The -$\alpha^{3.7}$ deletion in α-globin genes increases the concentration of fetal hemoglobin and hemoglobin A_2 in a Saudi Arabian population

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Abstract. The regions of Al-Qatif and Al-Ahssa in the Eastern Province of Saudi Arabia are known for their high prevalence of hemoglobinopathies, including β-thalassemia and sickle cell anemia. Previously, the α-gene deletion has been demonstrated as highly prevalent among populations residing in these two regions. The present study was conducted in order to investigate the implications of the α-globin gene deletion on fetal hemoglobin (HbF) and hemoglobin α_2 (HbA_2) concentrations in patients with transfusion-dependent β-thalassemia. A total of 166 Saudi patients with transfusion-dependent β-thalassemia and 337 healthy Saudi patients were included in the study. The -$\alpha^{3.7}$, -$\alpha^{4.2}$, --^{FIL}, --^{SEA}, --^{MED} and --$^{(20.5)}$ gene deletions were identified using multiplex α-globin deletion polymerase chain reaction. The present study revealed that the -$\alpha^{3.7}$ gene deletion is the most prevalent (43.5%) in the Saudi populations that were analyzed and is characterized by the deletion of 3,804 base pairs. Numerous genotypes, namely $^{-3.7}\alpha_2/\alpha_1\alpha_2$, $^{-3.7}\alpha_2/\alpha_1\alpha_{12}$, $^{-3.7}\alpha_2/^{-3.7}\alpha_2$, $^{-3.7}\alpha 2^{HphI}/\alpha_1\alpha_2^{HphI}$, $^{-3.7}\alpha_2/\alpha_1^{-4.2}$, $^{-3.7}\alpha_2/\alpha_1^{polyA-1}\alpha_2$, $^{-3.7}\alpha_{12}/\alpha_1\alpha_{12}$, $^{--FIL}/^{-3.7}\alpha_2$ and $^{-3.7}\alpha_2/^{-3.7}\alpha_2^{Hb\ Villiers\ le\ Bel}$ were also identified in the investigated population. Furthermore, a gradual increase in the concentration of HbF and HbA_2 in patients with β-thalassemia and the number of α-gene deletions was demonstrated; whereas in healthy patients the level of HbA_2 was demonstrated to decrease as the number of α-gene deletions increased. Therefore, it can be concluded that the high HbF concentration in the present study is predominantly associated with other mutations associated with β-thalassemia rather than α-globin deletions. Furthermore, the results of the present study also revealed novel α-gene deletion genotypes prevalent in the population studied, namely $\alpha_1\alpha_2/\alpha_1\alpha_2^{HphI}$, $\alpha_1\alpha_2^{HphI}/\alpha_1\alpha_2^{HphI}$, $\alpha_1\alpha_2/\alpha_1\alpha_2^{Hb\ Handsworth}$, $^{-3.7}\alpha_2^{HphI}/\alpha_1\alpha_2^{HphI}$, $^{-3.7}\alpha_2/^{-3.7}\alpha_2^{Hb\ Villiers\ le\ Bel}$ and $^{--MED}/\alpha_1\alpha_2^{HphI}$.

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Introduction

Hemoglobinopathies, including sickle cell anemia and β-thalassemia, are highly prevalent monogenic gene disorders in Saudi Arabia (1-10). β-thalassemia is caused by point sequence variations or large sequence deletions, that are heritable and either prevent the synthesis of the β-globin chain completely (β^0 variants) or alters the function of β-globin chain (β^+ variants) (1-10). The phenotype of β-thalassemia in the Saudi population is highly varied, ranging from asymptomatic to severe transfusion-dependent anemia (1,4,9,11). Furthermore, it has been previously demonstrated that the α-globin gene deletions, gene conversion [hemoglobin-α 12 (HBA12)] and point mutations, are also highly prevalent in the Saudi population, particularly in the densely populated regions of Qatif and Al-Ahssa in the Eastern Province (13.41% of sickle cell disease carriers; 5.9% β-thalassemia carriers) of Saudi Arabia (1-6,9,10,12,13). Previous studies have investigated the prevalence of the α-globin gene deletion in Arab populations, however, very little has been determined with regards to the prevalence of the α-globin gene deletion in Saudi patients with

thalassemia (14-20). It has previously been revealed that the coinheritance of α-thalassemia in patients with heterozygous β-thalassemia results in an increase of the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) blood concentration, however, carriers of β-thalassemia mutations have an increased blood concentration of hemoglobin A_2 (HbA_2) (21). However, not all carriers of β-thalassemia mutations exhibit this phenotype, and some may in fact exhibit normal HbA_2 blood concentrations (22), which has previously been attributed to a number of factors, including α-globin gene deletions (23). A pre-marriage screening for β-thalassemia mutations in these regions depends on the determination of HbA_2 blood concentrations (24,25). The most common α globin gene deletion is the 3.7 kb rightward deletion ($-\alpha^{3.7}$), which is caused by the breakage of DNA molecules in the α globin genes (HBA2 and HBA1) region and rejoining of the broken ends by leaving α globin genes region with single functional gene. The present study aimed to determine the effect of α-globin deletion on fetal hemoglobin (HbF) and HbA_2 blood concentrations in Saudi populations.

Materials and methods

Patient enrollment. A total of 503 Saudi individuals (Age 12.98±11.08; 80 female and 86 male patients with transfusion-dependent β-thalassemia, and 133 female and 204 male healthy patients) attending major hospitals in the Eastern Province of Saudi Arabia were included in the present study. The study was performed over a 5-year period between February 2012 and February 2017. The study was approved by the University of Dammam Institutional Review Board and Committee for Biological and Medical Ethics (CBME2012032; IRB-2013-08-030; Dammam, Saudi Arabia).

Determination of hematological parameters. Following receipt of informed consent from all participants, blood samples (5 ml) were collected in EDTA-coated vacutainers. VARIANT™ II Hemoglobin Testing System (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and Coulter Micro Diff II (Beckman Coulter, Inc., Brea, CA, USA) were used in order to measure all the hematological parameters.

DNA extraction and polymerase chain reaction (PCR). DNA was extracted (QIAamp DNA blood mini kit; Qiagen GmbH, Hilden, Germany) from the blood samples, and the $-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{FIL}$, $--^{SEA}$, $--^{MED}$ and $--^{(20.5)}$ gene deletions were identified using multiplex α-globin deletion PCR as described previously (26,27). Samples positive for the $-\alpha^{3.7}$ deletion were subjected to amplification of the region around the deletion using primers according to methods previously described (28). Forward and reverse primers were used separately for PCR using the BigDye Terminator Cycle Sequencing Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA), and then purified and electrophoresed using the Series Genetic Analyzer 3500 (Thermo Fisher Scientific, Inc.). From the total number of samples, PCR analysis revealed that 5% of the samples were positive for the $-\alpha^{3.7}$ deletion, and this was confirmed by Sanger sequencing at the Department of Genetic Research, Institute for Research and Medical Consultation, Imam Abdulrahman Bin Faisal University (Dammam, Saudi Arabia). Hemoglobin subunit α1 (HBA1) and hemoglobin subunit α2 (HBA2) genes were also sequenced as previously described (10). Electropherograms were analyzed using DNA sequencing analysis software v5.3 (Applied Biosystems; Thermo Fisher Scientific, Inc.). A multiple alignment program (MAFFT, v.7; https://mafft.cbrc.jp/alignment/server/) was used for HBA1, HBA2 and 3.7 fusion gene sequence alignment. Patients were classed into four groups [αα/αα, $-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{3.7}/-\alpha^{3.7}$ and $--/-\alpha^{3.7}$ where (--, indicates the SEA, MED or FIL deletion)] depending on the presence of their $-\alpha^{3.7}$ deletion genotypes (Fig. 1).

Statistical analysis. Statistical analyses between two groups were performed using the Student's t test (SPSS statistical package version 19; IBM, Corp, Armonk, NY, SA). The data are presented as the mean ± standard deviation. Analysis of variance (ANOVA) combined with post hoc Tukey test, Bonferroni and Holm multiple comparison tests; were performed in order to demonstrate statistically significant differences between multiple groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

The results of the present study demonstrated that the $-\alpha^{3.7}$ gene deletion is the most prevalent (43.5%) among the population of the Eastern province of Saudi Arabia. The second most prevalent gene deletion was the HBA_2: c.95+2_95+6het_delTGAGG (α_2^{HphI}) deletion, with a prevalence score of 24.3%. The $-\alpha^{3.7}$ gene deletion is characterized by the deletion of 3,804 base pairs (Fig. 2). Further α-globin gene mutations revealed to be present in the tested population were: α_1-$^{4.2}$ (1.78%), $\alpha^{1polyA\text{-}1}\alpha_2$ 1.78% and double gene deletions were at a prevalence rate of 1.39% for $-\alpha_2^{(20.5)}$ and <1% for $^{--FIL}$ and $^{--MED}$. The prevalence of the recently identified α12 (HBA12) allele was demonstrated to be 3.78% in the investigated population. A number of genotypes, namely $-^{3.7}\alpha_2/\alpha_1\alpha_2$, $-^{3.7}\alpha_2/\alpha_1\alpha_{12}$, $-^{3.7}\alpha_2/-^{3.7}\alpha_2$, $-^{3.7}\alpha_2^{HphI}/\alpha_1\alpha_2^{HphI}$, $-^{3.7}\alpha_2/\ \alpha_1-^{4.2}$, $-^{3.7}\alpha_2/\alpha_1^{polyA\text{-}1}\alpha_2$, $-^{3.7}\alpha_{12}/\alpha_1\alpha_{12}$, $--^{FIL}/-^{3.7}\alpha_2$ and $-^{3.7}\alpha_2/-^{3.7}\alpha_2^{Hb\ Villiers\ le\ Bel}$ were observed in the population. In addition, >10% of the total population carried other types of α-gene deletion, namely $-\alpha_2^{(20.5)}/\alpha_1\alpha_2$, $--^{MED}/\alpha_1\alpha_2^{HphI}$, $\alpha_1\alpha_2^{init}/\alpha_1^{polyA\text{-}1}\alpha_2$, α_1-$^{4.2}/\alpha_1\alpha_1$, $--^{MED}/\alpha_1\alpha_2$, $--^{FIL}/\alpha_1\alpha_2$, $--^{FIL}/\alpha_1^{polyA\text{-}1}\alpha_2$, $\alpha_1\alpha_2/\alpha_1\alpha_2^{HphI}$, $\alpha_1\alpha_2^{HphI}/\alpha_1\alpha_2^{HphI}$, $\alpha_1\alpha_2/\alpha_1\alpha_2^{Hb\ Handsworth}$ and $\alpha_1^{polyA\text{-}1}\alpha_2/\alpha_1\alpha_2$.

The concentrations of HbF and HbA_2 in the blood are presented in Table I and Fig. 1. A gradual increase in the levels of both HbF and HbA_2 in the β thalassemia patient groups was demonstrated as the number of α-gene deletions increased (Fig. 1 and Table II; $P<0.05$). However, in healthy patients, the concentration of HbA_2 was revealed to decrease as the number of gene deletions increased. The post-hoc Tukey, Bonferroni and Holm multiple comparison tests revealed significant differences with regards to the blood concentration of HbF (f-ratio=3.42806; P=0.020334 for ANOVA of all data groups) and HbA_2 (f-ratio=9.72308; P=0.000012 for ANOVA of all data groups; Table III), in different patient groups.

Discussion

The high prevalence of these disorders has previously been attributed to the high endemicity of malaria in affected areas (29). β-thalassemia disorders represent a group of

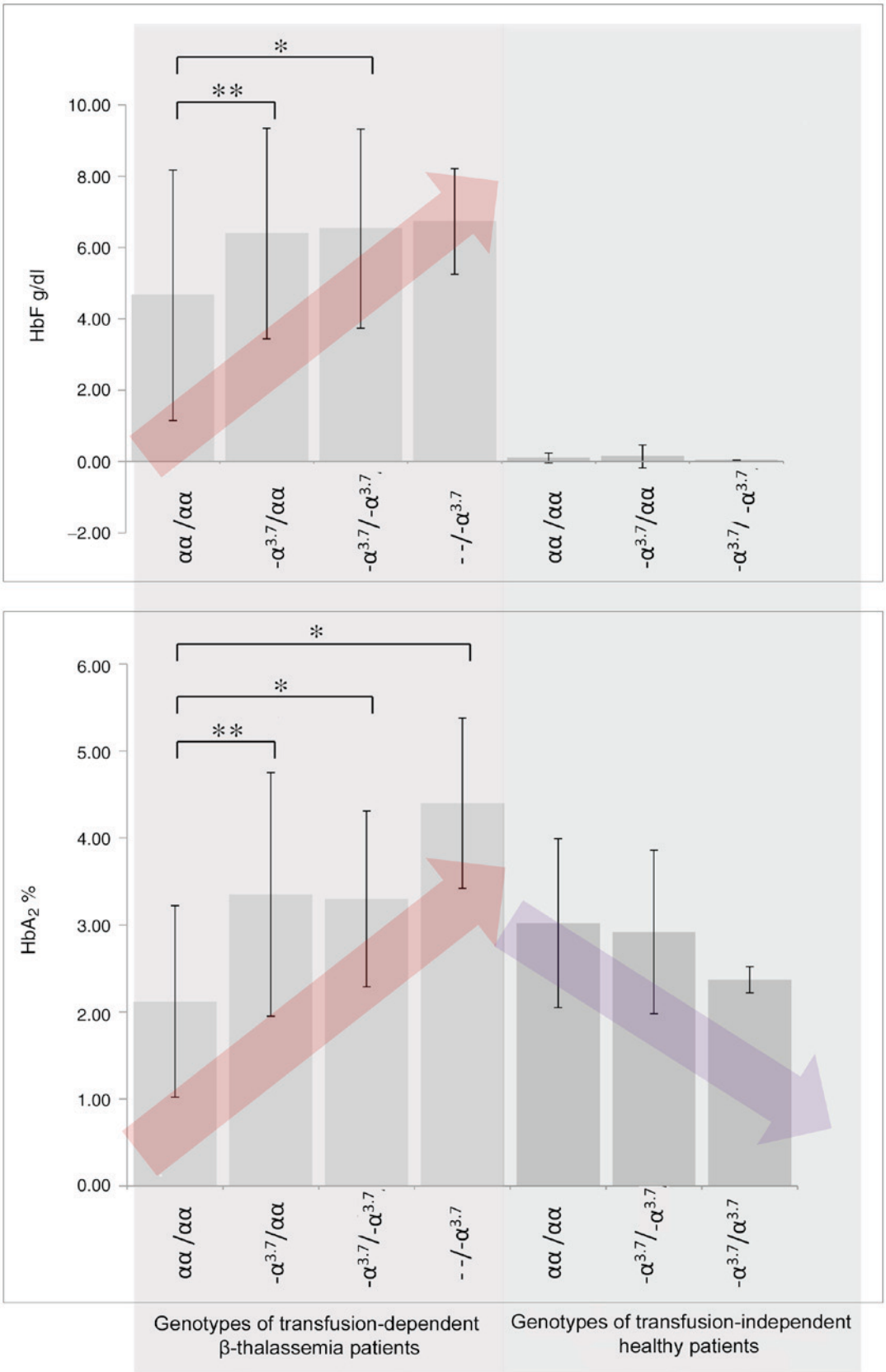

Figure 1. Effect of -$\alpha^{3.7}$ deletion on HbF (g/dl) and HbA2 (%). -$\alpha^{3.7}$, α-globin 3.7 single gene deletion; *P<0.05, **P<0.0001. HbF, fetal hemoglobin; HbA_2, hemoglobin α_2; --, indicates the $--^{FIL}$ or $--^{MED}$ deletions.

heterogeneous hemoglobin disorders, characterized by either the absence or reduced synthesis of the β-globin chain. Such disorders can be classified into three groups according to the severity of their associated clinical representation: β-thalassemia carrier (low severity), thalassemia intermedia (moderate severity) and thalassemia major (high

Figure 2. Schematic of -$\alpha^{3.7}$ deletion and associated fasta sequence in the investigated populations. Left: HBA2 gene sequences are colored purple; HBA1 gene sequences are colored red. Right: 3,804 base pair long sequence corresponding to the -$\alpha^{3.7}$ deletion region in the investigated populations. National Center for Biotechnology Information reference sequence used for the illustration: NC_000016.10. HBA2, hemoglobin subunit α 2; HBA1, hemoglobin subunit α 1.

severity). An excess of α-globin chain production, which aggregates in red blood cell precursors forming inclusion bodies, characterizes thalassemia major. This results in the destruction of red blood cells in the bone marrow and ineffective erythropoiesis, which results in the development of anemia as well as intense proliferation and expansion of the bone marrow (30).

The phenotypic variation exhibited by patients with β-thalassemia is due to the heterogeneity in genetic mutations associated with the disease. However, the phenotype can also be modified by other genetic factors. In addition to the influence of HbF concentration on the β-thalassemia phenotype, deletions of α-globin genes have also been demonstrated as having a significant effect on patient phenotype (20,21).

Furthermore, it has been revealed that the coinheritance of α-thalassemia in individuals heterozygous for β-thalassemia results in elevated levels of MCV and MCH (31). This most frequently occurs when two α-globin genes are silenced due to gene deletion or another type of mutation (32). These individuals also exhibit an increased concentration of HbA_2: A characteristic that is exploited for the identification of β-thalassemia carrier state.

Previously, we have demonstrated that the α-globin gene deletion is highly prevalent in the populations of the Al-Qatif and Al-Ahssa regions (4,9,10). Furthermore, it has previously been demonstrated that the association of the α-gene deletion with β-thalassemia and sickle cell anemia leads to amelioration of disease severity (33).

The results of the present study suggest that the frequency of α-gene deletions is increased in both normal and β-thalassemia populations in the Eastern province compared with other provinces, which is in agreement with the results of previous studies, including reports on African populations (34). Furthermore, previous studies have demonstrated that the prevalence of the α-gene deletion in Arab populations is varied, ranging from 28% in Kuwait and the United Arab Emirates to as high as 75% in Lebanon (14-20,35). This difference in the reported prevalence frequencies of α-globin gene deletions may be attributed to variation in the sample size as well as the inclusion criteria of different studies. Genome-wide association studies have revealed that the α-globin gene deletions have a significant effect on the blood concentrations of HbA and HbA_2, whereas it has no significant effect on the concentration of HbF (36). The results of the present study suggest that there is an association between the -$\alpha^{3.7}$ deletion and elevated concentrations of HbA_2, and is therefore in agreement with the aforementioned study. In addition, the β-thalassemia mutations were demonstrated

Table I. Genotypes of $-\alpha^{3.7}$ deletion and blood concentration of HbF and HbA_2.

Genotype	Number of patients	HbF (g/dl)	HbA_2 (%)
Patients with transfusion-dependent β-thalassemia			
αα/αα	63	4.66±3.51	2.12±1.1
$-\alpha^{3.7}/\alpha\alpha$	55	6.39±2.95	3.35±1.4
$-\alpha^{3.7}/-\alpha^{3.7}$	11	6.53±2.79	3.3±1.01
$--/-\alpha^{3.7}$	3	6.73±1.48	4.4±0.98
Healthy patients			
αα/αα	162	0.1±0.14	3.02±0.97
$-\alpha^{3.7}/\alpha\alpha$	142	0.14±0.32	2.92±0.94
$-\alpha^{3.7}/-\alpha^{3.7}$	4	0.04±0.006	2.37±0.15
$--/-\alpha^{3.7}$	0	-	-

Values are presented as the mean ± standard deviation. HbF, fetal hemoglobin; HbA_2, hemoglobin α_2.

Table II. Significance of $-\alpha^{3.7}$ deletion on blood concentrations of HbF and HbA_2.

Comparison between associated groups				
Group 1	Group 2	Number of patients	HbF, P-value	HbA_2, P-value
$-\alpha^{3.7}/\alpha\alpha$ transfused	αα/αα transfused	55 vs. 63	0.007609[a]	<0.00001[b]
$-\alpha^{3.7}/-\alpha^{3.7}$ or $-\alpha^{3.7}/-\alpha^{4.2}$ transfused	αα/αα transfused	11 vs. 63	0.048726[a]	0.001384[a]
$--/-\alpha^{3.7}$ transfused	αα/αα transfused	3 vs. 63	0.207171	0.001937[a]
$-\alpha^{3.7}/\alpha\alpha$ healthy patients	αα/αα healthy patients	144 vs. 162	0.110116	0.211731
$-\alpha^{3.7}/-\alpha^{3.7}$ or $-\alpha^{3.7}/-\alpha^{4.2}$ healthy patients	αα/αα healthy patients	4 vs. 162	0.226603	0.071323

[a]P<0.05; [b]P<0.0001 vs. group 1. HbF, fetal hemoglobin; HbA_2, hemoglobin α_2.

Table III. Statistical analyses using multiple comparison tests.

Treatment pair	Bonferroni and Holm TT-statistic	Bonferroni P-value	Bonferroni inference	Holm P-value	Holm inference
HbF					
A vs. B	2.5587	0.0364182	Not significant	0.0364182	P<0.05
A vs. C	2.4523	0.0482490	Not significant	0.0321660	P<0.05
A vs. D	0.9074	1.0996299	Not significant	0.3665433	Not significant
HbA_2					
A vs. B	4.8279	0.0000156	P<0.01	0.0000156	P<0.01
A vs. C	2.7093	0.0239577	P<0.05	0.0159718	P<0.05
A vs. D	2.6306	0.0297893	Not significant	0.0099298	P<0.01

A, αα/αα transfused; B, $-\alpha^{3.7}/\alpha\alpha$ transfused; C, $-\alpha^{3.7}/-\alpha^{3.7}$ or $-\alpha^{3.7}/-\alpha^{4.2}$ transfused; D, $--/-\alpha^{3.7}$ transfused; HbF, fetal hemoglobin; HbA_2, hemoglobin α_2.

as being associated with an increased concentration of HbF (36-38). Therefore, it can be concluded that the elevated HbF concentration in the present study is predominantly associated with β-thalassemia mutations as opposed to α-gene deletions. Furthermore, the results of the present study also revealed new α-gene deletion genotypes prevalent in the studied populations, namely: $\alpha_1\alpha_2/\alpha_1\alpha_2^{HphI}$, $\alpha_1\alpha_2^{HphI}/\alpha_1\alpha_2^{HphI}$, $\alpha_1\alpha_2/\alpha_1\alpha_2^{Hb\ Handsworth}$, $-^{3.7}\alpha_2^{HphI}/\alpha_1\alpha_2^{HphI}$, $-^{3.7}\alpha_2/-^{3.7}\alpha_2^{Hb\ Villiers\ le\ Bel}$ and $--^{MED}/\alpha_1\alpha_2^{HphI}$, which, to the best of our knowledge, have not previously been reported.

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