



## Commentary: Do Complement factors “connect the dots” in schizophrenia?

Paulo Lizano<sup>a</sup>, Konasale M. Prasad<sup>b,\*</sup>, Matcheri S. Keshavan<sup>a</sup>

<sup>a</sup> Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Ave KS253, Boston, MA 02215, United States of America

<sup>b</sup> Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, United States of America



“...everything connects to everything else...”

[– Leonardo da Vinci]

Schizophrenia is proposed to be the result of complex interaction between multiple genetic and environmental factors that alter healthy brain development (Insel, 2010). Typical onset of schizophrenia is during adolescence and young adulthood, and the presence of neurocognitive deficits and social impairments before the onset of schizophrenia suggest pronounced premorbid neurodevelopmental abnormalities (Millan et al., 2016) characterized by decreased neuronal volume, dendritic spine loss and increased packing density. The precise etiology of these deficits is unknown, although genetic factors have been associated with the risk for this illness. The contribution of inflammation has recently gained significant traction.

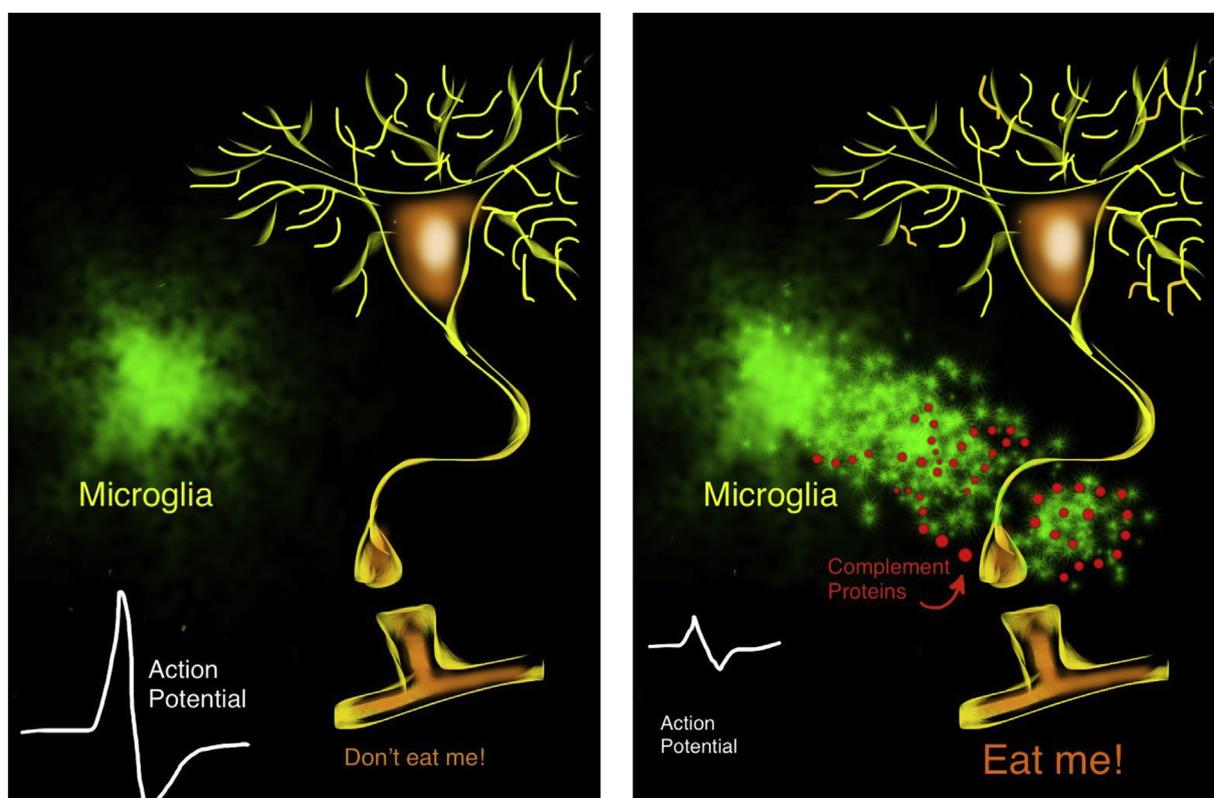
Over the decades, several inflammatory mediators were examined for their association with schizophrenia including complement proteins. Complement proteins are part of the innate immunity and potentially were important. Initial studies focused on these proteins because of their role in inflammation, thus “connecting the dots” between inflammation and schizophrenia. However, levels of complement proteins in peripheral blood in schizophrenia patients have not shown a consistent pattern of alteration. In that light, recent genome-wide association studies (GWAS) in large samples providing adequate power to detect variants with genome-wide significance, revealed 108 significantly associated genetic loci with the risk of schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). The strongest risk was repeatedly identified on the Human Leukocyte Antigen (HLA) region, which is a gene-rich region with complex linkage disequilibrium patterns. After painstaking experiments, Sekar et al. reported that a copy number variant consisting of four genes (denoted as R-C-C-X for *STK19* (*RP1*), *C4* (*C4A* or *C4B*), *CYP21A1* or *CYP21A2*, and *TNXB*) accounted for a portion of the risk associated with the HLA region (Sekar et al., 2016). It is important to note that *C4A* variants associated with schizophrenia and synaptic pruning are not single nucleotide polymorphisms on a candidate gene, but they are gene copy numbers that result from a recombination site at *CYP21A2* that leads to mono-, bi-, or trimodular cassettes. These

cassettes generate multiple functional copies of *C4A/C4B*. Intronic insertion of human endogenous retroviral (HERV) elements affects the transcription of *C4A/C4B*. Thus, Sekar et al. reported that both *C4A* and *C4B* protein expression was increased proportional to copy numbers in key regions of postmortem brain tissues of schizophrenia patients; however, *C4A* but not *C4B* copy numbers correlated with schizophrenia risk. Further, *C4*-deficient mice showed decreased synaptic pruning (Sekar et al., 2016). Complement proteins have in recent years been implicated as serving a role in the phagocytosis of redundant (or ineffective) synapses during adolescence, a critical period of risk for schizophrenia onset (Stephan et al., 2012). Together, these important observations implicated genetically mediated alterations in complement activity as underlying alterations in synaptic pruning and schizophrenia risk (Fig. 1, also on Front Cover).

Based on multiple supportive evidence from postmortem, sleep and EEG studies, Irwin Feinberg originally proposed that aberrant peri-adolescent pruning of synapses (resulting in either too much or too little pruning) as underlying the pathogenesis of schizophrenia (Feinberg, 1982). Subsequently, it was postulated that the neurobiological abnormalities observed in schizophrenia suggest an exaggerated pruning of synapses during adolescence/young adulthood which could explain the adolescence onset (Keshavan et al., 1994). While no direct evidence of excessive pruning is yet to emerge in schizophrenia, several “dots” are getting connected to yield a coherent view. First, gray matter loss in first episode schizophrenia and among at-risk individuals for psychosis evidenced by imaging (Cannon et al., 2015) may reflect synaptic pruning, though gray matter metrics are composite physical measures of synapses and other neural elements, glia, interneuronal space, and microvasculature. Second, phosphorus magnetic resonance spectroscopy (<sup>31</sup>P MRS) studies on first episode schizophrenia showed greater neuropil contraction compared to controls (Pettegrew et al., 1991). More recently a <sup>31</sup>P MRS study in young adult-onset, adolescent-onset schizophrenia patients and controls showed that increasing *C4A* and also *C4B* copy numbers were associated with greater neuropil contraction, suggesting a gene dosage effect on neuropil contraction (Prasad et al., 2018). Although <sup>31</sup>P MRS does not directly assess the synapses, relative signals from variations in membrane phospholipid metabolites are comparatively more specific and sensitive to changes in the neuropil, of which synapses are integral parts. Finally, there is support for decreased dendritic spine density in post-mortem brains of schizophrenia patients (Garey et al., 1998; Glantz and Lewis, 2000) compared to healthy subjects and patients with major depression. It is notable that

\* Corresponding author at: Ste 279, 3811 O'Hara St, Pittsburgh, PA 15213, United States of America.

E-mail addresses: [plizano@bidmc.harvard.edu](mailto:plizano@bidmc.harvard.edu) (P. Lizano), [prasadkm@upmc.edu](mailto:prasadkm@upmc.edu) (K.M. Prasad).



**Fig. 1.** The synaptic pruning hypothesis of schizophrenia. The figure on the left illustrates a healthy neuron (with a robust action potential) and microglia; the figure on the right shows a weak action potential and an activated microglia “eating up” a dysfunctional dendritic process via complement proteins.

decreased dendritic spine density was primarily confined to basilar dendrites in deep layer 3 of the frontal and temporal cortex. However, decreased dendritic spine density may not be only due to excessive synaptic pruning, but related to decreased formation or maintenance of dendritic spines.

Despite these challenges, the publication of Sekar et al. study renewed interest in examining complement proteins to elucidate factors associated with synaptic pruning. In recent publications in this *Journal*, Laskaris et al. (Laskaris et al., 2018), examined alterations in peripheral blood of C1q, C3, and C4 in ultra-high-risk, first episode psychosis and chronic schizophrenia patients. They found increased levels of C3 and C4 among ultra-high-risk subjects, no change in first-episode patients, and elevated C4 levels in chronic schizophrenia patients. While this is not a longitudinal study, these findings suggest elevated complement levels before the onset of schizophrenia with possible “normalization” at first-break, followed by elevation during a prolonged course of schizophrenia. Pathophysiological implications of these findings are, thus, unclear. This finding, if replicated in longitudinal follow-up studies, suggests that elevations in selected complement proteins may tip the balance toward conversion to psychosis and thus be a useful predictive biomarker.

Kopczynska et al. (2017) showed, in another recent study in this *Journal*, no differences in C3 or C4 among first episode schizophrenia patients. This finding is similar to Laskaris et al. study for first episode patients. Although it is tempting to propose an association of complement protein variations with increased synaptic pruning in ultra-high-risk subjects, it is important to note that peripheral complement proteins do not cross intact blood-brain barrier, and that complement proteins in the brain are synthesized locally in the microglia and neurons. Therefore, peripheral levels of C4 are not a proxy for complement activity in the brain, but a study by Yang et al. (Yang et al., 2003) demonstrated that serum C4 protein concentrations correlate with C4 gene size, which would suggest that an increase in C4 copy number could predict increased levels of C4 both centrally and in the periphery. Finally, the

authors did not evaluate the possibility that specific isoforms within this complement cascade may be altered at various stages of a psychotic illness, such as C4A or C4B (Sekar et al., 2016).

In summary, while the idea that complements alterations may prove to be an etiologically meaningful and therapeutically actionable biomarker in schizophrenia, many questions remain. First, it is important to note that there are other gene variants associated with schizophrenia risk, implicating other synaptic proteins and the development of new tools are making it more accessible to better understand the role of the complement system on synaptic pruning. For example, integrating genetics with *in vivo* brain imaging (Prasad et al., 2018) is a promising approach. Gene-environment interactions could also impact pathogenic complement protein processes in schizophrenia. Second, the relation between serum and CNS complement proteins is unclear, and further studies need to examine cerebrospinal fluid samples as well, across prodromal, early and chronic psychotic states; CSF and serum biomarker profiling has been found to characterize disease state in immune disorders such as multiple sclerosis (Ingram et al., 2012) and systemic lupus erythematosus (Jongen et al., 2000). Lastly, innovative imaging methods (e.g. Radhakrishnan et al., 2017) are needed to directly demonstrate increased pruning in schizophrenia. Clearly, more needs to be learnt to “connect the dots” between these tantalizing observations to move us toward a more complete understanding of at least one pathophysiological pathway to schizophrenia.

#### Acknowledgements

Paulo Lizano: Sydney R Baer Jr Foundation Grant, Dupont Warren Fellowship at Harvard Medical School.

#### References

- Cannon, T.D., Chung, Y., He, G., Sun, D., Jacobson, A., van Erp, T.G.M., McEwen, S., Addington, J., Bearden, C.E., Cadenhead, K., Cornblatt, B., Mathalon, D.H., McGlashan, T., Perkins, D., Jeffries, C., Seidman, L.J., Tsuang, M., Walker, E., Woods, S.W., Heinssen, R., North American Prodrome Longitudinal Study Consortium, 2015.

- Progressive reduction in cortical thickness as psychosis develops: a multisite longitudinal neuroimaging study of youth at elevated clinical risk. *Biol. Psychiatry* 77, 147–157. <https://doi.org/10.1016/j.biopsych.2014.05.023>.
- Feinberg, I., 1982. Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence? *J. Psychiatr. Res.* 17, 319–334.
- Garey, L.J., Ong, W.Y., Patel, T.S., Kanani, M., Davis, A., Mortimer, A.M., Barnes, T.R., Hirsch, S.R., 1998. Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. *J. Neurol. Neurosurg. Psychiatry* 65, 446–453.
- Glantz, L.A., Lewis, D.A., 2000. Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch. Gen. Psychiatry* 57, 65–73.
- Ingram, G., Hakobyan, S., Hirst, C.L., Harris, C.L., Loveless, S., Mitchell, J.P., Pickersgill, T.P., Robertson, N.P., Morgan, B.P., 2012. Systemic complement profiling in multiple sclerosis as a biomarker of disease state. *Mult. Scler.* 18 (10), 1401–1411 (2012 Oct).
- Insel, T.R., 2010. Rethinking schizophrenia. *Nature* 491:7422 (468), 187–193 (2012).
- Jongen, P.J., Doesburg, W.H., Ibrahim-Stappers, J.L., Lemmens, W.A., Hommes, O.R., Lamers, K.J., 2000 Feb. Cerebrospinal fluid C3 and C4 indexes in immunological disorders of the central nervous system. *Acta Neurol. Scand.* 101 (2), 116–121.
- Keshavan, M.S., Anderson, S., Pettegrew, J.W., 1994. Is schizophrenia due to excessive synaptic pruning in the prefrontal cortex? The Feinberg hypothesis revisited. *J. Psychiatr. Res.* 28, 239–265.
- Kopczynska, M., Zelek, W., Touchard, S., Gaughran, F., Di Forti, M., Mondelli, V., Murray, R., O'Donovan, M.C., Morgan, B.P., 2019. Complement system biomarkers in first episode psychosis. *Schizophr. Res.* 204, 16–22.
- Laskaris, L., Zalesky, A., Weickert, C.S., Di Biase, M.A., Chana, G., Baune, B.T., Bousman, C., Nelson, B., McGorry, P., Everall, I., Pantelis, C., Cropley, V., 2019. Investigation of peripheral complement factors across stages of psychosis. *Schizophr. Res.* 204, 30–37.
- Millan, M.J., Andrieux, A., Bartzokis, G., Cadenhead, K., Dazzan, P., Fusar-Poli, P., Gallinat, J., Giedd, J., Grayson, D.R., Heinrichs, M., Kahn, R., Krebs, M.-O., Leboyer, M., Lewis, D., Marin, O., Marin, P., Meyer-Lindenberg, A., McGorry, P., McGuire, P., Owen, M.J., Patterson, P., Sawa, A., Spedding, M., Uhlhaas, P., Vaccarino, F., Wahlestedt, C., Weinberger, D., 2016. Altering the course of schizophrenia: progress and perspectives. *Nat. Rev. Drug Discov.* 15, 485–515. <https://doi.org/10.1038/nrd.2016.28>.
- Pettegrew, J.W., et al., 1991. Alterations in brain high-energy phosphate and membrane phospholipid metabolism in first-episode, drug-naïve schizophrenics. A pilot study of the dorsal prefrontal cortex by in vivo phosphorus 31 nuclear magnetic resonance spectroscopy. *Arch. Gen. Psychiatry* 48, 563–568. <https://doi.org/10.1001/archpsyc.1991.01810300075011>.
- Prasad, K.M., Chowdari, K.V., D'Aiuto, L.A., Iyengar, S., Stanley, J.A., Nimgaonkar, V.L., 2018. Neuropil contraction in relation to Complement C4 gene copy numbers in independent cohorts of adolescent-onset and young adult-onset schizophrenia patients—a pilot study. *Transl. Psychiatry* 8, 134. <https://doi.org/10.1038/s41398-018-0181-z>.
- Radhakrishnan, R., Skosnik, P., Finnema, S., Rotolo, R., Forselius-Bielen, K., Creatura, G., Nabulsi, N., Carson, R., Souza, D.D., 2017. 962. In-vivo evidence of decreased synaptic density in schizophrenia: a [<sup>11</sup>C]UCB-J PET imaging study. *Biol. Psychiatry* 81, S389. <https://doi.org/10.1016/j.biopsych.2017.02.688>.
- Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 491:7422 (511), 421–427. <https://doi.org/10.1038/nature13595> (2012).
- Sekar, A., Bialas, A.R., de Rivera, H., Davis, A., Hammond, T.R., Kamitaki, N., Tooley, K., Presumey, J., Baum, M., Van Doren, V., Genovese, G., Rose, S.A., Handsaker, R.E., Schizophrenia Working Group of the Psychiatric Genomics Consortium, Daly, M.J., Carroll, M.C., Stevens, B., McCarroll, S.A., 2016. Schizophrenia risk from complex variation of complement component 4. *Nature* 491:7422 (530), 177–183. <https://doi.org/10.1038/nature16549> (2012).
- Stephan, A.H., Barres, B.A., Stevens, B., 2012. The complement system: an unexpected role in synaptic pruning during development and disease. *Annu. Rev. Neurosci.* 35, 369–389.
- Yang, Y., Chung, E.K., Zhou, B., Blanchong, C.A., Yu, C.Y., Füst, G., Kovács, M., Vatay, Á., Szalai, C., Karádi, I., Varga, L., 2003. Diversity in intrinsic strengths of the human complement system: serum C4 protein concentrations correlate with C4 gene size and polygenic variations, hemolytic activities, and body mass index. *J. Immunol.* 171, 2734–2745. <https://doi.org/10.4049/jimmunol.171.5.2734>.