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Whole transcriptome profiling of the human hippocampus suggests an involvement of the KIBRA rs17070145 polymorphism in differential activation of the MAPK signaling pathway

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Abstract

The rs17070145-T variant of the *WWCI* gene, coding for the KIBRA protein, has been associated with both increased episodic memory performance and lowered risk for late onset Alzheimer's disease, although the mechanism behind this protective effect has not been completely elucidated.

To achieve a better understanding of the pathways modulated by rs17070145 and its associated functional variant(s), we used laser capture microdissection (LCM) and RNA-sequencing to investigate the effect of rs17070145 genotypes on whole transcriptome expression in the human hippocampus (HP) of 22 neuropathologically normal individuals, with a specific focus on the dentate gyrus (DG) and at the pyramidal cells (PC) of CA1 and CA3 sub-regions.

Differential expression analysis of RNA-seq data within the HP based on the rs17070145 genotype revealed an overexpression of genes involved in the MAPK signaling pathway, potentially driven by the T/T genotype. The most important contribution comes from genes dysregulated within the DG region. Other genes significantly dysregulated, and not involved in the MAPK1 pathway (Adj P < 0.01 and Fold Change > |1.00|) were: *RSPO4* (HP); *ARC*, *DUSP5*, *DNAJB5*, *EGR4*, *PPP1R15A*, *WBP11P1*, *EGR1*, *GADD45B* (DG); *CH25H*, *HSPA1A*, *HSPA1B*, *TNFSF9* and *NPAS4* (PC).

Several evidences suggested that the MAPK signaling pathway is linked with memory and learning processes. In non-neuronal cells, the KIBRA protein is phosphorylated by ERK1/2 (involved in the MAPK signaling) in cells as well as *in vitro*. Several of the other dysregulated genes are involved in memory and learning processes, as well as in Alzheimer's Disease.

In conclusion, our results suggest that the effect of the *WWCI* rs17070145 polymorphism on memory performance and Alzheimer's disease might be due to a differential regulation of the MAPK signaling, a key pathway involved in memory and learning processes.

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Keywords

RNAseq; memory; dentate gyrus; pyramidal cells; *WWCI*

INTRODUCTION

The *WWCI* gene is located on chromosome 5q35.1 and encodes for the KIBRA protein, a scaffold or adaptor-like protein implicated in a variety of cellular processes and functions, expressed in the brain (particularly in cerebral cortex and hippocampus) lung, and kidney. The first evidence of involvement of *WWCI* in memory and cognition was reported with the association of T allele of the rs17070145 Single Nucleotide Polymorphism (SNP) with human memory performance (Papassotiropoulos et al., 2006). After this evidence, a considerable number of studies investigated the role of rs17070145 in cognition, using populations from different ethnic backgrounds (reviewed in: Schneider et al., 2010), sometimes reporting conflicting results. A meta-analysis of 13,605 individuals with Caucasian background confirmed the significant association with both episodic and working memory of the T allele (Milnik et al., 2012), explaining the 0.5% and the 0.1% of the variance for episodic and working memory tasks, respectively. Also more recent studies investigated the role of rs17070145 in memory performance in different population samples, reporting contrasting results. (Muse et al., 2014; Franks et al., 2014; Boraxbekk et al., 2015; Witte et al., 2016). Muse et al. (2014) studied the correlation between rs17070145 and scores in cognitive tests in a Caucasian sample, detecting an association between better delayed recall performance in the T-carriers versus the C/C carries. Furthermore, they found a significantly relationship between recall score (immediate and delayed) and increased aging between the two genotype groups. Franks et al. (2014) considered a sample of 386 individuals from the Tasmanian Healthy Brain Project, correlating the rs17070145 genotypes using multiple measures of verbal and visual episodic memory. The results did not suggest any significant association between genotypes and episodic memory. Boraxbekk et al. (2015) investigated the association between rs17070145, memory performance and hippocampal volume in two independent Australian cohorts of older adults. The results did not show any relationship with longitudinal memory decline or hippocampal atrophy. Witte et al. (2016) studied 140 subject aged 50–80 genotyping the rs17070145 SNP and testing memory with Auditory Verbal Learning Task. Moreover, volume, microstructural integrity and functional connectivity were assessed using functional magnetic resonance imaging (fMRI). The results showed the association of T-carriers with better memory performance. The fMRI results globally indicated that older individuals carrying the T-allele showed larger volumes and better microstructure in hippocampus subfields (CA2/3 and CA4/DG).

The rs17070145 SNP has been associated with Alzheimer's Disease (AD) (Corneveaux et al., 2008; Rodríguez-Rodríguez et al., 2009; Wang et al., 2013). All studies reported associations for T- or C-carriers, not indicating any differential phenotypic effect of heterozygous genotypes. Studies involving null-mutant mice confirmed that KIBRA is involved in memory performance because deletion of the protein causes decreased learning in contextual fear learning, memory (Makuch et al., 2011) and spatial memory tasks (Vogt-Eisele et al., 2014).

The hippocampus plays a key role in human memory (Scoville and Milner, 1957), especially in declarative memory (Cohen, and Squire, 1980), the ability to remember everyday facts and events. The main neuronal path in hippocampus is represented by the trisynaptic loop, starting from the layer 2 of the entorhinal cortex (medial temporal lobe) and involving granular cells of the dentate gyrus and then pyramidal cells of the CA1 and CA3 regions. The “Standard Model” proposed for the hippocampus involvement in episodic memory (a type of declarative memory), considers the CA3 area the “core” of the hippocampal memory system, with an important contribution of the dentate gyrus area (Marr et al., 1971).

Considering the key role of the hippocampus in memory formation, this study aimed to evaluate the influence of the *WWC1* rs17070145 SNP on total RNA expression levels within the human hippocampus (HP), particularly within the dentate gyrus (DG) and the pyramidal cells (PC) located in CA1 and CA3 sub-regions, obtained with Laser Capture Microscopy (LCM) technology. We also assessed the *WWC1* RNA expression by qPCR in the entorhinal cortex (EC), considered its role in memory and the anatomical connections with the hippocampus (Takehara-Nishiuchi, 2014). To look for extreme effects of rs17070145 on the hippocampus transcriptome, we considered individuals with T/T and C/C genotypes, not including C/T heterozygous in our study setup.

MATERIALS AND METHODS

Neuropathological methods

The subjects used in this study were autopsied and the brain sampled for complete neuropathological examination according to the standard protocol of the Brain and Body Donation Program at Banner Sun Health Research Institute, Sun City AZ (Beach et al. 2015; <https://www.brainandbodydonationprogram.org>). The right frozen HP (n = 23) and EC (n = 13) from non demented and neuropathologically normal controls was dissected at the level of the midthalamus and sectioned in a Leica CM3050 S cryostat at a thickness of 10µm and stored at –80°C until use. The 13 samples from EC were a sub-group of the 23 individuals sampled for the HP. Genotyping was done on extracted DNA using LGC Genomics (Hertz, UK), using the KASP genotyping technology.

Laser Capture Microdissection (LCM) and RNA isolation for DG and PC

Frozen sections were defrosted stained within 5 min using H & E staining. In short, sections were hydrated in a series of 100%, 95% and 80% alcohol and H₂O, followed by Hematoxylin, Acid Alcohol (1%) and Eosin. Sections were then dehydrated in an ethanol series (95%, 100% ethanol, and 100% xylene) and dried. All aqueous steps and stains contained a 1X ProtectRNA™ RNase Inhibitor (Sigma-Aldrich, St. Louis, MO). Immediately after staining, the DG region and PC from CA1–CA3 were captured in AdhesiveCap tubes (Carl Zeiss, Oberkochen, Germany) with a PALM microbeam LCM (Axiovert 200M, Carl Zeiss, Oberkochen, Germany). For samples which more than 300 cells were isolated, RNA was extracted using the PicoPure RNA Isolation Kit (Life Technologies, Carlsbad, CA). For the entire HP the section was scraped from the slide and RNA was isolated using TRIzol and purified using the PureLink RNA Mini Kit (Life Technologies, Carlsbad, CA), but not LCM was performed. The same RNA isolation and purification

methods were also used for the EC. Finally, the RNA extracted from all regions was quantified using the Quant-iT™ RiboGreen® RNA Assay Kit (Thermo Fisher Scientific, MA).

Transcriptome sequencing

For the DG and PC, RNA was prepared for RNA-seq with the ovation RNA Seq System V2 (NuGEN Technologies Inc, San Carlos, CA) and then fragmented to the appropriate size and ligated into a sequencing library using the Encore Rapid Library System (NuGEN Technologies Inc, San Carlos, CA). For the whole HP scrapes, libraries were prepared using the Truseq RNA sample prepV2 (Illumina Inc, San Diego, USA). Samples were barcoded and multiplexed before sequencing was performed on an Illumina HiSeq 2000 instrument using 2*50bp reads (Illumina, San Diego, CA, USA).

Data Analysis

The sequenced reads contained in the FASTQ files were aligned to the reference genome using Tophat (Trapnell et al., 2009) by generating the corresponding BAM files. FASTQ is the standard file format for storing the output of high-throughput sequencing. Specifically, they are text-based and include both the raw sequences letters and the quality scores. BAM files (Binary Alignment Map) are compressed binary files containing the sequenced reads aligned to the reference genome. *BAM* files were used to build a read count table at the gene level using the R package EasyRNASeq (Delhomme et al., 2012). Low quality samples were removed after inspection of Principal Component Analysis and density plots, resulting in 48 total samples included in the following analysis (Table 1). Differential analysis between T/T and C/C carriers was performed for all the three regions separately using the R package DESeq2 (Love et al., 2014). The statistical method implemented in DESeq2 fits a generalized linear model (GLM) for each gene, modeling reads counts following a negative binomial distribution. The Logarithmic Fold Change (FC), expressed as Log2 Fold Change, is estimated with an Empirical Bayes procedure, whereas the significance is assessed with a Wald Test. The P values were corrected with the False Discovery Rate (FDR) method (Benjamini and Hochberg, 1995) and transcripts were annotated using the R-Biomart package (Durinck et al., 2005), retrieving Ensemble ID and HGNC gene symbols according the GRCh37.p13 assembly.

Enrichment analysis was performed by the means of ToppGene web tool (Chen et al., 2007) using as input the gene lists obtained in the three differential analyses, using the hypergeometric statistics with the probability density method function. Enrichment for all gene lists was computed using the information from KEGG (Kanehisa et al., 2000), Reactome (Croft et al. 2014; Milacic et al. 2012) and Panther (Mi et al., 2016; Mi et al., 2009) databases, updated to Feb 24, 2015. All P values obtained from the enrichment analysis were corrected using the Bonferroni method (Bonferroni, 1936; Dunn, 1959).

qPCR expression analysis

Quantitative PCR (qPCR) was performed for the *WWC1* gene on the HP as well as the EC. Three housekeeping genes were used as references (*GAPDH*, *YWHAZ*, *B2M*). qPCR was done using the Superscript III platinum SYBR green one-step PCR qRT-PCR kit (Life

Technologies, Carlsbad, CA) and ran on a lightcycler480 instrument (Roche, Basel, Ge). Data were imported and analyzed using qBasePlus (Biogazelle, Ghent, Be). Expression levels were normalized against 3 reference genes and scaled to the minimum and compared between C/C and T/T genotypes using a t-test.

RESULTS

Sample preparation and quality controls

A total of 23 frozen human hippocampus samples were originally available for this study. A summary of the sample preparation steps, including initial and final sample sizes is reported in Fig. S1. First, DG and the PC regions were selected for RNA isolation and sequencing via an LCM procedure. For the DG, the LCM procedure was effective for 22 samples (> 300 cells were obtained). After RNA extraction and quality assessment all samples were included in the sequencing experiment, successful for all of them (Table 1). For the PC, the LCM procedure was successful for 17 samples (> 300 cells were obtained). After RNA extraction and quality assessment 7 additional samples were removed because of a low RNA concentration, resulting in a final sample size of 10 included in the sequencing experiment, which was successful for all the samples (Table 1).

Last, we extracted RNA from the remaining samples available from whole HP sections following the LCM procedure. After RNA extraction and quality assessment, 4 samples were removed from the dataset because of the low RNA concentration. After the sequencing, 3 other samples were removed because the low number of reads, for a final sample size of 16 (Table 1). We were not able to repeat the sample preparation because the limited amount of brain sections available, and some of them were almost fully depleted during LCM and therefore were in limited quantity available for whole hippocampus analysis. However, 22 samples (T/T = 10 and C/C = 12) were suitable for the qPCR conducted in order to assess the expression levels of the KIBRA gene. For the EC, we were able to obtain the RNA to use for the qPCR from all the 13 brain sections available (T/T = 4 and C/C = 9).

Expression level of *WWC1* gene in the different brain regions

A total of 48 samples were used in the differential expression analysis, including 16 samples from HP, 22 from the DG and 10 from PC. We first checked in the RNA sequencing data the expression levels of the *WWC1* gene associated with rs17070145 genotype in each brain region (HP, DG and PC). We did not find a significant difference, although in all comparisons the trend was toward an overexpression in the T/T carriers (Fig. 1).

We next used qPCR to assess the expression level of *WWC1* in the HP, including the additional samples not suitable for the RNA sequencing but usable for a qPCR (n = 22), and we found a significant overexpression in T/T carriers (P = 0.013; FC = 0.629) (Fig. 2). In the EC, *WWC1* expression was also higher in T/T homozygotes, however without reaching the statistical significance (P = 0.284; FC = 0.733) (Fig. 2).

Transcriptomic changes driven by rs17070145 genotypes in the different brain regions

We compared the whole transcriptome in T/T versus C/C carriers for each brain region. In the HP, we obtained 8 significant differentially expressed genes, 4 overexpressed and 4 underexpressed in T/T carriers (Table 2 and Fig. S2). The most significant gene (Adj P = 2.89E-06; FC = -0.700) was *KRCC1* (lysine-rich coiled-coil 1). However, the gene with a highest FC (Adj P = 3.26E-04; FC = 1.132) was *RSPO4* (R-spondin 4).

In the DG, we found a total of 66 genes differentially expressed, specifically 57 genes overexpressed and 6 underexpressed in T/T carriers (Tables 3 and S1, Fig. S3). The top gene for Adjusted P value was *OTUD* (OTU deubiquitinase 4), with Adj P = 7.29E-04 and FC = -0.536; the gene with the highest FC was instead *DUSP5* (dual specificity phosphatase 5; Adj P = 2.93E-03; FC = 1.956).

Finally, in the PC we found the *GRIK3* gene underexpressed (glutamate receptor, ionotropic, kainate 3) and the remaining 19 overexpressed in T/T (Table 4 and Fig. S4). The genes with the most significant adjusted P value (All with Adj P = 9.04E-05) were *CH25H* (cholesterol 25-hydroxylase), *HSPA1A* (heat shock 70kDa protein 1A) and *ENSG00000258437*, the last one also with the largest Fold Change (FC = 3.780).

Enrichment analysis

We performed the enrichment analysis, and after Bonferroni correction we observed a significant enrichment only for the differentially expressed genes detected in the dentate gyrus. We detected the KEGG “MAPK signaling pathway” (Bonferroni adj P = 0.015) including the following 6 genes: *MAPK8IP1*, *GADD45B*, *DUSP2*, *DUSP5*, *PRKCG*, and *NR4A1*. The same pathway was confirmed when we considered all the significant genes detected in the 3 regions, for a total of 10 genes involved (Bonferroni adj P = 3.659E-5; Genes: *MAPK8IP1*, *GADD45B*, *DUSP2*, *DUSP5*, *PRKCG*, *NR4A1*, *MAPK11*, *HSPA1A*, *HSPA1B* and *HSPB1*). The 4 additional genes were from the PC (n = 3) and HP region (n = 1). The graphical representation of the KEGG MAPK1 pathway with all genes differentially expressed in the 3 regions is reported in Fig. 3, whereas the total list of differentially expressed genes involved in the pathway is reported in Table 5.

DISCUSSION

In this study we analyzed the effect of the different genotypes of the *WWC1* polymorphism rs17070145 (T/T and C/C) on the transcriptome expression in the human HP, DG, and PC located in the CA1 and CA3 regions.

The genotype of rs17070145 seems to have a significant but modest effect on the *WWC1* mRNA expression levels in the hippocampus region. This supports data originally reported at the protein level (Papassotiropoulos et al., 2006). The expression changes in the EC are not significant between genotypes, but they follow the same trend of overexpression in T/T carriers.

When looking at the effect of rs17070145 on the transcriptome of the three regions analyzed, our results seem to suggest an activation of the MAPK pathway (Genes:

MAPK8IP1, *GADD45B*, *DUSP2*, *DUSP5*, *PRKCG*, *NR4A1*, *MAPK11*, *HSPA1A*, *HSPA1B* and *HSPB1*), in particular with the contribution of genes dysregulated in the DG region, and located in the different functional sections of the pathway (the classical, JNK-p38, and ERK5). This pathway is involved in memory and learning (Ciccarelli and Giustetto, 2012; Peng et al., 2010). Experimental evidence demonstrates that ERK activation, Long Term Potentiation, learning and memory are closely linked (Reviewed in Peng, 2010). In non-neuronal cells, KIBRA protein and MAPK-ERK pathway seem connected, as demonstrated by Yang et al. (2014). In particular, they demonstrated that ERK1/2 phosphorylates KIBRA at Ser⁵⁴⁸ and that RSK1/2 specifically phosphorylates KIBRA at two highly conserved sites (Thr⁹²⁹ and Ser⁹⁴⁷) *in vitro* and in cells. In a separate study Hilton et al. (2008) demonstrated that KIBRA plays a positive role in the collagen-induced activation of the ERK1/2 kinases in breast epithelial cells. However, no supporting data have been still reported concerning the interaction of KIBRA and MAPK1-ERK in neuronal cells. As far as we know, this is the first study reporting a potential interaction between KIBRA protein and MAPK1 signaling in neurons.

Beside the MAPK pathway, we observed other differentially expressed genes linked to the rs17070145 genotype with a relevant function. In the hippocampus, the gene *RSPO4* (R-spondin 4) is member of R-spondin family and is included in the Wnt/ β -catenin pathway with an activation role (Sharma et al., 2014; de Lau et al., 2012; Kim et al., 2008). The increase of activity of the Wnt/ β -catenin canonical pathways is correlated with memory consolidation (Fortress et al., 2013; Xu et al., 2014). Next, we found *CLK4* underexpressed in T/T homozygotes. *CLK4* belongs to the CLK family, including *CLK1*, involved in the pathophysiology of AD by phosphorylating the serine residue in SR proteins (Jain et al., 2014), and it is retained a target to inhibit as potential treatment. In the DG, the *ARC* gene (activity-regulated cytoskeleton-associated protein) has been widely associated with memory and synaptic plasticity (Day and Shepherd, 2015) and is a transcriptional target of early growth response (Egr) transcription factors (Li et al., 2005). Interestingly, we found *EGR1* and *EGR4* also upregulated in the same subregion. Finally, deletions of *Gadd45b* in mice determine deficits in hippocampus-dependent long-term memory (Leach et al., 2012). In the PC, the top differentially expressed gene is *CH25H*, a cholesterol metabolism gene that has been related to AD in different studies and confirmed after meta-analysis (Wollmer et al., 2010). *Npas4* has been found decreased in hippocampus of memory-impaired aged wild-type 11 β -HSD1 mice, and accordingly we found this gene overexpressed in carriers of the T/T genotype. Moreover in Tet1 KO mice, exhibiting abnormal hippocampal long-term depression and impaired memory extinction, *Npas4* was found downregulated in the brain (Rudenko et al., 2013). *GRIK3* is one of the kainate/AMPA-type ionotropic glutamate receptors (GRIK = glutamate receptor, ionotropic, kainate), which plays a critical role in synaptic potentiation, an essential process for learning and memory (Schmitz et al., 2001). In particular, *GRIK3* –/– deficient mice showed marked impaired short and long-term potentiation (Pinheiro et al., 2007). Last, *Dnab1* was upregulated in the rat amygdala post ICSS (Kadar et al., 2011) and *FOSB* was also involved in learning and memory processes in animal models (Solecki et al., 2008; Eagle et al., 2015).

The effect of rs17070145 on hippocampus transcriptome we observed in our study should be considered in relation to the specific characteristics of the analyzed sample, composed mostly by males and with an average age of 77.9 ± 10.6 years.

In conclusion, our data suggests a relationship between the *WWC1* polymorphism rs17070145 and the activation of MAPK signaling. Further assessments are needed to confirm the involvement of KIBRA protein in perturbing this pathway at the hippocampus level, since this interaction has been only reported in non-neuronal cells. Furthermore, other relevant genes were identified in our analysis that could be influenced by the KIBRA protein and play a key role in the memory processes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Expression levels of *WWC1* gene in T/T and C/C rs17070145 carriers in HP, DG and PC (RNA sequencing data). Expression values are reported as normalized counts.

Figure 2. Expression levels of *WWC1* in T/T and C/C rs17070145 carriers in HP and EC (qPCR data). Expression values are reported as expression rate of *WWC1* versus the reference genes (2^{-CT})

Figure 3. KEGG MAPK signaling pathway (KEGG ID: hs04010). The genes differentially expressed in HP, DG and PC are highlighted in grey.

Description of the samples for which the RNA sequencing was successful, then used for the differential expression analysis (Total n = 48). Sex distribution was assessed using Fisher's Exact Test, age differences were assessed with t-test with equal variance, Post Mortem Interval differences were assessed with Wilcoxon rank sum test.

Table 1

	Hippocampus			Dentate gyrus			Pyramidal cells		
	CC	TT	P	CC	TT	P	CC	TT	P
<i>Sample Size</i>	10	6		12	10		7	3	
<i>Males</i>	7	6		9	8		5	2	
<i>Females</i>	3	0	0.250	3	2	1.000	2	1	1.000
<i>Age</i>	77.7 ± 10.7	76.2 ± 13.6	0.805	77.75 ± 9.67	78.6 ± 11.52	0.852	82.43 ± 6.75	70.0 ± 15.39	0.099
<i>Post Mortem Interval</i>	3.147 ± 1.650	3.207 ± 0.870	0.744	2.983 ± 1.555	3.107 ± 0.989	0.574	3.474 ± 1.775	3.317 ± 0.558	0.909

Table 2

Differentially expressed transcripts between T/T and C/C rs17070145 carriers in the hippocampus. Data were analyzed using the method implemented in the R-package Deseq2, assessing the significance with a Wald Test (Love et al., 2014).

FC: Logarithmic (base 2) Fold Change

SE: Standard Error Fold Change

Ensemble ID	Gene	Description	Av. Exp.	FC	SE	Test Statistic	P	Adj P
ENSG00000172086	<i>KRCC1</i>	lysine-rich coiled-coil 1	1193.9	-0.700	0.110	-6.379	1.8E-10	2.9E-06
ENSG00000101282	<i>RSPO4</i>	R-spondin 4	136.6	1.132	0.206	5.490	4.0E-08	3.3E-04
ENSG00000130935	<i>NOL11</i>	nucleolar protein 11	870.1	-0.554	0.117	-4.747	2.1E-06	1.1E-02
ENSG00000126005	<i>MMP24-AS1</i>	MMP24 antisense RNA 1	2400.2	0.610	0.138	4.407	1.0E-05	3.6E-02
ENSG00000185386	<i>MAPK11</i>	mitogen-activated protein kinase 11	1847.7	0.752	0.171	4.395	1.1E-05	3.6E-02
ENSG00000178585	<i>CTN/NBPI</i>	catenin, beta interacting protein 1	1815.5	0.597	0.138	4.316	1.6E-05	4.3E-02
ENSG00000113240	<i>CLK4</i>	CDC-like kinase 4	2293.6	-0.467	0.110	-4.253	2.1E-05	4.5E-02
ENSG00000188641	<i>DPYD</i>	dihydropyrimidine dehydrogenase	474.2	-0.689	0.163	-4.240	2.2E-05	4.5E-02

Table 3

Differentially expressed transcripts (Adj P < 0.01) between T/T and C/C rs17070145 carriers in the dentate gyrus. Complete list of all significant transcripts detected in dentate gyrus is reported in Table S1. Data were analyzed using the method implemented in the R-package Deseq2, assessing the significance with a Wald Test (Love et al., 2014).

FC: Logarithmic (base 2) Fold Change

SE: Standard Error Fold Change

Ensemble ID	Gene	Description	Av. Exp.	FC	SE	Test Statistic	P	Adj P
ENSG00000164164	<i>OTUD4</i>	OTU deubiquitinase 4	434.7	-0.536	0.099	-5.416	6.1E-08	7.3E-04
ENSG00000198576	<i>ARC</i>	activity-regulated cytoskeleton-associated protein	63.8	1.669	0.329	5.069	4.0E-07	1.5E-03
ENSG00000138166	<i>DUSP5</i>	dual specificity phosphatase 5	15.1	1.956	0.404	4.844	1.3E-06	2.9E-03
ENSG00000120868	<i>APAF1</i>	apoptotic peptidase activating factor 1	204.9	-0.797	0.168	-4.736	2.2E-06	3.3E-03
ENSG00000137094	<i>DNAJB5</i>	DnaJ (Hsp40) homolog, subfamily B, member 5	168.8	1.267	0.273	4.637	3.5E-06	3.9E-03
ENSG00000178233	<i>TMEM151B</i>	transmembrane protein 151B	283.0	0.926	0.200	4.622	3.8E-06	4.0E-03
ENSG00000126583	<i>PRKCG</i>	protein kinase C, gamma	239.7	0.685	0.149	4.586	4.5E-06	4.2E-03
ENSG00000135625	<i>EGR4</i>	early growth response 4	66.3	1.363	0.297	4.586	4.5E-06	4.2E-03
ENSG00000087074	<i>PPP1R15A</i>	protein phosphatase 1, regulatory subunit 15A	38.8	1.371	0.302	4.534	5.8E-06	4.5E-03
ENSG00000260389	<i>WBPI1P1</i>	WW domain binding protein 11 pseudogene 1	8.0	1.493	0.330	4.519	6.2E-06	4.6E-03
ENSG00000120738	<i>EGR1</i>	early growth response 1	290.3	1.281	0.284	4.508	6.5E-06	4.7E-03
ENSG00000099860	<i>GADD45B</i>	growth arrest and DNA-damage-inducible, beta	48.4	1.262	0.285	4.423	9.7E-06	6.6E-03

Table 4

Differentially expressed transcript between TT and CC rs17070145 carriers detected in the Pyramidal Cells. Data were analyzed using the method implemented in the R-package Deseq2, assessing the significance with a Wald Test (Love et al., 2014).
FC: Logarithmic (base 2) Fold Change.
SE: Standard Error Fold Change.

Ensemble ID	Gene	Description	Av. Exp.	FC	SE	Test Statistic	P	Adj P
ENSG00000138135	<i>CH25H</i>	cholesterol 25-hydroxylase	34.1	3.780	0.661	5.719	1.1E-08	9.0E-05
ENSG00000204389	<i>HSPA1A</i>	heat shock 70kDa protein 1A	1540.9	2.161	0.376	5.748	9.0E-09	9.0E-05
ENSG00000258437			33.6	3.859	0.678	5.693	1.2E-08	9.0E-05
ENSG00000204388	<i>HSPA1B</i>	heat shock 70kDa protein 1B	1287.2	2.066	0.392	5.271	1.4E-07	7.4E-04
ENSG00000233878			27.6	3.691	0.712	5.184	2.2E-07	9.4E-04
ENSG00000125657	<i>TNFSF9</i>	tumor necrosis factor (ligand) superfamily, member 9	65.4	3.227	0.637	5.068	4.0E-07	1.5E-03
ENSG00000174576	<i>NPA54</i>	neuronal PAS domain protein 4	100.7	3.217	0.651	4.939	7.9E-07	2.3E-03
ENSG00000259884			47.7	2.882	0.586	4.918	8.7E-07	2.3E-03
ENSG00000163873	<i>GRIK3</i>	glutamate receptor, ionotropic, kainate 3	90.7	-2.700	0.597	-4.525	6.0E-06	1.3E-02
ENSG00000100219	<i>XBP1</i>	X-box binding protein 1	206.1	2.148	0.485	4.425	9.6E-06	1.8E-02
ENSG00000132002	<i>DNAJB1</i>	DnaJ (Hsp40) homolog, subfamily B, member 1	808.6	1.994	0.451	4.421	9.8E-06	1.8E-02
ENSG00000125740	<i>FOSB</i>	FBJ murine osteosarcoma viral oncogene homolog B	114.6	2.643	0.604	4.377	1.2E-05	2.0E-02
ENSG00000063438	<i>AHRR</i>	aryl-hydrocarbon receptor repressor	75.9	2.490	0.574	4.341	1.4E-05	2.2E-02
ENSG00000267131			7.1	3.160	0.737	4.289	1.8E-05	2.6E-02
ENSG00000162236	<i>STX5</i>	syntaxin 5	56.8	2.276	0.545	4.179	2.9E-05	4.0E-02
ENSG00000106211	<i>HSPB1</i>	heat shock 27kDa protein 1	226.3	1.573	0.380	4.139	3.5E-05	4.2E-02
ENSG00000259358			49.6	1.985	0.480	4.139	3.5E-05	4.2E-02
ENSG00000261379			6.0	2.989	0.732	4.084	4.4E-05	4.8E-02
ENSG00000144063	<i>MALL</i>	mal, T-cell differentiation protein-like	46.4	2.181	0.536	4.071	4.7E-05	4.8E-02
ENSG00000202252	<i>SNORD14C</i>	small nucleolar RNA, C/D box 14C	15.6	2.210	0.545	4.056	5.0E-05	4.9E-02

Table 5

List of genes differentially expressed in hippocampus, dentate gyrus and pyramidal cells involved in the MAPK signaling pathway. The genes are ordered by Logarithmic (base 2) Fold Change (FC).
SE: fold change standard error.

Ensemble ID	Gene	Description	Av. Exp.	FC	SE	Adj P	Region
ENSG000000204389	<i>HSPA1A</i>	heat shock 70kDa protein 1A	1540.9	2.161	0.376	9.0E-05	PC
ENSG000000204388	<i>HSPA1B</i>	heat shock 70kDa protein 1B	1287.2	2.066	0.392	7.4E-04	PC
ENSG000000138166	<i>DUSP5</i>	dual specificity phosphatase 5	15.1	1.956	0.404	2.9E-03	DG
ENSG000000106211	<i>HSPB1</i>	heat shock 27kDa protein 1	226.3	1.573	0.380	4.2E-02	PC
ENSG000000158050	<i>DUSP2</i>	dual specificity phosphatase 2	8.6	1.501	0.403	4.2E-02	DG
ENSG000000153234	<i>NR4A2</i>	nuclear receptor subfamily 4, group A, member 2	85.7	1.277	0.344	4.3E-02	DG
ENSG000000099860	<i>GADD45B</i>	growth arrest and DNA-damage-inducible, beta	48.4	1.262	0.285	6.6E-03	DG
ENSG000000121653	<i>MAPK8IP1</i>	mitogen-activated protein kinase 8 interacting protein 1	159.4	0.970	0.250	3.0E-02	DG
ENSG000000185386	<i>MAPK11</i>	mitogen-activated protein kinase 11	1847.7	0.752	0.171	3.6E-02	H
ENSG000000126583	<i>PRKCG</i>	protein kinase C, gamma	239.7	0.685	0.149	4.2E-03	DG