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JAMA Psychiatry. Author manuscript; available in PMC 2017 February 03.

Published in final edited form as:

JAMA Psychiatry. 2015 October ; 72(10): 1029–1036. doi:10.1001/jamapsychiatry.2015.1309.

Hippocampal activation, memory performance in young and old, and the risk for sporadic Alzheimer's disease converge genetically to calcium signaling

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Abstract

Importance—Human episodic memory performance is linked to the function of specific brain regions, including the hippocampus, declines as a result of increasing age, and is markedly disturbed in Alzheimer's disease (AD), an age-associated neurodegenerative disorder affecting

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Author contributions: A.H., M.F., H.B., F.J., H.K., W.M., S.R-H, M.W., S.We., D. J.-F. d. Q. & A.P. designed and conceptualized the study. A.H., M.F., B.A., D.C., L.G., V.F., F.J., H.K., A.M., M.P., S.R-H, C.V., M.W., S. We., S. Wo. & A.P. did the data analysis. F.H., H.B., W.M., M.P., K.S., M.W., S.We. & S.Wo. were responsible for data collection and preparation. D. J.-F. d. Q. & A.P. supervised the project. All authors contributed to the writing of the manuscript.

Conflicts of interest: There are no conflicts of interest to declare.

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primarily the hippocampus. Exploring the molecular underpinnings of human episodic memory is key to the understanding of hippocampus-dependent cognitive physiology and pathophysiology.

Objective—To determine whether biologically defined groups of genes are enriched in episodic memory performance across ages, in memory encoding-related brain activity and in AD.

Design, Setting, and Participants—In this multicenter collaborative study, gene set enrichment analysis was done by using primary and meta-analysis data from 57968 participants. The Swiss cohorts consisted of 3043 healthy young adults assessed for episodic memory performance. In a subgroup (1119 participants) of one of these cohorts, functional magnetic resonance imaging (fMRI) was used to identify gene set-dependent differences in brain activity related to episodic memory. The German AgeCoDe cohort consisted of 763 non-demented elderly participants assessed for episodic memory performance. The International Genomics of Alzheimer's Project (IGAP) case-control sample consisted of 54162 participants (17008 patients with sporadic AD, 37154 controls).

Main Outcomes and Measures—Gene set enrichment analysis in all samples was done by using genome-wide single nucleotide polymorphism (SNP) data. Episodic memory performance in the Swiss and AgeCoDe cohorts was quantified by picture and verbal delayed free recall tasks. In the fMRI experiment, activation of the hippocampus during encoding of pictures served as the phenotype of interest. In the IGAP sample, diagnosis of sporadic AD served as the phenotype of interest.

Results—We detected significant and consistent enrichment for genes constituting the Calcium Signaling Pathway (KEGG entry: hsa04020), especially those related to the elevation of cytosolic calcium (P=0.0002). This enrichment was observed in episodic memory performance in young and old, in hippocampal activation, and in the risk for sporadic AD.

Conclusion and Relevance—By detecting consistent significant enrichment in independent cohorts of young and elderly participants, this study identifies calcium signaling as a central player of hippocampus-dependent human memory processes, both in cognitive health and disease and contributes to the understanding -and hopefully treatment- of

Introduction

Episodic memory (EM), i.e. the ability to encode and retrieve a particular event along with its contextual information,[1] is a polygenic cognitive trait, characterized by large interindividual variability and substantial heritability.[2–5] (for review see[5]) As a consequence of physiological ageing processes in such brain regions as the hippocampus and the medial temporal lobe, performance in EM tasks declines with age.[6–9] Pathological EM impairment is a behavioral hallmark of age-related neurodegenerative conditions, such as Alzheimer’s disease (AD).[10,11]

Genome-wide studies utilizing single-marker statistics have been successful in identifying single loci linked to intact and impaired EM.[5,12–14] Triggered by statistical approaches for the analysis of gene expression, gene-set enrichment analysis (GSEA) has recently become available. By taking into account prior biological knowledge, GSEA examines whether test statistics for a group of related genes have consistent deviation from chance.[15,16] As shown

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recently in studies on working memory,[17] autism,[18] bipolar disorder,[19–21], ADHD[22] and schizophrenia.[21,23,24] GSEA can identify convergent molecular pathways relevant to neuropsychiatry.

Here we studied the enrichment of biologically defined gene sets in EM across ages, in EM-related brain activity, and in an EM-related neurodegenerative disorder (Fig. 1). Genome-wide GSEA of EM performance was performed in multiple independent data sets of young and aged cognitively healthy subjects (n=3806). In a large case-control sample (n=54162) we also performed GSEA for the risk of sporadic AD.

Methods

Samples

Discovery sample—This sample is part of an ongoing, continuously recruiting behavioral genetics study in the city of Basel, Switzerland. For the purposes of this study (data lock August 2013), data from 1458 healthy young Swiss adults were available (eTable 1). Subjects were free of any neurological or psychiatric condition and did not take medication at the time of the experiment. All participants gave written informed consent before participation and completed a picture delayed free recall task, which reflects EM performance. For a detailed description of the procedure please refer to the online-only material.

Replication sample—This sample is part of an ongoing, continuously recruiting imaging genetics study in the city of Basel, Switzerland. For the purposes of this study (data lock August 2013), data from 1176 healthy young Swiss adults were available (eTable 1). Subjects were free of any neurological or psychiatric condition and did not take medication at the time of the experiment. All participants gave written informed consent before participation and, while undergoing fMRI acquisition, completed a similar picture delayed free recall task as in the discovery sample. For a detailed description of the procedure please refer to the online-only material.

The ethics committee of the Canton of Basel approved the study protocol.

Zurich sample—We recruited 409 healthy young Swiss adults for a behavioral genetics study in the city of Zurich, Switzerland (eTable 1). Subjects were free of any neurological or psychiatric condition and did not take medication at the time of the experiment. All participants gave written informed consent before participation and completed a picture delayed free recall task. For a detailed description of the procedure please refer to the online-only material.

The ethics committee of the Canton of Zurich approved the study protocol.

Healthy elderly sample—This sample consisted of 763 healthy elderly participants of the German Study on Ageing, Cognition and Dementia in primary care patients (AgeCoDe, total n=3327) (eTable 1). The AgeCoDe study is an ongoing primary care-based prospective longitudinal study on early detection of mild cognitive impairment and dementia established

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by the German Competence Network Dementia.[25] Please also refer to the online-only material.

Genetic heterogeneity—For each of the four cognitively healthy samples, the genomic control inflation factor lambda (λ_{GC}) was calculated to assess admixture.[26] λ_{GC} showed a range between 1.0046 and 1.0449, indicating the absence of noteworthy admixture in these samples (eFigures 2,3,4,5).

Array-based SNP genotyping—Samples were processed as described in the Genome-Wide Human SNP Nsp/Sty 6.0 User Guide (Affymetrix). SNP calls and array quality control were performed using the command line programs of the Affymetrix Power Tools package (version: apt-1.14.4.1). According to the manufacturer's recommendation, contrast QC was chosen as QC metric, using the default value of greater or equal than 0.4. All samples passing QC criteria were subsequently genotyped using the Birdseed (v2) algorithm. Mean Call Rate for all samples averaged >98.5%. The sex-check in PLINK led to the exclusion of one individual in the discovery sample and three individuals in the replication sample. IBD analysis (PLINK) indicated the absence of duplicates within and between samples. For a detailed description of the procedure please refer to the online-only material.

Brain imaging

fMRI preprocessing and first level analyses—Preprocessing and data analysis was performed using SPM8 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging; http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB R2011b (MathWorks). The functional and structural images were spatially normalized by applying DARTEL, which leads to an improved registration between subjects.[27,28] For a detailed description please refer to the online-only material.

fMRI group statistics—The first-level contrast parameters were used for behavioral analyses in a random effects model (second-level analysis). We used a regression model to analyze associations between brain activation differences (meaningful vs scrambled pictures) and the multi-allelic score. Age and sex were included as covariates. A one-sample t-test was computed to assess the significance of task-related activation (meaningful vs scrambled pictures) at the group level. The analysis was focused on the left and right hippocampi, defined using the template-based hippocampal ROIs (see below: Construction of a Population-Average Anatomical Probabilistic Atlas). For a detailed description please refer to the online-only material.

Statistical genetic analysis

Genome-wide association analyses—For each genome-wide analysis (Genome Reference Consortium GRCh37, hg19), *P* values of effectively genotyped (i.e. not imputed) variants were obtained using linear regression analyses as implemented in PLINK (number of SNPs: discovery sample: 730540 SNPs, replication sample: 736286 SNPs, Zurich sample: 737533 SNPs, AgeCoDe sample: 683072 SNPs).[29] Sex and age were included as covariates. We applied the following quality control criteria: Non-significant deviation from Hardy-

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Weinberg equilibrium (HWE; *P*(HWE>0.0001)) and a minor allele frequency (MAF)>0.01. Mean per SNP call rate was >99%.

Pathway analysis—GSEA was performed using MAGENTA.[30] Briefly, the method first maps SNPs onto genes. In this study, only intragenic SNPs were used for SNP-to-gene mapping (i.e., ±0kb of the annotated gene according to Genome Reference Consortium GRCh37, hg19). We chose the ±0kb threshold to avoid potentially significant biases, which are introduced by overlapping signals especially in gene-rich regions. After SNP-to-gene mapping MAGENTA assigns each gene a SNP association score (i.e. the maximum SNP *P* value). The analysis is corrected for gene size, number of SNPs, number of independent SNPs, number of recombination hotspots, linkage disequilibrium (LD) and genetic distance. Lastly, a gene-set enrichment-like statistical test is applied to determine if a gene set is enriched for highly ranked *P* values compared to a gene-set of identical size, randomly drawn from the genome. False-discovery rate (FDR) based on the 75th percentile of association *P* values from all genes was used for multiple testing correction. As recommended, we used the 75th percentile cutoff because it yields optimal power for weak genetic effects that are expected for highly polygenic traits (e.g., EM performance).[30] The utilized gene sets are extracted and curated from the MSigDB v3.1 database (http://www.broadinstitute.org/gsea/msigdb, downloaded in February 2013), including gene-sets from different online databases (KEGG: http://www.genome.jp/kegg/, Gene Ontology GO: http://geneontology.org/, BioCarta: http://www.biocarta.com/ and Reactome: http://www.reactome.org/).[31,32] We used a gene set size ranging between 20 and 200 genes to avoid both overly narrow and broad functional gene-set categories, resulting in 1’411 to be analyzed gene-sets.

We also applied INRICH (http://atgu.mgh.harvard.edu/inrich/), a software tool that examines enrichment of association signals for genetic gene-sets.[33] Unlike MAGENTA, which uses each gene’s lowest *P* value of association with the phenotype to quantify gene set enrichment, INRICH defines LD-independent genomic regions (i.e. intervals) that contain the top GWAS hits. These intervals are then tested for overlaps with gene targets. A permutation-based resampling method is used to obtain the null distribution by random data shuffling and calculating the overlap between random test intervals of equal size as the observed ones and the gene target sets.

Multilocus genetic score calculation—To capture the multi-allelic effect of the Calcium Signaling Pathway gene set, we generated an individual multilocus genetic score.[17] The score comprises variants of the Calcium Signaling Pathway gene-set (one SNP per gene) of the discovery sample that remained significant ($P < 0.05$) after correction for number of independent SNPs per gene, gene size, genetic distance and recombination hotspots (Table e3). The algorithm calculates the score by summing up the individual number of reference alleles over all SNPs, and finally averages the score by the number of non-missing SNPs. We weighted each SNP by the direction of effect on EM performance (i.e., with “1” (the reference allele enhances performance) or “−1” (the reference allele decreases performance)). This procedure harmonizes the single variant effects and avoids bias due to overestimation of accidentally large SNP effects.

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Results

GSEA of EM in young healthy adults

Discovery sample (*n*=1458)—GSEA was performed with MAGENTA.[30] Among the 1411 database-derived gene-sets, MAGENTA identified significant enrichment (FDR < 0.05; multiple testing-corrected) for one gene set, the Calcium Signaling Pathway gene set (KEGG entry: hsa04020) (Table 1). The Calcium Signaling Pathway gene set was also significant when applying INRICH,[33] an alternative GSEA method. Of 864 independent intervals that contained the best genome-wide association signals, INRICH identified 26 intervals overlapping with the target genes of the Calcium Signaling Pathway gene set. Subsequent permutation analysis was done by generating a null distribution through calculation of multiple random intervals of the same size as the observed ones and computation of the overlap with the target gene-sets. This analysis revealed significant enrichment for the Calcium Signaling Pathway gene set ($P_{empirical}$=0.021).

Replication sample (*n*=1176)—Next, GSEA of the identical task (picture free recall task, see Methods) was performed in an independently recruited replication sample. The Calcium Signaling Pathway gene set was enriched significantly (P=0.015).

Calcium Signaling Pathway allelic load correlates with hippocampal activation

In an additional experiment, conducted in a subgroup (n=1119) of the replication sample, functional magnetic resonance imaging (fMRI) was used to identify gene set-dependent differences in brain activity related to EM (see Methods). We focused our search on the hippocampus because i) the Calcium Signaling Pathway gene set was associated with EM, which depends on the hippocampus,[34–37] and ii) genes of this gene set are part of the signaling cascade involved in the formation of hippocampus-dependent memory in vertebrates.[38–42] Thus, the left and right hippocampi served as regions of interest (ROI). Independently of allelic load, we detected highly robust picture encoding-related activation (contrast: meaningful vs scrambled pictures) in the hippocampus (Fig. e1). To capture the multi-allelic effect of the Calcium Signaling Pathway gene set on hippocampal activity, we generated an individual multilocus genetic score[17] (see Methods). Of note, the multiallelic score for this sample was calculated by using only those SNPs (including the respective directions of effect) that proved significant in the independent discovery sample (see Methods). This procedure prevented model overfitting.

Genetic score-dependent analysis revealed a significant positive correlation between genetic score values and activation in the right hippocampus (peak at [33 −16.5 −16]; t=3.35; $P_{uncorrected}$=0.0004, $P_{small\ volume\ correction\ (SVC)}$<0.05, Fig. 2; genotype-independent task-related activation at this coordinate t=42.51). The peak association in the left hippocampus [−24.75 −13.75 −28] did not survive small volume correction (t=2.36; $P_{uncorrected}$=0.009, P_{SVC}> 0.05). No significant effect of the multi-allelic score on gray matter volume was found within the hippocampal ROI.

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EM core gene set

GSEA tests for statistical enrichment at gene set level. Thus, a certain gene set might prove significant in two different samples without any overlap of the gene set components, which gave rise to the significant enrichment (i.e. significantly associated genes), between samples. We tested this possibility by comparing the significant components of the Calcium Signaling Pathway gene set between the discovery and the replication sample. The overlap was significant (P=0.007; exact hypergeometric probability). Of the 144 Calcium Signaling Pathway genes, 26 genes contributed to gene set significance in both samples, whereas 66 genes did not contribute to gene set significance in either sample. The remaining 52 genes contributed to gene set significance in one of the two samples. Thus, the former group of 26 genes was defined as the replicated EM core gene set (Table 2, Fig. 3, Table e2, Table e4). Further exploratory analysis (see Methods) revealed that the EM core gene set was highly significantly enriched with genes involved in the elevation of cytosolic calcium (42.3% of the genes, $P=8.9 \times 10^{-18}$). In comparison, the enrichment of the group of 66 non-contributing genes with molecules involved in the elevation of cytosolic calcium (10.6% of the genes, $P=2.7 \times 10^{-7}$) was 10 orders of magnitude weaker.

GSEA in additional samples

Zurich sample (n=409)—Participants performed a picture free recall task similar to the task used in the discovery and replication samples (see Methods). The EM core gene set was significantly enriched (P=0.038, Fig. 3). No significant enrichment (P=0.704, Fig. 3) was found for the set of 66 genes, which did not contribute to the significance of the Calcium Signaling Pathway gene set in any of the discovery and the replication samples.

GSEA in non-demented elderly subjects, AgeCoDe sample (n=763)—This sample of cognitively healthy elderly individuals was included to investigate whether the observed association of the EM core gene set with EM performance can be also observed in older adults. In analogy to the discovery, replication, and the Zurich sample, genome-wide P values for association with EM performance (delayed verbal free recall, see Methods) under the additive genetic model were used for GSEA. MAGENTA revealed significant enrichment (P=0.004, Fig. 3) of the EM core gene set. Also in this sample, no significant enrichment (P=0.284, Fig. 3) was found for the set of 66 genes, which did not contribute to the significance of the Calcium Signaling Pathway gene set.

GSEA in Alzheimer's disease

EM deficits represent a behavioral hallmark of AD[11] and are observed early in the course of the disease. We investigated the enrichment of the EM core gene set in a large AD case-control sample (for a detailed study description, please refer to the online-only material).

International Genomics of Alzheimer's Project (IGAP) case-control sample[43] (n=54162; n_{cases}=17008, $n_{controls}$=37154)—7036050 autosomal SNP P values of association with sporadic AD served as input for MAGENTA. MAGENTA was run with the identical parameters as in the studies of cognitively healthy subjects. The EM core gene set was significantly enriched (P=0.013, Fig. 3). Also in this sample, no significant enrichment (P=0.384, Fig. 3) was found for the set of 66 genes, which did not contribute to the

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significance of the Calcium Signaling Pathway gene set in the EM samples. No significant enrichment was observed for the entire Calcium Signaling pathway gene set (P=0.155).

Discussion

We detected consistent and robust associations between Calcium Signaling Pathway genes and human EM performance. In particular, a core gene set comprising 26 genes was significantly enriched in 4 independent cohorts of young and elderly cognitively healthy individuals (n=3806). This finding is compatible with the critical role of calcium signaling in molecular processes underlying memory, as shown in model organisms and *in vitro* studies:[44] For example, increases in intracellular calcium are causally related to the induction of long-term potentiation (LTP) and long-term depression (LTD),[45–47] two cellular correlates of learning and memory.[48,49] The results of the present study suggest that genes involved in calcium signaling are also related to human episodic memory throughout adulthood.

Moreover, fMRI data revealed that the individual Calcium Signaling-related allelic load correlated with hippocampal activity measured during memory encoding. Animal studies have amply demonstrated that calcium signaling genes are crucial for the formation of hippocampus-dependent memory.[38–42] Our findings suggest that calcium signaling genes are related to the formation of hippocampus-dependent memory also in humans.

Interestingly, the EM core gene set was also significantly enriched in a large case-control study of sporadic AD. Calcium signaling dysregulation has been repeatedly observed in cell culture and animal models of AD.[50,51] Our results argue in favor of a role for calcium signaling genes in AD and support recently published studies, which identified significant enrichment of the Calcium Signaling Pathway in AD.[52,53]

Interestingly, the EM core gene set was significantly enriched with genes involved in the elevation of cytosolic calcium. A local increase in calcium concentrations results in a number of short-term and long-term synapse-specific alterations that are essential for dendritic development, neuronal survival, synaptic plasticity, learning, and memory.[54,55] A genetic profile favoring the local increase in calcium concentrations might therefore be related to better cognitive capacities and consequently to a delayed clinical manifestation of cognitive decline in AD patients. On the other hand, a sustained increase in intracellular calcium can, in the long run, lead to neurodegeneration and cell death.[56–58] In this case, a genetic profile favoring the local increase in calcium concentrations could also increase AD risk. Thus, given the complexity of the phenotypic, biological, and temporal relationship between cognition, physiological brain aging, and neurodegeneration,[59,60] it is not possible to make definite inferences regarding the direction of effect of the results presented herein.

We stress that genes not contributing significantly to pathway enrichment cannot be excluded from being related to human EM. This is due to a number of possible reasons. For example, some genes might be associated with other forms or stages of EM as those investigated in the present study. Some genes may not be related to variability of memory performance because their expression in the brain might be too tightly regulated and

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independent of common genetic variability. Further, the results of the present study must not lead to the erroneous assumption of an exclusive role of calcium signaling genes in human EM and AD. Given the ubiquity and versatility of calcium signaling,[61] it is apparent that it must play a role in a variety of neurocognitive traits.[17] Nonetheless, by showing robust and consistently significant enrichment in independent cohorts of young and elderly participants, our study identifies calcium signaling as a central player of hippocampus-dependent human memory processes, both in cognitive health and disease and thereby contributes to the understanding -and hopefully treatment- of hippocampus-dependent cognitive pathology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Role of the Funder/Sponsor: The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

We thank Elmar Merkle, MD, Christoph Stippich, MD, and Oliver Bieri, MD for granting access to the fMRI facilities of the University Hospital Basel.

This work was funded by the Swiss National Science Foundation (Sinergia grant CRSII1_136227 to D.Q. and A.P.), by the 7th framework programme of the European Union (ADAMS project, HEALTH-F4-2009-242257), and by the German Research Network on Dementia (KND) and the German Research Network on Degenerative Dementia (KNDD), German Federal Ministry of Education and Research grants 01GI0420 and 01GI0711.

We also thank the International Genomics of Alzheimer's Project (IGAP) for providing summary results data for these analyses. The investigators within IGAP contributed to the design and implementation of IGAP and/or provided data but did not participate in analysis or writing of this report. IGAP was made possible by the generous participation of the control subjects, the patients, and their families. The i–Select chips was funded by the French National Foundation on Alzheimer's disease and related disorders. EADI was supported by the LABEX (laboratory of excellence program investment for the future) DISTALZ grant, Inserm, Institut Pasteur de Lille, Université de Lille 2 and the Lille University Hospital. GERAD was supported by the Medical Research Council (Grant n° 503480), Alzheimer's Research UK (Grant n° 503176), the Wellcome Trust (Grant n° 082604/2/07/Z) and German Federal Ministry of Education and Research (BMBF): Competence Network Dementia (CND) grant n° 01GI0102, 01GI0711, 01GI0420. CHARGE was partly supported by the NIH/NIA grant R01 AG033193 and the NIA AG081220 and AGES contract N01–AG–12100, the NHLBI grant R01 HL105756, the Icelandic Heart Association, and the Erasmus Medical Center and Erasmus University. ADGC was supported by the NIH/NIA grants: U01 AG032984, U24 AG021886, U01 AG016976, and the Alzheimer's Association grant ADGC–10–196728.

Angela Heck had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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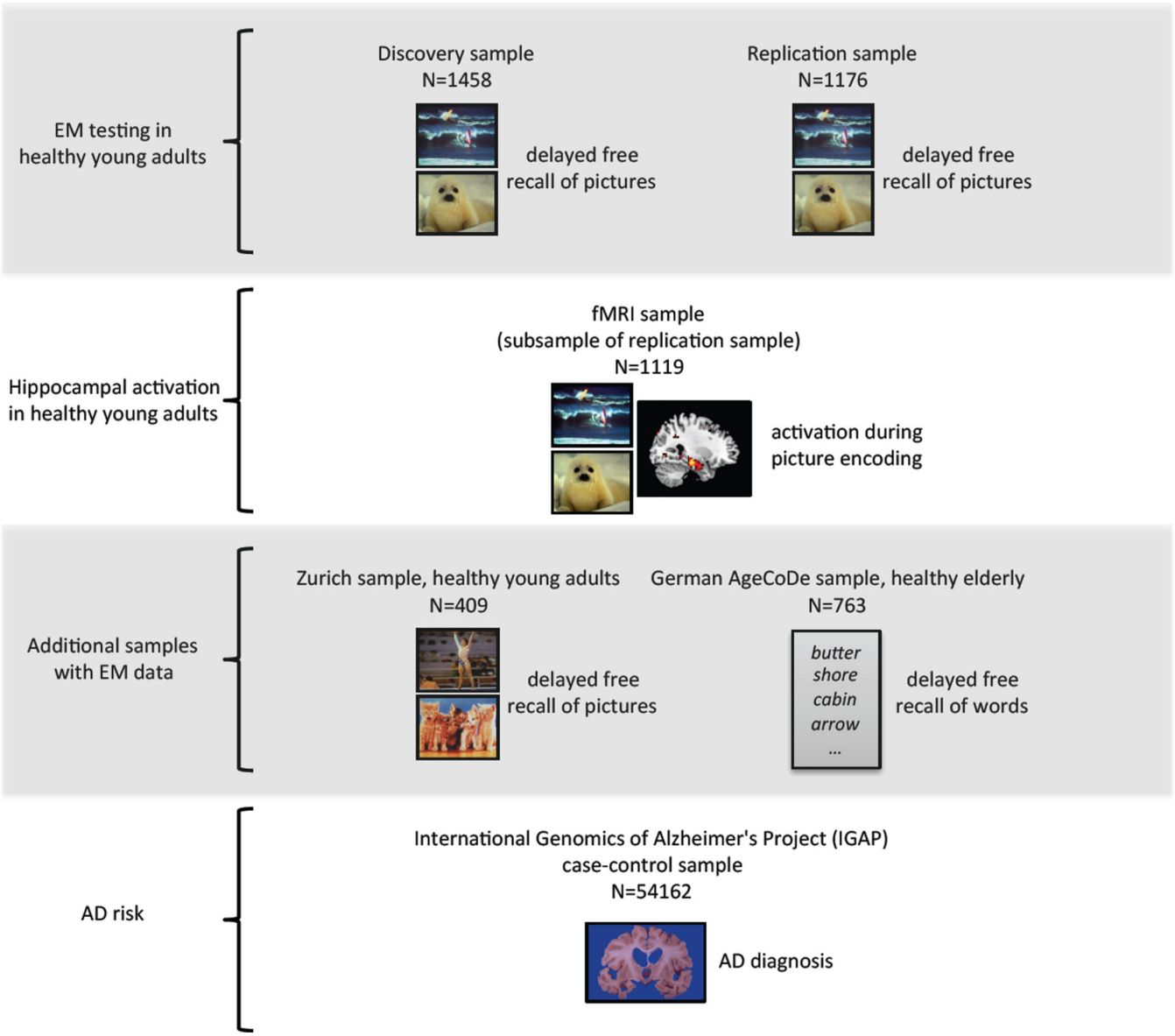

Fig. 1.

Schematic description of study workflow and included samples. First row, top: Testing for EM performance in the discovery sample (n=1458) and the replication sample (n=1176) of healthy young adults was done by quantifying the delayed free recall of IAPS pictures. The identical picture set was presented in both samples. Second row: This fMRI sample of healthy young adults (n=1119) was a subsample of the replication sample and was used for quantification of hippocampal activation during encoding of IAPS pictures (identical picture set as before). Third row: These additional samples of 409 healthy young and 763 healthy elderly adults were tested for EM performance by quantifying the delayed free recall of IAPS pictures (young sample, different picture set compared to the discovery and replication samples) or of words (elderly sample). Fourth row, bottom: This large case-control sample (n=54162) was used to test for enrichment of the episodic memory (EM) core gene set. Phenotype of interest was the diagnosis of Alzheimer's disease.

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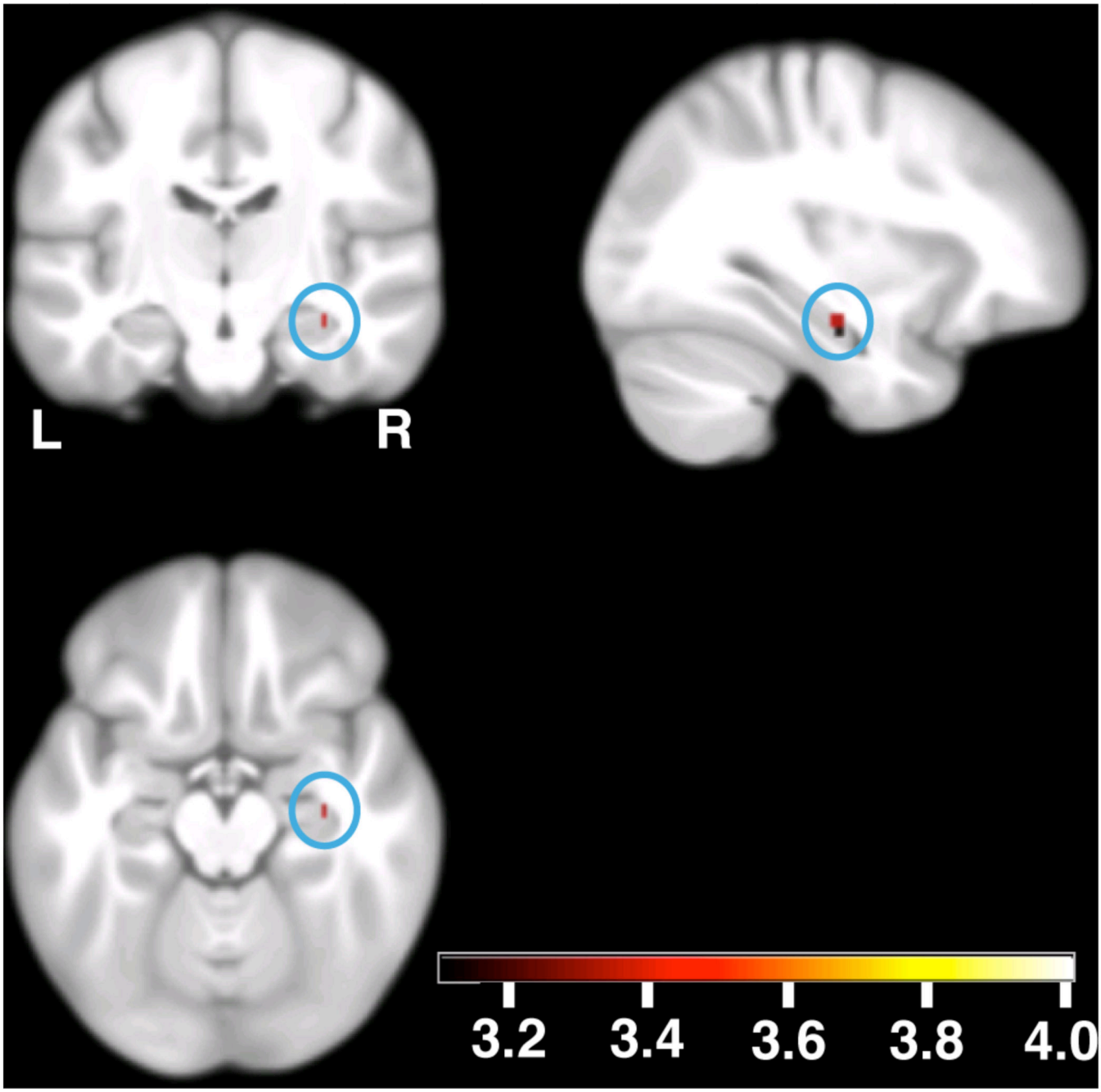

Fig. 2.

Allelic load-dependent increases in EM-related brain activity (n=1119). The blue circles show the activation in the right hippocampus. The local maximum is located at [33 −16.5 −16]; t=3.35, $P_{\text{uncorrected}}$=0.0004, $P_{\text{small volume correction (SVC)}} < 0.05$. Activations are overlaid on coronal (upper left), sagittal (upper right) and axial (lower left) sections of the study specific group template (see Methods); displayed at $P_{uncorrected}$=0.001, using color-coded t values. L, left side of the brain; R, right side of the brain.

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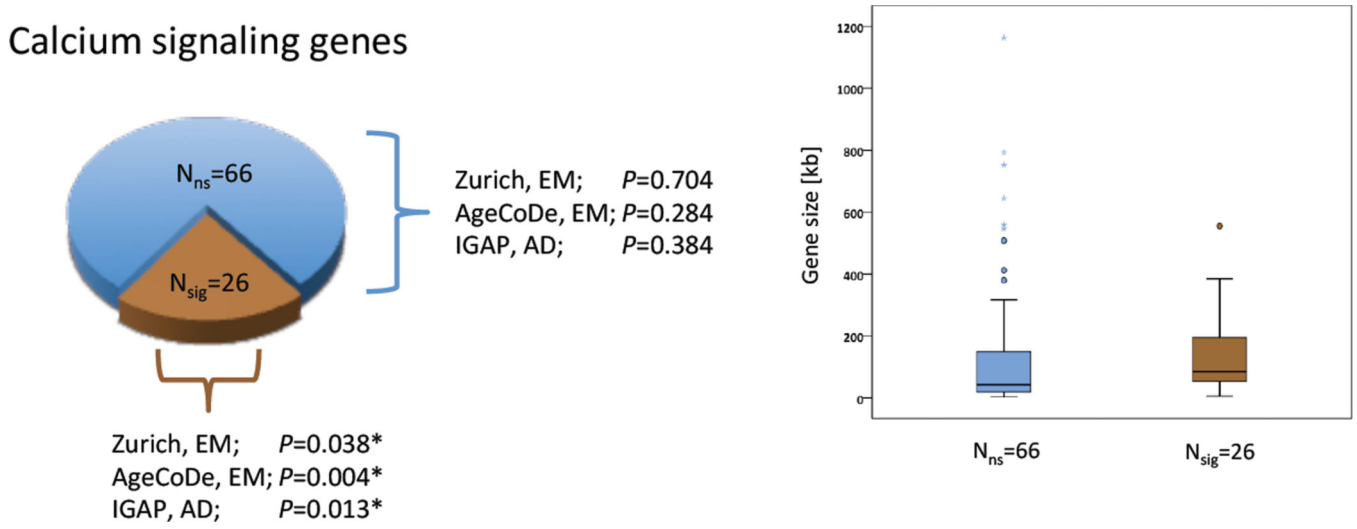

Fig. 3.

GSEA results of two different gene sets: EM core gene set (brown color), group of 66 non-significant genes (blue color). Left panel: enrichment *P* values of the respective gene sets in three samples (Zurich sample, AgeCoDe sample, IGAP sample). Right panel: Box-plot of gene size distribution stratified by gene set. Filled circles indicate outliers, stars indicate extreme values. The average length (± S.D.) of the 26 significant genes is 144.6 kb ± 139.7 kb. The average length of the 66 non-significant genes is 143.8 kb ± 226.9 kb. The difference is not significant (*P*=0.9).

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Table 1

GSEA results (FDR q < 0.25) in the discovery sample.

Database	Gene-set ID	Gene set name	Number of genes	Number of significant genes	P-value	FDR q value
KEGG	hsa4020	calcium signaling pathway	178	56	0.0002	0.024
Gene Ontology	GO:0015837	amine transport	38	17	0.0013	0.110
Gene Ontology	GO:0015171	amine transmembrane transporter activity	41	18	0.0006	0.122
Gene Ontology	GO:0043566	structure specific DNA binding	56	21	0.0024	0.140
Gene Ontology	GO:0008271	active transmembrane transporter activity	122	48	0.0007	0.142
Gene Ontology	GO:0045935	positive regulation of nucleobase-nucleoside-nucleotide and nucleic acid metabolic process	154	38	0.0017	0.148
Gene Ontology	GO:0045893	positive regulation of transcription-DNA dependent	118	24	0.0025	0.175
Gene Ontology	GO:0045944	positive regulation of transcription from RNA polymerase II promoter	65	38	0.001	0.184
Gene Ontology	GO:0051252	positive regulation of RNA metabolic process	120	48	0.0039	0.187
Gene Ontology	GO:0004930	G protein coupled receptor activity	191	16	0.004	0.193
Gene Ontology	GO:0017171	serine hydrolase activity	46	16	0.0059	0.195
Gene Ontology	GO:0008236	serine type peptidase activity	45	52	0.0064	0.195
Gene Ontology	GO:0008233	peptidase activity	176	10	0.0027	0.197
Gene Ontology	GO:0015082	di_tri_valent inorganic cation transmembrane transporter activity	22	17	0.0098	0.199
Gene Ontology	GO:0008081	phosphoric diester hydrolase activity	40	17	0.0047	0.207
Gene Ontology	GO:0005792	microsome	42	17	0.0049	0.209
Gene Ontology	GO:0042598	vesicular fraction	44	16	0.0095	0.248

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Table 2

GSEA results in the discovery and replication samples. Genes shown in this table were identified by GSEA as significant constituents of the Calcium Signaling Pathway in the respective sample. Genes highlighted bold are members of the EM core gene set (i.e. significant in the discovery and the replication sample). All gene symbols according to HGNC nomenclature.

Discovery sample (Basel 1, n=1458)								
ADCY2	*ADCY4*	***ADCY8***	*ADCY9*	***ADRA1A***	*ADRA1B*	*ADRA1D*	*ATP2A2*	*ATP2B2*
ATP2B4	***AVPR1A***	*BST1*	*CACNA1A*	*CACNA1B*	***CACNA1E***	***CACNA1G***	***CACNA1S***	***CAMK2G***
CCKBR	*CHRM1*	*CHRM3*	***CHRM5***	*EGFR*	***GNA15***	*GNAQ*	*GRIN2A*	*GRM1*
HTR2A	***ITPKB***	***ITPR1***	*ITPR2*	*ITPR3*	*LHCGR*	*MYLK*	*MYLK2*	*NOS3*
NTSR1	*OXTR*	*P2RX5*	***P2RX7***	*PDE1B*	***PDGFRA***	***PDGFRB***	***PLCB2***	***PLCD4***
PLCG2	***PPP3CA***	***PPP3R1***	***PRKCB***	*PTAFR*	***PTGER3***	***RYR3***	*SLC8A1*	*SLC8A3*
TACR1	*TRPC1*							
Replication sample (Basel 2, n=1176)								
ADCY3	***ADCY8***	***ADRA1A***	***ATP2B4***	***AVPR1A***	***CACNA1E***	***CACNA1G***	*CACNA1I*	***CACNA1S***
CAMK2A	*CAMK2B*	***CAMK2G***	*CCKAR*	***CCKBR***	*CHP2*	***CHRM5***	*CHRNA7*	*EDNRA*
EDNRB	*GNA14*	***GNA15***	*GNAL*	*HRH2*	***HTR2A***	*HTR5A*	***ITPKB***	***ITPR1***
NOS1	*NOS2*	*P2RX4*	***P2RX7***	***PDGFRA***	***PDGFRB***	***PLCB2***	***PLCD4***	*PLCE1*
PLCG2	*PLCZ1*	*PLN*	***PPP3CA***	***PPP3R1***	***PRKCB***	***PTGER3***	*PTK2B*	***RYR3***
TACR1	*TNNC2*	*VDAC2*						