Title: Neuropsin in Mental Health

Lina Bukowski[a,b], Ana Chernomorchenko[a,b], Anna Starnawska[a,b,c], Ole Mors [a,d], Nicklas Heine Staunstrup[a,b,c,e], Anders D. Børglum[a,b,c] and Per Qvist[a,b,c]

a) iPSYCH, The Lundbeck Foundation Initiative for Integrative Psychiatric Research, Denmark b) Department of Biomedicine, Aarhus University, Aarhus, Denmark c) Center for Genomics and Personalized Medicine, Aarhus University, Aarhus, Denmark d) Psychosis Research Unit, Aarhus University Hospital, Aarhus, Denmark e) Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

Corresponding author: Nicklas Heine Staunstrup, Assistant Professor
Department of Biomedicine, Aarhus University, Høegh-Guldbergs Gade 10, DK-8000 Aarhus, Denmark, Tel: (+45) 53838875, Email: nhs@clin.au.dk

Contact information all authors:

Lina Bukowski: lina.b@biomed.au.dk, Ana Chernomorchenko: au298932@uni.au.dk, Anna Starnawska: as@biomed.au.dk, Ole Mors: ole.mors@clin.au.dk, Nicklas Heine Staunstrup: nhs@clin.au.dk, Anders D. Børglum: anders@biomed.au.dk, Per Qvist: per.q@biomed.au.dk

List of abbreviations: AD = Alzheimer's disease, AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, BD = bipolar disorder, CAMKII = calcium/calmodulin dependent protein kinase II, ECM = extracellular matrix, E-LTP = early long-term potentiation, EphB2 = Eph receptor B2, GWAS = genome-wide association study, KLK8 = neuropsin gene, LOF = loss of function, LTP = long-term potentiation, L-LTP = late long-term potentiation, L1CAM = cell adhesion molecule L1, MDD = major depressive disorder, MRD = major

recurrent disorder, NMDA = N-methyl-D-aspartate, NRG-1 = neuregulin-1, PKA = protein kinase A, SNP = single nucleotide polymorphism, SZ = schizophrenia, WT = wildtype

ABSTRACT:

Neuropsin is a brain-expressed extracellular matrix serine protease that governs synaptic plasticity through activity-induced proteolytic cleavage of synaptic proteins. Its substrates comprise several molecules central to structural synaptic plasticity, and studies in rodents have documented its role in cognition and the behavioral and neurobiological response to stress. Intriguingly, differential usage of *KLK8* (neuropsin gene) splice forms in the fetal and adult brain has only been reported in humans, suggesting that neuropsin may serve a specialized role in human neurodevelopment. Through systematic interrogation of large-scale genetic data, we review *KLK8* regulation in the context of mental health and provide a summary of clinical and preclinical evidence supporting a role for neuropsin in the pathogenesis of mental illness.

Key words: Neuropsin, *KLK8*, Mental disorders, Mental health, Biomarker, Human, Depression, Extracellular Matrix, LTP, Gene expression

INTRODUCTION:

The extracellular matrix (ECM) serine protease neuropsin (also known as NP, PRSS19, BSP1 or TADG14), was named after its apparent neuronal expression and its sequence homology to trypsin [1]. Neuropsin possesses the complete triplet (His-Asp-Ser) of the serine protease domain and exhibits proteolytic activity with a trypsin-like substrate specificity [2]. Identified neuropsin substrates comprise: vitronectin [3]; fibronectin [2]; the cell adhesion molecule L1 (L1CAM) [4]; neuregulin-1 (NRG1) [3]; and the Eph receptor B2 (EphB2) [5], all of which localize to the synapse [6]. Accordingly, and like reported for other trypsin-like serine proteases [7, 8], accumulating evidence support a central role for neuropsin in peri-synaptic proteolysis and structural synaptic plasticity [9]. As several of its proteolytic targets have been linked to neurodevelopmental and mental disorders [10, 11], it is thus conceivable that neuropsin governs psychiatry-related synaptic signaling. Here, we review data on regulation of the neuropsin gene *KLK8* in the context of mental health and provide a summary of clinical and preclinical evidence supporting a role for neuropsin in the pathogenesis of mental illness.

KLK8 REGULATION IN THE BRAIN:

Human *KLK8* mRNA is detected in numerous brain tissues under non-pathological conditions [12] (Fehler! Verweisquelle konnte nicht gefunden werden.). Expression is abundant in the cerebellum throughout life, whereas it peaks postnatally in cortical and limbic tissues, namely in childhood and adulthood [12] (Fehler! Verweisquelle konnte nicht gefunden werden.). Age-dependent regional variation in *Klk8* expression and translation has additionally been reported in mice. Here, *Klk8* mRNA as well as protein levels were shown to decrease in the cerebral cortex with age, while peaking in adults in the olfactory bulb and the hippocampus [13]. *Klk8* is susceptible to transcriptional regulation by steroids, as demonstrated by corticosterone in primary cultured hippocampal neurons and in vivo [14, 15], and by estradiol in neuronal and

microglial cells [16]. Potentially related to this regulation, a sex bias in hippocampal *KLK8* expression has been reported at the protein level, with higher expression in healthy adult woman than in men [16]. This, however, is not mirrored at the mRNA level in the Brainspan atlas of the developing human brain [12] (Fehler! Verweisquelle konnte nicht gefunden werden.) or in adult hippocampal tissue from healthy donors [17]. In the developing mouse cortex and midbrain, the primary sources of *Klk8* mRNA are endothelial cells and microglia [18, 19], whereas its expression has been reported in pyramidal neurons of the hippocampal CA1–3 subfields and magnocellular neurons of the lateral/basolateral amygdaloid nucleus on the basis of *in situ* hybridization immunohistochemistry [1]. Here, its expression is activity dependent and can be evoked by neuronal stimuli [1, 20]. Similarly, its expression is induced in oligodendrocytes of the spinal cord following injury [21]. A re-analysis of data from the most comprehensive human brain single cell studies, representing several brain tissues and developmental stages, indicate that *KLK8* is predominantly expressed in neuronal cells, but generally only in a small fraction of cells (**Supplementary Table A.1**). Notably, *KLK8* expression was seen in cells derived from temporal cortical tissue in several studies [19, 22], whereas it was not detected in hippocampus-derived cells [17].

While orthologs of *KLK8* are found in many species, *KLK8* isoforms (type 2-6, **Figure 2A**), expressed due to alternative splicing, have only been reported in humans [23-26]. Unlike type 1 *KLK8*, which is predominantly expressed in the fetal human brain [23], type 2, resulting from an in frame splice site in exon 3, is preferentially expressed in human adult brain and particularly in in the amygdala and the CA1-3 regions of the hippocampus [23]. Specifically, a single human-specific T-to-A substitution at position 79 in the coding region (**Figure 2A)** leads to a novel GAA-containing motif in exon 3, which functions as splicing enhancer and creates a novel splicing site 8 bp upstream of the locus [27]. Type 2 *KLK8* is thus identical with type 1 except from a 135 bp (45 amino acid) insert located in exon 3 (**Figure 2A**) [23].

Interestingly human type 1 and type 2 *KLK8* mRNA transcripts produce the same active protease, as the type 2 specific 45 amino acid insertion is located outside the peptide sequence of the mature functional neuropsin protein [28]. It has been speculated that type 2 *KLK8* may be important for the adult brain plasticity, whereas *KLK8* in general may be necessary for the development of the human nervous system [23]. *KLK8* translation results in a preproneuropsin containing an serine protease peptide, an activity masking peptide, and a signal peptide [29] (**Figure 3A**). The signal peptide destines the preproneuropsin for translocation to the endoplasmatic reticulum and eventually for secretion as a proneuropsin following its removal. Cell-type specific secretion of neuropsin type 1 and 2 has been reported [28] which might be explained by differences in the signal peptide sequences between type 1 and 2. The non-active proneuropsin is stored in the extracellular matrix (ECM), probably mostly in the synaptic cleft [30]. Activation of extracellular proneuropsin is facilitated by removal of the activity masking peptide (QEDK) [2]. The responsible activating endoprotease has not yet been identified, but activation of proneuropsin follows various stimuli in mice, such as kindling epileptogenesis, long-term potentiation (LTP) and application of drugs that can depolarize synaptic activity [30]. Consequently, no or only a little protease activity has been detected in unstimulated brain tissues [2]. Interestingly an extra endoprotease site has been identified in the type 2-specific 45 amino acid region (**Figure 3B**) causing an intermediate protein form during the activation process [28]. Since protease activation of neuropsin only occurs after cleavage of the activity masking peptide, this intermediate form shows very low amidolytic activity, like other proneuropsin forms [28].

THE NEUROBIOLOGY OF NEUROPSIN:

Acting at the synapse, neuropsin has through its proteolytic activity been implicated with several molecular mechanisms underlying synaptic plasticity and associated cognitive and

behavioral traits. Neuropsin has a regulatory effect on Schaffer-collateral LTP [31], which is important for the acquisition of hippocampus-associated memory. LTP describes a persistent strengthening of synapses based on recent patterns of activity and consists of two phases – the temporary early LTP (E-LTP) and the long-lasting late LTP (L-LTP). Whereas L-LTP requires protein kinase A (PKA) activation leading to altered gene expression and novel protein biosynthesis [32], E-LTP comprises an interaction between the ECM and synaptic membranes and thus mechanically modulates synaptic plasticity. It requires N-methyl-D-aspartate (NMDA) receptor and calcium/calmodulin dependent protein kinase II (CAMKII) activation, which in turn increases the number of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors at synapses [33]. Post-synaptic NMDA receptor activation following synaptic stimuli in E-LTP has been shown to induce a rapid activation of the precursor form of neuropsin [4].

Active neuropsin cleaves the extracellular domain of L1CAM and produces a neuropsin-specific 180 kDa fragment [4, 34] (depicted in **Figure 4**). The function of this neuropsin-specific L1CAM fragment has not been investigated yet, but the role of cell adhesion molecules in synaptic plasticity [35-38] suggests that neuropsin-specific L1CAM cleavage decreases synaptic adhesion and subsequently increases the flexibility of synaptic structures. Neuropsin is involved in the synaptogenesis of L1CAM expressing orphan and small synaptic boutons in pre-synaptic membranes of the Schaffer-collateral pathway in the hippocampal CA1 substructure [34]. In accordance, *Klk8*-deficient mice are characterized by abnormalities of synapses and neurons in the CA1 subfield of the hippocampus [39] and show significantly impaired E-LTP and memory acquisition, but not memory retention [9]. Similarly, i*n vivo* inhibition of neuropsin in wild type (WT) mice leads to impaired E-LTP [9], and delivery of recombinant neuropsin to the hippocampus, conversely, to increased synaptic transmission and AMPA phosphorylation at an E-LTP activating site [9] (depicted in **Figure 4**). Collectively,

this implies that neuropsin-dependent L1CAM processing might modulate activity-dependent structural changes involved in E-LTP, and it has been suggested that neuropsin facilitates the transformation of E-LTP to L-LTP in a process referred to as synaptic tagging [40].

Another pathway through which neuropsin may contribute to the modulation of synaptic plasticity involves NRG1, its receptor ErbB4 and GABAergic transmission in the CA1-3 subfields of the hippocampus [3] (depicted in **Figure 4**). Activated by neuronal activity, neuropsin cleaves and releases NRG1 from its position in the ECM, which subsequently binds to its receptor ErbB4, leading to its phosphorylation [3]. ErbB4 is expressed in parvalbumin-positive GABAergic interneurons within the CA1 region and its phosphorylation is critical for GABAergic transmission [3]. Impairment of GABAergic transmission in *Klk8*-deficient mice leads to excessive post-synaptic excitation, which prevents the induction of NMDA receptor dependent LTP [41].

Linking the function of neuropsin to behavioral regulation, mice exposed to acute or chronic stress show increased *Klk8* mRNA expression in hippocampal tissue accompanied by depressive-like behavior [15]. This upregulation of *Klk8* after stress exposure was shown to be dependent on the stress hormone corticosterone in primary cultured hippocampal neurons as well as *in vivo* [14, 15]. Corticosterone exposure causes impairment in spatial memory, neurogenesis, dendritic morphology and demyelination in WT mice, while mice lacking *Klk8* (knockout mice as well as knockdown of *Klk8* by viral vectors) appear protected against these effects as well as the development of depressive-like behavior [15]. In line with this, overexpression of *Klk8* by viral vectors led to an increased impairment in spatial memory and depressive-like behavior. Neuropsin has further been demonstrated to play a critical role in stress-related plasticity in the amygdala, where stress-induced neuropsin-dependent EphB2 cleavage leads to the dissociation of EphB2 from the NR1-subunit of the NMDAR [5]. This changes the dynamics of the EphB2–NMDA-receptor interaction in mice, enhances NMDAR

current and induces anxiety-like behavior [5]. *Klk8*-deficient mice, however, are protected against stress-induced EphB2 cleavage and the associated behavioral changes [5].

KLK8 IN MENTAL DISORDERS:

Despite its documented role in synaptic signaling and importance in neurobiology and behavior, no clinical data directly link neuropsin to any brain disorder, including mental illnesses. However, there are genetic and epigenetic findings that support its implication in human mental health. This includes clinical data from individuals carrying structural genetic variants encompassing *KLK8*, among which >60% present with intellectual disability [42]. Other associated mental health phenotypes include specific learning disabilities, seizures and autism [42]. However, *KLK8* is one of fifteen kallikrein subfamily members located in tandem in a gene cluster on chromosome 19, so phenotypes cannot be specifically attributed to changes in *KLK8* copy number. Although a rare (<0.001% [43]) missense Val286Ile mutation has been identified in a schizophrenia (SZ) case [44] (Fehler! Verweisquelle konnte nicht gefunden werden.**)**, no other disruptive rare *KLK8* variants have been reported in any of the major exome or copy number variation studies performed on SZ or autism spectrum disorder (ASD) cases [45]. Thus, supporting the finding that *KLK8* is tolerant to loss of function (LOF) variants [42, 43]. A candidate study assessing genetic association between *KLK8* polymorphisms and BD and SZ, identified an association between BD and three SNPs, as well as a two-marker haplotype (Fehler! Verweisquelle konnte nicht gefunden werden.) [44]. Interestingly, healthy individuals carrying the BD risk allele of *KLK8*, rs1612902, showed a lower score in attention/concentration and verbal IQ [44]. However, in terms of common variation, no genome-wide significant association could be found between variants in the *KLK8* locus and mental disorders in the currently largest major depressive disorder (MDD), SZ, bipolar disorder

(BD), ASD or attention deficit hyperactivity disorder (ADHD) genome-wide association studies (GWASs) [46-50] (**Figure 5** and **Supplementary Figure A.1**).

Assessment of *KLK8* mRNA levels in peripheral blood from 186 patients diagnosed with major recurrent depression (MRD) compared to 105 healthy subjects, revealed significantly higher *KLK8* expression in patients [51]. Furthermore, it was shown that *KLK8* mRNA is significantly more abundant in blood samples from patients affected by MRD, compared to patients suffering from first episode depression. The observed increase in *KLK8* expression was associated with diminished interpersonal abilities in depressive patients [52]. An association between depression symptomatology score in the general population and blood DNA methylation levels in the promoter region of *KLK8* was recently identified in a large cohort of monozygotic Danish twins [53], supporting the implication of *KLK8* in depression symptomatology. However, a screening of *KLK8* expression data from RNA sequencing expression experiments on post-mortem brain tissues from MDD, BD and SZ patients, did not reveal significant differences between patients and healthy controls in any of the identified studies (**Supplementary Figure A.3**). *KLK8* expression levels has, on the other hand, been reported to be 11.5 fold increased in the hippocampus of patients suffering from the neurodegenerative mental disorder Alzheimer´s disease (AD) compared to controls [54]. In line with this, RNA sequencing in post-mortem lateral temporal lobe tissue revealed a tendency of higher *KLK8* expression in aged AD patients compared to age-matched healthy individuals (GSE104704, **Supplementary Figure A.3**).

CONCLUSION:

The proteolytic specificity of neuropsin directed at synaptic proteins implicated in mental disorders, makes it an interesting candidate in molecular psychiatry. Brain specific splicing of *KLK8* mRNA is only reported in humans and its expression and activation characteristics links

it to neuronal activity and a neuro-molecular response to stress. Under non-pathological conditions, *KLK8* expression in the CNS is, however, weak and appears to be restricted to only a small subset of cells. Through systematic interrogation of available large scale genetic and postmortem brain transcriptomic studies, we do not find compelling clinical data that support *KLK8* dysregulation in mental illness. However, the endoprotease and molecular machinery responsible for neuropsin activation remains to be characterized and it is possible that post-translational regulation of neuropsin may be the dominating molecular mechanism of activity regulation in the mature brain.

Interestingly, *KLK8* expression measured in blood as well as cerebrospinal fluid is a promising early biomarker for AD as well as mild cognitive impairment due to AD [55]. Blood *KLK8* levels have, furthermore, successfully been established as a biomarker for cancer diagnosis [56]. It is, thus, possible that *KLK8* blood parameters levels may accordingly serve as diagnostic biomarkers in mental disorders. This is especially interesting when considering that both blood *KLK8* mRNA and methylation status have been associated with symptomatology [51, 53].

ACKNOWLEDGEMENTS:

This work was supported by The Lundbeck Foundation Initiative for Integrative Psychiatric Research and Jascha Fonden (case 7662).

COMPETING INTERESTS STATEMENT:

The authors report no biomedical financial interests or potential conflicts of interest.

REFERENCES:

1. Chen, Z.L., et al., *Expression and activity-dependent changes of a novel limbic-serine protease gene in the hippocampus.* J Neurosci, 1995. **15**(7 Pt 2): p. 5088-97.
2. Shimizu, C., et al., *Characterization of recombinant and brain neuropsin, a plasticity-related serine protease.* J Biol Chem, 1998. **273**(18): p. 11189-96.
3. Tamura, H., et al., *Processing of neuregulin-1 by neuropsin regulates GABAergic neuron to control neural plasticity of the mouse hippocampus.* J Neurosci, 2012. **32**(37): p. 12657-72.
4. Matsumoto-Miyai, K., et al., *NMDA-dependent proteolysis of presynaptic adhesion molecule L1 in the hippocampus by neuropsin.* J Neurosci, 2003. **23**(21): p. 7727-36.
5. Attwood, B.K., et al., *Neuropsin cleaves EphB2 in the amygdala to control anxiety.* Nature, 2011. **473**(7347): p. 372-5.
6. Hillen, A.E.J., J.P.H. Burbach, and E.M. Hol, *Cell adhesion and matricellular support by astrocytes of the tripartite synapse.* Prog Neurobiol, 2018. **165-167**: p. 66-86.
7. Xi, G., G. Reiser, and R.F. Keep, *The role of thrombin and thrombin receptors in ischemic, hemorrhagic and traumatic brain injury: deleterious or protective?* J Neurochem, 2003. **84**(1): p. 3-9.
8. Sheehan, J.J. and S.E. Tsirka, *Fibrin-modifying serine proteases thrombin, tPA, and plasmin in ischemic stroke: a review.* Glia, 2005. **50**(4): p. 340-50.
9. Tamura, H., et al., *Neuropsin is essential for early processes of memory acquisition and Schaffer collateral long-term potentiation in adult mouse hippocampus in vivo.* J Physiol, 2006. **570**(Pt 3): p. 541-51.
10. Mostaid, M.S., et al., *Neuregulin-1 and schizophrenia in the genome-wide association study era.* Neurosci Biobehav Rev, 2016. **68**: p. 387-409.
11. Linneberg, C., et al., *L1cam-mediated developmental processes of the nervous system are differentially regulated by proteolytic processing.* Sci Rep, 2019. **9**(1): p. 3716.
12. *Brainspan - Atlas of the developing human brain*.
13. Konar, A. and M.K. Thakur, *Neuropsin Expression Correlates with Dendritic Marker MAP2c Level in Different Brain Regions of Aging Mice.* Mol Neurobiol, 2015. **51**(3): p. 1130-8.
14. Harada, A., et al., *Acute stress increases neuropsin mRNA expression in the mouse hippocampus through the glucocorticoid pathway.* Neurosci Lett, 2008. **436**(2): p. 273-7.
15. Chang, S., et al., *Neuropsin Inactivation Has Protective Effects against Depressive-Like Behaviours and Memory Impairment Induced by Chronic Stress.* PLoS Genet, 2016. **12**(10): p. e1006356.
16. Keyvani, K., et al., *Higher levels of kallikrein-8 in female brain may increase the risk for Alzheimer's disease.* Brain Pathol, 2018. **28**(6): p. 947-964.
17. Consortium, G.T., *The Genotype-Tissue Expression (GTEx) project.* Nat Genet, 2013. **45**(6): p. 580-5.
18. Zhang, Y., et al., *An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex.* J Neurosci, 2014. **34**(36): p. 11929-47.
19. La Manno, G., et al., *Molecular Diversity of Midbrain Development in Mouse, Human, and Stem Cells.* Cell, 2016. **167**(2): p. 566-580 e19.
20. Momota, Y., et al., *Blockade of neuropsin, a serine protease, ameliorates kindling epilepsy.* Eur J Neurosci, 1998. **10**(2): p. 760-4.
21. Terayama, R., et al., *Differential expression of neuropsin and protease M/neurosin in oligodendrocytes after injury to the spinal cord.* Glia, 2004. **48**(2): p. 91-101.
22. Darmanis, S., et al., *A survey of human brain transcriptome diversity at the single cell level.* Proc Natl Acad Sci U S A, 2015. **112**(23): p. 7285-90.
23. Mitsui, S., et al., *A novel form of human neuropsin, a brain-related serine protease, is generated by alternative splicing and is expressed preferentially in human adult brain.* Eur J Biochem, 1999. **260**(3): p. 627-34.

24. Magklara, A., et al., *The human KLK8 (neuropsin/ovasin) gene: identification of two novel splice variants and its prognostic value in ovarian cancer.* Clin Cancer Res, 2001. **7**(4): p. 806-11.
25. Sher, Y.P., et al., *Human kallikrein 8 protease confers a favorable clinical outcome in non-small cell lung cancer by suppressing tumor cell invasiveness.* Cancer Res, 2006. **66**(24): p. 11763-70.
26. Planque, C., et al., *Alternative splicing variant of kallikrein-related peptidase 8 as an independent predictor of unfavorable prognosis in lung cancer.* Clin Chem, 2010. **56**(6): p. 987-97.
27. Lu, Z.X., J. Peng, and B. Su, *A human-specific mutation leads to the origin of a novel splice form of neuropsin (KLK8), a gene involved in learning and memory.* Hum Mutat, 2007. **28**(10): p. 978-84.
28. Lu, Z.X., Q. Huang, and B. Su, *Functional characterization of the human-specific (type II) form of kallikrein 8, a gene involved in learning and memory.* Cell Res, 2009. **19**(2): p. 259-67.
29. Shiosaka, S. and Y. Ishikawa, *Neuropsin--a possible modulator of synaptic plasticity.* J Chem Neuroanat, 2011. **42**(1): p. 24-9.
30. Shiosaka, S., *Serine proteases regulating synaptic plasticity.* Anat Sci Int, 2004. **79**(3): p. 137-44.
31. Komai, S., et al., *Neuropsin regulates an early phase of schaffer-collateral long-term potentiation in the murine hippocampus.* Eur J Neurosci, 2000. **12**(4): p. 1479-86.
32. Kandel, E.R., *The molecular biology of memory storage: a dialogue between genes and synapses.* Science, 2001. **294**(5544): p. 1030-8.
33. Herring, B.E. and R.A. Nicoll, *Long-Term Potentiation: From CaMKII to AMPA Receptor Trafficking.* Annu Rev Physiol, 2016. **78**: p. 351-65.
34. Nakamura, Y., et al., *Role of neuropsin in formation and maturation of Schaffer-collateral L1cam-immunoreactive synaptic boutons.* J Cell Sci, 2006. **119**(Pt 7): p. 1341-9.
35. Benson, D.L., et al., *Making memories stick: cell-adhesion molecules in synaptic plasticity.* Trends Cell Biol, 2000. **10**(11): p. 473-82.
36. Nagappan-Chettiar, S., E.M. Johnson-Venkatesh, and H. Umemori, *Activity-dependent proteolytic cleavage of cell adhesion molecules regulates excitatory synaptic development and function.* Neurosci Res, 2017. **116**: p. 60-69.
37. Jang, S., H. Lee, and E. Kim, *Synaptic adhesion molecules and excitatory synaptic transmission.* Curr Opin Neurobiol, 2017. **45**: p. 45-50.
38. Sytnyk, V., I. Leshchyns'ka, and M. Schachner, *Neural Cell Adhesion Molecules of the Immunoglobulin Superfamily Regulate Synapse Formation, Maintenance, and Function.* Trends Neurosci, 2017. **40**(5): p. 295-308.
39. Hirata, A., et al., *Abnormalities of synapses and neurons in the hippocampus of neuropsin-deficient mice.* Mol Cell Neurosci, 2001. **17**(3): p. 600-10.
40. Ishikawa, Y., et al., *Neuropsin (KLK8)-dependent and -independent synaptic tagging in the Schaffer-collateral pathway of mouse hippocampus.* J Neurosci, 2008. **28**(4): p. 843-9.
41. Moore, S.D., D.S. Barr, and W.A. Wilson, *Seizure-like activity disrupts LTP in vitro.* Neurosci Lett, 1993. **163**(1): p. 117-9.
42. Firth, H.V., et al., *DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources.* Am J Hum Genet, 2009. **84**(4): p. 524-33.
43. Lek, M., et al., *Analysis of protein-coding genetic variation in 60,706 humans.* Nature, 2016. **536**(7616): p. 285-91.
44. Izumi, A., et al., *Genetic variations of human neuropsin gene and psychiatric disorders: polymorphism screening and possible association with bipolar disorder and cognitive functions.* Neuropsychopharmacology, 2008. **33**(13): p. 3237-45.
45. al., P.e. *Genebook*. 2014 [cited 2019; Available from: http://atgu.mgh.harvard.edu/~spurcell/genebook/genebook.cgi.

46. Howard, D.M., et al., *Genome-wide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions.* Nat Neurosci, 2019. **22**(3): p. 343-352.
47. Demontis, D., et al., *Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder.* Nat Genet, 2019. **51**(1): p. 63-75.
48. Grove, J., et al., *Identification of common genetic risk variants for autism spectrum disorder.* Nat Genet, 2019. **51**(3): p. 431-444.
49. Stahl, E.A., et al., *Genome-wide association study identifies 30 loci associated with bipolar disorder.* Nat Genet, 2019. **51**(5): p. 793-803.
50. Schizophrenia Working Group of the Psychiatric Genomics, C., *Biological insights from 108 schizophrenia-associated genetic loci.* Nature, 2014. **511**(7510): p. 421-7.
51. Bobinska, K., et al., *Human neuropsin gene in depression.* Psychiatr Danub, 2017. **29**(2): p. 195-200.
52. Talarowska, M., Bobińska, K., Gałecka, E., Szemraj, J., Gałecki, P., *Human neuropsin gene and social cognition in depression.* Neuropsychiatry, 2016.
53. Starnawska A., T.Q., Soerensen M., McGue M., Mors O., Børglum A. D., Christensen K., Nyegaard M., Christiansen L., *Epigenome-wide association study of depression symptomatology in elderly monozygotic twins.* Translational Psychiatry, 2019.
54. Shimizu-Okabe, C., et al., *Expression of the kallikrein gene family in normal and Alzheimer's disease brain.* Neuroreport, 2001. **12**(12): p. 2747-51.
55. Teuber-Hanselmann, S., et al., *CSF and blood Kallikrein-8: a promising early biomarker for Alzheimer's disease.* J Neurol Neurosurg Psychiatry, 2019.
56. Tailor, P.D., et al., *Diagnostic and prognostic biomarker potential of kallikrein family genes in different cancer types.* Oncotarget, 2018. **9**(25): p. 17876-17888.
57. *Smart Servier Medical Art*. 2019 [cited 2019 06.09.]; Available from: https://smart.servier.com/.
58. George Paxinos, K.B.J.F., *The Mouse Brain in Stereotaxic Coordinates*. second edition ed., Academic Press: Academic Press.

TABLES:

Table 1: Descriptive overview of human studies, in which *KLK8* was associated to mental health phenotypes.

Phenotype	Tissue	Parameters assessed	Main finding	Reference
Schizophrenia	Peripheral blood	**Singe nucleotide polymorphisms** (genotyping assay)	Rare Val286Ile missense mutation in exon 6 detected in a patient with schizophrenia	Izumi, Iijima et al., 2008
Bipolar disorder	Peripheral blood	**Singe nucleotide polymorphisms** (genotyping assay)	Significant allelic association between several SNPs and bipolar disorder: rs1722550 (P=0.019), rs1701946 (P=0.018), rs1612902 (P=0.002), rs1701946 plus rs1612902 (P=0.0068)	Izumi, Iijima et al., 2008
Depression	Peripheral blood	**Expression** levels (mRNA) of *KLK8* (RT-PCR), patients diagnosed with major recurrent depression and first episode depression	Higher *KLK8* expression in patients with recurrent depression compared to first episode patients	Talarowska, Bobinska et al., 2016
	Peripheral blood	**Expression** levels (mRNA) of *KLK8* (RT-PCR), patients diagnosed with major recurrent depression and healthy subjects	Higher *KLK8* expression in patients with depression compared to controls	Bobinska, Mossakowska-Wojcik et al., 2017
	Peripheral blood	**Methylation** levels of *KLK8* (450K methylation array), depression symptomatology score in monozygotic twins	Methylation levels in the promotor region of *KLK8* is associated with depression symptomatology in general population	Starnawska et al., 2019
Alzheimer's disease	Hippocampal and parietal cortex	**Expression** (RT-PCR), of *KLK8* in Alzheimer's disease (AD) and control tissue	11.5-fold increase in *KLK8* mRNA levels in AD hippocampus compared to controls	Shimizu-Okabe, Yousef et al., 2001

FIGURES:

Figure 1

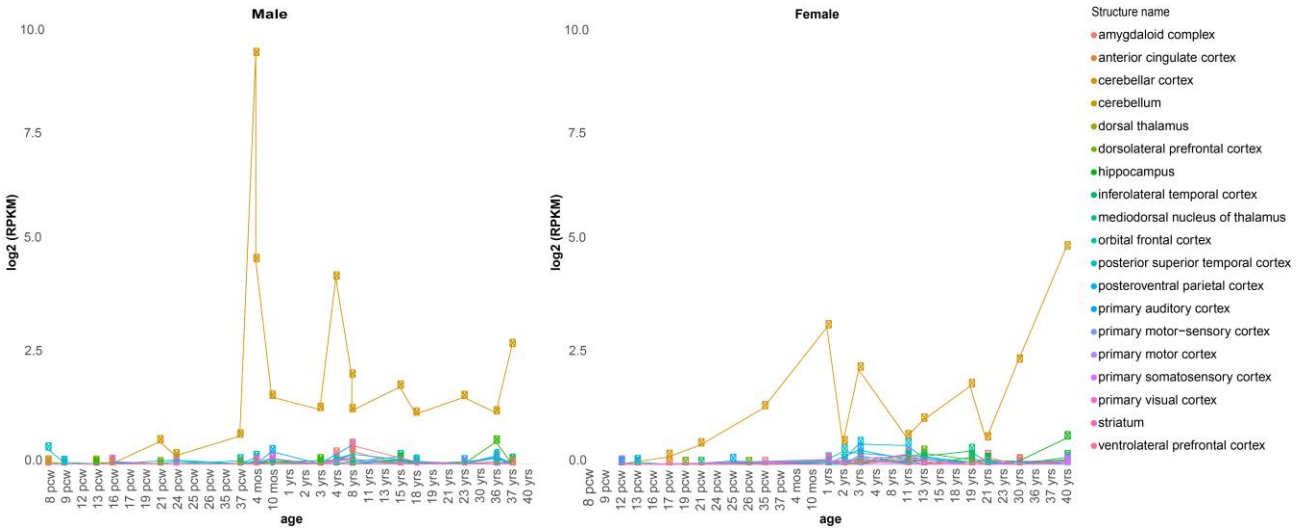

Figure 1: *KLK8* expression across lifespan in human. Shown is the normalized expression of *KLK8* in various human brain tissues across lifespan (from postconceptional week (pcw) 8 to year 40 for females and year 37 for males). Data obtained from [12].

Figure 2

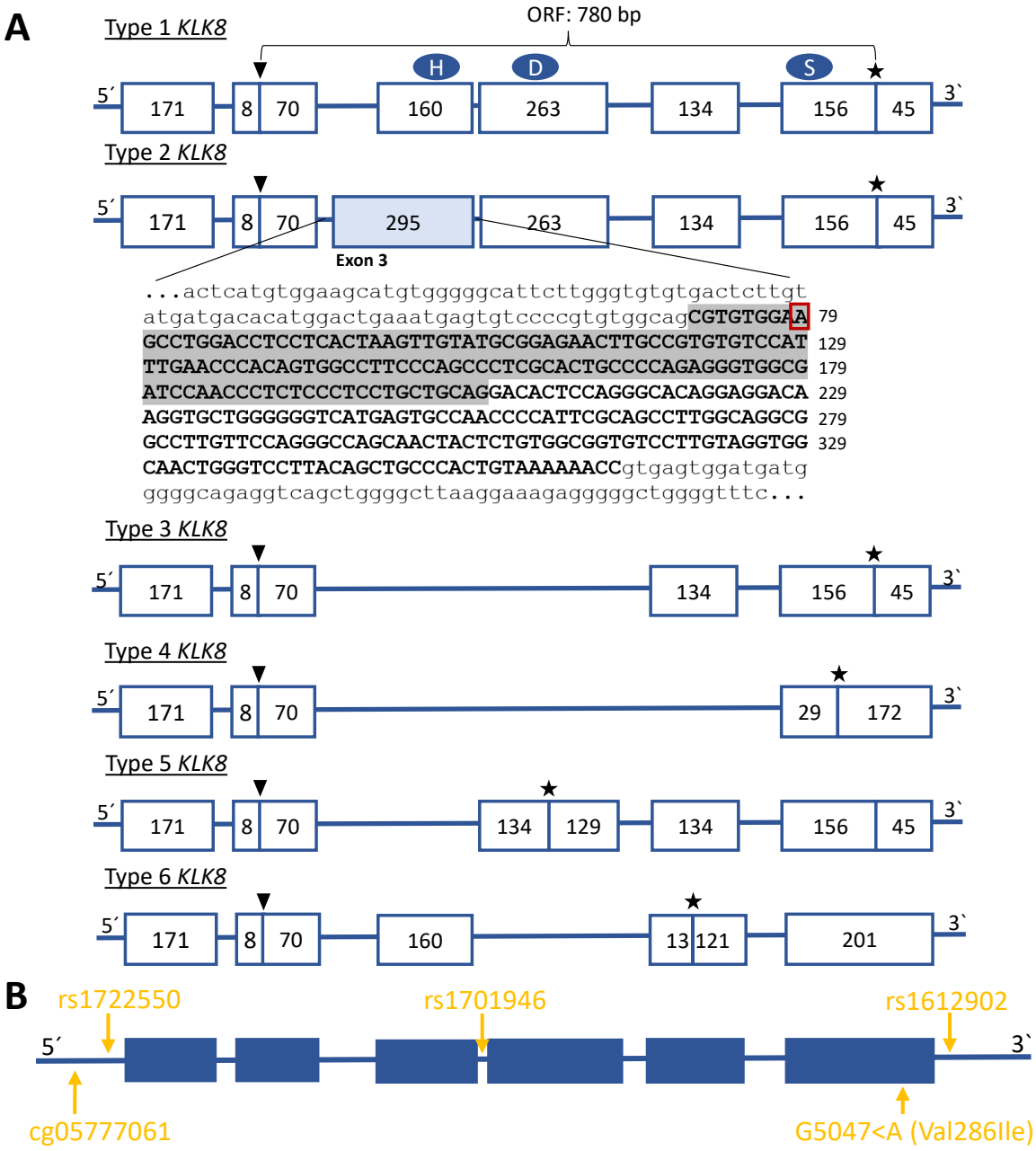

Figure 2: Genomic structures of human *KLK8* transcripts and loci reported in genetic association studies of mental disorders. A) *KLK8* spans 5.4 kb and is located at the long arm of chromosome 19 in human (19q13.4) and is comprised of 6 exons of which the first is non-coding. *KLK8* cDNA contains a single open reading frame of 780 bp, resulting in a protein comprising 260 amino acids. Shown are exons (boxes) and their length in base pairs, approximate amino acid locations of the characteristic catalytic triad of serine proteases (H, D, and S) as well as start codon (arrow) and stop codon (star). The base pair sequence of exon 3 is shown in upper-case letters (numbers indicate position in coding sequence) and flanking intron sequence in lower-case letters. The base pair sequence specific for type 2 *KLK8* is highlighted in grey and contains the 79 T>A variant (red box). **B)** Depicted

are identified *KLK8* single nucleotide polymorphisms associated with bipolar disorder (BD), *KLK8* missense mutation identified in schizophrenia, as well as *KLK8* CpG site linked to depression symptomatology in the general population (yellow arrows).

Figure 3

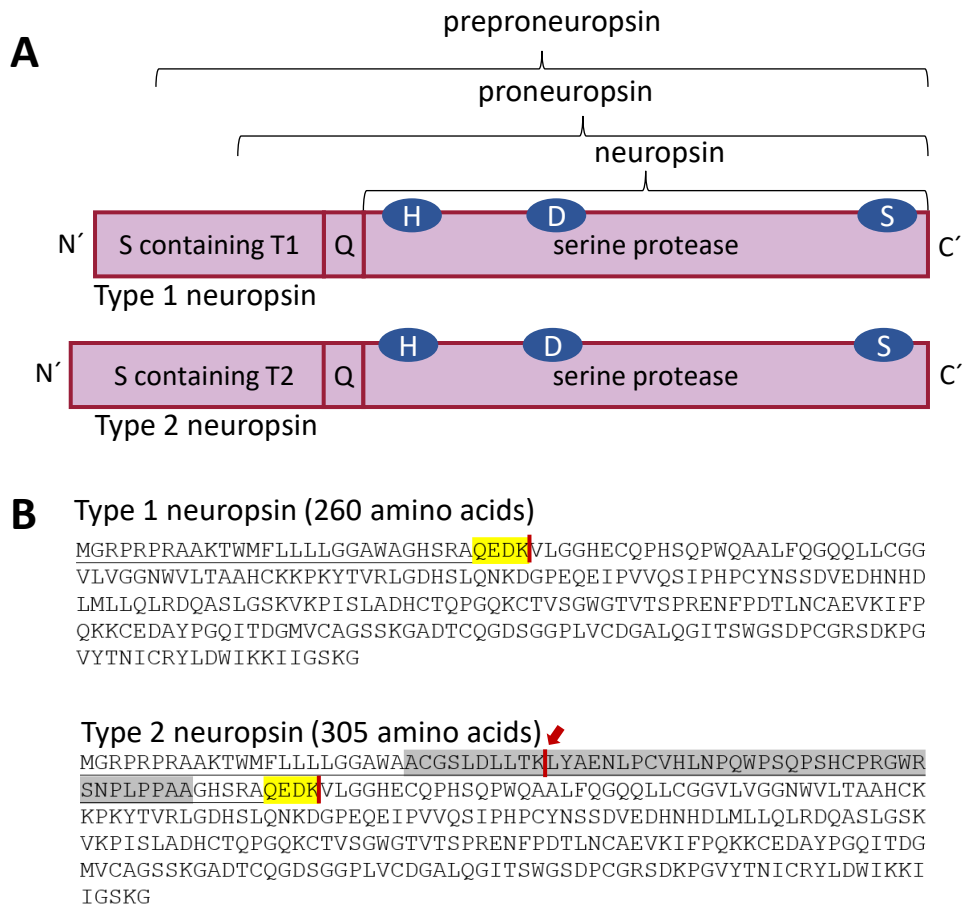

Figure 3: A) Human neuropsin preproprotein structure. Shown are: the signal sequence (S) (containing either the type 1 specific amino acid sequence (T1) or type 2 specific amino acid sequence (T2)); the activity masking peptide (Q) (QGSK in human and QDEK in mice); and the approximate amino acid locations of the characteristic catalytic triad of serine proteases (H, D, and S) **B)** Human neuropsin preprotein amino acid sequence showing endoprotease splice sites (red line). Type 2 neuropsin shows a novel splice site in the type 2 specific amino acid sequence which is highlighted in grey (red arrow).

Figure 4

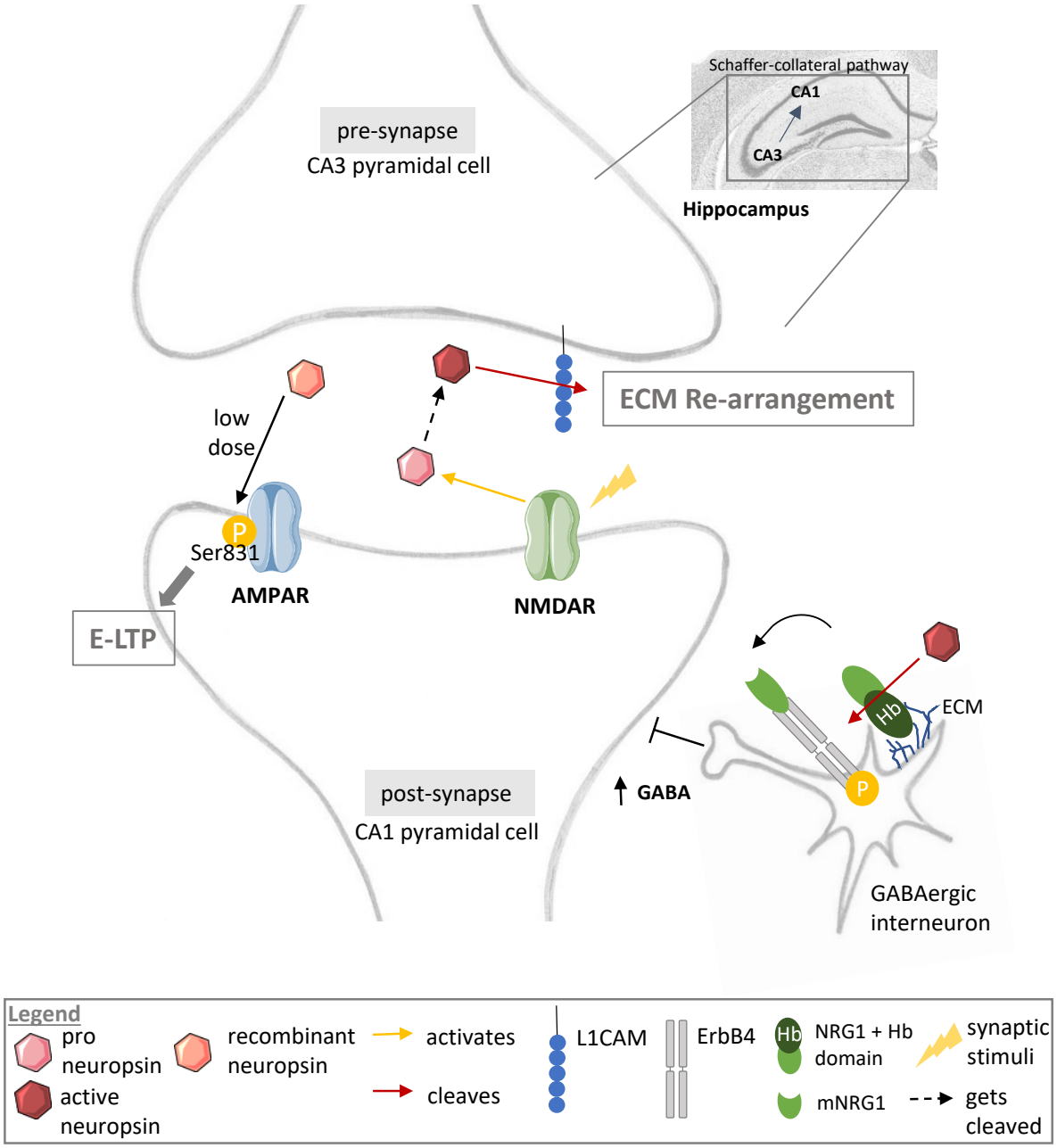

Figure 4: Neuropsin pathways implicated in synaptic plasticity. When an action potential arrives at the pre-synapse, this signal will be transmitted to the post-synapse by activating the NMDA receptor (NMDAR). NMDAR activation leads to the removal of the activity masking peptide of proneuropsin (light red) and results in neuropsin activation. Active neuropsin (dark red) then cleaves (red arrow) its substrate L1CAM (blue), and NRG1 (green). L1CAM cleavage might lead to an increased flexibility of synaptic structures. After removal of the heparin binding domain (dark green, Hb) mature NRG1 (mNRG1, light green) binds to its receptor ErbB4 (grey), which consequently gets phosphorylated (yellow circle). ErbB4 phosphorylation is critical for GABAergic inhibitory

transmission. Thus neuropsin-dependent NRG1 cleavage modulates E-LTP through the modulation of inhibitory projections into CA1 pyramidal cells in the hippocampus. Recombinant neuropsin furthermore leads to phosphorylation of the AMPA receptor (AMPAR) at a phosphorylation site specific for E-LTP (Ser831). Elements downloaded from [57], picture of mice brain taken from mouse brain atlas [58].

Figure 5

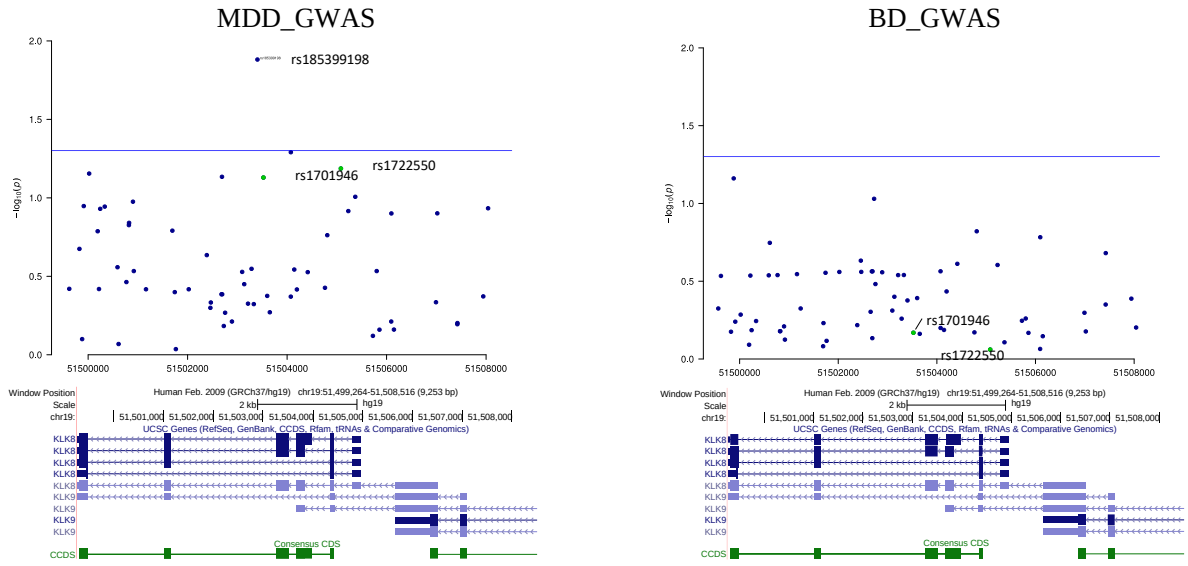

Figure 5: Single nucleotide polymorphisms (SNPs) associated with mental disorders. Shown are SNPs identified in MDD and BD GWASs [46, 49] as well as two SNPs (rs rs1722550 and rs1701946) associated with bipolar disorder [44] in relation to the UCSC genomic sequences of *KLK8* splice variants in blue. Blue line: nominal significant threshold of $p < 0.05$. Asterix indicate SNP located 3' to *KLK8*.