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Hearing function and thresholds: a genome-wide association study in European isolated populations identifies new loci and pathways

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Abstract

Background Hearing is complex trait, but until now only few genes are known to contribute to variability of this process. In order to discover genes and pathways that underlie auditory function, a Genome Wide Association Study was carried out within the International Consortium G-EAR.

Methods Meta-analysis of GWAS data from 6 isolated populations of European ancestry for an overall number of 3417 individuals.

Results Eight suggestive significant loci ($p<10^{-7}$) were detected with a series of genes expressed within the inner ear such as: *DCLK1*, *PTPRD*, *GRM8*, *CMIP*. Additional biological candidates marked by a SNP with a suggestive association ($p<10^{-6}$) were identified, as well as loci encompassing "gene desert regions", genes of unknown function or genes whose function has not be linked to hearing so far. Some of these new loci map to already known hereditary hearing loss loci whose genes still need to be identified. Data have also been used to construct a highly significant "in silico" pathway for hearing function characterized by a network of 49 genes, 34 of which are certainly expressed in the ear.

Conclusion These results provide new insights into the molecular basis of hearing function and may suggest new targets for hearing impairment treatment and prevention.

Keywords

Molecular basis of hearing system, GWAS, meta-analysis, isolated populations, "in silico" pathways

Introduction

The hearing system is characterized by three structures: a) the outer part, b) the middle ear, and c) the inner ear, that all play a role in hearing function. The hearing system is difficult to study through biochemical routes, due to the small amounts of tissue available for analysis and by key molecules that may be present in only a few tens of copies per cell, thus compounding the difficulty[1]. Hair cells of the inner ear are constituted by stereocilia arranged in bundles at their upper surface, packed with actin filaments, and deflected by the vibrations of sound[1]. This activity opens ion channels modulating potential within the cell. Cell activation releases neurotransmitters to synaptic junctions between hair cells and neural fibers of the auditory nerve. The neural spike subsequently propagates in the auditory nerve fiber. Nerve impulses are finally perceived by the brain, primarily in the temporal lobe, where they can be processed and assigned meaning.

Little is known about the molecular basis of variation of normal hearing function. Several molecules have been identified as having a role in auditory function and hair cell transduction because they are specifically expressed in or around the stereocilia and mutations in their genes lead to hearing impairments in either humans or mice models[2]. These dysfunctional proteins are involved in impaired molecular-physiologic processes of potassium and calcium homeostasis, apoptotic signaling, stereocilia linkage, mechano-electric transduction, electromotility, and many other processes[3]. Briefly, these molecules include myosins which represent one of the largest group of deafness associated molecules, adhesion protein such as cadherins, member of the ferlin family, components of the

tectorial membrane, genes involved in ion homeostasis such as connexins[4] and many others[5]. According to the Hereditary Hearing Loss homepage (see HHL), more than 140 loci for nonsyndromic HHL have been mapped, and approximately 80 genes identified in humans. In animal models ~70 loci for nonsyndromic HHL and ~60 genes have been so far described.

Hearing loss can also be multifactorial or complex in causality such as age-related hearing impairment (ARHI)[6] and noise-induced hearing loss (NIHL)[7], reflecting the interaction of a number of genetic and environmental factors. Despite some relevant efforts done to identify the molecular bases of these conditions, up to now, only a few genes have been associated with both ARHI[6] and NIHL[7]. Almost all genes so far identified in all forms of hearing loss are those directly related to hearing as qualitative traits (i.e. disease genes mainly involved in monogenic inherited forms), while almost nothing is known about genes implicated in defining hearing as quantitative trait (i.e. thresholds) in normal hearing.

The use of isolated populations to reduce heterogeneity of complex and/or quantitative traits has already proven very useful in identifying DNA polymorphisms associated with these traits[8]. Nevertheless, the argument about the advantage of the use of such population is still an open issue[9]. In principle, the inbreeding, typical of small communities, reduces genetic heterogeneity, increases homozygosity, and reduce environmental factors providing greater power for detection of susceptibility genes[8,10]. Moreover, such populations could be extremely useful to detect rare variants.

Here, we combine the power of studying isolated populations with a) a general screening of hearing function, b) high-throughput SNP analysis, c) Genome Wide Association Studies (GWAS), and d) up-to-date *in silico* pathways construction, to analyze hearing as a continuous trait and detect underlying genes and networks. To reach this goal, we analyzed

different hearing thresholds as well as Pure Tone Averages (PTA) and principal components from a Principal Component (PC) analysis of hearing traits performing a large meta-analysis on isolated populations coming from different European regions. Results have been then used to construct a highly significant biological pathway.

Materials and Methods

Subjects

Within the International consortium called G-EAR, we recruited 3417 subjects coming from several isolated villages: Carlantino located in South Eastern Italy (267)[11], Friuli Venezia Giulia Genetic Park[12], characterized by 6 villages located in North Eastern Italy (968), Korcula[13], an island in the Adriatic sea (Croatia) (795), Campora and Cardile two isolated villages located in the Cilento National's park , characterized by 2 different isolated villages in South Western Italy (421)[11], and, Talana (469), an isolated village from Ogliastra Genetic Park in central part of Sardinia, Italy[14], plus Split (497) an outbred not isolated population located on the Dalmatian coast (Croatia). All tests were performed using standard audiometers. Subjects underwent pure-tone audiometry, tympanogram, and acoustic reflex testing in both ears. Measurements were all obtained after any acoustically obstructing wax had been removed. The analysis of hearing function was done calculating the pure-tone average of air-conduction (PTA at the lower 0.25, 0.5 and 1 kHz, medium 0.5,1 and 2 kHz, and high frequencies 4,8 kHz).

A questionnaire to obtain socio-demographic information, as well as data on physical activity (i.e. job, sport, etc.), lifestyle (e.g smoking, alcohol consumption, coffee intake, diet including taste and food preferences, etc.), clinical examinations (psicological,

neurological, cardiological, etc.) clinical chemistry including blood count and more than 20 parameters, drugs, diseases and other information regarding the health status (BMI, bone density, blood pressure, etc.) have been collected for each subject. Only subjects aged 18 or older were included in the analysis. Clear familial forms of severe hearing loss have been excluded from the study.

Phenotypes

For the analyses we used only the threshold from the best ear, defined as the ear with lowest value of hearing loss for each individual. Since age explains a big part of the hearing variance, for this reason each trait was first linearly regressed against age. Residuals from this regression were cleaned from outliers (mean +/- 6 SD) and, since their distributions were skewed, normalized using rank normal transformation and used for the association analysis using sex as a covariate. The following quantitative traits have been tested:

- Pure Tone Average at low, medium and high frequencies (PTAL, PTAM, PTAH)
- Seven different thresholds (250 Hz, 500 Hz, 1 KHz, 2 KHz, 4 KHz, and 8 KHz)
- The first 3 components from PCs (PC1, PC2, PC3) estimated on all of the frequencies as previously described[15]. We did not regress out sex or age before running PCA analysis and we checked that none of the components used reflected either one. Each component describes a different pattern in the data[15]: PC1 is a "size variable" that represents an overall measure of a subject's hearing ability, PC2 and PC3 are instead "shape variables". In particular PC2 shows the ratio of hearing between the high and the low frequencies and is a measure of the slope of the audiogram, PC3 contrasts the middle frequencies with the lower and higher frequencies and can be considered a measure of the concavity of an audiogram.

DNA sampling and genotyping

All studies had appropriate ethical consent and consent forms for clinical and genetic studies have been signed by each participant in the study. Blood samples were collected and used to extract DNA using standard protocols. After measuring quantity and quality of DNA, samples were genotyped with Illumina 370k platform (Carlantino, FVG Genetic Park, Cilento, Korcula and Split) or Affymetrix 500K (Talana). Genotype quality control and data cleaning were conducted independently by each study group and are summarized in supplementary Table 2. Genotypes were then imputed to the 2.5M HapMap CEU SNP set v22 (summary of imputation can be found in Supplementary Material). The same map was also used to look at regions of Linkage Disequilibrium.

Statistical analysis

Association analysis was carried out through a mixed model linear regression where the variance/covariance matrix is the genomic kinship. The analysis was implemented in GenABEL[16] package for genotyped SNPs and ProbABEL[17] for imputed data. Meta-analysis was conducted using the inverse variance model as implemented in the MetABEL[16] R library. For PCs traits fixed effects meta-analysis was conducted where Z-scores were estimated from p-values and weighted on the sample-size as implemented in METAL (see METAL). SNPs with imputation quality (Rsq in MACH) less than 0.3 or with less than 30 copies of an allele in each population, were excluded. P-values were estimated using the Wald test. After quality control, 3417 subjects and approximately 2.2 million SNPs were used for meta-analysis.

Pathway analysis

From each GWA result all SNPs with P-values lower than $1x10^{-4}$ have been selected. After

removing duplicate loci, tagged by different SNPs, and keeping only the most significant ones, all genes that could be in linkage disequilibrium (LD) with these SNPs based on the HapMap CEU population have been taken into account, generating a list of 1276 genes. Identification of molecular network interactions and pathway analysis of most significant GWAS loci was completed using the Ingenuity Pathway Analysis (IPA)[18] tools from Ingenuity Systems® (Redwood City, California, USA; http://www.ingenuity.com). Briefly, the top 48 genes (cut-off of 5x10-5) arising from GWAS data were functionally characterized using IPA. Networks with a maximum of 70 genes or proteins were constructed, and scores were computed based on the likelihood of the genes being connected together due to random chance. A score of 2 indicates that there is a 1/100 chance that these genes are connected in a network due to random chance. Therefore, any networks with a score of 2 or above are considered statistically significant (with >99% confidence). IPA then map these genes to a global molecular network developed from information contained in the Ingenuity knowledge base (a manually curated database of experimentally proven molecular interactions from published literature). IPA determines the most significantly enriched biological functions and/or related diseases by calculating the P-value using Fisher's exact test. Using similar methods, significantly represented canonical pathways in a set of focus genes were also determined using IPA. To run the analysis only direct interactions were taken into account. After the definition of the most significant pathways, expression data within the ear have been obtained by searching the following databases: NCBI Gene for human and mouse, Eurexpress and Jackson Laboratory for mouse.

Results

The meta-analysis has succeeded in identifying some genome-wide suggestive loci associated

with hearing traits, plus several additional ones strongly suggestive. All these trait-locus associations represent novel findings.

For all quantitative traits the top SNPs resulting from this analysis are listed in Table 1 (PTA), Table 2 (PCs), Table 3 and Table 4 (thresholds). Eight loci were strongly associated with the analyzed traits (max $p=2.1x10^{-7}$), while many others were in a group showing a maximum p-value of $1x10^{-6}$. Table 1 reports the most suggestive significant GWAS data obtained on PTA. For PTAL the strongest association signal was given by rs248626 (P-value $3.1x10^{-6}$) located on chromosome 5, in a region containing *DIAPH1* and a cluster of protochaderin genes[19,20]. Suggestive significant association with this locus has been also obtained at 500Hz threshold. Another interesting SNP is rs4603971 (chromosome 3) that is in LD with *KCNMB2* a potassium large conductance calcium-activated channel[21]. It is known that several potassium channels are essential for hearing pathways[4]. Moreover, *KCNMB2* interacts with *KCNMA1* that plays a key role in controlling the tuning of hair cells in the cochlea, regulation of transmitter release and innate immunity[22].

For PTAM, rs898967 (chromosome 16) is located within *CMIP* gene, which is expressed in the ear (see NCBI). Suggestive significant associations with this gene have been also found at 250 Hz, 1 KHz and at 2 KHz. Another positive SNP is rs641113 located on chromosome 10 is in LD with *OPTN*, an important gene required for myosin VI localization at the Golgi complex[23]. *Myosin VI* is essential for auditory and vestibular function in mammals and genetic mutations lead to hearing impairment and vestibular dysfunction in both humans and mice[23]. Finally, for PTAH, there are two interesting SNPs: rs6673959 (chromosome 1) that is in LD with the *DFFB* gene, a pro-apoptotic related gene expressed in the cochlear cells[24] and rs10936160 located in the *MFSD1* gene whose expression in the ear is already known[25]. The full list of SNPs with a P-

value lower than $1x10^{-6}$ for the three PTAs, is reported in Table1.

Concerning PCs a significant association was detected at PC1 (rs2687481, P-value 3.2 $x10^{-7}$) with *GRM8* gene, a glutamate receptor that inhibits adenylyl cyclase decreasing the formation of cAMP[26]. Additional associations were detected with several SNPs located within the *FGF14* gene, a member of the fibroblast growth factors family and rs7182802 (P-value 9.9 $x10^{-6}$) located inside *GABRG3*, a ion channel gene differentially expressed in the aging ear [27,28].

Concerning PC2, rs6669265 (P-value 6 $x10^{-7}$), a SNP in LD with *NR0B2* gene, whose protein is an unusual orphan receptor that contains a putative ligand-binding site, has been identified[29]. The protein encoded by *NR0B2*, has been shown to interact with retinoid and thyroid hormone receptors, inhibiting their ligand-dependent transcriptional activation[30]. Two additional loci at P-value$<10^{-7}$ have been detected: one on chromosome 11 and the other one on chromosome 17.

Additional interesting SNPs are in LD with *OTX2* (P-value 5 $x10^{-6}$) that acts as a transcription factor and may play a role in brain and sensory organ development[18]. Another relevant SNP is rs3783041 on chromosome 13 (P-value 5 $x10^{-6}$) that is in LD with *DIAPH3*. This gene belongs to the diaphanous family and promotes actin polymerization. It is required for cytokinesis, stress fiber formation, and transcriptional activation of the serum response factor[19].

Finally, regarding the different thresholds (Table 3), rs10815873, a SNP within *PTPRD* gene was found to be strongly associated at 125 Hz threshold. Data available from the literature show that some members of this gene family play an important role in the hearing system[31]. The SNP rs248626, already detected for PTAL (see PTA results section and Table 1), was also found associated with 500 Hz. As already mentioned, it is in LD with a cluster of protochaderins genes. Similarly, rs898967 located within *CMIP* gene (see PTA results and Table 1) was found to be significantly associated with 1KHz. Another interesting SNP at the 250 Hz is rs1849287 on chromosome 15 located in *NMB* gene; a member of this family (*NMB1*) blocks

mechanotransducing ion channels in cochlear hair cells[32].

Others very interesting data have been obtained at 4KHz. The SNP rs9574464 (chromosome 13) shows an association (P-value $3.2x10^{-7}$). This SNP is in LD with *DCLK1* (see NCBI), a member of the protein kinase superfamily and the doublecortin family which is expressed in the inner ear. Additional suggestive SNPs are: a) rs2660178, located on chromosome 10, which is in LD with *PCDH15*, a gene already involved in causing hearing loss[5]; b) rs1719101, in LD with *GRM8*, a glutamate receptor already detected at PC1 and c) rs16939415 in LD with *PXMP3* a gene related with *GATA3* transcription factor, an early regulator of auditory system development[33]. At 8 KHz we identified rs9899183, on chromosome 17, that is in LD with *TNFS*; some members of the *TNF* family are important players in the initiation of acute cochlear apoptosis[24].

Some of the genes/loci here described map to already known HLL loci such as DFNA30, DFNA37, DFNA42 and DFNA54 for dominant forms and DFNB17, DFNB48, DFNA57, DFNB60 for recessive forms (see HHL) whose genes are not yet characterized.

The pathway analysis was carried out using Ingenuity software and 48 seed molecules arising from GWAS data. The analysis was able to generate only one significant pathway (Figure 1) characterized by a very high P-value ($1x10^{-79}$) and 49 different molecules. This score reflects the extremely low probability that genes were represented in this particular network by chance alone. Thirty out of them are present within the output of our GWAS further strengthening the impact of this work, while the remaining 19 (added by the software) are other proteins that interact with them at different levels to create this predicted pathway. We then searched for different human and mouse expression databases demonstrating that 34 genes (69%) contained within this pathway are expressed in the ear (in color in Figure 1). These

findings suggest that most of the genes here identified might have an interesting role in studying normal variation of hearing function. Finally, this hypothetical pathway is also characterized by a series of IPA canonical pathways, i.e. standardized pathways very well known in the literature having a molecular role in a biological system. The most represented in terms of members are "colorectal cancer metastasis signaling", "glucocorticoid receptor signaling" and "G-protein coupled receptor".

Discussion

GWAS became the tool of choice for the identification of genes for complex and quantitative traits, since they are able to analyze large amounts of data[34]. Despite recent progress, almost nothing is known about hearing thresholds and the molecular bases of variation of normal hearing, apart from genes identified as being directly involved in HHL (see HHL). Here, we present the first GWAS performed on hearing traits.

Our scan resulted in the identification of genes that have a realistic biological role in hearing function and that might be considered as good candidates for further research activities. Among them, *DCLK1* (4KHz), doublecortin-like kinase 1, a member of the protein kinase superfamily and the doublecortin family which is expressed in the inner ear. The encoded protein is involved in several different cellular processes, including neuronal migration in the developing brain and in maturation of nervous system. A possible role of *DCLK-1* in the maturation of the nervous system could be interesting on hypothesizing an important role of this gene in the development of sensitive neurons that we know essential for the hearing function. Another interesting gene is *PTPRD* (125 Hz), a member of the protein tyrosine phosphatase (*PTP*) family. *PTPs* are known to be signaling molecules that regulate a variety of cellular processes including cell growth,

differentiation, mitotic cycle, and oncogenic transformation. Some members of this family play an important role in the hearing system. For example, studies of homologous genes in others species suggest the role of *PTPs* family in promoting neurite growth and regulating neurons axon guidance, both mechanisms important for neuronal development. A third gene is *GRM8* (PC1), a member, together with *GRM7*, of the group III of metabotropic glutamate receptors family which has been divided into three groups on the basis of sequence homology, putative signal transduction mechanisms, and pharmacologic properties[26,34]. GRM8 and GRM7 proteins show 87% of homology and 76% of identity using BLAST analysis. Very interestingly, both associations with GRM8 and GRM7 have been detected at PC1 in two independent studies, one case controls focused on ARHI[6] (qualitative trait) and the present one on genetic bases of variation of normal hearing function (quantitative trait). A fourth significant candidate is *NR0B2 (PC2)*, whose product interacts with retinoid and thyroid hormone receptors, inhibiting their ligand-dependent transcriptional activation[29]. Nuclear receptors for thyroid hormone and retinoic acid are expressed in the developing sensory epithelia of the inner ear and their ligands play roles in hair cell development[30]. The last candidate is *CMIP* at 1kHz (*c-MAF* inducing protein), a gene also expressed in the inner ear.

Additional strong biological candidates arose from results of SNPs within a group showing a maximum p-value of $1x10^{-6}$. In particular, we should mention *FGF14* (PC1)[27]. It has been demonstrated that Fibroblast growth factors have been implicated in a wide range of cellular processes. Although the role of FGF signaling in the maintenance of normal auditory function remains to be elucidated, some members of this family play a dosage-sensitive role in the differentiation of the auditory sensory epithelium and have a fundamental function in cochlea development. Moreover, the inhibition of *FGFs* signaling could cause a reduction in hair cells and support cells and an alteration of *FGFRS*, which signal are produced by auditory neurons,

could modify the right development of the cochlea[27]; ii) *LPRP4* (PC3) and a quite long list of other genes such as *PCHD15*, *KCNMB2*, *PIBF1*, *OPTN*, *DFFB*, *MFSD1*, *NMB*, *PXMP3*, and *TNFSF12* gene. They are good candidates for variation of normal hearing function, and could be eventually considered as candidates for both NIHL and ARHI.

Since previous studies on molecular basis of inherited hearing loss have detected loci/genes which show, as expected, a minimum overlap with those here reported, present data describe additional genes which might be involved in hearing function.

These results led to "in silico" building of an extremely interesting pathway characterized by a network of genes in which the vast majority of them are expressed in the inner ear. Despite it represents an hypothetical network, it is characterized by a series of significant functional relationships amongst proteins and molecules with 3 main canonical networks: Colorectal cancer metastasis signaling, Glucocorticoid receptor signaling and G-protein coupled receptor.

As regards to the first network, recently has been found that *DFNA5* (a gene causing a non syndromic autosomal dominant type of hearing loss) is also involved in some type of cancer such as colorectal one[35]. Concerning the second one, although the exact mechanism of glucocorticoid action on the inner ear are not known, the inner ear of both humans and experimental animals demonstrate an abundance of glucocorticoid receptors in both neuronal and non-neuronal tissues. Moreover, glucocorticoids are widely used to treat different hearing disorders[36]. Finally, as regards to the last network, some members of G-protein coupled receptor family are known to play a relevant role in hearing function.

Moreover, among the molecules added by the Ingenuity software to build the final pathway we should mention *BMP4*, *VDR*, and *PTHLH*. *Bone Morphogenetic Protein 4* (*BMP4*) is a member of the *TGF-β* superfamily and is known to be important for the normal

development of many tissues and organs, including the inner ear. Recent studies have been demonstrated that *Bmp4* heterozygous null (*Bmp4*+/−) mice are viable and some adults exhibit an inner ear defect[37]. Regarding *VDR* (*Vitamin D receptor*), it seems to be important with a wide range of things and it has several important biological roles. *VIT D* deficiency, *VDR* malfunction, hypoparathyroidism and hypervitaminosis have been suggested to be potential caused of sensorineural hearing loss[38]. As a matter of fact, the *VDR* knock-out mouse shows a severe calcification in the thalamus causing an alteration of the connection between inferior colliculus and auditory cortex. As mentioned before, an alteration of expression of parathyroid hormone is related with hearing and, to be more precise, this alteration could contribute to the abnormal bone turnover in otoscleorosis; in this light *PTHLH* is another strong candidate[39].

Of course, all the proteins present in the pathway can be taken into account as a basis for further *in vitro* functional experiments.

In conclusion, here we report a first series of data on hearing quantitative traits (i.e. normal hearing function). Candidate genes located in positive GWAS regions belong to several different gene families that show only a small overlap with those already identified as causing hearing impairments. However, present findings should be further confirmed and/or replicated in other populations/cohorts. Anyway, these results increase our knowledge of the molecular basis of normal hearing function and might open new perspectives for preventive and therapeutic strategies for hearing impairments.

Conflict of interest

The authors declare no conflict of interest

Supplemental Data

Supplemental data include three tables that can be found with this article online.

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Web Resources

The URLs for data presented herein are as follows:

Database of human genes: Genecards, http://www.genecards.org/

Meta-analysis: METAL, http://www.sph.umich.edu/csg/abecasis/Metal/

U.S. National Institutes of Health (NIH) free digital archive of biomedical and life sciences: http://www.ncbi.nlm.nih.gov/ (NCBI)

Hereditary Hearing loss Homepage (HHL), http://hereditaryhearingloss.org/

Ingenuity software for pathway analysis, http://www.ingenuity.com/

Eurexpress, http://www.eurexpress.org/ee/

Jackson Laboratory, http://www.informatics.jax.org/

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Figure 1. Ingenuity functional pathway

Ingenuity functional pathway with a score of $1x10^{-79}$ represents 49 selected genes directly interacting each other. Genes colored in red are expressed in humans, those in blue are expressed in mouse, while those with both colors are expressed in both human and mouse.

Table 1. Top SNPs resulting from the mixed-model analysis for PTAL (low), PTAM (medium) and PTAH (high). Top SNPs obtained after meta-analysis of GWAS data are listed according different PTAs. Direction of effect in: Cilento, Split, Korcula, Carlantino, Friuli Venezia Giulia, Talana. Betas for single population have not been reported in value but in direction only. Abbreviations: Chr., chromosome, e.a., effect allele., SE ß., standard error ß.

Table 2. Top SNPs resulting from the mixed-model analysis for PC1, PC2 and PC3. Top SNPs obtained after meta-analysis of GWAS data are listed according different PCs. Direction of effect in: Cilento, Split, Korcula, Carlantino, Friuli Venezia Giulia, Talana. Betas for single population have not been reported in value but in direction only. Abbreviations: Chr., chromosome, e.a., effect allele., SE ß., standard error ß.

Table 3. Top SNPs resulting from the mixed-model analysis for 125Hz, 250Hz, 500Hz, 1kHz, 2kHz, 4kHz, 8kHz . Top SNPs obtained after meta-analysis of GWAS data are listed according different tresholds. Direction of effect in: Cilento, Split, Korcula, Carlantino, Friuli Venezia Giulia, Talana. Betas for single population have not been reported in value but in direction only. Abbreviations: Chr., chromosome, e.a., effect allele., SE ß., standard error ß.

Supplemetary tables 1., 2., 3.

Table 1.

PTA Low								
name	***chr***	***position***	***e.a***	***ß***	***SEß***	***direction of effect***	***p***	***Gene region***
rs248626	5	141077909	G	0.12	0.03	++++++	3.06E-06	PCDH-cluster; ARAP3; KIAA0141; RNF14; DIAPH1; GNPDA1; RELL2; CENTD3; FCHSD1; HDAC3
rs1541925	19	58798359	G	-0.12	0.03	-----?	5.31E-06	DPRX
rs4603971	3	179385161	G	0.32	0.07	++??+?	7.57E-06	KCNMB2
rs2325487	13	72305430	C	-0.11	0.02	---+--	7.66E-06	PIBF1; RP11-11C5.2; DIS3; C13orf34; PIBF1
rs9564920	13	72296946	G	0.11	0.02	+++-++	7.78E-06	

PTA Medium								
name	***chr***	***position***	***e.a***	***ß***	***SEß***	***direction of effect***	***p***	***Gene region***
rs898967	16	80124281	C	0.16	0.03	+??+++	3.07E-06	CMIP
rs713115	16	7528039	G	0.16	0.03	++++++	3.35E-06	A2BP1
rs641113	10	13177871	G	0.18	0.04	-++++?	3.66E-06	OPTN; CCDC3
rs17816387	15	30880026	C	0.13	0.03	++++++	4.41E-06	FORMIN-1
rs11629595	15	30876862	G	-0.13	0.03	------	4.53E-06	
rs2123068	15	30878176	G	0.13	0.03	++++++	4.65E-06	
rs9806641	15	30883187	C	-0.12	0.03	---+--	8.11E-06	
rs11077197	16	7535921	C	-0.16	0.04	------	8.67E-06	
rs11631339	15	30885186	C	-0.12	0.03	---+--	8.91E-06	
rs12906330	15	30885406	G	0.12	0.03	+++-++	9.29E-06	

PTA High								
name	***chr***	***position***	***e.a***	***ß***	***SEß***	***direction of effect***	***p***	***Gene region***
rs10767583	11	26783286	C	-0.16	0.04	-----	3.90E-06	BBOX1; TMEM16C;

								SLC5A12; FIBIN; MUC15
rs7926469	11	26781579	C	0.16	0.04	+++++	3.92E-06	
rs7936609	11	26781203	G	-0.16	0.04	-----	3.98E-06	
rs11739446	5	115648123	G	0.51	0.11	??++?	4.99E-06	COMMD10
rs6673959	1	4022977	G	0.39	0.09	???++	7.97E-06	DFFB; KIAA0562; C1orf174
rs10936160	3	160018645	C	-0.16	0.04	+----	8.72E-06	MFSD1

Table 2.

PC1						
name	***chr***	***position***	***e.a***	***Direction of ß***	***p***	***Gene region***
rs2687481	7	125656358	t	------	3.22E-07	GRM8
rs2521030	7	125656552	c	------	4.56E-07	GRM8
rs2687479	7	125655251	a	++++++	4.87E-07	GRM8
rs1673365	7	125677843	t	------	1.45E-06	GRM8
rs1673369	7	125684818	t	++++++	1.46E-06	GRM8
rs1719089	7	125675233	t	------	1.50E-06	GRM8
rs1719090	7	125672107	t	------	1.57E-06	GRM8
rs1719091	7	125671577	a	------	1.58E-06	GRM8
rs1719093	7	125671126	t	++++++	1.60E-06	GRM8
rs1673373	7	125660694	a	++++++	1.87E-06	GRM8
rs6544889	2	46430803	a	+-----	2.18E-06	EPAS1
rs6544887	2	46430716	t	-+++++	2.18E-06	EPAS1
rs6544888	2	46430769	a	-+++++	2.19E-06	EPAS1
rs1562452	2	46433948	a	+-----	2.61E-06	EPAS1
rs11671149	19	58862280	t	++??++	2.95E-06	DPRX
rs585747	11	79377793	a	------	3.08E-06	
rs3765620	11	102100702	a	++++++	3.35E-06	MMP27; MMP8
rs1036987	17	72403766	a	++++++	3.45E-06	MGAT5B
rs6756667	2	46432913	a	++++++	3.73E-06	EPAS1
rs1562453	2	46433978	t	++++++	3.76E-06	EPAS1
rs4953356	2	46432428	t	------	3.79E-06	EPAS1
rs17705835	15	91463287	t	?--?--	3.82E-06	RGMA; CHD2
rs6743991	2	46436739	a	------	4.27E-06	EPAS1
rs6735812	2	46434578	c	++++++	4.47E-06	EPAS1
rs4953358	2	46434957	c	++++++	4.56E-06	EPAS1
rs4953359	2	46435147	t	------	4.63E-06	EPAS1
rs10848114	12	129545308	t	------	4.75E-06	RIMBP2
rs6585804	10	123970098	a	------	5.33E-06	TACC2
rs17687720	13	101410000	a	++++++	5.76E-06	FGF14
rs7987221	13	101409767	a	++++++	5.77E-06	FGF14

rs17687714	13	101409041	a	++++++	5.78E-06	FGF14
rs17687732	13	101410068	a	++++++	5.83E-06	FGF14
rs17631289	13	101410431	a	++++++	6.35E-06	FGF14
rs12430805	13	101410771	t	------	6.50E-06	FGF14
rs1073341	7	125651040	a	------	6.52E-06	GRM8
rs12430821	13	101410824	a	------	6.66E-06	FGF14
rs6742663	2	20258641	a	++++++	6.68E-06	SDC1
rs4772428	13	101411719	c	++++++	6.74E-06	FGF14
rs12427431	13	101412944	t	------	6.88E-06	FGF14
rs17631205	13	101405423	t	++++++	6.89E-06	FGF14
rs12856393	13	101413146	a	------	7.02E-06	FGF14
rs12427457	13	101413321	a	------	7.07E-06	FGF14
rs6707241	2	46438356	t	+-----	7.32E-06	EPAS1
rs6740096	2	46439392	a	-+++++	7.34E-06	EPAS1
rs4350703	2	20263750	a	------	7.35E-06	SDC1
rs6756290	2	20259092	t	------	7.98E-06	SDC1
rs10191091	2	46426714	t	+-----	8.15E-06	EPAS1
rs2348475	2	20260595	t	------	8.28E-06	SDC1
rs11895809	2	20262535	a	------	8.30E-06	SDC1
rs978091	14	42414308	a	---?--	8.86E-06	
rs7182802	15	24929952	t	---+--	9.94E-06	GABRG3

PC2						
name	***chr***	***position***	***e.a***	***Direction of ß***	***p***	***Gene region***
rs7212433	17	27107800	t	------	2.07E-07	SUZ12; LRRC37B; C17orf79; RAB11FIP4; UTP6
rs11219981	11	124614837	a	---?--	2.86E-07	PKNOX2
rs10790729	11	124605332	t	+++?++	5.96E-07	PKNOX2
rs6669265	1	27233806	a	++++++	5.967E-07	C1orf172; FAM46B; SFN; ZDHHC18; ATPBD1B; NUDC; LOC388610; NR0B2; PIGV; ARID1A; GPATCH3
rs2276957	4	122480925	t	------	4.85E-06	GPR103
rs1952587	14	56230970	a	+++?++	4.98E-06	C14orf101; OTX2
rs12499677	4	122482588	a	------	5.05E-06	GPR103
rs10857080	4	122483595	a	++++++	5.09E-06	GPR103

rs3783041	13	59486892	a	??----	5.14E-06	DIAPH3
rs3783040	13	59486531	t	??----	5.18E-06	DIAPH3
rs17864603	2	51842904	t	--+---	5.27E-06	
rs11843015	13	59485201	t	??++++	5.28E-06	DIAPH3
rs11219971	11	124584492	t	++++++	5.35E-06	PKNOX2
rs11841515	13	59476294	t	??++++	5.83E-06	DIAPH3
rs13144655	4	122488810	t	------	6.57E-06	GPR103
rs928108	14	56240227	a	---?--	7.45E-06	C14orf101; OTX2
rs11842701	13	59329690	a	??----	7.77E-06	DIAPH3
rs4240259	4	122489943	t	++++++	7.89E-06	GPR103
rs11844009	13	59336501	t	??----	8.74E-06	DIAPH3
rs10790728	11	124583221	t	++++-+	8.91E-06	PKNOX2
rs17292288	9	128098321	t	+++?++	8.95E-06	FAM125B
rs10893349	11	124581125	t	----+-	8.99E-06	PKNOX2
rs913741	14	56241026	a	---?--	9.28E-06	C14orf101; OTX2
rs17091976	14	56239864	a	---?--	9.76E-06	C14orf101; OTX2

PC3						
name	***chr***	***position***	***e.a***	***Direction of ß***	***p***	***Gene region***
rs712932	1	99424088	c	------	2.32E-07	PAP2D; LPPR4
rs712934	1	99421564	a	------	2.33E-07	PAP2D; LPPR4
rs712931	1	99426756	t	------	2.38E-07	PAP2D; LPPR4
rs819786	1	99427115	a	------	2.39E-07	PAP2D; LPPR4
rs819785	1	99428208	a	------	2.41E-07	PAP2D; LPPR4
rs712936	1	99419627	t	++++++	2.84E-07	PAP2D; LPPR4
rs712937	1	99418034	a	++++++	3.15E-07	PAP2D; LPPR4
rs664835	1	99436556	a	------	4.74E-07	PAP2D; LPPR4
rs819778	1	99437774	a	++++++	5.00E-07	PAP2D; LPPR4
rs645441	1	99442645	t	++++++	5.65E-07	PAP2D; LPPR4
rs687101	1	99410160	a	------	6.25E-07	PAP2D; LPPR4
rs638723	1	99414257	a	++++++	6.59E-07	PAP2D; LPPR4
rs685267	1	99443937	a	++++++	8.37E-07	PAP2D;

						LPPR4
rs668206	1	99416551	t	------	9.64E-07	PAP2D; LPPR4
rs11485142	1	216899837	a	+++?++	1.41E-06	TGFB2
rs604876	1	99413608	t	++++++	1.91E-06	PAP2D; LPPR4
rs305256	8	137637434	t	++++++	2.37E-06	
rs3911410	1	99432733	a	++++++	2.44E-06	PAP2D; LPPR4
rs2581561	8	137614330	t	++++++	3.48E-06	
rs621215	1	99392012	a	------	3.73E-06	PAP2D; LPPR4
rs305255	8	137638184	a	------	3.95E-06	
rs305261	8	137631213	t	------	3.98E-06	
rs6756828	2	174627435	t	--+?--	4.23E-06	OLA1; SP3
rs2587847	8	137624757	t	++++++	4.28E-06	
rs305290	8	137655983	t	------	4.40E-06	
rs305289	8	137656252	t	------	4.49E-06	
rs2119234	8	137621985	t	++++++	4.53E-06	
rs305288	8	137656556	t	++++++	4.56E-06	CCDC81
rs587509	1	99388302	t	------	4.86E-06	PAP2D; LPPR4
rs7123014	11	85600090	a	------	4.95E-06	C11orf73; EED; MBIP
rs305286	8	137661228	a	++++++	5.23E-06	
rs10130121	14	35694502	t	+++?++	5.89E-06	BRMS1L; GARNL1; MBIP
rs594821	18	74846577	t	+++?++	6.07E-06	SALL3; MBIP
rs17461837	14	35714081	t	+++?++	6.29E-06	BRMS1L; GARNL1
rs10137274	14	35714290	a	---?--	6.35E-06	BRMS1L; GARNL1
rs305231	8	137673287	t	------	7.76E-06	
rs305237	8	137677000	a	++++++	8.53E-06	MBIP
rs305244	8	137684388	a	++++++	8.97E-06	
rs10139184	14	35719798	t	+++?++	9.57E-06	BRMS1L; GARNL1
rs305249	8	137688139	t	++++++	9.68E-06	
rs863686	1	99382741	a	++++-+	9.73E-06	PAP2D; LPPR4

Table 3.

125Hz						

name	***chr***	***position***	***e.a***	***ß***	***SEß***	***direction of effect***	***p***	***Gene region***
rs10815873	9	8426194	G	-0.32	0.06	--	3.35E-07	PTPRD
rs10758974	9	8425925	G	0.32	0.06	++	3.37E-07	
rs11263901	1	36733213	G	-0.48	0.10	--	2.04E-06	CSF3R
rs2241492	15	59934499	T	0.30	0.07	++	4.36E-06	VPS13C
rs2189693	3	61943483	C	-0.45	0.10	--	4.50E-06	PTPRG
rs7647959	3	61942076	C	-0.45	0.10	--	5.81E-06	
rs5009126	18	28023747	T	-0.28	0.06	--	7.37E-06	MEP1B; RNF125; RNF138
rs8092245	18	27999780	C	0.34	0.08	++	9.81E-06	

250Hz								
name	***chr***	***position***	***e.a***	***ß***	***SEß***	***direction of effect***	***p***	***Gene region***
rs12361953	11	24567706	C	-0.16	0.04	------	5.73E-06	LUZP2
rs1563655	16	80033433	G	-0.12	0.03	-----?	8.61E-06	CMIP
rs1849287	15	82904075	C	-0.14	0.03	------	9.09E-06	NMB; UBE2Q2P; WDR73; FLJ40113; SEC11A; ZNF592; SCAND2; ADAMTL 3;ALPK3; ZSCAN2; SLC28A1
rs2360243	8	53896908	C	0.14	0.03	+?++++	9.14E-06	RB1CC1; FAM150A

500Hz								
name	***chr***	***position***	***e.a***	***ß***	***SEß***	***direction of effect***	***p***	***Gene region***
rs248626	5	141077909	G	0.13	0.03	++++++	4.19E-06	PCDHcluster; ARAP3; KIAA0141; RNF14; DIAPH1; GNPDA1; RELL2;

								CENTD3; FCHSD1; HDAC3
rs833914	19	43165967	C	-0.19	0.04	-?----	5.13E-06	SIPA1L3
rs3809985	18	54335087	C	0.20	0.04	++++++	7.32E-06	ALPK2

1kHz								
name	***chr***	***position***	***e.a***	***ß***	***SEß***	***direction of effect***	***p***	***Gene region***
rs898967	16	80124281	C	0.16	0.03	+??+++	9.64E-07	C-MIP
rs6899078	5	101679772	G	0.12	0.03	++++++	3.24E-06	SLCO4C1; SLCO6A1
rs12933858	16	80123622	C	0.16	0.03	+??+++	3.62E-06	
rs4889334	16	80123683	C	0.16	0.03	+??+++	3.65E-06	
rs10055840	5	101752347	C	0.13	0.03	++++++	3.88E-06	
rs1901513	5	101739457	G	-0.13	0.03	-----?	4.02E-06	
rs7234534	18	21661434	G	0.15	0.03	+??+++	5.24E-06	PSMA8; SS18
rs5007492	18	21662551	C	0.15	0.03	+??+++	6.68E-06	
rs997243	18	21662168	C	0.15	0.03	+??+++	7.16E-06	
rs12608228	18	21658579	C	-0.12	0.03	------	7.39E-06	
rs12607678	18	21651977	C	-0.12	0.03	------	8.02E-06	
rs12608438	18	21653977	G	-0.11	0.03	------	8.74E-06	

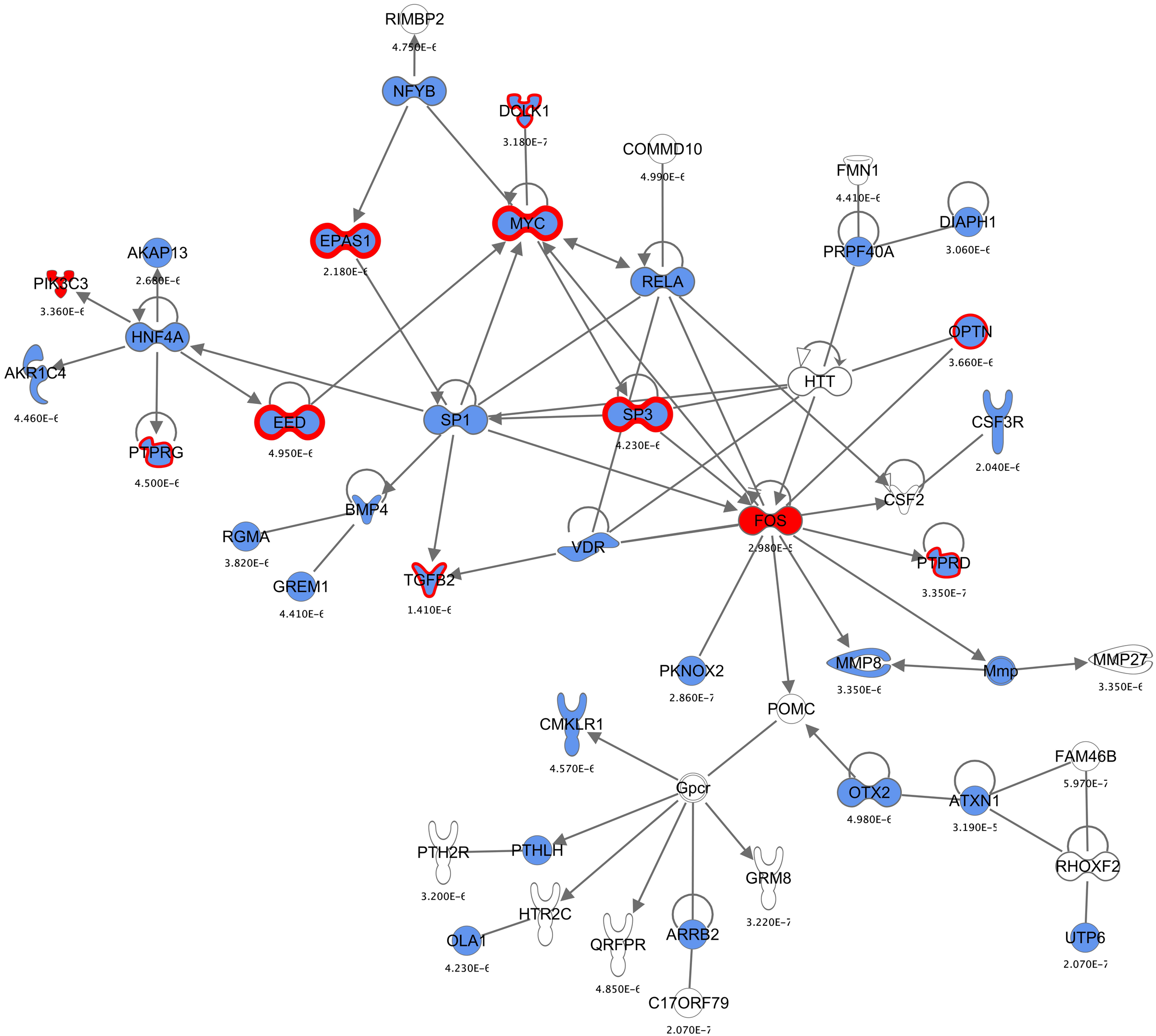
2kHz								
name	***chr***	***position***	***e.a***	***ß***	***SEß***	***direction of effect***	***p***	***Gene region***
rs1339521	10	130113247	G	-0.22	0.05	-??--?	2.58E-06	Desert zone
rs1533807	2	209576716	T	0.13	0.03	++?-++	3.20E-06	PTH2R
rs1563655	16	80033433	G	-0.12	0.03	---+-?	3.77E-06	C-MIP
rs17322627	12	107261161	C	-0.12	0.03	------	4.57E-06	CMKLR1
rs4964679	12	107263448	G	-0.12	0.03	------	4.62E-06	
rs502522	20	844299	C	0.18	0.04	+??+++	6.57E-06	ANGPT4
rs1110115	8	104167796	C	0.15	0.03	+??+++	9.54E-06	LOC100287262; ATP6V1C; FZD6; AZIN1; BAALC

4kHz								
name	***chr***	***position***	***e.a***	***ß***	***SEß***	***direction of effect***	***p***	***Gene region***
rs9574464	13	35212793	G	-0.17	0.03	------	3.18E-07	DCLK1
rs12429464	13	35212627	C	0.17	0.03	++++++	4.27E-07	
rs7326236	13	21801551	C	0.18	0.04	++++++	5.29E-07	

rs9574476	13	35215647	C	-0.16	0.03	------	9.88E-07	
rs9574471	13	35214940	C	-0.16	0.03	------	9.96E-07	
rs9574474	13	35215379	C	-0.16	0.03	------	1.00E-06	
rs9574483	13	35217579	C	0.16	0.03	++++++	1.02E-06	
rs7989263	13	21793358	C	-0.16	0.03	------	1.29E-06	
rs9574482	13	35217556	G	0.16	0.03	++++++	2.22E-06	
rs1719101	7	125663762	C	-0.15	0.03	------	2.57E-06	GRM8
rs2687473	7	125665980	C	0.15	0.03	++++++	2.58E-06	
rs1719094	7	125669335	G	0.15	0.03	++++++	2.60E-06	
rs1673371	7	125661306	G	0.15	0.03	++++++	2.61E-06	
rs2455561	15	84127352	G	-0.11	0.02	---+--	2.68E-06	KLHL25
rs1673365	7	125677843	C	-0.14	0.03	------	2.73E-06	
rs1673374	7	125697739	G	-0.15	0.03	------	2.75E-06	
rs1719107	7	125713864	G	0.15	0.03	++++++	2.78E-06	
rs1719108	7	125713550	C	0.15	0.03	++++++	2.79E-06	
rs1719089	7	125675233	C	-0.14	0.03	------	2.82E-06	
rs1673366	7	125679478	G	-0.15	0.03	------	2.85E-06	
rs1673369	7	125684818	C	0.14	0.03	++++++	2.91E-06	
rs1719090	7	125672107	C	-0.14	0.03	------	2.92E-06	
rs1719091	7	125671577	G	-0.14	0.03	------	2.93E-06	
rs1719093	7	125671126	G	0.14	0.03	++++++	2.94E-06	
rs1719105	7	125687939	C	-0.15	0.03	------	3.29E-06	
rs1673373	7	125660694	G	0.14	0.03	++++++	3.39E-06	
rs16939415	8	78160624	C	-0.11	0.02	------	5.18E-06	PXMP3
rs10090601	8	78159960	C	-0.11	0.02	------	5.27E-06	
rs6563133	13	35216869	G	0.11	0.02	++++++	5.38E-06	
rs471173	7	125635499	G	0.17	0.04	++++++	6.02E-06	
rs637306	7	125629887	C	0.17	0.04	++++++	6.07E-06	
rs660143	7	125628487	G	-0.17	0.04	------	6.26E-06	
rs2660178	10	55436513	G	0.12	0.03	-+++++	6.54E-06	PCDH15
rs2350502	3	141322209	C	0.16	0.04	+??+++	6.74E-06	CLSTN2
rs1419500	7	125719444	C	-0.14	0.03	------	7.81E-06	
rs10876286	12	51076461	C	0.19	0.04	++++++	8.46E-06	LOC100129509; KRT82; KRT84; KRT85
rs2926404	10	55423537	G	-0.12	0.03	+-----	8.59E-06	
rs2521027	7	125643654	G	0.16	0.04	++++++	9.51E-06	
rs17089512	13	72031104	G	0.14	0.03	++++++	9.77E-06	RP11-11C5.2; DIS3; C13orf34; PIBF1
rs9599975	13	72032378	C	0.14	0.03	++++++	9.77E-06	
8kHz								

name	***chr***	***position***	***e.a***	***ß***	***SEß***	***direction of effect***	***p***	***Gene region***
rs10084013	18	38077815	G	-0.19	0.04	-??--?	3.36E-06	PIK3C3
rs2398234	10	5300533	G	0.19	0.04	+++?++	4.46E-06	AKR1C4
rs17277310	7	36357772	C	0.18	0.04	++++++	6.11E-06	KIAA0895
rs7921115	10	5287642	C	0.19	0.04	+++?++	6.41E-06	
rs16922177	8	58517504	G	-0.24	0.05	-----?	6.47E-06	
rs1107822	10	5294823	C	-0.19	0.04	---?--	6.47E-06	
rs11996539	8	58518126	T	-0.24	0.05	-----?	6.76E-06	
rs7911824	10	5285247	T	-0.19	0.04	---?--	6.88E-06	
rs17635428	8	88647371	G	0.34	0.08	+??+++	9.37E-06	CNBD1
rs9899183	17	7393701	C	0.12	0.03	+++++-	9.75E-06	TNFS
rs7087937	10	5303492	G	0.19	0.04	+++?++	9.78E-06	

pathwayFinale47_JMG



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