramachandran, V., Tan, J.A.M.A., Teh, L.K., Othman, R., Hussein, N.H. and George, E., 2016. Analysis of α1 and α2 globin genes among patients with hemoglobin Adana in Malaysia. *Genetics and Molecular Research: GMR* 15.

Leung, K.Y., Lee, C.P., Tang, M.H.Y., Lau, E.T., Ng, L.K.L., Lee, Y.P., Chan, H.Y., Ma, E.S.K. and Chan, V., 2004. Cost-effectiveness of prenatal screening for thalassaemia in Hong Kong. *Prenatal Diagnosis* 24, 899-907.

Li, Q., Li, L.Y., Huang, S.W., Li, L., Chen, X.W., Zhou, W.J. and Xu, X.M., 2008. Rapid genotyping of known mutations and polymorphisms in β-globin gene based on the DHPLC profile patterns of homoduplexes and heteroduplexes. *Clinical Biochemistry* 41, 681-687.

Lin, M., Zhu, J.J., Wang, Q., Xie, L.X., Lu, M., Wang, J.L., Wang, C.F., Zhong, T.Y., Zheng, L., Pan, M.C. and Wu, J.R., 2012. Development and evaluation of a reverse dot blot assay for the simultaneous detection of common alpha and beta thalassemia in Chinese. *Blood Cells, Molecules, and Diseases* 48, 86-90.

Lorey, F., Charoenkwan, P., Witkowska, H.E., Lafferty, J., Patterson, M., Eng, B., Waye, J.S., Finklestein, J.Z. and Chui, D.H., 2001. Hb H hydrops foetalis syndrome: a case report and review of literature. *British Journal of Haematology* 115,72-78.

Mazaika, E. and Homsy, J., 2014. Digital droplet PCR: CNV analysis and other applications. *Current Protocols in Human Genetics* 7-24.

Megawati, D., Nainggolan, I.M., Swastika, M., Susanah, S., Mose, J.C., Harahap, A.R. and Setianingsih, I., 2014. Severe α-thalassemia intermedia due to a compound heterozygosity for the highly unstable Hb Adana (HBA2: c. 179G> A) and a novel codon 24 (HBA2: c. 75T> A) mutation. *Hemoglobin* 38,149-151.

Mills, A., 2014. Health care systems in low-and middle-income countries.*New England Journal of Medicine* 370, 552-557.

Miles, K.L., Norwich, J.T., Martinson, J.J. and Clegg, J.B., 2001. Polymerase chain reaction protocols for alpha globin haplotype polymorphisms. *British Journal of Haematology* 113, 694-698.

Modell, B. and Darlison, M., 2008. Global epidemiology of haemoglobin disorders and derived service indicators. *Bulletin of the World Health Organization* 86, 480-487.

Naing, L., Winn, T. and Rusli, B.N., 2006. Practical issues in calculating the sample size for prevalence studies. *Archives of Orofacial Sciences* 1, 9-14.

Nainggolan, I.M., Harahap, A. and Setianingsih, I., 2010. Hydrops fetalis associated with homozygosity for Hb Adana [α59 (E8) Gly→ Asp (α2)]. *Hemoglobin* 34, 394-401.

Nainggolan, I.M., Harahap, A., Ambarwati, D.D., Liliani, R.V., Megawati, D., Swastika, M. and Setianingsih, I., 2013. Interaction of Hb Adana (HBA2: c. 179G> A) with deletional and nondeletional α+-thalassemia mutations: diverse hematological and clinical features. *Hemoglobin* 37, 297-305.

Nemati, H., Rahimi, Z. and Bahrami, G., 2010. The Xmn1 polymorphic site 5′ to the Gγ gene and its correlation to the Gγ: Aγ ratio, age at first blood transfusion and clinical features in β-Thalassemia patients from Western Iran. *Molecular Biology Reports* 37, 159-164.

Old, J., Angastiniotis, M., Eleftheriou, A., Galanello, R. and Harteveld, C.L., 2013. Prevention of Thalassaemias and Other Haemoglobinopathies.Thalassaemia International Federation.

Origa, R., Paglietti, M.E., Sollaino, M.C., Desogus, M.F., Barella, S., Loi, D. and Galanello, R., 2014. Complexity of the alpha-globin genotypes identified with thalassemia screening in Sardinia. *Blood Cells, Molecules, and Diseases* 52, 46-49.

Oron-Karni, V., Filon, D., Shifrin, Y., Fried, E., Pogrebijsky, G., Oppenheim, A. and Rund, D., 2000. Diversity of a-globin mutations and clinical presentation of a-thalassemia in Israel. *American Journal of Hematology* 65, 196-203.

Padilla, C.D. and Therrell, B.L., 2007. Newborn screening in the Asia Pacific region. *Journal of Inherited Metabolic Disease* 30, 490-506.

Petrou, M., Brugiatelli, M., Old, J., Hurley, P., Ward, R.H.T., Wong K.P., Rodeck, C. and Modell, B., 1992. Alpha thalassaemia hydrops fetalis in the UK: the importance of screening pregnant women of Chinese, other South East Asian and Mediterranean extraction for alpha thalassaemia trait. BJOG: *An International Journal of Obstetrics & Gynaecology* 99, 985-989.

Piel, F.B. and Weatherall, D.J., 2014. The α-thalassemias. *New England Journal of Medicine* 371, 1908-1916.

Population Distribution and Basic Demographic Characteristics Report 2010. Department of Statistics Malaysia. Available at https://www.dosm.gov.my/v1/index.php?r=column/ctheme&menu_id=L0pheU43NWJwRWVSZklWdzQ4TlhUUT09&bul_id=MDMxdHZjWTk1SjFzTzNkRXYzcVZjdz09 . Accessed July 21, 2015.

Pornprasert, S., Phusua, A., Suanta, S., Saetung R., Sanguansermsri, T. 2008. Detection of alpha thalassaemia-1 Southeast Asian type using real-time gap-PCR with SYBR Green1 and high resolution melting analysis. *European Journal of Haematology* 80, 510-4

Pornprasert, S. and Prasing, W., 2014. Detection of alpha (0)-thalassemia South-East Asian - type deletion by droplet digital PCR. *European Journal of Haematology* 92, 244-248.

Pressley, L., Higgs, D.R., Clegg, J.B., Perrine, R.P., Pembrey, M.E. and Weatherall, D.J., 1980. A new genetic basis for hemoglobin-H disease. *New England Journal of Medicine* 303, 383-1388.

Quek, D.L., Ng, Y.Y., Wang, W., Tan, A.S., Tang-Lim, G.I., Ng, I.S., Law, H.Y. and Chong, S.S., 2007. Rapid carrier screening for β-thalassemia by single-step allele-specific PCR and detection. *Clinical Biochemistry* 40, 427-430.

Rosnah, B., Rosline, H., Wan Zaidah, A., Noor Haslina, M. N., Marini, R., Shafini,M.Y., Nurul Ain, F.A. 2012. Detection of Common Deletional Alpha-Thalassaemia Spectrum by Molecular Technique in Kelantan, Northeastern Malaysia. *International Scholarly Research Network* 1-3.

Sadiq, K., 2008. Paper Citizens: How illegal immigrants acquire citizenship in developing countries. Oxford University Press.

Schouten, J.P., McElgunn, C.J., Waaijer, R., Zwijnenburg, D., Diepvens, F., Pals, G. 2002. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Research* 30, e57.

Setianingsih, I., Harahap, A. and Nainggolan, I.M., 2003. Alpha thalassaemia in Indonesia: phenotypes and molecular defects. *In Tropical Diseases* 47-56.

Sharpe, J.A., Chan-Thomas, P.S., Lida, J., Ayyub, H., Wood, W.G. and Higgs, D.R., 1992. Analysis of the human alpha globin upstream regulatory element (HS-40) in transgenic mice. *The EMBO Journal* 11, 4565.

Sharpe, J.A., Summerhill, R.J., Vyas, P., Gourdon, G., Higgs, D.R. and Wood, W.G., 1993. Role of upstream DNase I hypersensitive sites in the regulation of human alpha globin gene expression. *Blood* 82, 1666-1671.

Sriiam, S., Leecharoenkiat, A., Lithanatudom, P., Munkongdee, T., Svasti, S.L. and Smith, D.R., 2012. Limit of sensitivity for melting curve screening for α-thalassemia. *Asian Biomedicine* 6, 105-109.

Steensma, D.P., Higgs, D.R., Fisher, C.A. and Gibbons, R.J., 2004. Acquired somatic ATRX mutations in myelodysplastic syndrome associated with α thalassemia (ATMDS) convey a more severe hematologic phenotype than germline ATRX mutations. *Blood* 103, 2019-2026.

Tan A.S., Quah, T.C., Low, P.S., Chong, S.S. 2001. A rapid and reliable 7-deletion multiplex polymerase chain reaction assay for alpha thalassaemia. *Blood* 98, 250-1

Tan, J. A. M. A., Kho, S. L., Ngim, C. F., Chua, K. H., Goh, A. S., Yeoh, S. L., & George, E., 2016. DNA studies are necessary for accurate patient diagnosis in compound heterozygosity for Hb Adana (HBA2: c. 179> A) with deletional or nondeletional α-thalassaemia. *Scientific Reports* 6, 26994.

Tabone, P., Henni, T., Belhani, M., Colonna, P., Verdier, G. and Godet, J., 1981. Hemoglobin H disease from Algeria: genetic and molecular characterization. *Acta Haematologica* 65, 26-31.

Thein, S.L., 1998. β-Thalassaemia. *Bailliere's Clinical Haematology* 11, 91-126.

Thein, S.L., 2004. Genetic Insights into the Clinical Diversity of β-Thalassaemia, Blackwell Publishing Ltd, *British Journal of Haematology* 124, 264-274.

Traeger-Synodinos, J., Kanavakis, E., Vrettou, C., Maragoudaki, E., Michael, T.H., Metaxotou-Mavromati, A. and Kattamis, C., 1996. The triplicated α-globin gene locus in β - thalassaemia heterozygotes: clinical, haematological, biosynthetic and molecular studies. *British Journal of Haematology* 95, 467-471.

Traeger-Synodinos, J. and Harteveld, C.L., 2014. Advances in technologies for screening and diagnosis of hemoglobinopathies. *Biomarkers* 8, 119-131.

Trent, R.J., 2006. Diagnosis of the haemoglobinopathies. *Clinical Biochemistry Reviews* 27, 27.

Tuan, D. and London, I.M., 1984. Mapping of DNase I-hypersensitive sites in the upstream DNA of human embryonic epsilon-globin gene in K562 leukemia cells. *Proceedings of the National Academy of Sciences* 81, 2718-2722.

Viprakasit, V., Kidd, A.M., Ayyub, H., Horsley, S., Hughes, J. and Higgs, D.R., 2003. De novo deletion within the telomeric region flanking the human α globin locus as a cause of α thalassaemia. *British Journal of Haematology* 120, 867-875.

Viprakasit, V., 2015. α−thalassaemia: a genotype–phenotype correlation and management. *ISBT Science Series* 10, 295-304.

Wang, W., Ma, E.S., Chan, A.Y., Prior, J., Erber, W.N., Chan, L.C., Chui, D.H. and Chong, S.S., 2003. Single-tube multiplex-PCR screen for anti-3.7 and anti-4.2 α-globin gene triplications. *Clinical Chemistry* 49, 1679-1682.

Weatherall, D. J., 1997. The thalassaemias. *British Medical Journal* 314, 1675-1678.

Weatherall, D.J., 1999. From genotype to phenotype: genetics and medical practice in the new millennium. Philosophical Transactions of the Royal Society of London B: *Biological Sciences* 354, 1995-2010.

Weatherall, D.J. and Clegg, J.B., 2001. The molecular pathology of the thalassaemias. The Thalassaemia Syndromes, Fourth Edition 33-191.

Weatherall, D.J., 2010. Thalassemia as a global health problem: recent progress toward its control in the developing countries. *Annals of the New York Academy of Sciences* 1202, 17-23.

Weatherall, D.J., 2011. The inherited disorders of haemoglobin: an increasingly neglected global health burden. *Indian Journal of Medical Research* 134, 493.

Wilkie, A.O.M., Buckle, V.J., Harris, P.C., Lamb, J., Barton, N.J., Reeders, S.T., Lindenbaum, R.H., Nicholls, R.D., Barrow, M., Bethlenfalvay, N.C. and Hutz, M.H., 1990. Clinical features and molecular analysis of the α thalassemia/mental retardation syndromes. 1. Cases due to deletions involving chromosome band 16p13. 3. *American Journal of Human Genetics* 46, 1112.

Wood, W.G., 2001. Hereditary persistance of fetal hemoglobin and 8~ thalassemia. *Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management* 356.