Association between DNA methylation of the CUB and sushi multiple domains 1 gene and schizophrenia

Mohd Asyraf Abdull Jalil[a], Nour El Huda Abd Rahim[a], Hanisah Mohd Noor[b], Noorul Amilin Harun[c], Norlelawati A. Talib[d]

[a]*Department of Basic Medical Sciences, Kulliyyah of Medicine, International Islamic University Malaysia, 25200 Kuantan, Pahang.*
[b]*Department of Psychiatry, Faculty of Medicine, Universiti Sultan Zainal Abidin, 23200 Kuala Terengganu, Terengganu.*
[c]*Department of Psychiatry and Mental Health, Hospital Tengku Ampuan Afzan, 25100 Kuantan, Pahang.*
[d]*Department of Pathology and Laboratory Medicine, Sultan Ahmad Shah Medical Centre, International Islamic University Malaysia, 25200 Kuantan, Pahang.*

Age of Onset

- Early adulthood
- Productive years

Employment

- 80% remain unemployed (*Dahlan et al, 2014*)
- Dependent on family

Economic loss

- RM 428 million = 0.04% GDP cost to Nation (*Teoh et al, 2017*)

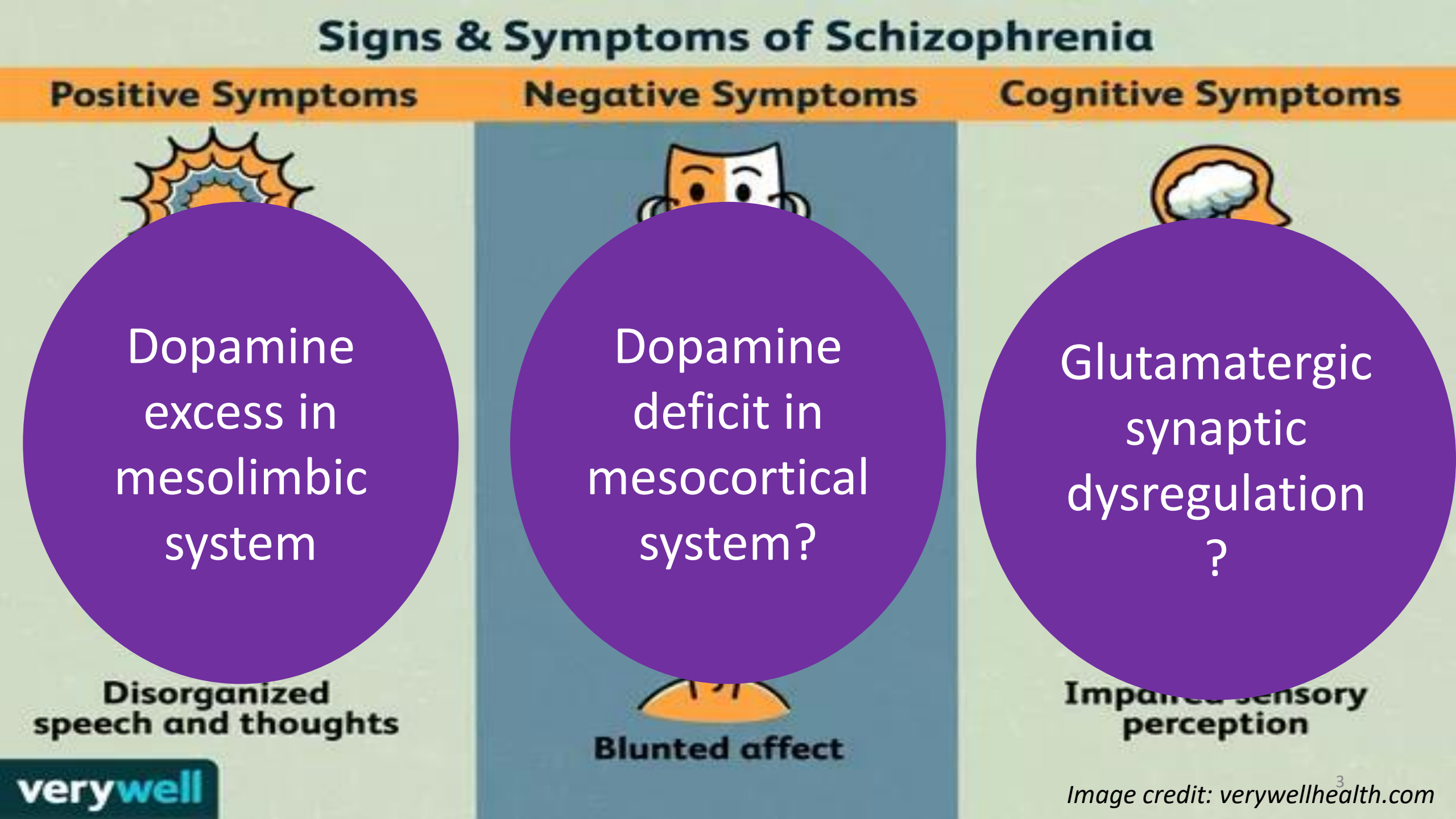
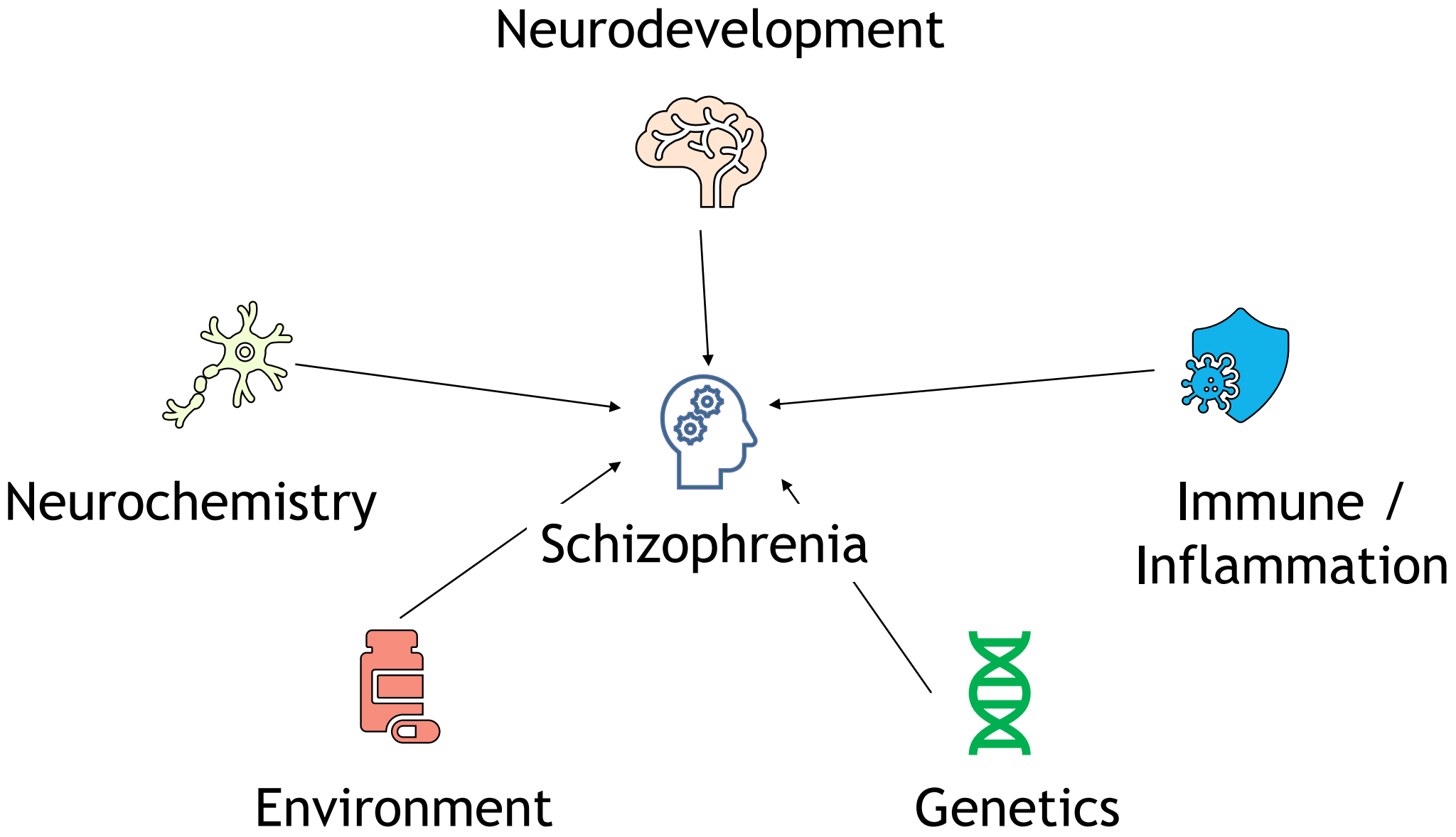
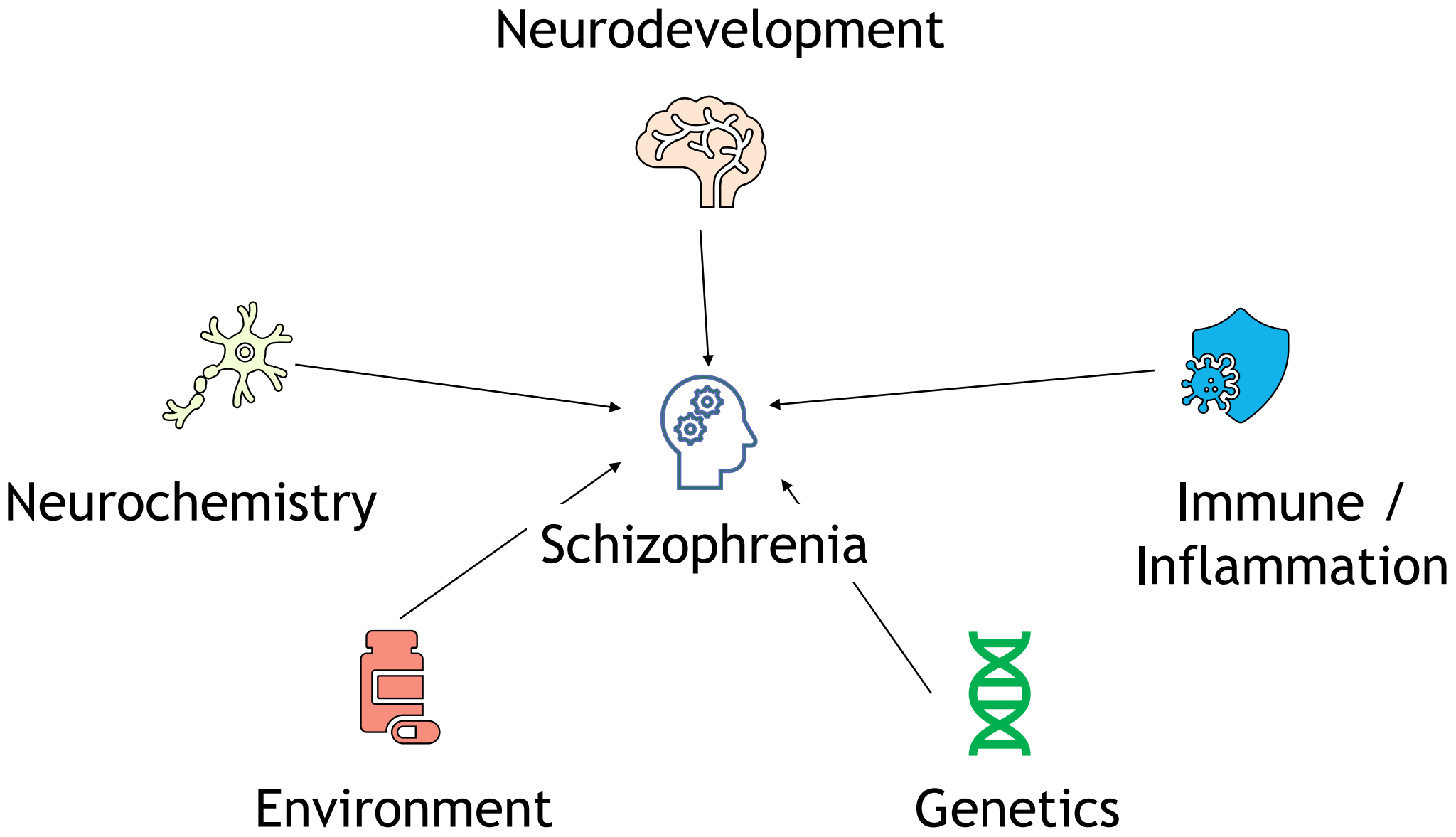


Image credit: verywellhealth.com


Neurodevelopment
Neurochemistry
Schizophrenia
Immune / Inflammation
Environment
Genetics

SYNAPSE PRUNING

- ❑ Developmental process of targeted synapse elimination
- ❑ Performed by microglia
- ❑ Complement system seems to be important mediators *(Stevens et al., 2007; Sekar et al., 2016; Comer et al., 2020)*
- ❑ Schizophrenia brains have fewer synapse from increased elimination by microglia *(Sellgren et al., 2019)*


Neurodevelopment
Neurochemistry
Schizophrenia
Immune / Inflammation
Environment
Genetics

CSMD1

- A large gene, about 2 Mb on Chr 8p23.2
- Potentially a tumour suppressor gene *(Gialeli et al., 2021)*
- Code for multiple CUB domains and complement control protein domains (sushi)
- Expressed mainly in the brain and testis *(Escudero-Esparza et al., 2013)*
- Inhibits deposition of C3b, co-factor for C3b degradation and membrane attack complex (MAC) assembly *(Escudero-Esparza et al., 2013)*

CSMD1 & Schizophrenia

- GWAS: association of SNPs with schizophrenia (*Schizophrenia Working Group of the Psychiatric Genomics, 2014*)
- SNPs associated with cognitive dysfunction *(Koiliari et al. 2014; Athanasiu et al., 2017; Stepanov et al., 2017;)*
- Low level of expression in schizophrenia *(Liu et al., 2019; El Gayed et al., 2021)*
- Deletion leads to altered behaviour similar to schizophrenia symptoms in mouse model *(Steen et al 2013)*
- Opposes complement action on neural tissues (*Baum et al., 2020)*

DNA METHYLATION

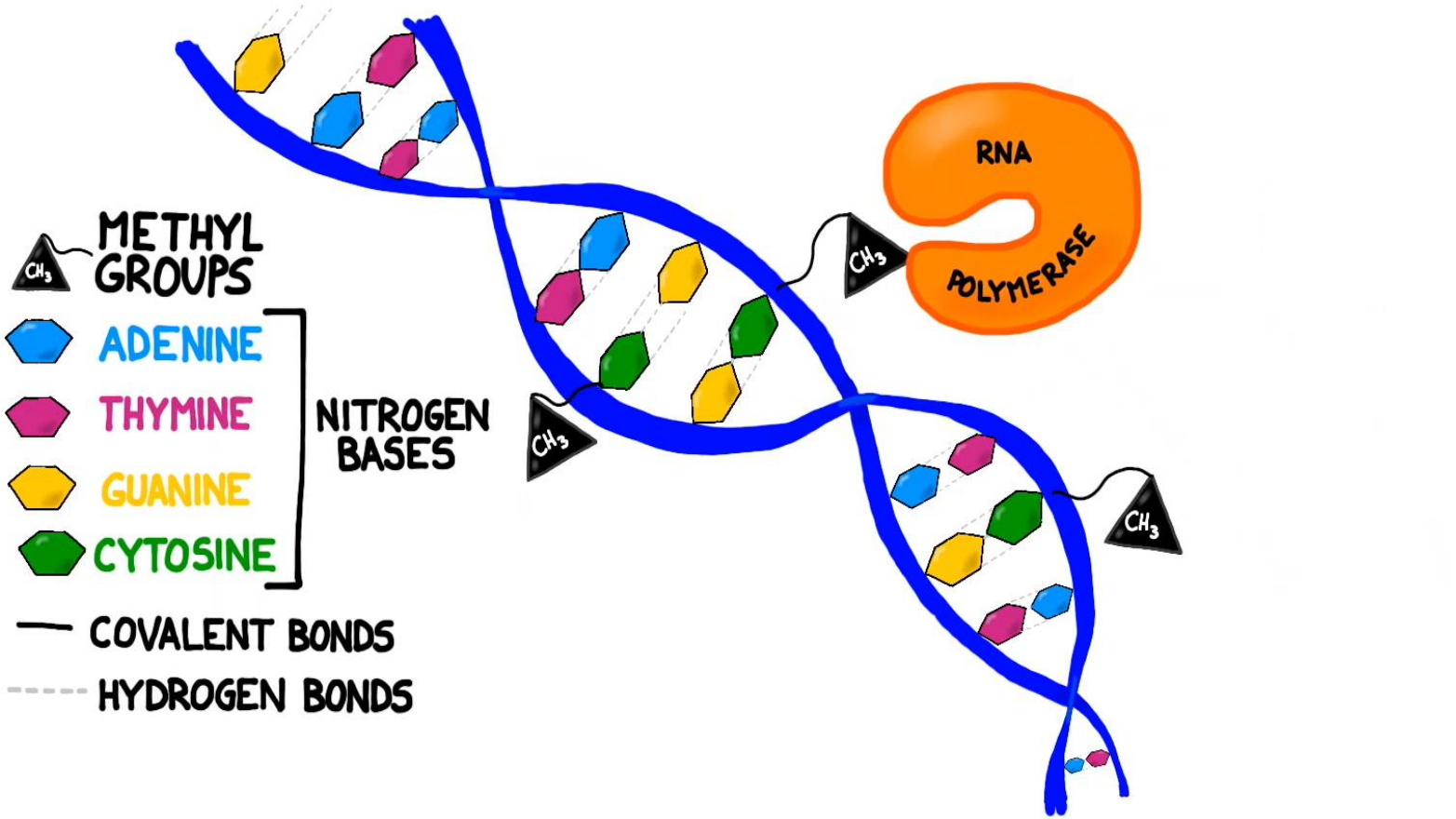
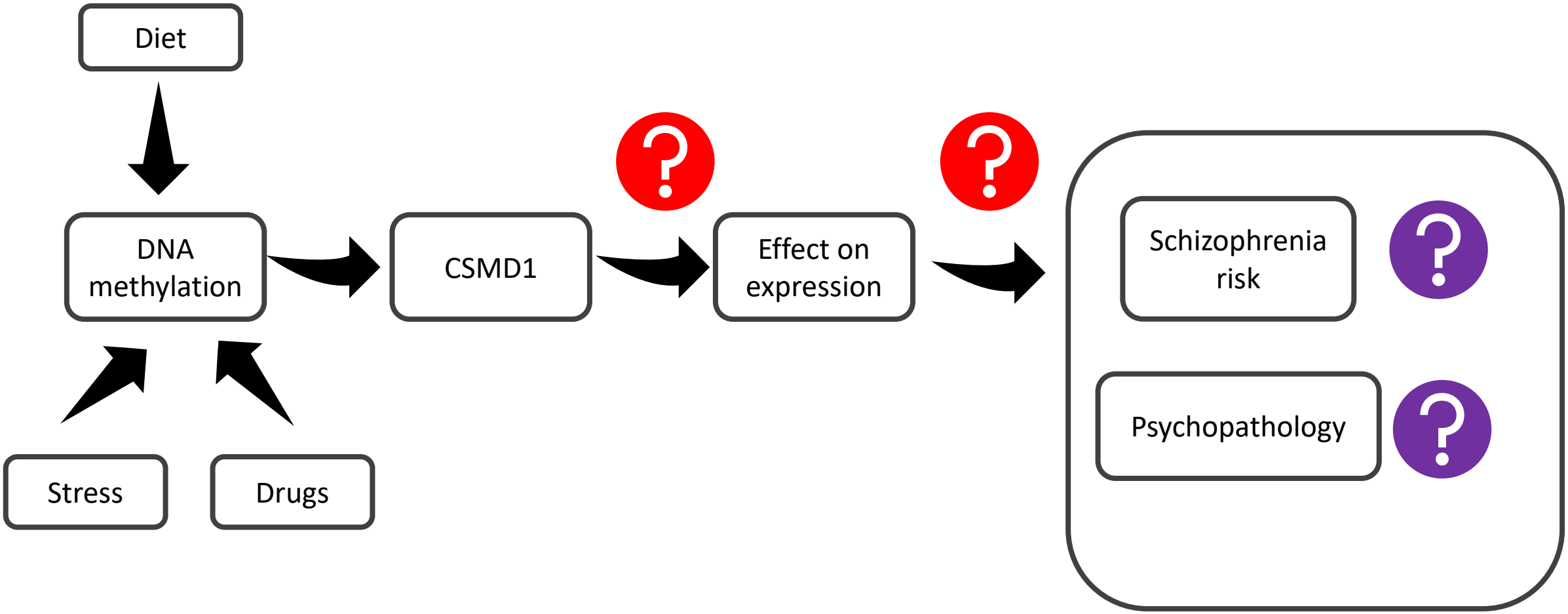
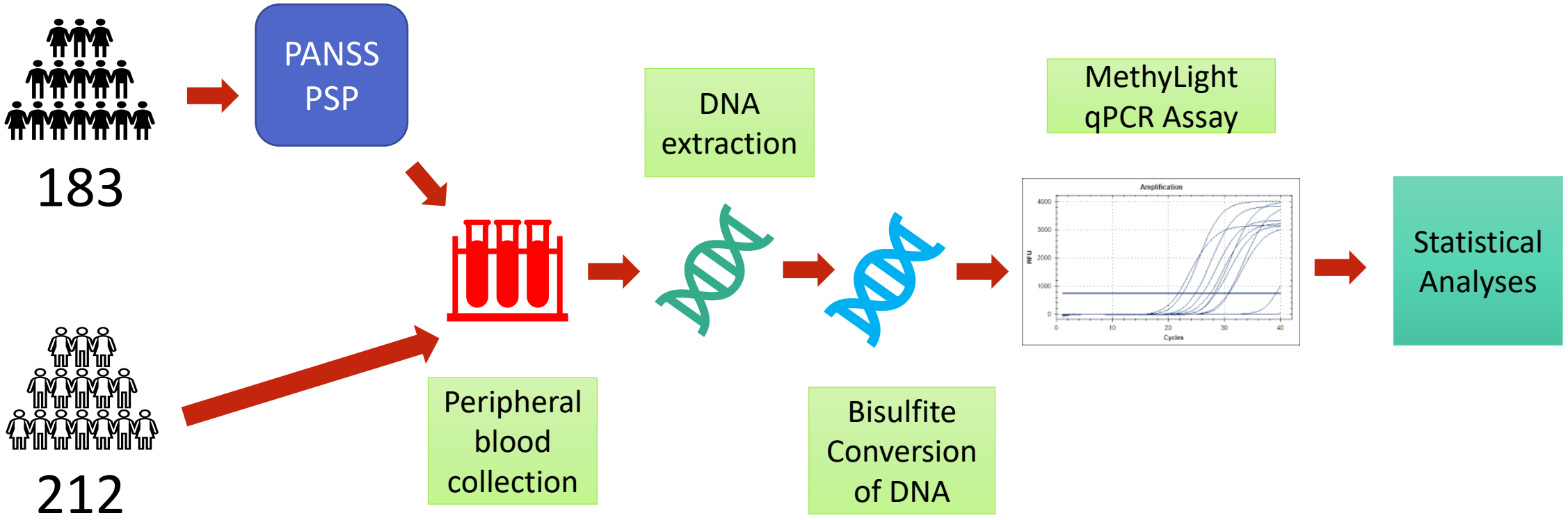


Image credit: BOGOBiology

DNA METHYLATION of CSMD1 in Schizophrenia



METHODOLOGY



Ethics approval: MREC NMRR-19-1911-47161; IREC 2020-089


Assembly exceptions
chromosome 8
Assembly exceptions
p23.1
p22
p12
q12.1
q21.13
q21.3
q22.1
q23.3
CSMD1
GC Percentage
0
20
40
60
80
0
200 bp
400 bp
600 bp
800 bp
1000 bp
CpG
Input Sequence
Bisulfite PCR primer
MSP Primer Set
Methylated-Specific
Unmethylated-Specific
CpG Island

PANSS & PSP

Positive and Negative Syndrome Scale (PANSS)	Personal and Social Performance Scale
Clinician administered	Clinician administered
30 items, 3 to 5 domains	4 domains
7 point Likert Scale	6 point Likert Scale
1 (absent) – 7 (extreme)	Absent to Very Severe
Score 30 (absent of symptom) – 210 (most severe symptom)	Score 0 (worst function) – 100 (best function)

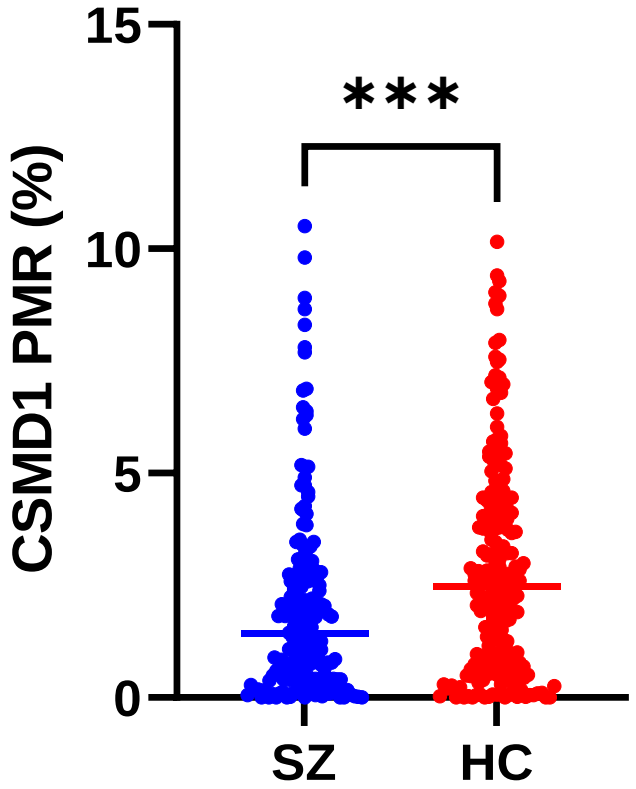
RESULTS - SOCIODEMOGRAPHICS

Table 1 Sociodemographic characteristics of study participants.

Sociodemographic Characteristics	Schizophrenia patients, n = 183	Healthy controls, n = 212	p-value
Age, Years median (IQR)	 39.0 (31.0 - 48.0)	 38.0 (31.0 – 45.0)	0.309[1]
Gender Male, % (n) Female, % (n)	 61.7 (113) 38.3 (70)	 61.3 (130) 38.7 (82)	0.931[2]
Ethnicity Malay, % (n) Chinese, % (n)	 79.2 (145) 20.8 (38)	 72.6 (154) 27.4 (58)	0.128[2]

*[1]Mann-Whitney U test; [2]Chi-square test; *statistically significant. n = number; IQR = interquartile range; BMI = Body Mass Index. p-value <0.05 is considered significant at 95% confidence interval.*

RESULTS – CSMD1 DNA METHYLATION in SZ vs HC



Median PMR Schizophrenia (*n* = 183): 1.45%
Median PMR Healthy Controls (*n* = 212): 2.48%
U = 15484.5, *p* = 0.001

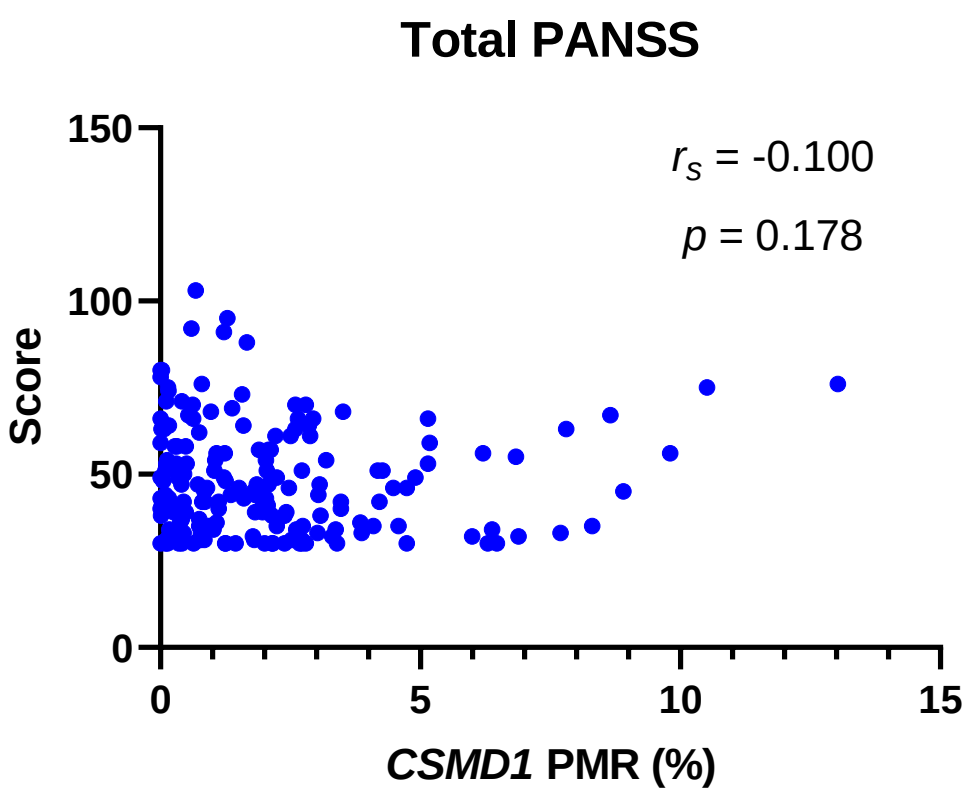
DISCUSSION

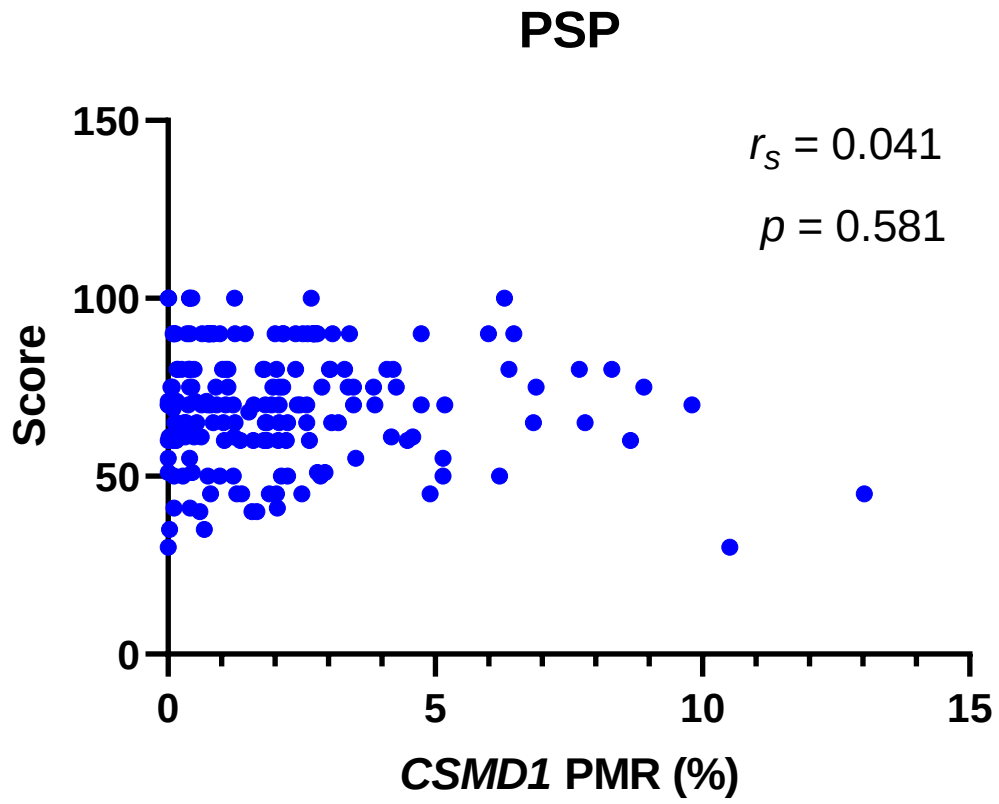
- Differential methylation of *CSMD1* gene or promoter has not been reported previously. The closest differentially methylated position reported by an EWAS is 700,000 bp downstream *(Hannon et al., 2021)*

- Higher methylation levels of *CSMD1* has been reported in several types of malignancy *(Kamal et al., 2017; Shull et al., 2013)* causing silencing of the gene.

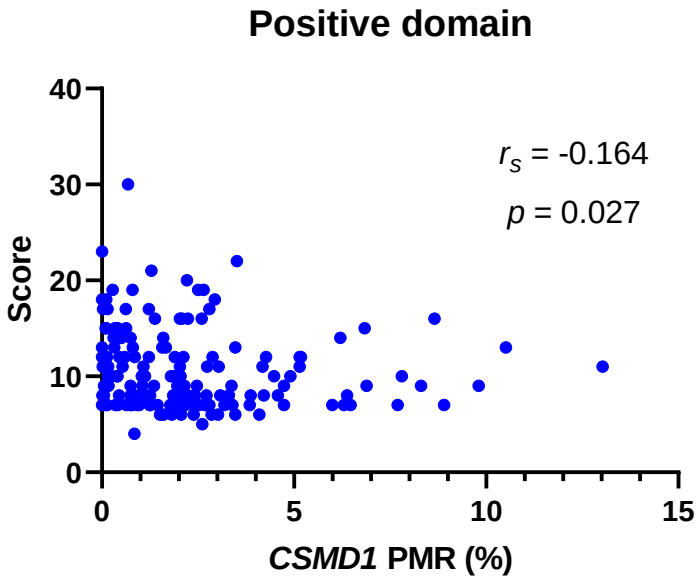
- DNA methylation of a may also cause increased expression *(Raluseviciute et al., 2020; Yang et al., 2014)*, and therefore is locus and context dependent *(Dhar et al., 2021)*

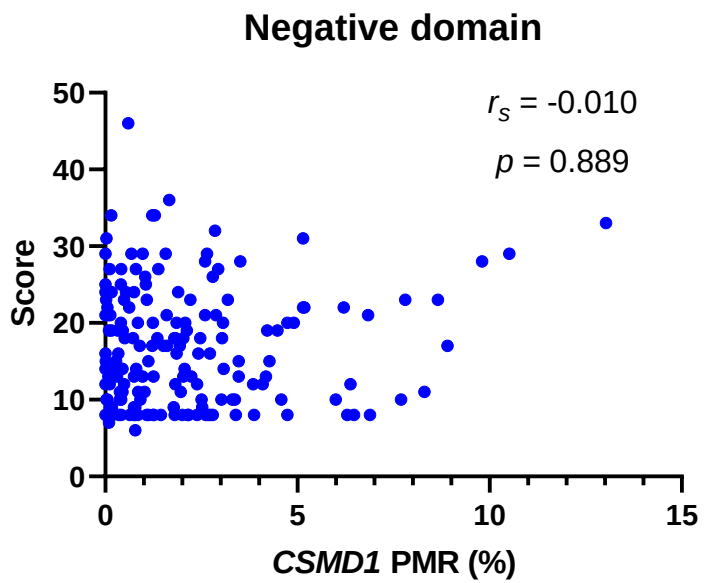
RESULTS - PSYCHOPATHOLOGY

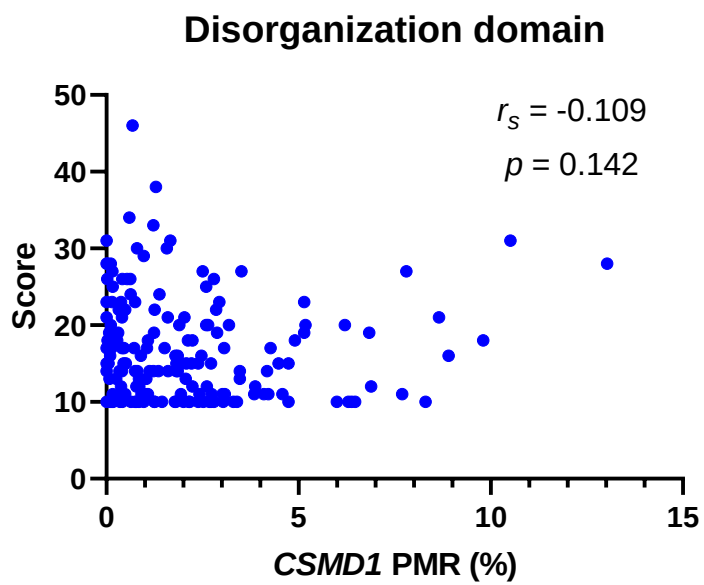


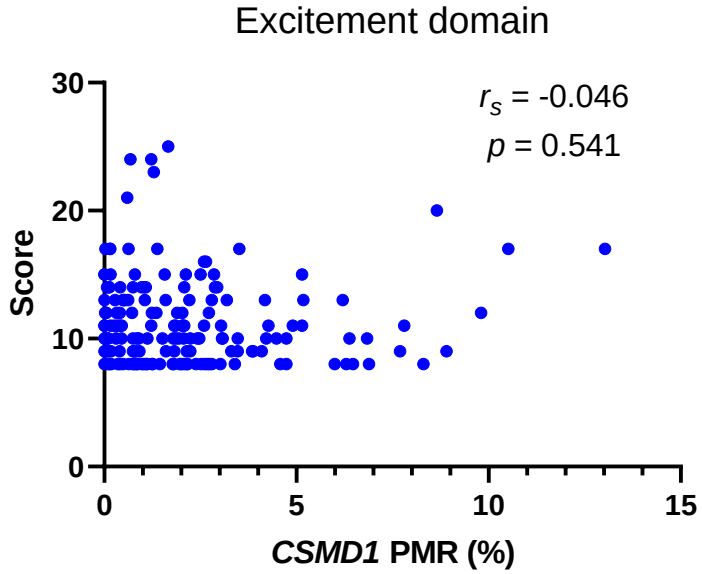


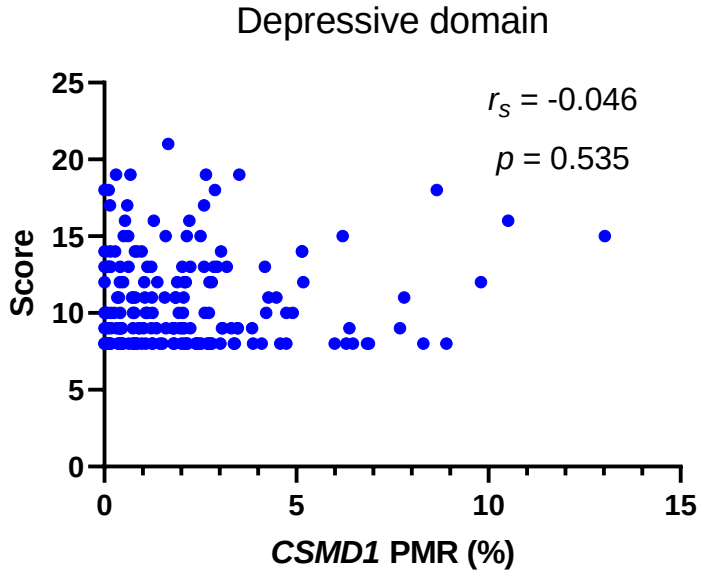
RESULTS - PSYCHOPATHOLOGY







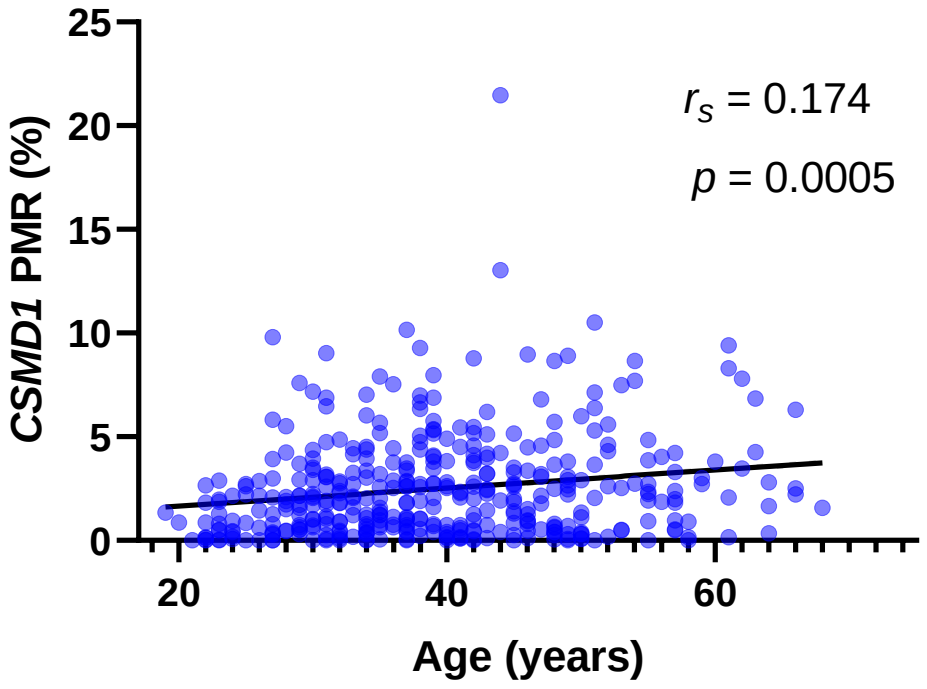


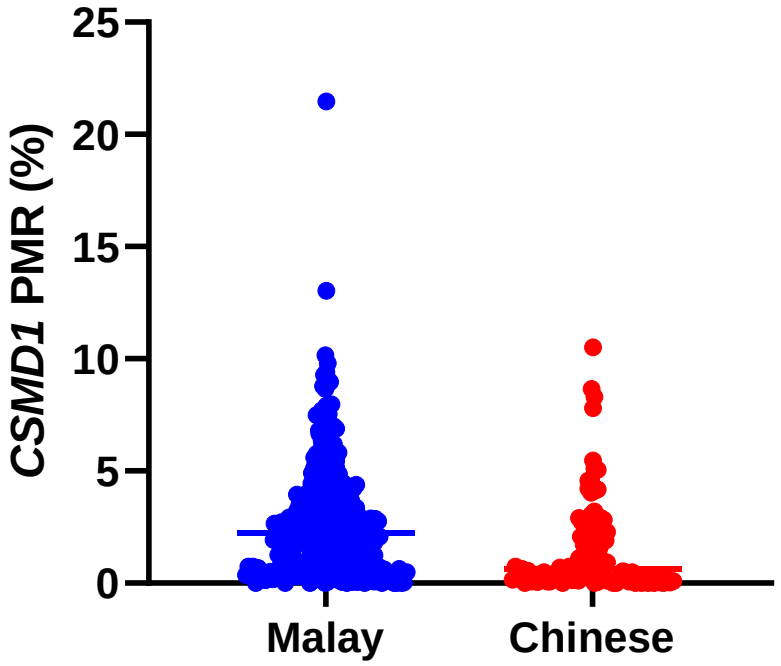


DISCUSSION

- ❑ Schizophrenia is a heterogenous and complex diagnosis. Multiple pathophysiology may be involved.
- ❑ Using PANSS can help identify the affected phenotypes. In this study, only positive symptoms were linked with *CSMD1* methylation.
- ❑ El Gayed et al. *(2021)* found lower expression of *CSMD1* associated with risk of psychosis
- ❑Other studies had found association between certain variants (SNP) of *CSMD1* and cognitive dysfunction *(Koiliari et al., 2014; Athanasiu et al., 2017; Stepanov et al. 2017)*
- ❑ Mice with *CSMD1* deletion shows altered behaviour akin to negative symptoms *(Steen et al., 2013)*

RESULTS – SOCIODEMOGRAPHICS vs CSMD1 DNA METHYLATION





Median PMR Malays (n = 299): 2.24%
Median PMR Chinese (n = 96): 0.63%
U = 8923, p < 0.001

DISCUSSION

- Age has been found to be correlated with methylation of many genes *(Jung & Pfeifer, 2015)*

- Differences in DNA methylation between different ethnicities could be explained by difference in ancestry or environmental exposures *(Galanter et al., 2017)*

- Since methylation is reversible, further exploration on the mechanism underlying this association could eventually be useful for novel treatment strategy in the future.

LIMITATIONS

- ❑ Results using peripheral blood may not reflect methylation in brain tissue
- ❑ We did not manage to test the association between DNA methylation and the expression of *CSMD1.*
- ❑ The DNA methylation of other CpG island inside *CSMD1* or other genes related to synapse pruning/ plasticity were not studied together.
- ❑ Recruited patients were mostly stable on medication, therefore the methylation levels during active/ relapse phase could not be evaluated.

CONCLUSION

- Lower DNA methylation of *CSMD1* may be involved in the pathogenesis of schizophrenia, particularly the positive symptoms.

- The cause-effect relationship and the exact mechanism need to be investigated in future studies.

REFERENCES

- Abd El Gayed, E. M., Rizk, M. S., Ramadan, A. N., & Bayomy, N. R. (2021). mRNA Expression of the CUB and Sushi Multiple Domains 1 (CSMD1) and Its Serum Protein Level as Predictors for Psychosis in the Familial High-Risk Children and Young Adults. *ACS Omega, 6*(37), 24128-24138. doi:10.1021/acsomega.1c03637
- Baum, M. L., Wilton, D. K., Muthukumar, A., Fox, R. G., Carey, A., Crotty, W., . . . Stevens, B. (2020). CUB and Sushi Multiple Domains 1 (CSMD1) opposes the complement cascade in neural tissues. *bioRxiv*, 2020.2009.2011.291427. doi:10.1101/2020.09.11.291427
- [BOGObiology]. (2017, October 11). Epigenetics: Nature vs. Nurture. [Video File]. Retrieved from https://youtu.be/Q8BMP6HDIco
- Comer, A. L., Jinadasa, T., Sriram, B., Phadke, R. A., Kretsge, L. N., Nguyen, T. P. H., . . . Cruz-Martin, A. (2020). Increased expression of schizophrenia-associated gene C4 leads to hypoconnectivity of prefrontal cortex and reduced social interaction. *PLoS Biol, 18*(1), e3000604. doi:10.1371/journal.pbio.3000604
- Dahlan, R., Midin, M., Shah, S. A., Nik Jaafar, N. R., Abdul Rahman, F. N., Baharudin, A., . . . Sidi, H. (2014). Functional remission and employment among patients with schizophrenia in Malaysia. *Compr Psychiatry, 55 Suppl 1*, S46-51. doi:10.1016/j.comppsych.2013.03.007
- Dhar, G. A., Saha, S., Mitra, P., & Nag Chaudhuri, R. (2021). DNA methylation and regulation of gene expression: Guardian of our health. *Nucleus (Calcutta), 64*(3), 259-270. doi:10.1007/s13237-021-00367-y
- Escudero-Esparza, A., Kalchishkova, N., Kurbasic, E., Jiang, W. G., & Blom, A. M. (2013). The novel complement inhibitor human CUB and Sushi multiple domains 1 (CSMD1) protein promotes factor I-mediated degradation of C4b and C3b and inhibits the membrane attack complex assembly. *FASEB J, 27*(12), 5083-5093. doi:10.1096/fj.13-230706
- Galanter, J. M., Gignoux, C. R., Oh, S. S., Torgerson, D., Pino-Yanes, M., Thakur, N., . . . Zaitlen, N. (2017). Differential methylation between ethnic sub-groups reflects the effect of genetic ancestry and environmental exposures. *Elife, 6*. doi:10.7554/eLife.20532

REFERENCES

- Gialeli, C., Tuysuz, E. C., Staaf, J., Guleed, S., Paciorek, V., Morgelin, M., . . . Blom, A. M. (2021). Complement inhibitor CSMD1 modulates epidermal growth factor receptor oncogenic signaling and sensitizes breast cancer cells to chemotherapy. *J Exp Clin Cancer Res, 40*(1), 258. doi:10.1186/s13046-021-02042-1
- Hannon, E., Dempster, E. L., Mansell, G., Burrage, J., Bass, N., Bohlken, M. M., . . . Mill, J. (2021). DNA methylation meta-analysis reveals cellular alterations in psychosis and markers of treatment-resistant schizophrenia. *Elife, 10*. doi:10.7554/eLife.58430
- Jung, M., & Pfeifer, G. P. (2015). Aging and DNA methylation. *BMC Biol, 13*, 7. doi:10.1186/s12915-015-0118-4
- Kamal, M., Holliday, D. L., Morrison, E. E., Speirs, V., Toomes, C., & Bell, S. M. (2017). Loss of CSMD1 expression disrupts mammary duct formation while enhancing proliferation, migration and invasion. *Oncol Rep, 38*(1), 283-292. doi:10.3892/or.2017.5656
- Koiliari, E., Roussos, P., Pasparakis, E., Lencz, T., Malhotra, A., Siever, L. J., . . . Bitsios, P. (2014). The CSMD1 genome-wide associated schizophrenia risk variant rs10503253 affects general cognitive ability and executive function in healthy males. *Schizophr Res, 154*(1-3), 42-47. doi:10.1016/j.schres.2014.02.017
- Liu, Y., Fu, X., Tang, Z., Li, C., Xu, Y., Zhang, F., . . . Zhu, C. (2019). Altered expression of the CSMD1 gene in the peripheral blood of schizophrenia patients. *BMC Psychiatry, 19*(1), 113. doi:10.1186/s12888-019-2089-4
- Rauluseviciute, I., Drablos, F., & Rye, M. B. (2020). DNA hypermethylation associated with upregulated gene expression in prostate cancer demonstrates the diversity of epigenetic regulation. *BMC Med Genomics, 13*(1), 6. doi:10.1186/s12920-020-0657-
- Schizophrenia Working Group of the Psychiatric Genomics, C. (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature, 511*(7510), 421-427. doi:10.1038/nature13595

REFERENCES

- Sekar, A., Bialas, A. R., de Rivera, H., Davis, A., Hammond, T. R., Kamitaki, N., . . . McCarroll, S. A. (2016). Schizophrenia risk from complex variation of complement component 4. *Nature, 530*(7589), 177-183. doi:10.1038/nature16549
- Sellgren, C. M., Gracias, J., Watmuff, B., Biag, J. D., Thanos, J. M., Whittredge, P. B., . . . Perlis, R. H. (2019). Increased synapse elimination by microglia in schizophrenia patient-derived models of synaptic pruning. *Nat Neurosci, 22*(3), 374-385. doi:10.1038/s41593-018-0334-7
- Shull, A. Y., Clendenning, M. L., Ghoshal-Gupta, S., Farrell, C. L., Vangapandu, H. V., Dudas, L., . . . Buckhaults, P. J. (2013). Somatic mutations, allele loss, and DNA methylation of the Cub and Sushi Multiple Domains 1 (CSMD1) gene reveals association with early age of diagnosis in colorectal cancer patients. *PLoS One, 8*(3), e58731. doi:10.1371/journal.pone.0058731
- Steen, V. M., Nepal, C., Ersland, K. M., Holdhus, R., Naevdal, M., Ratvik, S. M., . . . Havik, B. (2013). Neuropsychological deficits in mice depleted of the schizophrenia susceptibility gene CSMD1. *PLoS One, 8*(11), e79501. doi:10.1371/journal.pone.0079501
- Stepanov, V., Marusin, A., Vagaitseva, K., Bocharova, A., & Makeeva, O. (2017). Genetic Variants in CSMD1 Gene Are Associated with Cognitive Performance in Normal Elderly Population. *Genet Res Int, 2017*, 6293826. doi:10.1155/2017/6293826
- Stevens, B., Allen, N. J., Vazquez, L. E., Howell, G. R., Christopherson, K. S., Nouri, N., . . . Barres, B. A. (2007). The classical complement cascade mediates CNS synapse elimination. *Cell, 131*(6), 1164-1178. doi:10.1016/j.cell.2007.10.036
- Teoh, S. L., Chong, H. Y., Abdul Aziz, S., Chemi, N., Othman, A. R., Md Zaki, N., . . . Chaiyakunapruk, N. (2017). The economic burden of schizophrenia in Malaysia. *Neuropsychiatr Dis Treat, 13*, 1979-1987. doi:10.2147/NDT.S137140
- van der Gaag, M., Hoffman, T., Remijsen, M., Hijman, R., de Haan, L., van Meijel, B., . . . Wiersma, D. (2006). The five-factor model of the Positive and Negative Syndrome Scale II: a ten-fold cross-validation of a revised model. *Schizophr Res, 85*(1-3), 280-287. doi:10.1016/j.schres.2006.03.021
- Yang, X., Han, H., De Carvalho, D. D., Lay, F. D., Jones, P. A., & Liang, G. (2014). Gene body methylation can alter gene expression and is a therapeutic target in cancer. *Cancer Cell, 26*(4), 577-590. doi:10.1016/j.ccr.2014.07.028

ACKNOWLEDGEMENT

- This study was supported by The Ministry of Higher Education Malaysia via the Fundamental Research Grant Scheme (FRGS19-001-0609)

- Department of Psychiatry and Mental Health, Hospital Tengku Ampuan Afzan (HTAA), Kuantan

- Blood Bank, HTAA

THANK YOU