

Unraveling POLN germline mutations in familial nasopharyngeal carcinoma: Complementary evidence from gene association and molecular studies

Pei Pei Chong^{a,b}

^aSchool of Biosciences, Faculty of Health and Medical Sciences, Taylor's University, 47610 Selangor, Malaysia

^bDigital Health and Innovations Impact Lab, Taylor's University, 47610 Selangor, Malaysia



Familial clusters of nasopharyngeal carcinoma (NPC) are commonly reported in populations of both low and high incidences,¹ whereby a first-degree family history of NPC is associated with a marked increase in risk of NPC.² The high incidence of NPC among certain ethnic groups, including the Southern Han Chinese populations especially among the Cantonese, followed by the Inuits and Berbers, is widely known.³ Multiple genetic variants, rather than a single dominant susceptibility gene, coupled with shared environmental causes including Epstein–Barr virus (EBV) infection, were suggested to be the likely underlying causes associated with the observed mode of inheritance for NPC.⁴

The first case–control genome-wide association (GWAS) study of NPC patients and healthy controls had identified single nucleotide polymorphisms (SNPs) that were mapped to the *HLA-A* gene located in chromosome 6p21.3, in addition to two other genes, *GABBR1* and *HLA-F*.⁵ Multiple GWAS studies of sporadic NPC over decades have corroborated the finding of the association between NPC invariably caused by EBV and variation in the HLA genes.⁶ Nonetheless, polymorphisms in HLA genes could not account for the majority of sporadic NPC cases.

Furthermore, investigations of familial NPC cases have identified chromosomal regions linked to NPC carcinogenesis, although the results varied among different studies and remained inconclusive. A large-scale NPC multiplex family study followed by whole exome sequencing (WES) reported the identification of novel inherited rare genetic variants that most possibly function in biological pathways affecting EBV entry into epithelial cells, immune regulation, telomere maintenance, DNA repair, and Notch signalling.⁷ Nonetheless, most of the studies which investigated genetic determinants of NPC have only examined the possible association of the genetic variants versus wildtype genotypes through statistical regression analysis but did

not go beyond the basic genetic association studies to probe the functional roles of these variants at the protein level and their possible implications on EBV infection or on NPC carcinogenesis.

In a recent issue of eBioMedicine, Xiao and colleagues conducted the largest study of NPC multiplex families to date, made possible by a long-term nationwide cancer registry maximizing the representativeness of the NPC multiplex families evaluated. They found that germline mutations in a new susceptibility gene, DNA polymerase Nu (POLN) which encodes a DNA repair factor, contributed to risk of familial NPC.⁸ Specifically, they began by conducting WES on a small cohort of NPC families. This was expanded to a larger validation cohort totalling 6890 individuals comprising familial cases, sporadic cases, and healthy controls. They employed mutation filtering approaches and computational protein domain prediction tools to narrow down candidate mutations. They proceeded to perform a series of integrated bioinformatics and functional experiments to verify that three rare mutations in POLN impacted EBV infection in NPC cell lines compared to wildtype POLN. These POLN mutations (P577L, F545C, and R303Q) were subjected to further functional analysis. The intrinsic role of POLN in promoting EBV complete lytic replication was clarified through overexpression of POLN in two EBV-positive NPC cell lines which showed increased intracellular EBV copy number and increased progeny viral particles, whereas knock-down of POLN through siRNA gene silencing decreased the EBV copy number. To deduce the effects of the rare germline mutations on POLN function and EBV lytic replication, Xiao and co-workers overexpressed wildtype POLN and mutant POLN harbouring P577L, F545C, and R303Q in EBV+ NPC cell lines, using lentiviruses. They found that each of the mutations resulted in decreased extracellular viral particle and EBV copy number and impaired the inhibitory effect of POLN on cell proliferation. Like most EBV-associated epithelial cancers, NPC tumours are associated with incomplete or abortive lytic replication of EBV, which promoted carcinogenesis. This study showed that wild-type POLN reactivates lytic replication and EBV viral particles production, a condition which is not favourable to NPC carcinogenesis, while the POLN mutations suppressed

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*Corresponding author.

E-mail address: PeiPei.Chong@taylors.edu.my.

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EBV lytic replication, leading to increased viable NPC cells and carcinogenesis. The authors thus surmised that the three germline mutations in POLN had the overall effect of promoting NPC cell proliferation, and thereby play an important role in familial NPC carcinogenesis. They also suggested that the three POLN variants are useful as hereditary biomarkers that can be used in future for screening high-risk NPCs for early diagnosis and intervention.

There remain some unanswered research questions, including whether POLN has a direct mechanistic role in EBV DNA replication, since none of the three POLN mutations are found in catalytic or DNA-binding domains. Moreover, family-based genome-wide linkage studies have their limitations, as NPC is recognised to have multifactorial aetiology including environmental factors (smoking, consumption of salted and preserved food) aside from common genetic traits among family members. This study had unravelled rare POLN gene mutations for familial NPC and set a fine standard for future gene-disease linkage studies, demonstrating how molecular *in vitro* and *in silico* experiments could be useful tools for enhancing the value and impact of genetic association studies.

Declaration of interests

The author declares no conflict of interest.

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