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Mutations in the paralogous human α -globin genes yielding identical hemoglobin variants

Kamran Moradkhani · Claude Préhu · John Old · Shirley Henderson · Vera Balamitsa · Hong-Yuan Luo · Man-Chiu Poon · David H. K. Chui · Henri Wajcman · George P. Patrinos

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Abstract The human α -globin genes are paralogues, sharing a high degree of DNA sequence similarity and producing an identical α -globin chain. Over half of the α -globin structural variants reported to date are only characterized at the amino acid level. It is likely that a fraction of these variants, with phenotypes differing from one observation to another, may be due to the same mutation but on a different α -globin gene. There have been very few previous examples of hemoglobin variants that can be found at both *HBA1* and *HBA2* genes. Here, we report the results of a systematic multicenter study in a large multiethnic population to identify such variants and to analyze their differences from a functional and evolutionary perspective. We identified 14 different Hb variants resulting from identical mutations on either one of the two human α -globin paralogue genes. We also showed that the average percentage of hemoglobin variants due to a *HBA2* gene mutation ($\alpha 2$) is higher than the percentage of hemoglobin

variants due to the same *HBA1* gene mutation ($\alpha 1$) and that the $\alpha 2/\alpha 1$ ratio varied between variants. These α -globin chain variants have most likely occurred via recurrent mutations, gene conversion events, or both. Based on these data, we propose a nomenclature for hemoglobin variants that fall into this category.

Keywords α -Globin genes · Paralogues · Gene conversion · Hemoglobin variants · Mutations

Introduction

There are close to 1,000 human hemoglobin (Hb) variants reported to date [1, 2], and new ones are still being discovered. The vast majority of them are rare, while others, e.g., Hb S, Hb C, and Hb E, reach high frequencies in specific population groups [3]. Most Hb variants are

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clinically and hematologically silent, but some cause hemolytic anemia, e.g., Hb S, some are unstable Hbs, methemoglobinemia (Hbs M), and some cause cyanosis (Hb Kansas) or polycythemia due to their increased oxygen affinity (Hb Ypsilanti, Hb Malmö). There are still other Hb variants that also lead to thalassemic phenotype (Hb E, Hb Knossos).

The human α -like globin gene cluster resides on the telomeric region of chromosome 16. The *HBA2* and *HBA1* genes are paralogues, i.e., set of homologous genes that have evolved from gene duplication and can be associated with a subsequent divergence of function. Both genes have an almost identical DNA sequence due to sequence homogenization by a gene conversion event [4]. Each gene yields an identical 141-amino acid-long α -globin chain, which participates with a β -like globin chain to form the Hb tetramers. From the 332 α -globin chain variants identified to date, approximately 52% have been only characterized by protein chemistry methods, which creates uncertainty as to which α -globin gene is mutated (Table 1). It is likely that a fraction of these variants may result from the same mutation but on a different α -globin gene. Identification and comparative study of these variants would not only provide useful insights into α -globin gene transcription and translation but can also shed light into the mechanistic properties of sequence diversification of the α -like globin genes. There have been very few examples of Hb variants that have been previously identified at both *HBA1* and *HBA2* genes, namely Hb Frankfurt [5] and Hb I [6]. Moreover, as the vast majority of Hb variants are rare, identification and comparative analysis of heterozygous cases for these variants is problematic, even for laboratories with large sample volumes.

Here, we report our results from a multicenter study to identify and comparatively analyze Hb variants that result from the same mutation but on different α -globin gene paralogues in a large multiethnic population sample. In addition, we performed a systematic study in the HbVar database of Hb variants and thalassemia mutations (<http://globin.bx.psu.edu/hbvar>; [1, 3]) to reveal other globin chain variants that fall into this category. Based on these results, we propose a nomenclature for related Hb variants.

Table 1 Number of hemoglobin variants due to a mutant human α -globin gene

Globin genes	No. of Hb variants ^a
<i>HBA2</i>	95
<i>HBA1</i>	54
<i>HBA2</i> or <i>HBA1</i> unknown	173
Found in both <i>HBA2</i> or <i>HBA1</i>	14
Total	332

^a Data from HbVar database (<http://globin.bx.psu.edu/hbvar>, assessed in August 2007)

Materials and methods

Case selection

One hundred and one unrelated individuals, referred to the Unit of Biochemical Genetics, Henri Mondor Hospital (Créteil, France), National Haemoglobinopathy Reference Laboratory, Churchill Hospital, (Oxford, UK), Unit of Prevention of Thalassemia, Trikala General Hospital (Trikala, Greece), and Department of Pathology and Laboratory Medicine, Boston University (Boston, USA), were selected for this study. These subjects were previously identified as carriers of an Hb variant. Hematological indices were measured using a Coulter Cell Counter.

Additionally, 64 unrelated cases were identified in HbVar database of hemoglobin variants and thalassemia mutations (<http://globin.bx.psu.edu/hbvar>) and comparatively analyzed [7, 8]. Finally, additional data were extracted from Online Mendelian Inheritance in Man, where available (see Tables 2 and 3 for details).

Identification of Hb variants

Each sample was analyzed by cation-exchange high-performance liquid chromatography (HPLC) using the Hb Variant I or II systems with either the 6-min β -Thalassemia short or the Dual Kit program (BioRad Laboratories, Hercules, CA, USA). In addition, samples from the National Haemoglobinopathy Reference Laboratory were analyzed by isoelectric focusing, those from Trikala General Hospital were analyzed by alkaline agarose gel electrophoresis (Hydrigel Hemoglobin 7, SEBIA Norcross, GA, USA) and isoelectric focusing, and samples from Henri Mondor Hospital were characterized using a battery of electrophoretic and chromatographic tests (IEF, citrate agar gel electrophoresis, globin chain electrophoresis at acid and alkaline pH and in the presence of Triton X-100, CE-HPLC, RP-HPLC), matching perfectly with results obtained with Hb samples structurally identified as previously described [9].

DNA was isolated from blood leukocytes, and amplification of the *HBA2*, *HBA1*, and *HBB* genes was done using gene-specific primers [10]. DNA sequence analysis was performed with an automated sequencer (ABI 310, Applied Biosystems, Foster City, CA, USA, or CEQ 8000, Beckman Coulter, Fullerton, CA, USA). Detection of α -thalassemia deletion mutants was carried out by a gap-PCR procedure [11].

Results

Fourteen different Hb variants, resulting from identical mutations on either one of the two human α -globin

Table 2 Hematological indices and percentage of Hb variants in either the *HBA1* or *HBA2* genes and the $\alpha 2/\alpha 1$ ratio of the average percentage of the abnormal Hb for heterozygotes with a normal complement of α -globin genes ($\alpha\alpha/\alpha\alpha$)

Hb variant	Racial/ethnic background	Samples	Hb (g/dL)	MCV (fL)	Hb X (%)		Hb S (%)	$\alpha 2/\alpha 1$ ratio	References
					<i>HBA1</i>	<i>HBA2</i>			
p.N9S (c.29A>G)									
Hb Zurich-Hottingen	Portuguese	1	11.7	75	–	–	–	N.M.	[24]
Hb Zurich-Hottingen	Portuguese	1	13.8	78	–	–	–	N.M.	[24]
Hb Anadour	Moroccan	1	Normal	Normal	N.M.	–	–	–	HbVar (ID 2594) ^b
p.A12D (c.38C>A)									
Hb J-Paris-I	Asian ^c	4	12.7±1.8	89.2±6.5	26.5±1.2	–	–	0.98	This report
Hb J-Paris-I	N.A.	1	Normal	Normal	20.7	–	–	–	[14]
Hb J-Paris-I	Asian ^c	1	14.9	91.2	–	26	–	–	This report
Hb J-Paris-I	N.A.	2	Normal	Normal	–	24	–	–	[14]
p.H20Q (c.63C>A)									
Hb Le Lamentin	N.A.	1	Normal	Normal	–	28	–	1.10	[14]
Hb Le Lamentin	British	7	11.4±4.7	91.9±6.0	25.4±1.1	–	–	–	This report
Hb Le Lamentin	African	1	11.7	96.1	14.2	–	36.9	–	This report
p.E27D (c.84G>C)									
Hb Hekinan	Chinese (Macau)	1	12.8	94	13.4	–	–	N.A.	[25]
Hb Hekinan	Chinese (Macau)	1	10.8	92	13.7	–	–	–	[25]
Hb Hekinan	Chinese (Macau)	1	Normal	Normal	12.9	–	–	–	[25]
Hb Hekinan	Taiwanese	1	9.9	72.9	14.7	–	–	–	[26]
Hb Hekinan	French (Guyana)	1	Normal	Normal	–	–	13	–	[27]
p.D47H (c.142G>C)									
Hb Hasharon	Jewish (Brazilian)	1	12.6	79	18	–	–	1.15	This report
Hb Hasharon	Jewish (British)	1	12.1	83	18.9	–	–	–	This report
Hb Hasharon	Jewish (British)	10	14.4±3.8	87.5±6.0	–	21.2±0.9	–	–	This report
Hb Hasharon	Jewish	3	14.4	95	–	17.4	–	–	[14]
p.H50Q (c.153C>G)									
Hb Frankfurt	German	1	14.3	85	24	–	–	1.13	[5]
Hb Frankfurt	Corsican	1	Normal	Normal	–	27	–	–	[5]
p.G51R (c.154G>C)									
Hb Russ	Caucasian/Chinese	2	Normal	Normal	11.5	–	–	1.53	[28]
Hb Russ	British	1	11.2	88	14.6	–	–	–	This report
Hb Russ	British	1	12.9	87	–	22.3	–	–	This report
p.V55A (c.167T>C)									
Hb Gerland	French	1	15	81.7	–	15.9	–	1.67 ^d	[29], personal communication
Hb Gerland [A1]	Southeast Asian	1	11.5	71	9.5 ^d	–	–	–	[30]
p.N68H (c.205A>C)									
Hb St. Truiden	Belgian	1	15.6	90	–	24	–	1.28	HbVar (ID 2591) ^b
Hb Jeddah	Saudi Arabian	1	13.4	82.7	17.8	–	N.A.	–	[31]

Table 2 (continued)

Hb variant	Racial/ethnic background	Samples	Hb (g/dL)	MCV (fL)	Hb X (%) HB α 1	HB α 2	HBA ^a	Hb S (%)	α 2/ α 1 ratio	References
Hb Jeddah	Yemenite	1	11.6	90.3	19.6	–	–	–	–	[31]
Hb Jeddah	Emirati (Abu Dhabi)	1	12.6	72.9	N.A.	–	–	–	–	[31]
p.D75Y (c.226G>T)	American	10	12.9±1.3	90.7±6.8	14.1±0.6	–	–	–	1.3	[32]
Hb Winnipeg	French	38	13.0±1.9	91.2±7.3	13.0±0.7	–	–	–	–	This report
Hb Winnipeg	French	11	13.6±0.8	91.9±6.7	–	16.9±0.9	–	–	–	This report
Hb Winnipeg	Greek	1	13.6	88.9	–	16.1	–	–	–	This report
p.P77H (c.233C>A)	African	1	9.8	94.4	23	–	–	–	1.08	This report
Hb Toulon	British	1	13.3	91	–	26.3	–	–	–	This report
Hb Toulon	French	1	Normal	Normal	–	24	–	–	–	[33]
Hb Toulon	Canadian	1	Normal	Normal	–	25.3	–	–	–	[34]
Hb Toulon	Italian	1	13.1	91	–	24	–	–	–	[35]
p.N78K (c.237C>A)	Central African	5	Normal	Normal	–	–	N.A.	–	N.A.	[12]
Hb Stanleyville-II	French	6	Normal	Normal	–	–	24	–	–	[36]
Hb Stanleyville-II	Caucasian	1	11.0	88	–	15.3	–	–	–	This report
Hb Stanleyville-II	African	1	14.1	90	–	11.1	–	42.7	–	This report
p.D94N (c.283G>A)	Swedish/Finish	1	13.3	91	–	15.6	–	–	1.13	[37]
Hb Titusville	Swedish/Finish	1	13.6	88	–	16	–	–	–	[37]
Hb Titusville	Scottish	1	15.8	85.9	17	–	–	–	–	[38]
Hb Titusville	Scottish	1	14.5	91	11	–	–	–	–	[38]
Hb Titusville	Scottish	1	Normal	Normal	14	–	–	–	–	[38]
p.A120E (c.362C>A)	N.A.	1	Normal	Normal	18.4	–	–	–	1.22	[14]
Hb J-Meerut	Asian ^c	15	11.9±2.1	85.4±10	20.5±2.0	–	–	–	–	This report
Hb J-Meerut	N.A.	1	Normal	Normal	–	23	–	–	–	[14]

N.A. not available, N.M. not measurable, Normal cases where hematological data were normal but hematological indices were not available

^a This percentage was not included in the calculation of the α 2/ α 1 ratio of the average percentage of the abnormal Hb

^b Detailed information for these variants can be retrieved from HbVar from the URL: http://globin.bx.psu.edu/cgi-bin/hbvar/query_vars3?mode=output&display_format=page&f=ID, by replacing "ID" with the respective HbVar ID number

^c Pakistani and Indian

^d Average value from measurements of two independent samples from the same individual (8% and 11%). The variability is most likely due to the slight instability of Hb Gerland.

Table 3 Hematological indices and percentage of Hb variants in either the *HBA1* or *HBA2* genes for heterozygotes with an α -thalassemia phenotype ($-\alpha/\alpha\alpha$ or $-\alpha/\alpha$)

Hb variant	Racial/ethnic background	Samples	Hb (g/dL)	MCV (fL)	Hb X (%)		Hb E (%)	Number of α -globin genes	References
					<i>HBα1</i>	<i>HBα2</i>			
p.N9S (c.29A>G)									
Hb Zurich-Hottingen	Portuguese	1	16.5	91	–	N.M.	–	$-\alpha/\alpha\alpha$	[24]
p.E27D (c.84G>C)									
Hb Hekinan	N.A.	1	10.9	83.9	–	23	–	$-\alpha/\alpha\alpha$	[14]
Hb Hekinan	Asian	1	11.3	69	15.8	–	80	$-\alpha/\alpha\alpha$	This report
Hb Hekinan	Thai	1	10.2	83	27	–	10	$-\alpha/\alpha\alpha$	[39]
Hb Hekinan	Thai	1	8.2	68.6	26.5	–	9.1	$-\alpha/\alpha\alpha$	[39]
Hb Hekinan	Burmanese	1	11.2	68	25	–	–	$-\alpha/\alpha\alpha$	[40]
p.H50Q (c.153C>G)									
Hb Frankfurt	Portuguese	1	15.2	83	–	36	–	$-\alpha/\alpha\alpha$	[5]
p.V55A (c.167T>C)									
Hb Gerland	French	1	12.2	70.6	31	–	–	$-\alpha/\alpha\alpha$	[29]
Hb Gerland	French	1	13	76	31	–	–	$-\alpha/\alpha\alpha$	[29]
p.D75Y (c.226G>T)									
Hb Winnipeg	Greek	1	14.2	76.7	–	30.1	–	$-\alpha/\alpha\alpha$	This report
p.N78K (c.237C>G)									
Hb Stanleyville-II	Black	1	11.7	87	28.6	–	–	$-\alpha/\alpha\alpha$	This report
Hb Stanleyville-II	Black	1	9.3	89	25.2	–	–	$-\alpha/\alpha\alpha$	This report
Hb Stanleyville-II	Caucasian	1	15.1	80	–	27.4	–	$-\alpha/\alpha\alpha$	This report

The number of α -globin genes is also provided
N.A. not available, *N.M.* not measurable

paralogue genes, were identified during this study. From these, nine α -globin chain variants were identified in our study sample, and their hematological and DNA findings were comparatively analyzed with the same Hb variant carriers previously published and documented in HbVar, namely Hb Winnipeg (51 and ten cases, respectively), Hb J-Meerut (15 and two cases, respectively), Hb Hasharon (12 and three cases, respectively), Hb Lamentin (eight and one cases, respectively), Hb J-Paris (five and three cases, respectively), Hb Stanleyville-II (five and 11 cases, respectively), Hb Russ (two and two cases, respectively), Hb Toulon (two and three cases, respectively), and Hb Hekinan (one and nine cases, respectively). Also, five more Hb variants, resulting from identical mutations on either one of the two human α -globin paralogue genes, were selected from HbVar database and/or the literature and included in this study, namely Hb Zurich-Hottingen/Hb Anadour (four cases), Hb Frankfurt (three cases), Hb Gerland (four cases), Hb St. Truiden/Hb Jeddah (four cases), and Hb Titusville (five cases). The hematological indices of the subjects carrying these α -globin chain variants are provided in Tables 2 and 3. With the exception of the mildly unstable Hb Russ and Hb Gerland, the rest of the Hb variants were stable. Furthermore, all Hb variant were found in chromosomes bearing two α -globin genes, except Hb Frankfurt, where the variant was also found in the context of a $-\alpha^{3.7}$ thalassemia chromosome [5]. All heterozygous or com-

pound heterozygous (with $-\alpha^{3.7}$ thalassemia) cases were asymptomatic or present with a typical α -thalassemia carrier phenotype, respectively.

Interestingly, however, DNA analysis of the *HBA1* and *HBA2* genes revealed that in 23 unrelated Hb variant carriers, the mutated α -globin gene was different than the one previously described for each Hb variant. In particular, we identified Hb Hasharon and Hb Toulon resulting from an *HBA1*, instead of a *HBA2*, gene mutation; Hb Russ and Hb Winnipeg resulting from a *HBA2*, instead of a *HBA1*, gene mutation; while Hb Stanleyville-II mutation was found in both the *HBA1* or *HBA2* genes (Tables 2 and 3). The mutated gene for Hb Stanleyville-II was previously not known, as this variant was only characterized at the protein level [12].

Subsequently, we exploited the large number of unrelated Hb Winnipeg carriers bearing the c.226G>T mutation in either the *HBA2* or *HBA1* gene to address whether the percentage of the Hb variant can be correlated to the α -globin gene mutation. Statistical analysis, using SPSS 10 software, showed that Hb Winnipeg carriers cluster in two distinct groups, determined by the percentage of the Hb Winnipeg variant. In 38 Hb Winnipeg carriers bearing the *HBA1* gene mutation, Hb Winnipeg percentage varied from 10.3 to 14.2 (13.0 ± 0.7), while in 12 Hb Winnipeg carriers bearing the *HBA2* gene mutation, the percentage of the variant hemoglobin was between 15.6 and 18.4 (16.9 ± 0.9 ,

Fig. 1). The ratio of the average percentage of the abnormal Hb in heterozygotes with *HBA2* ($\alpha 2$) and *HBA1* mutations ($\alpha 1$) varied from 1.1 to 1.8, with a mean value of 1.3. These results showed that (1) Hb Winnipeg is produced at a higher rate (approx. 23%) when the underlying mutation is in the *HBA2* gene, and (2) the percentage of Hb variant could provide initial information regarding the α -globin gene molecular defect.

We then explored the HbVar database to identify other Hb variants with similar properties, e.g., identical mutations in different α -globin genes yielding the same α -globin chain variant. We identified 64 heterozygous and compound heterozygous, with $-\alpha^{3.7}$ thalassemia cases, originating from various ethnic backgrounds (Tables 2 and 3), creating a total of 14 different Hb variants that fall into this category. Comparative analysis of all 165 carriers, stratified on the basis of the Hb variant, showed that the $\alpha 2/\alpha 1$ average Hb variant percentage ranged from 0.98 to 1.67. However, these cases were extremely rare, and therefore, their comparative analysis does not provide statistical confidence compared to the Hb Winnipeg cases. Finally, in three cases, the variant Hb was only characterized by means of protein sequencing; therefore, the mutated α -globin gene could not be identified unambiguously, and these cases were excluded from further analysis.

Discussion

The present study reports the identification and a comparative analysis of the hematological and molecular properties of Hb variants resulting from the same mutation but residing on a different α -globin gene. Our effort was facilitated from the extensive and diverse sample and comprehensive data

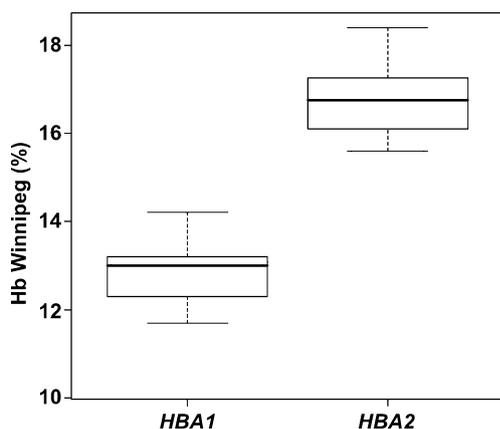


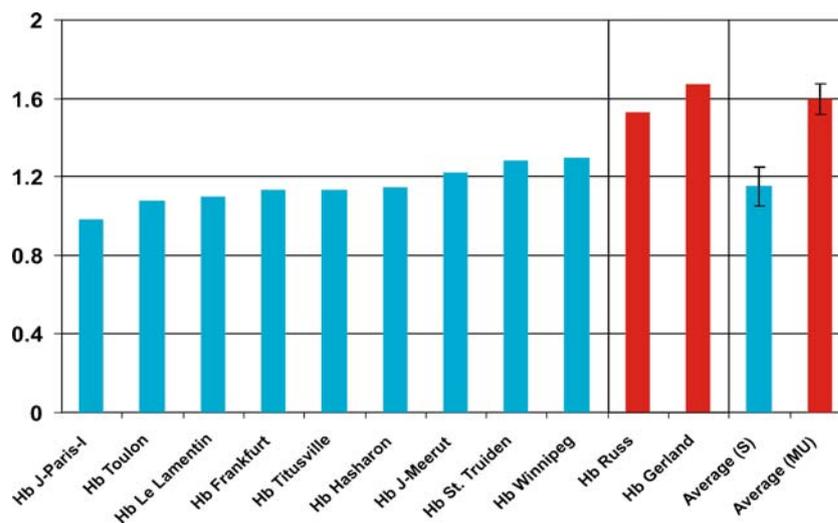
Fig. 1 Box plots ($p < 0.001$) indicating the range and mean values (thick black lines) of the Hb Winnipeg variant percentage between carriers bearing the *HBA1* or *HBA2* mutation. In our evaluation, the Hb Winnipeg/ α -thalassemia compound heterozygous case, as well as the cases described in Ref. [32], is excluded

recording of large diagnostic centers and reference laboratories participating in this study, most of which are members of the ITHANET network (<http://www.ithanet.eu>).

Structural variants have provided important insights into α -globin gene transcriptional regulation and translation. In particular, using in vitro translation experiments, it has been shown that the *HBA2* gene encodes two- to threefold more protein than the *HBA1* gene [13]. However, a more detailed study of heterozygous cases for an α -globin gene structural variant showed that the average abnormal Hb percentage in heterozygotes with *HBA2* and *HBA1* mutations correspond to a $\alpha 2/\alpha 1$ ratio of 1.19/1, respectively [14]. This ratio, however, was obtained from the cumulative analysis of all α -globin chain variants, and the relative position of the variant amino acid in the α -globin chain or the relative stability of the variant Hb was not taken into account. The latter may impact on the final amount of α -globin chain, particularly for unstable variants, the translational efficiency or the altered efficiency in hemoglobin tetramer formation. Although the Hb variant carriers reported herein are phenotypically silent, this study provides a much safer approach, since the comparison is more direct and performed between identical mutations in the α -globin genes, yielding identical globin chains. To our knowledge, this is the first attempt to comparatively analyze the mutations in gene paralogues leading to an identical protein product based on data collected from such a large multiethnic sample, not only for the α -globin but also for other gene families.

Our data show that the $\alpha 2/\alpha 1$ ratio of the average percentage of the abnormal Hb in heterozygotes with *HBA2* and *HBA1* mutations, respectively, varies from 0.98 (Hb J-Paris-I) to 1.67 (Hb Gerland; Tables 2 and 3). Moreover, comparison of the $\alpha 2/\alpha 1$ ratio of the average percentage of the abnormal Hbs showed that stable and mildly unstable abnormal Hbs nicely cluster in two distinct groups with average $\alpha 2/\alpha 1$ ratios of 1.15 ± 0.10 and 1.60 ± 0.09 , respectively (Fig. 2). Notably, in all cases summarized in Tables 2 and 3, the percentage of variant Hb has been calculated using cation-exchange HPLC. Therefore, although these values come from different centers and published reports, the use of similar analytical method, apparatus, and analysis software makes these values virtually comparable. Also, the large number of Hb Winnipeg cases studied in this context statistically strengthens the above claims (Table 2). These data are comparable with previous results from heterozygous cases for 24 different α -globin chain variants [14] but sharply contradicts with a previous study [13], which indicated that a mutated *HBA2* gene yielding a variant α -globin chain is expressed at a two- to threefold higher level than a mutated *HBA1* gene. The main supporting data for this claim were the Hb levels of Hb J-Oxford (40%) and Hb Hasharon (35%), which are comparable to levels expected for Hb variant/ α -thalassemia compound heterozygotes (see

Fig. 2 Comparison of the calculated $\alpha 2/\alpha 1$ ratio of the average percentage of the stable (*S*, shown in blue bars) and mildly unstable abnormal Hbs (*MU*, shown in red bars) reported in this paper



also Table 3). However, these data were obtained from in vitro translation experiments, and hence, this discrepancy is most likely due to the experimental approach rather than directly measuring Hb variant levels in vivo.

How do these variants occur? Although recurrent mutational events can be a likely cause for some of them, interallelic gene conversion event is the most plausible cause that might have resulted in the same mutation being “transferred” into different genomic contexts. A handful of examples also exist in the human β -like globin genes (reviewed in [15]), as well as in other human multigene families [16]. In favor for this assumption is the fact that 13 out of 14 Hb variants described herein are within exons 1 and 2. These exons, and not exon 3, have been shown to be involved in gene conversion events, as the 3′ end of the human α -globin gene conversion tract is located in intron II (Fig. 3).

Importantly, however, the ethnic background of the family or the individual where the mutation is found cannot be always indicative for the likely mechanism that resulted in the mutation in question. For example, the plethora of Hb Winnipeg observed in the French population may indicate recurrent gene conversion events. However, in the cases of Hb

Gerland and Hb Stanleyville-II, although the variants have been reported in two families from France and Southeast Asia and in Caucasian and Black families, respectively, recurrent mutations and (recurrent) gene conversion events are equally likely mechanisms to have generated the mutations in the *HBA2* and *HBA1* genes, and thorough haplotype analysis is required to favor one mechanism over the other.

Apart from gene conversion events, recurrent unequal crossovers cannot be completely ruled out as a likely cause of these Hb variants. Unequal crossovers have been previously shown to be frequent in the human α -globin gene family [17, 18]. In particular, two successive unequal crossover events, i.e., from a normal ($\alpha\alpha$) to a rearranged [e.g., deletional ($\alpha-$) or triplicated ($\alpha\alpha\alpha$)] chromosome and back, will be undistinguishable from a gene conversion event and have the same consequence, e.g., transfer of a DNA segment from one α -globin gene to another. The rearranged (intermediate) chromosome bearing the variant nucleotide can either persist in the population and, therefore, be found, as in the case of Hb Frankfurt [5] or can subsequently be lost.

In general, gene conversion events can be identified from neighboring to the mutation paralogous sequence

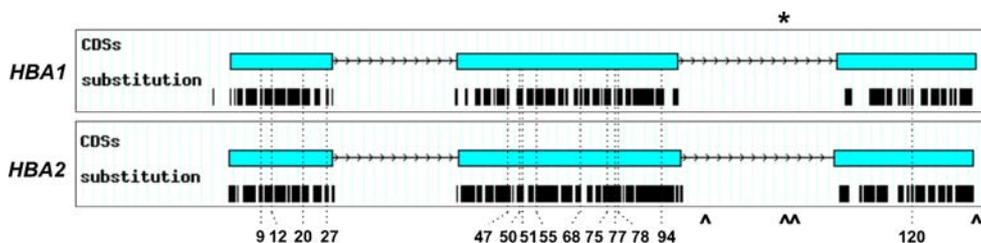


Fig. 3 Schematic drawing of the 14 α -globin chain variants resulting from an identical mutation in either the *HBA1* or *HBA2* genes. Original graphs have been automatically generated by HbVar graphical display [8]. Thick lines under each coding sequence (CDS) represent the position of each substitution deposited in HbVar. Asterisk *** 3′ end of the human α -globin gene conversion tract [6], caret PSVs in

the promoter and coding sequences of the human α -globin genes (c.300+55G/T, c.301-35_29GGCCCTCdel, c.301-24C/G, c.*+15G/A, c.*+19A/G) deduced from sequence comparison between the *HBA2* and *HBA1* reference sequences (NG_000006.1; see also Supplementary data)

variants (PSVs) that are also transferred along with the mutation. Therefore, PSVs are valuable, as they provide insights regarding the length of the gene conversion tract. The Cretan type of non-deletional hereditary persistence of fetal hemoglobin stands as a representative example [19, 20]. *HBA2* and *HBA1* genes have several PSVs outside the 1,436-bp α -globin gene converted region, spanning from positions -868 to $+568$ relative to the genes' transcriptional initiation site [4], but very few PSVs within this DNA segment (depicted as “^” in Fig. 3; see also Supplementary data). Therefore, it is very difficult to unambiguously distinguish these recombinational events from recurrent mutations. At present, there is only one well-documented mutagenic gene conversion event in the human α -globin gene locus (Hb I; p.K16E) identified in both *HBA1* and *HBA2* genes in *cis* [6]. This resembles Hb F-Charlotte [*HBG1*:p.I75T and p.A136E; 21], contrary to Hb F-Waynesboro and Hb F-Lesvos [22], which resulted from the same mutation but in the *HBG2* gene (*HBG2*:p.I75T). Also, two variant α -globin gene alleles consisting of a small DNA segment from the other α -globin gene paralogue ($\alpha 212$ and $\alpha 121$) are most likely the result of a single crossover between a normal and a recombinant allele, although a non-reciprocal gene conversion event cannot be completely ruled out [23]. In all cases, extended haplotype analysis around these variants is an absolute requirement, although this is beyond the scope of the present study.

From a formal genetic point of view, despite the fact that these mutations lead to an identical globin chain, they must be considered as different mutational events, since the nucleotide change occurs in another gene. These situations often cause ambiguities when depositing relevant data in locus-specific databases, like HbVar [1]. The same situation is also reported for two mutations leading to α -thalassemia, namely the c.95+5G>A mutation, found on both α -globin genes and the c.1A>G (initiation codon mutation), found on both *HBA2* and *HBA1* genes and in a hybrid α -globin gene in the context of $\alpha^{-3,7}$ thalassemia chromosome. In addition, apart from the Hb variants reported herein, there are also Hb variants for which the amino acid change is identical, but the nucleotide change is different. Such Hb variants are clearly the products of independent mutational events, namely Hb G-Philadelphia (p.N68K; *HBA2*:c.207C>A or *HBA1*:c.207C>G), Hb J-Broussais (p.K90N; *HBA2*:c.273G>T or *HBA1*:c.273G>C), and Hb Manitoba (p.S102R; *HBA2*:c.307A>C or *HBA2*:c.309C>A or *HBA1*:c.309C>A; also documented as Hb Manitoba I, III, and II, respectively). Therefore, we propose the use of an appropriate suffix in the corresponding variant's name as a solution to this issue. We propose the use of the [A1] suffix for Hb variants due to *HBA1* gene and [A2] suffix for *HBA2* gene mutations, e.g., Hb Winnipeg [A1] and Hb Winnipeg [A2], referring to the *HBA1*:c.226G>C and

HBA2:c.226G>C mutations, respectively. This proposed nomenclature is different than the one used for δ -globin chain variants, e.g., Hb A₂-Yialousa, to avoid confusion.

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