AJHG, Volume *86*

Supplemental Data

Loss-of-Function Mutations in HPSE2

Cause the Autosomal Recessive Urofacial Syndrome

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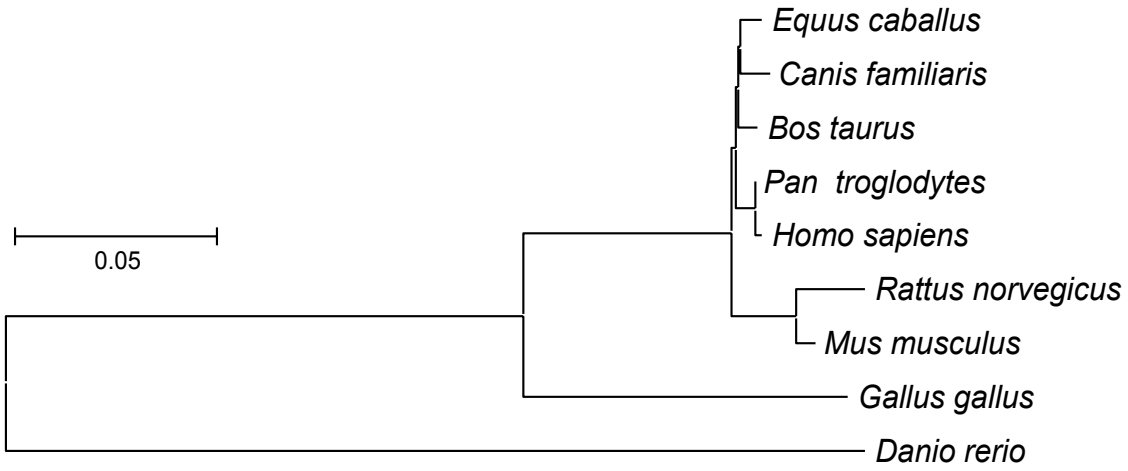

Figure S1. A Phylogenetic Tree Showing the Revolutionary Relationships of the *HPSE2* Gene between Different Species
The phylogenetic tree was generated using MEGA4.0 (Tamura, 2007) with p-distance model and constructed according to the calculation of the best match for the selected sequences. The distances for the tree branches are indicated in unit length as shown within the figure.

Tamura K, Dudley J, Nei M & Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0.Molecular Biology and Evolution 24: 1596-1599


Homo sapiens MRVLCAFPEAMPSSNSRPPACLAPGALYLALLLHLSLSSQAGDRRPLPVDRAAGLKEKTLILLDVSTKNPVRTVNE 76
Pan troglodytes MRVLCAFPEAMPSSNSRPPACLAPGALYLALLLHLSLSSQAGDRRPLPVDRAAGLKEKTLILLDVSTKNPVRTVNE 76
Canis familiaris MRVLCAFPEAMPSSNSRPPACLAPVALFLALLFHLSFSSQVGDRRPLPVDRAPGLKEKTLILLDVSTKNPVRTVNE 76
Equus caballus MRVLCAFPEAMPSSNSRPPACLTPVALFLALLLHLSLSSQAGDRRPLPVDRVPGLKEKTLILLDVSTKNPVRTVNE 76
Bos taurus MRVLCAFPEAMPSSNSRPPACLAPVALFLALLLHRSLSSQAGDRRPLPVDRAPGMKEKTLILLDVSTKNPVRTVNE 76
Mus musculus MRVLCAFPEAMASSSSRPPSCLALVALFLALLLHLSLSFHAGNRRPLPVDRATGLKEKTLILLDVSTKNPVRTVNE 76
Rattus norvegicus MRVLCAFPEAMSSSSSRPPSCLALVALFLALLLPLSLSFHAGNRRPLPVDRATGLKEKTLILLDVSTKNPVRTVSE 76
Gallus gallus ..........MPCSISCPPGLLALMAPGLALMATFSLPSQAGDRRALPVEKSPGVKGRTLILLDVNTKSPVRIISE 66
Danio rerio ....MPRQFFCP...VFGSSLWLALILQSLISSAVTYRRPVSGGKRQSFLERTLILLDVNTRSPVKVLND 63
Homo sapiens NFLSLQLDPSIIHDGWLDFLSSKRLVTLARGLSPAFLRFGGKRTDFLQFQNLRNPAKSRGGPGPDYYLKNYEDDIV 152
Pan troglodytes NFLSLQLDPSIIHDGWLDFLSSKRLVTLARGLSPAFLRFGGKRTDFLQFQNLRNPAKSRGGPGPDYYLKNYEDDIV 152
Canis familiaris NFLSLQLDPSIIHDGWLDFLSSKRLVTLARGLSPAFLRFGGKRTDFLQFQNLRNPAKSRGGPGPDYYLKNYEDDIV 152
Equus caballus NFLSLQLDPSIIHDGWLDFLSSKRLVTLARGLSPAFLRFGGKRTDFLQFQNLRNPAKSRGGPGPDYYLKNYEDDIV 152
Bos taurus NFLSLQLDPSIIHDGWLDFLSSKRLVTLARGLSPAFLRFGGKRTDFLQFQNLRNPAKSRGGPGPDYYLKNYEDDIV 152
Mus musculus NFLSLQLDPSIIHDGWLDFLSSKRLVTLARGLSPAFLRFGGKRTDFLQFQNLRNPAKSRGGPGPDYYLKNYEDDIV 152
Rattus norvegicus NFLSLHLDPSIIHDGWLDFLSSKRLVTLARGLSPAFLRFGGKRTDFLQFQNLRNPAKSRGGPGPDYYLKNYEDDII 152
Gallus gallus NFLSLQLDPSIIHDGWLDFLSSRRLVTLARGLAPAFLRFAGKRTDFLQFHNVKNPAKSRGGPGPDYYLKNYEDDIV 142
Danio rerio NFLSLQLDPSIIKDGWLDFLSSKRLVTLARGLSPAYLRFGGKRTDFLQFHNLKNLAKFRG.PGPDYYLKNYEDDIV 138
Homo sapiens RSDVALDKQKGCKIAQHPDVMLELQREKAAQMHLVLLKEQFSNTYSNLILT.........ARSLDKLYNFADCSGL 219
Pan troglodytes RSDVALDKQKGCKIAQHPDVMLELQREKAAQMHLVLLKEQFSNTYSNLILT.........ARSLDKLYNFADCSGL 219
Canis familiaris RSDVALDKQKGCKIAQHPDVMLELQREKAAQMHLVLLKEQFSNTYSNLILT.........ARSLDKLYNFADCSGL 219
Equus caballus RSDVALDKQKGCKIAQHPDVMLELQREKAAQMHLVLLKEQFSNTYSNLILT.........ARSLDKLYNFADCSGL 219
Bos taurus RSDVALDKQKGCKIAQHPDVMLELQREKAAQMHLVLLKEQFSNTYSNLILTGKAFLCFFPARSLDKLYNFADCSGL 228
Mus musculus RSDVALDKQKGCKIAQHPDVMLELQREKASQMHLVLLKEQYSNTYSNLILT.........ARSLDKLYNFADCSGL 219
Rattus norvegicus KSDICLHLSLPCLFLKHPDIYLDLKKKKKKKYHLLLLAEEYCKTQSYRILS.........ARSLDKLYNFADCSGL 219
Gallus gallus RSDIALDKQKGCKIAQHPDIMLELQREKAAQMHLVLLKEQFSNTYSNLTLT.........ARSLDKLYNFADCSGL 209
Danio rerio RSDFALDKQKGCKLASHPDMMLELQREKAAQMQQVLLKEQLSNIYSDVTLT.........ARSLDKLYNFADCAGL 205
Homo sapiens HLIFALNALRRNPNNSWNSSSALSLLKYSASKKYNISWELGNEPNNYRTMHGRAVNGSQLGKDYIQLKSLLQPIRI 295
Pan troglodytes HLIFALNALRRNPNNSWNSSSALSLLKYSASKKYNISWELGNEPNNYRTMHGRAVNGSQLGKDYIQLKSLLQPIRI 295
Canis familiaris HLIFALNALRRNPNNSWNSSSALSLLKYSASKKYNISWELGNEPNNYRTMHGRAVNGSQLGKDYIQLKSLLQPIRI 295
Equus caballus HLIFALNALCRNPNNSWNSSSALSLLKYSASKKYNISWELGNEPNNYRTMHGRAVNGSQLGKDYIQLKSLLQPIRI 295
Bos taurus HLIFALNALRRNPNNSWNSSSALSLLKYSASKKYNISWELGNEPNNYRTMHGRAVNGSQLGKDYIQLKSLLQPIRI 304
Mus musculus HLIFALNALRRNPNNSWNSSSALSLLKYSASKKYNISWELGNEPNNYRSIHGRAVNGSQLGKDYIQLKSLLQPIRV 295
Rattus norvegicus HLIFALNALRRNPNNSWNSSSALSLLKYSASKKYNISWELGNEPNNYRSIHGRAVNGSQLGKDYIQLRSLLQPIRI 295
Gallus gallus HLIFALNALRRNPNNSWNSSNALSLLKYSASKKYNISWELGNEPNNYRTLIGRSVNGSQLGKDYIQLRSLLQLIRT 285
Danio rerio HLIFGLNALHRNPDNSWNATSALSLLKYSAGKKYNISWELGNEPNSYRIMAARSVNSSRLAQDYTLLKTLLQSVRF 281
Homo sapiens YSRASLYGPNIGRPRKNVIALLDGFMKVAGSTVDAVTWQHCYIDGRVVKVMDFLKTRLLDTLSDQIRKIQKVVNTY 371
Pan troglodytes YSRASLYGPNIGRPRKNVIALLDGFMKVAGSTVDAVTWQHCYIDGRVVKVMDFLKTRLLDTLSDQIRKIQKVVNTY 371
Canis familiaris YSRASLYGPNIGRPRKNVIALLDGFMKVAGSTVDAVTWQHCYIDGRVVKVMDFLKTRLLDTLSDQIRKIQKVVNTY 371
Equus caballus YSRASLYGPNIGRPRKNVIALLDGFMKVAGSTVDAVTWQHCYIDGRVVKVMDFLKTRLLDTLSDQIRKIQKVVNTY 371
Bos taurus YSRASLYGPNIGRPRKNVIALLDGFMKVAGSTVDAVTWQHCYIDGRVVKVMDFLKTRLLDTLSDQIRKIQKVVNTY 380
Mus musculus YSRASLYGPNIGRPRKNVIALLDGFMKVAGSTVDAVTWQHCYIDGRVVKVMDFLKTRLLDTLSDQIRKIQKVVNTY 371
Rattus norvegicus YSRAGLYGPSIGRPRKNVIALLDGFLKVAGSTVDAVTWQHCYIDGRVVKVMDFLKTRLLDTLSDQIRKIQKVVNTY 371
Gallus gallus YSRANLYGPNIGRPRKNVMALLEGFMKVAGSTVDAVTWQHYYIDGRVAKVTDFLKTRLLDTLSDQIRKIQKVVNAY 361
Danio rerio YSRANLYGPNIGRPRKNAILLLDGFMKNGGSVVDAVTWQHYYIDRRVTKVEDFMKTRLLDTLSEQITKVLKIVQTH 357
Homo sapiens TPGKKIWLEGVVTTSAGGTNNLSDSYAAGFLWLNTLGMLANQGIDVVIRHSFFDHGYNHLVDQNFNPLPDYWLSLL 447
Pan troglodytes TPGKKIWLEGVVTTSAGGTNNLSDSYAAGFLWLNTLGMLANQGIDVVIRHSFFDHGYNHLVDQNFNPLPDYWLSLL 447
Canis familiaris TPGKKIWLEGVVTTSAGGTNNLSDSYAAGFLWLNTLGMLANQGIDVVIRHSFFDHGYNHLVDQNFNPLPDYWLSLL 447
Equus caballus TPGKKIWLEGVVTTSAGGTNNLSDSYAAGFLWLNTLGMLANQGIDVVIRHSFFDHGYNHLVDQNFNPLPDYWLSLL 447
Bos taurus TPGKKIWLEGVVTTSAGGTNNLSDSYAAGFLWLNTLGMLANQGIDVVIRHSFFDHGYNHLVDQNFNPLPDYWLSLL 456
Mus musculus TPGKKIWLEGVVTTSAGGTNNLSDSYAAGFLWLNTLGMLANQGIDVVIRHSFFDHGYNHLVDQNFNPLPDYWLSLL 447
Rattus norvegicus TPGKKIWLEGIVTTSAGGTNNLSDSYAAGFLWLNTLGMLANQGIDVVIRHSFFDHGYNHLVDQNFNPLPDYWLSLL 447
Gallus gallus TPGKKIWLEGVGATSAGGMNNLSDSYAAGFLWLNTLGLLASQGIDVVVRHSFLDHGHNHLVDQNFNPLPDYWLSLL 437
Danio rerio APEKHVWLGGVGPAWAGGTNNLSDTFAAGFLWLNTLGIAAAHGIDVVLRHSFFDYGYNHLVDQNFNPLPDYWVSLL 433
Homo sapiens YKRLIGPKVLAVHVAGLQRKPRPGRVIRDKLRIYAHCTNHHNHNYVRGSITLFIINLHRSRKKIKLAGTLRDKLVH 523
Pan troglodytes YKRLIGPKVLAVHVAGLQRKPRPGRVIRDKLRIYAHCTNHHNHNYVRGSITLFIINLHRSRKKIKLAGTLRDKLVH 523
Canis familiaris YKRLIGPKVLAVHVAGLQRKPRPGRVIRDKLRIYAHCTNHHNHNYVRGSITLFIINLHRSRKKIKLAGTLRDKLVH 523
Equus caballus YKRLIGPKVLAVHVAGLQRKPRPGRVIRDKLRIYAHCTNHHNHNYVRGSITLFIINLHRSRKKIKLAGTLRDKLVH 523
Bos taurus YKRLIGPKVLAVHVAGLQRKPRPGRVIRDKLRIYAHCTNHHNHNYVRGSITLFIINLHRSRKKIKLAGTLRDKLVH 532
Mus musculus YKRLIGPKVLAVHVAGLQRKPRPGRVIRDKLRIYAHCTNHHNHNYVRGSITLFIINLHRSRKKIKLAGTLRDKLVH 523
Rattus norvegicus YKRLIGPKVLAVHVAGLQRKPRPGRVIRDKLRIYAHCTNHHNHNYVRGSITLFIINLHRSRKKIKLAGTLRDKLVH 523
Gallus gallus YKRLIGPKVLAIHVAGLQRKPRPGRVIRDKLRIYAHCTSSHNHNYVRGSITLYIINLHRSRKKIKLAGTLRDKIVH 513
Danio rerio FKHLVGPRVLAVHVAGLQRKPRPGRVIRDKLRIYAHCTSFHNHDYVRGSVTIYIINLHRSRKKIKLAGTLRNKTVH 509
Homo sapiens QYLLQPYGQEGLKSKSVQLNGQPLVMVDDGTLPELKPRPLRAGRTLVIPPVTMGFYVVKNVNALACRYR 592
Pan troglodytes QYLLQPYGQEGLKSKSVQLNGQPLVMVDDGTLPELKPRPLRAGRTLVIPPVTMGFYVVKNVNALACRYR 592
Canis familiaris QYLLQPYGQEGLKSKSVQLNGQPLVMVDDGTLPELKPRPLRPGRTLVIPPVTMGFYVVKNVNALACRYR 592
Equus caballus QYLLQPYGQEGLKSKSVQLNGQPLVMVDDGTLPELKPRPLRAGRTLVIPPVTMGFYVVKNVNALACRYR 592
Bos taurus QYLLQPYGQEGLKSKSVQLNGQPLVMVDDGTLPELKPRPLRAGRTLVMPPVTMGFYVVKNVNALACRYR 601
Mus musculus QYLLQPYGQEGLKSKSVQLNGQPLVMVDDGTLPELKPRPLRAGRTLVIPPVTMGFYVVKNVNALACRYR 592
Rattus norvegicus QYLLQPYGQEGLKSKSVQLNGQPLVMVDDGTLPELKPRPLRAGRTLVIPPVTMGFYVVKNVNALACRYR 592
Gallus gallus QYLLQPYGKDGLHSKSVQLNGQPLAMVDDGTLPELKPRPLRAGRTLVIPPLTMSFYVVKNVNALACRYR 582
Danio rerio QYLLQPFGTEGLQSTSVQLNGEPLRMVDNETFPELKPRTLRAGRTIAMPPVSIGFYVIKNINAYACR.R 577
100%
75%
50%

Figure S2. HPSE2 Amino Acid Sequence Alignment Showing Evolutionary Conservation between Diverse Species

Amino acid sequence alignment was carried out using the DNAMAN 6.0 software (Lynnon Corporation). Genbank accession numbers for HPSE2 in each species are: *Danio rerio*, XP_691045; *Gallus gallus*, XP_421704; *Rattus norvegicus*, NP_001129234; *Mus musculus*, NP_001074726; *Homo sapiens*, NP_068600; *Canis familiaries*, XP_850311; *Bos taurus*, XP_617505; *Equus caballus*, XP_001501153; *Pan troglodytes*, XP_001166372.

Table S1. Primers Used for Mutation Screening for the *HPSE2* Gene

HPSE2exon1F:	5'-CGG ACA GAC ACA CAC TTT AG -3'
HPSE2exon1R:	5'-AGT TTC TGA AAT GCC TTC TG -3'
HPSE2exon2F:	5'-CTT TAG CGC CGT GCT CGT AG -3'
HPSE2exon2R:	5'-ACA AAC ACA GCG GGT GCT TG -3'
HPSE2exon3F:	5'-GAT GTG TCA TGG AGT TGG AG -3'
HPSE2exon3R:	5'-TGA GAA GAA AAC ACA TGC TG -3'
HPSE2exon4F:	5'-TCC CGA CCT TAG GTG ATC TG -3'
HPSE2exon4R:	5'-AAA AGG CAG CTC AGG CTG TG -3'
HPSE2exon5F:	5'-AAA GGC AGA GAG ATC TGT GG -3'
HPSE2exon5R:	5'-AAC CCA TCC TAG AGA TTG TG -3'
HPSE2exon6F:	5'-GAT GTG TGG GGA GCT GGA AG -3'
HPSE2exon6R:	5'-AGC CTA TGG GAA AAC AGT GG -3'
HPSE2exon7F:	5'-CAT CAA TTC CAG CAA GTA AG -3'
HPSE2exon7R:	5'-CGC TTT AAA CCT TGA TAT GG -3'
HPSE2exon8F:	5'-CAT GGC TTT TAG GGA GTA CG -3'
HPSE2exon8R:	5'-AAT CTT TGC TCT GAT GCC TG -3'
HPSE2exon9F:	5'-TTA GGC TTA CAA ATG GCT AG -3'
HPSE2exon9R:	5'-GTG TTG AAG CCA AAA GTC TG -3'
HPSE2exon10F:	5'-TGA CTC CAG GTA GGA AGT GA -3'
HPSE2exon10R:	5'-CTC ATG GGC ATT ACA TCA AC- 3'
HPSE2exon11F:	5'-ATG GCA GGA AGT GGC TAT CA -3'
HPSE2exon11R:	5'-GGC CAA GGA TGC TAA ACA GC -3'
HPSE2exon12_1F:	5'-TCA AAC CCT GGA CCT CCA AG- 3'
HPSE2exon12_1R:	5'-GAG TGG AGG AGT GGA AGC AG -3'
HPSE2exon12_2F:	5'-CTG CCG CTA CCG ATA AGC TA -3'
HPSE2exon12_2R:	5'-CAT CAG CCG GGA AAT CAT AC -3'