Supplementary Information SI9

Alignment of AHR protein sequences

Alignment of the modern human (Ensembl ID: ENSP00000242057.4) and Neanderthal AHR proteins encoded by the mRNA sequences used in this study (based on ENST00000242057.8), and the modern human and Neanderthal AHR proteins studied by Hubbard et al. (2016), illustrating that the AHR protein variants compared in both studies are identical. To depict the substantial amino acid sequence divergence, in addition to the Ala381Val polymorphism, of the modern human "Val-type" and ancient hominin "Ala-type" AHR proteins versus the rodent "Ala-type" AHRs, the amino acid sequences of the mouse and rat AHR proteins from the Ensemble database (Release 99 - January 2020) are also included. Amino acid residues that differ from the modern human AHR amino acid sequence encoded by the modern human *AHR* reference transcript (ENST00000242057.8) are highlighted in red. Alignment was performed using the ClustalW module of Lasergene DNAstar (version 10) with default parameter settings.

<u>Reference:</u>

Hubbard TD, Murray IA, Bisson WH, Sullivan AP, Sebastian A, Perry GH, Jablonski NG, Perdew GH. 2016. Divergent Ah Receptor Ligand Selectivity during Hominin Evolution. *Mol Biol Evol* 33(10):2648-2658.

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                                          10        20        30        40        50
Human_AHR-201_ENSP00000242057.4.pro       MNSSSANI TYASRKRRKPVQKTVKPI PAEGI KSNPSKRHRDRLNTEL DRL  50
Neanderthal_AHR_this study.pro            MNSSSANI TYASRKRRKPVQKTVKPI PAEGI KSNPSKRHRDRLNTEL DRL  50
Human_AHR_Hubbard.pro                     MNSSSANI TYASRKRRKPVQKTVKPI PAEGI KSNPSKRHRDRLNTEL DRL  50
Neanderthal_AHR_Hubbard.pro               MNSSSANI TYASRKRRKPVQKTVKPI PAEGI KSNPSKRHRDRLNTEL DRL  50
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    - MSSGANI TYASRKRRKPVQKTVKPI PAEGI KSNPSKRHRDRLNTEL DRL  49
Rat_Ahr-201_ENSRNOP00000006618.5.pro      - MSSGANI TYASRKRRKPVQKTVKPVPAEGI KSNPSKRHRDRLNTEL DRL  49

                                          60        70        80        90       100
Human_AHR-201_ENSP00000242057.4.pro       ASLLPFPQDVI NKLDKLSVLRLSVSYLRAKSFFDVALKSSPTERNGGQDN  100
Neanderthal_AHR_this study.pro            ASLLPFPQDVI NKLDKLSVLRLSVSYLRAKSFFDVALKSSPTERNGGQDN  100
Human_AHR_Hubbard.pro                     ASLLPFPQDVI NKLDKLSVLRLSVSYLRAKSFFDVALKSSPTERNGGQDN  100
Neanderthal_AHR_Hubbard.pro               ASLLPFPQDVI NKLDKLSVLRLSVSYLRAKSFFDVALKSSPTERNGGQDN  100
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    ASLLPFPQDVI NKLDKLSVLRLSVSYLRAKSFFDVALKSTPADRNGGQDQ  99
Rat_Ahr-201_ENSRNOP00000006618.5.pro      ASLLPFPQDVI SKLDKLSVLRLSVSYLRAKSFFDVALKSTPADRSRGQDQ  99

                                          110       120       130       140       150
Human_AHR-201_ENSP00000242057.4.pro       CRAANFREGLNLQEGEFLLQALNGFVLVVTTDALVFYASSTI QDYLGFQQ  150
Neanderthal_AHR_this study.pro            CRAANFREGLNLQEGEFLLQALNGFVLVVTTDALVFYASSTI QDYLGFQQ  150
Human_AHR_Hubbard.pro                     CRAANFREGLNLQEGEFLLQALNGFVLVVTTDALVFYASSTI QDYLGFQQ  150
Neanderthal_AHR_Hubbard.pro               CRAANFREGLNLQEGEFLLQALNGFVLVVTTDALVFYASSTI QDYLGFQQ  150
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    CRAQ-IRDWQDLQEGEFLLQALNGFVLVVTADALVFYASSTI QDYLGFQQ  148
Rat_Ahr-201_ENSRNOP00000006618.5.pro      CRAQ-VRDWQDLQEGEFLLQALNGFVLVVTADALVFYASSTI QDYLGFQQ  148

                                          160       170       180       190       200
Human_AHR-201_ENSP00000242057.4.pro       SDVI HQSVYELI HTEDRAEFQRQLHWALNPSQCTESGQGI EEATGLPQTV  200
Neanderthal_AHR_this study.pro            SDVI HQSVYELI HTEDRAEFQRQLHWALNPSQCTESGQGI EEATGLPQTV  200
Human_AHR_Hubbard.pro                     SDVI HQSVYELI HTEDRAEFQRQLHWALNPSQCTESGQGI EEATGLPQTV  200
Neanderthal_AHR_Hubbard.pro               SDVI HQSVYELI HTEDRAEFQRQLHWALNPSQCTESGQGI EEATGLPQTV  200
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    SDVI HQSVYELI HTEDRAEFQRQLHWALNP----DSAQGVDEAHGPPQAA  194
Rat_Ahr-201_ENSRNOP00000006618.5.pro      SDVI HQSVYELI HTEDRAEFQRQLHWALNPSQCTDSAQGVDETHGLPQPA  198

                                          210       220       230       240       250
Human_AHR-201_ENSP00000242057.4.pro       VCYNPDQI PPENSPLMERCFI CRLRCLLDNSSGFLAMNFQGKLKYLHGQK  250
Neanderthal_AHR_this study.pro            VCYNPDQI PPENSPLMERCFI CRLRCLLDNSSGFLAMNFQGKLKYLHGQK  250
Human_AHR_Hubbard.pro                     VCYNPDQI PPENSPLMERCFI CRLRCLLDNSSGFLAMNFQGKLKYLHGQK  250
Neanderthal_AHR_Hubbard.pro               VCYNPDQI PPENSPLMERCFI CRLRCLLDNSSGFLAMNFQGKLKYLHGQK  250
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    VYYTPDQLPPENASFMERCFRCRLRCLLDNSSGFLAMNFQGRLKYLHGQN  244
Rat_Ahr-201_ENSRNOP00000006618.5.pro      VYYTPDQLPPENTAFMERCFRCRLRCLLDNSSGFLAMNFQGRLKYLHGQN  248

                                          260       270       280       290       300
Human_AHR-201_ENSP00000242057.4.pro       KKGKDGSI LPPQLALFAI ATPLQPPSI LEI RTKNFI FRTKHKLDFTPI GC  300
Neanderthal_AHR_this study.pro            KKGKDGSI LPPQLALFAI ATPLQPPSI LEI RTKNFI FRTKHKLDFTPI GC  300
Human_AHR_Hubbard.pro                     KKGKDGSI LPPQLALFAI ATPLQPPSI LEI RTKNFI FRTKHKLDFTPI GC  300
Neanderthal_AHR_Hubbard.pro               KKGKDGSI LPPQLALFAI ATPLQPPSI LEI RTKNFI FRTKHKLDFTPI GC  300
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    KKGKDGAL LPPQLALFAI ATPLQPPSI LEI RTKNFI FRTKHKLDFTPI GC  294
Rat_Ahr-201_ENSRNOP00000006618.5.pro      KKGKDGAL LPPQLALFAI ATPLQPPSI LEI RTKNFI FRTKHKLDFTPI GC  298
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                                          310       320       330       340       350
Human_AHR-201_ENSP00000242057.4.pro       DAKGRI VLGYTEAELCTRGSGYQFI HAADMLYCAESHI RMI KTGESGMI V  350
Neanderthal_AHR_this study.pro            DAKGRI VLGYTEAELCTRGSGYQFI HAADMLYCAESHI RMI KTGESGMI V  350
Human_AHR_Hubbard.pro                     DAKGRI VLGYTEAELCTRGSGYQFI HAADMLYCAESHI RMI KTGESGMI V  350
Neanderthal_AHR_Hubbard.pro               DAKGRI VLGYTEAELCTRGSGYQFI HAADMLYCAESHI RMI KTGESGMI V  350
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    DAKGQLI LGYTEVELCTRGSGYQFI HAADILHCAESHI RMI KTGESGMTV  344
Rat_Ahr-201_ENSRNOP00000006618.5.pro      DAKGQLI LGYTEVELCNKGSGYQFI HAADMLHCAESHI RMI KTGESGMTV  348

                                          360       370       380       390       400
Human_AHR-201_ENSP00000242057.4.pro       FRLLTKNNRWTWVQSNARLLYKNGRPDYI I VTQRPLTDEEGTEHLRKRNT  400
Neanderthal_AHR_this study.pro            FRLLTKNNRWTWVQSNARLLYKNGRPDYI I ATQRPLTDEEGTEHLRKRNT  400
Human_AHR_Hubbard.pro                     FRLLTKNNRWTWVQSNARLLYKNGRPDYI I VTQRPLTDEEGTEHLRKRNT  400
Neanderthal_AHR_Hubbard.pro               FRLLTKNNRWTWVQSNARLLYKNGRPDYI I ATQRPLTDEEGTEHLRKRNT  400
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    FRLLAKHSRWRWVQSNARLIYRNGRPDYI I ATQRPLTDEEGREHLQKRST  394
Rat_Ahr-201_ENSRNOP00000006618.5.pro      FRLLAKHSRWRWVQSNARLIYRNGRPDYI I ATQRPLTDEEGREHLQKRSM  398

                                          410       420       430       440       450
Human_AHR-201_ENSP00000242057.4.pro       KLPFMFTTGEAVLYEATNPFPAI MDPLPLRTKNGTSGKDSATTSTLSKDS  450
Neanderthal_AHR_this study.pro            KLPFMFTTGEAVLYEATNPFPAI MDPLPLRTKNGTSGKDSATTSTLSKDS  450
Human_AHR_Hubbard.pro                     KLPFMFTTGEAVLYEATNPFPAI MDPLPLRTKNGTSGKDSATTSTLSKDS  450
Neanderthal_AHR_Hubbard.pro               KLPFMFTTGEAVLYEATNPFPAI MDPLPLRTKNGTSGKDSATTSTLSKDS  450
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    SLPFMFATGEAVLYEISSPFSPI MDPLPIRTKSNTSRKDWAPQSTPSKDS  444
Rat_Ahr-201_ENSRNOP00000006618.5.pro      TLPFMFATGKAVLYEISSPFSPI MDPLPIRTKSNTSRKDWAPQSTPSKDS  448

                                          460       470       480       490       500
Human_AHR-201_ENSP00000242057.4.pro       LNPSSLLAAMMQQDESI YLYPASSTSSTAPFENNFFNESMNECRNWQDNT  500
Neanderthal_AHR_this study.pro            LNPSSLLAAMMQQDESI YLYPASSTSSTAPFENNFFNESMNECRNWQDNT  500
Human_AHR_Hubbard.pro                     LNPSSLLAAMMQQDESI YLYPASSTSSTAPFENNFFNESMNECRNWQDNT  500
Neanderthal_AHR_Hubbard.pro               LNPSSLLAAMMQQDESI YLYPASSTSSTAPFENNFFNESMNECRNWQDNT  500
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    FHPSSLMSALIQQDESI YLCPPS---SPALLDSHFLMGSVSKCGSWQDSF  491
Rat_Ahr-201_ENSRNOP00000006618.5.pro      FHPNSLMSALIQQDESI YLCPPS---SPAPLDSHFLMDSMSECGSWQGSF  495

                                          510       520       530       540
Human_AHR-201_ENSP00000242057.4.pro       APMGNDTILKHEQI DQPQDVN-SFAGGHPGLFQDSKNSDLYSI MKNLGI D  549
Neanderthal_AHR_this study.pro            APMGNDTILKHEQI DQPQDVN-SFAGGHPGLFQDSKNSDLYSI MKNLGI D  549
Human_AHR_Hubbard.pro                     APMGNDTILKHEQI DQPQDVN-SFAGGHPGLFQDSKNSDLYSI MKNLGI D  549
Neanderthal_AHR_Hubbard.pro               APMGNDTILKHEQI DQPQDVN-SFAGGHPGLFQDSKNSDLYSI MKNLGI D  549
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    AAAGSEAALKHEQI GHAQDVNLALSGGPSELFPDNKNNDLYSI MRNLGI D  541
Rat_Ahr-201_ENSRNOP00000006618.5.pro      AAASNEALLKHEEI RHTQDVNLTLSGGPSELFPDNKNNDLYSI MRNLGI D  545

                                          550       560       570       580       590
Human_AHR-201_ENSP00000242057.4.pro       FEDI RHMQNEKFFRNDF---SGEVDFRDI DLTDEI LTYVQDSLSKSPFI PS  597
Neanderthal_AHR_this study.pro            FEDI KHMQNEKFFRNDF---SGEVDFRDI DLTDEI LTYVQDSLSKSPFI PS  597
Human_AHR_Hubbard.pro                     FEDI RHMQNEKFFRNDF---SGEVDFRDI DLTDEI LTYVQDSLSKSPFI PS  597
Neanderthal_AHR_Hubbard.pro               FEDI KHMQNEKFFRNDF---SGEVDFRDI DLTDEI LTYVQDSLSKSPFI PS  597
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    FEDI RSMQNEEFFRTDSTAAGEVDFKDI DITDEI LTYVQDSLNNSTLLNS  591
Rat_Ahr-201_ENSRNOP00000006618.5.pro      FEDI RSMQNEEFFRTDS---SGEVDFKDI DITDEI LTYVQDSLNNSTLLNS  593

                                          600       610       620       630       64
Human_AHR-201_ENSP00000242057.4.pro       DYQQQQSLALNSSCMVQEHLHLEQQQQHHQK---QVVVEPQQQLCQ-----  640
Neanderthal_AHR_this study.pro            DYQQQQSLALNSSCMVQEHLHLEQQQQHHQK---QVVVEPQQQLCQ-----  640
Human_AHR_Hubbard.pro                     DYQQQQSLALNSSCMVQEHLHLEQQQQHHQK---QVVVEPQQQLCQ-----  640
Neanderthal_AHR_Hubbard.pro               DYQQQQSLALNSSCMVQEHLHLEQQQQHHQK---QVVVEPQQQLCQ-----  640
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    ACQQQ-PVTQHLSCMLQERLQLEQQQQLQQP-PPQALEPQQQLCQMVCPQ  639
Rat_Ahr-201_ENSRNOP00000006618.5.pro      ACQQQ-PVSQHLSCMLQERLQLEQQQQLQQQHPTQTLEPQRQLCQVEVPQ  642

                                          650       660       670       680
Human_AHR-201_ENSP00000242057.4.pro       -----KMKHMQVNGMFENWNSNQFVPFNCPQQDPQQYNVFTDLHGI SQEF  685
Neanderthal_AHR_this study.pro            -----KMKHMQVNGMFENWNSNQFVPFNCPQQDPQQYNVFTDLHGI SQEF  685
Human_AHR_Hubbard.pro                     -----KMKHMQVNGMFENWNSNQFVPFNCPQQDPQQYNVFTDLHGI SQEF  685
Neanderthal_AHR_Hubbard.pro               -----KMKHMQVNGMFENWNSNQFVPFNCPQQDPQQYNVFTDLHGI SQEF  685
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    QDLG--PKHTQINGTFASWNPTPPVSFNCPQQELKHYQLFSSLQGTAQEF  687
Rat_Ahr-201_ENSRNOP00000006618.5.pro      HELGQKTKHMQVNGMFASWNPAPPVSFSCPQQERKHYSLFSGLQGTAQEF  692

                                          690       700       710       720       730
Human_AHR-201_ENSP00000242057.4.pro       PYKSEMDSMPYTQNFI SCNQPVLPQHSKCTELDYPMGSFEPSPYPTTSSL  735
Neanderthal_AHR_this study.pro            PYKSEMDSMPYTQNFI SCNQPVLPQHSKCTELDYPMGSFEPSPYPTTSSL  735
Human_AHR_Hubbard.pro                     PYKSEMDSMPYTQNFI SCNQPVLPQHSKCTELDYPMGSFEPSPYPTTSSL  735
Neanderthal_AHR_Hubbard.pro               PYKSEMDSMPYTQNFI SCNQPVLPQHSKCTELDYPMGSFEPSPYPTTSSL  735
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    PYKPEVDSVPYTQNFAPCNQPLLPEHSKSVQLDFPGRDFEPSLHPTTSNL  737
Rat_Ahr-201_ENSRNOP00000006618.5.pro      PYKSEVDSMPYTQNFAPCNQSLLPEHSKGTQLDFPGRDFERSLHPNASNL  742
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                                          740       750       760       770       780
Human_AHR-201_ENSP00000242057.4.pro       EDFVTCLQLPENQKHGLNPQSAIITPQTCYAGAVSMYQCQPEPQHTHVGQ 785
Neanderthal_AHR_this study.pro            EDFVTCLQLPENQKHGLNPQSAIITPQTCYAGAVSMYQCQPEPQHTHVGQ 785
Human_AHR_Hubbard.pro                     EDFVTCLQLPENQKHGLNPQSAIITPQTCYAGAVSMYQCQPEPQHTHVGQ 785
Neanderthal_AHR_Hubbard.pro               EDFVTCLQLPENQKHGLNPQSAIITPQTCYAGAVSMYQCQPEPQHTHVGQ 785
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    D-FVSCLQVPENQSHGINSQSAMVSPQAYYAGAMSMYQCQPGPQRTPVDQ 786
Rat_Ahr-201_ENSRNOP00000006618.5.pro      EDFVSCLQVPENQRHGINSQSAMVSPQAYYAGAMSMYQCQAGPQHTPVDQ 792

                                          790       800       810       820       830
Human_AHR-201_ENSP00000242057.4.pro       MQYNPVLPGQQAFLNKFQN-GVLNETYPAELNNINNTQTTTHLQPLHHPS 834
Neanderthal_AHR_this study.pro            MQYNPVLPGQQAFLNKFQN-GVLNETYPAELNNINNTQTTTHLQPLHHPS 834
Human_AHR_Hubbard.pro                     MQYNPVLPGQQAFLNKFQN-GVLNETYPAELNNINNTQTTTHLQPLHHPS 834
Neanderthal_AHR_Hubbard.pro               MQYNPVLPGQQAFLNKFQN-GVLNETYPAELNNINNTQTTTHLQPLHHPS 834
Mouse_Ahr-202_ENSMUSP00000112137.2.pro    TQYSSEIPGSQAFLSKVQS                                805
Rat_Ahr-201_ENSRNOP00000006618.5.pro      MQYSPEIPGSQAFLSKFQSPSILNEAYSADLSSIGHLQTAAHLP---RLA 839

                                          840
Human_AHR-201_ENSP00000242057.4.pro       EARPFPDLTSSGFL                                     848
Neanderthal_AHR_this study.pro            EARPFPDLTSSGFL                                     848
Human_AHR_Hubbard.pro                     EARPFPDLTSSGFL                                     848
Neanderthal_AHR_Hubbard.pro               EARPFPDLTSSGFL                                     848
Mouse_Ahr-202_ENSMUSP00000112137.2.pro                                                       805
Rat_Ahr-201_ENSRNOP00000006618.5.pro      EAQPLPDITPSGFL                                     853
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