

# Should we screen for globin gene mutations in blood samples with mean corpuscular volume (MCV) greater than 80 fL in areas with a high prevalence of thalassaemia?

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## Abstract

**Aims**—To investigate whether it is worthwhile, in areas where thalassaemia is common, to screen for globin gene mutations in subjects with a mean corpuscular volume (MCV) above 80 fL, especially in partners of known thalassaemia carriers.

**Methods**—Blood samples from 95 subjects with MCV between 80 and 85 fL were screened for the presence of  $\alpha$  globin gene mutations and the haemoglobin (Hb) E mutation.

**Results**—Thirty four subjects harboured globin gene mutations. Of these, 31 had deletions of one  $\alpha$  globin gene, one had Hb Constant Spring, and three had Hb E mutations.

**Conclusion**—Based on the above figures and known prevalence rates of thalassaemia carriers, it would seem worthwhile to screen for globin gene mutations in partners of known thalassaemia carriers, regardless of MCV, to identify pregnancies at risk of Hb H disease or Hb E/ $\beta$  thalassaemia.

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Current screening strategies for thalassaemia carriers based solely on an erythrocyte mean corpuscular volume (MCV) < 80 fL or erythrocyte mean corpuscular haemoglobin (MCH) are aimed at identifying individuals with clinically relevant mutations.<sup>1-4</sup> This is to enable the identification of couples at risk of pregnancies affected by hydrops fetalis and Cooley's anaemia, in which genetic counselling and prenatal diagnosis may be offered. Factors responsible for the success of programmes in the reduction of these severe forms of thalassaemia include public education, a cost effective

screening strategy, and adequate antenatal services.<sup>5-7</sup>

However, it is well known that an MCV > 80 fL, which is considered normal by screening criteria, does not preclude the presence of single globin gene mutations, especially in areas with a high prevalence of thalassaemia.<sup>8,9</sup> These globin gene mutations are primarily single  $\alpha$  globin gene deletions; point mutations of  $\alpha$  and  $\beta$  genes with normal MCVs are much less common. These carriers with MCV values > 80 fL have been ignored under the current screening protocol, mainly because of the costs and impracticability of screening the whole population regardless of MCV, as well as the generally accepted view that Hb H disease is not an indication for prenatal diagnosis. We believe, however, that screening for such mutations in areas with a high prevalence of thalassaemia (for example, southern China and Thailand) may be justified—depending on the prevalence of the mutations and in the light of recent case reports of severe forms of Hb H disease.<sup>10</sup>

## Methods

### SAMPLES

Ninety five samples with MCV values between 80 and 85 fL were identified for genotypic analysis after review of the results of 1727 stored blood samples from a previous study on thalassaemia carrier screening involving 2640 subjects in Hong Kong.<sup>11</sup>

Blood samples from parents of eight patients with Hb H disease under the care of the University Paediatric Unit, Queen Mary Hospital, Hong Kong were also obtained after informed consent.

Full blood counts and erythrocyte indices on all blood samples were determined by Technicon H\*1 and H\*2 blood analysers (Technicon, Tarrytown, New York, USA) within 12 hours of venesection.

### MOLECULAR ANALYSES

DNA was extracted from peripheral blood leucocytes according to standard protocols. The following genotypes were determined.

- (1) Deletional  $\alpha$  globin genotypes: polymerase chain reaction (PCR) based diagnostic strategies were used to detect either the ( $-\alpha^{3,7}$ ) or the ( $-\alpha^{4,2}$ ) types of single  $\alpha$  globin gene deletion as well as the ( $--^{SEA}$ ) type of  $\alpha$  thalassaemia deletion involving two  $\alpha$  globin genes in cis.<sup>12,13</sup>

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Table 1 Prevalence of single  $\alpha$  globin gene mutations and Hb E in carriers with mean corpuscular volume 80-85 fl

Type	Deletional	Number	Percentage
$\alpha$	-3.7	23/1727	1.3
	-4.2	8/1727	0.5
	( $--^{SEA}$ )	0/1727	0
	Non-deletional		
	$\alpha^{CS}\alpha$	1/1727	0.06
	$\alpha^{QS}\alpha$	0/1727	0
	$\Delta$ GAG	0/1727	0
Hb E		3/1727	0.2

Hb, haemoglobin.

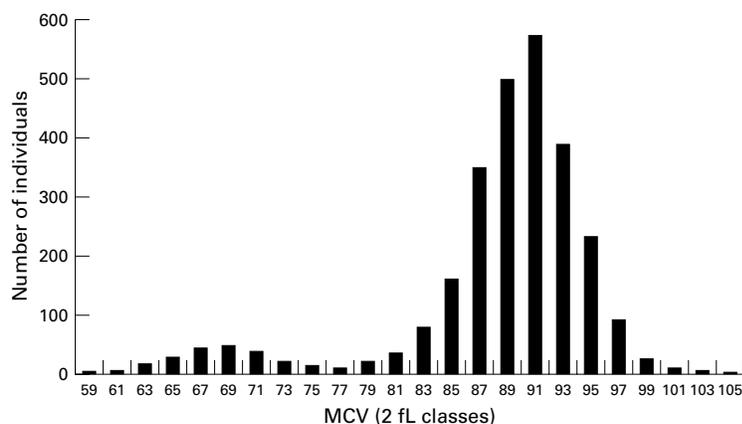


Figure 1 The distribution of 2640 subjects according to their erythrocyte mean corpuscular volume (MCV) at 2 fL intervals.

Table 2 Studies of the parents of patients with Hb H disease

Relationship	Age (years)	Genotype	Hb (g/l)	MCV (fL)	MCH (pg)
Mother I	33	( $-\alpha^{3.7}/\alpha\alpha$ )	117	92	29
Father I	35	( $-\alpha^{SEA}/\alpha\alpha$ )	136	74	22
Father II	43	( $-\alpha^{3.7}/\alpha\alpha$ )	146	81	28
Mother II	39	NA	111	68	22
Father III	36	( $-\alpha^{4.2}/\alpha\alpha$ )	158	87	28
Mother III	36	( $-\alpha^{SEA}/\alpha\alpha$ )	116	73	22
Father IV	51	( $\alpha^{CS}/\alpha\alpha$ )	147	86	27
Mother IV	47	( $-\alpha^{SEA}/\alpha\alpha$ )	112	68	20
Mother V	36	( $\alpha^{CS}/\alpha\alpha$ )	135	79	26
Father V	40	( $-\alpha^{SEA}/\alpha\alpha$ )	141	70	22
Mother VI	37	( $\alpha^{QS}/\alpha\alpha$ )	129	76	25
Father VI	40	( $-\alpha^{SEA}/\alpha\alpha$ )	134	69	22
Father VII	41	( $\alpha^{QS}/\alpha\alpha$ )	133	79	25
Mother VII	NA	NA	NA	NA	NA
Mother VIII	34	( $\alpha^{codon 30 \Delta GAG}/\alpha\alpha$ )	124	82	26
Father VIII	41	NA	135	71	22

MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; NA, not available.

- (2) Hb Constant Spring ( $\alpha 2^{codon 142 TAA \rightarrow CAA}$ ) and Hb Quong Sze ( $\alpha 2^{codon 125 CTG \rightarrow CCG Leu \rightarrow Pro}$ ): the  $\alpha 2$  globin gene was amplified by PCR with appropriate oligonucleotide primers, and subjected to dot blotting for hybridisation with allele specific oligonucleotide (ASO) non-radioactive probes.<sup>14-16</sup>
- (3) Deletion of GAG (glutamic acid) in codon 30 of the  $\alpha 2$  globin gene ( $\Delta GAG$ ): this mutation was detected by PCR based heteroduplex analysis by which the amplified products of the  $\alpha 2$  globin genes were subjected to electrophoresis in 8% polyacrylamide non-denaturing gel.<sup>17</sup>
- (4) Hb E ( $\beta^{codon 26 GAG \rightarrow AAG Glu \rightarrow Lys}$ ) mutation: this was detected by dot blotting of PCR amplified products for hybridisation with ASO non-radioactive probes.<sup>11</sup>

## Results

Figure 1 shows the MCV distribution of all 2640 subjects. It follows a bimodal distribution with clear delineation between the microcytic (MCV < 80 fL) and the normal (MCV > 80 fL) population. Ninety five of 1727 individuals with stored samples available for study (5.5%) had MCVs between 80 and 85 fL. Thirty one of these had single  $\alpha$  globin gene mutations (table 1), giving a prevalence rate of 1.8% (31 of 1727), which is almost four times greater than the prevalence rate of 0.5% seen in the microcytic population (MCV < 80 fL).<sup>11</sup>

Table 3 Projected number of pregnancies at risk of Hb H disease

Type	MCV 80–85 fL*	<80 fL†
Deletional	57	16
Non-deletional	2	0

\*Calculated using values from table 1 and a prevalence rate<sup>11</sup> of 82/1800 for ( $-\alpha^{SEA}$ ) as follows: for deletional Hb H disease, 70 000 births/year  $\times$  (31/1727  $\times$  82/1800); for non-deletional Hb H disease, 70 000  $\times$  (1/1727  $\times$  82/1800).

†Calculated using values from Lau et al,<sup>11</sup> where the prevalence rate of the ( $-\alpha^{SEA}$ ) deletion is 82/1800, the prevalence rate of single  $\alpha$  gene deletion is 9/1800, and the prevalence rate of single non-deletion  $\alpha$  gene mutations is 0/1800; for deletional Hb H, 70 000  $\times$  (82/1800  $\times$  9/1800); for non deletional Hb H, 70 000  $\times$  (82/1800  $\times$  0/1800).

MCV, mean corpuscular volume.

Among the 31 individuals identified, the ( $-\alpha^{3.7}$ ) rightward deletion was twice as common as the ( $-\alpha^{4.2}$ ) leftward deletion. An individual with an MCV of 81 fL was identified as a carrier of Hb Constant Spring, a non-deletional form of  $\alpha$  globin mutation. Hb Quong Sze,  $\Delta GAG$ , and the ( $-\alpha^{SEA}$ ) type of  $\alpha$  thalassaemia deletion were not detected.

Three carriers of Hb E were identified, and their MCVs were 81, 82, and 82 fL. The four most common  $\beta$  thalassaemia mutations in Hong Kong (codons 41–42 (-CTTT), IVSII-654 (C→T), nt-28 (A→G), and codon 17 (A→T)) were not screened for because these are always associated with MCVs < 76 fL.<sup>11</sup>

## STUDY OF THE PARENTS OF PATIENTS WITH Hb H DISEASE

To explore further the range of MCVs in individuals who are carriers of single  $\alpha$  globin mutations, parents of eight patients with Hb H disease were studied. Either parent should be an obligate carrier of a deletion or point mutation involving a single  $\alpha$  globin gene. Table 2 details their haematological findings and  $\alpha$  globin genotypes. These results clearly illustrate that the MCVs of individuals with a single  $\alpha$  globin gene deletion, or mutation such as  $\Delta GAG$ ,  $\alpha^{CS}$  or  $\alpha^{QS}$ , vary between 76 and 92 fL. In contrast, the MCVs of individuals with deletion of two  $\alpha$  globin genes in cis are always < 80 fL.

## PROJECTED NUMBER OF PREGNANCIES AT RISK OF Hb H DISEASE

The incidence of Hb H disease is more accurately defined when the true prevalence rate of ( $-\alpha^{SEA}$ ) thalassaemia deletion and single  $\alpha$  globin gene deletions are known. Our results show that when the cut off value of MCVs is extended to 85 fL, there is a 3.5 fold increase in the projected number of pregnancies at risk of Hb H disease when compared with a cut off of MCV < 80 fL (table 3). This includes two cases of non-deletion Hb H disease associated with severe phenotypes that would otherwise be missed if an MCV cut off point of < 80 fL were adopted.

## Discussion

Screening strategies for thalassaemia carriers are usually based on cut off values for red blood cell indices (either MCV or MCH). There is evidence for a higher sensitivity for MCH < 27 pg as a criterion for  $\beta$  thalassaemia screening in pregnant women.<sup>3</sup> However, as long as blood samples are analysed fresh (within 12 hours of collection), and the automated cell counter used is under stringent quality control procedures, either the MCV or the MCH is a suitable parameter for screening.<sup>18</sup> Our study of single globin gene mutations in the normal population with MCVs between 80 and 85 fL shows that many carriers of single  $\alpha$  gene mutations cannot be detected using the cut off value of MCV < 80 fL as a criterion for thalassaemia screening. By extending the cut off point to an MCV of 85 fL, the combined prevalence rate of single  $\alpha$  gene mutations is increased from a rate of 0.5% in the microcytic population to 2.3%, which represents a fourfold increase. These single  $\alpha$  globin gene mutations are deletions, with only one individual being identified as a carrier of Hb Constant Spring. None of the 95 samples with an MCV between 80 and 85 fL had the ( $-\alpha^{SEA}$ ) type of  $\alpha$  gene deletion. We believe that this is an underestimate of the true prevalence because many carriers of a single  $\alpha$  thalassaemia deletion might have MCVs > 85 fL, as illustrated by the results of parents of children with Hb H disease. The true prevalence, however, of single  $\alpha$  gene deletions ( $-\alpha^{3,7}$  or  $-\alpha^{4,2}$ ) and the non-deletional  $\alpha$  gene mutations (Hb Constant Spring, Hb Quong Sze,  $\alpha 2$  codon 30 ( $\Delta GAG$ ), and  $\alpha 2$  codon 59 ( $G \rightarrow A$ ))<sup>19</sup> in our community remains to be determined.

Our study also reveals that Hb E, probably the next most common human haemoglobin variant after sickle haemoglobin, and present primarily in people of southeast Asian origin, can be detected in individuals with MCVs between 80 and 85 fL. Our results are consistent with the observation that many Hb E carriers have MCVs above 80 fL.<sup>20, 21</sup> In addition to encoding a haemoglobin variant, Hb E, the  $\beta^{\text{codon } 26} \text{GAG} \rightarrow \text{AAG}$  mutation also creates a cryptic RNA splice site, thus leading to a  $\beta$  thalassaemia like phenotype. The clinical presentation of patients who are compound heterozygous for Hb E/ $\beta$  thalassaemia mutations varies considerably, but often they have pronounced anaemia conforming to either  $\beta$  thalassaemia intermedia or major phenotypes.<sup>9, 22</sup>

To screen for thalassaemia carriers in the whole population, regardless of the MCV value, is costly and impractical. We propose that, in areas where thalassaemia is common, globin gene mutations should be screened for in partners of known thalassaemia carriers, regardless of MCV results. If one partner is found to be a carrier of a  $\beta$  thalassaemia mutation, it is advisable to look for Hb E in the other partner, even if the MCV is within the normal range. This is particularly relevant in other southeast Asian regions such as Thailand, where the prevalence rate of Hb E is much higher than in southern China. The goal is to

identify and counsel those couples who are at risk of conceiving a fetus with Hb E/ $\beta$  thalassaemia so that they can be fully informed of the potential health care problem of the condition as mentioned above. In practice, this strategy could be implemented by performing Hb pattern analysis using high performance liquid chromatography (HPLC), regardless of MCV in partners of  $\beta$  thalassaemia carriers. This test, which costs HK\$150 or £12 for each case, will detect both  $\beta$  thalassaemia trait (in which Hb  $A_2$  is increased) as well as Hb E. Although "silent"  $\beta$  thalassaemia trait with normal MCV and normal Hb  $A_2$  values has been reported in other countries,<sup>9</sup> this has not been well documented in China.

Similarly, in partners of those who have the ( $-\alpha^{SEA}$ ) type of  $\alpha$  globin gene deletion, we propose screening for single  $\alpha$  gene deletions and non-deletional  $\alpha$  gene mutations even if the MCV is normal. In this way, couples at risk of conceiving a child with Hb H disease can be identified and counselled. Currently, Hb H disease is generally thought not to be an indication for prenatal diagnosis because patients with Hb H disease have anaemia but usually not severe enough to warrant regular transfusions.<sup>8, 9, 22</sup> However, exceptions do occur such as Hb H hydrops fetalis syndrome,<sup>10, 16, 23</sup> Hb H disease with non-deletional  $\alpha$  thalassaemia mutation,<sup>8, 22, 24</sup> or Hb H disease with concomitant erythroid enzymopathies or membrane protein abnormalities. The detection of single  $\alpha$  gene deletional or non-deletional  $\alpha$  gene mutations would require the use of molecular diagnostic techniques. In practice, single  $\alpha$  gene deletions can be detected by a PCR based method, whereas the four non-deletional  $\alpha$  gene mutations as described above can be detected by dot blot hybridisation with allele specific oligonucleotide probes. The estimated cost of detecting a single  $\alpha$  gene deletion or mutation is HK\$900 or £70.

It is imperative that both parents should be screened by blood counts early on during the pregnancy, or better still, before conception. With proper genetic counselling, the couple should not be unnecessarily alarmed and anxious. Making parents aware of the risks before conception (or antenatally) might help alleviate the distress and guilt that results from the diagnosis of a hereditary disorder in a newborn infant. At present, there is little information on the psychological and social effects on the parents of children with inherited conditions such as a Hb H disease, which are not associated with early deaths and yet might have morbidity. Studies on these issues in the antenatal setting are urgently needed at this time because of the increasing public awareness of the human genome project and its impact on disease diagnosis and management.

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