7. Appendices

Appendix 1: NCBI ob gene annotation (updated 1/11/2005)

Annotated sequence file:

LOCUS    7 26351 bp DNA HTG 1-NOV-2005
DEFINITION Homo sapiens chromosome 7 NCBI35 partial sequence
          127470282..127496632 reannotated via Ensembl
ACCESSION chromosome:NCBI35:7:127470282:127496632:1
VERSION  chromosome:NCBI35:7:127470282:127496632:1
KEYWORDS .
SOURCE   Human
ORGANISM Homo sapiens
          Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
          Mammalia; Eutheria; Euarchontoglires; Primates; Catarrhini;
          Hominidae; Homo.
COMMENT  This sequence was annotated by the Ensembl system. Please visit
          The Ensembl web site, http://www.ensembl.org/ for more
          information.
COMMENT  All feature locations are relative to the first (5') base of the
          sequence in this file. The sequence presented is always the
          forward strand of the assembly. Features that lie outside of the
          sequence contained in this file have clonal location coordinates
          in the format: .:. .
COMMENT  The /gene indicates a unique id for a gene,
          /note="transcript_id=..." a unique id for a transcript,
          /protein_id             a unique id for a peptide and
          note="exon_id=..." a unique id for an
          exon. These ids are maintained wherever possible between versions.
COMMENT  All the exons and transcripts in Ensembl are confirmed by
          similarity to either protein or cDNA sequences.
FEATURES Location/Qualifiers
          source          1..26351
          /organism="Homo sapiens"
          /db_xref="taxon:9606"
          gene            5001..21351
          /gene=ENSG00000174697
          /note="transcript_id=ENST00000308868"
          mRNA            join(5001..5029,15714..15885,18127..21351)
          /gene="ENSG00000174697"
          /note="transcript_id=ENST00000308868"
          /protein_id="ENSP00000312652"
          /db_xref="HUGO:LEP"
          /db_xref="CCDS:CCDS5800.1"
          /db_xref="Uniprot/SWISSPROT:OB_HUMAN"
          /db_xref="RefSeq_peptide:NP_000221.1"
          /db_xref="RefSeq_dna:NM_000230.1"
          /db_xref="Uniprot/SPTREMBL:Q4TVR7_HUMAN"
          /db_xref="Uniprot/SPTREMBL:Q6NT58_HUMAN"
          /db_xref="EntrezGene:3952"
          /db_xref="AgilentCGH:A_14_P126175"
          /db_xref="AgilentProbe:A_32_P112263"
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NLRLHVLAFSKSCHLPSWASGLETLSLGGVEASGERVSTEVVALSRLQGSLQDMWQL
LDLSPGC"

exon 18127..21351
/note="exon_id=ENSE0001208755"

exon 5001..5029
/note="exon_id=ENSE0001422382"

exon 15714..15885
/note="exon_id=ENSE0001303768"

ORIGIN

1 – 4200 continuous_sequence

4201 gcctaccaca ccactcacag gcctgtatga caaaaacgtg gcctatg\tctg gcctacatgcctacccc (4248) (c/g) (dbSNP:rs791619)
4261 ccaacccccca ctttgtatgt ag\tgaatatag gatgtgagga tggagaccca cagtatgtcctcc (4283) (g/a) (dbSNP:rs6976701)
4321 agatgtgccc caaggggccc a gttgcaccct cttggcccttg cagagagctg gcctcctggtgc cctgcggcagtgc\tgcgcggtgc (4341) (c/t) (dbSNP:rs10262600)
18001 tggtagggga gggttgaagg aggcagcc

18061 cagagggctc tgagag^cgt tctctccaca tgcgtgacac ttggtctccc tcttctctct
(18077) (c/t) (dbSNP:rs17151914) ob-Exon3 (18262) (g/a) (dbSNP:rs17151919) TOP
18301 CTTCTTCAC^G TGCTGGCCCT CTCCAAGAGC TCCTGGCCCA GTGCGCTGAG
(18310) (g/a) (dbSNP:rs1800564)
18361 ACCTGGGACA GCTCGGGGGA TGTCCTGGAA GCTCACCACT GCTGCTGAGCC
18421 CTGACGGGAT CAGCCGGGCT TCTCCCACTT CACGGCGCGC AGCTGGACCT
(18486) (g/t) (dbSNP:rs17151922)
18541 TTTCCAGGTAT CTCCAGGATT GAAGAGCATT

18601 AATTTCCTCG ACTCCTCTAA GCCACTCT^TC CAAGAAGCCAA AGACACTTCTAAG CCTCCTTTTG
(18628-18823) (STS:78152)
18861 CTTGAAACGCAAGATATATA CACTCACTCC TTTTCTCACC AGGAAAGGGC TCCACCAGCC
18721 AAGAGTGTCGG CGAGACTCTGG GAGGTGTCCT GCTGCTTTCCT CTCCATTTGA
18781 TGTCGCCCTCT TGCCACCATCT CCCCTCCACT GCTACGCTAAG AGCTGGAGGT
18841 AGAGAGGGGA GAGGGGTTAG CAGGGCCTTT CAGTGACGAG AACA^GATTTC CCTCTGAGAA
(18886) (g/t) (dbSNP:rs17151922)
18901 TTCCAGGGAG TTCCAGTGAAG ACCA^CAGCCGAAAG CACTCACTCCAGC AACCAAGCT

18961 GGAAGCCATG GTTATTATTAT TCTCCATTGTT ATCTGAGGTTAGG TATTTGGAGCA AAGACCAAGC
19021 CTCTCTCAAGG CTTGTTGGG GACTCGCCAGG CCAGGGTGGT CCGTGCTTACAG AAGCACTC
19081 TCCCATAGAT GGTGCTGCTG TGGTGCCAGG CATTTTTGAGG TACTGGGAAGG TGGCTGTCAT
19141 GTGGCAGGAAG CTGGGAAAGG GCTGCTTCTCC AATTGGTCTGC AGTTCTTCTG
19201 TACTGGTACTGATGTTTACAT CAGAAGGGTTTT GCAGGTTGGTT TGCCCTGAGAGA

19260 – 26352 continuous sequence

82
Appendix 2: NCBI obR gene sequence annotation (updated 1/11/2005)

Annotated sequence file:

LOCUS NC_000001 232191 bp DNA linear CON 25-OCT-2004
DEFINITION Homo sapiens chromosome 1, complete sequence.
ACCESSION NC_000001 REGION: 65593007..65825197
VERSION NC_000001.8 GI:51511461
KEYWORDS HTG.
SOURCE Homo sapiens (human)
ORGANISM Homo sapiens
  Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
  Mammalia; Eutheria; Euarchontoglires; Primates; Catarrhini;
  Hominidae; Homo.
REFERENCE 1 (bases 1 to 232191)
AUTHORS International Human Genome Sequencing Consortium.
TITLE Finishing the euchromatic sequence of the human genome
PUBMED 15496913
COMMENT GENOME ANNOTATION REFSEQ: Features on this sequence have been
produced for build 35 version 1 of the NCBI's genome annotation
[see documentation].
On Aug 24, 2004 this sequence version replaced gi:42406218.
The DNA sequence is part of the third release of the finished
human reference genome. It was assembled from individual clone
sequences by the Human Genome Sequencing Consortium in
consultation with NCBI staff.

FEATURES Location/Qualifiers
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    /mol_type="genomic DNA"
    /db_xref="taxon:9606"
    /chromosome="1"
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    /note="FISH-mapped clone"
  gene <1..567
    /gene="DNAJC6"
    /note="Derived by automated computational analysis using
gene prediction method: GNOMON. Supporting evidence
includes similarity to: 2 mRNAs, 21 ESTs"
    /db_xref="GeneID:9829"
    /db_xref="MIM:608375"
  mRNA <1..567
    /gene="DNAJC6"
    /product="DnaJ (Hsp40) homolog, subfamily C, member 6"
    /note="Derived by automated computational analysis using
gene prediction method: GNOMON. Supporting evidence
includes similarity to: 2 mRNAs, 21 ESTs"
    /transcript_id="XM_375737.1"
    /db_xref="GI:42655781"
    /db_xref="GeneID:9829"
    /db_xref="MIM:608375"
  misc_feature <1..18123
    /standard_name="RP5-992N21"
    /note="FISH-mapped clone"
  misc_feature <1..147615
    /standard_name="RP4-630A11"
    /note="FISH-mapped clone"
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    /gene="OBRGRP"
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/mRNA
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/gene="OBRGRP"
/product="leptin receptor gene-related protein"
/note="Derived by automated computational analysis using
gene prediction method: BestRefseq. Supporting evidence includes similarity to: 1 mRNA"
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/db_xref="GeneID:54741"
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/go_process="biological_process unknown [pmid 9207021]"
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/product="leptin receptor gene-related protein"
/protein_id="NP_059996.1"
/db_xref="GI:8923785"
/db_xref="GeneID:54741"
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/gene="LEPR"
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/db_xref="MIM:601007"
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189736..193451,193599,194645..194804,
194912..194998,200706..200922,202662..202844,
204626..204721,206051..206156,207604..207679,
214900..215120,217850..220126,220889..222191)
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ORIGIN
1 - 4920 continuous sequence

1 4920 4921 gggggccggg tcgggacagc cagggccggc ctctgagagt tgggggcgga attctgggcc
4981 ggtgagggct ggggcgggac cagcagggga ggcctccgcg agctagggga ggagctctgc
5041 attgtctggg gcggggcttc gagtagcggt agcgaggtct ggagccgcag gcgctgcctc
5101 cgcgaggtag gggaggagct ctgactgtc aggggcgggg ctctgagtag cgaggccggg
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5221 gggttgtgag tggtgggggc ggggtctgga gcagcaggcg ccgcgtttgc gagctaagtg
5281 cggagttctg caccgccggt ggcgggggtc tcgctgagct tcgctcgccg agcatcgcggt ccccggctcg
5341 cggagttctg caccgccggt ggcgggggtc tcgctgagct tcgctcgccg agcatcgcggt ccccggctcg
5401 obR-Exon1(5333-5438 -> 106bp)
5461 cttgtcgtgt ggtggggttg ccaacctcctg tccggtcaag cttgaggggtc gcgcctccgc
84
5521 gcgccgttgg ggaacggcct cacaccacct cccgcctctc cggttcggga ggcgatcgac
gctcccttc gtcccttggg gtgtgggtgg agcggcgttt cgggggagcc ttggcccttt
9721 aatttgggac ctttttttct ttctttttta ttttagttaa tttttttatt gataaatgat
9781 tcacacacca taaaatatac atctttaaag tgaagatttt catggttttt agtatatttc
9841 gaaggtttag cagccatcac tactatctaa ttccagaaaa tttactcatc gcgcaaaaga
9901 atccgtggct tattttaaag catctcctct cccctccacac cccctcagtt cgtacaacct
obR-Exon2 (10001-10076 -> 76bp)
9961 ctactgtggg aa^ctttaact tttggcttta tttttcacag CTCTCGTGGC ATTATCCTTC
(9973) (branch site - YNYRAY)
10021 AGTGGGGGCTA TTGGACTGAC TTTTCTTATG CTGGGATGTG CCTTAGAGGA TTATGGgtaa
10081 gttatcattt caaaaagaac tattcctctt tctgtgtctt tgtcactatt agtatgggtg
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10201 atgaacacag cttcctttgtg ggtcaggtgt acttttgcag agggctctgt taaaaattga
14221 gacactctac tattttactt agggaaggca gaaatgcaca aatctgctat attcctctta
14281 agaaaattgtg gctcagtggt acagaaaaca gtaaagtttc taggttatat attataataq
14341 gccttcttaa caacaatataa gctacctcaca tagtattatt taaaatatctc ctatctctctc
14401 attccggtgta gaaatgcata tccttcttaa taattctatatt taaaaattat cttatcctca
14461 atcatttaaa tagcattgaat tttgagtttt ggtcacagtt cagccccctt ttctttcttt ctatcctca
14521 tattttttttt tttgcagtttc gcctgggtcc aactgacag GTTTACTGGC CCTTATTCGT
14581 CTAGTTTTCT TACAGCTACT TCCCCATCCC CTTTTCATTC GTACCTCATT
14641 CTTCAGTGGCA ACCAGAATGTG CCTGCGAGGA ACTGGCATAT TTCTCCTACT CGTGAATGG
14701 GTGTTTCTGCC TTTGGATTTCC TCTGTTCTTC GTGGTGTGGT GCCTGTTgtaa gtttttttttt
14761 ctatgttttt gcccaacccctgtctgcctt attcctcaggg cttcgctcctt ggcacccctt
14821 tctatccttc ctactgtgct ttcttcttct ctatctcttt cttctcgctcctc tctatccttcctc
tttagatctag aatctcacttt cttgaggtt gttttttttt tataaatgga
OBRGRP-Exon3 (14560-14746 -> 187bp)
14521 tattttttttt tttgcagtttc gcctgggtcc aactgacag GTTTACTGGC CCTTATTCGT
14581 CTAGTTTTCT TACAGCTACT TCCCCATCCC CTTTTCATTC GTACCTCATT
14641 CTTCAGTGGCA ACCAGAATGTG CCTGCGAGGA ACTGGCATAT TTCTCCTACT CGTGAATGG
14701 GTGTTTCTGCC TTTGGATTTCC TCTGTTCTTC GTGGTGTGGT GCCTGTTgtaa gtttttttttt
14761 ctatgttttt gcccaacccctgtctgcctt attcctcaggg cttcgctcctt ggcacccctt
14821 tctatccttc ctactgtgct ttcttcttct ctatctcttt cttctcgctcctc tctatccttcctc
tttagatctag aatctcacttt cttgaggtt gttttttttt tataaatgga
15001 - 16320 continuous sequence
15601 ATCAAATGGG GAGCCTGCGG CCTTGTGTTG GCAGGCAATG CAGTCATTTT CCTTACAATT
15661 CATGCGTGTTT TTCTTATATT TGGAAGAGGA GATGATTTTA GCTGGGAGCA GTGG^TAGCAC
stop
16221 TTTATTCTGA TTACAGTGCA TTGAATTTCT TAGAACTCAT ACTATCTGTA TACATGTGCA
16281 CTTGCGGCGAT TTATACCTAG AATTTAATAT GCTGCTCTCT TAAATACCTT TATATATCAT
16741 GTTCACTTTTT AGAAAGACCT CATAGATTAG AGATGAGTTT TTATCTCAGC AAATAGACCT
16801 GTCAAATTTT CTAATATTTTT CTAATATTTTT CTAATATTTTT CTAATATTTTT CTAATATTTTT
16861 GTGCTCTCAG AAAATATATT AACCCAGCTT TTAGGGACAC TGCCACCTTA TGCACTGTCAT
16921 CGAAACCTTT TGTCTTTTGGG^A TGTGCTTTTGA GAGGGCTAGA AGCTGGAAGC AGGCGCTCTCA
16981 TGACCCAGGA AGGGCGGGGT GAGATCCTTCT TTGTGTGT^GTA GTCCATGCTA TTAAAGTGTG
17041 GGGGGGCGCA CCAAGGGCTT CATGAGCGCT CACCCTTTCT CTTGAGATTG CAGATCTCC
17101 GCCCGACCTCT GGGCGCCAGAA CATTCTTGTG ATGCTCAGAA AGTCAAGAAG C^ACATGAGAA
(17152-17203) (branch site - YNYRAY)
17161 TTTGAGAAGC ATCATCATAG AGAAGTAAAC ATCACACCCA ACTTCCTTAT CTTGAGAGCT
17221 GTTAAACCCCA TATACCTCTTT GGTGGTGTAC CTGGCTACTT GGTTAAAAA AAAAAAAAAG
17281 tcctcctcct cttcctccttt aggcaacagt cagatgtctt cagcctataa attttaggcy
tttagattttta taatgtaatg cttattatatt taaaatatttt cttattatatttt cttattatatttt
tttagatctag aatctcacttt cttgaggttt gtttttttttt tataaatgga
17461 aggataaatt gatgtatat ttaattttttt tataaatattt cttgaggtg ctgctttctcata
17521 – 150000 continuous sequence

150001 taagagcaac ttggctaaa attttttctga cttttttttaaatgatagtta agataggtat
150061 cgctacagaaaa ttaaatgtgttt agctttctcat atagttatcag atagttacttag
150121 agatcgctcatg agatcatct aatcctttgc tctctctttttt gcctttccag
150181 acaacattata tatagtgtag tttggtttttg ttggtgatggct ggttatttttag ttaaccccttttc
(150193) (branch site - YNYYRAY)

obR-Exon3 (150244-150303 -> 60bp)

150241 cagGTGTACT TCTCTGAAGT AAG^ATGATTT GTCAAAAATT CTGTGTGGTT TTGTTACATT

start

150351 ggggtaatgg attttcgctat tttgtttttt aatgccctta aacatggtag agatgttggcc
150361 tttaagagtt cttgcttatttt ttagacttta cagatacttttt taatctcctag taggaattct
150421 catacagctct tatgtatat aataaacaac atagatataggt gtagttcgat tggaaacgaa
150481 aacctcttccc aacctccccca agacacactctt atggtgttagc ctcctcctccttc

150541 – 155040 continuous sequence

155041 agacactttac tcagagcttaa gaaagtttaa ataatattag cactacatgtg tttttatctc
155151 agatacttttt gagaaggttttga aataaatctccttttctagttctag tttttttttttt
155219 ggggtaagtt attttcgctat tttgtttttt aatgccctta aacatggtag agatgttggcc
155301 ggtggactct ctaaaatccttt ttaatttaga aaaaagaaa ctggagggcca gagggactaa
155361 ggtatgggca aacgcatgcc actagttaaa gcttgttcttt tttctctcag atagattttgc
155421 agatggtttt tgagatttca tgtagttgtt tttttttttttt tttttttttttt tttttttttttt
155481 aataattgaa ttttaattcttt ttacttttaga aaaaagaaa ctggagggcca gagggactaa
155541 ggtggactct ctaaaatccttt ttaatttaga aaaaagaaa ctggagggcca gagggactaa
155601 ggtatgggca aacgcatgcc actagttaaa gcttgttcttt tttctctcag atagattttgc
155661 aataattgaa ttttaattcttt ttacttttaga aaaaagaaa ctggagggcca gagggactaa
155719 ggtggactct ctaaaatccttt ttaatttaga aaaaagaaa ctggagggcca gagggactaa
155781 ggtggactct ctaaaatccttt ttaatttaga aaaaagaaa ctggagggcca gagggactaa
155841 ggtggactct ctaaaatccttt ttaatttaga aaaaagaaa ctggagggcca gagggactaa
155901 ggtggactct ctaaaatccttt ttaatttaga aaaaagaaa ctggagggcca gagggactaa
155961 ggtggactct ctaaaatccttt ttaatttaga aaaaagaaa ctggagggcca gagggactaa
156021 ggtggactct ctaaaatccttt ttaatttaga aaaaagaaa ctggagggcca gagggactaa
156081 ggtggactct ctaaaatccttt ttaatttaga aaaaagaaa ctggagggcca gagggactaa
156141 ggtggactct ctaaaatccttt ttaatttaga aaaaagaaa ctggagggcca gagggactaa
156201 ggtggactct ctaaaatccttt ttaatttaga aaaaagaaa ctggagggcca gagggactaa
156261 ggtggactct ctaaaatccttt ttaatttaga aaaaagaaa ctggagggcca gagggactaa
156321 ggtggactct ctaaaatccttt ttaatttaga aaaaagaaa ctggagggcca gagggactaa
156381 – 177180 continuous sequence

177181 acctgcattgc atcttttatttagtctttttttttttttctttaaagtattgggtt tttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
obR

- Exon 7 (181146 - 181291 -> 146bp)

- Exon 8 (183358 - 183502 -> 145bp)

- Exon 9 (186090 - 186380 -> 291bp)

- Exon 10 (186541 - 186658 -> 118bp)
214801 cctgccttaa catatagtat ccagtgtgac tcagatacta t^tgatatttt
(214852)(branch site - YRYRAY)

**obR-Exon20 transcript variant 3** (214900-215120 -> 221bp)

214861 tactccctac tgttttacta taatctttct tttaaaccag TGGTTGAGG GACGAGGTTC
214921 GTTAAAGGTC ATCCACACTC CTAATCTCA AGTACCGGG GACCAAAAAA CTCGGGGAGGG
214981 CCAACAGCCT CTCTGCTTAG GAAAAACAGA GACCTTTGGT CACTGTGCTGAC
215041 CTCCCTCCGC TTATGGCTCA TGGACCTGCC AAATCCCCCT CTGTGAAGAAA CACCCAGAGA
215101 ^TGACAAATAA AAAAAAAA gaagaaaaaa aaatcttttc tctttttttgt tggattgtaat
(215103-215106)(STS:tv3-Stop)
215161 ttctcaagtag gaattcggag tggttcttttt ttatattatat ttgagaaga
215221 ctcctctcatt ctatgtatgt gataattttc gaaatttttc gcttttttca
215281 tctctcttctt tctctcttctc gatctctgt tgggatatg gacccctctg

215341 - 217620 continuous sequence

217621 attaaagctg tccttagagt gtgatattaa acattcattt aagaaaaatg gagaagccac
217681 cttatgccttaa aatgggcccc tttccccaaa ggtatgctta tgtgacacta cacaacaaat
217741 ttatgcttca gaccacctgtc tttggaattc ttttacttttt tttctttttgt

**obR-Exon20 transcript variant 2** (217850-220126 -> 2277bp)

217801 agtgatttttt tattttgctt tcttatttttg ttttatttta tctaaacagA GAACGGACAT
217861 TCTT^TGAAGT CTAATCATGA TCACTACAGA TGAACCCAAT GTGCCAACTT CCCAACAGTC
(217867-217870)(STS:tv2-Stop)
217921 TATAGGATAT TAGAAGATTG TTACATTTTG AAGAAGGGGA GCAAATCTAA AAAAAATTCA
217981 GTTGAACTTC TGAGAGTTAA CATATGGTGG ATTATGTTGA TTTAGAACTT AAAATAGATG
218041 TGTTAAATGTG GGTCAAAATGT GTGAAACTG GTGGAAGAACA

21801 - 220740 continuous sequence

220741 tatatgtatt ttatgttaaa aactattttt tttggttttgga tttagttctt ttattattatt
220801 ttatgccacat ttatctttct gcctagtaga ccattgagc caaatttttt taaataaat
(220848)(branch site - YNYRAY)

**obR-Exon20 transcript variant 1** (220889-222191 -> 1303bp)

220861 gagccttaaa atgtgtcttt tcttttagCC AGAAACGTTT GAGCATCTTT TTATCAAGCA
220921 TACAGCATCA GTGACATGTG GTCCTCTTCT TTTGGAGCCT GAAACAATTT CAGAAGATAT
220981 CAGTGTTGAT ACAT^CATGGA AAAATAAAGA TGAGATGAT^G CCAACAACTG TGGTCTCTCT
(220995-221497)(STS:LEPR-2), (221020-221257)(STS:PMC209419P1)
221041 ACTTTCAACA ACAGATCTTTG AAAAGCTCTC> TTTGTATTTG AGTGACAGT TCAACAGGT

22101 - 220740 continuous sequence

220741 tatatgtatt ttatgttaaa aactattttt tttggttttgga tttagttctt ttattattatt
220801 ttatgccacat ttatctttct gcctagtaga ccattgagc caaatttttt taaataaat
(220848)(branch site - YNYRAY)

**obR ex20F**

221101 TAATCTCTCTC GAGGCTGAGG GTACTGAGGT AACCTATGAG GACGAAAGCC AGAGACACC
221161 CTCTGGTAAA TA^GCCACGCC TGATCGACCA CTCTAAACCA AGTGAAGATCT GTGAAGAAC
(221173)(c/t) (dbSNP:1805095)
221221 AGGGCTTATA AATAGT^CA TGCAAGAGTG TCTCTCTCTC AAAAAATTTC GAT^CTGAGGA
(216239)(a/g) (dbSNP:6413506) , (216272)(a/g) (dbSNP:1805096)

221281 TTCTTTCTC TACTAATGCTG AGGGAGATGA GGCCACCGCA TTTTTATATAT TATCAGATCA

**obR ex20R**

221341 GCTCGGCCC CTGCTATGAGT GACCGAGGGT AACATTTTCTT GCTCAGATGG GAGTAAGAGA
221401 ATGGAGAGGA AATTCTTTCTG AAGAAAATG TGTAAATTTT TTTTTTTTTTG
221461 CACCCCAATCC AAAAGAGAG AGAGTGGTG GTGTGGCTGG CAGACGGCA GGAATGTGCG
221521 CCAATCCCA CCTCCCTCCTT TATTCAGCGA CCTCAGAACA GATCCTCCA
221581 CTTTCTCAGA AATAATATCA ACCTAGGGAC TTCTAGTGAAG AAAGATTTTT GTCTCCTACA
221641 GCTTCAATCTC CAAACTCTCC CTTACAGAC TCAATAGAC ATGAAAACCA AGATGGCTGG
221701 CACTAGTGGT ^TAATTTTACT GAAGAAACCT TGCAAGTTGC TTATATGG TAAATATATAT
221761 GTGTAATAAG TATAGTTTGT GGTTGGAGGA GAGAAAGAAA ACCAGACCTAA CTATTGAAA

221821 - 222670 continuous sequence
Appendix 3: Study cohort ethical approval (C99/025)
Appendix 4: Patient consent form template
Appendix 5: Patient questionnaire template
Appendix 6: Control cohort ethical approval (C050/2001)
Appendix 7: DNA extraction method (protocol on p. 101)

Extraction Buffers and chemicals used:

**Nuclear Lysis Buffer** (1l, pH 8.2 set with 1M NaOH, store at 4°C)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris-Cl</td>
<td>1.211g</td>
<td>0.01M</td>
</tr>
<tr>
<td>NaCl</td>
<td>23.4g</td>
<td>0.4M</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.6g</td>
<td>0.002M</td>
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</tbody>
</table>

**Cell Lysis Buffer** (1l, pH 7.4 set with concentrated HCl, store at 4°C)

<table>
<thead>
<tr>
<th>Component</th>
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<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄Cl</td>
<td>8.3g</td>
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<tr>
<td>KHCO₃</td>
<td>1.1g</td>
<td>0.01M</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.03g</td>
<td>0.0001M</td>
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</tbody>
</table>

**Phosphate Buffered Saline (PBS)** (1l, store at 4°C)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>2g</td>
<td>0.027M</td>
</tr>
<tr>
<td>NaCl</td>
<td>8g</td>
<td>0.137M</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>1.14g</td>
<td>0.008M</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.2g</td>
<td>0.0015M</td>
</tr>
</tbody>
</table>

**10% SDS** (100ml, store at room temperature to prevent precipitation)

10g SDS

Work in vapour hood

**Proteinase K** (store at -20°C)

10mg/ml dissolved in dH₂O, aliquot in 1.5mL Eppendorf tubes

**6M NaCl** (500ml, saturated solution)

175.32g NaCl
**Genomic DNA Extractions from Blood:**

- Place ~10ml blood in a 50ml Falcon tube
- Add 30ml cold Lysis Buffer
- Place on ice for 15 min, shaking each 5 min interval
- Centrifuge @ 1500rpm for 10min
- Pour off supernatant , keeping pellet
- Add 10ml PBS and mix
- Centrifuge @ 1500rpm for 10min
- Pour off supernatant keeping pellet
- Dissolve pellet in : 30ml Cell – Lysis buffer
  
  30μl Proteinase K (10mg/ml)

  300μl 10% SDS

- Mix well and incubate overnight in 55°C waterbath

---

**Day 2:**

- Add 1ml 6M NaCl and shake for 1min
- Centrifuge @ 3500rpm for 30 min
- Transfer supernatant to new tube and shake for 15s
- Centrifuge @ 2500rpm for 15 min
- Transfer supernatant to new tube without foam or pellet
- Add 2 volumes ice cold 100% ethanol to precipitate DNA
- Scoop out DNA and place in Eppendorf tube with 500μl 70% ethanol
- Centrifuge @ 14 000rpm for 10 min @ 4°C
- Dissolve pellet in 200-800μl ddH₂O/TE buffer depending on pellet size
Appendix 8: Rapid DNA extraction protocol

Rapid DNA Isolation from 300μl Whole Blood:
*PureGene® DNA Isolation Kit (Gentra Systems™, Minneapolis, USA)

Rapid 25 Minute Protocol, does not include RNase treatment

Cell Lysis:
1. Add 300μl whole blood to 900μl RBC Lysis Solution and incubate for 1min at room temperature; invert gently 10 times during incubation.

2. Centrifuge for 20s at 13 000 –16 000g, Remove supernatant (leave approximately 10μl liquid) and vortex to resuspend the pellet.

3. Add 300μl Cell Lysis Solution and pipette up and down.

Protein Precipitation:
4. Add 100μl Protein Precipitation Solution and Vortex at high speed for 20s
   Centrifuge for 1 min at 13 000 –16 000g (dark brown pellet forms)

DNA Precipitation:
5. Transfer supernatant to a clean Eppendorf tube containing 300μl 100% isopropanol and mix by inverting samples 50 times.

6. Centrifuge at 13 000 – 16 000g for 60s (get small white pellet)

7. Pour off supernatant and drain tube on clean absorbent paper.
   Add 300μl 70% ethanol and invert tube several times to wash pellet.

8. Centrifuge at 13 000- 16 000g for 60s
   Carefully pour off ethanol and invert and drain tube for 30 min on clean towel paper.

DNA Hydration:
9. Add 20μl DNA Hydration Solution and dissolve for 30 min. Store at 4°C.
Appendix 9:  Multiphor SSCP/HD protocol (description on p. 104)

Multiphor Solutions:

40% acrylamide PDA solution: 1l
(keep in foil in fridge)
- 396g acrylamide
  - 4g Piperazine diacrylamide
  - bring to volume (1l) with dH2O

0.75M TRIS- Formate Buffer: 1l
- 90.8g TRIS
  - 600ml dH2O (dissolve)
  - pH = 9.0 with formic acid
  - bring to volume (1l) with dH2O

41% Glycerol: 100mL
- 41ml glycerol
  - 59ml dH2O

TRIS- Borate Buffer: 1l (pH = 9.0)
- 125.9g TRIS
  - 17.3g Boric acid
  - 700ml dH2O (dissolve)
  - 50μl Bromophenol Blue (4% solution)
  - bring to volume (1l) with dH2O

SSCP loading Dye: 50ml
- 47.5ml 95% Formamide
  - 0.16g 100mM NaOH
  - 0.125g 0.25% Bromophenol Blue
  - 0.125g 0.25% Xylene cyanol
  - bring to volume (50ml) with dH2O

10% APS: 1.5mL
- 0.15g Ammonium Persulphate
  - bring to volume (1.5ml) with dH2O

Gel mix: X10 (12% non-denaturing)
- 53ml 40% acrylamide-PDA
  - 85ml Tris-Formate Buffer
  - 30ml 41% Glycerol
  - keep in foil in fridge

Per 1 Gel:
- 15ml Gel Mix
- 150μl 10% APS
- 15μl TEMED

Plate Glue
- 50μl 0.25% plate glue (3-(Trimethoxysilyl) propyl methacrylate
diluted in 100% ethanol

Silver Staining solutions:

Solution I: 1l
- 0.1% silver nitrate = 0.3g silver nitrate
  - 500ml dH2O
  - keeps max 2 days in the dark

Solution II: 1l
- 4.5g NaOH
  - 1L dH2O
  - 3ml 40% Formaldehyde
**Gel Plates preparation**  (do everything in quick succession)

- Wash plates (118mm x 220mm x 3mm) 4X with 70% ethanol
- Every 2 runs clean plate with blocks with 80µl Repelcote (Merck, Darmstadt, Germany) especially over blocks
- Put 80µl plateglue and 6µl 10% acetic acid on plate without blocks
- Wipe well until resistance is felt and wash a few times with ethanol
- Clean spacers and place them on plates
- Camp well on both short sides and long area close to blocks and then place upside down on bench
- Prepare gel mix and pour quickly using a syringe
- Allow to set at room temp for 30min
- Separate plates (gel should be on one without blocks)
- Put water on surface of multiphor apparatus
- Don’t get water on plates when placing on multiphor apparatus
- Slide plate facing upwards over water (no bubbles)
- Use 3 buffer strips/gel-side/2h run soaked in TRIS-borate buffer
- Place strips on both sides of gel close to the wells
- Wash electrodes of multiphor gently with 70% ethanol each time before use

**PCR Products:**

- Take required volume of PCR product and add 3µl SSCP loading dye (all on ice)
- Digest @ 95°C for 5min in PCR thermocycler
- Place on ice immediately for 3min
- Load 3µl (skipping ends of gel) onto gel and run @ 12°C first (if resolution not good, run at 9°C)
- 2-2.5h at 355V

**Visualization of bands:**

- remove gel from apparatus
- rinse X2 in dH₂O (remove buffer because it interferes with the stain)
- incubate for 10min @room temp in solution I (silver nitrate intercalates with DNA)
- rinse X2 with dH₂O
- incubate for 10min @room temp in solution II
- rinse X2 with dH₂O
- blot dry with paper towel
- cover with filter paper
- leave overnight, then put paper off glass and label paper with dried gel
Appendix 10: DNA purification protocols

DNA Purification by Centrifugation:

*Wizard® SV Gel and PCR Clean-Up System (Promega, Wi, USA)*

1. Assemble SV Minicolumn in collection tube for each sample
2. Transfer all of PCR product to column and incubate at room temperature for 1min
3. Centrifuge assembly for 1min at 14 000rpm and discard liquid in collection tube
4. Add 700μl Membrane Wash Solution to the SV Minicolumn and repeat Step 3
5. Add 500μl Membrane Wash Solution to the SV Minicolumn and centrifuge for 5min at 14 000rpm.
6. Discard flowthrough and recentrifuge the assembly for 1min at 14 000rpm
7. Transfer SV Minicolumn to clean 1.5ml microcentrifuge tube
8. Add 50μl Nuclease-Free water directly to center of the column and incubate at room temperature for 1min
9. Centrifuge for 1min at 14 000rpm
10. Store sample at 4°C

Column-free protocol

* Bioline SureClean® (Bioline, UK)*

1. Add an equal volume of Bioline SureClean® to the amplified nucleic acid sample and incubate at room temperature for 10min
2. Centrifuge at 13 000rpm for 10min and remove supernatant
3. Add 100μl of 70% ethanol and vortex for 30 seconds
4. Centrifuge at 13 000 rpm for 10min, remove supernatant and air-dry
5. Resuspend in Nuclease-free water (starting volume)
Appendix 11: Polyacrylamide Gel Electrophoresis (PAGE) protocol

10% APS

1g APS + 10ml dH₂O

5%C, 40% stock

38g Acylamide
2g Bis-acrylamide
Dissolve in ~60ml dH₂O
Bring to volume (100ml)

Mix for 2 gels

6ml 5%C, 40% stock of PAA
4ml 5x TBE
10ml dH₂O
300μl 10% APS
30μl TEMED

Run @ 250V for 40 min
Appendix 12: Fetal Growth Chart (specific for Tygerberg Hospital patients)