α2CD31 AGG→AAG, Arg→Lys causing non-deletional α-thalassemia in a Chinese family with HbH disease

We present an unusual HbH disease caused by —^{SEA} deletion combined with an α2CD31 AGG→AAG,Arg→Lys point mutation, which was found by PCR-SSCP and confirmed by direct sequencing of the α2 globin gene. Heterozygous carriers of this mutation showed slight anemia with lower mean corpuscular volume and mean corpuscular hemoglobin.

HbH disease usually occurs in individuals who are double heterozygotes for α^0-thalassemia (thal) and α^+-thal or a non-deletional form, and occasionally in homozygotes for point mutations in both α2 genes.[1] A novel point mutation, α2CD31 AGG→AAG Arg→Lys, has recently been reported in addition to four types of non-deletional α-thal that have been characterized in China.[2,3] Here we describe another new case of this mutation.

The seven-year old boy was from a Chinese family from Guangdong province of South China. He visited our genetic consultation clinic because of moderate anemia, jaundice, and hepatosplenomegaly for years. His growth and development were, however, normal and he had never received a blood transfusion. Hematologic data were obtained with a cell counter (Model Cell-DYN-3500, ADBOTP, USA) and hemoglobin electrophoresis was carried out with a REP system (Helena Laboratory, Beaumont, USA). Gap-PCR assays were performed to detect the --^{SEA} deletion of α^0-thal and the -$\alpha^{3.7}$ and -$\alpha^{4.2}$ determinants of α^+-thal as reported elsewhere.[4,5] A PCR-reverse dot blot (RDB) method was employed to investigate the four-types of α-thal point mutations reported in the Chinese population.[6] The PCR-SSCP analysis used a modification of the technique of Harteveld *et al*.[7] Both the whole α2 globin gene fragments of the propositus and his father were sequenced. Hematologic data are listed in Table 1. Microcytosis and hypochromia were obvious (MCV 56 fL; and MCH 16.7 pg) in the child propositus while anemia was just moderate. Hematologic findings in his father and sister were decreased HbA_2 levels (2.1% and 2.3%, respectively), and low values of MCV (63.9 fL and 60.9 fL, respectively) and MCH (20.3 and 19.1pg, respectively). These results indicated they might be heterozygous for α^0-thal due to their normal or nearly normal hemoglobin level. However, his mother and his brother, though with approximately normal HbA_2 levels and very mild clinical conditions, also had low values of MCV and MCH, indicating the possibility of the two subjects being carriers of deletional or non-deletional α^+-thal. The propositus, the father and sister were proven to be carriers of --^{SEA} deletion of α^0-thal, whereas neither -$\alpha^{3.7}$ nor -$\alpha^{4.2}$ deletion of α^+-thal determinants were found in any member of the affected family. Moreover, the four known mutations of non-deletional α-thal in the Chinese population were excluded in all five subjects of the family. PCR-SSCP analysis of the nest amplified fragment I (250bp in length) of α2-globin gene showed a distinguishable gel profile in the propositus. The DNA samples from the obligate individuals were subsequently analyzed by DNA sequencing. Figure 1 illustrates representative DNA sequences of the selective amplified α2 globin gene fragments from genomic DNA of the propositus and his father. There was a G to A base transition near the conserved donor sequence GT of IVS-I. The mutant sequence was submitted on January 31, 2000 to GenBank (AF230076).

The clinical conditions of all family members were in accordance with their genotype. However, further work is needed to elucidate the molecular mechanism causing the α-thal, i.e., more detailed Hb electrophoresis tests and mutant mRNA function studies. This mutation might affect mRNA splicing, since it is located adjacent to the conserved donor sequence GT of IVS-I in the α2 globin gene. Unfortunately, we have not found any

Table 1. Hematologic data of the members of the HbH disease family.

Family Member	Propositus	Father	Mother	Sister	Brother	Ref. Value
Age (years)	7	32	34	8	5	–
RBC (×10^{12}/L)	4.68	6.45	4.83	5.17	4.79	4.3~5.5
HGB (g/L)	78	13`	113	99	109	120~160
HCT	0.262	0.412	0.349	0.315	0.343	0.4~0.5
MCV (fL)	56	63.9	72.3	60.9	71.6	82~95
MCH (pg)	16.7	20.3	23.4	19.1	22.8	27~31
MCHC (g/L)	298	318	324	314	318	320~360
RDW	21.1	16.7	18.5	18.2	15.2	<15.0
Ret	5.3	0.5	0.5	1.0	0.8	0.5~1.5
Hb (A+F)	82.5	97.9	97.5	97.7	97.1	94.5~96.5
HbA2	1.0	2.1	2.5	2.3	2.9	2.5~3.5
Hb H	10.7	–	–	–	–	0
Hb Bart's	5.9	–	–	–	–	0
Genotype	$\alpha\alpha^{CD31}/--^{SEA}$	$--^{SEA}/\alpha\alpha$	$\alpha\alpha^{CD31}/\alpha\alpha$	$--^{SEA}/\alpha\alpha\alpha\alpha^{CD31}/\alpha\alpha$		$\alpha\alpha/\alpha\alpha$

– Undetectable.

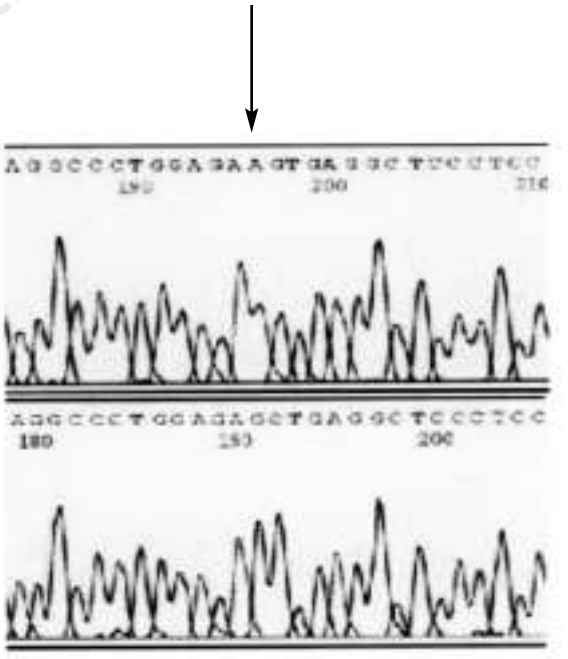

Figure 1. DNA sequence results of the selective amplified α2 globin gene. Top: from the propositus, bottom: wild type control equivalent from his father. The arrow indicate the mutation site. The figure illustrates G to A transition (detailed sequence variation data can also be seen in GenBank, access number AF230076).

abnormal mRNA splicing products using RT-PCR analysis. There are two possible reasons: the influence is small, or the abnormal splicing product is very unstable. If normal splicing occurs, an Arg→Lys replacement would be expected to happen, which might cause the production of a highly unstable α-globin chain or hemoglobin. Functional studies of the mutant gene in a eukaryotic expression system might give an explanation.

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