AJHG*, Volume *86
Supplemental Data

Homozygosity Mapping Reveals Mutations of GRXCR1 as a Cause of Autosomal Recessive Nonsyndromic Hearing Impairment DFNB25

Margit Schraders, Kwanghyuk Lee, Jaap Oostrik, Patrick L.M. Huygen, Ghazanfar Ali, Lies H. Hoefsloot, Joris A. Veltman, Frans P.M. Cremers, Sulman Basit, Muhammad Ansar, Cor W.R.J. Cremers, Henricus P.M. Kunst, Wasim Ahmad, Ronald J.C. Admiraal, Suzanne M. Leal, and Hannie Kremer

<u>This document contains six elements:</u>

Figure S1, which shows the pedigrees and genotypes of families with homozygous mutations in *GRXCR1*.

Figure S2, which shows the pedigree and haplotypes for family DEM 4265

Figure S3, which shows the pedigree and haplotypes for family DEM 4349

Figure S4, which shows an alignment of the GRXCR1 protein of different species.

Table S1, which gives primer sequences and PCR conditions

Table S2, which gives genotyping data of SNPs for families W98-053 and W07-0122

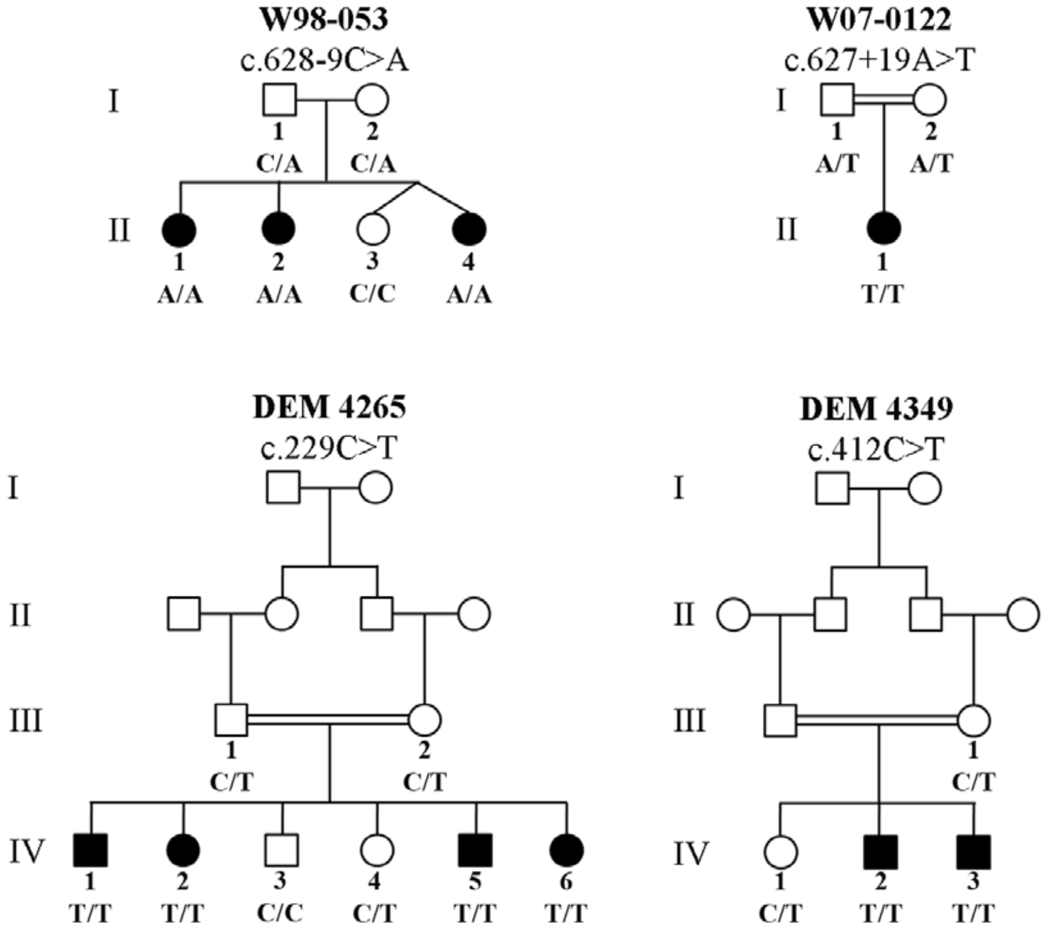


Figure S1. Pedigrees and Genotypes of Families with Homozygous Mutations in *GRXCR1*

The mutations co-segregate with the hearing impairment.

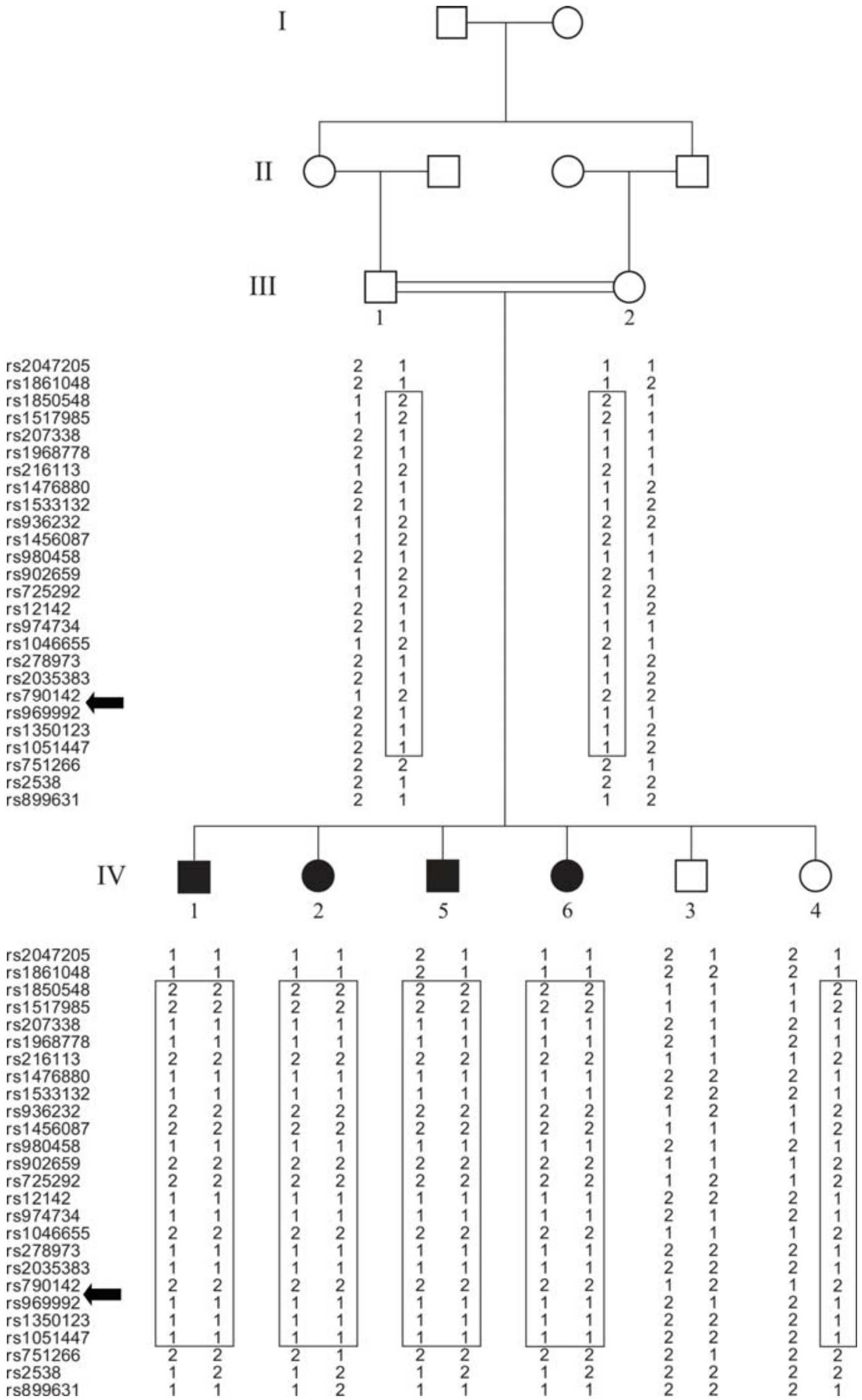


Figure S2. Critical Region for Family DEM 4265

Pedigree and haplotypes for SNP markers within and flanking the linkage interval are shown. The disease haplotype is boxed and the critical region is flanked by rs1861048 and rs751266. The location of the *GRXCR1* gene is indicated with a black arrow.

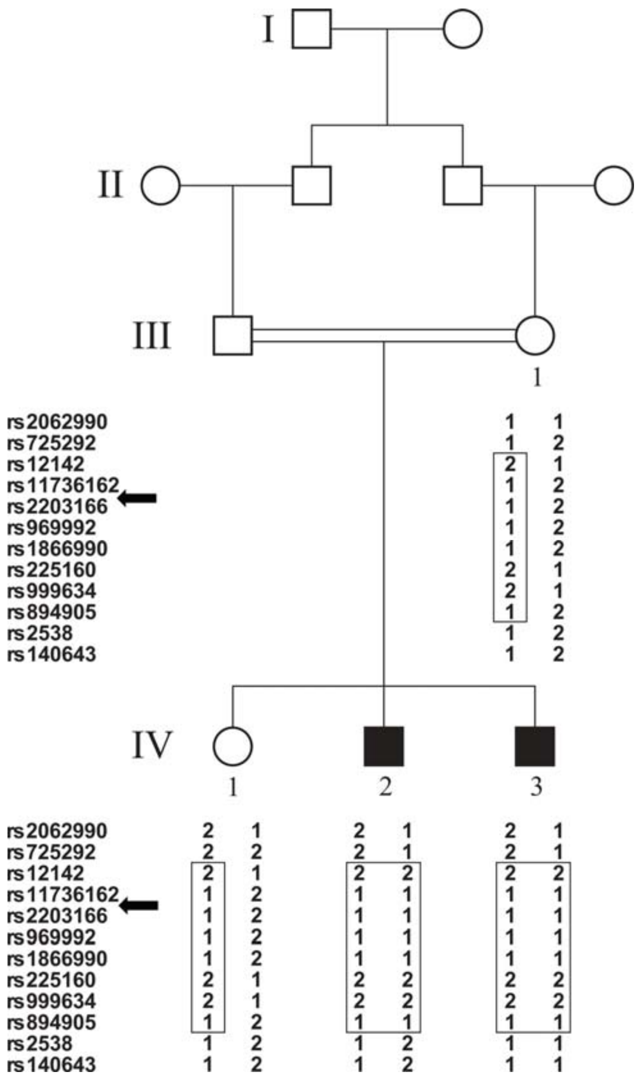
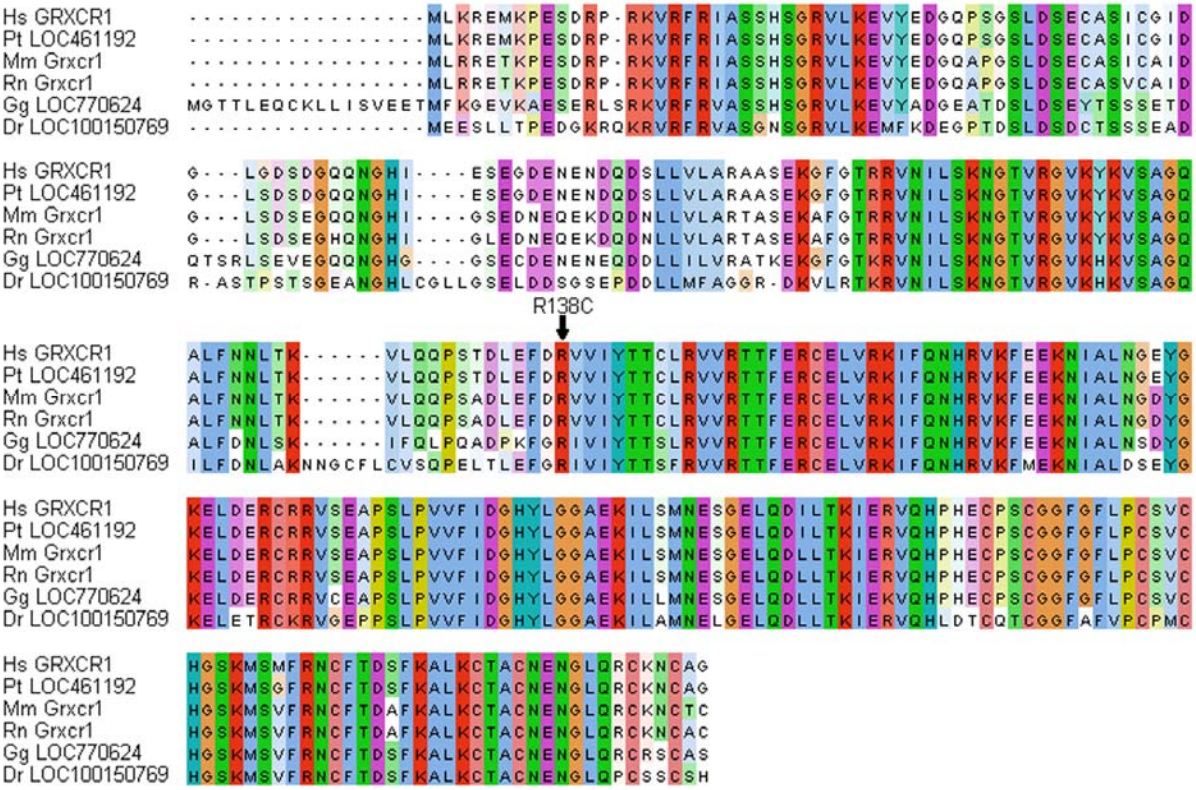


Figure S3. Critical Region for Family DEM 4349

Pedigree and haplotypes for SNP markers within and flanking the critical region are shown. The disease haplotype is boxed and the critical region is flanked by rs725292 and rs2538. The location of the *GRXCR1* gene is indicated with a black arrow.



```
Hs GRXCR1          -----------------MLKREMKPESDRP-RKVRFRIASSHSGRVLKEVYEDGQPSGSLDSECASICGID
Pt LOC461192       -----------------MLKREMKPESDRP-RKVRFRIASSHSGRVLKEVYEDGQPSGSLDSECASICGID
Mm Grxcr1          -----------------MLRRETKPESDRP-RKVRFRIASSHSGRVLKEVYEDGQAPGSLDSECASICAID
Rn Grxcr1          -----------------MLRRETKPESDRP-RKVRFRIASSHSGRVLKEVYEDGQAPGSLDSECASVCAID
Gg LOC770624       MGTTLEQCKLLISVEETMFKGEVKAESERLSRKVRFRVASSHSGRVLKEVYADGEATDSLDSEYTSSSETD
Dr LOC100150769    -----------------MEESLLTPEDGKRQKRVRFRVASGNSGRVLKEMFKDEGPTDSLDSDCTSSSEAD

Hs GRXCR1          G---LGDSDGQQNGHI----ESEGDENENDQDSLLVLARAASEKGFGTRRVNILSKNGTVRGVKYKVSAGQ
Pt LOC461192       G---LSDSDGQQNGHI----ESEGDENENDQDSLLVLARAASEKGFGTRRVNILSKNGTVRGVKYKVSAGQ
Mm Grxcr1          G---LSDSEGQQNGHI----GSEDNEQEKDQDNLLVLARTASEKAFGTRRVNILSKNGTVRGVKYKVSAGQ
Rn Grxcr1          G---LSDSEGHQNGHI----GLEDNEQEKDQDNLLVLARTASEKAFGTRRVNILSKNGTVRGVKYKVSAGQ
Gg LOC770624       QTSRLSEVEGQQNGHG----GSECDENENEQDDLLILVRATKEKGFGTKRVNILSKNGTVRGVKHKVSAGQ
Dr LOC100150769    R-ASTPSTSGEANGHLCGLLGSELDDSGSEPDDLLMFAGGR-DKVLRTKRVNILSKNGTVRGVKHKVSAGQ

                                             R138C
                                               ↓
Hs GRXCR1          ALFNNLTK------VLQQPSTDLEFDRVVIYTTCLRVVRTTFERCELVRKIFQNHRVKFEEKNIALNGEYG
Pt LOC461192       ALFNNLTK------VLQQPSTDLEFDRVVIYTTCLRVVRTTFERCELVRKIFQNHRVKFEEKNIALNGEYG
Mm Grxcr1          ALFNNLTK------VLQQPSADLEFDRVVIYTTCLRVVRTTFERCELVRKIFQNHRVKFEEKNIALNGDYG
Rn Grxcr1          ALFNNLTK------VLQQPSADLEFDRVVIYTTCLRVVRTTFERCELVRKIFQNHRVKFEEKNIALNGDYG
Gg LOC770624       ALFDNLSK------IFQLPQADPKFGRIVIYTTSLRVVRTTFERCELVRKIFQNHRVKFEEKNIALNSDYG
Dr LOC100150769    ILFDNLAKNNGCFLCVSQPELTLEFGRIVIYTTSFRVVRTTFERCELVRKIFQNHRVKFMEKNIALDSEYG

Hs GRXCR1          KELDERCRRVSEAPSLPVVFIDGHYLGGAEKILSMNESGELQDILTKIERVQHPHECPSCGGFGFLPCSVC
Pt LOC461192       KELDERCRRVSEAPSLPVVFIDGHYLGGAEKILSMNESGELQDILTKIERVQHPHECPSCGGFGFLPCSVC
Mm Grxcr1          KELDERCRRVSEAPSLPVVFIDGHYLGGAEKILSMNESGELQDLLTKIERVQHPHECPSCGGFGFLPCSVC
Rn Grxcr1          KELDERCRRVSEAPSLPVVFIDGHYLGGAEKILSMNESGELQDLLTKIERVQHPHECPSCGGFGFLPCSVC
Gg LOC770624       KELDERCRRVCEAPSLPVVFIDGHYLGGAEKILLMNESGELQDLLTKIEKVQHPHECPSCGGFGFLPCSVC
Dr LOC100150769    KELETRCKRVGEPPSLPVVFIDGHYLGGAEKILAMNELGELQDLLTKIERVQHLDTCQTCGGFAFVPCPMC

Hs GRXCR1          HGSKMSMFRNCFTDSFKALKCTACNENGLQRCKNCAG
Pt LOC461192       HGSKMSGFRNCFTDSFKALKCTACNENGLQRCKNCAG
Mm Grxcr1          HGSKMSVFRNCFTDAFKALKCTACNENGLQRCKNCTC
Rn Grxcr1          HGSKMSVFRNCFTDAFKALKCTACNENGLQRCKNCAC
Gg LOC770624       HGSKMSVFRNCFTDSFKALKCTACNENGLQRCRSCAS
Dr LOC100150769    HGSKMSVFRNCFTDSFKALKCTACNENGLQPCSSCSH
```

Figure S4. Alignment of the GRXCR1 Protein of Different Species

An alignment of GRXCR1 orthologs was made using ClustalW and Jalview. (Waterhouse et al. Bioinformatics 25(9): 1189-1191 (2009) and Larkin et al. Bioinformatics 23(21):2947-2948 (2007). Hs, Homo sapiens; Pt, Pan troglodytes ; Mm, Mus musculus ; Rn, Rattus norvegicus; Gg, Gallus gallus; Dr, Danio rerio. Accession numbers of the various protein sequences: Hs GRXCR1: NP_001073945.1, Pt LOC461192: XP_517170.2, Mm Grxcr1: NP_001018019.1, Rn Grxcr1: XP_573590.2, Gg LOC770624: XP_001233963.1, Dr LOC100150769: XP_001919567.1. The colors of the amino acids show the degree of conservation.

Table S1. Sequences of Primers for Amplifications of Exons, Intron-Exon Boundaries and Transcripts of *GRXCR1*

Fragment	Oligonucleotides	Size (bp)	Annealing Temperature (°C)
Primers for PCR and sequence analysis			
GRXCR1 exon 1	Forward: gccatcactagaattggctc	584	58
	Reverse: tgcaaataagagaggcttgc		
GRXCR1 exon 2	Forward: attcaattttattgcatggc	490	56
	Reverse: aaaaccagcaagtgttccag		
GRXCR1 exon 3	Forward: caaagggaagaactgtgctc	308	56
	Reverse: tgccacatgtatcctattgc		
GRXCR1 exon 4	Forward: tgaccactcacagttcagaaag	498	56
	Reverse: gaaaccagggtctagcacag		
ATP8A1 exon 1	Forward: tgcctttctcctcctcctgtcg	330	58
	Reverse: aggtaggatgcggtctcttc		
primers for ARMS PCR			
wildtype exon 2	Forward: attaccttggggtaagtaagctgcccagta	154	57
c.627+19A>T	Forward: attaccttggggtaagtaagctgcccagtt	154	57
	Reverse: aagtgctccaatccctttgg		
Primers for analysis *GRXCR1* transcripts			
GRXCR1 exon 2-3	Forward: aaacatagccctgaatggtg	204	58
	Reverse: gagggacactcatgtggatg		
seminested	Reverse: ggttaggatgtcttgcagttctcc	166	58
Primers for Q-PCR			
GRXCR1 exon 2-3	Forward: cttccctccctgttgtgttc	93	60
	Reverse: ggatgtcttgcagttctcctg		
GUSB	Forward: agagtggtgctgaggattgg	80	60
	Reverse: ccctcatgctctagcgtgtc		

Table S2. Genotypes of Families W98-053 and W07-0122 for Markers within the DFNB25 Locus

			W98-053			W07-0122	
SNP_ID	rs_ID	Genomic position on chr. 4	II.4	II.2	II.1	II.1	
SNP A-2110311	rs2612510	42432622	BB	BB	BB	AA	
SNP A-2073163	rs1380749	42433229	AA	AA	AA	BB	
SNP A-2082601	rs2612516	42435421	BB	BB	BB	AA	
SNP A-1976944	rs10517048	42443021	BB	BB	BB	AA	
SNP A-1916015	rs16854904	42443048	AA	AA	AA	BB	
SNP A-1976945	rs2345475	42443300	AA	AA	AA	BB	
SNP A-1976946	rs10517049	42443415	BB	BB	BB	AA	
SNP A-1976947	rs10517050	42443702	BB	BB	BB	AA	
SNP A-4196984	rs12640399	42490920	AA	AA	AA	AA	
SNP A-1821662	rs2575515	42495213	BB	BB	BB	AA	
SNP A-2269519	rs2575516	42495646	AA	AA	AA	AA	
SNP A-1919620	rs1450912	42504394	AA	AA	AA	BB	
SNP A-1803110	rs4861224	42510046	AA	AA	AA	BB	
SNP A-1976949	rs2218827	42515001	BB	BB	BB	BB	
SNP A-4239046	rs4036048	42525873	AA	AA	AA	AA	
SNP A-4203389	rs12508994	42526579	BB	BB	BB	BB	
SNP A-1893657	rs1450926	42535340	BB	BB	BB	BB	
SNP A-2046909	rs16855009	42535400	AA	AA	AA	AA	
SNP A-2033735	rs12642552	42542658	AA	AA	AA	BB	
SNP A-1958182	rs13142169	42548809	AA	AA	AA	AA	
SNP A-1839559	rs10938223	42549707	AA	AA	AA	AA	
SNP A-2276302	rs13142149	42562218	AA	AA	AA	BB	
SNP A-2240952	rs41493449	42590472	BB	BB	BB	BB	
SNP A-1976955	rs1450911	42609322	AA	AA	AA	BB	**GRXCR1**
SNP A-2104688	rs4861237	42609397	AA	AA	AA	BB	
SNP A-2282630	rs1376313	42616757	BB	BB	BB	AA	
SNP A-4192751	rs1450916	42620388	BB	BB	BB	AA	
SNP A-1976956	rs17534598	42620779	BB	BB	BB	AA	
SNP A-1976957	rs10517062	42628253	AA	AA	AA	AA	
SNP A-1877660	rs1450934	42634215	BB	BB	BB	AA	
SNP A-4240696	rs2345759	42634449	BB	BB	BB	AA	
SNP A-2133212	rs1597565	42634794	AA	AA	AA	AA	
SNP A-4207671	rs17450262	42641640	BB	BB	BB	AA	
SNP A-1954996	rs13105520	42652288	AA	AA	AA	AA	
SNP A-2009753	rs4346680	42653743	BB	BB	BB	BB	
SNP A-2140147	rs992133	42655425	BB	BB	BB	AA	
SNP A-2102398	rs1595188	42656122	BB	BB	BB	AB	
SNP A-2076559	rs7664576	42676268	BB	BB	BB	AA	
SNP A-1909795	rs9994547	42691897		AA	AA	AA	
SNP A-4226359	rs1561147	42693025		BB	BB	BB	
SNP A-4239243	rs16855182	42696452	BB	BB	BB	BB	
SNP A-2137904	rs10805107	42709288	AB	AB	AB	BB	
SNP A-1875106	rs16855222	42714046	BB	BB	BB	BB	
SNP A-2255620	rs10008544	42717608	BB	BB	BB	BB	
SNP A-2273039	rs10017415	42719903	BB	BB	BB	BB	
SNP A-2084004	rs10938230	42720012	AA	AA	AA	BB	
SNP A-1976958	rs10517066	42734363	BB	BB	BB	BB	
SNP A-1976959	rs16855262	42734802	AA	AA	AA	AA	
SNP A-4228792	rs11736676	42735775	BB	BB	BB	BB	
SNP A-1976960	rs10517069	42735820	BB	BB	BB	BB	
SNP A-1976962	rs920153	42737163	BB	BB	BB	AA	
SNP A-4198005	rs4512035	42737733	BB	BB	BB	BB	
SNP A-2235122	rs9291227	42738007	BB	BB	BB	BB	
SNP A-2073972	rs10938235	42744002	AA	AA	AA	BB	
SNP A-1976963	rs720961	42749414	AA	AA	AA	BB	
SNP A-2128185	rs2043640	42751838	AA	AA	AA	AA	
SNP A-2074955	rs6844568	42762326	AA	AA	AA	AA	
SNP A-4237356	rs6447209	42762467	AA	AA	AA	AA	
SNP A-1976964	rs1436472	42783059	BB	BB	BB	AA	
SNP A-2183044	rs10031221	42799900	AA	AA	AA	AA	
SNP A-2072621	rs7663681	42804500	AA	AA	AA	BB	
SNP A-2307022	rs6447228	42804528	BB	BB	BB	AA	
SNP A-1837172	rs1017429	42808275	AA	AA	AA	AA	
SNP A-2076542	rs1436485	42819467	AA	AA	AA		
SNP A-4196631	rs1436487	42819777	BB	BB	BB	AB	
SNP A-4198461	rs6447236	42820381	BB	BB	BB	AA	
SNP A-1901356	rs4361423	42858360	AA	AA	AA	AA	
SNP A-2155340	rs4309873	42905245	AA	AA	AA	AA	

In this table SNP genotypes of part of the overlapping homozygous regions are listed encompassing *GRXCR1*. The heterozygous calls for the SNPs in *GRXCR1* and telomeric to the gene have confidence level just above the threshold.