**Supplementary Material**

Delta-aminolevulinic acid dehydratase (ALAD) and vitamin D receptor (VDR) genes polymorphisms in children residing in an abandoned lead-zinc mine area

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**Supplementary Material**

The δ-ALAD and VDR Genes nucleotide sequence, primer sets, and genotypes with fragment sizes after RFLP

**ALAD**

Primers: CAGAGCATGGACAGGGAGCAA / TAGGCAGCGGTGGAGGCATCTCT

[product length = 917]

AGACAGACATTAGCTCAGTAGAGGAAAGGGTTTCCCCGGGGCCAGAGCTGTTCCACAGTGGAAGGGGCAGCCCCATAAAGTAAAGAGCTACCCATCACCCGAGACGTCGTGGCAGAGGCTGTTGCAGAAGGGAGCTGAACTGCAGATGGGAGTTCAAAAAGAGGGCCTCGAAGGAGCCTTCCACAGCCGAATTC↓CGGAGCTCTGCTACTCAGGGCCTCAGTCTTCCCTCCTATTTAGTGGATGCATCCCTGCCCCTTCTGTCCTGGGGGCTTGAGCCCTCCTGGTGCCATATGCAGCTTGGTTTCTAACAGAGGCACACAGTGTGGTGGGGTC↓CGGAGGACCGTTGCCTGGGACCTGCCTTCCTTCAACCCCTCTACCCACACCCACACAGGTATGGTGTGAAG↓CGGCTGGAAGAGATGCTGAGGCCCTTGGTGGAAGAGGGCCTACGCTGTGTCTTGATCTTTGGCGTCCCCAGCAGAGTTCCCAAGGTGAAGAATCAAAGGAAGGGCTAAGAAGGGAGGTTGCGCTCACGCCCGTAATCCCAGCACTTTGGGAGGCCAAAGTGGGTGGATCACTTGAGCCCAGGATTTTGAGACCAGCCTGGACAACATGGCAAAACCCATCTCTACAAAAAATACAAAAGTTAGCTGGGTGTGGGGGTATGTGCCTGTAGTCCCAGCTACTCGGGAGGTGGAGAGGTGGGAGGATTGCTTGAGCCCAGAAAGTCGAGGCTGCAGTGAGCCAAAATCGCGCCAGTGCACTCTAGCCTGGGTGACAGAGCAAGACCCTGTCTCCAATACAAACAGAAAAAGGAAGGGAGGTTGGGCAAAGGTGGACTGAGGGTCCACACTGACTGCACCCTCACTCCCACATTGTGCTGGCCCTGGGGCCACAGGTGAATGGACGTGGTCTTTGCC

MspI: (rs1800435): G to C polymorphism exists (AAG to AAC)

Msp-I-cutsite_1

|  |  |  |  |
| --- | --- | --- | --- |
| Wild type | ALAD 1-1 (GG) | 3 fragments | 584 +158 + 139 bp |
| Heterozygous | ALAD 1-2 (GC) | 4 fragments | 584 + 513 + 158 + 139 bp |
| Homozygous mutant | ALAD 2-2 (CC) | 3 fragments | 513 + 158 + 139 bp |

**VDR – FokI**

Primers: AGCTGGCCCTGGCACTGACTCTGGCT / ATGGAAACACCTTGCTTCTTCTCCCTC [product length = 267]

AGCTGGCCCTGGCACTGACTCTGGCTCTGACCGTGGCCTGCTTGCTGTTCTTACAGGGATGGAGGCAATG↓GCGGCCAGCACTTCCCTGCCTGACCCTGGAGACTTTGACCGGAACGTGCCCCGGATCTGTGGGGTGTGTGGAGACCGAGCCACTGGCTTTCACTTCAATGCTATGACCTGTGAAGGCTGCAAAGGCTTCTTCAGGTGAGCCCTCCTCCCAGGCTCTCCCCAGTGGAAAGGGAGGGAGAAGAAGCAAGGTGTTTCCAT

FokI: (rs2228570): T to C polymorphism exists (ATG to ACG)

Fok-I-cutsite_1

|  |  |  |  |
| --- | --- | --- | --- |
| Wild type | TT | 2 fragments | 197 + 70 bp |
| Heterozygous | TC | 3 fragments | 267 + 197 + 70 bp |
| Homozygous mutant | CC | 1 fragment | 267 bp |

**VDR - BsmI**

Primers: GACCTGTGGCAACCAAGACT / AACCAGCGGAAGAGGTCAAG

[product length = 831]

GACCTGTGGCAACCAAGACTACAAGTACCGCGTCAGTGACGTGACCAAAGGTATGCCTAGACTCCACCTCCTGGGGAGTCTTTTTCAGCTCCCAGATTCTGGCTCCACCCGTCCTGGGGTTTGGCTCCAATCAGATACATGGGAGGGAGTTAGGCACCAACAGGGAGAGAAGGGCGAGGGTCAGACCCATGGGGTTGGAGGTGGGTGGGCGGCTCCTCAGCTCTGCCCGCAGTACCTGGCCATTGTCTCTCACAGCCGGACACAGCCTGGAGCTGATTGAGCCCCTCATCAAGTTCCAGGTGGGACTGAAGAAGCTGAACTTGCATGAGGAGGAGCATGTCCTGCTCATGGCCATCTGCATCGTCTCCCCAGGTATGGGGCCAGGCAGGGAGGAGCTCAGGGACCTGGGGAGCGGGGAGTATGAAGGACAAAGACCTGCTGAGGGCCAGCTGGGCAACCTGAAGGGAGACGTAGCAAAAGGAGACACAGATAAGGAAATACCTACTTTGCTGGTTTGCAGAGCCCCTGTGGTGTGTGGACGCTGAGGTGCCCCTCACTGCCCTTAGCTCTGCCTTGCAGAGTGTGCAGGCGATTCGTAGGGGGGATTCTGAGGAACTAGATAAGCAGGGTTCCTGGGGCCACAGACAGGCCTGCG↓CATTCCCAATACTCAGGCTCTGCTCTTGCGTGAACTGGGCTCAACATTCCTGTTATTTGAGGTTTCTTGCGGGCAGGGTACAAAACTTTGGAGCCTGAGAGATGGTTCTGCCTATATAGTTTACCTGATTGATTTTGGAGGCAATGTGCAGTGACCCTTGACCTCTTCCGCTGGTT

BsmI: (rs1544410) [G>A polymorphism]

|  |  |  |  |
| --- | --- | --- | --- |
| Wild type | GG | 2 fragments | 655 + 176 bp |
| Heterozygous | GA | 3 fragments | 831 + 655 + 176 bp |
| Homozygous mutant | AA | 1 fragment | 831 bp |

Bsm-I-cutsite_1

**VDR - ApaI**

Primers: CAGAGCATGGACAGGGAGCAA / TAGGCAGCGGTGGAGGCATCTCT

[product length = 832]

CAGAGCATGGACAGGGAGCAAGGCCAGGCAGGGACAGGGCCAGGTGCGCCCATGGAAGGACCTAGGTCTGGATCCTAAATGCACGGAGAAGTCACTGGAGGGCTTTGGGGCCAGGCAGTGGTATCACCGGTCAGCAGTCATAGAGGGGTGGCCTAGGGGGTGCTGCCGTTGAGTGTCTGTGTGGGTGGGGGGTGGTGGGATTGAGCAGTGAGGGGCC↓CAGCTGAGAGCTCCTGTGCCTTCTTCTCTATCCCCGTGCCCACAGATCGTCCTGGGGTGCAGGACGCCGCGCTGATTGAGGCCATCCAGGACCGCCTGTCCAACACACTGCAGACGTACATCCGCTGCCGCCACCCGCCCCCGGGCAGCCACCTGCTCTATGCCAAGATGATCCAGAAGCTAGCCGACCTGCGCAGCCTCAATGAGGAGCACTCCAAGCAGTACCGCTGCCTCTCCTTCCAGCCTGAGTGCAGCATGAAGCTAACGCCCCTTGTGCTCGAAGTGTTTGGCAATGAGATCTCCTGACTAGGACAGCCTGTGGCGGTGCCTGGGTGGGGCTGCTCCTCCAGGGCCACGTGCCAGGCCCGGGGCTGGCGGCTACTCAGCAGCCCTCCTCACCCCGTCTGGGGTTCAGCCCCTCCTCTGCCACCTCCCCTATCCACCCAGCCCATTCTCTCTCCTGTCCAACCTAACCCCTTTCCTGCGGGCTTTTCCCCGGTCCCTTGAGACCTCAGCCATGAGGAGTTGCTGTTTGTTTGACAAAGAAACCCAAGTGGGGGCAGAGGGCAGAGGCTGGAGGCAGGGCCTTGCCCAGAGATGCCTCCACCGCTGCCTA

**ApaI**: (rs7975232) [G>T polymorphism]

Apa-I-cutsite_1

|  |  |  |  |
| --- | --- | --- | --- |
| Wild type | GG | 2 fragments | 615 + 217 bp |
| Heterozygous | GT | 3 fragments | 832 + 615 + 217 bp |
| Homozygous mutant | TT | 1 fragment | 832 bp |

**VDR - TaqI**

Primers: CAGAGCATGGACAGGGAGCAA / TAGGCAGCGGTGGAGGCATCTCT

[product length = 832]

CAGAGCATGGACAGGGAGCAAGGCCAGGCAGGGACAGGGCCAGGTGCGCCCATGGAAGGACCTAGGTCTGGATCCTAAATGCACGGAGAAGTCACTGGAGGGCTTTGGGGCCAGGCAGTGGTATCACCGGTCAGCAGTCATAGAGGGGTGGCCTAGGGGGTGCTGCCGTTGAGTGTCTGTGTGGGTGGGGGGTGGTGGGATTGAGCAGTGAGGGGCCCAGCTGAGAGCTCCTGTGCCTTCTTCTCTATCCCCGTGCCCACAGATCGTCCTGGGGTGCAGGACGCCGCGCTGATTGAGGCCATCCAGGACCGCCTGTCCAACACACTGCAGACGTACATCCGCTGCCGCCACCCGCCCCCGGGCAGCCACCTGCTCTATGCCAAGATGATCCAGAAGCTAGCCGACCTGCGCAGCCTCAATGAGGAGCACTCCAAGCAGTACCGCTGCCTCTCCTTCCAGCCTGAGTGCAGCATGAAGCTAACGCCCCTTGTGCT↓CGAAGTGTTTGGCAATGAGATCTCCTGACTAGGACAGCCTGTGGCGGTGCCTGGGTGGGGCTGCTCCTCCAGGGCCACGTGCCAGGCCCGGGGCTGGCGGCTACTCAGCAGCCCTCCTCACCCCGTCTGGGGTTCAGCCCCTCCTCTGCCACCTCCCCTATCCACCCAGCCCATTCTCTCTCCTGTCCAACCTAACCCCTTTCCTGCGGGCTTTTCCCCGGTCCCTTGAGACCTCAGCCATGAGGAGTTGCTGTTTGTTTGACAAAGAAACCCAAGTGGGGGCAGAGGGCAGAGGCTGGAGGCAGGGCCTTGCCCAGAGATGCCTCCACCGCTGCCTA

**TaqI**: (rs731236): T to C polymorphism exists (ATT to ATC)

Taq-I-cutsite_1

|  |  |  |  |
| --- | --- | --- | --- |
| Wild type | TT | 2 fragments | 494 + 338 bp |
| Heterozygous | TC | 4 fragments | 494 + 338 + 293 + 201 bp |
| Homozygous mutant | CC | 3 fragment | 338 + 293 + 201 bp |

**Table S1**

Blood lead levels in environmentally exposed children with different genotypic combinations of VDR gene

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Group | Genotype combination |  | BLL (μg/dL) | | |
| N | Mean ± SD | *p* value | Range |
| G1 | bbFfAATT | 5 | 37.8 ± 14.9 | Ref | 24.1 – 60.8 |
| G2 | bbFFAATT | 6 | 23.4 ± 10.5 | NS | 10.5 – 35.1 |
| G3 | bbFFAATt | 12 | 19.8 ± 10.2 | \* | 4.7 – 42.2 |
| G4 | bbFFAaTT | 20 | 18.0 ± 11.3 | \* | 4.5 – 41.8 |
| G5 | bbFFaaTT | 11 | 24.6 ± 7.9 | NS | 11.1 – 34.8 |
| G6 | bbFFAaTt | 11 | 12.7 ± 6.3 | \*\* | 4.9 – 24 |
| G7 | bbFfAaTT | 8 | 15.2 ± 8.5 | \*\* | 3.4 – 26.2 |
| G8 | bbFfAATt | 4 | 14.2 ± 10.1 | \* | 3.9 – 24.5 |
| G9 | bbFfAaTt | 4 | 18.8 ± 18.4 | NS | 5.1 – 45.8 |
| G10 | BbFFAATT | 4 | 22.9 ± 13.4 | NS | 4.4 – 33.5 |
| G11 | BbFFAaTT | 6 | 21.0 ± 11.4 | NS | 10.7 – 40.4 |
| G12 | BbFFAATt | 9 | 17.6 ± 6.4 | \*\* | 5.9 – 23.2 |
| G13 | BbFFAaTt | 5 | 20.9 ± 9.1 | NS | 8.8 – 30.4 |
| G14 | BbFFAAtt | 5 | 17.9 ± 10.7 | NS | 4.7 – 34.1 |
| G15 | BbFfAATT | 6 | 13.9 ± 2.9 b | \*\* | 10.8 – 19.2 |
| G16 | BbFfAaTT | 3 | 16.7 ± 12.8 | NS | 1.65 – 25.1 |
| G17 | BbFfAATt | 6 | 17.2 ± 12.6 | NS | 1.65 – 33.5 |
| G18 | BbFfAaTt | 4 | 16.7 ± 5.1 | \* | 10.2 – 21.2 |
| G19 | bbFfaaTT | 2 | 30.3 ± 6.6 |  | 25.6, 34.9 |
| G20 | BbffAATt | 2 | 29.8 ± 6.4 |  | 25.3, 34.3 |
| G21 | bbffAaTT | 1 | 6.7 |  |  |
| G22 | bbffAATt | 1 | 24.8 |  |  |
| G23 | bbffAaTt | 1 | 19.3 |  |  |
| G24 | bbFFaaTT | 1 | 32.4 |  |  |
| G25 | BBFFAATT | 1 | 7.6 |  |  |
| G26 | BBFFAATt | 1 | 26.6 |  |  |
| G27 | BBFfAATT | 1 | 17 |  |  |
| N: Number of children; BLL: Blood lead level; Ref: Reference; NS: Not significant  \* = p < 0.05; \*\* = p < 0.01; Nonparametric Wilcoxon analysis for comparison for each pair for sample numbers with greater than three | | | | | |