Supplementary Material

ORIGINAL ARTICLE

Role of Genomic Biomarkers in Increasing Fetal Hemoglobin Levels Upon Hydroxyurea Therapy and in -Thalassemia Intermedia: A Validation Cohort Study

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Supplementary Table 1. *p* Values resulting from Fisher’s exact test for the previous studies (Tafrali *et al.* [1], Gravia *et al.* [2], Chalikiopoulou *et al.* [3]; personal communications from I. Papantoni *et al.*, A. Stratopoulos *et al.*, A. Skarpathioti *et al.*, Janaury, 2019), performed for patients of Greek descent, the present study and the total results, taking into account both studies. (Bold *p* values are statistically significant.)

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | *FLT1* | *ARG2* | *MAP3K5* | *NOS2A* | *PDE7B* | *NOS1* | *ASS1* |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | rs2182008 | rs10483801 | rs10483802 | STR 5'>3'a | rs9376230 | rs9483947 | rs944725 | rs1137933 | rs2327669 | rs11154849 | rs9376273 | rs816361 | rs7977109 | rs10793902 | rs7860909 | rs10901080 |
| b | C-TDT | 1.00 | 0.039 | NF | **0.011** | 0.873 | 0.955 | 0.736 | NF | 0.603 | 0.512 | NF | NF | NF | 0.44 | 0.522 | NF |
| b | C-NTDT | **0.039** | NF | 0.521 | 0.26 | 0.599 | 0.396 | 0.897 | NF | 0.28 | 0.823 | NF | NF | NF | 0.899 | 0.514 | 1.00 |
| b | TDT-NTDT | 0.054 | NF | NF | **0.03** | 0.454 | 0.426 | 0.581 | NF | 0.352 | 1.00 | NF | NF | NF | 0.620 | 0.779 | NF |
| b | Rs-NRs 20.0% | 1.00 | **0.05** | NF | 0.233 | **0.002** | **0.026** | 0.591 | 0.748 | 0.261 | 0.124 | 0.663 | **<0.001** | 0.737 | **0.046** | 0.514 | **<0.0001** |
| b | Rs-NRs 3-fold | 1.00 | 0.715 | 0.645 | 0.06 | 0.799 | 0.169 | 0.4 | 0.643 | 0.9 | 0.896 | 1.00 | 1.00 | 0.474 | 0.683 | 0.345 | 0.72 |
| c | C-TDT | NF | 1.00 | NF | 1.00 | 0.43 | 1.00 | 0.162 | 1.00 | 0.382 | 0.78 | NF | 0.09 | NF | **0.006** | 0.337 | 1.00 |
| c | C-NTDT | NF | 0.69 | NF | 0.818 | 0.659 | 0.427 | 0.415 | 0.809 | 0.444 | 0.129 | 0.64 | 1.00 | NF | 0.604 | 0.343 | 0.756 |
| c | TDT-NTDT | NF | 0.689 | NF | 0.785 | 0.232 | 0.333 | 0.794 | 1.00 | 0.382 | 0.2 | NF | 0.543 | NF | 0.758 | 0.448 | 0.999 |
| c | Rs-NRs 20.0% | NF | **0.015** | NF | 0.547 | 0.579 | 0.466 | 0.557 | 1.00 | 0.152 | 0.151 | **0.05** | 0.879 | 0.256 | 0.905 | 0.905 | 0.374 |
| c | Rs-NRs 3-fold | NF | 0.639 | NF | 0.094 | 0.397 | 0.769 | **0.008** | 0.749 | 0.875 | 0.254 | 0.625 | 0.866 | 1.00 | 0.098 | 0.323 | 0.75 |
| d | C-TDT | 0.342 | 0.128 | NF | 0.544 | 0.497 | 0.984 | 0.815 | 0.278 | 0.323 | 0.641 | NF | 0.173 | NF | 0.071 | 0.37 | 0.373 |
| d | C-NTDT | **0.004** | **0.001** | NF | 0.288 | 0.176 | 0.12 | 0.575 | 0.788 | 0.238 | 0.684 | NF | 0.655 | NF | 0.868 | 0.77 | 0.735 |
| d | TDT-NTDT | 0.135 | **0.015** | 0.148 | 0.189 | 0.093 | 0.123 | 0.519 | 0.577 | 0.425 | 0.568 | NF | 0.932 | NF | 0.832 | 0.522 | 0.391 |
| d | Rs-NRs 20.0% | NF | **0.005** | 0.609 | 0.559 | 0.177 | 0.768 | 0.56 | 1.00 | 0.269 | 0.143 | 0.104 | 0.619 | 0.157 | 0.681 | 0.517 | 0.096 |
| d | Rs-NRs 3-fold | NF | 0.774 | 0.296 | **0.004** | 0.367 | 0.297 | **0.015** | 0.573 | 0.421 | 0.433 | 0.624 | 0.699 | 0.8 | 0.143 | 0.788 | 0.411 |

NF: not feasible (according to the requirements of the statistical software use); C-TDT: control-transfusion-dependent -thalassemia (-thal); C-NTDT: control-non transfusion-dependent -thal; TDT-NTDT: transfusion-dependent -thal-non transfusion-dependent -thal; Rs: responders; NRs: non responders (also see text for criteria).

aSTR-5'-GCGCG-3'.

b*p* Value of previous studies.

c*p* Value of present study.

d*p* Value of both studies.

Supplementary Table 2. *HBB* pathogenic variants of non transfusion-dependent -thalassemia patients and their Hb F levels (%).

|  |  |  |
| --- | --- | --- |
| Case | Hb F (%) | *HBB* Pathogenic Variants |

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | Conventional Nomenclature | HGVS Nomenclature |
| HB\_TH\_2 | 12.3 | IVS-I-6(T>C)/IVS-I-6(T>C) | *HBB*: c.92+6T>C/*HBB*: c.92+6T>C |
| HB\_TH\_3 | 12.0 | IVS-I-6(T>C)/IVS-I-6(T>C) | *HBB*: c.92+6T>C/*HBB*: c.92+6T>C |
| HB\_TH\_5 | 73.6 | IVS-I-110(G>A)/IVS-I-110(G>A) | *HBB*: c.93-21G>A/*HBB*: c.93-2G>A |
| HB\_TH\_6 | 51.4 | FSC 6(–A)/IVS-I-6(T>C) | *HBB*: c.20delA/*HBB*: c.92+6T>C |
| HB\_TH\_8 | 20.9 | IVS-I-6(T>C)/IVS-I-6(T>C) | *HBB*: c.92+6T>C/*HBB*: c.92+6T>C |
| HB\_TH\_34 | 51.4 | IVS-I-110(G>A)/codon 39(C>T) | *HBB*: c.93-21G>A/*HBB*: c.118C>T |
| HB\_TH\_57 | NA | IVS-I-6(G>A)/IVS-I-110(G>A) | *HBB*: c.92+5G>A/*HBB*: c.93-21G>A |

NA: not available; FSC: frameshift codon.

Supplementary Table 3. Hb F levels (%) before and after hydroxyurea administration in relation to *HBB* pathogenic variants of sickle cell disease and -thalassemia compound heterozygotes.

|  |  |  |  |
| --- | --- | --- | --- |
| Case | Hb F (%) | Hb F (%) | *HBB* Pathogenic Variants |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | (before HU) | (after HU) | Conventional Nomenclature | HGVS Nomenclature |
| HB\_TH\_7 | 15.5 | 32.6 | codon 6(A>T)/codon 39(C>T) | *HBB*: c.20A>T/*HBB*: c.118C>T |
| HB\_TH\_HU\_8 | 9.5 | 16.4 | codon 6(A>T)/codon 39(C>T) | *HBB*: c.20A>T/*HBB*: c.118C>T |
| HB\_TH\_HU\_11 | 11.3 | 17.5 | codon 6(A>T)/IVS-I-110G>A | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_12 | 3.6 | 7.8 | codon 6(A>T)/codon 39(C>T) | *HBB*: c.20A>T/*HBB*: c.118C>T |
| HB\_TH\_HU\_13 | 8.2 | 31.8 | codon 6(A>T)/codon 39(C>T) | *HBB*: c.20A>T/*HBB*: c.118C>T |
| HB\_TH\_HU\_14 | 11.6 | 21.4 | codon 6(A>T)/IVS-II-6(G>A) | *HBB*: c.20A>T/*HBB*: c315+1G>A |
| HB\_TH\_HU\_15 | 24.9 | 32.9 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_16 | 1.2 | 9.9 | codon 6(A>T)/codon 39(C>T) | *HBB*: c.20A>T/*HBB*: c.118C>T |
| HB\_TH\_HU\_21 | 9.5 | 27.4 | codon 6(A>T)/IVS-II-745(C>G) | *HBB*: c.20A>T/*HBB*: c.316-106C>G |
| HB\_TH\_HU\_24 | 8.7 | 12.8 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_25 | 1.1 | 5.9 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_26 | 9.0 | 12.3 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_27 | 6.7 | 12.1 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_28 | 1.9 | 13.4 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_29 | 9.6 | 18.2 | codon 6(A>T)/codon 39(C>T) | *HBB*: c.20A>T/*HBB*: c.118C>T |
| HB\_TH\_HU\_30 | 14.1 | 30.8 | codon 6(A>T)/codon 39(C>T) | *HBB*: c.20A>T/*HBB*: c.118C>T |
| HB\_TH\_HU\_31 | 17.1 | 38.2 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_33 | 5.7 | 7.1 | codon 6(A>T)/IVS-II-745(C>G) | *HBB*: c.20A>T/*HBB*: c.316-106C>G |
| HB\_TH\_HU\_35 | 8.0 | 28.4 | codon 6(A>T)/IVS-I-1(G>A) | *HBB*: c.20A>T/*HBB*: c.92+1G>A |
| HB\_TH\_HU\_37 | 6.9 | 19.8 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_40 | 4.8 | 4.0 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_46 | 3.2 | 7.6 | codon 6(A>T)/IVS-II-745(C>G) | *HBB*: c.20A>T/*HBB*: c.316-106C>G |
| HB\_TH\_HU\_47 | 14.1 | 27.7 | codon 6(A>T)/codon 39(C>T) | *HBB*: c.20A>T/*HBB*: c.118C>T |
| HB\_TH\_HU\_50 | 3.9 | 20.8 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_52 | 17.3 | 28.3 | codon 6(A>T)/FSC 8(–AA) | *HBB*: c.20A>T/*HBB*: c.25\_26delAA |
| HB\_TH\_HU\_54 | 13.2 | 55.1 | codon 6(A>T)/IVS-II-745(C>G) | *HBB*: c.20A>T/*HBB*: c.316-106C>G |
| HB\_TH\_HU\_56 | 18.0 | 26.6 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_57 | 2.9 | 9.3 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_58 | 9.1 | 11.2 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_59 | 7.0 | 12.1 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_61 | 2.1 | 9.8 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_62 | 2.2 | 11.3 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_65 | 5.3 | 15.1 | codon 6(A>T)/IVS-I-1(G>A) | *HBB*: c.20A>T/*HBB*: c.92+1G>A |
| HB\_TH\_HU\_66 | 2.2 | 15.2 | codon 6(A>T)/IVS-I-6(T>C) | *HBB*: c.20A>T/*HBB*: c.92+6T>C |
| HB\_TH\_HU\_67 | 11.6 | 17.5 | codon 6(A>T)/codon 39(C>T) | *HBB*: c.20A>T/*HBB*: c.118C>T |
| HB\_TH\_HU\_69 | 7.2 | 8.3 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_70 | 8.3 | 14.8 | codon 6(A>T)/IVS-I-1(G>A) | *HBB*: c.20A>T/*HBB*: c.92+1G>A |
| HB\_TH\_HU\_72 | 20.2 | 6.9 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_73 | 9.6 | 22.3 | codon 6(A>T)/codon 39(C>T) | *HBB*: c.20A>T/*HBB*: c.118C>T |
| HB\_TH\_HU\_74 | 6.6 | 14.9 | codon 6(A>T)/IVS-I-110(G>A) | *HBB*: c.20A>T/*HBB*: c.93-21G>A |
| HB\_TH\_HU\_76 | 20.0 | 28.7 | codon 6(A>T)/IVS-I-5(G>A) | *HBB*: c.20A>T/*HBB*: c.92+5G>A |
| AS004 | 3.8 | 23.1 | codon 6(A>T)/IVS-I-1(G>A) | *HBB*: c.20A>T/*HBB*: c.92+1G>A |

FSC: frameshift codon.

Supplementary Table 4. Genotype frequencies of  type hemoglobinopathies patients and healthy individuals of Hellenic origin for the rs10483801 and rs10483802 *ARG2* gene variants. Data were analyzed separately for the present study and collectively, also including the patients from a previous study (personal communication from A. Stratopoulos *et al.*, January, 2019).

|  |  |
| --- | --- |
| *ARG2* Gene | Genotype Frequencies (%) |

|  |  |  |
| --- | --- | --- |
|  | Present Study | Previous and Present Study |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| rs10483801 | CC | AA | CA | CC | AA | CA |  |  |  |  |  |  |  |
| Control | 61.0 | 8.0 | 31.0 | 41.0 | 4.0 | 55.0 |  |  |  |  |  |  |  |
| TDT | 62.0 | 8.0 | 30 | 48.0 | 9.0 | 42.0 |  |  |  |  |  |  |  |
| NTDT | 71.0 | 14.0 | 14.0 | 86.0 | 7.0 | 7.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 35.0 | 6.0 | 59.0 | 29.0 | 4.0 | 68.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 75.0 | 0.0 | 25.0 | 64.0 | 0.0 | 36.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 50.0 | 0.0 | 50.0 | 46.0 | 0.0 | 54.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 62.0 | 3.0 | 34.0 | 52.0 | 2.0 | 45.0 |  |  |  |  |  |  |  |
| rs10483802 | TT | CC | TC | TT | CC | TC |  |  |  |  |  |  |  |
| Control | 17.0 | 0.0 | 83.0 | 39.0 | 1.0 | 60.0 |  |  |  |  |  |  |  |
| TDT | 89.0 | 0.0 | 11.0 | 68.0 | 0.0 | 32.0 |  |  |  |  |  |  |  |
| NTDT | 86.0 | 14.0 | 0.0 | 71.0 | 7.0 | 21.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 100.0 | 0.0 | 0.0 | 70.0 | 0.0 | 30.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 88.0 | 0.0 | 13.0 | 63.0 | 0.0 | 37.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 100.0 | 0.0 | 0.0 | 59.0 | 0.0 | 41.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 91.0 | 0.0 | 9.0 | 73.0 | 0.0 | 27.0 |  |  |  |  |  |  |  |

TDT: transfusion-dependent thalassemia; NTDT: non transfusion-dependent thalassemia; Rs: responders; NRs: non responders.

Supplementary Table 5. Genotype frequencies of  type hemoglobinopathies patients and healthy individuals of Hellenic origin for the rs7860909 and rs10793902 and rs10901080 *ASS1* gene variants. Data were analyzed separately for the present study and collectively, also including the patients from a previous study by Chalikiopoulou *et al.* [3].

|  |  |
| --- | --- |
| *ASS1* Gene | Genotype Frequencies (%) |

|  |  |  |
| --- | --- | --- |
|  | Present Study | Previous and Present Study |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| rs7860909 | AA | GG | AG | AA | GG | AG |  |  |  |  |  |  |  |
| Control | 37.0 | 10.0 | 53.0 | 29.0 | 13.0 | 57.0 |  |  |  |  |  |  |  |
| TDT | 23.0 | 21.0 | 56.0 | 27.0 | 21.0 | 53.0 |  |  |  |  |  |  |  |
| NTDT | 14.0 | 0.0 | 86.0 | 39.0 | 11.0 | 50.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 47.0 | 6.0 | 47.0 | 35.0 | 13.0 | 52.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 40.0 | 12.0 | 48.0 | 35.0 | 23.0 | 42.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 58.0 | 0.0 | 42.0 | 33.0 | 23.0 | 43.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 37.0 | 13.0 | 50.0 | 37.0 | 16.0 | 47.0 |  |  |  |  |  |  |  |
| rs10793902 | CC | TT | CT | CC | TT | CT |  |  |  |  |  |  |  |
| Control | 7.0 | 31.0 | 62.0 | 11.0 | 45.0 | 44.0 |  |  |  |  |  |  |  |
| TDT | 5.0 | 68.0 | 27.0 | 6.0 | 60.0 | 34.0 |  |  |  |  |  |  |  |
| NTDT | 0.0 | 47.0 | 47.0 | 6.0 | 53.0 | 41.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 12.0 | 47.0 | 43.0 | 7.0 | 50.0 | 43.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 12.0 | 40.0 | 48.0 | 11.0 | 39.0 | 50.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 25.0 | 42.0 | 33.0 | 18.0 | 39.0 | 43.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 3.0 | 43.0 | 53.0 | 4.0 | 46.0 | 50.0 |  |  |  |  |  |  |  |
| rs10901080 | GG | TT | GT | GG | TT | GT |  |  |  |  |  |  |  |
| Control | 70.0 | 5.0 | 25.0 | 59.0 | 3.0 | 38.0 |  |  |  |  |  |  |  |
| TDT | 74.0 | 3.0 | 24.0 | 55.0 | 1.0 | 44.0 |  |  |  |  |  |  |  |
| NTDT | 86.0 | 0.0 | 14.0 | 71.0 | 0.0 | 29.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 75.0 | 6.0 | 19.0 | 61.0 | 4.0 | 36.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 88.0 | 0.0 | 12.0 | 81.0 | 2.0 | 17.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 82.0 | 0.0 | 18.0 | 70.0 | 0.0 | 30.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 83.0 | 3.0 | 13.0 | 77.0 | 4.0 | 19.0 |  |  |  |  |  |  |  |

TDT: transfusion-dependent thalassemia; NTDT: non transfusion-dependent thalassemia; Rs: responders; NRs: non responders.

Supplementary Table 6. Genotype frequencies of  type hemoglobinopathies patients and healthy individuals of Hellenic origin for the rs9376230 and rs9483947 and promoter short tandem repeat *MAP3K5* gene variants. Data were analyzed separately for the present study and collectively, also including the patients from a previous study by Tafrali *et al.* [1].

|  |  |
| --- | --- |
| *MAP3K5* Gene | Genotype Frequencies (%) |

|  |  |  |
| --- | --- | --- |
|  | Present Study | Previous and Present Study |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| rs9376230 | CC | AA | CA | CC | AA | CA |  |  |  |  |  |  |  |
| Control | 8.0 | 45.0 | 47.0 | 10.0 | 45.0 | 45.0 |  |  |  |  |  |  |  |
| TDT | 13.0 | 53.0 | 34.0 | 15.0 | 43.0 | 43.0 |  |  |  |  |  |  |  |
| NTDT | 0.0 | 29.0 | 71.0 | 0.0 | 33.0 | 67.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 29.0 | 24.0 | 47.0 | 29.0 | 35.0 | 35.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 32.0 | 36.0 | 32.0 | 17.0 | 27.0 | 56.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 25.0 | 50.0 | 25.0 | 17.0 | 41.0 | 41.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 33.0 | 27.0 | 40.0 | 24.0 | 26.0 | 50.0 |  |  |  |  |  |  |  |
| rs9483947 | CC | TT | CT | CC | TT | CT |  |  |  |  |  |  |  |
| Control | 8.0 | 51.0 | 41.0 | 12.0 | 46.0 | 42.0 |  |  |  |  |  |  |  |
| TDT | 8.0 | 53.0 | 39.0 | 11.0 | 46.0 | 43.0 |  |  |  |  |  |  |  |
| NTDT | 0.0 | 29.0 | 71.0 | 0.0 | 33.0 | 67.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 6.0 | 29.0 | 65.0 | 10.0 | 37.0 | 53.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 12.0 | 44.0 | 44.0 | 6.0 | 34.0 | 60.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 8.0 | 50.0 | 42.0 | 10.0 | 43.0 | 47.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 10.0 | 33.0 | 57.0 | 6.0 | 30.0 | 64.0 |  |  |  |  |  |  |  |
| STR (5'>-3') | 4/4 | 5/5 | 4/5 | 4/4 | 5/5 | 4/5 |  |  |  |  |  |  |  |
| Control | 44.0 | 8.0 | 49.0 | 45.0 | 12.0 | 43.0 |  |  |  |  |  |  |  |
| TDT | 45.0 | 5.0 | 50.0 | 38.0 | 17.0 | 45.0 |  |  |  |  |  |  |  |
| NTDT | 57.0 | 0.0 | 43.0 | 44.0 | 0.0 | 56.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 24.0 | 6.0 | 71.0 | 39.0 | 3.0 | 58.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 40.0 | 8.0 | 52.0 | 38.0 | 10.0 | 52.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 58.0 | 0.0 | 42.0 | 58.0 | 0.0 | 42.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 23.0 | 10.0 | 67.0 | 25.0 | 13.0 | 63.0 |  |  |  |  |  |  |  |

TDT: transfusion-dependent thalassemia; NTDT: non transfusion-dependent thalassemia; Rs: responders; NRs: non responders; STR (5'>3'): short tandem repeat (5'-GCGCG-3').

Supplementary Table 7. Genotype frequencies of  type hemoglobinopathies patients and healthy individuals of Hellenic origin for the rs23277669, rs9376173 and rs11154849 *PDE7B* gene variants. Data were analyzed separately for the present study and collectively, also including the patients from a previous study by Tafrali *et al.* [1].

|  |  |
| --- | --- |
| *PDE7B* Gene | Genotype Frequencies (%) |

|  |  |  |
| --- | --- | --- |
|  | Present Study | Previous and Present Study |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| rs2327669 | CC | GG | CG | CC | GG | CG |  |  |  |  |  |  |  |
| Control | 13.0 | 53.0 | 34.0 | 13.0 | 48.0 | 39.0 |  |  |  |  |  |  |  |
| TDT | 13.0 | 37.0 | 50.0 | 16.0 | 38.0 | 46.0 |  |  |  |  |  |  |  |
| NTDT | 14.0 | 29.0 | 57.0 | 6.0 | 33.0 | 61.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 0.0 | 76.0 | 24.0 | 16.0 | 52.0 | 32.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 16.0 | 52.0 | 32.0 | 15.0 | 36.0 | 49.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 8.0 | 58.0 | 33.0 | 17.0 | 33.0 | 50.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 10.0 | 63.0 | 27.0 | 15.0 | 48.0 | 38.0 |  |  |  |  |  |  |  |
| rs9376173 | AA | CC | AC | AA | CC | AC |  |  |  |  |  |  |  |
| Control | 16.0 | 11.0 | 73.0 | 10.0 | 13.0 | 78.0 |  |  |  |  |  |  |  |
| TDT | 0.0 | 21.0 | 79.0 | 2.0 | 14.0 | 84.0 |  |  |  |  |  |  |  |
| NTDT | 0.0 | 14.0 | 86.0 | 0.0 | 15.0 | 85.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 24.0 | 0.0 | 76.0 | 17.0 | 10.0 | 73.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 16.0 | 28.0 | 56.0 | 9.0 | 30.0 | 61.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 17.0 | 25.0 | 58.0 | 7.0 | 26.0 | 67.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 20.0 | 13.0 | 67.0 | 15.0 | 19.0 | 66.0 |  |  |  |  |  |  |  |
| rs11154849 | CC | TT | CT | CC | TT | CT |  |  |  |  |  |  |  |
| Control | 56.0 | 17.0 | 28.0 | 37.0 | 15.0 | 39.0 |  |  |  |  |  |  |  |
| TDT | 47.0 | 17.0 | 28.0 | 47.0 | 15.0 | 39.0 |  |  |  |  |  |  |  |
| NTDT | 29.0 | 0.0 | 71.0 | 39.0 | 11.0 | 50.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 47.0 | 0.0 | 53.0 | 35.0 | 6.0 | 58.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 44.0 | 20.0 | 36.0 | 49.0 | 15.0 | 36.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 33.0 | 25.0 | 42.0 | 37.0 | 17.0 | 47.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 50.0 | 7.0 | 43.0 | 48.0 | 8.0 | 44.0 |  |  |  |  |  |  |  |

TDT: transfusion-dependent thalassemia; NTDT: non transfusion-dependent thalassemia; Rs: responders; NRs: non responders.

Supplementary Table 8. Genotype frequencies of  type hemoglobinopathies patients and healthy individuals of Hellenic origin for the rs944725 and rs1137933 *NOS24* gene variants. Data were analyzed separately for the present study and collectively, also including the patients from a previous study by Chalikiopoulou *et al.* [3].

|  |  |
| --- | --- |
| *NOS24* Gene | Genotype Frequencies (%) |

|  |  |  |
| --- | --- | --- |
|  | Present Study | Previous and Present Study |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| rs944725 | CC | TT | CT | CC | TT | CT |  |  |  |  |  |  |  |
| Control | 33.0 | 0.0 | 67.0 | 32.0 | 12.0 | 56.0 |  |  |  |  |  |  |  |
| TDT | 26.0 | 8.0 | 66.0 | 32.0 | 16.0 | 52.0 |  |  |  |  |  |  |  |
| NTDT | 14.0 | 0.0 | 86.0 | 19.0 | 13.0 | 69.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 18.0 | 6.0 | 76.0 | 20.0 | 10.0 | 70.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 32.0 | 4.0 | 64.0 | 27.0 | 17.0 | 56.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 58.0 | 0.0 | 42.0 | 41.0 | 14.0 | 45.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 13.0 | 7.0 | 80.0 | 13.0 | 15.0 | 73.0 |  |  |  |  |  |  |  |
| rs1137933 | GG | AA | GA | GG | AA | GA |  |  |  |  |  |  |  |
| Control | 56.0 | 8.0 | 36.0 | 25.0 | 5.0 | 70.0 |  |  |  |  |  |  |  |
| TDT | 57.0 | 8.0 | 35.0 | 37.0 | 6.0 | 56.0 |  |  |  |  |  |  |  |
| NTDT | 71.0 | 0.0 | 29.0 | 29.0 | 0.0 | 71.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 53.0 | 6.0 | 41.0 | 58.0 | 3.0 | 39.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 56.0 | 4.0 | 40.0 | 56.0 | 6.0 | 38.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 50.0 | 8.0 | 42.0 | 50.0 | 7.0 | 43.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 57.0 | 3.0 | 40.0 | 61.0 | 4.0 | 35.0 |  |  |  |  |  |  |  |

TDT: transfusion-dependent thalassemia; NTDT: non transfusion-dependent thalassemia; Rs: responders; NRs: non responders.

Supplementary Table 9. Genotype frequencies of  type hemoglobinopathies patients and healthy individuals of Hellenic origin for the rs8166361 and rs7977109 *NOS1* gene variants. Data were analyzed separately for the present study and collectively, also including the patients from a previous study by Chalikiopoulou *et al.* [3]; (personal communication from A. Stratopoulos *et al.*, January, 2019).

|  |  |
| --- | --- |
| *NOS1* Gene | Genotype Frequencies (%) |

|  |  |  |
| --- | --- | --- |
|  | Present Study | Previous and Present Study |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| rs8166361 | CC | GG | CG | CC | GG | CG |  |  |  |  |  |  |  |
| Control | 37.0 | 3.0 | 60.0 | 43.0 | 8.0 | 49.0 |  |  |  |  |  |  |  |
| TDT | 45.0 | 16.0 | 39.0 | 39.0 | 16.0 | 45.0 |  |  |  |  |  |  |  |
| NTDT | 43.0 | 0.0 | 57.0 | 47.0 | 13.0 | 40.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 41.0 | 6.0 | 53.0 | 38.0 | 5.0 | 57.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 48.0 | 4.0 | 48.0 | 47.0 | 10.0 | 43.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 42.0 | 0.0 | 58.0 | 43.0 | 0.0 | 57.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 47.0 | 7.0 | 47.0 | 43.0 | 8.0 | 49.0 |  |  |  |  |  |  |  |
| rs7977109 | GG | AA | GA | GG | AA | GA |  |  |  |  |  |  |  |
| Control | 4.0 | 0.0 | 96.0 | 18.0 | 13.0 | 69.0 |  |  |  |  |  |  |  |
| TDT | 0.0 | 3.0 | 97.0 | 40.0 | 6.0 | 54.0 |  |  |  |  |  |  |  |
| NTDT | 0.0 | 14.0 | 86.0 | 0.0 | 13.0 | 88.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 6.0 | 6.0 | 88.0 | 10.0 | 7.0 | 83.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 20.0 | 16.0 | 64.0 | 24.0 | 13.0 | 62.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 17.0 | 8.0 | 75.0 | 18.0 | 7.0 | 75.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 13.0 | 13.0 | 73.0 | 20.0 | 13.0 | 67.0 |  |  |  |  |  |  |  |

TDT: transfusion-dependent thalassemia; NTDT: non transfusion-dependent thalassemia; Rs: responders; NRs: non responders.

Supplementary Table 10. Genotype frequencies of  type hemoglobinopathies patients and healthy individuals of Hellenic origin for the rs2182008 *FLT1* gene variants. Data were analyzed separately for the present study and collectively, also including the patients from a previous study (personal communication from I. Papantoni *et al.*, January, 2019).

|  |  |
| --- | --- |
| *FLT1* Gene | Genotype Frequencies (%) |

|  |  |  |
| --- | --- | --- |
|  | Present Study | Previous and Present Study |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| rs2182008 | GG | AA | AG | GG | AA | AG |  |  |  |  |  |  |  |
| Control | 100.0 | 0.0 | 0.0 | 99.0 | 0.0 | 1.0 |  |  |  |  |  |  |  |
| TDT | 97.0 | 0.0 | 3.0 | 97.0 | 0.0 | 3.0 |  |  |  |  |  |  |  |
| NTDT | 100.0 | 0.0 | 0.0 | 89.0 | 0.0 | 11.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 100.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 100.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 100.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 100.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |  |  |  |  |  |  |  |

TDT: transfusion-dependent thalassemia; NTDT: non transfusion-dependent thalassemia; Rs: responders; NRs: non responders.

Supplementary Table 11. Genotype frequencies of  type hemoglobinopathies patients and healthy individuals of Hellenic origin for the rs826729 *TOX* gene variants. Data were analyzed separately for the present study and collectively, also including the patients from a previous study (personal communication from A. Skarpathioti *et al.*, January, 2019).

|  |  |
| --- | --- |
| *TOX* Gene | Genotype Frequencies (%) |

|  |  |  |
| --- | --- | --- |
|  | Present Study | Previous and Present Study |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| rs826729 | GG | AA | GA | GG | AA | GA |  |  |  |  |  |  |  |
| Control | 0.0 | 52.0 | 48.0 | 5.0 | 64.0 | 31.0 |  |  |  |  |  |  |  |
| TDT | 10.0 | 54.0 | 36.0 | 10.0 | 58.0 | 32.0 |  |  |  |  |  |  |  |
| NTDT | 14.0 | 43.0 | 43.0 | 12.0 | 71.0 | 18.0 |  |  |  |  |  |  |  |
| Rs (20.0%) | 6.0 | 47.0 | 47.0 | 4.0 | 46.0 | 50.0 |  |  |  |  |  |  |  |
| NRs (20.0%) | 4.0 | 56.0 | 40.0 | 11.0 | 56.0 | 33.0 |  |  |  |  |  |  |  |
| Rs 3-fold | 0.0 | 58.0 | 42.0 | 4.0 | 48.0 | 48.0 |  |  |  |  |  |  |  |
| NRs 3-fold | 7.0 | 50.0 | 43.0 | 11.0 | 55.0 | 34.0 |  |  |  |  |  |  |  |

TDT: transfusion-dependent thalassemia; NTDT: non transfusion-dependent thalassemia; Rs: responders; NRs: non responders.

References

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[3] Chalikiopoulou C, Tavianatou AG, Sgourou A, *et al.* Genomic variants in the *ASS1* gene, involved in the nitric oxide biosynthesis and signaling pathway, predict hydroxyurea efficacy in compound sickle cell disease/-thalassemia patients. Pharmacogenomics. 2016;17(4):393–403.