Male-specific epistasis between WWC1 and TLN2 genes is associated with Alzheimer's disease

Elena S. Gusareva a,*, Jean-Claude Twizere b, Kristel Sleegers c,d, Pierre Dourlen e,f,

Jose F. Abisambra g,h,i,j, Shelby Meier g,h, Ryan Cloyd g, Blaine Weiss g, Bart Dermaut e,f,

Kyrylo Bessonov a, Sven J. van der Lee k, Minerva M. Carrasquillo l, Yuriko Katsumata m,

Majid Cherkaoui b, Bob Asselbergh c,d, M. Arfan Ikram n,o, Richard Mayeux p,

Lindsay A. Farrer q, Jonathan L. Haines r, Margaret A. Pericak-Vance s,

Gerard D. Schellenberg t, on behalf of Genetic and Environmental Risk in Alzheimer's

Disease 1 consortium (GERAD1), Alzheimer's Disease Genetics Consortium (ADGC),

The European Alzheimer Disease Initiative Investigators (EADI1 Consortium)1, Rebecca Sims u, Julie Williams u, Philippe Amouyel f, Cornelia M. van Duijn k,

Nilüfer Ertekin-Taner l,v, Christine Van Broeckhoven c,d, Franck Dequiedt b,

David W. Fardo m,w, Jean-Charles Lambert f, Kristel Van Steen a,x,y

a Medical Genomics Research Unit, GIGA-R, University of Liège, Belgium

b Molecular Biology of Diseases Research Unit, GIGA-R, University of Liège, Belgium

c Neurodegenerative Brain Diseases group, Center for Molecular Neurology, VIB, Antwerp, Belgium

d Institute Born-Bunge, University of Antwerp, Antwerp, Belgium

e U1167-RID-AGE, Facteurs de risque et déterminants moléculaires des maladies liées au vieillissementa, Universite de Lille Nord de France, Lille, France f NSERM U1167, Institut Pasteur de Lille, Universite de Lille Nord de France, Lille, France

g Sanders-Brown Center on Aging, University of Kentucky, College of Medicine, Lexington, KY, USA

h Department of Physiology, University of Kentucky, College of Medicine, Lexington, KY, USA

i Epilepsy Center, University of Kentucky, College of Medicine, Lexington, KY, USA

j Spinal Cord and Brain Injury Research Center, University of Kentucky, College of Medicine, Lexington, KY, USA

k Department of Epidemiology, Erasmus University Medical center, Rotterdam, the Netherlands

l Department of Neuroscience, Mayo Clinic Florida, Jacksonville, FL, USA

m Department of Biostatistics, College of Public Health, University of Kentucky, Lexington, KY, USA n Department of Neurology, Erasmus University Medical center, Rotterdam, the Netherlands

n Department of Radiology, Erasmus University Medical center, Rotterdam, the Netherlands

p Department of Neurology, Gertrude H. Sergievsky Center, Taub Institute on Alzheimer's Disease and the Aging Brain, Columbia University, New York, NY, USA q Departments of Biostatistics, Medicine (Genetics Program), Ophthalmology, Neurology, and Epidemiology, Boston University,

Boston, MA, USA r Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH, USA

s Department of Human Genetics, The John P. Hussman Institute for Human Genomics, Dr John T. Macdonald Foundation, University of Miami, Coral Gables, FL, USA

t Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA

u Medical Research Council Centre for Neuropsychiatric Genetics and Genomics, Institute of Psychological Medicine and Clinical Neurosciences, Cardiff University School of Medicine, Cardiff, UK

v Department of Neurology, Mayo Clinic Florida, Jacksonville, FL, USA

w Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY, USA

x Walloon Excellence in Life sciences and BIOtechnology (WELBIO), Belgium

y Department of Human Genetics, KU Leuven, Leuven, Belgium

Abstract

Systematic epistasis analyses in multifactorial disorders are an important step to better characterize complex genetic risk structures. We conducted a hypothesis-free sex-stratified genome-wide screening for epistasis contributing to Alzheimer's disease (AD) susceptibility. We identified a statistical epistasis signal between the single nucleotide polymorphisms rs3733980 and rs7175766 that was associated with AD in males (genome-wide significant pBonferroni-corrected¼0.0165). This signal pointed toward the genes WW and C2 domain containing 1, aka KIBRA; 5q34 and TLN2 (talin 2; 15q22.2). Gene-based meta-analysis in 3 independent consortium data sets confirmed the identified interaction: the most significant (pmeta-Bonferroni-corrected¼9.02*10□3) was for the single nucleotide polymorphism pair rs1477307 and rs4077746. In functional studies, WW and C2 domain containing 1, aka KIBRA and TLN2 coexpressed in the temporal cortex brain tissue of AD subjects (b¼0.17, 95% CI 0.04 to 0.30, p¼0.01); modulated Tau toxicity in Drosophila eye experiments; colocalized in brain tissue cells, N2a neuroblastoma, and HeLa cell lines; and coimmunoprecipitated both in brain tissue and HEK293 cells. Our finding points toward new AD-related pathways and provides clues toward novel medical targets for the cure of AD.

1. Introduction

Alzheimer's disease (AD) is a progressive, irreversible neurode-generative disorder characterized by the development of amyloid plaques and neurofibrillary tangles, the loss of connections between neurons, and nerve cell death. AD is highly heritable and genetically heterogeneous with 58%e79% of risk attributed to genetic factors (Gatz et al., 2006; Sims and Williams, 2016). Although genome-wide association studies have strongly improved our knowledge of AD genetics (Ridge et al., 2013), genetic risk factors explain no more than 30% of heritability (Cuyvers and Sleegers, 2016). In this contribution, we focus on late-onset AD, the most common form of the disease with onset age >65 years. The most established genetic factor for AD, apolipoprotein E gene ([APOE] gene, 19q13), exhibits allelic hetero-geneitydAPOE's ε4 allele is a risk enhancer, whereas the ε2 allele is protective (Bertram et al., 2007).

AD presents notable sexual dimorphism (Mielke et al., 2014). Records exist of sex differences in the brain, such as in brain anat-omy, age-related declines in brain volume and brain glucose metabolism (Carter et al., 2012), and sex hormones influencing AD progression (Musicco, 2009). Risk associated

with the APOE-e4 allele is stronger in females than in males, and loss of chromosome Y have been associated with increased AD risk in males (Dumanski et al., 2016). These data support complex interplay between sex and genetic background regarding AD predisposition.

Gene regulatory and biochemical networks create dependencies among genes that are realized as gene-gene interactions (epistasis) (Templeton, 2000). Although epistasis has been well studied in model organisms using biological experiments (Miko, 2008), hypothesis-free discovery of biological epistasis via statistical methods remains challenging in humans. This is in part due to the conceptual discrepancy between statistical and biological epistasis (Moore, 2005), the utility of oversimplified population-level models to capture complex individual phenomena, insufficient power, and the gross multiple testing burden inherent in genome-wide epistasis screening. Therefore, most evidence for epistasis in AD is hypothesis-driven, using prior biological or statistical knowledge (Ebbert et al., 2015). The same holds for sex-specific searches for coinvolvement of multiple genetic loci in AD (Medway et al., 2014).

Gusareva et al. published the first replicable interaction associated with AD using a genome-wide exhaustive screening approach that combines strengths over different analytic approaches (Gusareva and Van Steen, 2014), identified a statistical interaction between KHDRBS2 (rs6455128) and CRYL1 (rs7989332), and exhibited down-stream functional consequences (Gusareva et al., 2014). Here, we used the same European AD Initiative Investigators (EADI1) consortium cohort (Lambert et al., 2009) (2259/6017 AD cases/controls) and an adapted hypothesis-free genome-wide exhaustive epistasis screening protocol to identify sex-specific interactions with AD. We identified AD-associated male-specific statistical interaction between variants of the genes WWC1 (WW and C2 domain containing 1 or kidney and brain expressed protein, aka KIBRA; locus 15q22.2) and TLN2 (talin 2, locus 15q22.2). This novel statistical epistasis signal was replicated in 2 of 3 independent consortium data sets via gene-based replication strategy (Gusareva and Van Steen, 2014). Extensive biological validation studies (subcellular colocalization and immunoprecipitation [IP] analyses, transcriptome analysis, experiments in model organisms [Drosophila melanogaster], as well as in silico protein docking and molecular dynamics assessments) further helped elucidate the epistatic relationship.

2. Methods

2.1. Study populations

The discovery cohort consisted of a sample of 2259 late-onset AD patients and 6017 controls from 3 cities in France (Bordeaux, Dijon, and Montpellier), as part of EADI1. Follow-up statistical analyses used data from 3 AD consortia: (1) the Genetic and Environmental Risk for AD consortium (GERAD1) including cohorts from Germany, UK, and the USA (Harold et al., 2009); (2) the Rotterdam Study (RS), a prospective cohort study that started in 1990 in Rotterdam (the Netherlands) (Hofman et al., 2013); and (3) the AD Genetic Consortium (ADGC) that collects genetic data from over 30 studies in the US (Naj et al., 2011). Data collection quality control procedures have been described in the corresponding references. Only subjects with complete information on sex and age were included in the analyses. Sex-specific sample size distributions and age characteristics are given in the Table S1.

2.2. Genotyping

The EADI1 and RS samples were genotyped by Illumina Human 610-Quad BeadChip (Hofman et al., 2013; Lambert et al., 2009), the GERAD1 samples by Illumina 610-quad chip and by Illumina HumanHap550 Beadchip (Harold et al., 2009), the ADGC subjects by Illumina or Affymetrix high-density single nucleotide poly-morphism (SNP) microarrays (Naj et al., 2011). Applied genotype

filtering procedure as described in the Note S2 leaving 312,064 SNPs for epistasis analyses with EADI1. Replication cohorts used only directly genotyped SNPs.

2.3. Statistical discovery and replication analysis

Following guidelines in Gusareva et al. (Gusareva and Van Steen, 2014), we tested for all pairwise statistical interactions between SNPs in association to AD in sex-stratified samples within EADI1. Two different analytic techniques both parametric (customized version of the BOolean Operation-based Screening and Testing [BOOST] (Wan et al., 2010) with stringent Bonferroni correction) and nonparametric (model-based multifactor dimensionality reduction [MB-MDR]) (Cattaert et al., 2011; Van Lishout et al., 2013) that uses permutation-based gammaMAXT algorithm for multiple testing correction (Lishout et al., 2015) were adopted in this study with default options (Note S3). Statistical epistasis signals at the genome-wide significance level of 0.05 were followed up with a logistic regression analyses adjusting for age at time of subject examination and the first 4 SNP-based principal components (to adjust for confounding by shared genetic ancestry). Evidence of interaction was based on a likelihood-ratio test statistic with 4 degrees of freedom to reflect 2 SNPs with 3 genotypes each (in the absence of missing multilocus genotypes). Main effect single-SNP associations were assessed via Cochrane-Armitage trend test in SVS Version 7.5 software (Golden Helix, Inc).

For replication analysis, we selected 68 and 98 SNPs assigned to WWC1 (5q34: 167651670-167829334 bp) and TLN2 (15q22.2: 60726802-60920733 bp), respectively, according to NCBI B36 genome assembly (SNP list is provided in Table S2). We did not consider SNPs from any regulatory regions outside WWC1 and TLN2 genes. Thus, all the SNPs falling into the boundaries of WWC1 and TLN2 genes and typed in all the study cohorts (discovery EADI1 and the 3 replication cohorts: GERAD1, RS, and ADGC) were exhaustively tested for 2-way intergenic interactive association with AD, in males and females separately. We used logistic regression adjusted for age and genetic population stratification as before. The number of in-dependent tests (Nyholt, 2004) was 1564 (of 6664 total). All obtained p-values (not corrected for multiple testing pnominal)forEADI1, GERAD1, RS, and ADGC were meta-analyzed using Fisher'scombined p-value (Fisher, 1948) and Stouffer's Zscore(Stouffer et al., 1949) methods, giving rise to meta-analysis p-values (pmeta). Details on the applied significance criteria are described in the Note 4.

2.4. Functional analysis and biological validation

We used transcriptome analysis to assess coexpression of WWC1 and TLN2 in temporal cortex and cerebellum human brain regions with data from the brain expression GWA study (eGWAS) (Allen et al., 2012; Zou et al., 2012)(Note 5). The laboratory fruit fly Drosophila melanogaster was used to further explore the role of WWC1 and TLN2 in model organisms (Note S6). In addition, formalin-fixed temporal cortexes of male AD patients were used to perform brain immunohistochemistry (Note S7). The latter was performed in 2 independent labs to robustly establish reproducibility. To assess subcellular localization of WWC1 and TLN2, we performed immunofluorescence and confocal microscopy analyses (Note S8). We also investigated the presence of WWC1 and TLN2 in the same complex via IP analysis (Note S9). Molecular mechanisms of interaction be-tween WWC1 and TLN2 were modeled via protein docking (Note S10) and molecular dynamics in silico experiments (Note S11).

The entire analysis protocol is described in Fig. 1.

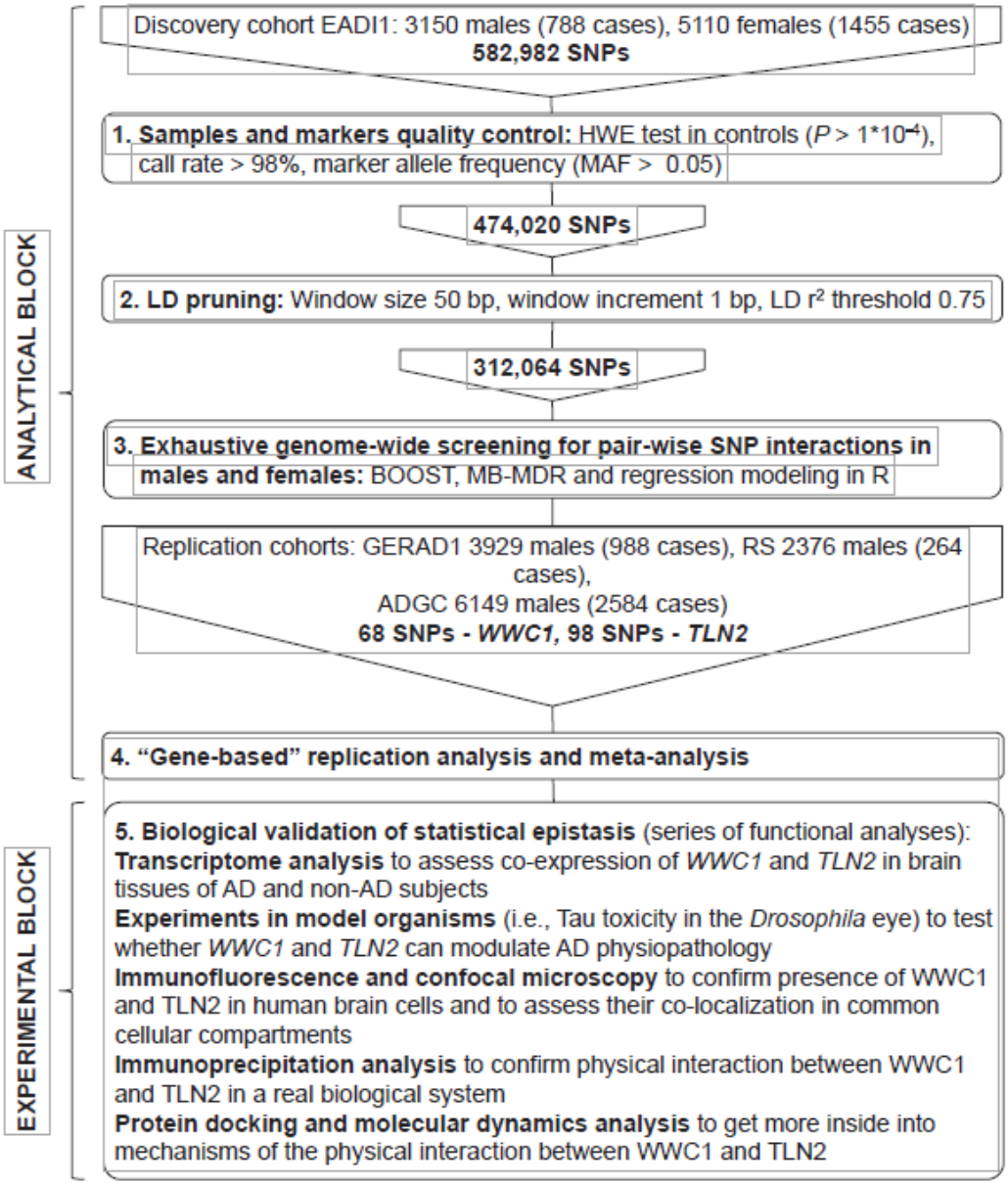
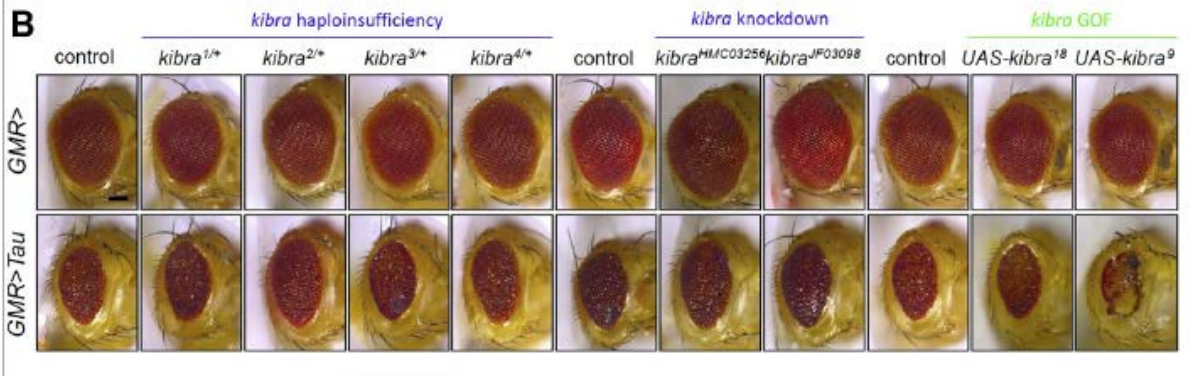
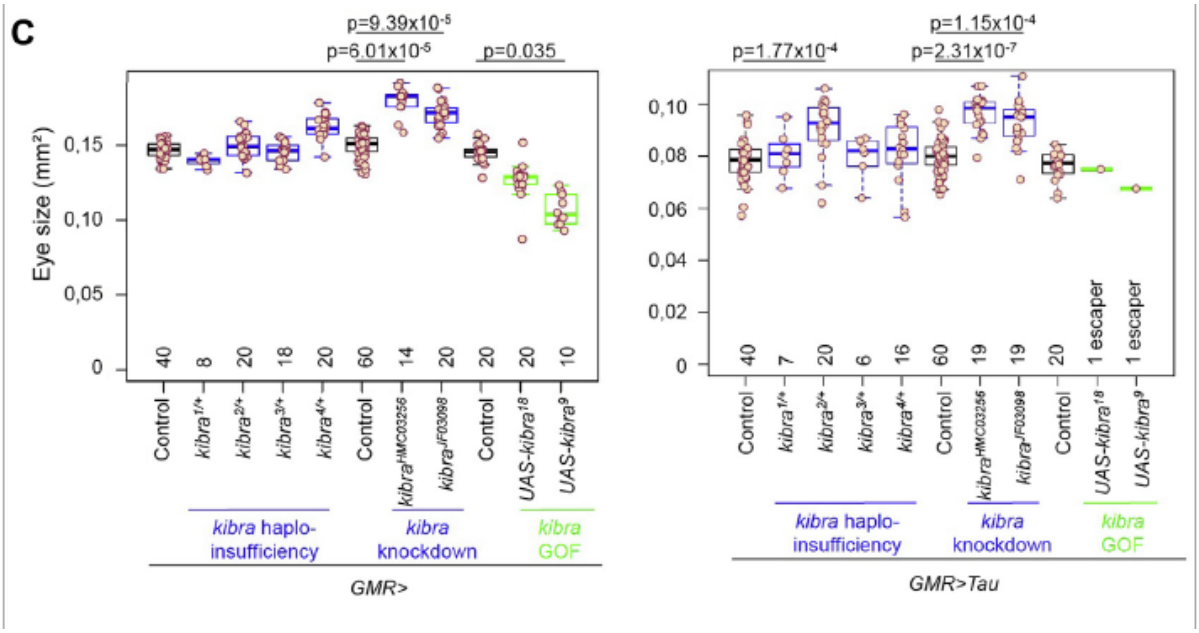


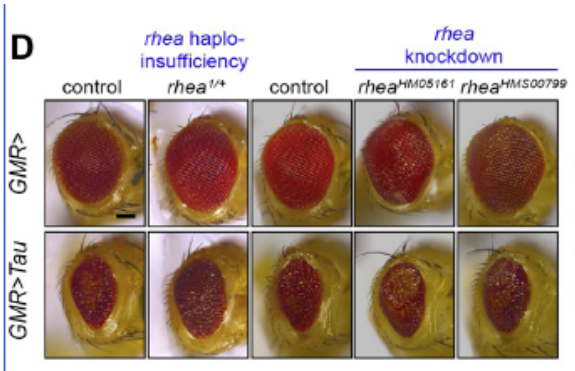
Fig. 1. Analysis protocol including genome-wide association interaction (analytical block) and biological validation of epistasis (experimental block).

A

Human gene	Fly gene	DIOPT score	Identity (%)	Similarity (%)
WWC1	*kibra*	8	34	52
TLN2	*rhea*	9	47	67







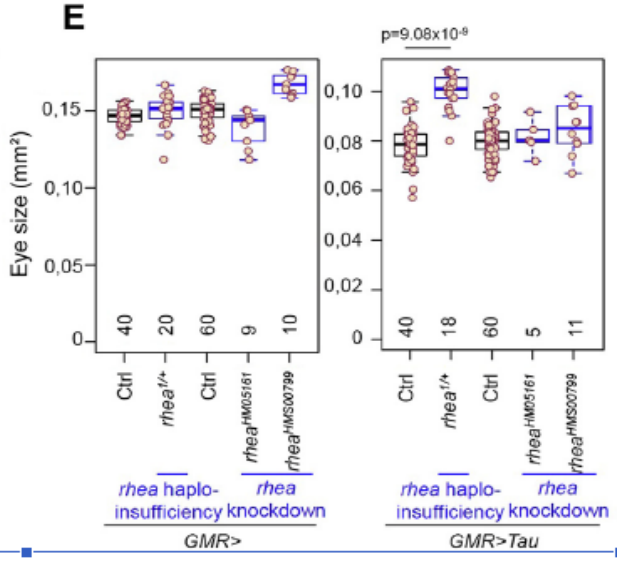


Fig. 2. Genetic interaction between *kibra*, *rhea*, and human Tau in the eye of *Drosophila*. (A) Table presenting the homology of *WWC1* and *TLN2* with their *Drosophila* orthologs. (B and C) Image and size quantification of fly eyes expressing the 2N4R Tau isoform (*GMR>Tau*) in loss-of-function (in blue) and gain-of-function ([GOF], in green) *kibra* conditions (scale bar 0.1 mm). The *GMR>* images correspond to the same *kibra* conditions without Tau expression. Numbers above the x axis in the graphs indicate the number of eyes that were quantified. Knockdown (overexpression) of *kibra* rescued partially (enhanced) Tau toxicity in the eye (C. right graph). This was likely an additive effect of the modulation of *kibra* with Tau as knockdown (overexpression) of *kibra* alone increased (decreased) the size of the eyes (C. left graph). However, 1 haploinsufficient condition,

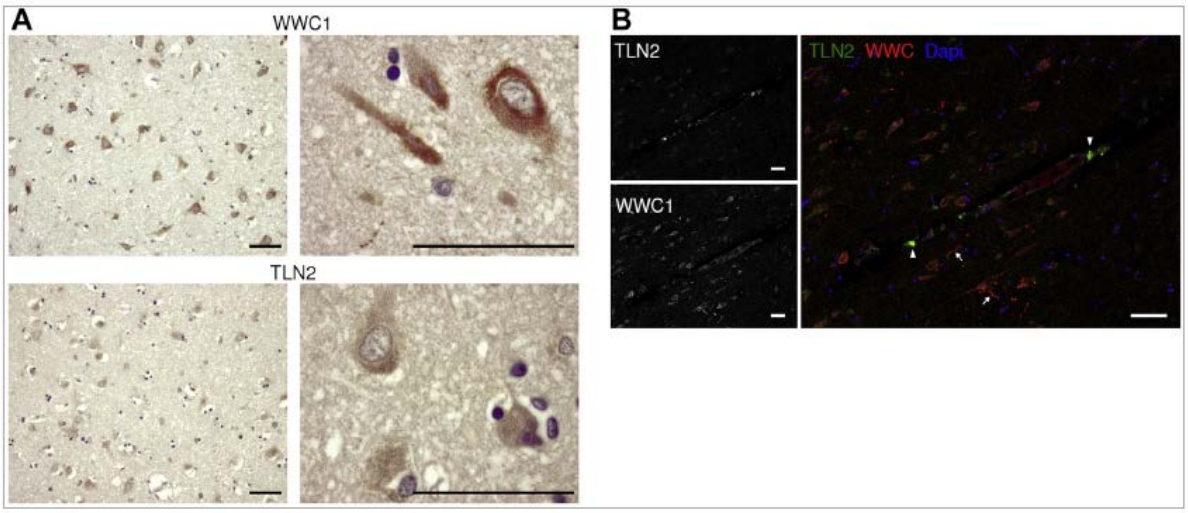


Fig. 3. Presence and localization of WWC1 and TLN2 in the temporal cortex of an AD patient. (A) Single immunostaining with chromogenic detection reveals in neuronal cytoplasm a moderate to strong WWC1 staining and low TLN2 expression. (B) Fluorescence double immunostaining confirms the presence of WWC1 and TLN2 in neuronal cells. Strong neuritic WWC1 accumulations are highlighted with arrows; blood vessel endothelial cells with high TLN2 signal are marked with arrowheads. Scale bar = 50 µm. Abbreviations: AD, Alzheimer's disease; *WWC1*, WW and C2 domain containing 1, aka KIBRA.

3. Results

3.1. Synergy between variants of WWC1 and TLN2 in association to AD

Both parametric (BOOST) and nonparametric (MB-MDR) analyses highlighted epistasis between the SNPs rs3733980 and rs7175766 (minor allele frequencies¼0.365, 0.307 in EADI1, respectively) as genome-wide significant in males (BOOST: pBonfer-roni-corrected¼0.018, MB-MDR: ppermutation-based¼0.005). Case/control distributions within the 9 multilocus genotype combinations and MB-MDR "high risk"/"low risk" labeling are in the Table S3. Only rs3733980 also showed a main effect (pnominal¼0.015, trend test), which would not withstand stringent multiple testing correction. The identified epistasis signal remained statistically significant in a logistic regression model accounting for age and the first 4 PCs (pBonferroni-corrected¼0.0165). The APOE gene did not confound the identified interaction because we found no dependence between the APOE ε4 AD-risk allele and the 9-level categorical SNP pair for these SNPs (p-value¼0.999,c28). No female-specific epistasis was identified (BOOST, MB-MDR p>0.05).

3.2. Statistical replication of epistasis between WWC1 and TLN2

We considered all pairwise intergenic interactions between the directly genotyped 68 SNPs of WWC1 and 98 SNPS of TLN2 (Table S2) for follow-up replication analysis in both sexes with the GERAD1, RS, and ADGC data sets. In males, the SNP pair rs3733980 and rs7175766 was significant in a single study (EADI1: pBonferroni-corrected¼5.29*10□10). Rs7175766 appeared 4 times in the top 10 male-specific meta-analysis results but did not show any marginal association with AD (pnominal¼0.546, trend test). Interaction be-tween rs1477307 and rs4077746 was found in 3 study populations (EADI1: pnominal¼0.040, RS: pnominal¼9.37*10□4, and ADGC: pnominal¼5.06*10□5, but not in GERAD1: pnominal¼0.544; Fisher's combined pmeta-Bonferroni-corrected¼2.74*10□3, and Stouffer's Z score pmeta-Bonferroni-corrected¼9.02*10□3; Table S4). In females, similar meta-analysis gave no replicable epistasis signals (Table S5).

3.3. Functional analysis and biological validation

Transcriptome analysis revealed significant positive association between expression levels of WWC1 (probe ID - ILMN_1658619) and TLN2 (probe ID - ILMN_1700042) in temporal cortex brain samples from autopsied AD subjects (b¼0.17, p¼0.01) and from combined autopsied AD and non-AD subjects (b¼0.20, p¼0.0003). These associations were mostly driven by females (temporal cortex from autopsied AD females: b¼0.28, p¼0.005, combined autopsied AD and non-AD females b¼0.20, p¼0.016) but were not prominent in males. This association was only marginally significant for autopsied non-AD subjects (b¼0.19, p¼0.05). In the cerebellar tissue, no significant associations between expression levels of WWC1 and TLN2 genes were observed (Table S6).

We also tested whether WWC1 and TLN2 could modulate AD physiopathology in human Tau (2N4R)-expressing Drosophila, an in vivo model of AD (review (Gistelinck et al., 2012)). Kibra, ortholog of WWC1 (Fig. 2AeC), and rhea, ortholog of TLN2 (Fig. 2A and D and E), were tested as modifiers of Tau toxicity in Drosophila eye. In Drosophila, kibra belongs to the growth controlling Hippo pathway. Gain (loss) of kibra results in smaller (bigger) eyes (Baumgartner et al., 2010), which we also observed (Fig. 2A and B). Expression of human Tau (2N4R) in the eye with the GMR driver resulted in smaller rough eyes. The eye size was partially restored in kibra2/þ haploinsufficient background, on RNAi-mediated knockdown of kibra (Fig. 2B and C) and in rhea1/þ haploinsufficient background (Fig. 2D and E). Coexpression of kibra with Tau resulted in lethality and the only escapers that we obtained had smaller eyes. For kibra knockdown and kibra overexpression, the effect may be additive as in both conditions without Tau expression, fly eyes are respectively bigger and smaller (Fig. 2A and B). For kibra haploinsufficiencies, only 1 of 4 independent null mutations restored the eye size precluding us to firmly conclude that kibra interacts with Tau in Drosophila eye. The result in the rhea1/þ haploinsufficient background (Fig. 2D and E) suggested that rhea interacted functionally with human Tau in Drosophila eye.

Immunohistochemistry of the brain of a male autopsied AD patient indicated strong expression of WWC1 in the soma of neuronal cells throughout the temporal lobe of the cerebral cortex (Fig. 3). In these neurons, WWC1 presented in the cytoplasm with presumed membrane and/or cytoskeleton associations and strong neuritic accumulations in some cells. TLN2 also presented in the cytoplasm of neuronal cells, although immunoreactivity was low. In addition to the weak neuronal signal, a strong TLN2 signal was detected in the endothelial cells of blood vessels.

We also performed coimmunofluorescent staining analyses of human Braak I and Braak VI brains (Braak and Braak, 1991)(Fig. 4). After performing quantitative pixel intensity spatial correlation analysis (extracting Pearson's, Manders', and Costes' parameters [autothreshold and randomization] (Bolte and Cordelieres, 2006)), we determined that TLN2 (Talin2) and WWC1 (aka KIBRA) colocalized in all cases. Interestingly, WWC1 staining appeared to be more cellular in Braak I compared to Braak VI tissue, where the staining appeared stronger and more widely distributed.

In complement, we confirmed colocalization of WWC1 and TLN2 in HeLa cell lines and in mouse N2a neuroblastoma cells. When overexpressed in HeLa cells, WWC1 displayed diffuse cytoplasm localization and small perinuclear rings (Fig. 5, Flag-WWC1), and TLN2-GFP displayed cytoplasmic focal adhesion localization with elongated fibrillar adhesions through the cell body (Fig. 5, TLN2-GFP), consistent with previous studies (Kremerskothen et al., 2003; Praekelt et al., 2012). Coexpression of both WWC1 and TLN2 dramatically changed TLN2 localization. In the presence of WWC1,

kibra$^{2/+}$, partially rescued Tau toxicity (C. right graph) without affecting the eye on its own (C. left graph). (D and E) Image and size quantification of fly eyes expressing the 2N4R Tau isoform (*GMR>Tau*) in loss-of-function (in blue) *rhea* conditions (scale bar 0.1 mm). Expression of Tau in the haploinsufficient *rhea*$^{1/+}$ background resulted in bigger eyes (E. right graph), whereas haploinsufficient *rhea*$^{1/+}$ flies have similar eye size than control (E. left graph), suggesting a genetic interaction between Tau and *rhea*. Abbreviation: *WWC1*, WW and C2 domain containing 1, aka KIBRA. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

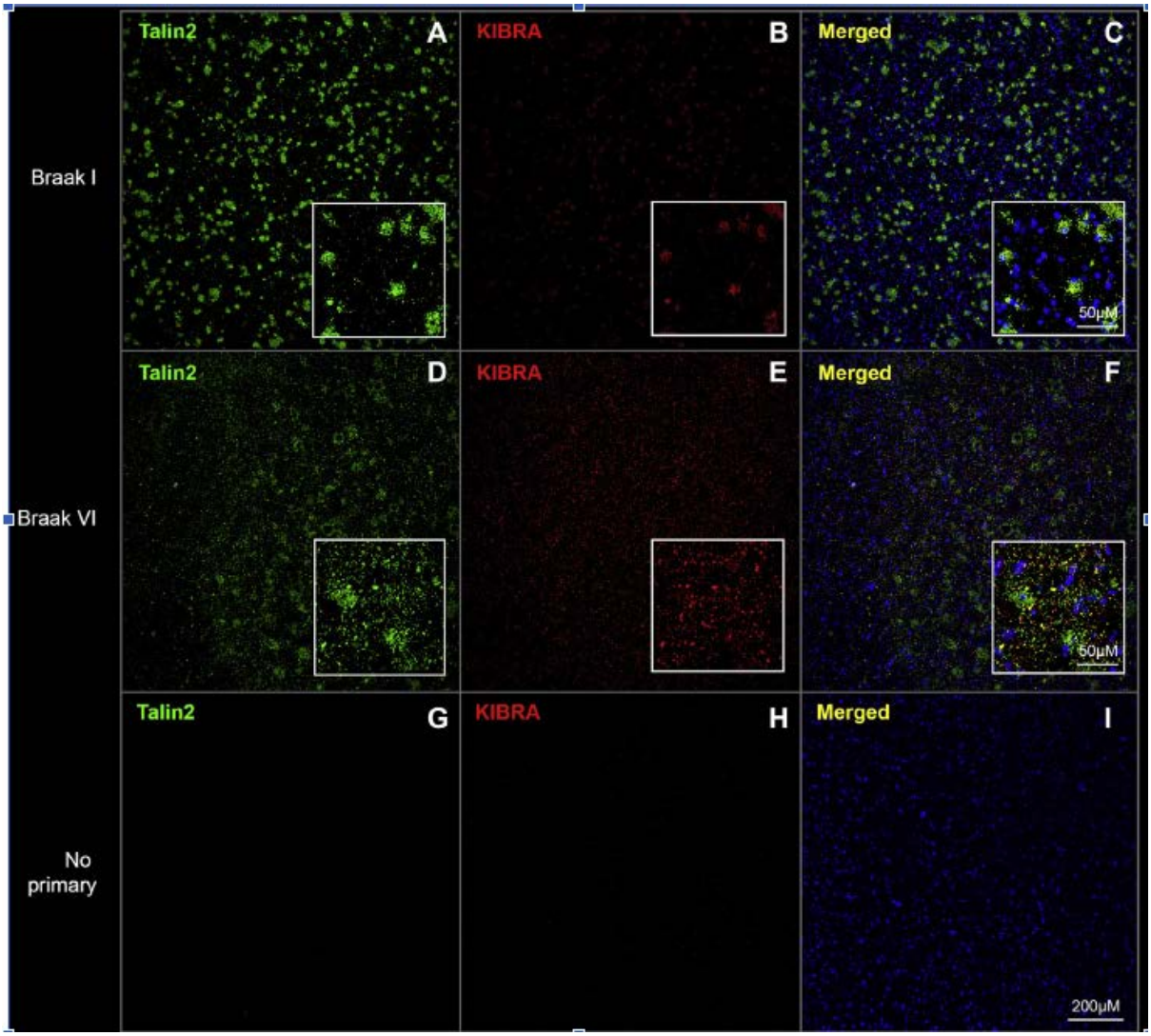


Fig. 4. TLN2 and WWC1 (aka KIBRA) colocalize in AD and control brains. Representative images of healthy (Braak I, A–C) and late-stage AD (Braak VI, D–F) brains that were immunofluorescently labeled with anti-Talin2 (green) and anti-KIBRA (red) antibodies. Colocalization analysis was performed on positive immunofluorescent signals from multiz-stack confocal microscopy images. Braak I (A–C) and VI (D–F) brains showed positive colocalization between both signals (C and F). DAPI (blue) was used to reveal cell nuclei. (G–I) Representative images of brain sections incubated with only secondary, but not primary, antibodies to reveal non-specific staining. Three Braak I and 3 Braak VI brains were imaged. A total of 9 sets of confocal z-stacked images were obtained for each condition (Braak I and VI). Abbreviations: AD, Alzheimer's disease; *WWC1*, WW and C2 domain containing 1, aka KIBRA. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

TLN2-GFP appeared concentrated in cytoplasmic foci (Fig. 5, compare GFP staining for TLN2 and WWC1þTLN2) surrounded by Flag-WWC1 rings (Fig. 5,WWC1þTLN2-GFP, merge image). In N2a cells, WWC1 and TLN2 were found to colocalize in cytoplasm and in filopodia-like protrusions (Fig. S1). However, different colocalization patterns observed in N2a cells may be due to different levels of the proteins expressions.

Furthermore, IP analysis both in human brain samples and in HEK293 cells indicated the presence of WWC1 and TLN2 in the same protein complex. The levels of the 2 proteins were variable in all conditions and brain regions queried (Braak I and Braak VI brains (Braak and Braak, 1991), Fig. 6A [upper panel]). WWC1 coimmunoprecipitated with the anti-TLN2 antibody (Fig. 6A [lower panel]); as expected, TLN2 bands were evident in the Western blot. Interestingly, when the WWC1 antibody was used, TLN22 bands were absent (Fig. 6A [lower panel]). These data suggest that the anti-WWC1

antibody could competitively disrupt the TLN2 and WWC1 interaction. In HEK293 cells, TLN2-GFP specifically copurified with Flag-WWC1 when both proteins were overexpressed together (Fig. 6B).

To model molecular mechanisms of interaction between WWC1 and TLN2, we performed protein docking and molecular dynamics in silico experiments. We determined the top 10 ranked WWC1/TLN2 poses (Fig. S2) via ClusPro 2.0 docking server (Comeau et al., 2004a,b; Kozakov et al., 2006). Poses 2 and 7 showed the most favorable conditions for complex formation as their average MM/PBSA protein-ligand binding free energies (dGbind) were amongst the most negative showing the lowest dispersion over the course of the 50 ns aqueous simulations. In all 50 ns molecular dynamics simulations, WWC1 and TLN2 remained physically associated in a complex throughout the entire course of simulation. The average dGbind remained negative for all 10 poses (dGbind ranged from -16 to -227 kJ/mol indicating the size of the binding affinity between the 2 proteins; Table S7 and Fig. S3).

4. Discussion

This is the first contribution showing (sex-specific) biological epistasis in AD between genes identified via exhaustive genomic epistasis analysis: WWC1 (WW and C2 domain containing 1 or kidney and brain expressed protein, aka KIBRA) and TLN2 (talin 2). WWC1 is expressed in brain regions responsible for learning and memory (hippocampus and cortex) and is involved in maintaining of synaptic plasticity (Vogt-Eisele et al., 2014). TLN2 expression is restricted to the heart, skeletal muscle, and brain (synapses and focal adhesions) (Di Paolo et al., 2002). It plays an important role in the assembly of actin filaments (particularly affecting actin dynamics and clathrinmediated endocytosis at neuronal synapses (Morgan et al., 2004)) and in spreading and migration of various cell types. WWC1 has already been associated with memory-related disorders including AD (Burgess et al., 2011; Corneveaux et al., 2010; Papassotiropoulos et al., 2006; Rodriguez-Rodriguez et al., 2009), whereas TLN2 has not. However, in our study, rhea (ortholog of TLN2 in Drosophila) modulated Tau toxicity in Drosophila and thus may be involved in AD pathology. Interestingly, recent studies identified several other components of the cell adhesion pathway as modifiers of Tau toxicity in Drosophila (Dourlen et al., 2016; Shulman et al., 2014). Studying the mechanisms of the identified epistatic interaction, we performed comprehensive functional biological experiments. WWC1 and TLN2 were coexpressed in the temporal cortex brain tissue (responsible for learning and memory) of AD subjects, colocalized in both brain tissue cells, in neuroblastoma N2a and HeLa cell lines, and coimmunoprecipitated both in brain tissue and HEK293 cells. The physical interaction between WWC1 and TLN2 was also supported by in silico experiments where the binding affinity between the 2 proteins was pretty strong with favorable conditions for forming a stable protein complex.

We may speculate on the involvement of WWC1 and TLN2 in common signaling pathways connected to signal transduction via synapses that are impaired when dementia symptoms and AD progress. Because overexpression of WWC1 was previously associated with AD (Burgess et al., 2011), we speculate that impairment expression of WWC1 and/or TLN2 proteins may destabilize actin filaments. Additional work is required to further describe a functional interplay between WWC1 and TLN2 and to explain why we observed the interaction at an individual level for both sexes, whereas we could detect association with AD only in males at a population level (despite of the theoretical power loss for epistasis detection in a sample stratum of males). A few explanations are possible and should be investigated in detail: the influence of sex hormones on the epistasis manifestation, the involvement of a third interacting component (i.e., an interacting gene) linked to the sex chromosomes, other types of sex-specific variant(s) in WWC1 and TLN2, among others. Regardless, our findings provide impetus for an in-depth search of AD-related mutation(s) in WWC1 and TLN2 genes to better explore and grasp biological mechanisms underlying the identified sex-specific

epistasis signals. Targeted next-generation sequencing of the interacting genes may facilitate the identification of new functional mutations (either common or rare) that play a role in protein structure, stability, solubility, folding, and affinity of interaction with ligand(s), to name a few.

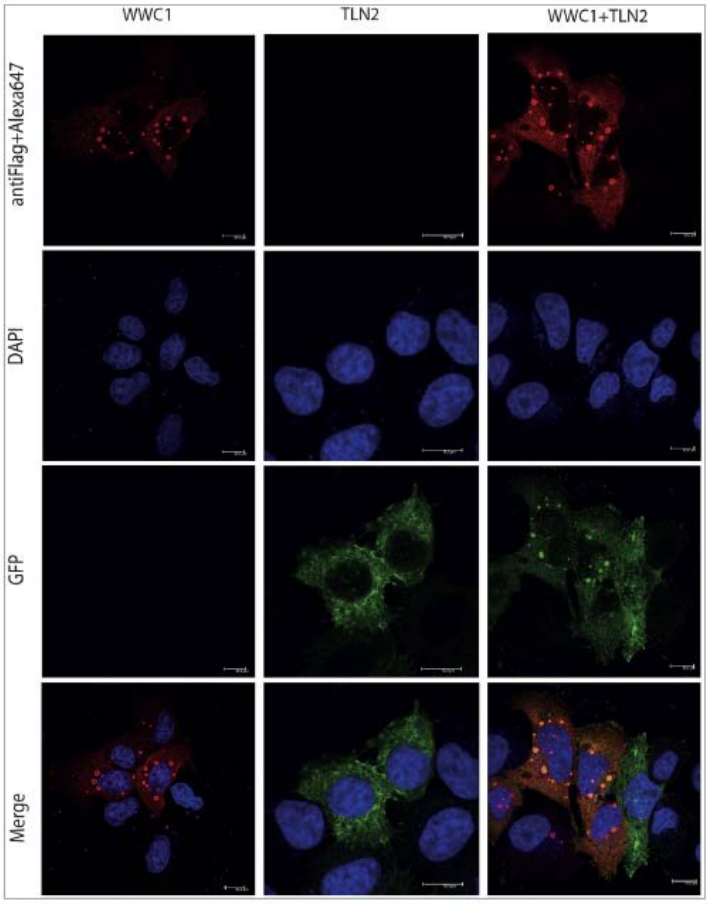


Fig. 5. WWC1 (aka KIBRA) and TLN2 colocalize in HeLa cells. HeLa cells were transfected with expressing vectors for TLN2-GFP and/or Flag-WWC1. Cells on glass coverslips were fixed, permeabilized and labeled with an anti-Flag M2 antibody followed by Alexa633-conjugated secondary antibody and Dapi nuclear staining. Images were analyzed using a confocal microscope. Abbreviation: *WWC1*, WW and C2 domain containing 1, aka KIBRA.

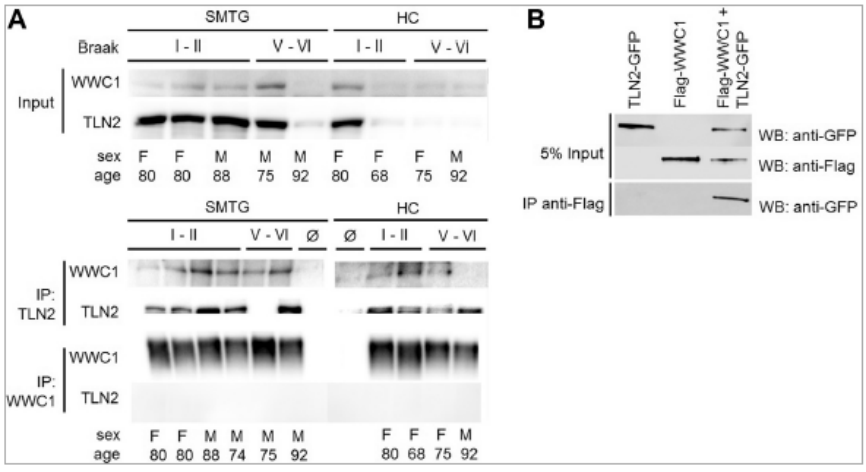


Fig. 6. WWC1 and TLN2 present in the same protein complex. (A) (Upper panel). Representative Western blot showing varying levels of TLN2 and WWC1 in SMTG and HC homogenates from Braak I and VI brains. (A) (Lower panel). Representative Western blots of co-IP showing that WWC1 associates with TLN2. TLN2, however, did not coimmunoprecipitate when anti-WWC1 antibodies were used. Ø represents brain homogenates that were not incubated with primary antibodies (only secondary). Ages and sex of each sample is shown. (B) HEK293 cells were transfected with expressing vectors for TLN2-GFP and/or Flag-WWC1 as indicated. Cell lysates were immunoprecipitated using anti-Flag M2 antibody followed by SDS-PAGE and Western blot using an anti-GFP antibody. Five percent of the amount of each lysate was used as positive control for protein expression. Abbreviations: HC, hippocampal; *WWC1*, WW and C2 domain containing 1, aka KIBRA; SMTG, superior medial temporal gyrus.

There is still a big divide between statistical epistasis and biological epistasis. The ambition in detecting statistical epistasis is to close this gap by improved analysis protocols and to formulate guidelines toward the interpretation of statistical findings in the context of epistasis. The field has evolved a lot over the last decade, in this sense. This does not change the fact that indeed, the power of a genome-wide epistasis screening (GWAI analysis) using a single study is much smaller than the power of a corresponding main effects GWA analysis using the same data (Gauderman, 2002). Our experience with large-scale epistasis studies is consistent with this, usually only giving rise to 1 or 2 reliable statistical findings (i.e., findings for which we can rule out numerical instability issues or strong main effects overtaking the joint effects of the loci involved). Regardless, results dating back from already suggested that bio-logical inference from statistical models is not a utopia (Moore, 2005).

5. Conclusion

In this research, we aimed to identify novel gene/protein targets to pave the way toward novel biochemical pathways related to AD via SNP panels as a starting point. By following a rigorous analytic genome-wide epistasis detection protocol (Gusareva and Van Steen, 2014), which minimizes false positive findings and enhances functional relevance, the statistically replicable epistasis was identified. A series of biological experiments indicated novel protein-protein interaction between WWC1 and TLN2 that can potentially be a medical target for the cure of AD. To our knowledge, this is the first report in AD where a hypothesis-free screening led to evidence for replicable statistical interaction and where functional studies were performed beyond the transcriptome.

Disclosure statement

The authors have no actual or potential conflicts of interest.

Acknowledgements

We thank all participating subjects of this study.

We thank Dr Jixin Dong (University of Nebraska Medical Center, Omaha, Nebraska, USA) and Dr Richard O. McCann (Mercer University School of Medicine, Macon, GA, USA) for providing plasmid DNA constructs, as well as the GIGA-Research technology platforms (Interactome and Imaging) for technical assistance. We thank Dr Pedro Vera and the Lexington VA Medical Center for microscopy support, as well as Ela Patel and Sonya Anderson with their assistance accessing the human brain tissue. We thank Ivy Cuijt for technical support and Bavo Heeman for helpful discussions (both at VIB Department of Molecular Genetics, University of Antwerp, Belgium).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2018. 08.001.

References

Allen, M., Zou, F., Chai, H.S., Younkin, C.S., Miles, R., Nair, A.A., Crook, J.E., Pankratz, V.S., Carrasquillo, M.M., Rowley, C.N., Nguyen, T., Ma, L., Malphrus, K.G., Bisceglio, G., Ortolaza, A.I., Palusak, R., Middha, S., Maharjan, S., Georgescu, C., Schultz, D., Rakhshan, F., Kolbert, C.P., Jen, J., Sando, S.B., Aasly, J.O., Barcikowska, M., Uitti, R.J., Wszolek, Z.K., Ross, O.A., Petersen, R.C., Graff-Radford, N.R., Dickson, D.W., Younkin, S.G., Ertekin-Taner, N., 2012. Glutathione S-transferase omega genes in Alzheimer and Parkinson disease risk, age-at-diagnosis and brain gene expression: an association study with mech-anistic implications. Mol. Neurodegener. 7, 13.

Baumgartner, R., Poernbacher, I., Buser, N., Hafen, E., Stocker, H., 2010. The WW domain protein Kibra acts upstream of Hippo in Drosophila. Dev. Cell 18, 309e316.

Bertram, L., McQueen, M.B., Mullin, K., Blacker, D., Tanzi, R.E., 2007. Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. Nat. Genet. 39, 17e23.

Bolte, S., Cordelieres, F.P., 2006. A guided tour into subcellular colocalization analysis in light microscopy. J. Microsc. 224 (Pt 3), 213e232.

Braak, H., Braak, E., 1991. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. 82, 239e259.

Burgess, J.D., Pedraza, O., Graff-Radford, N.R., Hirpa, M., Zou, F., Miles, R., Nguyen, T., Li, M., Lucas, J.A., Ivnik, R.J., Crook, J., Pankratz, V.S., Dickson, D.W., Petersen, R.C., Younkin, S.G., Ertekin-Taner, N., 2011. Association of common KIBRA variants with episodic memory and AD risk. Neurobiol. Aging 32, 557.e1e557.e9.

Carter, C.L., Resnick, E.M., Mallampalli, M., Kalbarczyk, A., 2012. Sex and gender differences in Alzheimer's disease: recommendations for future research.J. Womens Health 21, 1018e1023.

Cattaert, T., Calle, M.L., Dudek, S.M., Mahachie John, J.M., Van Lishout, F., Urrea, V., Ritchie, M.D., Van Steen, K., 2011. Model-based multifactor dimensionality reduction for detecting epistasis in case-control data in the presence of noise. Ann. Hum. Genet. 75, 78e89.

Comeau, S.R., Gatchell, D.W., Vajda, S., Camacho, C.J., 2004a. ClusPro: a fully automated algorithm for protein-protein docking. Nucleic Acids Res. 32 (Web Server issue), W96eW99.

Comeau, S.R., Gatchell, D.W., Vajda, S., Camacho, C.J., 2004b. ClusPro: an automated docking and discrimination method for the prediction of protein complexes. Bioinformatics 20, 45e50.

Cdocking and discrimination method for the prediction of protein complexes. Bioinformatics 20, 45e50.

Bandy, D., Lee, W., Chen, K., Beach, T.G., Mastroeni, D., Grover, A., Ravid, R., Sando, S.B., Aasly, J.O., Heun, R., Jessen, F., Kolsch, H., Rogers, J., Hutton, M.L., Melquist, S., Petersen, R.C., Alexander, G.E., Caselli, R.J., Papassotiropoulos, A., Stephan, D.A., Huentelman, M.J., 2010. Evidence for an association between KIBRA and late-onset Alzheimer's disease. Neurobiol. Aging 31, 901e909.

Cuyvers, E., Sleegers, K., 2016. Genetic variations underlying Alzheimer's disease: evidence from genome-wide association studies and beyond. Lancet Neurol. 15, 857e868.

Di Paolo, G., Pellegrini, L., Letinic, K., Cestra, G., Zoncu, R., Voronov, S., Chang, S., Guo, J., Wenk, M.R., De Camilli, P., 2002. Recruitment and regulation of phos-phatidylinositol phosphate kinase type 1 gamma by the FERM domain of talin. Nature 420, 85e89.

Dourlen, P., Fernandez-Gomez, F.J., Dupont, C., Grenier-Boley, B., Bellenguez, C., Obriot, H., Caillierez, R., Sottejeau, Y., Chapuis, J., Bretteville, A., Abdelfettah, F., Delay, C., Malmanche, N., Soininen, H., Hiltunen, M., Galas, M.C., Amouyel, P., Sergeant, N., Buee, L., Lambert, J.C., Dermaut, B., 2016. Functional screening of Alzheimer risk loci identifies PTK2B as an in vivo modulator and early marker of Tau pathology. Mol. Psychiatry 22, 874e883.

Dumanski, J.P., Lambert, J.C., Rasi, C., Giedraitis, V., Davies, H., Grenier-Boley, B., Lindgren, C.M., Campion, D., Dufouil, C., European Alzheimer's Disease Initiative, I., Pasquier, F., Amouyel, P.,

Lannfelt, L., Ingelsson, M., Kilander, L., Lind, L., Forsberg, L.A., 2016. Mosaic loss of chromosome Y in blood is associated with Alzheimer disease. Am. J. Hum. Genet. 98, 1208e1219.

Ebbert, M.T., Ridge, P.G., Kauwe, J.S., 2015. Bridging the gap between statistical and

biological epistasis in Alzheimer's disease. Biomed. Res. Int. 2015, 870123. Fisher, R., 1948. Combining independent tests of significance. Am. Stat. 2, 30. Gatz, M., Reynolds, C.A., Fratiglioni, L., Johansson, B., Mortimer, J.A., Berg, S., Fiske, A.,

Pedersen, N.L., 2006. Role of genes and environments for explaining Alzheimer disease. Arch. Gen. Psychiatry 63, 168e174.

Gauderman, W.J., 2002. Sample size requirements for association studies of gene gene interaction. Am. J. Epidemiol. 155, 478e484.

Gistelinck, M., Lambert, J.C., Callaerts, P., Dermaut, B., Dourlen, P., 2012. Drosophila models of tauopathies: what have we learned? Int. J. Alzheimers Dis. 2012, 970980.

Gusareva, E.S., Carrasquillo, M.M., Bellenguez, C., Cuyvers, E., Colon, S., Graff- Radford, N.R., Petersen, R.C., Dickson, D.W., Mahachie John, J.M., Bessonov, K., Van Broeckhoven, C., Consortium, G., Harold, D., Williams, J., Amouyel, P., Sleegers, K., Ertekin-Taner, N., Lambert, J.C., Van Steen, K., Ramirez, A., 2014. Genome-wide association interaction analysis for Alzheimer's disease. Neurobiol. Aging 35, 2436e2443.

Gusareva, E.S., Van Steen, K., 2014. Practical aspects of genome-wide association interaction analysis. Hum. Genet. 133, 1343e1358.

Harold, D., Abraham, R., Hollingworth, P., Sims, R., Gerrish, A., Hamshere, M.L., Pahwa, J.S., Moskvina, V., Dowzell, K., Williams, A., Jones, N., Thomas, C., Stretton, A., Morgan, A.R., Lovestone, S., Powell, J., Proitsi, P., Lupton, M.K., Brayne, C., Rubinsztein, D.C., Gill, M., Lawlor, B., Lynch, A., Morgan, K., Brown, K.S., Passmore, P.A., Craig, D., McGuinness, B., Todd, S., Holmes, C., Mann, D., Smith, A.D., Love, S., Kehoe, P.G., Hardy, J., Mead, S., Fox, N., Rossor, M., Collinge, J., Maier, W., Jessen, F., Schurmann, B., van den Bussche, H., Heuser, I., Kornhuber, J., Wiltfang, J., Dichgans, M., Frolich, L., Hampel, H., Hull, M., Rujescu, D., Goate, A.M., Kauwe, J.S., Cruchaga, C., Nowotny, P., Morris, J.C., Mayo, K., Sleegers, K., Bettens, K., Engelborghs, S., De Deyn, P.P., Van Broeckhoven, C., Livingston, G., Bass, N.J., Gurling, H., McQuillin, A., Gwilliam, R., Deloukas, P., Al-Chalabi, A., Shaw, C.E., Tsolaki, M., Singleton, A.B., Guerreiro, R., Muhleisen, T.W., Nothen, M.M., Moebus, S., Jockel, K.H., Klopp, N., Wichmann, H.E., Carrasquillo, M.M., Pankratz, V.S., Younkin, S.G., Holmans, P.A., O'Donovan, M., Owen, M.J., Williams, J., 2009. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat. Genet. 41, 1088e1093.

Hofman, A., Darwish Murad, S., van Duijn, C.M., Franco, O.H., Goedegebure, A., Ikram, M.A., Klaver, C.C., Nijsten, T.E., Peeters, R.P., Stricker, B.H., Tiemeier, H.W., Uitterlinden, A.G., Vernooij, M.W., 2013. The Rotterdam Study: 2014 objectives and design update. Eur. J. Epidemiol. 28, 889e926.

Kozakov, D., Brenke, R., Comeau, S.R., Vajda, S., 2006. PIPER: an FFT-based protein docking program with pairwise potentials. Proteins 65, 392e406. Kremerskothen, J., Plaas, C., Buther, K., Finger, I., Veltel, S., Matanis, T., Liedtke, T., Barnekow, A., 2003. Characterization of KIBRA, a novel WW domain-containing protein. Biochem. Biophys. Res. Commun. 300, 862e867.

Lambert, J.C., Heath, S., Even, G., Campion, D., Sleegers, K., Hiltunen, M., Combarros, O., Zelenika, D., Bullido, M.J., Tavernier, B., Letenneur, L., Bettens, K., Berr, C., Pasquier, F., Fievet, N., Barberger-Gateau, P., Engelborghs, S., De Deyn, P., Mateo, I., Franck, A., Helisalmi, S., Porcellini, E., Hanon, O., ,

European Alzheimer's Disease Initiative, I., de Pancorbo, M.M., Lendon, C., Dufouil, C., Jaillard, C., Leveillard, T., Alvarez, V., Bosco, P., Mancuso, M., Panza, F., Nacmias, B., Bossu, P., Piccardi, P., Annoni, G., Seripa, D., Galimberti, D., Hannequin, D., Licastro, F., Soininen, H., Ritchie, K., Blanche, H., Dartigues, J.F., Tzourio, C., Gut, I., Van Broeckhoven, C., Alperovitch, A., Lathrop, M., Amouyel, P., 2009. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat. Genet. 41, 1094e1099.

Lishout, F.V., Gadaleta, F., Moore, J.H., Wehenkel, L., Steen, K.V., 2015. gammaMAXT: a fast multiple-testing correction algorithm. BioData Min. 8, 36.

Medway, C., Combarros, O., Cortina-Borja, M., Butler, H.T., Ibrahim-Verbaas, C.A., de Bruijn, R.F., Koudstaal, P.J., van Duijn, C.M., Ikram, M.A., Mateo, I., Sanchez Juan, P., Lehmann, M.G., Heun, R., Kolsch, H., Deloukas, P., Hammond, N., Coto, E., Alvarez, V., Kehoe, P.G., Barber, R., Wilcock, G.K., Brown, K., Belbin, O., Warden, D.R., Smith, A.D., Morgan, K., Lehmann, D.J., 2014. The sex-specific associations of the aromatase gene with Alzheimer's disease and its interac-tion with IL10 in the Epistasis Project. Eur. J. Hum. Genet. 22, 216e220.

Mielke, M.M., Vemuri, P., Rocca, W.A., 2014. Clinical epidemiology of Alzheimer's disease: assessing sex and gender differences. Clin. Epidemiol. 6, 37e48.

Miko, I., 2008. Epistasis: gene interaction and phenotype effects. Nat. Education 1,197.

Moore, J.H., 2005. A global view of epistasis. Nat. Genet. 37, 13e14.

Morgan, J.R., Di Paolo, G., Werner, H., Shchedrina, V.A., Pypaert, M., Pieribone, V.A., De Camilli, P., 2004. A role for talin in presynaptic function. J. Cell Biol. 167, 43e50. Musicco, M., 2009. Gender differences in the occurrence of Alzheimer's disease.Funct. Neurol. 24, 89e92.

Naj, A.C., Jun, G., Beecham, G.W., Wang, L.S., Vardarajan, B.N., Buros, J., Gallins, P.J., Buxbaum, J.D., Jarvik, G.P., Crane, P.K., Larson, E.B., Bird, T.D., Boeve, B.F., Graff-Radford, N.R., De Jager, P.L., Evans, D., Schneider, J.A., Carrasquillo, M.M., Ertekin-Taner, N., Younkin, S.G., Cruchaga, C., Kauwe, J.S., Nowotny, P., Kramer, P., Hardy, J., Huentelman, M.J., Myers, A.J., Barmada, M.M., Demirci, F.Y., Baldwin, C.T., Green, R.C., Rogaeva, E., St George-Hyslop, P., Arnold, S.E., Barber, R., Beach, T., Bigio, E.H., Bowen, J.D., Boxer, A., Burke, J.R., Cairns, N.J., Carlson, C.S., Carney, R.M., Carroll, S.L., Chui, H.C., Clark, D.G., Corneveaux, J., Cotman, C.W., Cummings, J.L., DeCarli, C., DeKosky, S.T., Diaz-Arrastia, R., Dick, M., Dickson, D.W., Ellis, W.G., Faber, K.M., Fallon, K.B., Farlow, M.R., Ferris, S., Frosch, M.P., Galasko, D.R., Ganguli, M., Gearing, M., Geschwind, D.H., Ghetti, B., Gilbert, J.R., Gilman, S., Giordani, B., Glass, J.D., Growdon, J.H., Hamilton, R.L., Harrell, L.E., Head, E., Honig, L.S., Hulette, C.M., Hyman, B.T., Jicha, G.A., Jin, L.W., Johnson, N., Karlawish, J., Karydas, A., Kaye, J.A., Kim, R., Koo, E.H., Kowall, N.W., Lah, J.J., Levey, A.I., Lieberman, A.P., Lopez, O.L., Mack, W.J., Marson, D.C., Martiniuk, F., Mash, D.C., Masliah, E., McCormick, W.C., McCurry, S.M., McDavid, A.N., McKee, A.C., Mesulam, M., Miller, B.L., Miller, C.A., Miller, J.W., Parisi, J.E., Perl, D.P., Peskind, E., Petersen, R.C., Poon, W.W., Quinn, J.F., Rajbhandary, R.A., Raskind, M., Reisberg, B., Ringman, J.M., Roberson, E.D., Rosenberg, R.N., Sano, M., Schneider, L.S., Seeley, W., Shelanski, M.L., Slifer, M.A., Smith, C.D., Sonnen, J.A., Spina, S., Stern, R.A., Tanzi, R.E., Trojanowski, J.Q., Troncoso, J.C., Van Deerlin, V.M., Vinters, H.V., Vonsattel, J.P., Weintraub, S., Welsh-Bohmer, K.A., Williamson, J., Woltjer, R.L., Cantwell, L.B., Dombroski, B.A., Beekly, D., Lunetta, K.L., Martin, E.R., Kamboh, M.I., Saykin, A.J., Reiman, E.M., Bennett, D.A., Morris, J.C., Montine, T.J., Goate, A.M., Blacker, D., Tsuang, D.W., Hakonarson, H., Kukull, W.A., Foroud, T.M., Haines, J.L., Mayeux, R., Pericak-Vance, M.A., Farrer, L.A., Schellenberg, G.D., 2011. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat. Genet. 43, 436e441.

Nyholt, D.R., 2004. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am. J. Hum. Genet. 74, 765e769.

Papassotiropoulos, A., Stephan, D.A., Huentelman, M.J., Hoerndli, F.J., Craig, D.W., Pearson, J.V., Huynh, K.D., Brunner, F., Corneveaux, J., Osborne, D., Wollmer, M.A., Aerni, A., Coluccia, D., Hanggi, J., Mondadori, C.R., Buchmann, A., Reiman, E.M., Caselli, R.J., Henke, K., de Quervain, D.J., 2006. Common Kibra alleles are associated with human memory performance. Science 314, 475e478.

Praekelt, U., Kopp, P.M., Rehm, K., Linder, S., Bate, N., Patel, B., Debrand, E., Manso, A.M., Ross, R.S., Conti, F., Zhang, M.Z., Harris, R.C., Zent, R., Critchley, D.R., Monkley, S.J., 2012. New isoform-specific monoclonal antibodies reveal different sub-cellular localisations for talin1 and talin2. Eur. J. Cell Biol. 91, 180e191.

Ridge, P.G., Mukherjee, S., Crane, P.K., Kauwe, J.S., Alzheimer's Disease Genetics, C.,2013. Alzheimer's disease: analyzing the missing heritability. PLoS One 8, e79771.

Rodriguez-Rodriguez, E., Infante, J., Llorca, J., Mateo, I., Sanchez-Quintana, C., Garcia Gorostiaga, I., Sanchez-Juan, P., Berciano, J., Combarros, O., 2009. Age-dependent association of KIBRA genetic variation and Alzheimer's disease risk. Neurobiol. Aging 30, 322e324.

Shulman, J.M., Imboywa, S., Giagtzoglou, N., Powers, M.P., Hu, Y., Devenport, D., Chipendo, P., Chibnik, L.B., Diamond, A., Perrimon, N., Brown, N.H., De Jager, P.L., Feany, M.B., 2014. Functional screening in Drosophila identifies Alzheimer's disease susceptibility genes and implicates Tau-mediated mechanisms. Hum. Mol. Genet. 23, 870e877.

Sims, R., Williams, J., 2016. Defining the genetic Architecture of Alzheimer's disease: where next. Neurodegener Dis. 16, 6e11.

Stouffer, S., DeVinney, L., Suchmen, E., 1949. The American Soldier: Adjustment during Army Life. Princeton University Press, Princeton, NY.

Templeton, A.R., 2000. Epistasis and Complex Traits. Oxford University Press, New York.Van Lishout, F., Mahachie John, J.M., Gusareva, E.S., Urrea, V., Cleynen, I., Theatre, E.,Charloteaux, B., Calle, M.L., Wehenkel, L., Van Steen, K., 2013. An efficient algorithm to perform multiple testing in epistasis screening. BMC Bioinformatics 14, 138.

Vogt-Eisele, A., Kruger, C., Duning, K., Weber, D., Spoelgen, R., Pitzer, C., Plaas, C., Eisenhardt, G., Meyer, A., Vogt, G., Krieger, M., Handwerker, E., Wennmann, D.O., Weide, T., Skryabin, B.V., Klugmann, M., Pavenstadt, H., Huentelmann, M.J., Kremerskothen, J., Schneider, A., 2014. KIBRA (KIdney/BRAin protein) regulates learning and memory and stabilizes Protein kinase Mzeta. J. Neurochem. 128, 686e700.

Wan, X., Yang, C., Yang, Q., Xue, H., Fan, X., Tang, N.L., Yu, W., 2010. BOOST: a fast approach to detecting gene-gene interactions in genome-wide case-control studies. Am. J. Hum. Genet. 87, 325e340.

Zou, F., Chai, H.S., Younkin, C.S., Allen, M., Crook, J., Pankratz, V.S., Carrasquillo, M.M., Rowley, C.N., Nair, A.A., Middha, S., Maharjan, S., Nguyen, T., Ma, L., Malphrus, K.G., Palusak, R., Lincoln, S., Bisceglio, G., Georgescu, C., Kouri, N., Kolbert, C.P., Jen, J., Haines, J.L., Mayeux, R., Pericak-Vance, M.A., Farrer, L.A., Schellenberg, G.D., Alzheimer's Disease Genetics, C., Petersen, R.C., Graff-Radford, N.R., Dickson, D.W., Younkin, S.G., Ertekin-Taner, N., 2012. Brain expression genome-wide association study (eGWAS) identifies human disease-associated variants. PLoS Genet. 8, e1002707.