Correlation between the level of hemoglobin variants (HbS, C and E) and alpha-thalassemia

Hemostasis, transfusion medicine, vascular, laboratory medicine, benignhematology

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Objectives

This retrospective study aims to analyze the different types of α-thalassemia mutations, the levels of abnormal hemoglobin, and the hematological indices in a cohort of Viollier patients carrying hemoglobins S, C, or E, with or without α-thalassemia.

In this context, the study addresses the following questions :

- Which α-thalassemia mutations are associated with abnormal hemoglobins S, C, and E ?
- From what percentage of hemoglobin S, C, or E can the presence of α-thalassemia be confirmed with a high probability (current threshold at Viollier: <40%) ?

Introduction

Hemoglobin consists of four globin chains bound to an iron-containing porphyrin. In adults, the predominant form is HbA, with minor components HbA_2 and residual HbF.

Hemoglobin disorders may be qualitative, quantitative, or both, typically arising from gene mutations or deletions. Common qualitative variants include HbS, HbC, and HbE, resulting from mutations in the β-globin chain. Quantitative defects involve either α- or β-globin gene expression.

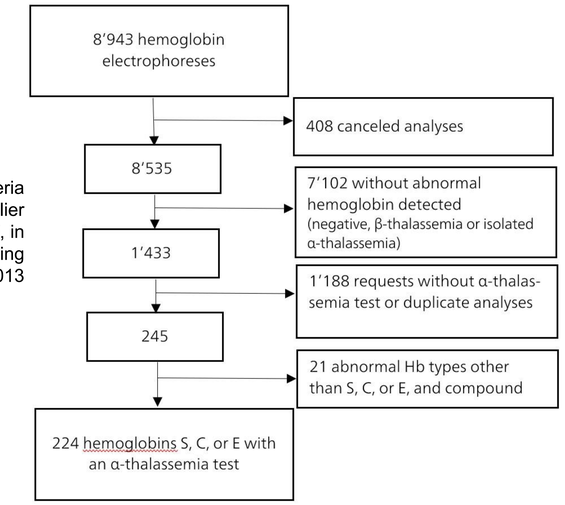
These abnormalities often co-exist and interact, contributing to a wide spectrum of hematologic phenotypes and clinical complications such as anemia and vaso-occlusive crises. Their identification is critical for genetic counseling and clinical management.

Diagnosis relies on a combination of hematological indices, hemoglobin analysis (e.g., electrophoresis or HPLC), and molecular techniques. Due to increased global migration, these genetic conditions - once endemic to regions like the Mediterranian, Africa, Middle East, and Asia - are now increasingly observed in Europe, presenting a growing public health challenge.

Materials and Methods

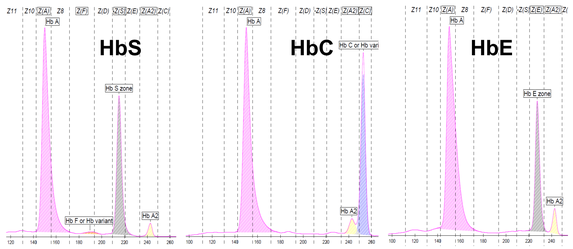
Patient cohort

The flowchart describes the exclusion criteria to select patient samples from the Viollier database with an hemoglobins S, C, and E, in combination with an α-thalassemia screening test, performed between September 2013 and October 2024.



Identification of abnormal hemoglobins

The identification of abnormal hemoglobins was carried out by capillary electrophoresis (Minicap Flex-Piercing system, Sebia).
Hemoglobin fractions separate according to their electrophoretic mobility, determined by the charge-to-mass ratio of the molecules. This method allows for the identification of hemoglobin S (confirmed by the Emmel sickling test), hemoglobin C, and hemoglobin E (confirmed by HPLC).



Identification of α-thalassemia mutations

α-thalassemia mutations were was detected using DNA extracted from EDTA whole blood (Maxwell RSC kit, Promega). The PCR-amplified products were hybridized on a nitrocellulose membrane, the α-Globin StripAssay (ViennaLab), using the AutoLipa 48 automated system (Fujirebio).

This method allows for the detection of the following mutations :

- Single deletions : -3.7, -4.2
- Double deletions: -20.5 kb, --MED, --SEA, --THAI, --FIL
- Alpha1 gene: cd 14 G>A, cd 59 G>A (Hb Adana)
- Alpha2 gene: init cd T>C, cd 19 -G, IVS 1 -5nt, cd 59 G>A, cd 125 T>C, cd 142 T>C, cd 142 T>A, cd 142 A>T, cd 142 A>C, poly A-1 AATAAA>AATAAG, poly A-2 AATAAA>AATGAA
- Anti-3.7 gene triplication

Results and Discussion

Abnormal hemoglobins and α-thalassemia

		α-thalassemia				
Abnormal Hemoglobin	**None**	**-3,7**	**--MED**	**cd 19 -G**	**poly A-2**	**Total**
AS	52	76 { 58* 18** }	0	0	2 { 1* 1** }	130
SS	0	1*	0	0	0	1
AC	6	9 { 7* 2** }	0	0	0	15
CC	0	0	0	0	0	0
AE	49	11 { 8* 3** }	2	1*	0	63
EE	12	3*	0	0	0	15
Total	119	100	2	1	2	224

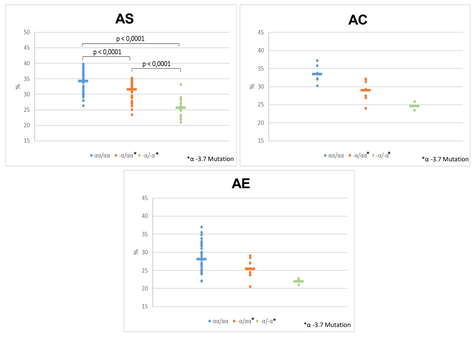
*Heterozygous; **Homozygous.

In the AS group with HbS <40%, an α-gene mutation is identified in 60% of the samples (78/130 pts). Almost exclusively the single deletions -3.7 was detected (97%, 76/78 pts.). It is well established that the sickle cell gene and the -3.7 α-thalassemia mutation frequently coexist in the same geographic regions, particularly in sub-Saharan Africa, due to their positive selection driven by Plasmodium falciparum infection.

In the AC group with HbC <40%, the -3.7 α-gene mutation is found in 60% of the samples (9/15 pts), which can be explained by the high joint prevalence of HbC and this α mutation in West Africa. This indicates a geographic influence on their co-occurrence.
In the AE and EE groups with HbE <40%, α-gene mutations are detected in 22% of the samples (17/78 pts.).

In addition to the -3.7 deletion, only a few other α-gene mutations such as --MED and cd19-G are detected. This genetic diversity most probably reflects the multiple origins of patients in Switzerland.

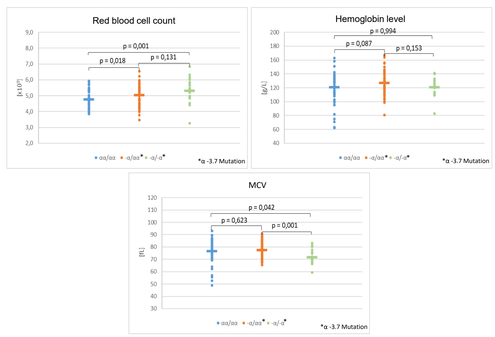
Percentage of abnormal hemoglobin



The percentage of abnormal hemoglobin decreases with the number of α genes, due to the preferential affinity of α-chains for normal β-chains.

	AS			AC			AE		
	αα/αα (N = 52)	-α/αα (N = 58)	-α/-α (N = 18)	αα/αα (N = 6)	-α/αα (N = 7)	-α/-α (N = 2)	αα/αα (N = 49)	-α/αα (N = 8)	-α/-α (N = 3)
Mean (%)	34.3	31.7	25.8	33.5	29.0	24.7	28.2	25.5	22.0
SD	3.63	2.65	2.94	2.35	2.82	1.20	3.76	2.71	0.76
Min	26.4	23.5	21.0	30.3	24.0	23.5	22.0	20.5	21.0
Max	39.8	35.3	33.2	37.2	32.1	25.9	37.0	29.1	22.8

Hematological indices (AS Group)



In the AS group, a significant increase in red blood cell count is observed as soon as one or two α-gene deletions are present, due to a compensatory mechanism related to the presence of α-thalassemia.

No significant variation in hemoglobin levels is observed between the different subgroups, confirming that α-gene deletions do not have a major impact on hemoglobin concentration, indicating stability in hemoglobin levels despite the presence of these deletions.

A significant decrease in MCV is observed when two α-genes are absent, highlighting the pronounced effect of α-thalassemia on microcytosis. This observation is consistent with the literature.
The observed MCV values are slightly lower than expected, likely due to the presence of iron deficiencies that were not systematically evaluated in the analysis.

Conclusions

- The -3.7 α-globin gene deletion accounts for 95% of mutations associated with hemoglobins S, C, and E, reflecting their common distribution in regions of origin such as sub-Saharan Africa, West Africa, and Asia.
- The percentage of abnormal hemoglobin decreases with the loss of α genes, confirming an association between the number of α-genes and the levels of HbS, HbC, and HbE.
- The current thresholds of 40% for HbS, HbC, and HbE to recommend α-thalassemia screening can be lowered to 36%, 33%, and 30%, respectively, to reduce α-thalassemia screening tests while maintaining optimal sensitivity and thus optimize the number of tests performed.
- α-thalassemia has no significant effect on hemoglobin levels but leads to a compensatory increase in red blood cell count and marked microcytosis in the case of double deletion.
- Perspectives: Systematic investigation of anemias and microcytosis is a key step in the diagnosis of hemoglobinopathies. A larger-scale study would help refine patient management and optimize screening strategies.

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