Postprint: Changes in Serum RAGE/NF- B Pathway Expression Levels and Their Relationship with Cognitive Function in Patients with Wilson' s Disease Complicated by Mild Cognitive Impairment

Authors: Ni Mingzhu, Wang Li, Xu Zhenjing, stone bridge, Hou Zhifeng, Hou Zhifeng

Date: 2025-05-29T17:24:28+00:00

Abstract

Background Wilson' s disease (WD) exhibits increased inflammatory levels that are closely associated with cognitive impairment. Studies have demonstrated that activation of the receptor for advanced glycation end products (RAGE)/nuclear factor B (NF- B) signaling pathway represents an important inflammatory mechanism mediating cognitive dysfunction; however, the expression levels of this pathway in the serum of WD patients with mild cognitive impairment (MCI) remain unclear. Objective To investigate alterations in serum RAGE/NF- B pathway expression levels in WD patients and their impact on MCI. Methods Twenty-three WD patients with MCI hospitalized at the Brain Disease Center of the First Affiliated Hospital of Anhui University of Chinese Medicine between January 2024 and January 2025 were enrolled as the WD-MCI group. Twenty-three healthy individuals with normal cognitive function, matched for age, gender, and education level during the same period, were selected as the control group. The Mini-Mental State Examination (MMSE), Hopkins Verbal Learning Test (HVLT), Boston Naming Test-Second Edition (BNT-2), Clock Drawing Test (CDT), Trail Making Test-A (TMT-A), and Instrumental Activities of Daily Living Scale (IADL) were employed to evaluate patients' global cognitive status and memory, language, visuospatial, executive, and daily living abilities. Enzyme-linked immunosorbent assay and qRT-PCR were applied to determine serum expression levels of key molecules in the RAGE/NF- B signaling pathway. Differences in cognitive function, serum RAGE levels, and NF- B p65 mRNA expression levels between the two groups were compared. Pearson or Spearman tests were used to analyze correlations between serum RAGE levels, NF- B p65 mRNA expression levels, and cognitive

function scores. Results Compared with the control group, the WD-MCI group exhibited decreased scores in MMSE, HVLT, BNT-2, and CDT, while TMT-A and IADL scores were increased (P<0.05). Serum RAGE levels and NF- B p65 mRNA expression levels in the WD-MCI group were both higher than those in the control group (P<0.05). Correlation analysis revealed that serum RAGE levels and NF- B p65 mRNA expression levels were negatively correlated with MMSE (r values of -0.866 and -0.729, respectively), HVLT (r values of -0.721 and -0.728, respectively), BNT-2 (rs values of -0.381 and -0.382, respectively), and CDT (rs values of -0.788 and -0.709, respectively) scores, and positively correlated with TMT-A (r values of 0.774 and 0.524, respectively) and IADL (rs values of 0.433 and 0.376, respectively) scores (P<0.05). Conclusion Serum RAGE/NF- B pathway expression levels are significantly elevated in WD patients and are significantly positively correlated with cognitive impairment.

Full Text

Preamble

Alterations in Serum RAGE/NF- B Pathway Expression Levels and Their Association with Cognitive Function in Wilson' s Disease Patients with Mild Cognitive Impairment

NI Mingzhu, WANG Li, XU Zhenjing, SHI Qiao, HOU Zhifeng*

Encephalopathy Center, First Affiliated Hospital of Anhui University of Chinese Medicine, Hefei 230031, Anhui Province, China

*Corresponding author: HOU Zhifeng, Chief physician; E-mail: houzf197711@163.com

Abstract

Background: Wilson' s disease (WD) is associated with elevated inflammatory levels and closely linked to cognitive impairment. Studies suggest that activation of the receptor for advanced glycation end products (RAGE)/nuclear factor kappa B (NF- B) signaling pathway is a critical inflammatory mechanism mediating cognitive dysfunction, yet its expression in serum of WD patients with mild cognitive impairment (MCI) remains unclear.

Objective: To investigate changes in serum RAGE/NF- B pathway expression and their impact on MCI in WD patients.

Methods: A total of 23 WD patients with MCI (WD-MCI group) hospitalized at the Encephalopathy Center of the First Affiliated Hospital of Anhui University of Chinese Medicine from January 2024 to January 2025 were enrolled. Age-, sex-, and education-matched healthy controls (n=23) were recruited. Cognitive function was assessed using the Mini-Mental State Examination (MMSE), Hopkins Verbal Learning Test (HVLT), Boston Naming Test-Second Edition (BNT-2), Clock Drawing Test (CDT), Trail Making Test-A (TMT-A), and Instrumental Activities of Daily Living Scale (IADL). Serum RAGE levels and

NF- B p65 mRNA expression were measured using enzyme-linked immunosorbent assay (ELISA) and qRT-PCR, respectively. Differences in cognitive scores, serum RAGE levels, and NF- B p65 mRNA expression between groups were compared. Pearson or Spearman correlation analysis evaluated associations between RAGE/NF- B p65 mRNA levels and cognitive scores.

Results: Compared with controls, the WD-MCI group showed significantly lower MMSE, HVLT, BNT-2, and CDT scores, and higher TMT-A and IADL scores ($P<0.05$). Serum RAGE levels and NF- B p65 mRNA expression were elevated in the WD-MCI group ($P<0.05$). Correlation analysis revealed that serum RAGE levels and NF- B p65 mRNA expression negatively correlated with MMSE ($r=-0.866$, -0.729), HVLT ($r=-0.721$, -0.728), BNT-2 ($rs=-0.381$, -0.382), and CDT ($rs=-0.788$, -0.709) scores, and positively correlated with TMT-A ($r=0.774$, 0.524) and IADL ($rs=0.433$, 0.376) scores ($P<0.05$).

Conclusion: WD patients exhibit increased serum RAGE/NF- B pathway activity, which is significantly associated with mild cognitive impairment.

[**Key words**] Wilson' s disease; Mild cognitive impairment; Inflammation; Receptor for advanced glycation end products; Nuclear factor kappa-B

[**Funding**] National Natural Science Foundation of China (82405176); Clinical Research Project of Anhui University of Chinese Medicine (2024YFYLCZX18); High-level Talent Introduction Program of Anhui University of Chinese Medicine (ahgccrcjb20240297)

[**Citation**] NI MZ, WANG L, XU ZJ, et al. Alterations in serum RAGE/NF- B pathway expression levels and their association with cognitive function in Wilson' s disease patients with mild cognitive impairment [J]. Chinese General Practice, 2025. DOI: 10.12114/j.issn.1007-9572.2025.0072. [Epub ahead of print]

Introduction

Wilson' s disease (WD), also known as hepatolenticular degeneration, is an autosomal recessive disorder of copper metabolism with a global prevalence of approximately 30 per million population. Mutations in ATP7B lead to impaired biliary copper excretion, causing copper accumulation in the liver, brain, and other organs, resulting in hepatic disease, movement disorders, and psychiatric symptoms. Recent clinical studies have confirmed that cognitive impairment represents a major psychiatric manifestation of WD. Although typically mild, WD-related cognitive deficits cause significant distress in patients'learning, work, and daily life.

The pathogenesis of cognitive impairment in WD remains incompletely understood. Our previous research and other studies have demonstrated that peripheral inflammatory responses play a key regulatory role in the pathogenesis

of cognitive impairments such as Alzheimer' s disease (AD) and aging-related cognitive decline. Notably, our prior clinical and animal studies found that inflammatory levels are significantly elevated in both WD patients and animal models, correlating with cognitive dysfunction. The receptor for advanced glycation end products (RAGE), an important pattern recognition receptor of the immunoglobulin superfamily, triggers inflammatory cascades and subsequent cognitive damage by activating the nuclear factor kappa B (NF- B) signaling pathway upon binding to advanced glycation end products (AGEs) at supraphysiological concentrations. Recent evidence has confirmed that copper dyshomeostasis (overload) promotes AGEs generation and upregulates RAGE expression, thereby mediating abnormal activation of the RAGE/NF- B signaling pathway. However, the expression levels of the RAGE/NF- B pathway in serum of WD patients with mild cognitive impairment (MCI) and their correlation with cognitive dysfunction remain unclear. This study aims to investigate changes in serum expression levels of key molecules in the RAGE/NF- B signaling pathway and their relationship with MCI in WD patients.

Methods

Study Subjects

We enrolled 23 WD patients with MCI (WD-MCI group) hospitalized at the Encephalopathy Center of the First Affiliated Hospital of Anhui University of Chinese Medicine between January 2024 and January 2025. Twenty-three age-, sex-, and education-matched healthy individuals with normal cognitive function were selected as the control group during the same period.

Diagnostic Criteria: (1) WD diagnosis followed the "Guidelines for Diagnosis and Treatment of Hepatolenticular Degeneration (2022 Edition)." (2) MCI diagnosis followed the "2018 Chinese Guidelines for Diagnosis and Treatment of Dementia and Cognitive Impairment," including: (a) cognitive complaints reported by patients or informants or identified by experienced clinicians; (b) objective evidence of impairment in one or more cognitive domains from neuropsychological testing; (c) minimal impairment in complex instrumental activities of daily living but preserved independent daily functioning; and (d) not meeting dementia criteria. (3) WD with MCI required fulfillment of both criteria (1) and (2).

Inclusion Criteria: (1) Age 18-50 years; (2) meeting diagnostic criteria for WD with MCI, confirmed by two attending neurologists with associate senior professional titles or higher; (3) providing informed consent.

Exclusion Criteria: (1) Other conditions causing cognitive impairment, including traumatic brain injury, cerebrovascular disease, AD, Parkinson' s disease, or dementia with Lewy bodies; (2) other metabolic or endocrine disorders, infectious or immune diseases such as vitamin B12 deficiency, hypothyroidism, or rheumatoid arthritis; (3) severe physical or psychiatric illness; (4) inability to complete assessments for any reason.

This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Anhui University of Chinese Medicine (2024AH-12), and all participants provided informed consent.

Since executive dysfunction is particularly prominent in WD patients, we used executive function scores as the primary outcome for sample size calculation. Based on preliminary results, the estimated mean executive function scores were 55.54 for controls and 79.62 for the WD-MCI group, with standard deviations of 9.23 and 10.17, respectively. Using G*Power 3.1.9.7 software (=0.05, power=0.90, two-tailed test) and assuming a 20% dropout rate, the required sample size was determined to be 23 participants per group.

Data Collection and Assessment

General Clinical Data: We recorded participants' sex, age, and education level.

Neuropsychological Assessment: Cognitive function was evaluated using the Mini-Mental State Examination (MMSE), Hopkins Verbal Learning Test (HVLT), Boston Naming Test-Second Edition (BNT-2), Clock Drawing Test (CDT), Trail Making Test-A (TMT-A), and Instrumental Activities of Daily Living Scale (IADL) to assess global cognition and memory, language, visuospatial ability, executive function, and daily living skills. The 24-item Hamilton Depression Rating Scale (HAMD) was used to evaluate depressive symptoms (participants with scores >8 suggesting possible depression were excluded). All assessments were completed by two attending neurologists.

Serum RAGE/NF- B Pathway Molecular Detection: Fasting venous blood (4 mL) was collected from all participants in the morning, allowed to clot for 15 minutes, then centrifuged at 4,000 rpm for 10 minutes (radius 10 cm). Serum was collected and stored at -80°C. Serum RAGE levels were measured by enzyme-linked immunosorbent assay (ELISA) (Wuhan Jiyinmei Biotechnology Co., Ltd., catalog No. JYM0140Hu). Serum NF- B p65 mRNA expression was detected by quantitative real-time polymerase chain reaction (qRT-PCR). Total serum RNA was extracted using TRIzol LS reagent (Life Technologies, 10296010CN), purity was determined by NanoDrop (A260/A280 ratio 1.8), and RNA integrity was verified by agarose gel electrophoresis. One microgram of RNA was reverse-transcribed to cDNA using PrimeScript RT reagent kit (Takara, RR047A). qPCR was performed using SYBR Green premix with the following primer sequences: NF- B p65 forward 5' -GCTCCTGTTCGAGTCTCCAT-3' , reverse 5' -TTGCGCTTCTCTTCAATCCG-3' ; -actin (internal control) forward 5' -AGTGTGACGTTGACATCCGT-3' , reverse 5' -TGCTAGGAGCCAGAGCAGTA-3' . Reactions were run on an ABI 7500 system: 95°C for 5 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 30 seconds. Relative NF- B p65 mRNA expression was calculated using the 2-ΔΔCt method.

Statistical Analysis

Data were analyzed using GraphPad Prism 8 software. Categorical variables are presented as number (%) and compared between groups using ^{2} test. Non-normally distributed continuous variables are expressed as median (P25, P75) and compared using Wilcoxon rank-sum test. Normally distributed continuous variables are presented as mean ± standard deviation and compared using independent samples t-test when variances were homogeneous. Correlations between serum RAGE levels, NF- B p65 mRNA expression, and cognitive function scores were analyzed using Pearson correlation (for normally distributed data) or Spearman rank correlation (for non-normally distributed data). $P<0.05$ was considered statistically significant.

Results

Baseline Characteristics

No significant differences were observed between the control and WD-MCI groups in sex distribution, age, years of education, or 24-HAMD scores ($P>0.05$) .

Cognitive Function Comparison

Compared with the control group, the WD-MCI group showed significantly lower MMSE, HVLT, BNT-2, and CDT scores, while TMT-A and IADL scores were significantly higher ($P<0.05$) .

Serum RAGE/NF- B Pathway Expression

Serum RAGE levels and NF- B p65 mRNA expression were significantly higher in the WD-MCI group compared with controls ($P<0.05$) .

Correlation Analysis

Serum RAGE levels negatively correlated with MMSE ($r=-0.866$, $P<0.001$), HVLT ($r=-0.721$, $P<0.001$), BNT-2 ($rs=-0.381$, $P=0.009$), and CDT ($rs=-0.788$, $P<0.001$) scores, and positively correlated with TMT-A ($r=0.774$, $P<0.001$) and IADL ($rs=0.433$, $P=0.003$) scores. Serum NF- B p65 mRNA expression also negatively correlated with MMSE ($r=-0.729$, $P<0.001$), HVLT ($r=-0.728$, $P<0.001$), BNT-2 ($rs=-0.382$, $P=0.009$), and CDT ($rs=-0.709$, $P<0.001$) scores, and positively correlated with TMT-A ($r=0.524$, $P<0.001$) and IADL ($rs=0.376$, $P=0.010$) scores.

Discussion

Wilson' s disease typically manifests insidiously during childhood or adolescence. In addition to hepatic damage, extrapyramidal dysfunction, and Kayser-Fleischer rings, studies show that over 50% of WD patients experience cognitive

impairment. Our Encephalopathy Center serves as a national diagnosis and treatment center for major rare diseases (hepatolenticular degeneration). Our previous cognitive assessments of WD patients revealed that although cognitive impairment in WD is relatively mild, it can affect multiple cognitive domains, particularly executive function. In this study, compared with healthy controls, the WD-MCI group demonstrated impairments in global cognitive function, memory, language, visuospatial ability, executive function, and daily living skills, with particularly significant deficits in global cognition, visuospatial ability, and executive function. Therefore, the inclusion of a healthy control group in this study further strengthens our previous research, and the findings further characterize the profile of cognitive dysfunction in WD patients.

Numerous clinical and animal studies have demonstrated that copper overload-driven inflammatory responses represent a key pathogenic mechanism of cognitive impairment in WD. RAGE is a multi-ligand pattern recognition receptor composed of 404 amino acids. Upon ligand binding, RAGE expression is markedly upregulated, initiating activation of the downstream inflammatory transcription factor NF- B signaling pathway and participating in inflammatory responses. Moreover, the RAGE promoter contains two NF- B response elements that can form a positive feedback loop activation mechanism, maintaining a chronic inflammatory state. Consequently, RAGE has become a key regulator of innate immune responses. The role of RAGE/NF- B pathway activation in cognitive impairment in AD has been extensively documented. Our previous research found that increased RAGE/NF- B pathway expression is closely associated with aging-related cognitive decline. Additionally, Giridharan et al. found that RAGE overexpression plays an important role in mediating cognitive impairment induced by meningitis. Since impaired copper excretion in WD leads to markedly elevated free copper levels and accelerated AGEs generation, RAGE expression is significantly upregulated upon binding to AGEs. No previous studies have reported changes in serum RAGE/NF- B pathway expression levels in WD patients and their impact on cognitive impairment. In this study, both RAGE levels and NF- B p65 mRNA expression were higher in WD patients than in controls. To explore the relationship between RAGE/NF- B pathway expression and cognitive impairment, we conducted correlation analyses.

The results demonstrated significant correlations between serum RAGE levels, NF- B p65 mRNA expression, and cognitive function scores. Specifically, serum RAGE levels and NF- B p65 mRNA expression negatively correlated with MMSE, HVLT, BNT-2, and CDT scores (where higher scores indicate better cognitive function), and positively correlated with TMT-A and IADL scores (where higher scores indicate worse cognitive function). These findings suggest that elevated serum RAGE levels and NF- B p65 mRNA expression may be associated with cognitive impairment in WD patients.

This study has several limitations. First, it is a single-center, small-sample clinical study that only collected observational indicators (RAGE/NF- B pathway expression and cognitive function) at a single time point. The findings require

validation through multi-center, large-sample, multi-time-point studies. Future research will explore the clinical significance of RAGE/NF- B pathway expression in WD with MCI through pharmacological interventions.

Conclusion

Serum RAGE/NF- B pathway expression levels are significantly elevated in WD patients and positively correlated with cognitive impairment, providing insights and potential therapeutic targets for treating WD-related cognitive impairment through anti-inflammatory interventions.

Author Contributions: NI Mingzhu conceived the study, designed the research protocol, and wrote the manuscript. NI Mingzhu, WANG Li, XU Zhenjing, and SHI Qiao collected and organized data, performed serum assays, and conducted statistical analysis. HOU Zhifeng was responsible for quality control and revision, and takes overall responsibility for the article. All authors approved the final manuscript.

Conflict of Interest: The authors declare no conflict of interest.

ORCID IDs: NI Mingzhu: https://orcid.org/0009-0008-3213-363X HOU Zhifeng: https://orcid.org/0009-0005-5872-8779

References

[1] SCHILSKY ML, ROBERTS EA, BRONSTEIN JM, et al. A multidisciplinary approach to the diagnosis and management of Wilson disease: Executive summary of the 2022 Practice Guidance on Wilson disease from the American Association for the Study of Liver Diseases[J]. Hepatology, 2023, 77(4): 1428-1455. DOI: 10.1002/hep.32805.

[2] SHRIBMAN S, MARJOT T, SHARIF A, et al. Investigation and management of Wilson' s disease: a practical guide from the British association for the study of the liver[J]. Lancet Gastroenterol Hepatol, 2022, 7(6): 560-575. DOI: 10.1016/S2468-1253(22)00012-5.

[3] WANG GQ, JIN P, MA XF, et al. Cognitive and gait in Wilson' s disease: a cognitive and motor dual-task study[J]. Front Neurol, 2023, 14: 1243122. DOI: 10.3389/fneur.2023.1243122.

[4] SHRIBMAN S, BURROWS M, CONVERY R, et al. Neuroimaging correlates of cognitive deficits in Wilson' s disease[J]. Mov Disord, 2022, 37(8): 1728-1738. DOI: 10.1002/mds.29123.

[5] FARD MT, SAVAGE KM, STOUGH CK. Peripheral inflammation marker relationships to cognition in healthy older adults - A systematic review[J]. Psychoneuroendocrinology, 2022, 144: 105870. DOI: 10.1016/j.psyneuen.2022.105870.

[6] XIE JH, VAN HOECKE L, VANDENBROUCKE RE. The impact of systemic inflammation on Alzheimer' s disease pathology[J]. Front Immunol, 2022, 12: 796867. DOI: 10.3389/fimmu.2021.796867.

[7] NI MZ, ZHANG YM, LI Y, et al. Environmental enrichment improves declined cognition induced by prenatal inflammatory exposure in aged CD-1 mice: Role of NGPF2 and PSD-95[J]. Front Aging Neurosci, 2022, 14: 1021237. DOI: 10.3389/fnagi.2022.1021237.

[8] 王丹. 基于高阶认知网络及经典焦亡途径探索 WD 伴认知功能障碍的机制及化痰祛瘀方的疗效研究 [D]. 合肥: 安徽中医药大学, 2023. DOI: 10.26922/d.cnki.ganzc.2023.000006.

[9] JIANG ZS, DONG T, WANG Y, et al. Gandouling alleviates cognitive dysfunction by regulates the p62/Nrf2 signaling pathway to reduce oxidative stress and autophagy in mice models of Wilson' s disease[J]. Arab J Chem, 2023, 16(2): 104477. DOI: 10.1016/j.arabjc.2022.104477.

[10] YUE Q, SONG Y, LIU Z, et al. Receptor for advanced glycation end products (RAGE): a pivotal hub in immune diseases[J]. Molecules, 2022, 27(15): 4922. DOI: 10.3390/molecules27154922.

[11] GIRIDHARAN VV, GENEROSO JS, COLLODEL A, et al. Receptor for advanced glycation end products (RAGE) mediates cognitive impairment triggered by pneumococcal meningitis[J]. Neurotherapeutics, 2021, 18(1): 640-653. DOI: 10.1007/s13311-020-00917-3.

[12] CHEN JL, MOOLDIJK SS, LICHER S, et al. Assessment of advanced glycation end products and receptors and the risk of dementia[J]. JAMA Netw Open, 2021, 4(1): e2033012. DOI: 10.1001/jamanetworkopen.2020.33012.

[13] LI W, WANG S, WANG H, et al. Astragaloside IV prevents memory impairment in D-galactose-induced aging rats via the AGEs/RAGE/NF- B axis[J]. Arch Med Res, 2022, 53(1): 20-28. DOI: 10.1016/j.arcmed.2021.05.005.

[14] DING B, LIN CH, LIU Q, et al. Tanshinone IIA attenuates neuroinflammation via inhibiting RAGE/NF- B signaling pathway in vivo and in vitro[J]. J Neuroinflammation, 2020, 17(1): 302. DOI: 10.1186/s12974-020-01981-4.

[15] AASETH J, SKALNY AV, ROOS PM, et al. Copper, iron, selenium and lipo-glycemic dysmetabolism in Alzheimer' s disease[J]. Int J Mol Sci, 2021, 22(17): 9461. DOI: 10.3390/ijms22179461.

[16] 中华医学会肝病学分会遗传代谢性肝病协作组. 肝豆状核变性诊疗指南 (2022 年版)[J]. 中华肝脏病杂志, 2022, 30(1): 9-20. DOI: 10.3760/cma.j.cn501113-20211217-00603.

[17] 中国痴呆与认知障碍诊治指南写作组, 中国医师协会神经内科医师分会认知障碍疾病专业委员会. 2018 中国痴呆与认知障碍诊治指南 (五): 轻度认知障碍的诊断与治疗 [J]. 中华医学杂志, 2018, 98(17): 1294-1301. DOI: 10.3760/cma.j.issn.0376-2491.2018.17.003.

[18] 倪明珠, 余锋, 刘文灿, 等. 皮质下缺血性血管病患者血清 ADMA 变化及其对轻度认知损害的影响 [J]. 安徽医科大学学报, 2018, 53(2): 279-283. DOI: 10.19405/j.cnki.issn1000-1492.2018.02.021.

[19] TIAN LW, DONG T, HU S, et al. Radiomic and clinical nomogram for cognitive impairment prediction in Wilson' s disease[J]. Front Neurol, 2023, 14: 1131968. DOI: 10.3389/fneur.2023.1131968.

[20] 王莉, 马守亮, 鲍远程, 等. 肝豆灵片对痰瘀互结型肝豆状核变性患者执行功能的影响 [J]. 现代中西医结合杂志, 2023, 32(8): 1115-1118. DOI: 10.3969/j.issn.1008-8849.2023.08.016.

[21] DONG T, WU MC, TANG LL, et al. GanDouLing promotes proliferation and differentiation of neural stem cells in the mouse model of Wilson's disease[J]. Biosci Rep, 2021, 41(1): BSR20202717. DOI: 10.1042/BSR20202717.

[22] DONG JJ, WANG X, XU CC, et al. Inhibiting NLRP3 inflammasome activation prevents copper-induced neuropathology in a murine model of Wilson' s disease[J]. Cell Death Dis, 2021, 12(1): 87. DOI: 10.1038/s41419-021-03397-1.

[23] 倪明珠. 丰富环境对产前母体免疫激活加速子鼠衰老相关认知衰退的改善作用及机制研究 [D]. 合肥: 安徽医科大学, 2023. DOI: 10.26921/d.cnki.ganyu.2023.000014.

(Received: April 10, 2024; Revised: May 26, 2025) (Edited by: MAO Yamin)

Note: Figure translations are in progress. See original paper for figures.