The American Journal of Human Genetics, Volume *93*

# Supplemental Data

# An X-Linked Cobalamin Disorder Caused by Mutations in Transcriptional Coregulator *HCFC1*

**Hung-Chun Yu, Jennifer L. Sloan, Gunter Scharer, Alison Brebner, Anita M. Quintana, Nathan P. Achilly, Irini Manoli, Curtis R. Coughlin II, Elizabeth A. Geiger, Una Schneck, David Watkins, Terttu Suormala, Johan L.K. Van Hove, Brian Fowler, Matthias R. Baumgartner, David S. Rosenblatt, Charles P. Venditti, and Tamim H. Shaikh**

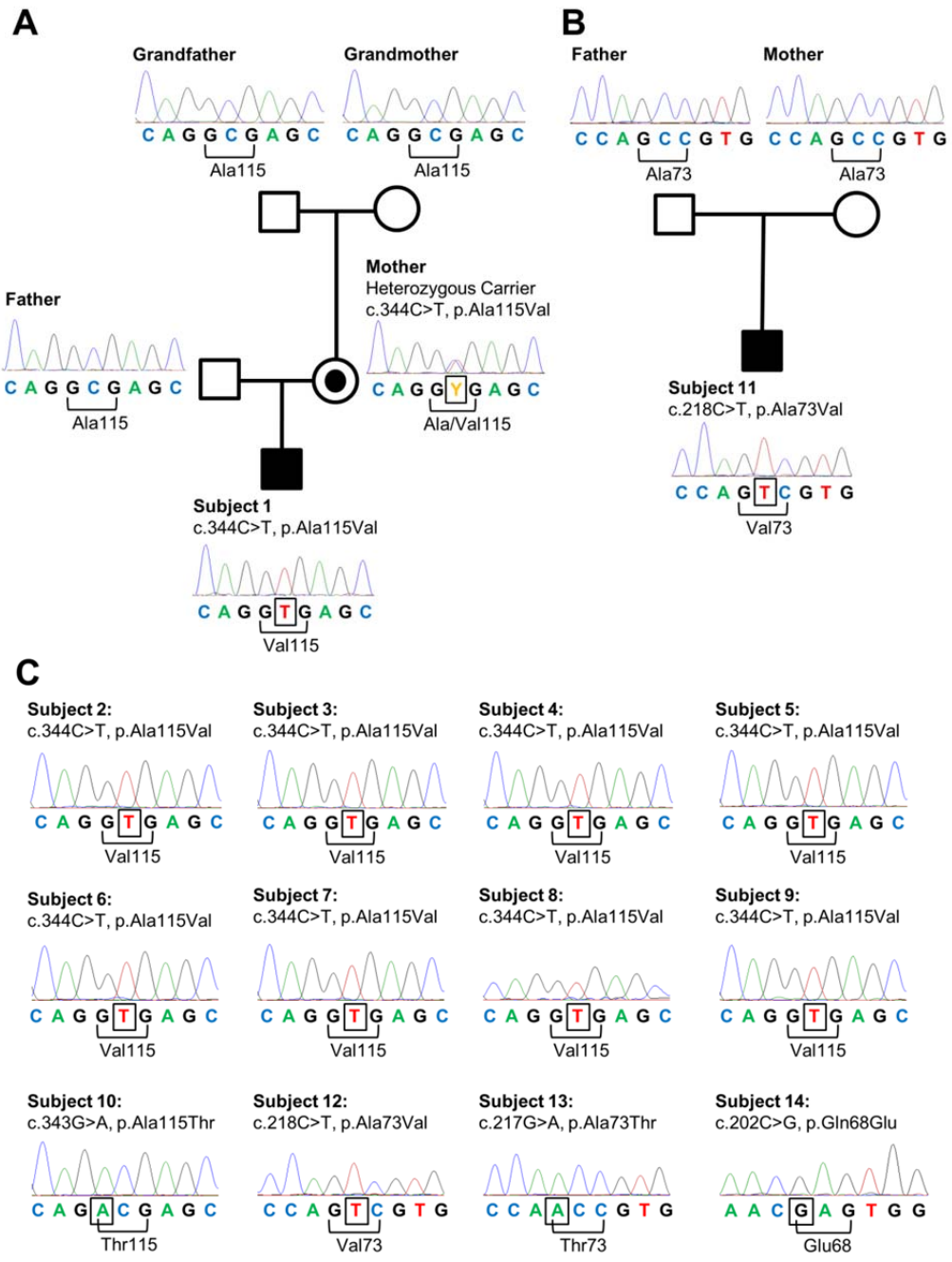


**Figure S1. Pathogenic Variants in *HCFC1* Verified by Sanger Sequencing**

(A) Partial chromatograms showing Sanger sequencing results of Exon 3 of *HCFC1* in the family of Subject 1. Subject 1 has a missense mutation c.344C>T (p.Ala115Val) (black box) inherited from his mother who is a heterozygous carrier with one copy each of the C and T (*de novo*) allele shown as Y (pyrimidine), due to dual peaks. (B) Partial chromatograms showing Sanger sequencing results of Exon 2 of *HCFC1* in Subject 11 and his parents. Subject 11 has a *de novo* mutation c.218C>T (p.Ala73Val) (black box). Both parents carry the normal C allele. (C) Subject 2-9 have c.344C>T (p.Ala115Val) mutation, Subject 10 has c.343G>A (p.Ala115Thr) mutation, Subject 12 has c.218C>T (p.Ala73Val) mutation, Subject 13 has c.217G>A (p.Ala73Thr) mutation, and Subject 14 has c.202C>G (p.Gln68Glu) mutation.

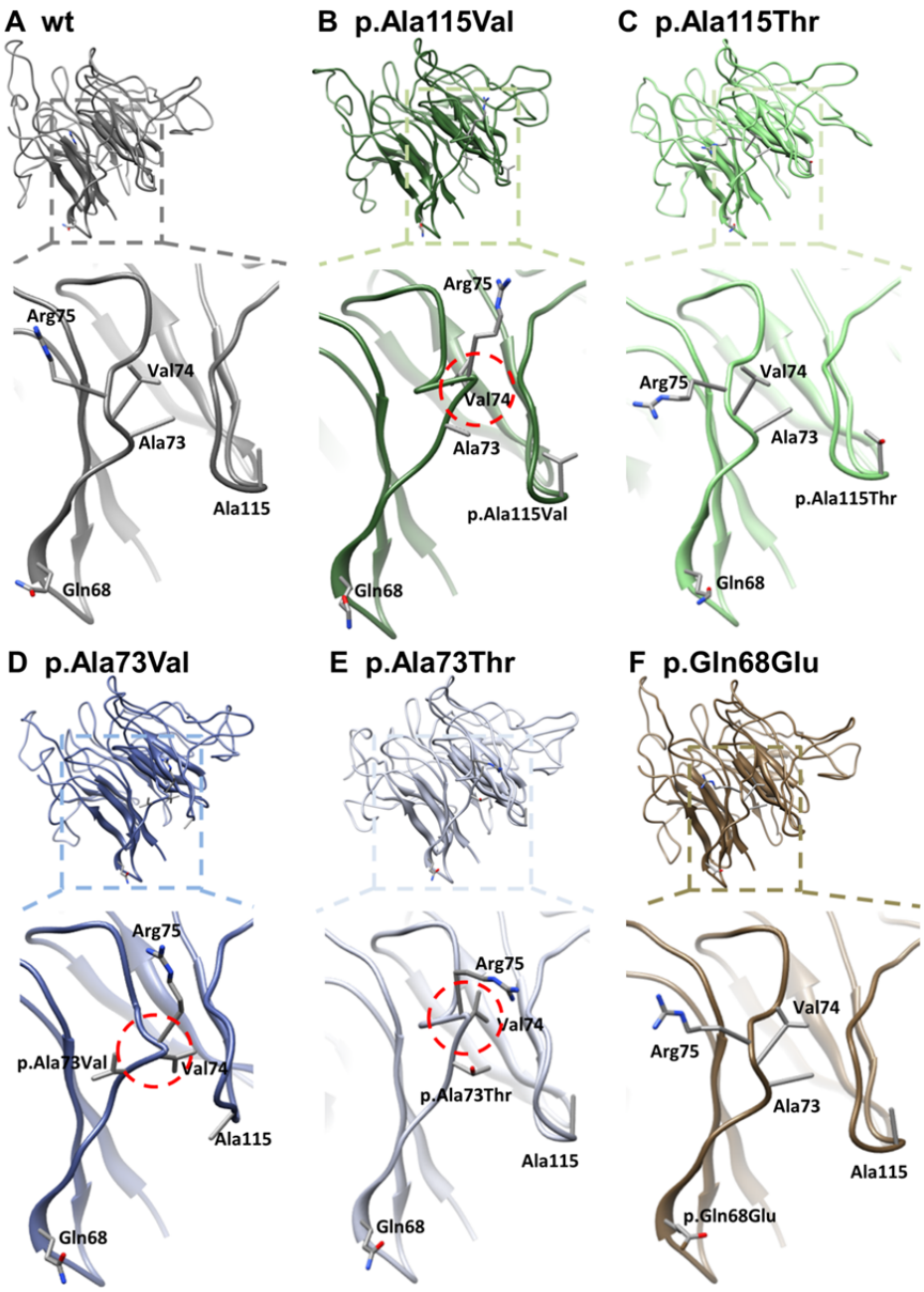


**Figure S2. Protein Modeling of Wild-type and Mutant HCFC1**

The entire protein is shown on the top and the expanded view of the region where the mutations have occurred in shown in the bottom. (A) Predicted structure of wild-type HCFC1 with a close-up of the region containing Gln 68, Ala73, Val74, Arg75, and Ala115 in the bottom panel. The side chains of these amino acids are also shown. The predicted structural changes due to the p.Ala115Val (B), p.Ala115Thr (C), p.Ala73Val (D), p.Ala73Thr (E) and p.Gln68Glu (F) mutations as predicted by Modeller[1]. In p.Ala115Val, p.Ala73Val and p.Ala73Thr mutations, the close-up view indicates that this amino acid change generates a conformational change resulting in a kink (red dashed circle) between Val74 and Arg75. The p.Gln68Glu mutation did not generate a similar conformational change.

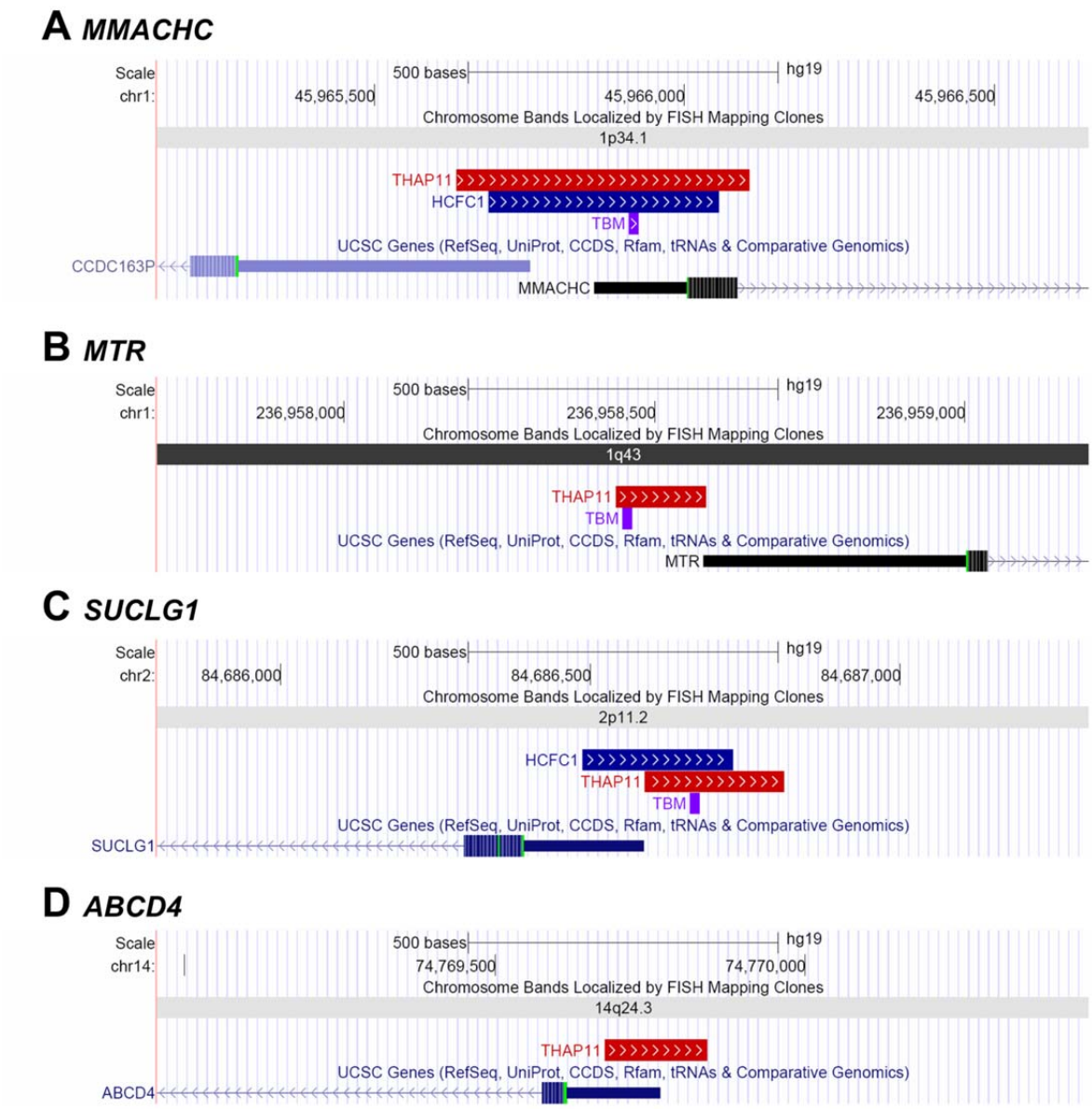


**Figure S3. HCFC1 and THAP11 Binding Regions**

Predicted binding sites for HCFC1 and THAP11 based on mouse ChIP-Seq data[2]. The mouse genome coordinates were converted to the human genome coordinates (NCBI build 37) using Liftover tool (http://genome.ucsc.edu/cgi-bin/hgLiftOver). The sequences bound to HCFC1 are shown in red and to THAP11 are shown in blue. The conserved THAP11 Binding Motif (TBM) is shown in purple. (A) The region of human chromosome 1 (1p34.1) containing the *MMACHC* gene is shown. (B) The region of human chromosome 1 (1q43) containing the *MTR* gene, (C) the region of human chromosome 2 (2p11) containing the *SUCLG1* gene, and (D) the region of human chromosome 14 (14q24) containing the *ABCD4* is shown.


**Figure S4. HCFC1 Expression in siRNA Knockdown Cells**

Western blot of *HCFC1* siRNA knockdown in HEK293 cells. Three biological replicates of *HCFC1* siRNA treated cells are shown in the left and replicates of scrambled siRNA treated HEK293cells are shown in the right. In the bottom panel, β–actin was used as a loading control.

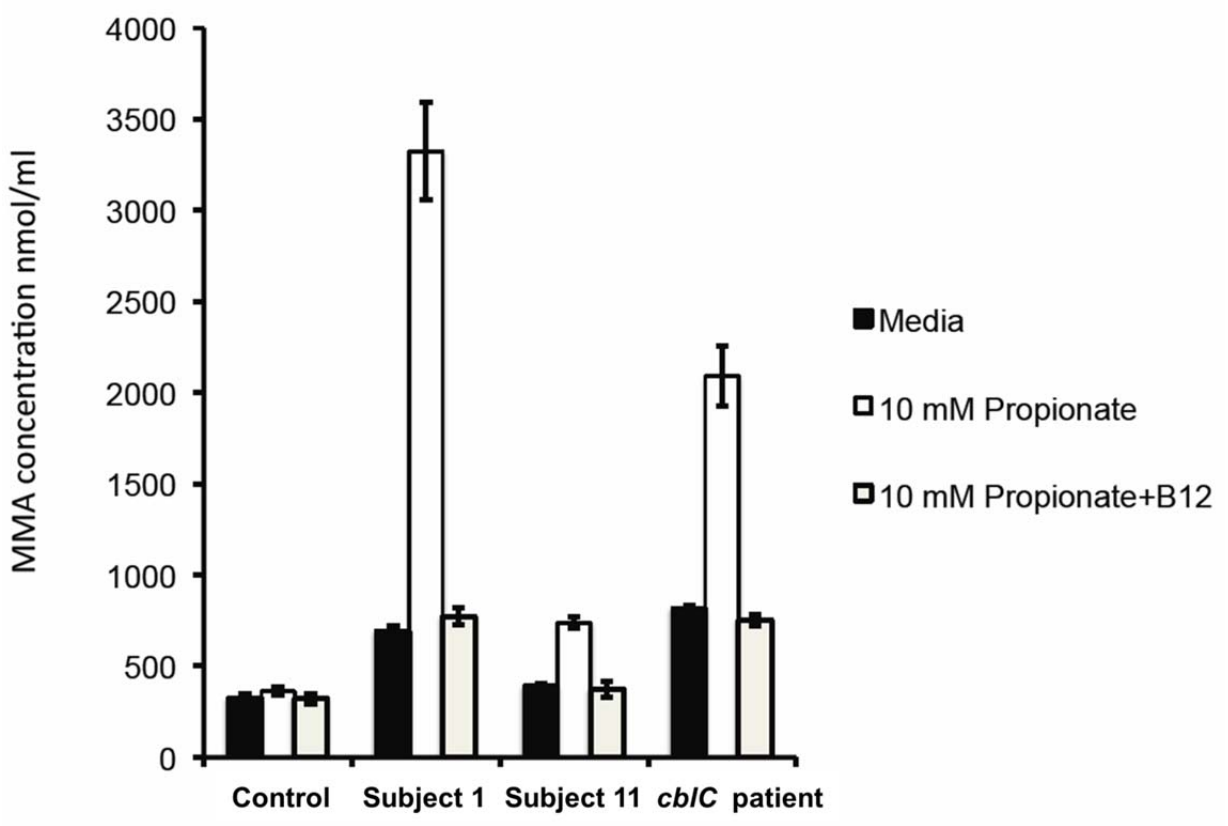


**Figure S5. MMA Production by *cblX* Fibroblasts in Cell Culture**

$5x10^5$ cells, plated in triplicate, were incubated in complete DMEM medium (solid black bar), medium with 10 mM propionate added (white bar) or medium with 10 mM propionate and 1 ug/ml hydroxocobalamin (grey bar) for 72 hours. The concentration of MMA (nmol/ml) was assayed in the medium. Fibroblasts from Subjects 1 and 11 show a 5.9- or 2.2-fold increase after incubation in propionic acid that was corrected with the addition of exogenous hydroxocobalamin to the medium, comparable to the response of the *cblC* cell line, which rises 2.6 fold after identical treatment and also responds to exogenous hydroxocobalamin. Error bars , +/- 1 s.d. (n=3 replicates per cell line).

**Table S1. PCR Primers**

| | 5’-3’ |
|---|---|
| Validation of WES result in Subject 1 | |
| HCFC1_validation_forward | CACCTTGGAATGTTGTTCTTTGGGTCCTCG |
| HCFC1_validation _reverse | TGACCTCTACGAACTCCAGGTAAAGCAGCC |
| TTN_validation _forward_1 | CAAGTACTTTGCAATTAACGGCCACAGACC |
| TTN_validation _reverse_1 | AGAGATCCTTGTGACCCTCCTGGACAACC |
| TTN_validation _forward_2 | GTGTTTTTATTGCTCTCACCCATCGCAGGC |
| TTN_validation _reverse_2 | AAGCAACACTTACATGGACACCTCCCTTGG |
| | |
| Mutation Analysis in other Subjects | |
| HCFC1_exon1_forward | ACCAGTCGGACTAGGGGCTTTCTTTTAACTAACG |
| HCFC1_exon1_reverse | ATGGAGTCGTCTACCGCTGTGTGAGAAACC |
| HCFC1_exon2_forward | TCTTTGCTTTGAGTCTCTTCCACTCCCACC |
| HCFC1_exon2_reverse | AGTGGCTAGATTCCCAGTAGCTGATGAGGG |
| HCFC1_exon3_forward | TGGGACTACCTCCAGACAGAGAGTCCTTGG |
| HCFC1_exon3_reverse | GACCTCTACGAACTCCAGGTAAAGCAGCC |
| HCFC1_exon4_forward | ATGACAAGAGGAAAGCACGAAGTGGTATGC |
| HCFC1_exon4_reverse | CCAGTTGGACTCATCCTAGGGAGCTTCTGG |
| HCFC1_exon5&6_forward | GAGGCTTTTGCACTTTTGGATGTTGGGC |
| HCFC1_exon5&6_reverse | GTACCTGAAGGAGCGCTATTGCTAGCCTGG |
| HCFC1_exon7&8_forward | CTACAACCCTTGGAGACAAGCCCAAAGC |
| HCFC1_exon7&8_reverse | TTGACAACAAAAGAACCAAGGGCAACCAGGG |
| HCFC1_exon9_forward | TATTAATTCCCACCATGACCTCGCAGGAGC |
| HCFC1_exon9_reverse | CTTTGGGCTTGTCTCCAAGGGTTGTAGGG |
| HCFC1_exon10_forward | AAAGCGATGGGGAAACCACCCAAGATGTCC |
| HCFC1_exon10_reverse | GGTCTGGCAGTAGAGAAAGCTGAGCCATGC |
| HCFC1_exon11&12_forward | GCTGAGGTCACCAGCTTCAGGATTGTTCCC |
| HCFC1_exon11&12_reverse | GAAGGTGAGTGTTGAGGCAGGAGCTATGGG |
| HCFC1_exon13&14_forward | TTAAGCACCACCTGGAACACCAGAGGAAAG |
| HCFC1_exon13&14_reverse | CATCCAGGTGAGCTCTCAGTCTTTGCACAT |
| HCFC1_exon15&16_forward | GACAGCCAAACCACGGACTATGACACAGGG |
| HCFC1_exon15&16_reverse | CACCTGCGAAAATCATCACTGCTGTCCC |
| HCFC1_exon17_forward_1 | CGACATGGCTGTAGTGGCAGTGTTGGTGG |
| HCFC1_exon17_reverse_1 | CCTCTTCCTCATCCCAGTTGCCTTTGAGG |
| HCFC1_exon17_forward_2 | CTCACATGGTGGGTTGGAGCAGACTTGG |
| HCFC1_exon17_reverse_2 | GCTGCTTCTCTTGTGACCTCGACTGTGG |
| HCFC1_exon17_forward_3 | AACTCATGTTGGAAGTGACAGTGGTGGCCG |
| HCFC1_exon17_reverse_3 | CTTTTGTGCAGTTGGCCCCTCTGAGC |
| HCFC1_exon18_forward | GTCTTATACAGGCTGCGGTGAAGTGACACG |
| HCFC1_exon18_reverse | CACTTCTTCCCACAGGACCGTGGAGTCTGG |
| HCFC1_exon19_forward | CCTGTTCTACACGGCAGTCTCTACTACTTGG |
| HCFC1_exon19_reverse | GAAAACTTAGGCCCACAAGGAAAGGTGAGG |
| HCFC1_exon20_forward | AGGCCTAGGAAGAAAGAAGGTGTCAGCG |
| HCFC1_exon20_reverse | CAGATGTGATGGGGAGGACGAGCACAGG |
| HCFC1_exon21&22_forward | TAACTATGGGCCAGAGGGAAAAGAGGTCAGC |
| HCFC1_exon21&22_reverse | CTCAACGGCCACTGAGAGTAAGCGACTGAGG |
| HCFC1_exon23_forward | CCCCCAAGTGGAAGGACTAAGACAAACTGG |

| | |
|---|---|
| HCFC1_exon23_reverse | GGGCTTACAGGAAACTCCTCCATCTTTGGG |
| HCFC1_exon24_forward | TTTCGGGCCTGGAAACCTACACTTTCCC |
| HCFC1_exon24_reverse | CCTTCCACTTGGGGGAAGATCTCTTTGAAGC |
| HCFC1_exon25&26_forward | GAAAGAGAAAGGGGAGAGGAGTGAACAGCG |
| HCFC1_exon25&26_reverse | TGAGTGGGTTTCTAACCTACGTCCAGAGGG |
| | |
| qPCR Expression Analysis | |
| HCFC1 left | ACACGCTGGCTTGTCTCAA |
| HCFC1 right | GGGATGTTGTCCTCCAGTGT |
| HCFC1 Universal Probe number | 14 |
| MMACHC left | GCTTCGAGGTTTACCCCTTC |
| MMACHC right | AGGCCAGGGTAGGTCCTG |
| MMACHC Universal Probe number | 19 |
| MMADHC left | GCTATTTTGCGAACTATCTTCTCC |
| MMADHC right | TCCTGGGAGATAGGAAACCA |
| MMADHC Universal Probe number | 21 |
| MTR left | CAGAGTGCTTAACGGCACAG |
| MTR right | TAACAGTGGCCAGCACGAT |
| MTR Universal Probe number | 35 |
| SUCLG1 left | TTCACCAAACAACGCAAGTT |
| SUCLG1 right | TAAAAGGATCACCTCCAATGC |
| SUCLG1 Universal Probe number | 43 |
| ABCD4 left | ATGCAGATTCGGGTGAATG |
| ABCD4 right | TCTGTCCTCATGTGCTCCAC |
| ABCD4 Universal Probe number | 40 |

## Table S2. Enzymatic Studies in Skin Fibroblasts and Biochemical Findings in *cblX* Patients

| Subject number | *HCFC1* variant | [$^{14}$C] propionate incorporation[a] Ref. range: 10.8±3.7 | | [$^{14}$C] methylTHF incorporation[b] Ref. range: 225±165 | | [$^{14}$C] formate incorporation into methionine[c] Ref. range: 2.2±0.6 | | AdoCbl% Ref.range: 15.3±4.2 | MeCbl% Ref. range: 58.0±6.7 | Urine Hcy[d] (nl=und.) | Propionylcarnitine[e] (nl<1.78) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | basal medium | medium + OHCbl | basal medium | medium + OHCbl | basal medium | medium + OHCbl | | | | |
| 1 | p.Ala115Val | 1.0 | 3.2 | 27 | 52 | nd | nd | 3.9 | 1.5 | nd | nd |
| 2 | p.Ala115Val | 1.8 | 2.6 | 49 | 61 | nd | nd | 6.5 | 2.8 | 61 | nd |
| 3 | p.Ala115Val | 2.4 | 5.9 | 50 | 74 | nd | nd | 7.1 | 10.6 | 56 | nd |
| 4 | p.Ala115Val | 1.9 | 4.1 | 47 | 60 | nd | nd | 3.8 | 6.7 | nd | nd |
| 5 | p.Ala115Val | 3.3 | 6.0 | 48 | 73 | nd | nd | 6.5 | 2.6 | nd | nd |
| 6 | p.Ala115Val | 1.5 | 3.9 | 23 | 32 | nd | nd | 9.8 | 15.9 | nd | nd |
| 7 | p.Ala115Val | 1.4 | 4.1 | 21 | 39 | nd | nd | 3.1 | 2.3 | nd | nd |
| 8 | p.Ala115Val | 1.2* | 8.0* | nd | nd | 0.3 | 1.9 | 12.9 | 5.1 | nd | nd |
| 9 | p.Ala115Val | 1.4* | 7.3* | nd | nd | 0.4 | 1.6 | 0 | 1.1 | nd | nd |
| 10 | p.Ala115Thr | 0.6 | 0.9 | 36 | 52 | nd | nd | 3.0 | 0.7 | nd | nd |
| 11 | p.Ala73Val | 2.0 | 4.7 | 48 | 54 | nd | nd | 2.7 | 15.6 | nd | 2.16 |
| 12 | p.Ala73Val | 3.7* | 11.4* | nd | nd | 0.6 | 1.3 | 1.0 | 1.6 | nd | nd |
| 13 | p.Ala73Thr | 1.9 | 3.0 | 38 | 44 | nd | nd | 5.2 | 16.7 | nd | nd |
| 14 | p.Gln68Glu | 2.5 | 4.0 | 61 | 87 | nd | nd | 9.6 | 6.9 | nd | nd |

Ref. range: Reference range(mean ± SD) ; OHCbl: hydroxycobalamin ; THF: tetrahydrofolate; AdoCbl: adenosylcobalamin; MeCbl: methylcobalamin; tHcy: total homocysteine; Hcy: homocystine; MMA: methylmalonic acid; nl: normal; nd: not determined; und, undetectable

a: (nmol/mg protein/18 hrs), * nmol/mg protein/16hrs in patients 8, 9 and 12 (reference range 11.7 ± 4.3); b: (pmol/mg protein/18h); c: (nmol/mg protein/16h); d: µmol/L; e: nmol/mL

**Table S3. Summary of Exome Variants and Test of Inheritance Models in Subject 1**

| | | Subject 1 | | |
|---|---|---|---|---|
| Total variants | | 76,191 | | |
| Coding variants | | 20,279 | | |
| Nonsynonymous, splice-site, InDel variants | | 9,249 | | |
| Rare variants | | 161 | | |
| Test of inheritance model | Dominant model | Recessive models | | |
| | *de novo* | Compound heterozygous | Homozygous | X-linked hemizygous |
| | 0 | 1 (*TTN*) | 0 | 1 (*HCFC1*) |

**Table S4. Annotation of Mutation Effect and Conservation Score by SeattleSeq134**

| cDNA variant | Amino acid | PolyPhen-2[a] | Grantham Score[b] | Conservation GERP[c] | Conservation phastCons[d] |
|---|---|---|---|---|---|
| c.344C>T | p.Ala115Val | 0.997 (Probably Damaging) | 64 | 5.25 | 0.981 |
| c.343G>A | p.Ala115Thr | 0.998 (Probably Damaging) | 58 | 5.25 | 0.998 |
| c.218C>T | p.Ala73Val | 0.991 (Probably Damaging) | 64 | 5.46 | 0.958 |
| c.217G>A | p.Ala73Thr | 0.731 (Possibly Damaging) | 58 | 5.46 | 0.952 |
| c.202C>G | p.Gln68Glu | 0.722 (Possibly Damaging) | 29 | 5.46 | 1 |

a: 1=Most Damaging, 0=Most Benign; b: 125=Most Damaging, 0=Most Benign; c: 6.17=Most Conserved, -12.3=Least Conserved; d: 1=Most Conserved, 0=Least Conserved.

**Table S5. Human Variation Panel Controls from Coriell Institute**

| | Sample ID | Gender* | | Sample ID | Gender* |
|---|---|---|---|---|---|
| Individuals of European Descent | NA17202 | F | African Americans | NA17101 | M |
| | NA17203 | M | | NA17102 | M |
| | NA17204 | F | | NA17103 | M |
| | NA17205 | M | | NA17104 | M |
| | NA17206 | F | | NA17105 | M |
| | NA17207 | M | | NA17106 | M |
| | NA17208 | F | | NA17107 | M |
| | NA17209 | F | | NA17108 | M |
| | NA17210 | F | | NA17109 | M |
| | NA17211 | M | | NA17110 | F |
| | NA17212 | M | | NA17111 | M |
| | NA17213 | F | | NA17112 | F |
| | NA17214 | M | | NA17113 | F |
| | NA17215 | F | | NA17114 | M |
| | NA17216 | F | | NA17115 | M |
| | NA17218 | F | | NA17116 | F |
| | NA17219 | M | | NA17117 | M |
| | NA17220 | M | | NA17118 | F |
| | NA17221 | F | | NA17119 | F |
| | NA17222 | M | | NA17120 | F |
| | NA17223 | M | | NA17121 | F |
| | NA17224 | F | | NA17122 | F |
| | NA17225 | M | | NA17123 | F |
| | NA17226 | M | | NA17124 | F |
| | NA17227 | M | | NA17125 | M |
| | NA17228 | M | | NA17126 | F |
| | NA17229 | F | | NA17127 | F |
| | NA17230 | F | | NA17128 | F |
| | NA17231 | M | | NA17129 | F |
| | NA17232 | M | | NA17130 | F |
| | NA17233 | F | | NA17131 | F |
| | NA17234 | F | | NA17132 | F |
| | NA17235 | M | | NA17134 | F |
| | NA17236 | F | | NA17135 | F |
| | NA17237 | M | | NA17137 | F |
| | NA17238 | F | | NA17138 | F |
| | NA17239 | F | | NA17149 | F |
| | NA17240 | M | | NA17150 | F |
| | NA17241 | M | | NA17151 | F |
| | NA17242 | F | | NA17152 | M |
| | NA17243 | M | | NA17154 | F |
| | NA17244 | M | | NA17156 | F |
| | NA17245 | F | | NA17157 | F |
| | NA17246 | F | | NA17164 | F |
| | NA17247 | F | | NA17167 | F |

| | | | | | |
|---|---|---|---|---|---|
| | NA17248 | M | | NA17173 | F |
| | NA17250 | F | | NA17176 | F |
| | NA17251 | F | | NA17181 | F |
| | NA17252 | F | | NA17185 | F |
| | NA17253 | M | | NA17189 | F |

*: M=Male, F=Female

## References


1. Eswar, N., Webb, B., Marti-Renom, M.A., Madhusudhan, M.S., Eramian, D., Shen, M.-Y., Pieper, U., and Sali, A. (2006). Comparative protein structure modeling using Modeller. Curr Protoc Bioinformatics *Chapter 5*, Unit 5.6.

2. Dejosez, M., Levine, S.S., Frampton, G.M., Whyte, W.A., Stratton, S.A., Barton, M.C., Gunaratne, P.H., Young, R.A., and Zwaka, T.P. (2010). Ronin/Hcf-1 binds to a hyperconserved enhancer element and regulates genes involved in the growth of embryonic stem cells. Genes Dev. *24*, 1479–1484.