HMGA1 deficiency: a pathogenic link between tau pathology and insulin resistance

Maria Mirabelli,[a] Eusebio Chiefari,[a] Biagio Arcidiacono,[a] Alessandro Salatino,[a] Angelo Pascarella,[b] Maurizio Morelli,[b] Sara C. Credendino,[c] Francesco S. Brunetti,[a] Anna Di Vito,[d] Adelaide Greco,[e] Vincent Huin,[f] Ferdinando Nicoletti,[g,i] Giovanna M. Pierantoni,[c] Monica Fedele,[h] Umberto Aguglia,[b] Daniela P. Foti,[d,**] and Antonio Brunetti[a,*]

[a]Department of Health Sciences, University of Catanzaro "Magna Græcia", Catanzaro, Italy
[b]Department of Medical and Surgical Sciences, University of Catanzaro "Magna Græcia", Catanzaro, Italy
[c]Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II", Naples, Italy
[d]Department of Experimental and Clinical Medicine, University of Catanzaro "Magna Græcia", Catanzaro, Italy
[e]Interdepartmental Centre of Veterinary Radiology, University of Naples "Federico II", Naples, Italy
[f]University of Lille, Inserm, CHU-Lille, Lille Neuroscience & Cognition, UMR-S1172, Team Alzheimer & Tauopathies, F-59000, Lille, France
[g]Department of Physiology and Pharmacology, University of Rome "Sapienza", Rome, Italy
[h]Institute of Experimental Endocrinology and Oncology, CNR, Naples, Italy
[i]IRCCS Neuromed, Pozzilli, Italy

eBioMedicine
2025;115: 105700
Published Online xxx
https://doi.org/10.1016/j.ebiom.2025.105700

Summary

Background Growing evidence links tau-related neurodegeneration with insulin resistance and type 2 diabetes (T2D), though the underlying mechanisms remain unclear. Our previous research identified HMGA1 as crucial for insulin receptor (INSR) expression, with defects in the *HMGA1* gene associated with insulin resistance and T2D. Here, we explore HMGA1 deficiency as a potential contributor to tauopathies, such as Alzheimer's disease (AD), and its connection to insulin resistance.

Methods Immunoblot analyses, protein-DNA interaction studies, ChIP-qPCR, and reporter gene assays were conducted in human and mouse neuronal cell models. Tau immunohistochemistry, behavioural studies, and brain glucose metabolism were analysed in *Hmga1*-knockout mice. Additionally, a case–control study investigated the relationship between HMGA1 and tau pathology in patients with tauopathy, carrying or not the *HMGA1* rs146052672 variant, known to reduce HMGA1 protein levels and increase the risk of insulin resistance and T2D.

Findings We show that HMGA1 regulates tau protein expression primarily through the specific repression of *MAPT* gene transcription. In both human neuronal cells and primary mouse neurons, tau mRNA and protein levels were inversely correlated with HMGA1 expression. This inverse relationship was further confirmed in the brain of *Hmga1*-knockout mice, where tau was overexpressed, INSR was downregulated, and brain glucose uptake was impaired. Additionally, the rs146052672 variant was more common in patients with tauopathy (12/69, 17.4%) than in controls (10/200, 5.0%) ($p = 0.001$), and carriers of this variant exhibited more severe disease progression and poorer therapeutic outcomes.

Interpretation These findings suggest that HMGA1 deficiency may drive tau pathology, linking tauopathies to insulin resistance and providing new insights into the relationship between metabolic and neurodegenerative disorders. Furthermore, our observation that over 17% of individuals with tauopathy exhibit a deficit in *HMGA1* protein production could have significant clinical implications, potentially guiding the development of therapeutic strategies targeting this specific defect.

Funding See acknowledgements section.

Keywords: Neurodegeneration; Insulin resistance; Glucose metabolism; Gene transcription; HMGA1

*Corresponding author. University of Catanzaro "Magna Græcia", Viale Europa, 88100, Catanzaro, Italy.
**Corresponding author. University of Catanzaro "Magna Græcia", 88100, Catanzaro, Italy.
E-mail addresses: brunetti@unicz.it (A. Brunetti), foti@unicz.it (D.P. Foti).

Research in context

Evidence before this study

Tauopathies, including Alzheimer's Disease (AD) and other forms of dementia, are a group of neurodegenerative disorders characterised by the abnormal accumulation of tau protein in brain cells, which leads to neuronal damage. These disorders are sometimes linked to mutations in the *MAPT* gene, which encodes the tau protein. However, how these mutations contribute to the development of tauopathies is not well understood. The HMGA1 nuclear protein has significant transacting effects on the insulin receptor gene (*INSR*). Variant of the *HMGA1* gene, particularly the *HMGA1* rs146052672 variant, have been associated with an increased risk of metabolic conditions, such as insulin resistance and type 2 diabetes, both of which are also linked to neurodegeneration. However, the potential role of HMGA1 in regulating the *MAPT* gene and its contribution to tauopathy risk has not been previously explored.

Added value of this study

In this study, we provide evidence that HMGA1 plays a role in regulating tau protein levels in neuronal cells. Using cultured neurons, we observed an inverse correlation between HMGA1 expression and tau levels, suggesting that HMGA1 may act to suppress tau production. We further confirmed these findings in a mouse model, where mice lacking the *Hmga1* gene showed increased tau accumulation and exhibited abnormal behavioural patterns compared to their wild-type counterparts. Our research also demonstrated that HMGA1 directly interacts with the *MAPT* gene, helping to regulate tau production through its suppressive effects. Genetic analysis revealed that the *HMGA1* rs146052672 variant occurs more frequently in patients with tauopathies compared to controls, and is associated with more severe disease outcomes, including greater brain damage and reduced treatment efficacy.

Implications of all the available evidence

Our findings suggest that tauopathies may not only arise from direct issues with tau protein accumulation, but could also result from disruptions in the regulatory function of HMGA1. When HMGA1 is absent or dysfunctional, tau accumulates excessively, increasing the vulnerability of brain cells and amplifying the risk of neurodegenerative diseases, such as AD. This new insight into HMGA1's regulatory role could open new avenues for therapeutic strategies targeting tauopathies.

Introduction

Tauopathies are an heterogenous group of degenerative disorders of the central nervous system (CNS), which are histopathologically characterised by the deposit of abnormal tau protein inclusions in neurons and glia.[1] Clinically, they are defined by signs and symptoms of dementia and/or parkinsonism. Alzheimer's disease (AD) is the most common dementing disorder with an underlying dysregulation of tau, whereas some other forms of dementia, such as frontotemporal dementia (FTD) and progressive supranuclear palsy (PSP) are also included among tauopathies, although less common than AD.[2]

The tau protein is predominantly located in the cytosol of neurons and glial cells, where it is required for the assembly of tubulin into microtubules and the maintenance of the overall axonal structural integrity, in addition to other diverse cellular functions, which involve, among others, signal transduction and cellular proliferation, neuroplasticity and synaptic function.[3] Under pathological conditions, tau becomes excessively phosphorylated. This reduces its ability to bind to microtubules, causing the formation of abnormal filaments known as neurofibrillary tangles, which are primarily composed of self-aggregated hyperphosphorylated tau.[4,5]

In the late 1990s, mutations in the microtubule-associated protein tau (*MAPT*) gene, which determined tau mRNA abnormal splicing and tau protein aggregates, were reported to cause rare hereditary familial forms of FTD.[6,7] Later studies evidenced that a number of *MAPT* gene mutations altering tau mRNA splicing, or affecting tau post-translational modifications, could also lead to tau dysfunction and microtubule instability, thereby contributing to the pathogenesis of more frequent, age-related forms of tauopathies, including AD.[8–11] The importance of *MAPT* gene diversity was supported by genome-wide association studies, confirming an association between susceptibility to tauopathies and variants at the *MAPT* locus.[11,12] In this regard, changes related to genetic or epigenomic variants in *MAPT cis*-acting regulatory elements, which could affect the transcription and expression of the *MAPT* gene, leading to alterations in tau expression levels, have been associated with major susceptibility to neurodegeneration in humans.[13,14] For example, it has previously been reported that polymorphisms in the human *MAPT* gene promoter, which affect binding of the transcription factors, Sp1 and AP2, can alter the transcriptional activity of *MAPT* gene,[15,16] thereby further supporting the notion that abnormalities in *MAPT* gene regulation might be involved in the pathogenesis of tauopathies.

The high-mobility group A1 (HMGA1) protein is an architectural non-histone chromatin factor that regulates gene transcription in vivo by controlling the assembly of stereospecific multiprotein complexes on the AT-rich sequences of certain DNA gene promoters.[17] By orchestrating the assembly and stability of stereospecific transcriptional regulatory factors into an enhanceosome

complex, HMGA1 can activate or repress a variety of mammalian genes.[17] We previously showed that HMGA1 is a key regulator of insulin receptor (*INSR*) gene expression and an important contributing factor in the transcriptional regulation of genes implicated in the maintenance of glucose homoeostasis and metabolic control.[18–21] Defects in *HMGA1* gene and protein expression have been related to systemic insulin resistance and increased susceptibility to T2D mellitus,[22–27] two conditions that have been widely associated with ageing-related neurodegenerative diseases, such as AD.[28,29] Prior investigations in this area of research have suggested that HMGA1 could be involved in the pathophysiology of degenerative disorders of the CNS. In particular, it has been shown that HMGA1 is responsible for the aberrant splicing of the pre-mRNA of presenilin 2, *a* protein which can trigger neuronal cell death in patients with inherited familial forms of AD and other dementia-associated disorders, even in the absence of mutations of the *presenilin 2* gene.[30,31] Furthermore, previous studies have reported that systemic insulin resistance and T2D were linked to increased abundance of hyperphosphorylated tau in the CNS of animals showing a series of psychomotor abnormalities, suggesting these dysmetabolic conditions as risk factors for neurodegenerative diseases,[32] thus leading researchers *to* hypothesize that abnormalities in insulin signalling and glucose metabolism and tauopathies may share common underlying pathogenic mechanisms, including molecular signatures and genetic risk factors.[33,34]

Based on these considerations, our present study aimed to investigate the role of HMGA1 in regulating tau expression and its implication in neurodegeneration. Interest in this nuclear factor was further stimulated by the observation that putative binding sites for HMGA1 were highly represented in the promoter region of the *MAPT* gene. Through a combination of experiments in human and mouse neuronal cell models, along with data from *Hmga1*-knockout mice and patients with tauopathy, we provide compelling evidence that HMGA1 is a key regulator of the *MAPT* gene, inhibiting its transcription and preventing tau expression. Consequently, HMGA1 deficiency, alongside its adverse effects on INSR signalling, glucose homoeostasis, and metabolic control, serves as an important causative factor in the aetiology of tauopathies. Furthermore, our findings offer a mechanistic explanation for the frequent co-occurrence of these metabolic and neurodegenerative disorders.

Methods

Cell culture conditions and neuronal differentiation, protein extracts and western blot

SH-SY5Y human neuroblastoma cells (American Type Culture Collection; RRID:CVCL_0019) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplied with 10% foetal bovine serum (FBS) (Corning), 100 U/mL penicillin and 100 μg/mL streptomycin and maintained at 37 °C, 5% CO_2 in a humidified atmosphere. Differentiation of SH-SY5Y cells was performed by lowering the FBS in culture medium to 1% and adding 10 μM retinoic acid (RA) every 3 days for 10 days. To validate the differentiation process, cell morphology of SH-SY5Y cells exposed to RA was examined by optical microscopy, and immunostaining was performed to confirm the expression of tau protein. Briefly, 1×10^5 cells were grown on a high-optical quality coverslip, serum starved for 24 h to synchronize the cell cycle, and treated with RA-enriched culture medium to induce neuronal differentiation. At the appropriate time points, cells were fixed in 10% formalin, permeabilized with 0.1% Triton X-100 (Sigma–Aldrich) and incubated overnight at 4 °C with the primary anti-tau antibody (AHB0042; Invitrogen Antibodies; RRID:AB_1502093). SH-SY5Y cells were then incubated with the secondary anti-mouse FITC-conjugated antibody (A16067; Thermo Fisher Scientific; RRID:AB_2534740) for 1 h at room temperature, and nuclei were counterstained with the DNA binding dye, 4,6-diamidino-2-phenylindole (DAPI, Sigma–Aldrich). Coverslips were mounted on glass microscope slides using the ProLong Gold mounting solution (Thermo Fisher Scientific), and images were acquired through the Leica DM6 FS microscopy system (Leica Microsystems GmbH). Mycoplasma contamination in cell cultures was excluded by DAPI staining.

Nuclear and total cellular protein extracts were prepared as previously described,[35] and final protein concentration in the extracts was determined by the Bradford protein assay kit (Bio-Rad Laboratories). Cellular protein (20 μg) was resolved on 10% or 12.5% SDS-PAGE, transferred to a nitrocellulose membrane (0.2 μm pore-size, Bio-Rad Laboratories), blotted for 1 h with blocking solution (5% non-fat dry milk), and incubated *overnight* at 4 °C with a primary antibody against either HMGA1 (sc-393213; Santa Cruz Biotechnology; RRID:AB_367174), tau (AHB0042; Invitrogen Antibodies; RRID:AB_1502093), p-tau Ser 400/Thr403/Ser404 (11837; Cell Signaling Technology; RRID:AB_3676175), p-tau Thr205 (49561; Cell Signaling Technology; RRID:AB_2799361), InsR (sc-711; Santa Cruz Biotechnology; RRID:AB_631835), insulin-like growth factor 1 receptor (Igf1R) (sc-713; Santa Cruz Biotechnology; RRID:AB_671792), glucose transporter, Glut3 (MA5-32697; Invitrogen Antibodies; RRID:AB_2809974), amyloid β precursor protein, APP (76600; Cell Signaling Technology; RRID:AB_2925222), glial fibrillary acidic protein, GFAP (G9269; Sigma–Aldrich; RRID:AB_477035), or actin (4967; Cell Signaling Technology; RRID:AB_330288). Immune complexes were visualized by enhanced chemiluminescence after incubation of the blots with HRP-conjugated secondary anti-mouse antibody (1706516;

Bio-Rad Laboratories; RRID:AB_2921252) or anti-rabbit antibody (1706515, Bio-Rad Laboratories; RRID:AB_11125142).

Secretome analysis

Equal numbers of undifferentiated and differentiated SH-SY5Y cells (1×10^6) were resuspended in 600 µL of PBS and incubated for 30 min at room temperature. After incubation, the cell suspensions were centrifuged, and the supernatant containing the secreted protein was collected. The protein fractions were concentrated by deoxycholate-trichloroacetic acid precipitation. The precipitated proteins were resuspended in SDS loading buffer and resolved by SDS-PAGE. Equal volumes of conditioned media from 24-h cultures of undifferentiated and differentiated SH-SY5Y cells were centrifuged at 1000*g* for 5 min to remove cell debris and then resolved on 11% SDS-PAGE. Western blot analysis was performed using a primary antibody against HMGA1 (sc-393213; Santa Cruz Biotechnology; RRID:AB_367174). The absolute amount of tau protein in the conditioned media was quantified by chemiluminescence using the CLEIA analyser Lumipulse G600 II (Fujirebio).

Assessment of oxidative stress in neuronal cultures

The generation of reactive oxygen species (ROS) was assessed in both undifferentiated and differentiated SH-SY5Y cells to compare oxidative stress levels between these conditions. Mitochondrial ROS levels were first visually evaluated using the MitoSOX Red Superoxide Indicator (Thermo Fisher Scientific), which selectively detects mitochondrial superoxide. Cells were cultured in low-serum DMEM (1% FBS) at a density of 1×10^5 cells per well for 24 h. After washing with PBS to remove phenol red, the cells were incubated with 5 µM MitoSOX Red reagent for 30 min at 37 °C. Live-cell imaging was conducted using a Leica DM6 FS microscope (Leica Microsystems GmbH) with a 590 nm emission filter. To confirm increased ROS production in differentiated SH-SY5Y neurons, characterised by elevated tau protein expression, hydrogen peroxide (H_2O_2) levels were quantified using the ROS-Glo™ H_2O_2 Assay (Promega). Cells were similarly cultured in low-serum DMEM for 24 h, then treated with 25 µM H_2O_2 substrate solution and incubated for 4 h at 37 °C. Following incubation, 50 µL of the culture medium was transferred to a white 96-well plate, mixed with an equal volume of ROS-Glo detection solution, and incubated for 20 min at room temperature. Luminescence was measured using the GloMax® Discover Microplate reader (Promega). To ensure accurate normalization of ROS levels, luminescence values were adjusted based on cell viability, which was determined by measuring adenosine triphosphate (ATP) content using the CellTiter-Glo® luminescent cell viability assay (Promega).

Transcription factor affinity prediction (TRAP), plasmid construction and transfections

Consensus transcription factor-binding sites within the *MAPT* promoter region were explored by computational analysis using the TRAP method, which calculates the affinity of transcription factors for DNA sequences based on a biophysical model.[36] Putative HMGA1 DNA binding sites were identified within the 5' regulatory region of both human and mouse *MAPT* genes, and initially characterised by electrophoretic mobility shift assay (EMSA), using sequence-specific biotin-labelled DNA fragments. Mouse genomic DNA was used to amplify the mouse *Mapt* promoter region extending from –2591 to –49 upstream of the ATG codon, using specific modified primers (Table S1). The PCR products were purified, sequenced, and cloned into the pGL3 basic vector (Promega), upstream of the firefly luciferase (Luc) reporter gene. The pGL3 Luc plasmid containing the human *MAPT* promoter region (–1879/+121), cloned upstream of the firefly Luc gene was previously described.[37] These mouse and human plasmids are denoted *mMapt*-Luc and *hMAPT*-Luc, respectively. A second human *MAPT*-Luc reporter construct containing the transcriptionally active repressor C region was kindly provided by Dr. R. de Silva (University College London). These plasmid constructs were transiently transfected into SH-SY5Y cells, using Lipofectamine 3000 (Invitrogen Life Technology Corporation), in the presence or absence of an effector vector for the HMGA1a isoform protein. Luc activity was assayed 48 h post-transfection, using the dual-luciferase reporter assay system (Promega). siRNA oligonucleotides targeting HMGA1 (sc-37115; Santa Cruz Biotechnology) and nonspecific siRNA controls with a similar GC content (Dharmacon) (100–200 pmol siRNA duplex) were transfected into cells at 40–50% confluency, and cells were analysed 48 h later. Renilla control vector was used as a control for transfection efficiency, together with measurements of protein expression levels.

Bioinformatics analysis using single-cell (single-nuclei) transcriptomic data from cortical middle temporal gyrus (MTG) samples collected from post-mortem AD donors in the Seattle Alzheimer's Disease Brain Cell Atlas (SEA-AD) consortium [https://portal.brain-map.org/explore/seattle-alzheimers-disease][38] was performed to explore the relationship between *MAPT* and *HMGA1* genes, and *MAPT* gene expression changes across AD progression. Details are provided in Figs. S1 and S2.

EMSA

A promoter portion of the mouse *Mapt* gene (between –1739 and –1531 from the ATG codon) containing the identified putative binding sites for HMGA1 was generated by PCR using specific oligonucleotide primers (Table S1), and the double-stranded DNA probe was biotin-labelled at the 3′ position using the Biotin 3' End DNA Labelling kit (Thermo Fisher Scientific).

Biotin-labelled DNA probe was incubated with nuclear extracts from undifferentiated and differentiated SH-SY5Y cells and DNA-protein binding was performed. Briefly, the DNA probe (5 nM) was incubated for 1 h with protein extract (1 μg) in 1x binding buffer at room temperature, as previously described.[19] The reaction mixture was then separated on 6% SDS-PAGE, transferred to Hybond N+ membranes, and signal of DNA-protein binding was detected using the LightShift chemiluminescent kit (Thermo Fisher Scientific). Antibody against HMGA1 (sc-393213; Santa Cruz Biotechnology; RRID:AB_367174) was used in supershift experiments. 1 μg Poly(dI-dC) was used to compete for nonspecific binding.

Chromatin immunoprecipitation (ChIP)

ChIP assay was done as described previously,[23] using undifferentiated SH-SY5Y cells treated or untreated with distamycin A (Sigma–Aldrich), and fully differentiated SH-SY5Y cells. Formaldehyde-fixed DNA-protein complex was immunoprecipitated with anti-HMGA1 specific antibody or IgG control. Primers for the human *MAPT* promoter sequence (between −638 and −287 from the ATG codon) (Table S1) were used for PCR amplification of immunoprecipitated DNA (35 cycles), using the ready-to-use MyTaq Red Mix (Meridian Bioscience). The PCR products were electrophoretically resolved on 1.5% agarose gel and visualized by ethidium bromide staining.

RNA isolation and reverse transcription

Total RNA was extracted from cultured cells and mouse brain tissue, using Trizol reagent (Invitrogen Life Technology Corporation), according to the manufacturer's instructions. RNA concentration was measured by a NanoDrop spectrophotometer (Thermo Fisher Scientific), and its quality confirmed on agarose gel. The High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) was used to synthesize cDNA from 1 μg of RNA sample. A reaction mixture contained 10x RT Buffer, 100 mM dNTP mix, 10x RT Random Primers, 0.50 U/μL Multiscribe Reverse Transcriptase. The cDNA thermal profile was 25 °C for 10 min, 37 °C for 120 min and the enzyme was inactivated at 85 °C for 5 min.

Quantitative real-time PCR (qRT-PCR)

Relative quantification was performed to measure HMGA1, InsR, and tau expression, using a real-time thermocycler (Eppendorf Mastercycler ep realplex ES). 1 μL of cDNA and 0.2 μmol/L each of sense and antisense primers were mixed with SYBR Green PCR MasterMix (Applied Biosystems). qRT-PCR reactions were done in triplicates, using the ribosomal protein S9 (RPS9) cDNA as an internal standard. Amplification conditions were: 2 min at 95 °C and three step-cycle of 95 °C for 15 s, 58 °C for 20 s and 68 °C for 20 s, for a total of 40 cycles. Primers for tau and InsR (Table S1) were designed according to sequences from the GenBank database.

Cultures of primary cortical mouse embryonic neurons and transfection studies

Cultures of primary cortical neurons were obtained from CD1 mice at embryonic day 14–15, as described previously.[39] Briefly, abdominal skin was washed with 70% ethanol, subsequently incised and the peritoneal cavity entered. Then, embryos were taken out from uterus and transferred to a 50 mL polypropylene falcon tube containing 30 mL dissecting medium on ice. The heads were decapitated by scissors and immediately transferred to ice-cold dissecting medium. Cerebral cortices were dissected and centrifuged at 1000*g* for 7 min. After centrifugation, cortices were dissected in Ca^{2+}/Mg^{2+} free buffer and mechanically dissociated. Cortical cells were plated at a density of 3×10^5 cells/well in a 24-well plate precoated with 0.1 mg/mL poly-D-lysine (Sigma–Aldrich) in Neurobasal B27 medium supplemented with 5% heat-inactivated horse serum, insulin (10 μg/mL), transferrin (100 μg/mL), putrescine (100 μM), progesterone (20 nM), selenium (30 nM), glutamine (2 mM), glucose (6 mg/mL), and penicillin/streptomycin mixture. To avoid proliferation of non-neuronal elements, 10 μM of Cytosine-D-arabinofuranoside was added to the culture 18 h after cell seeding and kept for 3 days before medium replacement. This method yields primary neuronal cultures of high purity (>99%), as judged by immunocytochemistry for GFAP and neuron-specific microtubule-associated protein 2. Cultures were grown at 37 °C in a humidified CO_2 atmosphere and experiments were performed using 8–12 days in vitro matured cells. After cell transfection, total cellular RNA was extracted, cDNA was prepared and employed in qRT-PCR.

Studies in animals, immunohistochemical staining (IHC), positron emission tomography with 18 F-labelled 2-fluoro-2-deoxy-D-glucose (FDG-PET) and behavioural testing

Male groups of *Hmga1*-deficient and wild-type mice (129/SvJ strain) aged 9–10 months were investigated. The generation and the physiological characteristics of these mice have been described previously.[23] Based on previous studies of insulin resistance and type 2 diabetes,[23] twelve mice from each genotype group were randomly selected and balanced by size and body weight. Sample size calculations were supported by the resource equation method.[40] All animal work was performed according to institutional and ARRIVE guidelines for animal care. After the mice were sacrificed, the brain was quickly removed and used for both RNA and protein extraction and IHC analysis. For IHC, whole brains were fixed in 4% paraformaldehyde, and embedded into paraffin, as described elsewhere.[41] 9-μm

sections were obtained, deparaffinized and rehydrated. Endogenous peroxidases were saturated with methanol and 1.5% oxygen peroxide, and tissues blocked in blocking solution [5% normal goat serum (Vector Laboratories); 3% BSA; 20 mM $MgCl_2$; 0.3% Tween 20 in PBS] 1 h at room temperature. Primary antibodies against tau (mouse monoclonal anti-tau antibody, AHB0042; Invitrogen Antibodies; RRID:AB_1502093), Hmga1 (mouse monoclonal anti-HMGA1 antibody, sc-393213; Santa Cruz Biotechnology; RRID:AB_367174), and Glut3 (recombinant rabbit monoclonal anti-GLUT3 antibody, MA5-32697; Invitrogen Antibodies; RRID:AB_2809974), were used for section staining overnight at 4 °C. Next, biotinylated anti-mouse (BP-9200; Vector Laboratories; RRID:AB_2827937) and anti-rabbit (BA-1000; Vector Laboratories; RRID:AB_2313606) IgG secondary antibodies were added for further staining, and samples were then analysed by the high-performance Leica DMD 108 Digital Microscope (Leica Microsystems GmbH) at different magnifications. For tau signal analysis, the EVOS FL Auto Imaging System (Life Technologies Corp.) was used. FDG-PET was performed on the YAP(S)PET small animal scanner (ISE Srl). FDG-PET studies were carried out in fasting anaesthetised *Hmga1*-deficient and wild-type mice, after administration of 12 MBq radiolabelled FDG into the tail vein, followed by intraperitoneal administration of rhIGF1 at a dose of 1 ng/gr body weight (Ipsen Pharma). For spontaneous behaviour analysis, the animals underwent an initial habituation phase before being individually subjected to novel cage test sessions. These sessions occurred three times a day at 3-h intervals over five consecutive days. The test sessions took place from 9:00 a.m. to 6:00 p.m. in a sound-isolated room, and were recorded using a vertically mounted camera. Analysis was conducted by observing video recordings, during which the cumulative duration of exploratory behaviours and instances of freezing were timed using a stopwatch. Both before and after each test session, the cages and instruments were cleaned with 70% ethanol to maintain hygiene and prevent contamination. All experiments involving animals were approved by the Italian Ministry of Health.

Study population

We enrolled 69 consecutive, unrelated patients of both sexes (male or female sex was self-reported by participants or confirmed during clinical visits and refers to biological characteristics) from the Neurology Unit at "Renato Dulbecco" University Hospital of Catanzaro, Italy, between 2018 and 2019 (prior to the COVID-19 pandemic). The patients had a clinical diagnosis of tauopathy: 34 with AD, 28 with FTD, and 7 with PSP presenting with Richardson syndrome (PSP-RS). Diagnoses were made by experienced neurologists specialising in neurodegenerative and motor neuron diseases. AD diagnosis followed the original NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) criteria[42] and its updates. PSP-RS diagnosis was based on the Movement Disorder Society Criteria for Progressive Supranuclear Palsy,[43] with inclusion restricted to patients meeting at least the probable diagnostic certainty level. FTD diagnosis adhered to the consensus criteria of Neary et al.[44] and subsequent revisions.[45] Only FTD patients with confirmed tau pathology via in vivo tau-PET imaging or those with the FTD-PSP phenotype, strongly associated with tau-related neurodegeneration, were included. FTD patients were grouped with those presenting with PSP-RS, supported by post-mortem findings indicating shared tau pathology, though with differences in regional distribution and severity of tau aggregates.[46] A control group of 200 subjects was identified during the same period from the outpatient clinics of the Endocrinology Unit at the same hospital. Control criteria included age ≥50 years and the absence of neurodegenerative diseases in both the subjects and their first-degree relatives. Clinical and biochemical parameters were assessed for both cases and controls. All subjects were recruited from Calabria, a region of Southern Italy known for its limited genetic diversity, which makes it an ideal model for studying neurodegenerative diseases.[47] The study was reviewed and approved by the Local Ethic Committee (protocol code n. 116, 14 May 2015) and was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to enrolment.

Genotyping of the *HMGA1* rs146052672 variant

Genomic DNA from peripheral blood cells of patients and controls was genotyped for the *HMGA1* rs146052672 variant using the fluorescence-based TaqMan allelic discrimination technique (Applied Biosystems), as reported previously.[24] The presence of the variant was confirmed by sequencing analysis of the exon 6 and adjacent introns of the *HMGA1* gene (Genebank n. NC_000006.11, http://www.ncbi,nlm.nih.gov), using a 3100 DNA Genetic Analyser (Applied Biosystems).

Tau and HMGA1 determination in cerebrospinal fluid (CSF)

A subset of 18 AD patients (6 carrying the *HMGA1* rs146052672 variant and 12 with wild-type alleles) from the original case cohort underwent further analyses, including CSF assessments of tau and HMGA1 protein levels. To minimize potential confounding factors, each AD patient carrying the *HMGA1* gene variant was matched individually with two non-carrier AD patients of the same sex and similar age (within a 6-years tolerance). The CSF was obtained by lumbar puncture at the time of diagnosis, samples were centrifuged at 8000*g* for 10 min at 4 °C, the supernatants aliquoted into

polypropylene tubes and stored at –80 °C until analysis. Total tau and p-tau (181p) were determined using the CLEIA analyser Lumipulse G600 II (Fujirebio). Cut-off values for normal protein concentrations were as follows: total tau 132–599 pg/mL; p-tau 35.84–66.26 pg/mL. For the determination of HMGA1 in CSF, equal-volume CSF samples from each group of AD patients were pooled, total protein content in CSF pools was determined using the Bradford method, and Western blots were normalized using Ponceau S staining of nitrocellulose membranes (Ponceau S solution, Sigma–Aldrich). For immunoprecipitation, a CSF pool sample (200 µL) from AD patients carrying the wild-type *HMGA1* alleles was incubated overnight with rotation at 4 °C with 2 µg of HMGA1 antibody-coupled protein A/G agarose beads. Beads were recovered by gentle centrifugation and washed three times with 500 µL of NETN buffer [0.1% NP-40, 150 mM NaCl, 1 mM EDTA, 50 mM Tris–HCl (pH 8.0)]. HMGA1 protein was eluted from the beads by boiling in sample buffer for 10 min and analysed by SDS-PAGE and immunoblotting.

Assessment of INSR expression and insulin binding studies in blood monocytes

Peripheral blood mononuclear cells (PBMC) were isolated from the selected AD patients by Ficoll-Hypaque gradient centrifugation (GE Healthcare), and monocytes were purified by adhesion on 6-well polystyrene plates (Corning) for 4 h in RPMI-1640 medium supplemented with 10% FBS. Then, adherent monocytes were resuspended in fresh medium and used for subsequent experiments. For immunofluorescence assays, monocytes were fixed in glutaraldehyde, permeabilized with 0.1% Triton X-100, and incubated with primary polyclonal rabbit anti-human INSR antibody (N-20, α chain, sc-710; Santa Cruz Biotechnology; RRID:AB_631106) for 2 h at room temperature. After washing with PBS, monocytes were incubated with anti-rabbit FITC-conjugated secondary antibody (A16118; Thermo Fisher Scientific; RRID:AB_2534790) for 1 h at room temperature, and the nuclei were counterstained with the DNA-binding dye DAPI (Sigma–Aldrich). Labelled monocytes were then resuspended in Vectashield mounting medium (Vector Laboratories), transferred to microscope slides and images acquired by confocal imaging (Leica Microsystems GmbH). To assess insulin binding, equal numbers of monocytes (1×10^4) from each *HMGA1* genotype were incubated for 90 min at 4 °C with biotinylated human insulin (Eagle Biosciences), then cells were fixed with paraformaldehyde, blocked with 2% BSA, and stained with the Alexa Fluor™ 488 streptavidin conjugate (Thermo Fisher Scientific) for 1 h at room temperature, before DAPI counterstaining (Invitrogen). Cells were imaged using confocal microscopy and the fluorescence signals were quantified using Image J software. Protein extracts were prepared from blood monocytes and analysed by SDS-PAGE and immunoblotting with anti-INSR (C-19, β chain, sc-711; Santa Cruz Biotechnology; RRID:AB_631835) and anti-actin (4967; Cell Signaling Technology; RRID:AB_330288) antibodies.

Statistics

Continuous variables are expressed as mean ± S.D., and categorical variables as number and percentage. Normality was assessed using the Shapiro–Wilk test, and the parametric or nonparametric tests were used accordingly. The two-tailed unpaired (two-samples) *t*-test, followed by Welch's correction in case of unequal variances, was used for comparisons of continuous variables with normal distribution, whereas the Mann–Whitney U-test was used for comparisons of non-normally distributed variables. The Chi-square test was employed for the comparison of proportions. A significance level of 0.05 was chosen for all statistical calculations. Logistic regression analysis, adjusted for appropriate covariates, including age, sex, body mass index (BMI), and homoeostatic model assessment of insulin resistance (HOMA-IR), was used to assess the association between the *HMGA1* rs146052672 variant and tauopathies, providing odds ratios with 95% confidence bounds. Because of the low frequency of the *HMGA1* rs146052672 variant, only the dominant genetic model was considered. All data were analysed with SPSS 20.0 software.

Role of funders

The funding sources were not involved in study design, data collection, data analyses, interpretation, or writing of the manuscript.

Results

HMGA1 and tau expression are inversely related in SH-SY5Y cells before and after neuronal differentiation

Expression and localization of tau protein depend on the developmental stage of neurons. We first examined the relationship between HMGA1 and tau protein in SH-SY5Y neuroblastoma cells, a human cell line commonly used as a model for neurodegenerative disorders.[48] SH-SY5Y cells were treated with retinoic acid (RA) to induce their differentiation into neuronal-like cells. Treatment was extended up to 10 days until full cell differentiation was obtained. To validate the differentiation protocol toward a neuronal phenotype, morphological and immunofluorescence analyses of SH-SY5Y cells were carried out. Optical microscopy images were acquired at different time points during the neuronal differentiation process, showing the progressive elongation and branching of neurites leading to dendrite formation (Fig. 1a). At the same time points, immunostaining was performed to track the increasing

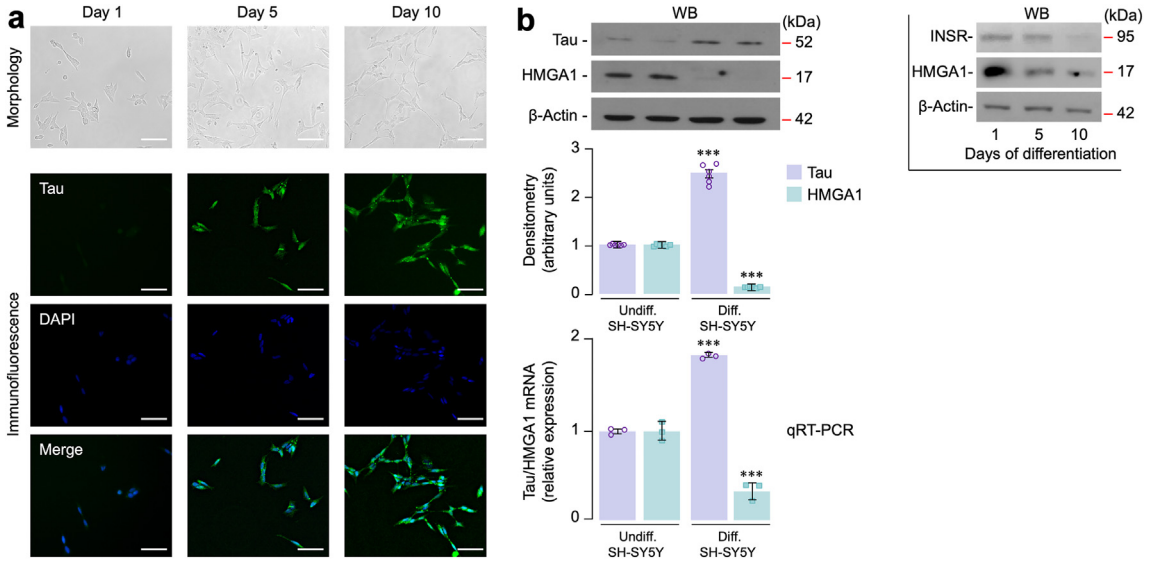

Fig. 1: **Neuronal differentiation of SH-SY5Y human neuroblastoma cells and HMGA1 and tau e**xpression. (**a**) Upper panels: representative optical microscopy images of SH-SY5Y cell morphology are shown at day 1, day 5, and day 10 after starting differentiation with RA. Lower panels: representative immunofluorescence microscopy images of tau protein (green fluorescence), nuclear DAPI (blue fluorescence), and merged signals at the same time points selected for the morphological analysis of SH-SY5Y cells, during differentiation. Scale bar = 20 μm. (**b**) HMGA1 and tau mRNA and protein expression were measured in both undifferentiated (Undiff.) and differentiated (Diff.) SH-SY5Y cells, using Western blot (WB) analysis and qRT-PCR. Values represent the mean ± s.e.m. of three independent experiments. ***$p < 0.001$ versus undifferentiated cells, in each assay condition. The inset shows the progressive changes in HMGA1 and INSR expression over time (day 1, day 5, and day 10) following the initiation of differentiation with RA, as measured by WB. (**c**) HMGA1 and tau protein levels in the secretome of SH-SY5Y cells before and after neuronal differentiation. Left, representative WB of HMGA1 and tau in cell suspension (PBS) of undifferentiated (Undiff.) and differentiated (Diff.) SH-SY5Y cells. Ponceau S, protein loading control. Right, WB of HMGA1 in 24-h conditioned medium samples from undifferentiated (lanes 1–2) and differentiated (lanes 3–4) SH-SY5Y cells. A representative WB out of six independent experiments is shown. The absolute values of tau protein content in 24-h conditioned medium from undifferentiated (Undiff.) and differentiated (Diff.) SH-SY5Y cells are shown in bar graph (mean ± s.e.m.), together with the levels of p-tau (inset). ***$p < 0.001$ versus undifferentiated cells. (**d**) Oxidative stress in undifferentiated (Undiff.) and differentiated (Diff.) SH-SY5Y cells. Left, Representative images of the MitoSOX Red Superoxide Indicator assay, showing red fluorescence, which indicates intracellular, mitochondrial-generated superoxide, overlaid with brightfield images. Scale bar = 100 μm. Right, Quantification of reactive oxygen species (ROS) production using the ROS-Glo™ H_2O_2 Assay. Data are expressed as mean ± s.e.m. of relative light units, normalized to the number of viable cells, from three independent experiments.

expression of tau protein, that is considered a marker of mature neurons (Fig. 1a).[48] Then, mRNA and protein expression levels of both HMGA1 and tau in differentiated SH-SY5Y cells were detected by qRT-PCR and Western blotting, respectively. As shown in Fig. 1b, a significant decrease in both mRNA and protein expression of HMGA1 was observed in fully differentiated SH-SY5Y cells as compared to undifferentiated cells ($p < 0.001$). Conversely, the mRNA and protein expression levels of tau were significantly higher in RA-differentiated SH-SY5Y neurons compared to undifferentiated SH-SY5Y neuroblastoma cells ($p < 0.001$) (Fig. 1b), thereby indicating an inverse relationship between HMGA1 and tau. Consistent with previous studies on the regulation of the *INSR* gene by HMGA1,[18,23] the progressive decline of HMGA1 during SH-SY5Y cell differentiation was accompanied by a decrease in INSR expression (Fig. 1b). This suggests that neuronal maturation may contribute to increased insulin resistance, in line with prior findings indicating impaired insulin sensitivity during SH-SY5Y cell differentiation.[49] The inverse relationship between HMGA1 and tau proteins was verified and confirmed within the cell secretome of both undifferentiated and differentiated SH-SY5Y cells. As shown in Fig. 1c, tau, its *phosphorylated* form (p-tau), and HMGA1 exhibit contrasting patterns of release into the extracellular milieu, suggesting that HMGA1 may play a dual role as both a chromatin regulator and a circulating secreted factor in tauopathies. Moreover, in differentiated SH-SY5Y cells, which have higher tau expression and lower HMGA1 levels compared to undifferentiated SH-SY5Y cells, signs of oxidative stress were observed. This was evidenced by a significant increase in reactive oxygen species (ROS) production, including mitochondrial superoxide (Fig. 1d). These findings are consistent with observations in human and mouse tauopathy models, where tau dysregulation contributes to the accumulation of oxidative stress in the brain.[50]

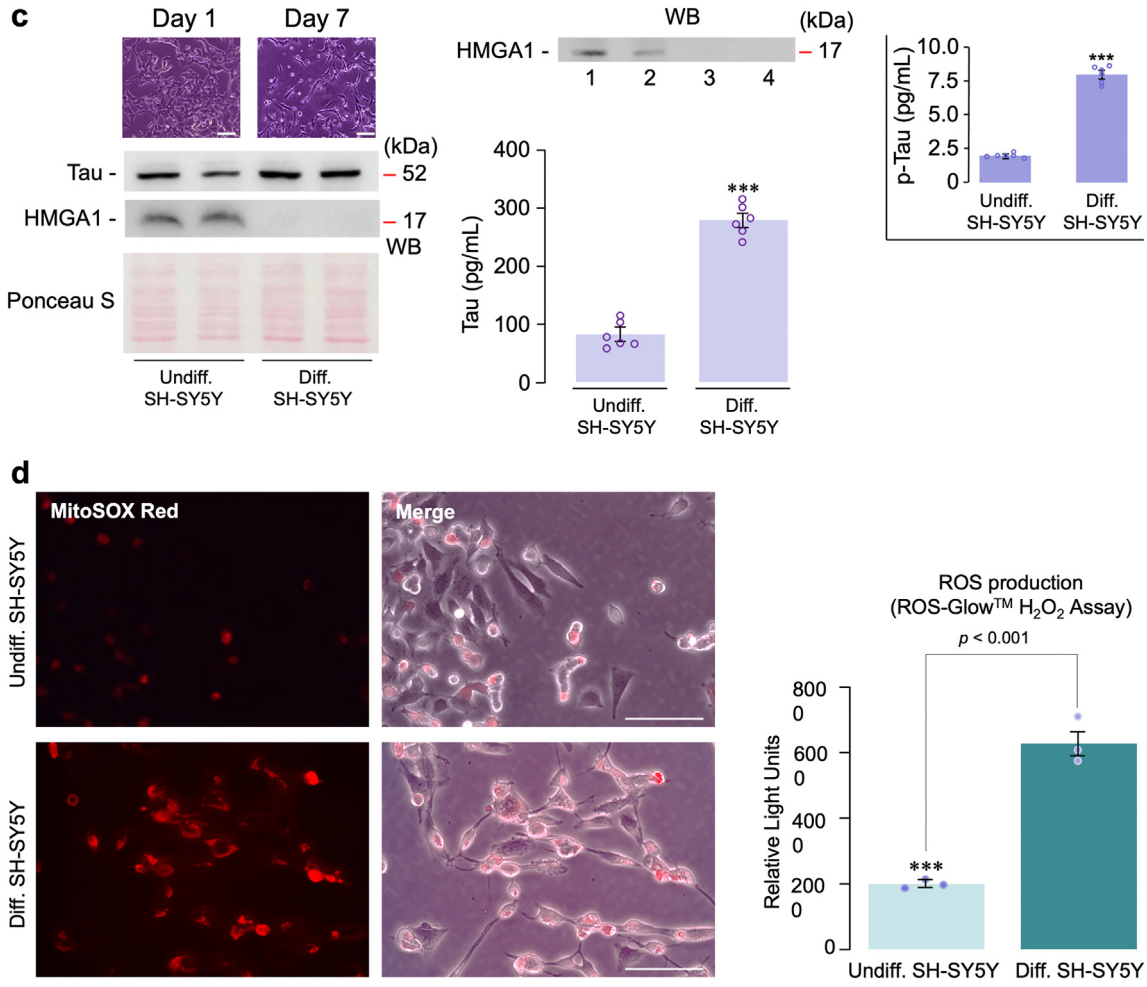

Fig. 1: Continued.

HMGA1 represses *MAPT* gene transcription

Human and mouse *MAPT* genes display an intrinsic variability with only few common points on their entire sequences.[51,52] However, there is evidence that both species share a transcriptionally active repressor region (C region), located 19 kb upstream of exon 1 and 47 kb downstream from a core promoter element,[53,54] which was previously shown to be a target of Sp1 and AP2 transcription factors.[15] This core promoter encompasses the exon 0 and drives constitutive *MAPT* gene transcription in the brain and other mammalian tissues (Fig. 2a).[37,51,53–55] An alternative regulatory region (~2 kb proximal to exon 1), with 50% homology between the two species, has been reported. This region regulates the activation of brain-specific *MAPT* transcripts that are overexpressed in some tauopathies.[37] Here, by using computational analysis of *cis*-acting elements embedded in this regulatory region of the *MAPT* gene (http://trap.molgen.mpg.de/cgi-bin/home.cgi), we have identified DNA binding sites for HMGA1 in both human and mice, confirming previous bioinformatic analysis restricted to the C region, which is known to exert repressive activities on the core promoter.[53,54]

Therefore, in order to explore the possibility that HMGA1 could be involved in the regulation of tau expression, we first performed functional studies using plasmid reporter constructs containing *tau* promoter regions of either human or mouse origin, which were transiently cotransfected with a HMGA1 expression vector or a small interfering RNA (siRNA) against HMGA1 into terminally differentiated SH-SY5Y cells that barely express endogenous HMGA1 following acquisition *of* a neuron-like phenotype. As shown in Fig. 2b, induced overexpression of HMGA1 markedly reduced mouse *Mapt*-Luc activity in differentiated SH-SY5Y cells, and this inhibitory effect was dose dependent. However, when SH-SY5Y cells under the same experimental conditions were cotransfected with siRNA targeting HMGA1, *Mapt*-Luc activity significantly increased compared to cells treated with control siRNA

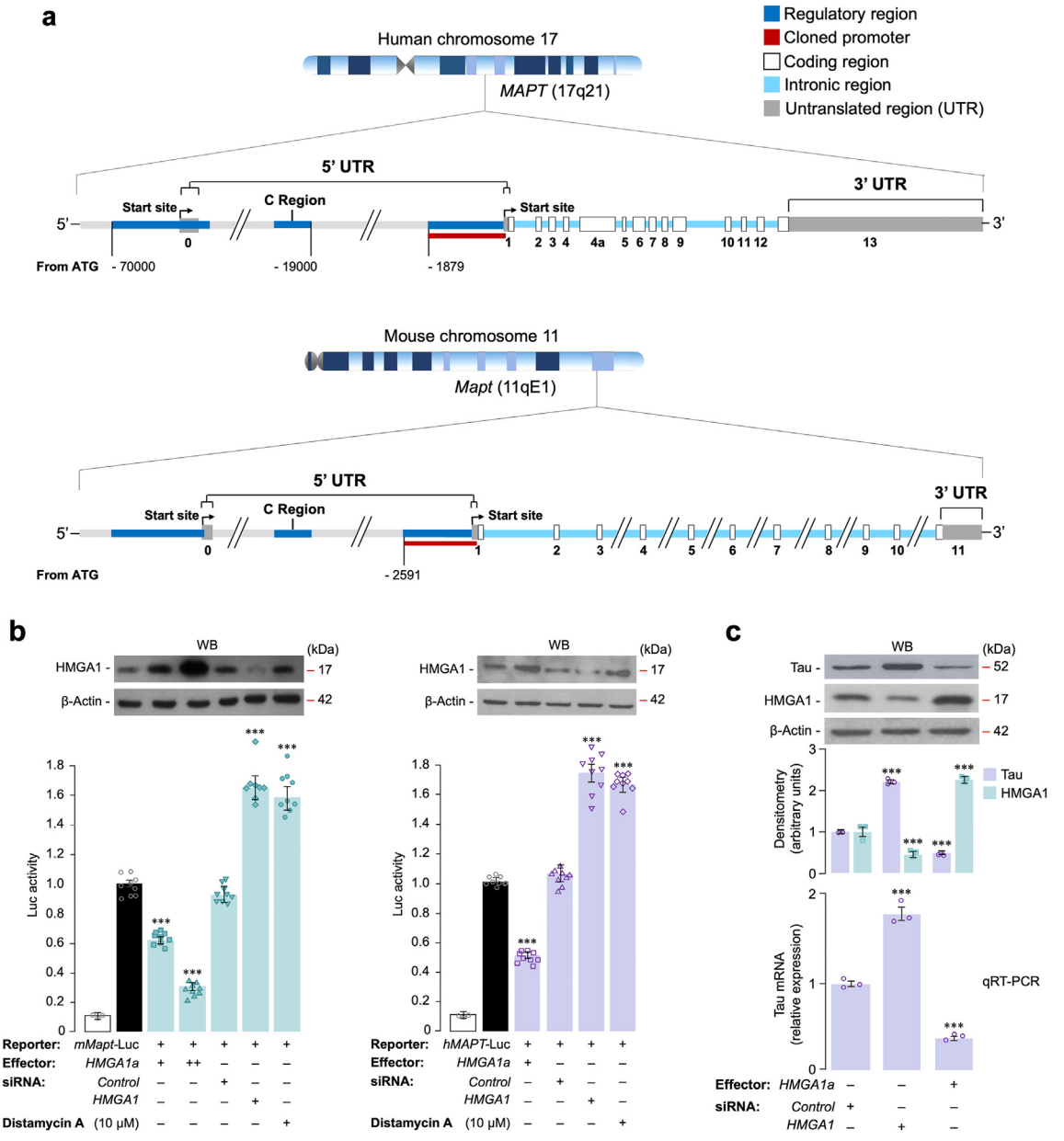

***Fig. 2:* Schematic representation of human and mouse *MAPT* genes and *MAPT* gene repression** by HMGA1. (**a**) The gene structure of human (upper) and mouse (bottom) *MAPT* genes is shown. UTRs, exons/introns, and regulatory regions are shown for each gene. The regulatory regions, including the core promoter, the repressor C region, and the alternative promoter proximal to exon 1, are depicted by blue boxes in a left-to-right (5′ to 3′) orientation. The promoter regions proximal to exon 1 (red boxes), containing putative DNA binding sites for HMGA1, were used in reporter gene analysis and DNA-protein interaction studies. (**b**) Mouse (*mMapt*-Luc, left) or human (*hMAPT*-Luc, right) reporter vector and increasing amounts (0, 0.3, 0.6 μg) of pcDNA3-*HMGA1a* expression plasmid were cotransfected into terminally differentiated SH-SY5Y cells, in the absence or presence of 10 μM distamycin A. After 6 h of transfection, cells were treated with anti-*HMGA1* siRNA (100 pmol), or a non-targeting control siRNA, and Luc activity was measured 48–96 h later. Values are expressed as the factors by which Luc activity increased or decreased as compared with the level of Luc activity obtained in transfections with the reporter vector alone (black bars), which is assigned an arbitrary value of 1. Open bar, pGL3-basic (vector without an insert). Data are the mean ± s.e.m. of triplicate assays from three independent experiments. ***$p < 0.001$ versus control (black bar). Representative Western blots (WB) of HMGA1 in each condition are shown in the autoradiograms. (**c**) pcDNA3-*HMGA1a* effector vector was transfected into differentiated SH-SY5Y cells. After 6 h post-transfection, cells were treated with siRNA targeting HMGA1 (100 pmol) or control siRNA and endogenous tau mRNA expression was measured by qRT-PCR 48–96 h later. ***$p < 0.001$ versus control siRNA. Representative WB of both tau and HMGA1 in each condition are shown in the autoradiograms, along with the densitometric analysis of the blots.

($p < 0.001$) (Fig. 2b). Similar to the results obtained with the mouse *Mapt* promoter gene, functional studies with human *MAPT*-Luc indicated that overexpression of HMGA1 in SH-SY5Y cells reduced Luc activity and this effect was abolished by pretreatment with siRNA against HMGA1 (Fig. 2b). Inhibition of mouse (*mMapt*) and human (*hMAPT*) promoter reporter genes by HMGA1 was also prevented by treating cells with distamycin A, a DNA ligand which selectively blocks DNA binding by HMGA1 (Fig. 2b).[56] Analogous results (data not shown) were obtained when the same experiments were performed with a human *MAPT*-Luc reporter construct, encompassing the repressor C region.

Therefore, these results indicate that HMGA1 plays a functional role in controlling the expression of tau, in either human or mouse, and this function appears to be mediated primarily through the specific repression of *MAPT* gene transcription. Consistently with these results, endogenous tau was markedly reduced at the mRNA and protein levels in differentiated SH-SY5Y cells induced to overexpress HMGA1, whereas it was increased in cells exposed to siRNA targeting HMGA1 (Fig. 2c).

Physical association of HMGA1 with the promoter region of mouse and human *MAPT*

To further support the functional role of HMGA1 in tau expression, we next carried out a series of EMSAs aimed at exploring the capability of HMGA1 to physically interact with the promoter region of the *MAPT* gene. As shown in Fig. 3a, DNA-protein binding activity of HMGA1 was detected when EMSAs were conducted using a biotinylated-labelled probe that contained a mouse *Mapt* promoter portion, in the presence of nuclear protein extract from undifferentiated SH-SY5Y cells, naturally expressing considerable amount of endogenous HMGA1. To determine the specificity of HMGA1 DNA-binding, competition assays were performed in preliminary studies (not shown), in which a 50 to 200-fold molar excess of unlabelled oligonucleotide containing the binding site for HMGA1 markedly decreased the binding of HMGA1 to labelled DNA. In contrast, a 200-fold molar excess of an unlabelled unrelated oligonucleotide containing the binding site for the Sp1 transcription factor had no effect on the HMGA1-DNA complex formation (Fig. 3a).[18] The existence of HMGA1 in a complex with the *Mapt* promoter region was investigated more deeply in supershift assays, in which nuclear protein extracts from SH-SY5Y cells were exposed to anti-HMGA1 specific antibody prior to incubation with labelled probe. As shown in Fig. 3a, the addition of anti-HMGA1 antibody resulted in the appearance of a clear supershift band, which was accompanied by a decrease in the intensity of the lower shifted band, indicating the presence of HMGA1 in this DNA-protein complex. Preincubation of the *Mapt* promoter probe with distamycin A considerably decreased the binding of HMGA1 to DNA, whereas HMGA1 DNA-binding activity was undetectable in fully differentiated SH-SY5Y cells (Fig. 3a).

As a further step toward deepening the association of HMGA1 with the human *MAPT* gene, we extended these in vitro studies to studies in living intact SH-SY5Y cells, either undifferentiated or differentiated into neurons, in order to detect and quantify the contact established between HMGA1 protein and the regulatory region of the *MAPT* gene. As measured by ChIP in whole cells, and subsequent qRT-PCR of ChIP-ed samples, binding of the *MAPT* gene promoter by endogenous HMGA1 was increased in undifferentiated SH-SY5Y cells compared to differentiated counterparts (Fig. 3b). Again, when the ability of HMGA1 to interact with DNA was prevented by distamycin A in in vivo cultured cells, binding of HMGA1 to this promoter region was considerably reduced (Fig. 3b).

Direct interaction between HMGA1 and tau proteins, was excluded by co-immunoprecipitation, using tau-enriched extracts from differentiated SH-SY5Y cells and purified HMGA1 (data not shown).

Studies in primary cultures of mouse neurons and in *Hmga1*-knockout mice

We extended the study to primary cultures of cortical neurons prepared from mouse embryos. Cultures were transiently transfected with plasmids expressing siRNA targeting HMGA1 or a vector expressing HMGA1 to examine the relationship between HMGA1 and tau protein in mouse neurons. We confirmed the inverse correlation between the amount of intracellular HMGA1 and tau expression levels. The transcript encoding tau protein was upregulated by a siRNA targeting HMGA1; in contrast, the overexpression of HMGA1 led to a reduction in both tau mRNA and protein levels (Fig. 4a). Consistent with previous results observed in patient-derived cells,[19,23,24] the diminished expression of HMGA1 in cortical neurons parallelled the decrease in InsR mRNA abundance. Conversely, HMGA1 overexpression significantly enhanced InsR mRNA transcript levels ($p = 0.043$) (Fig. 4a).

We next investigated the relationship between HMGA1 and tau protein expression in the intact brain of *Hmga1*-knockout mice, a model of obesity-independent insulin resistance and T2D that we have extensively studied before.[23] Tau mRNA and protein levels were found to be significantly increased in the brain of *Hmga1*-knockout mice. Tau phosphorylation was also enhanced in the brain of *Hmga1*-knockout mice at sites known to be linked with tau aggregation (Fig. 4b), suggesting that abnormalities in HMGA1 protein binding to consensus sequences within the *MAPT* gene may be relevant to the pathophysiology of AD and other tauopathies. Interestingly, in this case as well, an opposing pattern emerged in the levels of tau and InsR present in the brain of *Hmga1*-knockout and

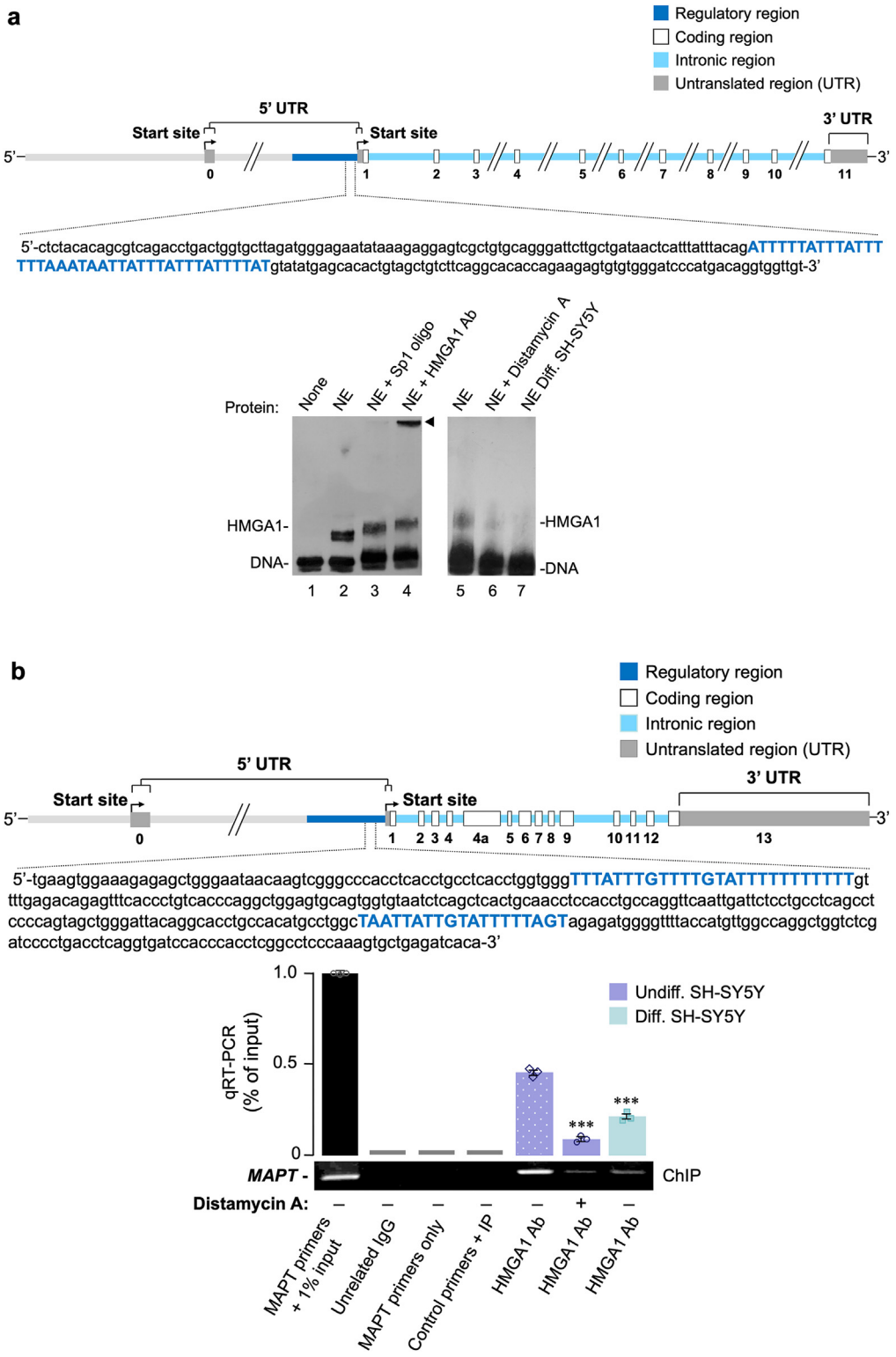

***Fig. 3:* Binding of HMGA1 to *MAPT* promoter.** (**a**) Top, schematic representation of the mouse *Mapt* gene with HMGA1 binding sites (blue uppercase) within the regulatory region (blue) of this gene. A 200 bp biotin-labelled *Mapt* promoter probe was used in EMSAs (bottom) with 1 µg of nuclear extracts (NE) from undifferentiated SH-SY5Y cells, in the presence of 1 µg poly (dI-dC) as nonspecific competitor. Lanes: 1, probe

wild-type mice, indicating a divergent modulation of *InsR* and *Mapt* gene expression by HMGA1 in brain tissue.

IHC expression of tau, behavioural studies, and brain glucose metabolism in *Hmga1*-knockout and control mice

Tau protein expression was subsequently verified in IHC staining of brain tissue slices of wild-type and *Hmga1*-knockout mice. IHC assessment using anti-mouse Hmga1 antibody confirmed that Hmga1 expression was detected within all brain regions of wild-type mice, whereas there was no specific signal in the brain of *Hmga1*-null mice, in which only IHC background staining was observed. As shown in Fig. 5a, illustrating the results of IHC analysis of cerebral cortical sections, the cerebral cortex of *Hmga1*-knockout mice was characterised by intense and diffuse cytoplasmic vacuolization, a morphological phenomenon indicative of cell stress and damage and a prelude to cell death.[57] Cerebral cortical tissue slices from *Hmga1*-knockout mice also showed an increased number of small blood-like vessels (Fig. 5a), which are considered a pathological hallmark of neuroinflammation in various mouse models of tauopathies, including AD.[58] Following up the in vitro and in vivo findings described above, IHC of the mouse cerebral cortex tissue using the anti-tau antibody indicated an increment of tau expression in *Hmga1*-knockout mice compared to the levels in wild-type animals (Fig. 5b). As evidenced in Fig. 5b, tau immunoreactivity was heightened in neuronal cell bodies. Furthermore, the cerebral cortex of *Hmga1*-null mice exhibited a highly eosinophilic extracellular matrix, which may relate with an enhanced expression of tau in animals lacking Hmga1. Changes in the perineuronal extracellular matrix are, indeed, considered to be consequences of the intracellular accumulation of tau and its extracellular propagation in mice that are genetically prone to tauopathies.[59] The APP amyloid β precursor protein is a key factor in genetic forms of AD.[60] APP generates amyloid β through proteolytic cleavage and is also known to facilitate tau aggregation and its propagation, contributing to both amyloid and tau pathologies.[61] Additionally, APP has been implicated in metabolic regulation, with increased expression observed in brain and adipose tissue under insulin-resistant conditions.[62] However, immunoblotting analysis of brain tissue showed no differences in APP levels when comparing *Hmga1*-knockout to wild-type mice (Fig. S3), suggesting that Hmga1 deficiency does not contribute to neurodegeneration via APP overexpression. Moreover, GFAP, a marker of reactive astrocytosis triggered by amyloidogenesis,[63] was less prominent in *Hmga1*-knockout mice (Fig. S3). Instead, increased tau immunoreactivity was also observed in the hippocampus of *Hmga1*-knockout mice (Fig. 5c), where tau accumulation was linked to hypertrophic neuronal cell bodies (Fig. S4). This response resembles the early stages of neurotoxic damage seen in human AD progression.[64]

To investigate the impact of Hmga1 deficiency on the psychomotor abilities of mice, a spontaneous behaviour analysis was conducted. The experimental protocol involved exposing mice to the novel cage test, where each mouse was placed individually in a new cage for a brief period (10 min). This test was repeated over 15 sessions, conducted three times per day for five consecutive days, to evaluate the exploratory responses of the mice to an unfamiliar environment. The results showed a significant decrease in the overall time spent exploring the floor, cage walls, or air via sniffing among *Hmga1*-knockout mice compared to wild-type mice ($p = 0.021$) (Fig. 5d). Furthermore, the *Hmga1*-knockout mice exhibited increased immobility, indicated by prolonged freezing behaviour (Fig. 5d). This observation suggests that Hmga1 deficiency, which is associated with an accumulation of brain tau and AD-like histopathological changes, can lead to behavioural abnormalities and reduced reactivity to novelty in *Hmga1*-null mice. This phenotype resembles the apathetic behaviour observed in tau transgenic mice and patients with tauopathies.[65] These findings underscore the potential of *Hmga1*-deficient mice as a model for studying human tauopathies.

The decrease in brain glucose metabolism in patients with AD is considered a critical driver of cognitive impairment.[66] The neuron-specific glucose transporter, GLUT3, is abundantly expressed in human and murine brain, and plays a crucial role in this process.[67] Downregulation of GLUT3-mediated glucose uptake in the brain represents an early event in the pathogenesis of AD, where decreased expression of GLUT3 negatively correlates with tau hyperphosphorylation.[68] Upregulation of glucose uptake through HMGA1-mediated *GLUT3* gene induction has been reported previously

alone; 2, probe with NE; 3, competition with unlabelled oligonucleotide *for* the unrelated Sp1 transcription factor; 4, supershifting of the HMGA1-DNA complex (arrowhead), in the presence of anti-HMGA1 specific antibody (Ab). Lanes 5–6: distamycin A (10 μM) was preincubated with *Mapt* promoter probe (15 min at room temperature) before NE was added. Lane 7, NE from differentiated SH-SY5Y cells. Representative EMSAs are shown. Experiments were performed three times with similar results. (**b**) Top, schematic representation of the human *MAPT* gene with HMGA1 binding sites (blue uppercase) within the regulatory region (blue) of this gene. Bottom, representative ChIP of the *MAPT* gene locus in undifferentiated (Undiff.) and differentiated (Diff.) SH-SY5Y cells, either untreated or pretreated with distamycin A (10 μM), using an anti-HMGA1 specific antibody (Ab). qRT-PCR of ChIP-ed samples is shown in each condition. ***$p < 0.001$ versus control (dotted bar).

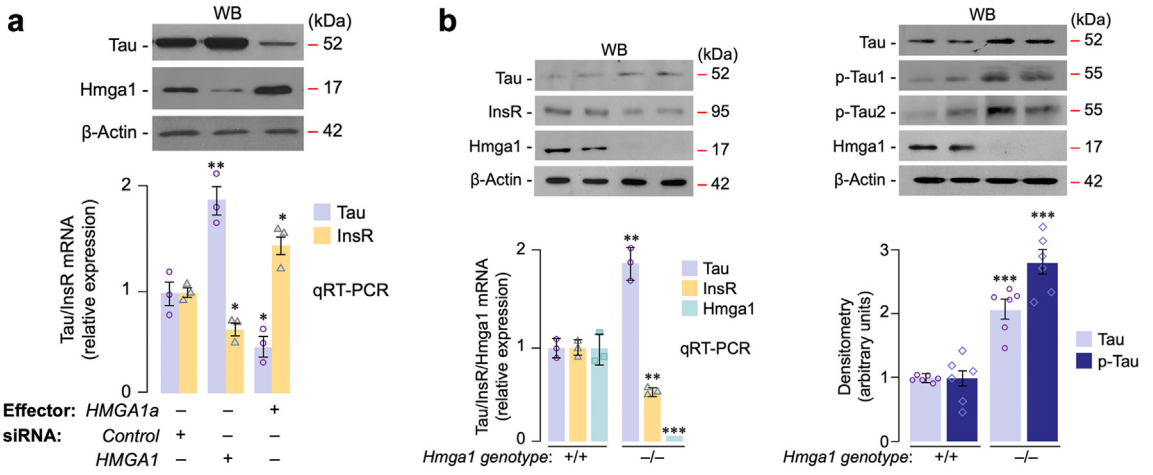

Fig. 4: **Hmga1, tau and InsR expression in primary cultures of mouse neurons and in *Hmga1*-deficient mice**. (**a**) Hmga1 and tau protein expression, as well as tau/InsR mRNA levels, were assessed in primary cultured neurons derived from wild-type mouse embryos. *pcDNA3-HMGA1a* expression plasmid or anti-*HMGA1* siRNA (100 pmol) was transfected into cultured neurons and Hmga1 protein and tau/InsR mRNA were measured 48–96 h later. *$p < 0.05$, **$p < 0.01$ versus control siRNA-transfected cells. Data are shown as the mean ± s.e.m. of three independent experiments. (**b**) Hmga1, InsR and tau mRNA and protein expression (left) in cerebral tissues from wild-type (+/+) and *Hmga1* knockout (–/–) mice. mRNA was measured by qRT-PCR and normalized to *Rps9*. Data from three independent assays from six animals per genotype are shown, together with representative Western blots (WB) of Hmga1, tau and p-tau (p-Tau1, Ser400/Thr403/Ser404; and p-Tau2, Thr205) (right) along with densitometric analysis of the blots. **$p < 0.01$, ***$p < 0.001$ versus wild-type mice.

in human colorectal cancer cell lines and tissues.[69] Given the striking sequence similarity between human and mouse *GLUT3* genes,[67] we investigated whether *Hmga1*-knockout mice exhibited changes in neuronal Glut3 protein expression, potentially affecting brain glucose uptake. As shown in Fig. 5e, IHC of brain sections revealed immunopositivity for Glut3 primarily in neuronal cell bodies of the cerebral cortex, where Glut3 protein expression levels were decreased in *Hmga1*-knockout mice compared to wild-type animals. The decrease in Glut3 protein expression was confirmed in Western blot analysis of whole brain tissues from wild-type and *Hmga1*-null mice (Fig. 5f). Molecular mechanisms regulating glucose uptake in vivo, with a focus on the role of HMGA1 in animals experiencing peripheral insulin resistance, have been previously elucidated.[20,23,70,71] These studies demonstrated that the peptide hormone, IGF1, by acting through its own IGF1 receptor (IGF1R), plays an essential role in maintaining glucose metabolism homoeostasis in vivo, under adverse metabolic conditions in which INSRs are reduced and insulin action is precluded. As shown in Fig. 5f, Western blot analyses demonstrated that in contrast to the expression of the closely related InsR (as described in Fig. 4b), brain expression of the Igf1R protein was similar in wild-type and *Hmga1*-knockout mice. Based on these observations, we next analysed in vivo brain glucose uptake. By using FDG-PET, we assessed the importance of IGF1 on brain glucose uptake following injection of recombinant human IGF1 (rhIGF1) in wild-type and *Hmga1*-knockout mice. As shown in Fig. 5f, there was a clear trend toward reduced brain glucose uptake in *Hmga1*-knockout mice, although this did not reach statistical significance, likely due to the limited sample size ($p = 0.054$). Interestingly, this reduction closely parallels the brain glucose hypometabolism characteristic of neurodegenerative diseases, such as AD and other tauopathies in humans.

Case-control association of *HMGA1* rs146052672 variant with tauopathy

We previously reported that a variant of the human *HMGA1* gene, namely the rs146052672 variant, resulted in reduced levels of HMGA1 mRNA and protein by affecting RNA splicing.[72] The negative impact of this variant on *HMGA1* gene functionality has been previously demonstrated using the minigene strategy, an effective approach for assessing the significance of intronic polymorphisms.[72] Of note, the decrease in HMGA1 levels was associated with a significant reduction in INSR expression and insulin signalling in insulin target cells and tissues of both humans and mice with insulin resistance and T2D,[19,23–26] two dysmetabolic conditions often occurring concurrently in patients with tauopathies and, in particular, with AD.[28,29,73] To investigate the potential link between this variant and these neurodegenerative diseases, a case–control study was conducted, including 34 patients with AD, 28 with FTD, 7 with PSP-RS, and 200 unrelated controls of similar age. Given the shared neuropathological features observed in post-mortem studies and common genetic risk factors,[46] FTD patients, as defined by the study's inclusion criteria, and PSP-RS patients were grouped together as non-AD tauopathy. Table 1 presents an

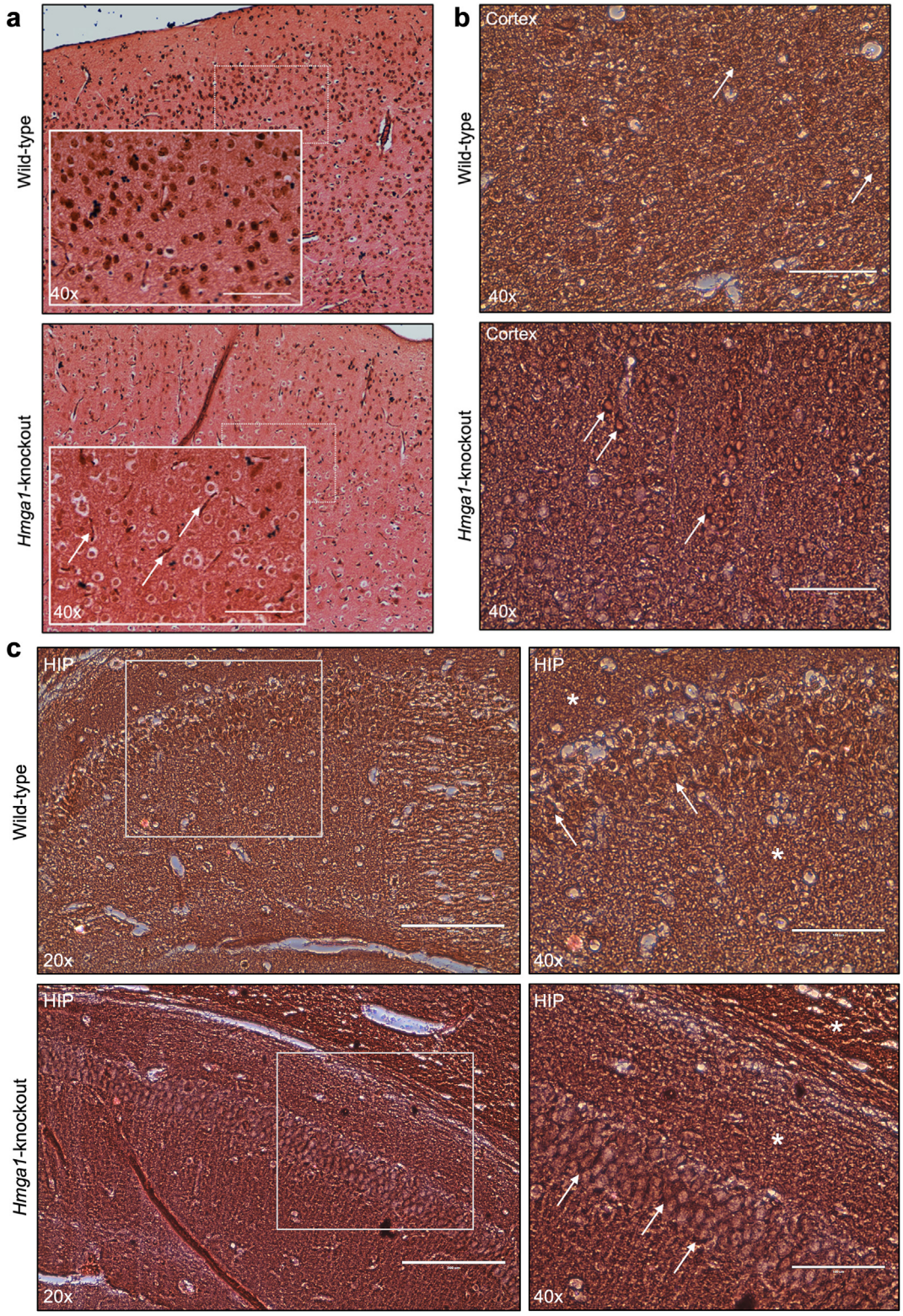

Fig. 5: **Brain IHC staining of tau and Glut3, behavioural studies, and FDG-PET in *Hmga1*-knockout and control mice**. (**a**) IHC staining of paraffin-embedded cerebral cortex sections with anti-Hmga1 specific antibody. Compared to wild-type mice, the cerebral cortex of the *Hmga1*-knockout mice displays diffuse neuronal cytoplasmic vacuolization and an increased number of small blood-like vessels (white arrows), in the

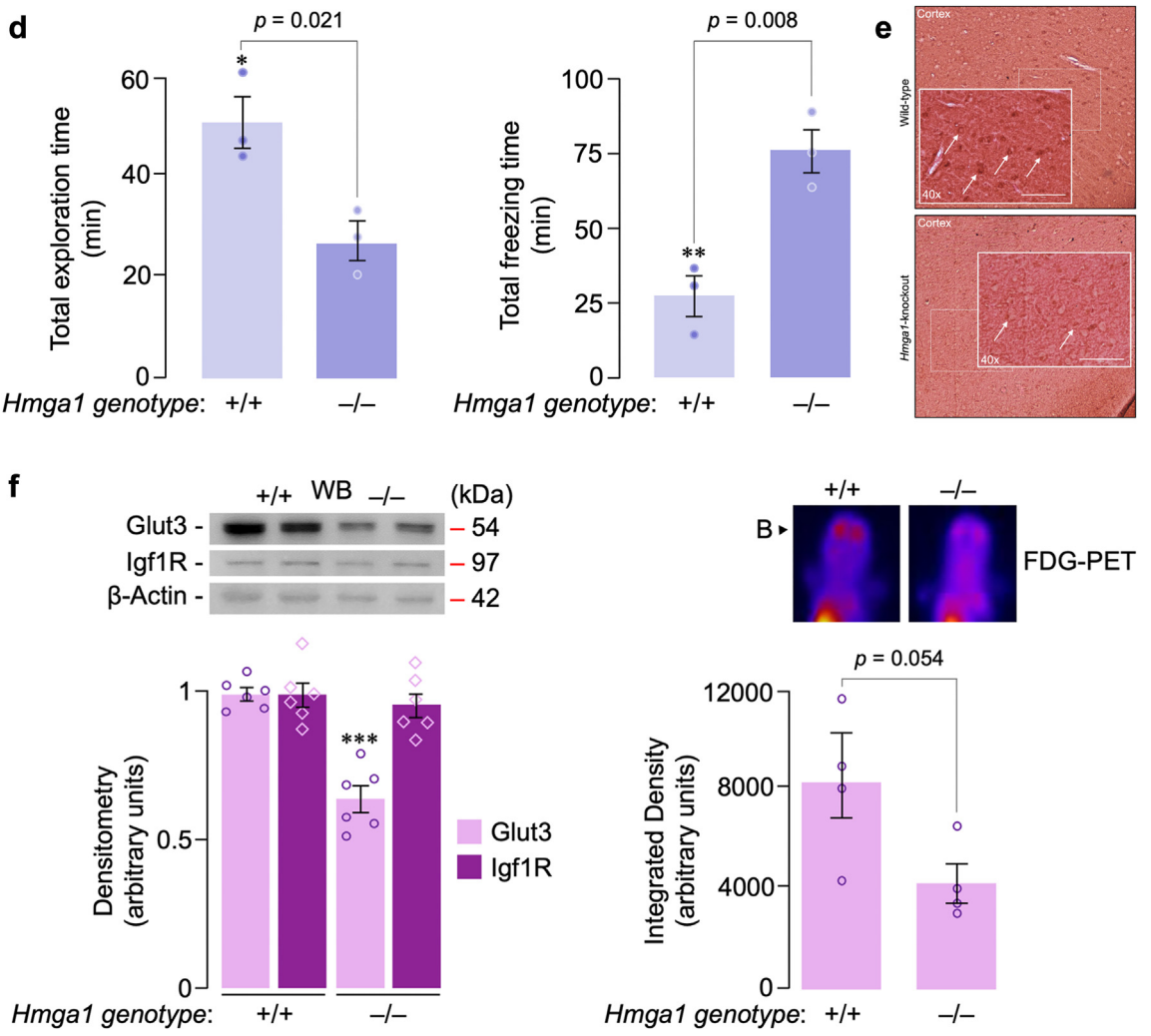

Fig. 5: Continued.

overview of the study participants, whose clinical and metabolic characteristics reflect those of the general older adult population in Calabria.[74] Genetic analysis revealed that the rs146052672 variant was significantly more common among patients with tauopathies compared to controls, with frequencies of 17.4% (12 of 69 patients) and 5.0% (10 of 200 patients), respectively (p = 0.001). Logistic regression analysis, adjusted for

absence of Hmga1 protein brown staining. Scale bar = 100 µm. (**b**) IHC staining of cerebral cortex sections using an anti-tau specific antibody. Enhanced tau immunoreactivity in neuronal cell bodies (white arrows), is shown in *Hmga1*-knockout mice. Scale bar = 100 µm. (**c**) IHC staining of the hippocampus (HIP) with anti-tau-specific antibody shows similar enhanced tau immunoreactivity in both neuronal cell bodies (white arrows) and neuropil (white asterisks), as observed in the cerebral cortex of *Hmga1*-knockout mice. Scale bar = 200 µm at 20x magnification; Scale bar = 100 µm at 40x magnification. (**d**) Exploratory and freezing behaviours in wild-type (+/+) and *Hmga1*-knockout (–/–) mice. Histograms with error bars represent means ± s.e.m. of the total time spent engaging in exploratory activity (left) or in immobility (right) across 15 test sessions, each lasting 10 min (n = 3 animals per genotype); *p < 0.05, **p < 0.01 versus wild-type mice. (**e**) Representative images of IHC staining of Glut3 in brain cortex. Compared to *Hmga1*-knockout mice, wild-type animals show enhanced Glut3 brown staining immunoreactivity in neuronal cell bodies (white arrows). Scale bar = 100 µm. (**f**) Left, Western blots (WB) of Glut3 and Igf1R proteins in brain tissue of wild-type (+/+) and *Hmga1*-knockout (–/–) mice. Representative autoradiograms of six mice per genotype group are shown. Values of quantitative densitometric analyses of Glut3 and Igf1R immunoblots are shown in bar graphs. ***p < 0.001 versus wild-type mice. Right, FDG-PET. Shown are scan images of a representative wild-type mouse (+/+) and a representative *Hmga1*-knockout mouse (–/–) 35 min after tracer administration. For each animal, quantitative assessment of whole brain FDG-uptake is indicated as the sum of integrated density of each slice, using a small region of interest positioned on the brain (B). Data are shown as the mean ± s.e.m. integrated density units, for four mice of each genotype.

age, sex, and BMI, showed a strong association between this variant and tauopathy [OR, 4.828 (95% CI, 1.865–12.500) p = 0.001]. This association remained significant even after additional adjustment for HOMA-IR, a marker of insulin resistance (Table 2). When comparing individual tauopathies to controls, the *HMGA1* rs146052672 variant was associated with both AD [OR, 5.833 (95% CI, 1.778–19.132) p = 0.004] and non-AD tauopathy [OR, 3.683 (95% CI, 1.122–12.096) p = 0.032]. No homozygotes for the *HMGA1* rs146052672 variant were observed (overall Hardy–Weinberg equilibrium's p = 0.480). Interestingly, adjusted linear regression analysis revealed that also in this small cohort of patients an association of the rs146052672 variant with insulin resistance indeed existed (β: 0.107, t: 1.985, p = 0.048), thus confirming previous observations with larger populations from different geographic regions.[24,25] Furthermore, as shown in Table S2, patients with tauopathy who carried the HMGA1 gene variant had a higher prevalence of T2D. Based on clinical examination of six selected AD patients with the rs146052672 variant and twelve age/sex matched AD patients without this variant, it was observed that patients carrying the variant had a significantly lower score on the neuropsychological Mini-Mental State Examination (MMSE) compared to non-carrier patients (MMSE score 14.7 ± 4.6 versus 19.7 ± 4.1 points, respectively; p = 0.030) (Table 3). This indicates a greater level of cognitive impairment and an increased risk of severe health deterioration in AD patients with the *HMGA1* gene variant.

When the therapeutic response to conventional drugs (acetylcholinesterase inhibitor and/or N-methyl-D-aspartate receptor antagonist) was monitored in these selected patients for 12–16 months using clinical cognitive tests, it was notable that AD patients without the rs146052672 variant showed the best responses (Table 3). In contrast, patients with the rs146052672 variant had the worst responses, exhibiting a progressive decline in Activities of Daily Living (ADL) scores, more severe functional impairments, or, in one case, death (Table 3). These findings further confirm that the rs146052672 variant is associated with greater clinical severity in AD and indicate that patients with the HMGA1 genotype have a differential phenotypic profile, showing the poorest therapeutic response to conventional therapy. Differences in receptor activity, glucose metabolism, oxidative stress, and inflammation may influence drug responses and represent a possible explanation for the reduced effectiveness of standard therapies in these patients.

Assessment of total tau and p-tau proteins in CSF

Increased levels of total tau or p-tau in CSF are considered reliable biomarkers of AD.[75,76] This view gains further support from a recent study showing a significant rise in CSF total tau concentrations occurring in AD patients up to 10 years before diagnosis.[77] Consequently, we sought to investigate the relationship between the HMGA1 rs146052672 variant and CSF tau and p-tau levels in AD patients. CSF tau and p-tau levels were quantified by chemiluminescence enzyme immunoassay in the selected AD patients, both carriers and non-carriers of the rs146052672 variant, who also underwent neuropsychological MMSE testing. As shown in Fig. 6a, AD patients carrying the *HMGA1* rs146052672 variant exhibited a significant elevation in total tau and p-tau levels compared to non-carriers (total tau, 947 ± 161 versus 753 ± 99 pg/mL, p = 0.031, respectively; p-tau, 103.1 ± 23.1 versus 78.7 ± 10.7 pg/mL, p = 0.049, respectively). Additionally, HMGA1 protein abundance in the CSF was assessed by Western blot analysis, confirming that HMGA1 levels were significantly reduced in AD patients with the rs146052672 variant compared to non-carrier AD patients (Fig. 6b). *The specificity of this* assay was ensured through immunoprecipitation of CSF followed by

	Patients with tauopathy n = 69	Controls n = 200	p-value
AD	34	–	–
Non-AD	35	–	–
Ethnicity	Caucasian	Caucasian	–
Female sex (n)	36 (52.2)	120 (60.0)	0.256
Age (yr)	74.7 ± 7.1	75.0 ± 6.4	0.658
BMI (Kg/m^2)	24.2 ± 2.7	26.2 ± 3.3	<0.001
Hypertension (n)	42 (60.9)	132 (66.0)	0.442
Type 2 diabetes (n)	18 (26.1)	51 (25.5)	0.923
FPG (mg/dL)	113.0 ± 29.4	104.4 ± 22.9	0.022
Insulin (μU/mL)	6.4 ± 2.7	5.9 ± 3.3	0.158
HOMA-IR index	1.9 ± 1.1	1.6 ± 1.1	0.031
HOMA-IR ≥ 1.8 (n)	31 (44.9)	58 (29.0)	0.015

Data are presented as mean ± SD or n (%). The two-tailed unpaired (two-samples) *t*-test followed by Welch's correction, or the Mann–Whitney U test, was used for distribution comparisons of quantitative variables, as appropriate. The Chi-square test was used for proportion comparisons between groups. AD, Alzheimer's disease; the term non-AD refers to 28 patients with tau-related frontotemporal dementia (FTD) and 7 patients with progressive supranuclear palsy presenting with Richardson syndrome (PSP-RS); BMI, body mass index; FPG, fasting plasma glucose; HOMA-IR, homoeostatic model assessment of insulin resistance, HOMA-IR values equal to or above 1.8 are indicative of insulin resistance in the studied population.[26]

Table 1: **Clinical and metabolic characteristics of participants in the case–control study.**

	–/– (%)	–/C (%)	C/C (%)	MAF (%)	Adjusted OR (95% CI)	p-value
Controls	190 (95.0)	10 (5.0)	0 (0)	2.50	–	–
Tauopathies	57 (82.6)	12 (17.4)	0 (0)	8.70	4.828 (1.865–12.500) 4.180 (1.593–10.971)[a]	0.001 0.004[a]
AD	28 (82.4)	6 (17.6)	0 (0)	8.82	5.833 (1.778–19.132)	0.004
Non-AD	29 (82.9)	6 (17.1)	0 (0)	8.57	3.683 (1.122–12.096)	0.032

Logistic regression analysis was performed to assess the independent role of the rs146052672 variant on tauopathies. Adjusted OR and p values respect to Controls are shown. Age, sex, and BMI were added as covariates. MAF, minor allele frequency. [a]Adjusted OR was calculated by adding HOMA-IR.

Table 2: **Association of the *HMGA1* rs146052672 variant with tauopathies.**

	AD, variant carriers n = 6	AD, non-carriers n = 12	*p*-value
Baseline			
MMSE score	14.7 ± 4.6	19.7 ± 4.1	0.030
ADL score	3.8 ± 1.7	4.4 ± 1.2	0.424
12–16 months			
MMSE score	10.9 ± 6.8[a]	18.8 ± 4.6	0.007
ADL score	2.5 ± 2.5[a]	4.1 ± 1.5	0.086
ADL score change	1.3 ± 1.2[a]	0.3 ± 0.5	0.041
Worsening ADL functionality or death[a] (n)	4 (66.7)	1 (8.3)	0.021

Data are presented as mean ± S.D. or n (%). The Mann–Whitney U test or the unpaired *t*-test was used for distribution comparisons of quantitative variables, as appropriate. MMSE, Mini-Mental State Examination; ADL, Activities of Daily Living. Worsening ADL functionality refers to a clinically significant decline from full functioning (ADL score of 5–6 points) to moderate impairment (ADL score of 3–4 points) or from moderate to severe impairment (ADL score of 2 or less). [a]Denotes the death of one patient with the rs146052672 variant, who experienced a severe form of AD. This patient was assigned an arbitrary score of 0 in neuropsychological and functional assessments at the 12–16 months follow-up.

Table 3: **Impact of the *HMGA1* rs146052672 variant on cognitive impairment and declining health status in AD patients.**

Western blot analysis for HMGA1 detection (Fig. 6b). However, the invasive nature of CSF collection renders this procedure unsuitable as a screening or early diagnostic tool. Also, it is well-established that tau plasma levels do not correlate with those in CSF. Collectively, our findings suggest that, besides its roles in *MAPT* gene transcription and the transcriptional regulation of glucose metabolism,[27] HMGA1 may also serve as a potential biomarker for the early diagnosis of AD and other tauopathies. As such, the identification of HMGA1 deficiency through straightforward genetic analysis or blood dosage holds promise as a valuable indirect method for early identification of individuals at risk of AD.[24]

Although neuropathological data specific to carriers of the *HMGA1* rs146052672 variant are currently unavailable, single-cell transcriptomic analysis of postmortem AD brain samples from the SEA-AD consortium revealed an inverse relationship between *HMGA1* and *MAPT* gene expression in the cortical MTG. This inverse pattern was particularly evident in various neuronal cell types, but less pronounced in non-neuronal cells, such as oligodendrocytes, and absent in endothelial cells and perivascular macrophages, which do not constitutively express tau (Fig. S1). Moreover, MAPT gene expression remained stable throughout the progression of AD in most neuronal and non-neuronal cell types (Fig. S2), suggesting that the constitutive transcriptional derepression of MAPT, due to HMGA1 gene defects, could ultimately promote tau accumulation over time. Collectively, these findings support a role for HMGA1 deficiency in the pathophysiology of AD, specifically through its contribution to tau dysregulation.

Functionality of the *HMGA1* rs146052672 variant

The biological plausibility of the *HMGA1* gene influencing insulin resistance and glucose metabolism has been documented in previous studies, supporting the results of the present work. The *HMGA1* gene plays a key role in the expression of the *INSR* gene and other genes involved in metabolic control.[18,20,23] In humans, the *HMGA1* gene variant, rs146052672, has been associated with T2D and certain components of metabolic syndrome.[24–26] In the present study, to further support the biological relevance of the rs146052672 variant, we performed immunofluorescence staining *combined with* confocal imaging and Western blot analysis of INSR expression and insulin binding in circulating blood monocytes from AD patients with and without the variant. The molecular functionality of the *HMGA1* variant and its impact on INSR expression were demonstrated by a significant decrease in INSR levels in blood monocytes from wild-type AD patients compared to AD patients with the rs146052672 variant. Based on cell surface immunofluorescence reactivity with an anti-INSR specific antibody, the variant group showed a considerable reduction in cell membrane INSR content (Fig. 7a). As a consequence of the reduction in cell surface INSR, blood monocytes from variant carriers exhibited decreased insulin binding compared to the wild-type group (Fig. 7b). These results were confirmed by Western blot analysis of cellular lysates from circulating monocytes, which showed a significant reduction in INSR protein expression in the variant group compared to the control group ($p < 0.001$) (Fig. 7b). These findings implicate the rs146052672 variant in pathogenicity, leading to impaired INSR expression and insulin resistance in patients with AD.

Therefore, we propose that a deficiency in HMGA1 expression and/or function may lead to tau overexpression and hyperphosphorylation, thereby increasing susceptibility to tau pathology (Fig. 8). Furthermore, our results consistently support the notion that HMGA1 deficiency could represent a common pathogenic link between insulin-resistant conditions and this heterogeneous group of neurodegenerative diseases.

Discussion

Despite the well-known role of tau dysfunction and its intracellular aggregation in a set of neurodegenerative brain diseases collectively known as tauopathies,[1,2] little is known concerning the mechanism(s) involved in the regulation of *MAPT* gene expression. The *MAPT* gene has a complex structural organization involving multiple transcription start sites, alternative promoter sequences, numerous repeated motifs, CpG islands/DNA methylation regions, a large inversion (H1/H2 haplotypes), and a variety of splicing patterns.[51] Also, whereas some sequences in the *MAPT* gene promoter are mainly involved in the repression of gene expression, other elements seem to play a major role in activating gene transcription.[15,37,53–55] So far, numerous mutations and polymorphisms have been identified in the *MAPT* gene,

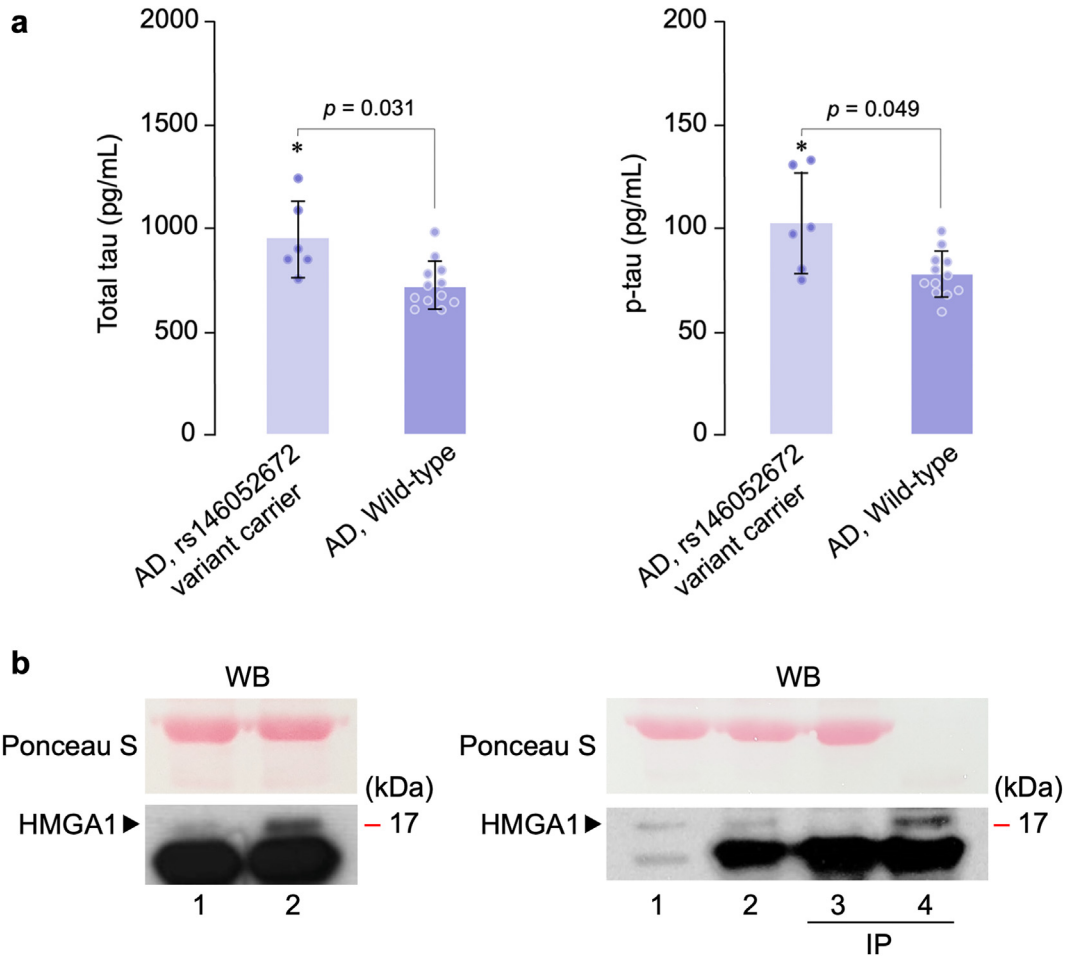

Fig. 6: **Total and p-tau and HMGA1 in CSF from patients with AD**. (**a**) Levels of total tau (left) and p-tau (right) were measured in CSF from patients with AD, carrying the rs146052672 variant (n = 6) and wild-type AD patients (n = 12). (**b**) Left panel: Western blot (WB) of HMGA1 was performed on the CSF pool derived from six AD patients with the rs146052672 variant (lane 1) and twelve age- and sex-matched wild-type AD patients (lane 2), all of whom underwent lumbar puncture at the time of diagnosis. Right panel: immunoprecipitation (IP) of the CSF pool obtained from wild-type AD patients. Lanes: 1, pure CSF; 2, CSF following incubation with protein A/G agarose beads; 3, immunodepleted CSF supernatant following incubation with HMGA1 antibody-coupled protein A/G agarose beads; 4, immunoprecipitated HMGA1 protein following protein A/G-mediated recovery of the immuno-complex. Ponceau S staining was used as total protein loading control. Representative WBs of three independent assays are shown.

some of which have been associated with tauopathies. A number of these pathogenic mutations associated with tauopathy have been found within the *cis*-acting regulatory elements of the *MAPT* gene, including the *MAPT* promoter region.[53,78,79]

The study herein represents the demonstration of a quantitative abnormality in a *trans*-acting regulatory element that regulates the level of expression of the *MAPT* gene. Based on the observations and results in this work, we propose a pathogenic mechanism whereby HMGA1 deficiency, by decreasing its ability to repress the *MAPT* gene promoter, leads to overexpression of tau, which may be hyperphosphorylated, thus promoting tau pathology. This interpretation was supported by experiments in vitro, in SH-SY5Y neuronal cells and in primary cultures of mouse neurons, in which endogenous expression of tau inversely correlated to the level of HMGA1 following cell transfection with either HMGA1 overexpression vector or siRNA against HMGA1. Consistent with these results, in vivo data revealed a significant increase in both mRNA and protein levels of tau in the brains of *Hmga1*-knockout mice in which, concomitant with this increase, there was a reduction in the expression of both InsR and Glut3 proteins. Furthermore, findings from our preliminary case–control study indicate that the *HMGA1* rs146052672 genotype, which has been linked to insulin resistance and T2D,[24–26] was also associated

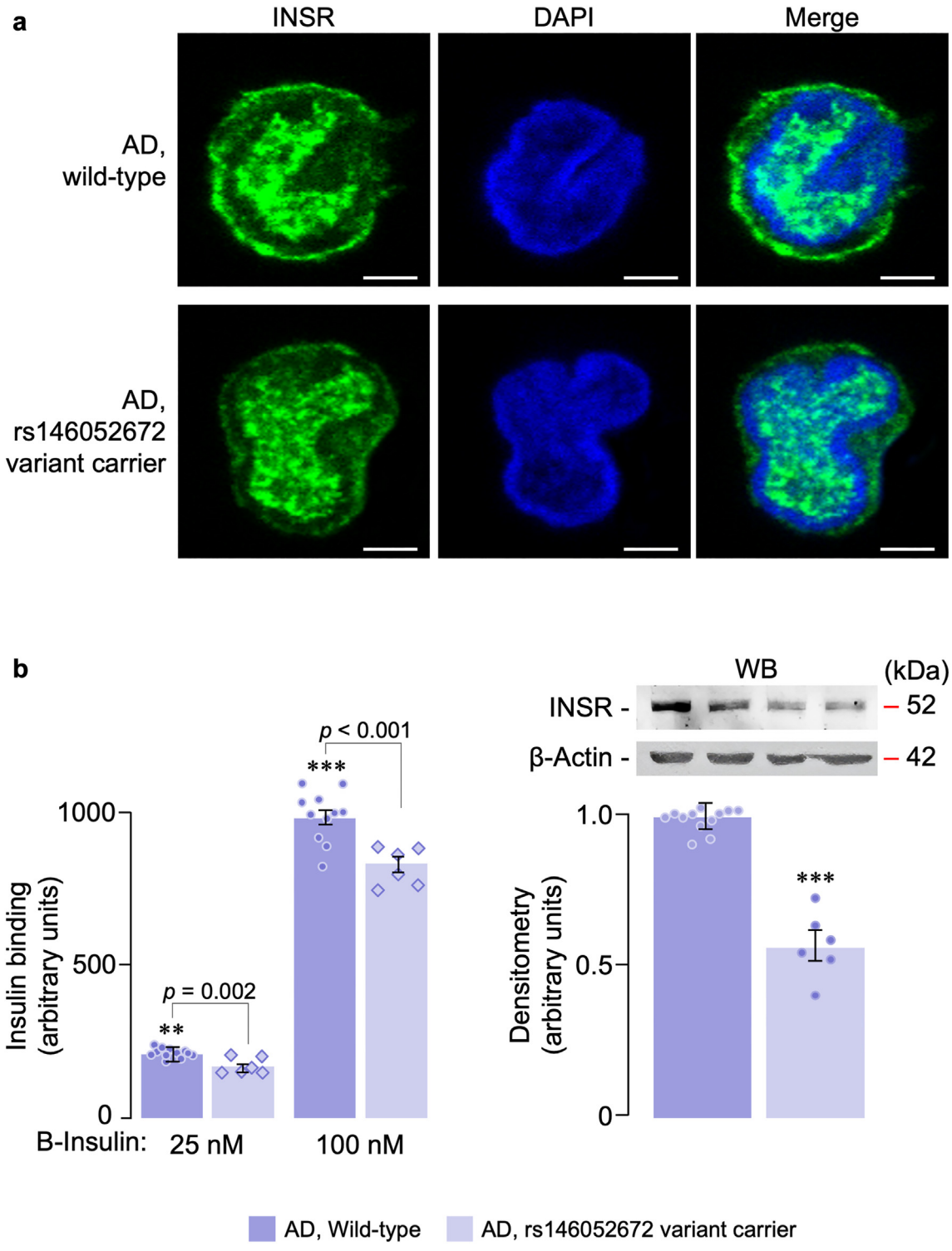

Fig. 7: **INSR expression and insulin binding in circulating blood** monocytes. (**a**) Immunofluorescence of blood monocytes from AD patients, with and without the rs146052672 variant, fixed and stained with anti-INSR antibody. Representative immunofluorescence microscopy images of cell surface INSR protein (green fluorescence), nuclear DAPI (blue fluorescence), and merged signals. Scale bar = 2 µm. (**b**) Left: biotinylated insulin (B-Insulin) binding to the INSR was measured in intact monocytes from AD patients carrying (n = 6) or not carrying (n = 12) the *HMGA1*

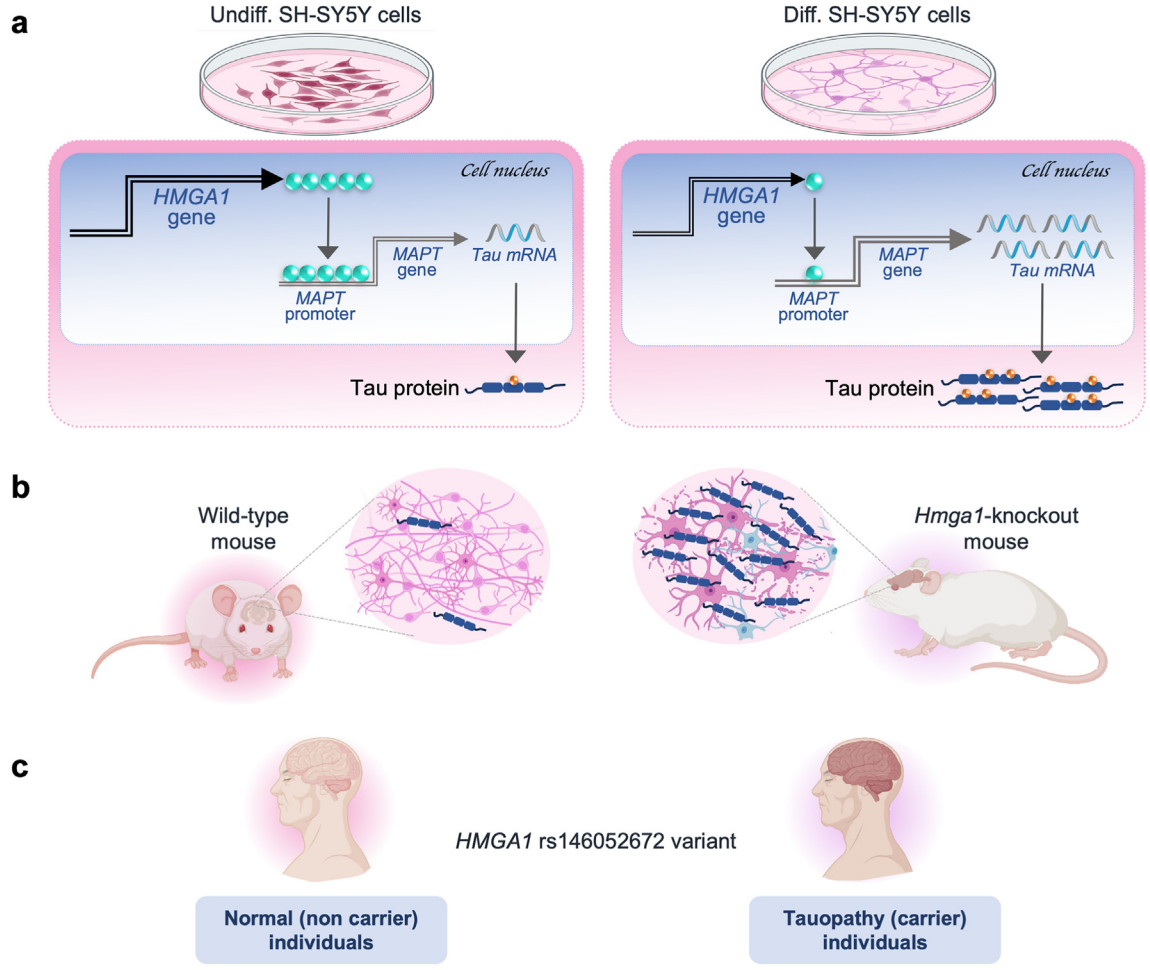

Fig. 8: **Schematic illustration of the proposed model for the role of HMGA1 deficiency in the development of tauopathies.** (**a**) The higher expression of HMGA1 in undifferentiated (Undiff.) SH-SY5Y cells turns off/slows down *MAPT* gene transcription, leading to a decline in tau mRNA and protein levels. Conversely, the decreased binding of HMGA1 to *MAPT* promoter in differentiated (Diff.) SH-SY5Y cells, ramps up *MAPT* gene transcription, with subsequent overexpression of total tau and tau hyperphosphorylation. (**b**) The in vitro model of *MAPT* gene regulation finds support in in vivo studies showing that tau protein expression is increased in the brain of *Hmga1*-knockout mice with signs of neuronal damage. (**c**) In humans, the *HMGA1* rs146052672 variant, capable of reducing the expression levels of HMGA1, is associated with increased risk of tauopathies.

with different tauopathies. Of importance, this association was independent of diabetes, as also supported by the fact that the prevalence of the rs146052672 variant in the investigated population with tauopathies was more than twice that reported previously in an Italian population of patients with T2D.[24] As a limitation of this case–control study –and of the overall study– we acknowledge that the small sample size of clinical participants may reduce the statistical power of our findings and limit the reliability of the results. This also restricts the ability to generalize the findings to a larger population. Despite this limitation, the observed association of the *HMGA1* gene variant with tauopathy was substantiated by clinical data. AD patients carrying the variant exihibited higher levels of total and p-tau in CSF and showed more severe disease progression compared to non-carrier AD patients, further strengthening the case for a genuine relationship between the *HMGA1* gene variant and tauopathy. The absence of non-Caucasian participants also limits the broader applicability of our

variant. Fluorescence intensities are shown in bar graph (mean ± s.e.m.) for each *HMGA1* genotype. Right, INSR expression was measured in monocytes from wild-type AD patient (n = 12) and AD variant carriers (n = 6), by Western blot (WB) analysis. A representative blot is shown, together with densitometric values (mean ± s.e.m). $^{**}p < 0.01$, $^{***}p < 0.001$.

findings. However, single-cell analysis of postmortem brain tissue from AD patients with diverse geographic backgrounds offers additional evidence of the *HMGA1* variant's relevance to tau expression. This suggests that the HMGA1-tau relationship may extend beyond a single population, partially supporting the generalizability of our findings. We perceive the association between HMGA1 deficiency and the risk of neurodegeneration as a strength of our work and a motivation for future research in this area.

To date, there have been only few attempts to investigate how genetic and/or diet-induced insulin resistance and diabetes may affect cognition and memory-related neuronal circuitry in animal models. An increased phosphorylation of tau, leading to the formation of neurofibrillary tangles, has been reported in mice fed a high fat diet.[80] This dietary model, known to mimic many metabolic features of human obesity and T2D, is associated with microscopic neuroanatomical abnormalities linked to tau hyperphosphorylation alongside psychomotor impairment.[81] However, not all rodent studies have consistently reported a correlation between altered glucose homoeostasis, psychomotor alterations, and memory deficits.[82] Possible explanations for this inconsistency include variations in the type or duration of dietary feeding, differences in animal strain or genetic background, as well as discrepancies in target behaviours or the sensitivity of specific tests employed. For instance, within the context of metabolic dysfunction, memory deficits have been documented in leptin-receptor mutant *db/db* mice,[83] but not in leptin-deficient *ob/ob* mice,[84] which exhibit concomitant insulin resistance and hypersensitivity to insulin in peripheral tissues.[85] In the present study, we observed that the *Hmga1*-knockout mice exhibit abnormal spontaneous behaviours, including apathetic traits and decreased reactivity to a novel environment. These behaviours mimic the clinical symptoms and neuropathological features of patients with tauopathy. Notably, these alterations in psychomotor function manifest at a relatively early stage of the animals' lifespan (9–10 months). This finding aligns with recent observations of psychomotor behavioural disturbances and changes in brain histology in older mice lacking the homeodomain-interacting protein kinase 2 (HIPK2), a serine/threonine kinase involved in phosphorylating HMGA1 and modulating its DNA-binding affinity.[86,87] In *Hipk2*-knockout mice, the reduced phosphorylation of HMGA1 and its consequent increased affinity for DNA may lead to the repression of the *MAPT* gene, thereby explaining the delayed appearance of neuronal morphological changes and loss.[86]

Previous studies have shown that heterozygous *presenilin 1* and *presenilin 2* gene mutations are associated with the autosomal-dominant familial forms of AD. *Presenilin* gene variants have also been reported in sporadic forms of AD,[88,89] in which some of these variants seemed to be associated to an increased formation of amyloid plaques, a histological hallmark of AD, beside neurofibrillary tangles and microvascular lesions.[89] In this context, it is interesting to note that HMGA1 plays a role in controlling presenilin 2 mRNA splicing, and there is evidence that overexpression of the HMGA1a isoform may lead to an aberrant splicing process of the presenilin 2 pre-mRNA, which has been involved in neurodegenerative disorders, including AD.[30] Our work further underlines the importance of HMGA1 in neuronal physiology and pathophysiology and suggests that decreased expression of HMGA1 may predispose to neurodegenerative disorders also through mechanisms independent of amyloid plaque formation. In this regard, it has been recently demonstrated that a rare gain-of-function variant in the *RELN* gene, by negatively affecting tau phosphorylation, reduced the accumulation of tau aggregates and neurofibrillary tangles in a patient with concomitant *PSEN1* mutation and excessive amyloid plaque formation, who developed an extreme phenotype of delayed AD.[90]

Currently, the treatment of tauopathies is still a major problem and a great challenge for public health, as demonstrated by the limited success of clinical trials exploring therapeutic interventions to reduce tau protein expression and aggregation.[91] This emphasizes the need for a deeper understanding of tau biology and the development of novel therapeutic strategies. One promising approach in this regard involves the use of antisense oligonucleotides targeting MAPT mRNA to reduce tau protein in neurons of affected patients. This innovative technique has shown promise in reducing CSF total and p-tau levels in both animal models of tauopathy and AD subjects.[92,93] As gene silencing therapy has been shown to be effective in reducing tau levels in neurological disorders, it becomes crucial to identify individuals who would benefit the most from this approach. This may entail investigating patients carrying the *HMGA1* rs146052672 variant, as they are predisposed to tau pathology. Moreover, research into innovative therapies for tauopathies could explore interventions aimed at increasing HMGA1 expression as a possible strategy to lower *MAPT* gene transcription. These considerations are supported by our observation that patients with tauopathy carrying the *HMGA1* rs146052672 variant exhibit worse clinical outcomes and respond differently to currently available therapies compared to non-carries.

In conclusion, our study highlights HMGA1 as a key regulator of *MAPT* and *INSR* genes in both human and mouse species. Furthermore, our data indicate that deficiencies in HMGA1 production or function, as observed in patients with the *HMGA1* rs146052672 variant, may lead to increased tau expression and phosphorylation, thereby contributing to tau pathology. While further research is needed to fully elucidate the causal relationship between HMGA1 deficiency and

tauopathies, our findings suggest a shared pathogenic link between tau pathology and insulin-resistant states through HMGA1 downregulation. This sheds light on a potential mechanism underlying the frequent clinical association between these metabolic and neurodegenerative disorders. We believe that further exploration aimed at unravelling still unexplored HMGA1-dependent mechanisms within the brain will not only advance our understanding of fundamental biology but also have implications for the diagnosis and treatment of these related conditions.

Contributors

M. Mirabelli and E. Chiefari contributed equally to this study and share first-authorship position. M. Mirabelli conducted transfection studies, Western blotting, secretome, oxidative stress and immunoprecipitation assays. E. Chiefari collected and analysed research data. Both, M. Mirabelli and E. Chiefari wrote the manuscript draft. B. Arcidiacono performed EMSA experiments and genotyping of patients. A. Salatino performed immunofluorescence staining of SH-SY5Y cells and ChIP experiments. A. Pascarella and M. Morelli provided clinical care to the study's participants and performed clinical and biochemical characterisation of patients. S.C. Credendino, A. Greco, A. Di Vito, G.M. Pierantoni and M. Fedele were involved in animal work and related experiments. F.S. Brunetti contributed to data analysis and manuscript writing. V. Huin and F. Nicoletti contributed materials and revised the manuscript draft. U. Aguglia contributed human samples. D.P. Foti and A. Brunetti jointly supervised the study and contributed to interpretation of results. A. Brunetti conceived the study and edited the manuscript. A. Brunetti, D.P. Foti, M. Mirabelli and E. Chiefari had access to the data and verified it. All authors read and approved the final version of the manuscript.

Data sharing statement

Detailed information on the experimental protocols and materials supporting the findings of this study is available upon request to the corresponding authors. To ensure participant privacy, human data from this study has not been deposited in external repositories nor made publicly accessible. However, anonymised raw data may be shared with qualified academic researchers upon reasonable request, solely for the purpose of replicating the findings described in this article. Data sharing is subject to compliance with local legislation and approval by the local Ethics committee.

Declaration of interests

The authors declare no competing interests.

Acknowledgements

We wish to thank Dr. R. de Silva (University College London) for kindly providing the human *MAPT*-Luc reporter plasmid containing the C region of the promoter gene. This research was supported by grants from the Italian Ministry of University and Research (MUR-PRIN 2022, prot. 202279JKW5, to A. Brunetti), and, in part, by the Italian Ministry of Health (WFR PNRR-MCNT2-2023-12377884, to A. Brunetti).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2025.105700.

References

1 Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. *Nature*. 1997;388(6645):839–840.
2 Delacourte A. Tau pathology and neurodegeneration: an obvious but misunderstood link. *J Alzheimers Dis*. 2008;14(4):437–440.
3 Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW. A protein factor essential for microtubule assembly. *Proc Natl Acad Sci U S A*. 1975;72(5):1858–1862.
4 Alonso AD, Cohen LS. Our tau tales from normal to pathological behavior. *J Alzheimers Dis*. 2018;64(s1):S507–S516.
5 Ballatore C, Lee VM, Trojanowski JQ. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nat Rev Neurosci*. 2007;8(9):663–672.
6 Hutton M, Lendon CL, Rizzu P, et al. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature*. 1998;393(6686):702–705.
7 Spillantini MG, Murrell JR, Goedert M, Farlow MR, Klug A, Ghetti B. Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc Natl Acad Sci U S A*. 1998;95(13):7737–7741.
8 Chen Q, Boeve BF, Schwarz CG, et al. Tracking white matter degeneration in asymptomatic and symptomatic MAPT mutation carriers. *Neurobiol Aging*. 2019;83:54–62.
9 Cruts M, Theuns J, Van Broeckhoven C. Locus-specific mutation databases for neurodegenerative brain diseases. *Hum Mutat*. 2012;33(9):1340–1344.
10 Spillantini MG, Goedert M. Tau pathology and neurodegeneration. *Lancet Neurol*. 2013;12(6):609–622.
11 Kouri N, Ross OA, Dombroski B, et al. Genome-wide association study of corticobasal degeneration identifies risk variants shared with progressive supranuclear palsy. *Nat Commun*. 2015;6:7247.
12 Chen J, Yu JT, Wojta K, et al. Genome-wide association study identifies *MAPT* locus influencing human plasma tau levels. *Neurology*. 2017;88(7):669–676.
13 Gallo D, Ruiz A, Sánchez-Juan P. Genetic architecture of primary tauopathies. *Neuroscience*. 2023;518:27–37.
14 Corces MR, Shcherbina A, Kundu S, et al. Single-cell epigenomic analyses implicate candidate causal variants at inherited risk loci for Alzheimer's and Parkinson's diseases. *Nat Genet*. 2020;52(11):1158–1168.
15 Gao L, Tucker KL, Andreadis A. Transcriptional regulation of the mouse microtubule-associated protein tau. *Biochim Biophys Acta*. 2005;1681(2–3):175–181.
16 de Silva R, Weiler M, Morris HR, Martin ER, Wood NW, Lees AJ. Strong association of a novel Tau promoter haplotype in progressive supranuclear palsy. *Neurosci Lett*. 2001;311(3):145–148.
17 Reeves R, Beckerbauer L. HMGI/Y proteins: flexible regulators of transcription and chromatin structure. *Biochim Biophys Acta*. 2001;1519(1–2):13–29.
18 Brunetti A, Manfioletti G, Chiefari E, Goldfine ID, Foti D. Transcriptional regulation of human insulin receptor gene by the high-mobility group protein HMGI(Y). *FASEB J*. 2001;15(2):492–500.
19 Chiefari E, Iiritano S, Paonessa F, et al. Pseudogene-mediated posttranscriptional silencing of HMGA1 can result in insulin resistance and type 2 diabetes. *Nat Commun*. 2010;1:40.
20 Chiefari E, Foti DP, Sgarra R, et al. Transcriptional regulation of glucose metabolism: the emerging role of the HMGA1 chromatin factor. *Front Endocrinol (Lausanne)*. 2018;9:357.
21 Chiefari E, Paonessa F, Iiritano S, et al. The cAMP-HMGA1-RBP4 system: a novel biochemical pathway for modulating glucose homeostasis. *BMC Biol*. 2009;7:24.
22 Semple RK. From bending DNA to diabetes: the curious case of HMGA1. *J Biol*. 2009;8(7):64.
23 Foti D, Chiefari E, Fedele M, et al. Lack of the architectural factor HMGA1 causes insulin resistance and diabetes in humans and mice. *Nat Med*. 2005;11(7):765–773.
24 Chiefari E, Tanyolaç S, Paonessa F, et al. Functional variants of the HMGA1 gene and type 2 diabetes mellitus. *JAMA*. 2011;305(9):903–912.
25 Pullinger CR, Goldfine ID, Tanyolaç S, et al. Evidence that an HMGA1 gene variant associates with type 2 diabetes, body mass index, and high-density lipoprotein cholesterol in a Hispanic-American population. *Metab Syndr Relat Disord*. 2014;12(1):25–30.
26 Chiefari E, Tanyolaç S, Iiritano S, et al. A polymorphism of HMGA1 is associated with increased risk of metabolic syndrome and related components. *Sci Rep*. 2013;3:1491.
27 Arce-Cerezo A, García M, Rodríguez-Nuevo A, et al. HMGA1 overexpression in adipose tissue impairs adipogenesis and prevents diet-induced obesity and insulin resistance. *Sci Rep*. 2015;5:14487.
28 Wei Z, Koya J, Reznik SE. Insulin resistance exacerbates Alzheimer disease via multiple mechanisms. *Front Neurosci*. 2021;15:687157.
29 Baglietto-Vargas D, Shi J, Yaeger DM, Ager R, LaFerla FM. Diabetes and Alzheimer's disease crosstalk. *Neurosci Biobehav Rev*. 2016;64:272–287.
30 Manabe T, Ohe K, Katayama T, et al. HMGA1a: sequence-specific RNA-binding factor causing sporadic Alzheimer's disease-linked

exon skipping of presenilin-2 pre-mRNA. *Genes Cells*. 2007;12(10):1179–1191.
31 Ohe K, Mayeda A. HMGA1a trapping of U1 snRNP at an authentic 5' splice site induces aberrant exon skipping in sporadic Alzheimer's disease. *Mol Cell Biol*. 2010;30(9):2220–2228.
32 Kim B, Figueroa-Romero C, Pacut C, Backus C, Feldman EL. Insulin resistance prevents AMPK-induced tau dephosphorylation through Akt-mediated increase in AMPKSer-485 phosphorylation. *J Biol Chem*. 2015;290(31):19146–19157.
33 Gonçalves RA, Wijesekara N, Fraser PE, De Felice FG. The link between tau and insulin signaling: implications for Alzheimer's disease and other tauopathies. *Front Cell Neurosci*. 2019;13:17.
34 Zhang Y, Wu KM, Yang L, Dong Q, Yu JT. Tauopathies: new perspectives and challenges. *Mol Neurodegener*. 2022;17(1):28.
35 Brunetti A, Foti D, Goldfine ID. Identification of unique nuclear regulatory proteins for the insulin receptor gene, which appear during myocyte and adipocyte differentiation. *J Clin Invest*. 1993;92(3):1288–1295.
36 Thomas-Chollier M, Hufton A, Heinig M, et al. Transcription factor binding predictions using TRAP for the analysis of ChIP-seq data and regulatory SNPs. *Nat Protoc*. 2011;6(12):1860–1869.
37 Huin V, Buée L, Behal H, Labreuche J, Sablonnière B, Dhaenens CM. Alternative promoter usage generates novel shorter MAPT mRNA transcripts in Alzheimer's disease and progressive supranuclear palsy brains. *Sci Rep*. 2017;7(1):12589.
38 Gabitto MI, Travaglini KJ, Rachleff VM, et al. Integrated multimodal cell atlas of Alzheimer's disease. *Nat Neurosci*. 2024;27(12):2366–2383.
39 Copani A, Condorelli F, Caruso A, et al. Mitotic signaling by beta-amyloid causes neuronal death. *FASEB J*. 1999;13(15):2225–2234.
40 Charan J, Kantharia ND. How to calculate sample size in animal studies? *J Pharmacol Pharmacother*. 2013;4(4):303–306.
41 Credendino SC, Lewin N, de Oliveira M, et al. Tissue- and cell type-specific expression of the long noncoding RNA Klhl14-AS in mouse. *Int J Genomics*. 2017;9769171.
42 McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of department of health and human services task force on Alzheimer's disease. *Neurology*. 1984;34(7):939–944.
43 Höglinger GU, Respondek G, Stamelou M, et al. Clinical diagnosis of progressive supranuclear palsy: the movement disorder society criteria. *Mov Disord*. 2017;32(6):853–864.
44 Neary D, Snowden JS, Gustafson L, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology*. 1998;51(6):1546–1554.
45 Rascovsky K, Hodges JR, Knopman D, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain*. 2011;134(Pt 9):2456–2477.
46 Sakae N, Josephs KA, Litvan I, et al. Neuropathologic basis of frontotemporal dementia in progressive supranuclear palsy. *Mov Disord*. 2019;34(11):1655–1662.
47 Bruno F, Laganà V, Di Lorenzo R, Bruni AC, Maletta R. Calabria as a genetic isolate: a model for the study of neurodegenerative diseases. *Biomedicines*. 2022;10(9):2288.
48 Agholme L, Lindström T, Kågedal K, Marcusson J, Hallbeck M. An in vitro model for neuroscience: differentiation of SH-SY5Y cells into cells with morphological and biochemical characteristics of mature neurons. *J Alzheimers Dis*. 2010;20(4):1069–1082.
49 Bagaméry F, Varga K, Kecsmár K, Vincze I, Szökő É, Tábi T. The impact of differentiation on cytotoxicity and insulin sensitivity in streptozotocin treated SH-SY5Y cells. *Neurochem Res*. 2021;46(6):1350–1358.
50 Bartolome F, Carro E, Alquezar C. Oxidative stress in tauopathies: from cause to therapy. *Antioxidants*. 2022;11(8):1421.
51 Caillet-Boudin ML, Buée L, Sergeant N, Lefebvre B. Regulation of human MAPT gene expression. *Mol Neurodegener*. 2015;10:28.
52 Hernández F, Merchán-Rubira J, Vallés-Saiz L, Rodríguez-Matellán A, Avila J. Differences between human and murine tau at the N-terminal End. *Front Aging Neurosci*. 2020;12:11.
53 Anaya F, Lees A, de Silva R. Tau gene promoter rs242557 and allele-specific protein binding. *Translat Neurosci*. 2011;2:176–205.
54 Myers AJ, Pittman AM, Zhao AS, et al. The MAPT H1c risk haplotype is associated with increased expression of tau and especially of 4 repeat containing transcripts. *Neurobiol Dis*. 2007;25(3):561–570.
55 Andreadis A, Wagner BK, Broderick JA, Kosik KS. A tau promoter region without neuronal specificity. *J Neurochem*. 1996;66(6):2257–2263.
56 Baron RM, Lopez-Guzman S, Riascos DF, et al. Distamycin A inhibits HMGA1-binding to the P-selectin promoter and attenuates lung and liver inflammation during murine endotoxemia. *PLoS One*. 2010;5(5):e10656.
57 Shubin AV, Demidyuk IV, Komissarov AA, Rafieva LM, Kostrov SV. Cytoplasmic vacuolization in cell death and survival. *Oncotarget*. 2016;7(34):55863–55889.
58 Govindpani K, McNamara LG, Smith NR, et al. Vascular dysfunction in Alzheimer's disease: a prelude to the pathological process or a consequence of it? *J Clin Med*. 2019;8(5):651.
59 Schmidt S, Holzer M, Arendt T, Sonntag M, Morawski M. Tau protein modulates perineuronal extracellular matrix expression in the TauP301L-acan mouse model. *Biomolecules*. 2022;12(4):505.
60 Tcw J, Goate AM. Genetics of β-amyloid precursor protein in Alzheimer's disease. *Cold Spring Harb Perspect Med*. 2017;7(6):a024539.
61 Takahashi M, Miyata H, Kametani F, et al. Extracellular association of APP and tau fibrils induces intracellular aggregate formation of tau. *Acta Neuropathol*. 2015;129(6):895–907.
62 Guo Y, Wang Q, Chen S, Xu C. Functions of amyloid precursor protein in metabolic diseases. *Metabolism*. 2021;115:154454.
63 Pereira JB, Janelidze S, Smith R, et al. Plasma GFAP is an early marker of amyloid-β but not tau pathology in Alzheimer's disease. *Brain*. 2021;144(11):3505–3516.
64 Iacono D, O'Brien R, Resnick SM, et al. Neuronal hypertrophy in asymptomatic Alzheimer disease. *J Neuropathol Exp Neurol*. 2008;67(6):578–589.
65 Robinson L, Dreesen E, Mondesir M, Harrington C, Wischik C, Riedel G. Apathy-like behaviour in tau mouse models of Alzheimer's disease and frontotemporal dementia. *Behav Brain Res*. 2024;456:114707.
66 Small GW, Ercoli LM, Silverman DH, et al. Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2000;97(11):6037–6042.
67 Nagamatsu S, Kornhauser JM, Burant CF, Seino S, Mayo KE, Bell GI. Glucose transporter expression in brain. cDNA sequence of mouse GLUT3, the brain facilitative glucose transporter isoform, and identification of sites of expression by in situ hybridization. *J Biol Chem*. 1992;267(1):467–472.
68 Liu Y, Liu F, Iqbal K, Grundke-Iqbal I, Gong CX. Decreased glucose transporters correlate to abnormal hyperphosphorylation of tau in Alzheimer disease. *FEBS Lett*. 2008;582(2):359–364.
69 Ha TK, Her NG, Lee MG, et al. Caveolin-1 increases aerobic glycolysis in colorectal cancers by stimulating HMGA1-mediated GLUT3 transcription. *Cancer Res*. 2012;72(16):4097–4109.
70 Cheng CM, Reinhardt RR, Lee WH, Joncas G, Patel SC, Bondy CA. Insulin-like growth factor 1 regulates developing brain glucose metabolism. *Proc Natl Acad Sci U S A*. 2000;97(18):10236–10241.
71 Di Cola G, Cool MH, Accili D. Hypoglycemic effect of insulin-like growth factor-1 in mice lacking insulin receptors. *J Clin Invest*. 1997;99(10):2538–2544.
72 Chiefari E, Ventura V, Capula C, et al. A polymorphism of HMGA1 protects against proliferative diabetic retinopathy by impairing HMGA1-induced VEGFA expression. *Sci Rep*. 2016;6:39429.
73 Arnold SE, Arvanitakis Z, Macauley-Rambach SL, et al. Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. *Nat Rev Neurol*. 2018;14(3):168–181.
74 https://www.cittadinanzattiva.it/multimedia/import/files/comunicati/salute/Seconda_Indagine_civica_sul_Diabete_Abstract_def.pdf. Accessed February 19, 2025.
75 Jack CR Jr, Bennett DA, Blennow K, et al. NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement*. 2018;14(4):535–562.
76 Hampel H, Blennow K. CSF tau and β-amyloid as biomarkers for mild cognitive impairment. *Dialogues Clin Neurosci*. 2004;6(4):379–390.
77 Jia J, Ning Y, Chen M, et al. Biomarker changes during 20 years preceding Alzheimer's disease. *N Engl J Med*. 2024;390(8):712–722.
78 Pastor P, Moreno F, Clarimón J, et al. MAPT H1 haplotype is associated with late-onset Alzheimer's disease risk in APOEε4 noncarriers: results from the dementia genetics Spanish Consortium. *J Alzheimers Dis*. 2016;49(2):343–352.
79 Niblock M, Gallo JM. Tau alternative splicing in familial and sporadic tauopathies. *Biochem Soc Trans*. 2012;40(4):677–680.
80 Kothari V, Luo Y, Tornabene T, et al. High fat diet induces brain insulin resistance and cognitive impairment in mice. *Biochim Biophys Acta Mol Basis Dis*. 2017;1863(2):499–508.

81 Arnold SE, Lucki I, Brookshire BR, et al. High fat diet produces brain insulin resistance, synaptodendritic abnormalities and altered behavior in mice. *Neurobiol Dis*. 2014;67:79–87.
82 Boitard C, Etchamendy N, Sauvant J, et al. Juvenile, but not adult exposure to high-fat diet impairs relational memory and hippocampal neurogenesis in mice. *Hippocampus*. 2012;22(11):2095–2100.
83 Li XL, Aou S, Oomura Y, Hori N, Fukunaga K, Hori T. Impairment of long-term potentiation and spatial memory in leptin receptor-deficient rodents. *Neuroscience*. 2002;113(3):607–615.
84 Finger BC, Dinan TG, Cryan JF. Leptin-deficient mice retain normal appetitive spatial learning yet exhibit marked increases in anxiety-related behaviours. *Psychopharmacology (Berl)*. 2010;210(4):559–568.
85 Chlouverakis C, White PA. Obesity and insulin resistance in the obese-hyperglycemic mouse (obob). *Metabolism*. 1969;18(12):998–1006.
86 De Biase D, Valente V, Conte A, et al. Phenotypic effects of homeodomain-interacting protein kinase 2 deletion in mice. *Int J Mol Sci*. 2021;22(15):8294.
87 Zhang Q, Wang Y. Homeodomain-interacting protein kinase-2 (HIPK2) phosphorylates HMGA1a at Ser-35, Thr-52, and Thr-77 and modulates its DNA binding affinity. *J Proteome Res*. 2007;6(12):4711–4719.
88 De Strooper B, Saftig P, Craessaerts K, et al. Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature*. 1998;391(6665):387–390.
89 Weggen S, Diehlmann A, Buslei R, Beyreuther K, Bayer TA. Prominent expression of presenilin-1 in senile plaques and reactive astrocytes in Alzheimer's disease brain. *Neuroreport*. 1998;9(14):3279–3283.
90 Lopera F, Marino C, Chandrahas AS, et al. Resilience to autosomal dominant Alzheimer's disease in a Reelin-COLBOS heterozygous man. *Nat Med*. 2023;29(5):1243–1252.
91 Samudra N, Lane-Donovan C, VandeVrede L, Boxer AL. Tau pathology in neurodegenerative disease: disease mechanisms and therapeutic avenues. *J Clin Invest*. 2023;133(12):e168553.
92 DeVos SL, Miller RL, Schoch KM, et al. Tau reduction prevents neuronal loss and reverses pathological tau deposition and seeding in mice with tauopathy. *Sci Transl Med*. 2017;9(374):eaag0481.
93 Mummery CJ, Börjesson-Hanson A, Blackburn DJ, et al. Tau-targeting antisense oligonucleotide MAPTRx in mild Alzheimer's disease: a phase 1b, randomized, placebo-controlled trial. *Nat Med*. 2023;29(6):1437–1447.