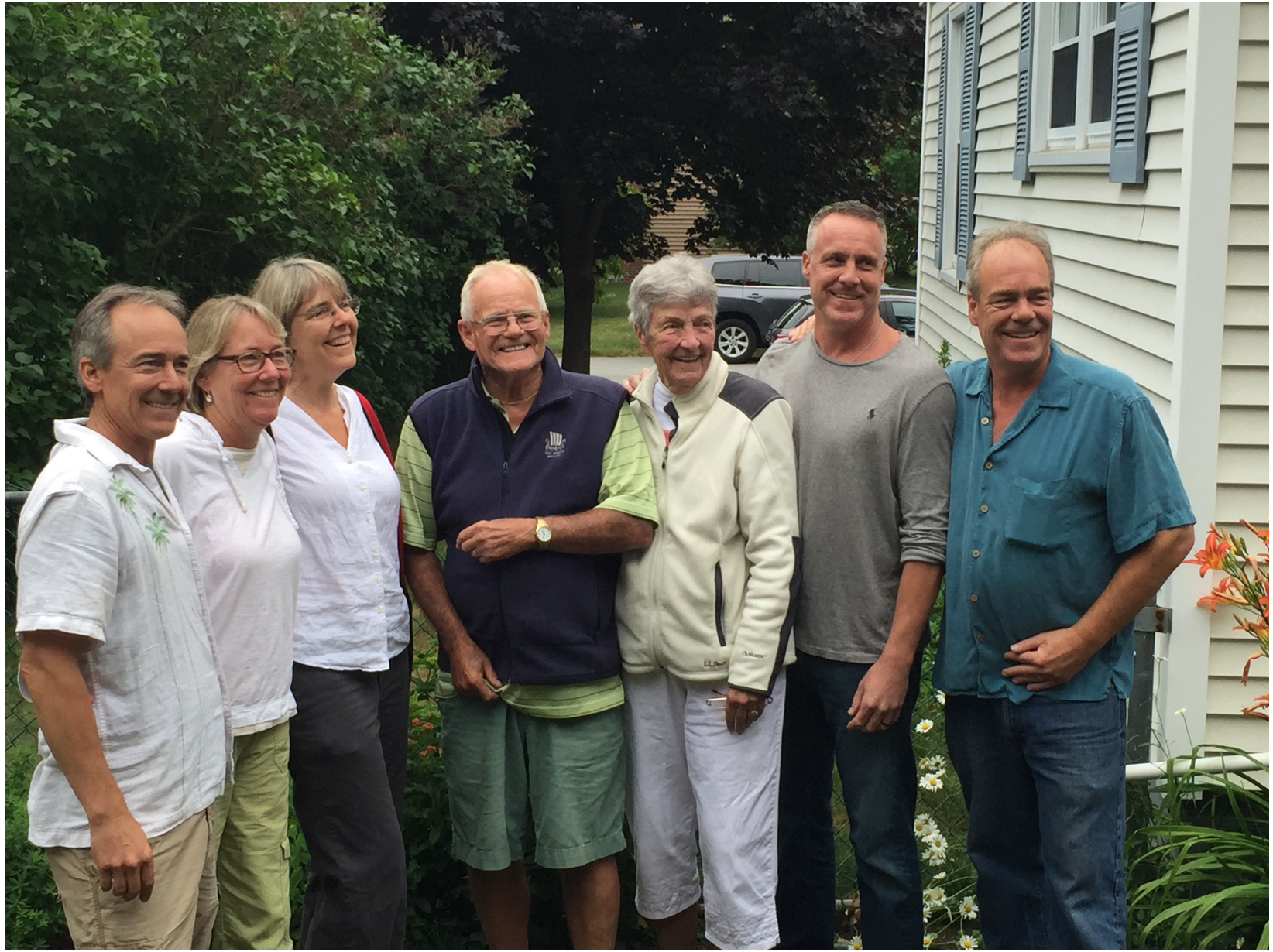
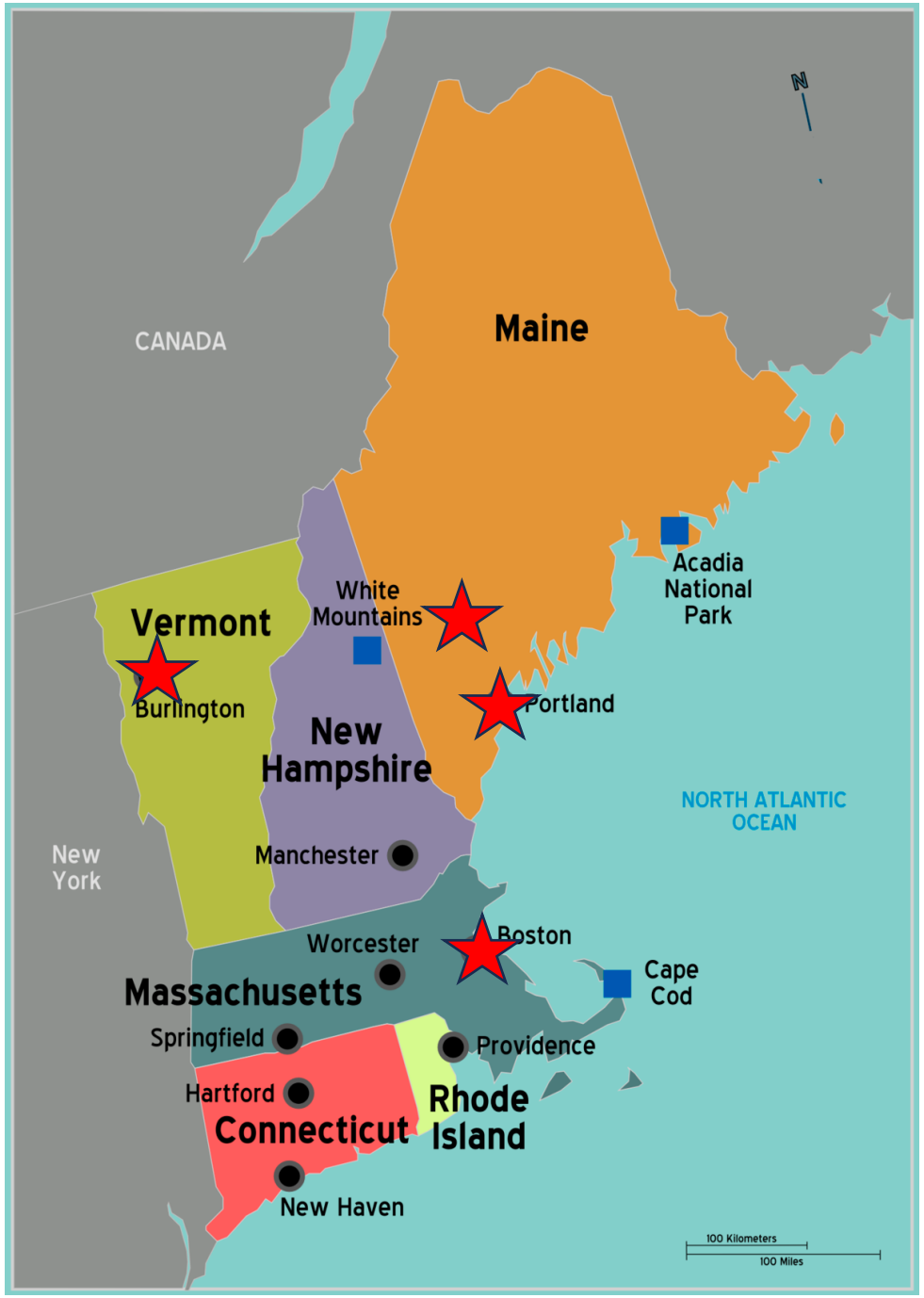
Wednesday June 5 Seminar





Portland Maine to Lewiston Maine
To Boston Massachusetts
To Burlington Vermont
To Boston Massachusetts
To Brunswick Maine….


United States of America
State Boundary
Road
River
National Capital
State Capital
City or Town
300 KM
300 Miles
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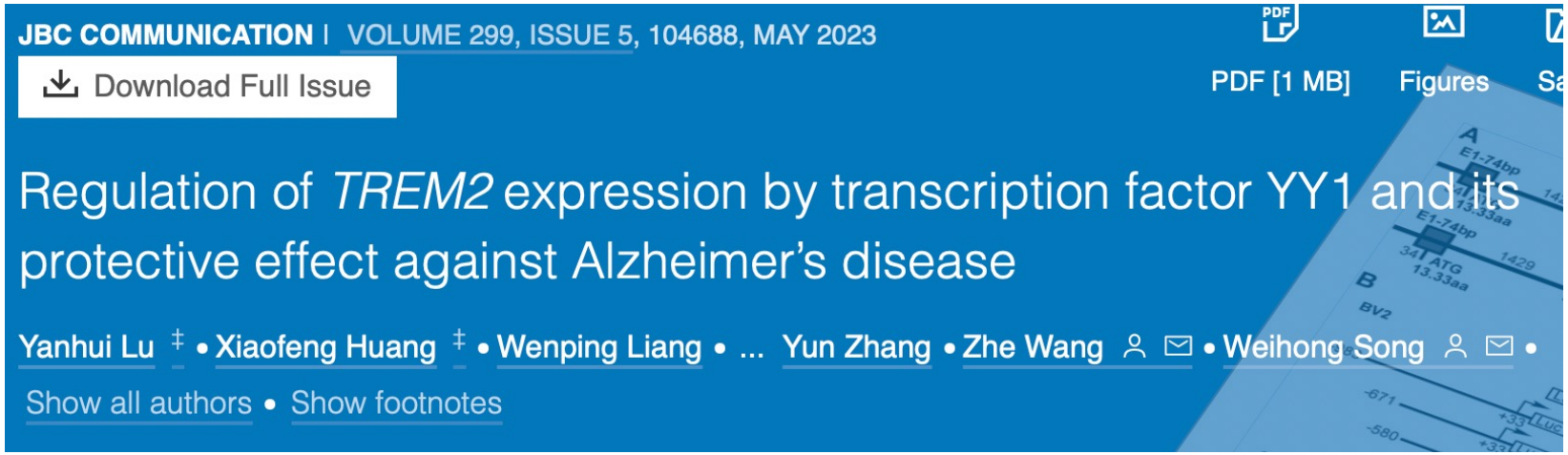
Hermosillo
Chihuahua
Saltillo
Monterrey
MEXICO
Pacific Ocean
Atlantic Ocean
Gulf of Mexico
Missouri
Mississippi
Colorado
Arkansas
Canadian
Red
Pecos
Rio Grande
Ohio
RUSSIA
ALASKA
CANADA
Anchorage
Juneau
500 KM
500 Miles
HAWAII
Honolulu
100 KM
100 Miles

Be Brave, Not Perfect

How to Read a Scientific Paper



Google:
Journal of Biological Chemistry TRSM2 2023

Primary Literature vs Review Article

- Original data done by that group
- Look for data figures
- Look for a Methods section
- Look for the sections of a research article

- A summary of the work on a topic from many groups
- No original data
- No Methods section
- Often lots of diagrams vs data figures

Understand the Structure of a Primary Literature Article

- **Title** - Key terms, the system/molecules/result
- **Abstract** – A summary of the paper
- **Introduction** – Background, current state of knowledge, the question being asked
- **Methods** – methods used for experiments
- **Results** – what was found
- **Discussion** – How these results fit into the current state of knowledge, how these results advance knowledge, and what the result means
- **Supplemental Materials**
- **References**

Understand Your Purpose

- Background knowledge?
- Understanding a method?
- Using a particular method?
- Replicating a result?

You will read a paper MULTIPLE TIMES.
You will NOT read it straight from beginning to end.

1. Skim the article. Get a sense of the terms, the structure. Read the abstract, the end of the intro, the end of the discussion
2. Reread the abstract. Read the Introduction. Read the Discussion, focusing on the beginning and the end.
    1. Note and look up terms you don't know
3. Go through the Results section, highlighting the Figures; look at the subheadings.
4. Look at the Methods, focusing only on the subheadings.
    1. Look up methods that are not familiar to you, and stay away from the nitty gritty details
5. Reread the abstract, focusing on the results statements, then read each Results section:
    1. Figure 1 text with Figure 1 images
    2. Figure 2 text with Figure 2 images, etc.
    3. You may be looking at Methods at this time too
    4. When you finish the results section, then read the entire Discussion.
6. Go back and now read the entire paper, focusing on the reasons for doing various experiments and the findings for each, and then the conclusions. Pay attention to the flow and the focus.

Make note of any questions you have AS YOU READ!

JBC COMMUNICATION

Regulation of *TREM2* expression by transcription factor YY1 and its protective effect against Alzheimer's disease

Received for publication, January 2, 2023, and in revised form, March 31, 2023 Published, Papers in Press, April 11, 2023,
https://doi.org/10.1016/j.jbc.2023.104688

Yanhui Lu[1,‡], Xiaofeng Huang[1,‡], Wenping Liang[1], Yu Li[1], Mengen Xing[2], Wenhao Pan[2], Yun Zhang[1], Zhe Wang[1,*], and Weihong Song[1,2,*]

From the [1]The National Clinical Research Center for Geriatric Disease, Xuanwu Hospital, Capital Medical University, Beijing, China; [2]Zhejiang Provincial Clinical Research Center for Mental Disorders, School of Mental Health and The Affiliated Wenzhou Kangning Hospital, Institute of Aging, Key Laboratory of Alzheimer's Disease of Zhejiang Province, Wenzhou Medical University, Oujiang Laboratory (Zhejiang Lab for Regenerative Medicine, Vision and Brain Health), Wenzhou, Zhejiang, China

Reviewed by members of the JBC Editorial Board. Edited by Craig Cameron

TREM2 **encoding the transmembrane receptor protein TREM2 is a risk gene of Alzheimer's disease (AD), and the impairment of TREM2 functions in microglia due to mutations in *TREM2* may significantly increase the risk of AD by promoting AD pathologies. However, how the expression of *TREM2* is regulated and the transcription factors required for *TREM2* expression are largely unknown. By luciferase assay, DNA pull-down, and *in silico* predictions, we identified Yin Yang 1(YY1) as a binding protein of the minimal promoter of the *TREM2* gene, and the binding was further confirmed by EMSA and DNA pull-down assay. shRNA-mediated *YY1* silencing significantly reduced the activity of the *TREM2* minimal promoter and TREM2 protein levels in the microglial cell line BV2 and the neuroblastoma Neuro2A. Furthermore, we found that the levels of TREM2 and YY1 were both downregulated in lipopolysaccharide-treated BV2 cells and in the brain of AD model mice. These results demonstrated that YY1 plays a crucial role in the regulation of *TREM2* expression. Our study suggests that microglial YY1 could be targeted to maintain *TREM2* expression for AD prevention and therapy.**

binding protein TYROBP (or DAP12) and is as such involved in a variety of cellular functions (3, 4). TREM2 in microglia is required for the regulation of immune responses and phagocytosis that are closely related to AD pathogenesis (5–8).

Case-control studies revealed several rare mutations in *TREM2* gene increase the risk of AD. The carriers of the best characterized p.Arg47His in *TREM2* are 2.83 times more prone to AD, although the association is only confirmed in European population (9, 10). Functional studies suggest that these *TREM2* gene variations cause loss-of-function of TREM2 protein, resulting in not only AD but also other disorders (11, 12). TREM2 can be cleaved by ADAM10 and ADAM17 in the extracellular/intraluminal domain to release the C terminally truncated soluble TREM2 (sTREM2) into the interstitial or cerebrospinal fluid in the brain (13). The increased sTREM2 in the cerebrospinal fluid of early stage of AD could be due to enhanced cleavage, which may reduce functional TREM2 at the cell surface (13–16).

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- TREM2 is expressed and that expression is regulated
- Expression regulated by YY1, which is a transcription factor
- Regulating that expression has a protective effect against Alzheimer's

- What is TREM2?
- How does YY1 regulate TREM2 expression?
- How do they measure a protective effect?

Abstract

TREM2 encoding the transmembrane receptor protein TREM2 is a risk gene of Alzheimer's disease (AD), and the impairment of TREM2 functions in microglia due to mutations in *TREM2* may significantly increase the risk of AD by promoting AD pathologies. However, how the expression of *TREM2* is regulated and the transcription factors required for *TREM2* expression are largely unknown. By luciferase assay, DNA pull-down, and *in silico* predictions, we identified Yin Yang 1(YY1) as a binding protein of the minimal promoter of the *TREM2* gene, and the binding was further confirmed by EMSA and DNA pull-down assay. shRNA-mediated *YY1* silencing significantly reduced the activity of the *TREM2* minimal promoter and TREM2 protein levels in the microglial cell line BV2 and the neuroblastoma Neuro2A. Furthermore, we found that the levels of TREM2 and YY1 were both downregulated in lipopolysaccharide-treated BV2 cells and in the brain of AD model mice. These results demonstrated that YY1 plays a crucial role in the regulation of *TREM2* expression. Our study suggests that microglial YY1 could be targeted to maintain *TREM2* expression for AD prevention and therapy.

Background info

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Background info

The Question, what is not known

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Methods

Background info

The Question, what is not known

Results

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Methods

Background info

The Question, or what is not known yet

Results

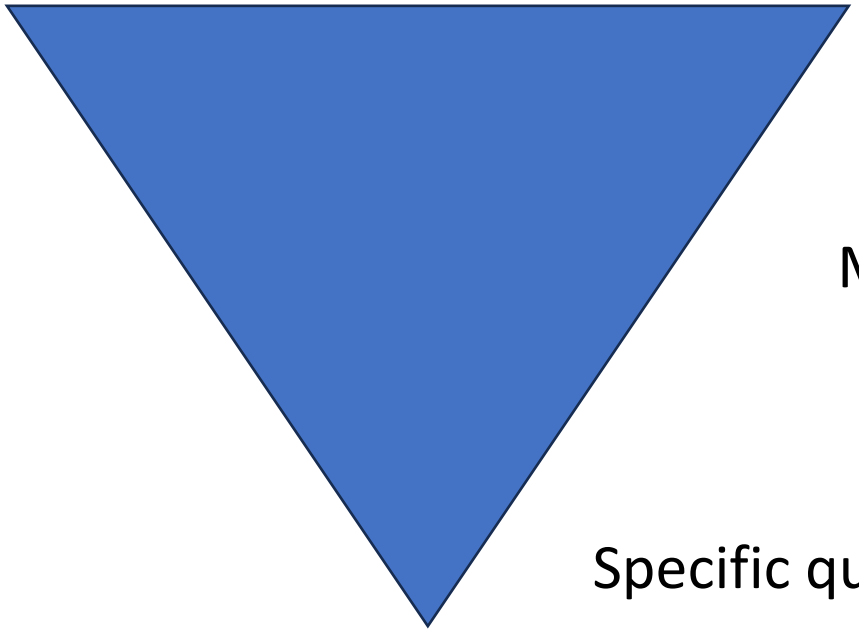
Conclusions and implications

TREM2 encoding the transmembrane receptor protein TREM2 is a risk gene of Alzheimer's disease (AD), and the impairment of TREM2 functions in microglia due to mutations in *TREM2* may significantly increase the risk of AD by promoting AD pathologies. However, how the expression of *TREM2* is regulated and the transcription factors required for *TREM2* expression are largely unknown. By luciferase assay, DNA pull-down, and *in silico* predictions, we identified Yin Yang 1(YY1) as a binding protein of the minimal promoter of the *TREM2* gene, and the binding was further confirmed by EMSA and DNA pull-down assay. shRNA-mediated *YY1* silencing significantly reduced the activity of the *TREM2* minimal promoter and TREM2 protein levels in the microglial cell line BV2 and the neuroblastoma Neuro2A. Furthermore, we found that the levels of TREM2 and YY1 were both downregulated in lipopolysaccharide-treated BV2 cells and in the brain of AD model mice. These results demonstrated that YY1 plays a crucial role in the regulation of *TREM2* expression. Our study suggests that microglial YY1 could be targeted to maintain *TREM2* expression for AD prevention and therapy.

Methods

Introduction

- Remember this is a persuasive essay
- Will provide background
- Knowledge of area to this point in time



Broad statements

More specific details

Specific question being asked

Introduction

Info on AD

Alzheimer's disease (AD) is the most common neurodegenerative disease leading to dementia in the elderly. The extracellular neuritic plaque with the amyloid protein (Aβ) as the major component and intracellular fibrillary tangle formed by the aggregation of hyperphosphorylated tau protein are the characteristic neuropathologies of AD (1). While how neuritic plaque and fibrillary tangle deposit in the brain of AD patients is not clear, impaired clearance of toxic components by the innate immune cells could contribute to the pathology (2).

Info on TREM2

Triggering receptor expressed on myeloid cells (TREM2) is a type I transmembrane receptor mainly expressed in myeloid lineage including microglia in the brain. Upon its binding with extracellular ligands on the plasma membrane, TREM2 initiates downstream signaling cascades through its cytoplasmic binding protein TYROBP (or DAP12) and is as such involved in a variety of cellular functions (3, 4). TREM2 in microglia is required for the regulation of immune responses and phagocytosis that are closely related to AD pathogenesis (5–8).

Connecting AD and TREM2 mutations

Case-control studies revealed several rare mutations in TREM2 gene increase the risk of AD. The carriers of the best characterized p.Arg47His in TREM2 are 2.83 times more prone to AD, although the association is only confirmed in European population (9, 10). Functional studies suggest that these TREM2 gene variations cause loss-of-function of TREM2 protein, resulting in not only AD but also other disorders (11, 12). TREM2 can be cleaved by ADAM10 and ADAM17 in the extracellular/intraluminal domain to release the C terminally truncated soluble TREM2 (sTREM2) into the interstitial or cerebrospinal fluid in the brain (13). The increased sTREM2 in the cerebrospinal fluid of early stage of AD could be due to enhanced cleavage, which may reduce functional TREM2 at the cell surface (13–16).

Low TREM2 levels may cause AD

Given the genetic and functional studies indicate that compromised TREM2 functions are correlated to AD, insufficient TREM2 expression could be a potential cause of AD (17). In vitro studies in microglial cells demonstrated that the expression of TREM2 is decreased by proinflammatory agents such as TNFα, IL1β, IFNγ, and lipopolysaccharide (LPS) (18, 19). However, how TREM2 expression is regulated, especially in the context of AD, remains elusive.

How TREM2 levels regulated unknown – this is the purpose of the study!

In this study, we identified Yin Yang 1 (YY1) as a transcription factor required for TREM2 expression. An evolutionarily conserved YY1 response element close to the transcription starting site (TSS) of TREM2 is indispensable for YY1-mediated TREM2 expression. In microglia cell challenged with LPS and in brains of AD model mice, both TREM2 and YY1 were significantly decreased. Therefore, microglial YY1 could be targeted to maintain TREM2 expression for AD prevention and therapy.

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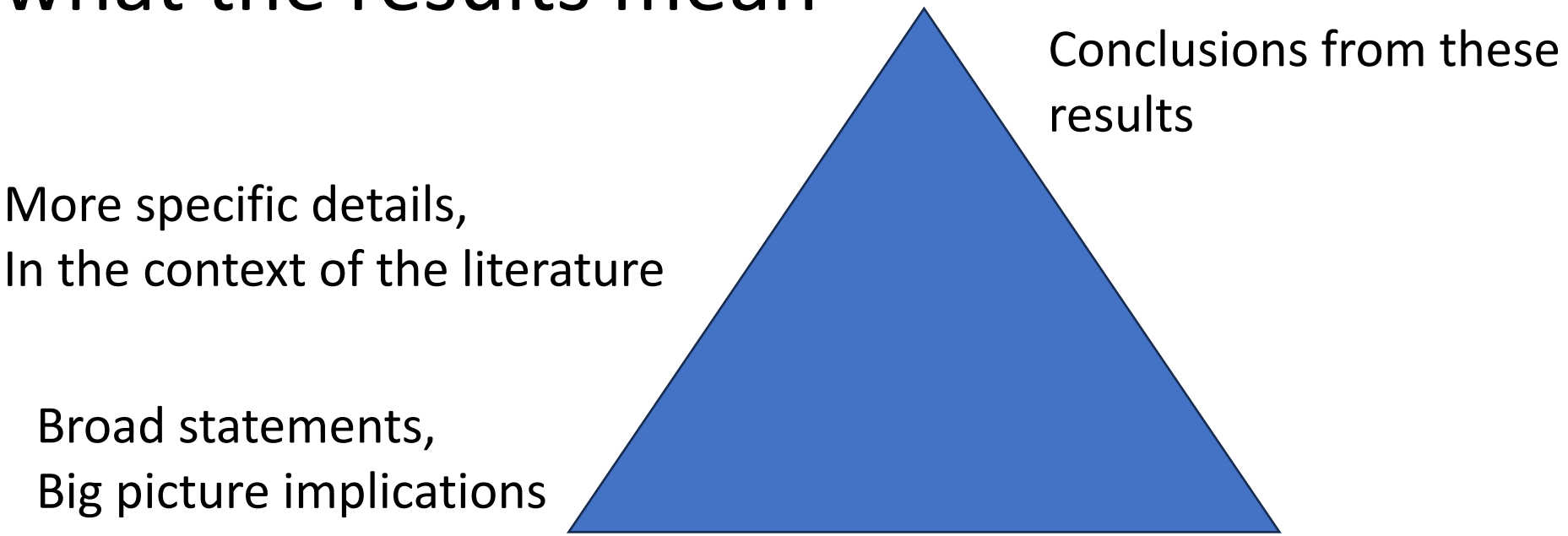
In this study,

1. we identified Yin Yang 1 (YY1) as a transcription factor required for TREM2 expression.
2. An evolutionarily conserved YY1 response element close to the transcription starting site (TSS) of TREM2 is indispensable for YY1-mediated TREM2 expression.
3. In microglia cell challenged with LPS and in brains of AD model mice, both TREM2 andYY1 were significantly decreased.

Therefore, microglial YY1 could be targeted to maintain TREM2 expression for AD prevention and therapy.

Discussion: What the Results Mean

- Will embed this new information in the context of what was known
- Articulates the answer to the question posed in the introduction
- Explains what the results mean



Resetting the stage here

Discussion

Deficiency in TREM2 significantly increases the risk of AD. In microglia, the major cell type in the brain to express TREM2, TREM2 participates in multiple cellular functions such as mediating the phagocytosis of Aβ, suppressing the inflammatory, and orchestrating lipid metabolism (23). All of these functions of microglia are compromised in AD (4). The mutations/SNPs in TREM2 correlated to AD may blunt some of these functions and as such increase the risk of AD (24–26). In nonmutation/SNP carriers, TREM2 may be involved in AD pathogenesis through altered expression (5, 27), and the decreased TREM2 expression compromises the functions of TREM2 (28). However, how TREM2 expression is regulated and the transcription factors required for TREM2 expression are largely unknown.

What they did

To identify the minimal promoter region of TREM2 and the transcription factors necessary for TREM2 expression, we generated a series of fragments upstream TSS of TREM2 gene. It was interesting to note that although the longest fragment (−983 +33) we tested showed little promoter activity, when it was truncated down to −370 +33, the promoter activity spiked up. Further truncation of −370 +33 to −118 +33 abolished promoter activity. Together, these data indicated that there could be repressing cis-element between −580 −370 and activating cis-element between −370 −118. We also noticed that the fragments pTREM2-E (0 −33) and G (−983 −303) in PGL3-Basic displayed even lower promoter activity than the empty PGL3-Basic vector. One possible explanation is that these fragments replaced the multiple cloning sites of PGL3-Basic that may have a weak promoter activity. If this is the case, the promoter activity of −370 +33 could have been under estimated.

What they found

YY1 is ubiquitously expressed in mammalian cells and serves as both transcription activator and repressor depending on its modifications, cofactors, chromatin structures, and target genes (29, 30). Some studies suggested that YY1 in neurons may increase Aβ by regulating the expression of proteins directly and indirectly involved in Aβ production (31–33). Biopsy examinations indicated that in the hippocampus and temporal cortex of AD patients, YY1 decreases and the proteolytic fragments of YY1 increases. Moreover, YY1 was also found to be reduced in the brains of patients with other neurodegenerative disease (34). We found that YY1 in BV2 is decreased by LPS, a condition to simulate neuroinflammation that is common for almost all neurodegenerative diseases. However, LPS was shown to increase YY1 activity in B cell in the periphery (35). Our results suggested that YY1 may directly promote TREM2 expression in microglia, which in turn enhances the clearance of Aβ, suppresses the immune responses, and maintains the cell homeostasis of microglia. Interestingly, YY1 was also reported to indirectly upregulate TREM2 through miRNA (36). Thus, to maintain microglial TREM2 expression by enhancing YY1 activity could be a potential strategy for AD prevention and diagnosis.

Info on YY1, not in intro

What they found

Related info in the literature

What their results mean, their conclusions

Given the genetic and functional studies indicate that compromised TREM2 functions are correlated to AD, insufficient TREM2 expression could be a potential cause of AD (17). In vitro studies in microglial cells demonstrated that the expression of TREM2 is decreased by proinflammatory agents such as TNFα, IL1β, IFNγ, and lipopolysaccharide (LPS) (18, 19). However, how TREM2 expression is regulated, especially in the context of AD, remains elusive.

From the Introduction

We found that YY1 in BV2 is decreased by LPS, a condition to simulate neuroinflammation that is common for almost all neurodegenerative diseases. However, LPS was shown to increase YY1 activity in B cell in the periphery (35). Our results suggested that YY1 may directly promote TREM2 expression in microglia, which in turn enhances the clearance of Aβ, suppresses the immune responses, and maintains the cell homeostasis of microglia.
Interestingly, YY1 was also reported to indirectly upregulate TREM2 through miRNA (36). Thus, to maintain microglial TREM2 expression by enhancing YY1 activity could be a potential strategy for AD prevention and diagnosis.

From the Discussion –
The “Edge of the World” has moved,
YY1 has been added to this system/response

Big picture application/context

Results – What they found

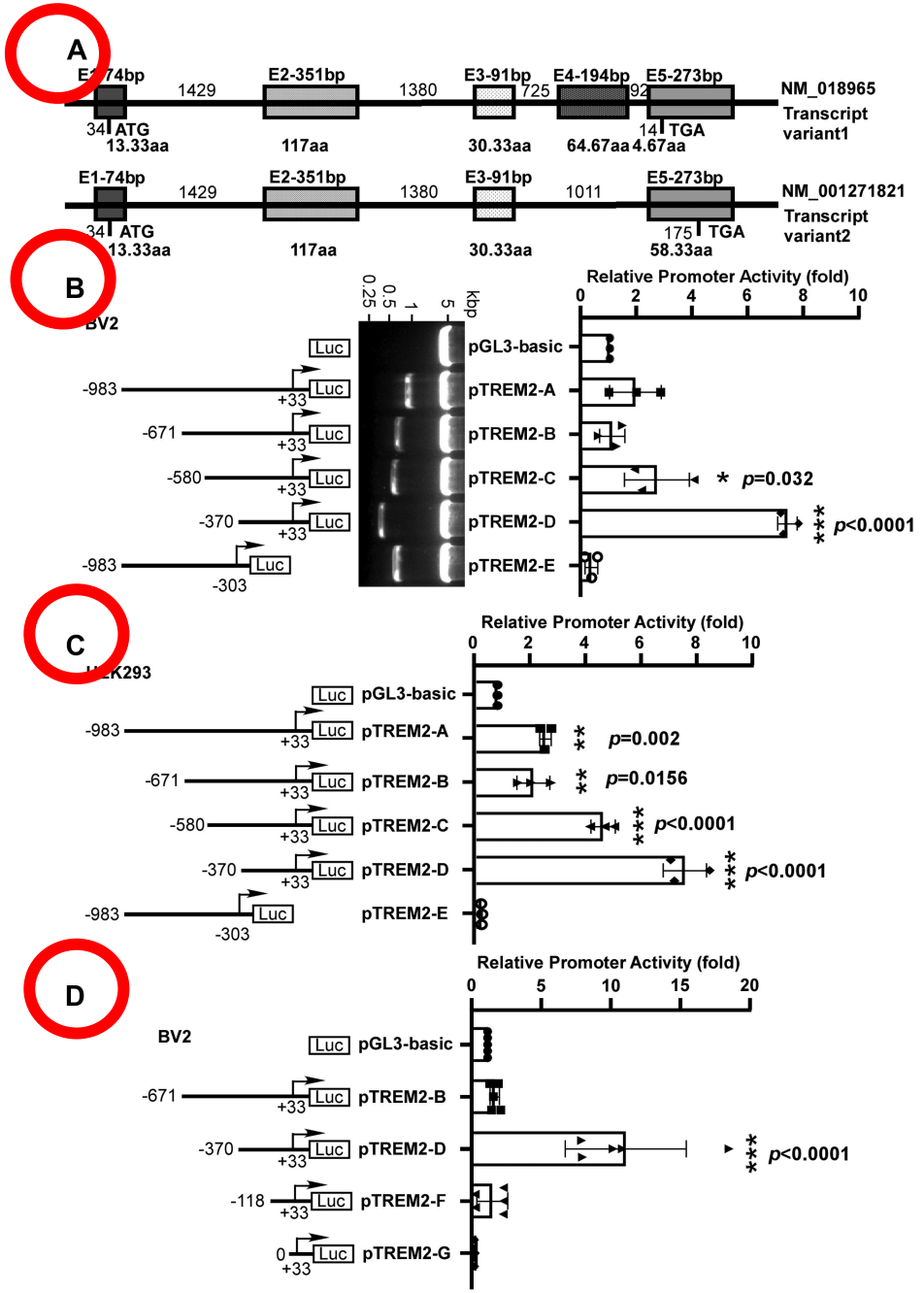
- Text will describe the results – the words
- Figures will show the results – the images
- You want to read them together

- Pay attention to subheadings!
- Look for topic sentences (what we're doing and why)
- Look for results statements (we found/the data show…)
- The results often flow from one to another, to tell a story
- Key results are often near the end. Results near the beginning often define/set up the system.

Identification of the minimal active region in TREM2 promoter

Human *TREM2* gene transcript can be spliced into two variants: the variant 1 is 693 bp in length and consists of five exons, whereas variant 2 is 660 bp in length and lacks exon 4 with larger exon 5. The two variants differ only in the 3' ends and share the identical TSS. To investigate the transcriptional activity of the human *TREM2* gene promoter, we extracted human genomic DNA and cloned a 983-bp fragment upstream the TSS (site 0, and the start codon ATG is +34–+36) (Fig. 1A). This fragment, and a series of 5' deletion fragments, were cloned into pGL3-Basic vector for luciferase reporter assay. As in the brain, TREM2 is highly expressed in microglia, we first transfected the plasmids into the microglial cell line BV2 and cotransfected the plasmid pCMV-RLuc to express *Renilla* luciferase under the strong ubiquitous promoter CMV as an internal control (Fig. 1B). The plasmid pTREM2-A and pTREM2-B, containing −983 +33 and −671 +33, respectively, displayed a similar promoter activity compared to pGL3-Basic. The promoter activity of pTREM2-C containing −580 +33 had slight increase by 2.679 ± 0.452 folds compared with vector. Further 5' truncation down to −370 +33 (pTREM2-D) significantly elevated the promoter activity by 6.391 ± 1.167 folds compared with pGL3-Basic. Another fragment −983 −303 (pTREM2-E) showed nearly zero promoter activity and the luciferase expression under this fragment is even lower than that in PGL3-Basic. Similar difference in the promoter activities of these fragments were also found in human embryonic kidney 293 (HEK293) cells (Fig. 1C). It appears that in the −983 +33 region, there are both positive and negative regulatory elements, with the latter within −580 −370 bp region.

To further narrow down the core promoter region, additional 5' deletion fragments −118 +33 and 0 +33 were cloned into PGL3-Basic to generate pTREM2-F and pTREM2-G. Both pTREM2-F and pTREM2-G had little promoter activity. These data suggested that there is a strong cis-acting element between −370 and −118 that spiked luciferase expression and could be crucial for *TREM2* expression (Fig. 1D).



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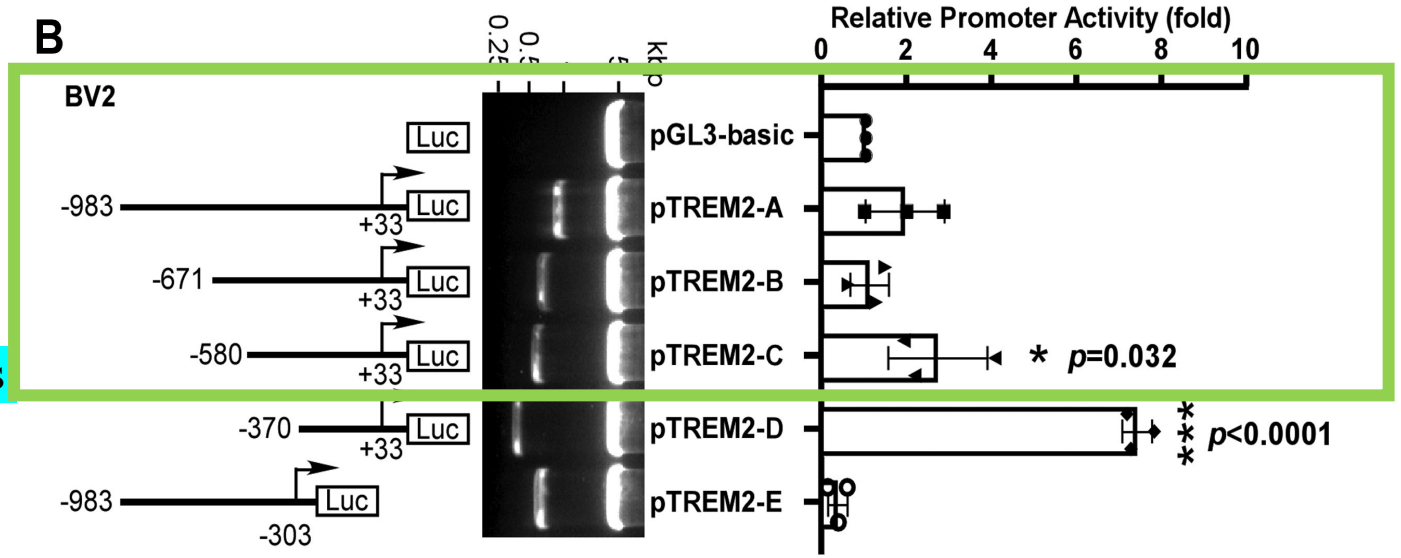

B, schematic illustration of human TREM2 promoter deletion constructs in pGL3-Basic vector. The arrow represents the direction of transcription and the number demonstrates the start and ending point of each construct insert relative to the transcription start site. TREM2 promoter deletion constructs were verified by restriction enzyme digestion, and the digested products were analyzed on 1.2 % agarose gel. The size of pGL3-Basic vector is 4.8 kb and inserts range from 337 bp to 1016 bp. The inserts were further confirmed by sequencing. The series of deletion constructs were cotransfected with pCMV-RLuc into BV2 cells. The cell lysates were harvested 24 h after transfection, and the luciferase activity was measured with a luminometer. The TREM2 promoter luciferase activity was normalized by pCMV-Luc luciferase activity for transfection efficiency and expressed in folds in comparison with the luciferase activity of pGL3-Basic vector.

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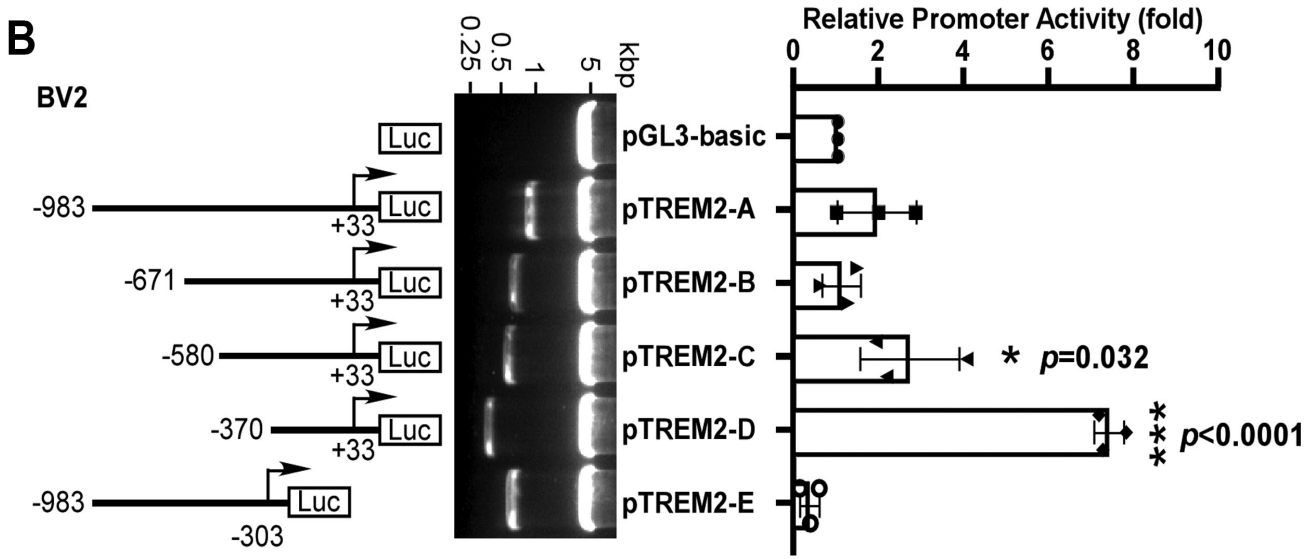

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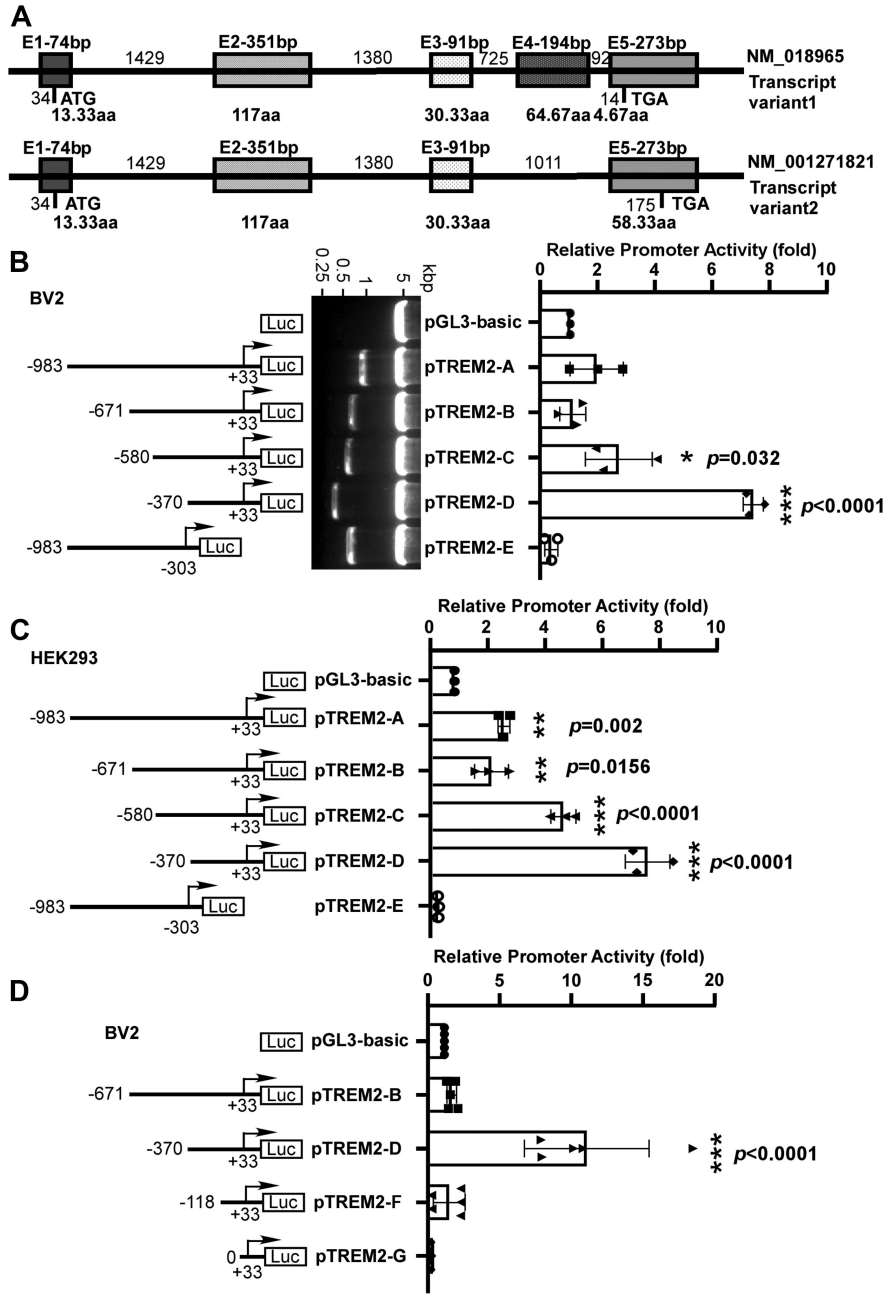

You can return to the abstract and the intro to look for highlights:

Abstract:

1. identified Yin Yang 1(YY1) as a binding protein of the minimal promoter of the *TREM2* gene,
2. shRNA-mediated *YY1* silencing significantly reduced the activity of the *TREM2* minimal promoter and
3. (shRNA-mediated *YY1* silencing significantly reduced) TREM2 protein levels in the microglial cell line BV2 and the neuroblastoma Neuro2A.
4. the levels of TREM2 and YY1 were both downregulated in lipopolysaccharide-treated BV2 cells and in the brain of AD model mice.

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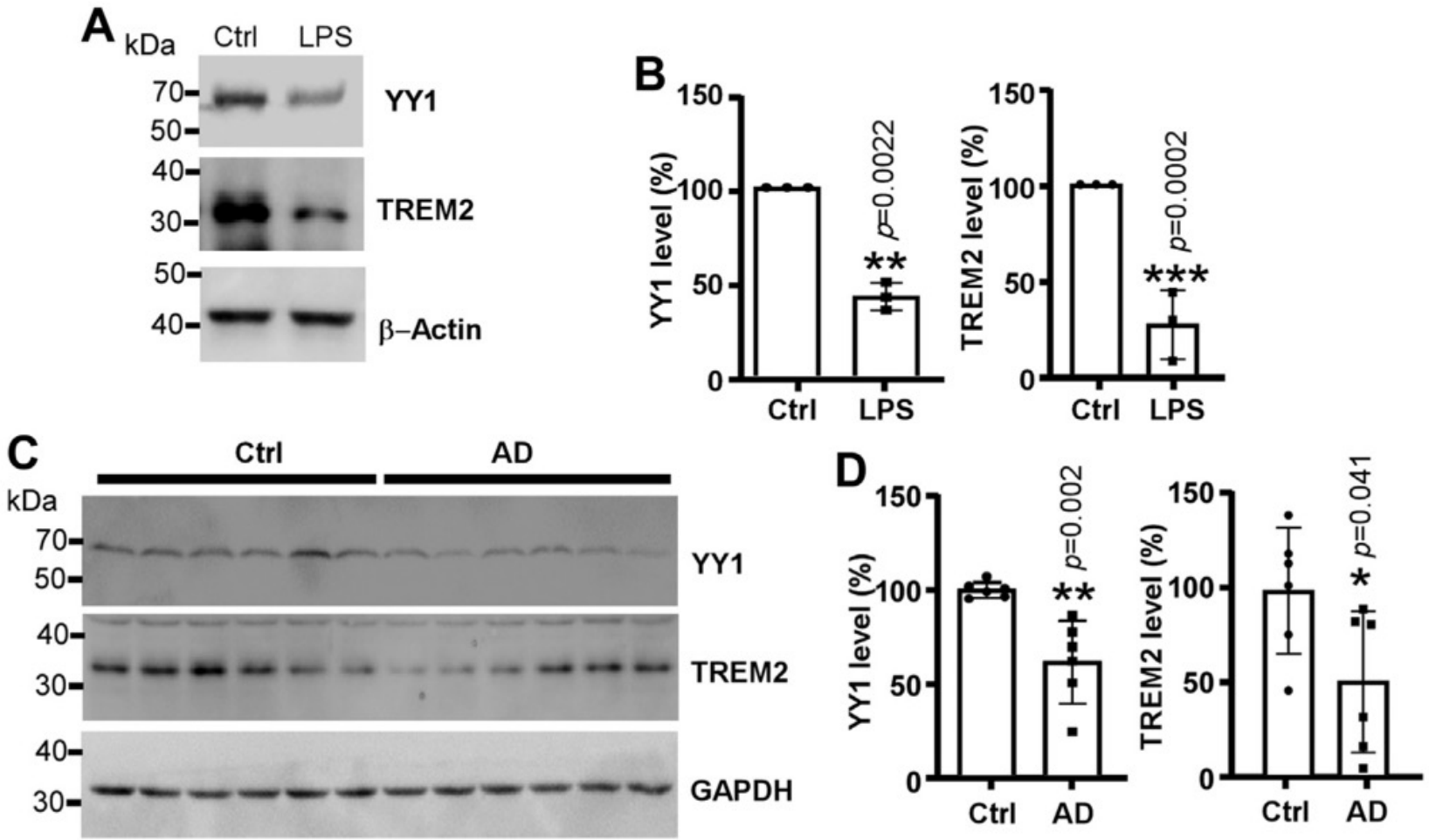
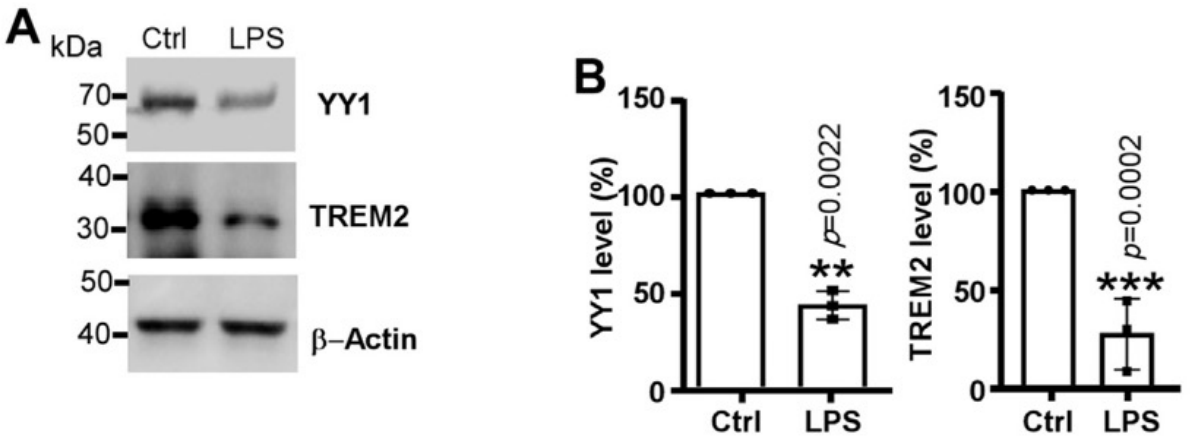


Figure 4. Both YY1 and TREM2 respond to inflammation and AD pathologies. *A*, BV2 cells were treated with 2 μg/ml LPS for overnight. TREM2 and YY1 were detected by immunoblotting. *B*, the protein level of TREM2 and YY1 was quantified in comparison with control. The values represent means ± SD, n= 3 independent repeats, *$p < 0.05$ *versus* control by Student's *t* test. *C* and *D*, the protein level of TREM2 and YY1 in WT mice and age matched AD mice were determined and quantified. The values represent means ± SD, n= 6 mice in each group, *$p < 0.05$ *versus* control by Student's t test. AD, Alzheimer's disease; LPS, lipopolysaccharide; YY1, Yin-Yang 1.



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Decreased YY1 and TREM2 expression by LPS and in AD transgenic mice

It has been well established that TREM2 in microglia is downregulated by the inflammation eliciting agent LPS (18, 21). We found that YY1 was also decreased by LPS at 2 μg/ml in BV2 cells (Fig. 4, A and B). Hence, it is possible that the reduction of TREM2 could be a consequence of YY1 suppression. We further tested if the overexpression of YY1 could rescue the decrease of TREM2 under the condition of LPS, however, the overexpression of functional YY1 in BV2, especially in the context of LPS, was extremely weak, and the increase of TREM2 protein, if any, was only marginal (data not shown).

YY1 may also be involved in TREM2 expression in vivo. Compared with age- and gender-matched WT mice, both YY1 and TREM2 in the APP/PS45 AD model mice (22) were decreased by 38.84 ± 9.339% and 49.25 ± 21.0%, respectively (Fig. 4, C and D).

Methods: What they did

- These and the figure legends give details that will allow the experiment to be replicated
- Pay attention to subheadings!
- For unfamiliar methods, look for videos and animations on YouTube
- Do NOT get bogged down in the details
  - Unless you are looking for experimental details to replicate the experiment

Cell culture, transfection, and luciferase reporter assay

HEK293 cells and BV2 cells were purchased from the American Tissue Culture Collection and were cultured in Dulbecco'smodified Eagle's medium containing 10% fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine (servicebio) at 37°C in a 5% CO2 and 95% air in an incubator. HEK293 cells and BV2 cells were cotransfected with 500 ng TREM2 promoter constructs and 1 ng pCMV-RLuc per well of 24-well plate with lipo8000 (Beyotime) for luciferase assay. Luciferase assay was performed according to technical manual of Dual-Luciferase Reporter Assay System (Promega). Cell lysates were harvested and lysed with 100 μl passive lysis buffer per well after 24 hours transfection. Firefly luciferase activities and *Renilla* luciferase activities were measured sequentially by the Dual-Luciferase Reporter Assay System (Promega). The firefly luciferase activity was normalized with *Renilla* luciferase activity and represented as relative folds in comparison with pGL3-Basic vector activity. The YY1 targeting sequence of the shRNA (50 GTGGTTGAAGAGCAGATCATTTTCAA-GAGAAATGATCTGCTCTTCAACCACTTTTTT 30)was cloned into pAV-U6-shRNA-CMV-intron-GFP vector for the expression under U6 promoter.

What is transfection?
What is cotransfection?

What is a luciferase assay?


Promega
Intro To Reporter Gene Assays

Supplemental Materials

- Additional data, not the key info but can be helpful
- Unless you are diving deep, don’t spend a lot of time here

References

- These allow you to move BACK in time
- Can be helpful if you are looking for more background information

If you are reading primarily for background info, focus on

- Title
- Abstract
- Intro
- Discussion
- Read the subheadings in the Results, look for key results statements
- Maybe for the key results mentioned in the abstract
- Skim the methods (maybe)

If you are reading for information about the experimental system, focus more on

- Results
- Methods

Keep an annotated bibliography

- Paper citation info
- A few sentences on the key info/findings
- Maybe a list of methods

Lu Y, Huang X, Liang W, Li Y, Xing M, Pan W, Zhang Y, Wang Z, Song W. Regulation of TREM2 expression by transcription factor YY1 and its protective effect against Alzheimer's disease. J Biol Chem. 2023 May;299(5):104688. doi: 10.1016/j.jbc.2023.104688. Epub 2023 Apr 11. PMID: 37044212; PMCID: PMC10193014.

- Identified a small region of the promoter of TREM2 that controls TREM2 expression
- Found that YY1 binds to the promoter
- TREM2 and YY1 were both downregulated in cultured cells when cells treated with inflammation activating compounds
- TREM2 and YY1 also downregulated in mouse brain of AD model mice

- Methods: cell culture, transfection, luciferase assay, DNA pulldown assay, mass spec, Electrophoretic mobility shift assay (EMSA), immunoblotting/Western blotting