The Genetic and Environmental Factors of Primary Membranous Nephropathy: An Overview from China

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Keywords

Autoimmune response · Disease susceptibility · Environment pollution · Genetic studies · Membranous nephropathy

Abstract

Background: Primary membranous nephropathy (pMN) is the most common cause of nephrotic syndrome in adults. The discovery of the 2 autoantigens, M-type phospholipase A2 receptor (PLA2R) and thrombospondin type-1 domain-containing 7A (THSD7A), has defined pMN as an autoimmune disease. A remarkable increase in the frequency of pMN in primary glomerular disease was witnessed in China. The genetic and environmental contributors to disease susceptibility have been investigated in these patients. **Summary:** We reviewed recent publications in genetic and environmental studies of pMN, focusing mainly on those undertaken in China. Following a genome-wide association study, the gene-gene interaction between the 2 most significant risk factors, PLA2R1 and DQA1, was validated in Chinese patients with MN. Fine mapping on human leukocyte antigen (HLA) locus found that DRB1*1501 and DRB1*0301 were risk alleles. Three amino acid residues on positions 13 and 71 of HLA-

DRβ1 chain may confer the susceptibility to pMN by presenting T-cell epitopes on PLA2R. Another study found that DRB3*0202 was the most likely culprit allele for the signal at DRB1*0301. One environmental risk factor for pMN has been identified as the long-term exposure to high levels of PM_{2.5} in Chinese patients with MN. Each 10 µg/m³ increase in PM_{2.5} concentration was associated with 14% higher odds for pMN in the regions with PM_{2.5} above 70 µg/m³. **Key Message:** A gene-environment interaction is suspected as an underlying mechanism for the increasing trend of pMN in China.

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Introduction

Primary membranous nephropathy (pMN) is the most common cause of nephrotic syndrome in adults [1, 2]. The histopathology is characterized by the presence of subepithelial immune complexes, diffuse thickening of glomerular basement membrane, and granular staining of IgG and complement C3 along the periphery of glo-

CSN/ERA EDTA Joint Session.

merular capillary loops [3]. In the landmark study in 2009 by Beck et al. [4] it was shown that 70% of pMN cases present autoimmune responses against the M-type transmembrane phospholipase A2 receptor (PLA2R), which can be detected on glomerular podocytes and in sub-epithelial immune deposits. A less common autoantigen is thrombospondin type-1 domain-containing 7A (THSD7A) [5], establishing pMN as a family of autoimmune disorders. Autoantigens or exogenous antigens in the rare situation of neonatal MN include neutral endopeptidase and bovine serum albumin [6, 7].

The annual incidence of pMN has been estimated as 1.2 per 100,000 adults in the United States and in European countries [8, 9]. It has been reported that the frequency of pMN in primary glomerular disease has been decreasing over the past 3 decades [9–13], with the frequencies in the UK and the United States decreasing from 37% and 38% in the 1970s to 26% and 15% in the 2000s, respectively [9, 10]. In China, there are little data from epidemiological studies all over the country. One recent study [14] retrospectively analyzed 6,049 renal biopsy patients from a diagnostic referral center and found that the proportion of pMN in primary glomerular disease increased from 17% in the period of 2003–2007 to 29% in the period of 2008–2012 in all age groups. This increase is mainly due to the increase in early stages of pMN in young patients (14–44 years of age). A similar increasing trend of pMN was observed in many centers from different geographic regions of China [15, 16]. A nationwide survey [17] has been conducted based on a commercial kidney biopsy database including 71,151 patients from 938 hospitals in 282 cities across China, encompassing all age groups and both tertiary and community hospitals. The frequency of MN doubled from 2004 (12.2%) to 2014 (24.9%), whereas the proportions of other major glomerulopathies remained stable. In average, the odds of MN increased by 13% annually. In China, the tertiary hospitals account for 6.5% of all hospitals but receive 37% of all inpatients. Tertiary hospitals in China are mandatorily and automatically submitting electronically the first page of medical records to a Hospital Quality Monitoring System data center. Among all hospitalized patients with primary glomerular disease, an increasing trend of pMN was revealed from 4.5% in 2010 to 8.8% in 2015 [unpubl. data]. All these findings demonstrated an increasing trend of pMN in China. One possible reason may be due to the increased awareness, better diagnosis, and improved access to health care in China; however, the mechanism underlying the increase in MN is worth further investigations.

As an autoimmune disease, both genetic susceptibility and environmental factors are essential for the mechanism of pMN. Couser and Johnson [18] observed the following points in their review. (1) Hereditary risk factors predispose certain individuals to responding to environmental factors in ways that can lead to a nephritogenic autoimmune response (first hit). (2) Exposure to etiologic agents in the environment occurs (second hit), which may be modified by epigenetic factors, and activates the innate immune system through interactions with toll-like receptors and complement. (3) Conversion of a non-antigen-specific innate immune response to an antigen-specific adaptive immune response directed at specific autoantigens can occur through several pathways that may operate simultaneously and in concert. (4) The adaptive immune response generates antigen-specific T and B cells, usually directed at antigens that are fixed or “planted” in the glomeruli. These immune reactants, usually through inflammatory effector cells and/or complement, mediate tissue damage.

The Autoimmune Disorders of pMN

In 1959, Heymann et al. [19] set up a rat model of MN by injection of suspension containing low-speed supernatant of blood-free rat kidney. The deposited immune complex forms *in situ* rather than in circulation [20, 21]. Then, the antigen was identified as a glycoprotein of podocyte and brush border membrane, magalin/gp330 [22–24].

In humans, 2 proteins, dipeptidyl-peptidase IV and enkephalinase, expressed on human podocytes, are involved in the immune deposits of MN [25]. In 2004, Debiec et al. [26] reported a fetus patient who prenatally developed severe MN. The mother was deficient in neutral endopeptidase and was immunized against the antigen at the time of or after an earlier miscarriage; the anti-neutral endopeptidase antibodies crossed the placenta and bound to fetal glomerular podocytes. Thus, neutral endopeptidase was defined as the first podocyte antigen responsible for human MN [6, 26]. In 2009, Beck et al. [4] identified PLA2R as the major antigen of human pMN, which is located on normal podocytes and in immune deposits in patients with pMN [4, 27]. Then, Tomas et al. [5] found that circulating autoantibodies to THSD7A occurred in 5–10% of PLA2R-negative patients. The structures of these 2 antigens are similar. They both are transmembrane glycoproteins containing large extracellular domains and short cytoplasmic tails [28]. The epitopes on

PLA2R targeted by anti-PLA2R antibodies were dominantly in the N-terminus, CysR-FnII-CTLD1 region [29]. Two peptides within a 31-amino acid sequence in the CysR domain were identified as producing 85% of the inhibition of autoantibody binding to PLA2R [30]. A subsequent study found that the CysR domain appears to be the primary dominant epitope with evidence of epitope spreading toward CTLD1 and CTLD7 domains [31].

Assays for circulating PLA2R antibodies rapidly changed the clinical practice in pMN. Circulating PLA2R antibodies seem to be specific for pMN [32–34]; however, they can also occur in secondary MN, especially the hepatitis B virus-related MN common in China [35]. The prevalence of positive antibodies varies between different diagnostic centers, from the highest prevalence of 82% [36] to the lower prevalences of 68% [37], 59% [38], and 69% [39], respectively, in China. The different results may be due to different approaches to antibody detection, including immunofluorescence, ELISA, or Western blot analysis. The antibody titer is associated with disease activity and predicts prognosis and response to treatment [27, 36, 38, 40–42], the disease course after treatment [36, 43], and recurrences after kidney transplantation [44]. A decrease in antibody titers during treatment often precedes proteinuria response [45] and can thus help to differentiate patients who respond to treatment from those who are refractory to treatment.

The finding of PLA2R on podocytes on renal biopsy can also aid in the diagnosis of pMN. Antibodies against another antigen, THSD7A, are of lower prevalence in pMN [5], while they are more frequently detectable in patients with PLA2R-negative pMN, with a prevalence of 7.5% [39] and 16.4% [37] in Chinese pMN patients without serum antibodies against PLA2R. Four cases processing dual positive antibodies to PLA2R and THSD7A have been reported; 2 of them are from China [37]. In particular, patients who are negative for PLA2R or THSD7A autoantibodies exhibit a high probability of spontaneous remission but should be screened for malignancy [46].

Genetic Studies of pMN

Family-Based Studies

MN is not a typical hereditary disease in Mendelian terms, apart from rare cases in which more than 1 family member is affected, which indicates a genetic contribution to the disease. In 1984, the Manchester royal infirmary reported 3 sets of brothers (containing a pair of twins) with renal biopsy-proven MN [47]. Elshihabi et al.

[48] summarized 9 families in which MN occurred in siblings, and several other pairs were reported [49–56]. Human leukocyte antigen (HLA) typing confirmed the association between certain HLA types and pMN [47, 53, 56]. In addition to B8, B18, DQ2, DQW1, and DQW2, DR3 was detected as the prime marker of susceptibility to MN [57]. The brothers having DR3 antigens [47] and the pair of father and son having DR3 antigens [56] strongly suggested the role of HLA in pMN.

Genome-Wide Association Studies

There is only 1 genome-wide association study (GWAS) on pMN [58], which included 3 independent European cohorts from French, Dutch, and British populations. This study examined 242,824 common single nucleotide polymorphisms (SNPs) of 556 patients with pMN as well as 2,338 controls and identified 2 significant genomic loci associated with pMN: chromosome 2q24 contains the gene encoding PLA2R1 (SNP rs4664308), and chromosome 6p21 contains the gene encoding HLA complex class II HLA-DQA1 chain (HLA-DQA1) (SNP rs2187668). Then, the study evaluated the effect of the 2 risk alleles on pMN. The odds ratio for being homozygous for the risk allele in DQA1 was 5 times higher than that for being homozygous for the risk allele in PLA2R1. The odds ratio for having this disease was as high as 78.5 for persons who were homozygous for the risk alleles at both HLA-DQA1 and PLA2R1.

Validation studies were conducted worldwide including China. They corroborated a highly significant association between pMN and the risk alleles of HLA-DQA1 and PLA2R1 [59–61]. Furthermore, in a Chinese cohort, Lv et al. [60] noted gene-gene interactions between the 2 risk alleles and discovered an association between carrying risk alleles and the presence of anti-PLA2R antibodies in serum as well as the expression of PLA2R in glomeruli. The odds ratios of risk genotypes were 1.5 in HLA-DQA1 and 3.4 in PLA2R1. The combination of risk genotypes of 2 genes (rs4664308/rs2187668: AA/GA+AA) conferred an 11.1-fold higher risk for the development of pMN compared with either protective genotype (GG/GG) at both loci. Anti-PLA2R antibodies could be detected in 74% of patients with the PLA2R1 high-risk genotype and 4% of patients with the PLA2R1 low-risk genotype. Autoantibodies were detectable in 73% of patients with both PLA2R1 and HLA-DQA1 high-risk genotypes and in none of the patients with both low-risk genotypes. This study with more than 2,000 participants represents the largest genetic study on pMN and made the new discovery of an association between risk alleles and anti-PLA2R

antibodies. A study of a Spanish cohort found that the 2 risk alleles could predict response to treatment and disease progression. The carriers of the pMN-susceptible genotypes (A/A and A/G for HLA-DQA1 or A/A for PLA2R1) showed a trend to respond to immunosuppressive therapies [59]. These studies provide functional clues for further investigations of these genes in the pathogenic contribution to pMN.

PLA2R is the major autoantigen of pMN. The results from the GWAS suggest that rare genetic variants within the coding region of PLA2R1 may contribute to antibody formation. Two studies from an Eastern population firstly revealed the association between PLA2R SNPs and MN, but the results differed [62, 63]. Kim et al. [62] in Korea examined 2 SNPs (rs35771982 and rs3828323) in PLA2R and found that subjects with the C/C genotype of rs35771982 (His300Asp) had a higher susceptibility to pMN. Liu et al. [63] in Taiwan investigated 2 SNPs (rs6757188 and rs35771982) in PLA2R1 and found that the G allele and the G/G genotype of rs35771982 were more common in pMN. A follow-up study sequenced all 30 exons of PLA2R1 from 95 white patients with biopsy-proven pMN to identify rare genetic variants. But no evidence was provided that rare variants within the coding region cause the proposed association between pMN and the PLA2R1 gene [64]. Gupta et al. [65] reviewed the genetics of MN and proposed that the genetics of PLA2R1 may control the possible enzyme fragmentation pattern of PLA2R1 by a change in amino acid, either by creating or destroying an enzyme cut site, by a change in splice sites, controlling the protein species available for fragmentation, or by a change in the level of transcript, leading to higher levels of peptides. The genetics of DQA1 will shape the amino acid structure of its receptor groove, thus defining and restricting the possible peptide sequences available from PLA2R1 that will fit the groove.

At variance with European and East Asian populations, no association was found in African Americans [66]. In a large analysis in Northern American, PLA2R1 variants were only associated with PLA2R-related MN in Caucasians but not associated with MN in African Americans with either PLA2R-positive or -negative MN. Similarly, HLA-DQA1 SNP rs2187668 was positively associated with PLA2R-positive MN and negatively associated with PLA2R-negative MN in Caucasians, while it was associated with PLA2R-positive but not with PLA2R-negative MN in African Americans [66]. Larsen et al. [67] found that APOL1 was a risk allele in African American patients. They included 120 PLA2R-related MN patients and screened the presence of APOL1 risk alleles. There

were 46 cases with 0, 51 cases with 1, and 23 cases with 2 APOL1 risk alleles. Besides, patients with 2 APOL1 risk alleles suffered from severer pathological damage, which suggests that the presence of APOL1 risk alleles might serve as an acceleration factor in this disease.

Association Studies Focused on HLA Loci

In the GWAS on pMN, variants within the HLA region conferred the greatest risk of disease. Polymorphism within the HLA is associated with almost every autoimmune disease studied to date, but the identity of causal variation in many diseases has been hampered by the strong linkage disequilibrium across disease-associated haplotypes. The HLA region resides on chromosome 6p21.3 and is among the most gene-dense portions of DNA, with gene products ranging from antigen-binding molecules and receptors to signaling factors. The region can be subdivided into class I, II, and III. Class I encompasses HLA-A, -B, and -C that function as presenters of peptides to cytotoxic T cells; class II consists of HLA-DR and HLA-DQ molecules that present epitopes to CD4⁺ T cells; and class III includes genes of several components in the complement system, such as C4, factor B and C2 [68, 69].

The first report [70] of genetic contributions of the HLA locus to the risk of pMN was published in the UK, in 1979, with the finding of HLA-DR3. This result was confirmed by a series of studies [71, 72]. In the European Caucasoid cohort, DRB1*0301 has the strongest association with British pMN, while the Greek pMN association with DRB1*0301 is weaker [73]. The top SNP in GWAS on pMN, rs2187668, in the gene HLA-DQA1, is a well-established tag SNP for DRB1*0301 in Northern European populations [74]. The haplotype B*0801-DRB1*0301-DQA1*0501-DQB1*0201 is highly conserved in Northern European populations and is associated with several autoimmune diseases, including pMN [75, 76]. Besides, TAP1 (lying within the MHC II region) was also associated with pMN [77]. The association between pMN and DRB1*1501 was discovered in a Japanese cohort, together with DR2 and DQ1 [78, 79]. They reported a risk haplotype DRB1*1501-DQB1*0602 in pMN [80].

Two studies from China provide novel insights into the role of specific HLA alleles in pMN [81]. Cui et al. [82] performed 4-digit resolution typing of HLA-DRB1, DQA1, DQB1, and DPB1 genes, followed by a case-control association analysis in 261 patients and 599 healthy controls, and pointed to 2 classical alleles, DRB1*1501 and DRB1*0301, with highly significant independent ef-

fects on the risk of pMN among Han Chinese. The DRB1*1501 and DRB1*0301 alleles had large, independent, and genome-wide significant effects with allelic odds ratios of 4.65 and 3.96, respectively. These alleles were also significantly associated with circulating anti-PLA2R antibodies. Similar to SNPs in GWAS, both HLA alleles exhibited statistical interaction with the PLA2R1 variant rs4664308. Notably, the homozygosity for risk alleles at the PLA2R1 locus combined with DRB1*1501 or DRB1*0301 positivity conferred an up to 30-fold increased risk of pMN. This synergistic effect is suggestive of a physical interaction between DR β 1 molecules and the PLA2R epitope(s) during the process of antigen presentation. The analysis of individual amino acid substitutions within HLA proteins further narrowed down the search to the amino acid positions 13 and 71 within the HLA-DR β 1 chain. Both of these positions participate in the formation of the fourth peptide-binding pocket of the HLA-DR β 1 chain. Furthermore, in silico modeling of PLA2R peptides presented by the susceptible HLA molecule revealed several candidate epitopes that require experimental validation. Interestingly, modeling sequence variants of PLA2R1 had little effect on T-cell epitope prediction, suggesting that coding variants in PLA2R1 are less likely to alter the immunogenicity of its gene product.

The second study by Le et al. [83] performed a comprehensive analysis by targeted high-throughput sequencing of all HLA genes in the region, including class I and class II genes, in 99 anti-PLA2R-positive pMN cases and 100 healthy controls. This study confirmed the association of DRB1*1501 with anti-PLA2R-positive pMN and suggested DRB3*0202 as the second independent risk factor, subsequently replicating both of these associations in an independent cohort of 293 cases and 285 controls. The effects were significantly larger, with allelic odds ratios of 24.9 and 17.7, respectively. These larger effects are likely explained by differences in the ascertainment of cases: the first study included all cases of pMN, but the second study used PLA2R-related pMN. Notably, DRB3 alleles were not typed in the first study, but DRB3*0202 resides on the same haplotype as DRB1*0301, thus the results of both studies are technically in full agreement. In fact, the second study suggests that DRB3*0202 is the most likely culprit allele explaining the reported signal at DRB1*0301, considering its stronger statistical significance and the conditional analysis that removes any residual signal at DRB1*0301 after accounting for both DRB1*1501 and DRB3*0202. Secondary genotype-phenotype correlation analyses revealed that DRB1*1501 was strongly associated with an earlier age at

disease onset. Among PLA2R-positive pMN cases, patients carrying DRB1*1501 presented at a median age of 35 years compared to a median age of 50 years for all other patients.

Environmental Studies of pMN

In the genetically susceptible individuals (first hit), the exposure to some environmental factors (second hit) is suspected to trigger the disease. China comprises 20% of the world population. With rapid developments in its economy and urbanization, especially during the past decade, air pollution has become a public health problem in some cities. From 2004 to 2014 [84], 3-year average levels of AOD-derived PM_{2.5}, the particulate matter of <2.5 μ m, have been increasing up to a plateau in 2008. In 2008, levels of PM_{2.5} exposure varied from 8.1 to 110.5 μ g/m³ with a mean of 55.6 μ g/m³. This level was much higher than that in many developed countries, such as the United States (mean, 12 μ g/m³), the UK (mean, 14 μ g/m³), and Japan (mean, 10 μ g/m³), and was comparable to developing countries, such as India (mean, 59 μ g/m³) [85].

Exposure to air pollution, especially PM_{2.5}, has been associated with increased death and incidence of cardiovascular events [86]. Animal studies have shown that exposure to fine particulate promotes the production of autoantibodies and immune complexes and results in immune dysregulation, which is implicated in the pathogenesis of some glomerulopathies [87, 88]. It has been hypothesized that cytokines generated in the airways in response to air pollution can spill over into the circulation, influencing autoimmune responses and distant events [89]. Air pollution increases the circulating levels of inflammation mediators, such as TNF- α , IL-6, and plasminogen activator inhibitor-1 [88, 90], and genetic polymorphisms in these cytokines are associated with the development of MN [91–95].

An increasing trend of pMN has been observed in many developing countries, such as Pakistan [96], Czech Republic [97], and India [98, 99]. The frequency of pMN in India increased almost by 2-fold during 1999–2008 [98, 99]. In an Indian cohort with high-concentration exposure to PM_{2.5}, during the period of 1971–2002, there was a steady increase in pMN prevalence [100]. On the contrary, in developed countries like France, the increasing trend of pMN happened in the 1990s and has been reversed since 1996 [101, 102], which could also be observed in Brazil [103] and the United States [104]. pMN was shown to be declining in other East Asian countries

with lower PM_{2.5} exposure levels, such as Japan [105] and Korea [106, 107], where the pMN prevalence was stable during the past 10 years.

Yang et al. [108] conducted a correlation study in Taipei and reported that the exposure to PM₁₀, but not PM_{2.5}, during the previous year was associated with renal function reduction. A recent study from Mainland China correlated the rising incidence of pMN with the deterioration of air pollution. Xu et al. [17] observed a remarkable rising trend in the frequency of pMN from 12.2% in 2004 to 24.9% in 2014. The frequency of pMN was higher in the Northern region of China, especially in Hebei province, the most polluted area in China. Most importantly, they found that long-term exposure to high levels of PM_{2.5} was associated with an increased risk of pMN after controlling for confounders, including age, gender, geographic region, level of hospital for biopsy, pathologic laboratory, clinical syndrome, and year of biopsy. The relationship appeared to be nonlinear: each 10 µg/m³ increase in PM_{2.5} concentration was associated with 14% higher odds for pMN in the regions with a PM_{2.5} concentration above 70 µg/m³. The curve was flat at PM_{2.5} below 70 µg/m³. The annual increase in odds for pMN was greater in the cities with a higher PM_{2.5} slope even after adjusting for geographic region. Assuming a causal relationship, 15.2% of pMN in China could be attributable to PM_{2.5} air pollution exposure. Another analysis [unpubl. data] using the national inpatient registration database of tertiary hospitals in China confirmed the increasing percentage of pMN among all primary glomerular nephropathy, doubling from 4.6% in 2010 to 8.8% in 2015. In North and Northeast China, it had exceeded the frequency of IgA nephropathy since 2014. The patients clustered in Hebei province and Guangxi province. The association between exposure to PM_{2.5} and increases in pMN was confirmed in Northern China demarcated by Yangtze River. However, in the South of China, a close association was observed between the frequency of pMN and the Zhuang population, the largest minority population of China, but not PM_{2.5}.

In addition to air pollution, some other environmental factors, such as bacterial infections and occupational exposure, also participate in the development of pMN. There are several studies on the association between *Helicobacter pylori* infection and MN. The infection rate of *H. pylori* was found to be significantly higher (66%) in MN patients than in age-matched healthy controls (44%) [109, 110]. A case report described a patient with MN whose nephrotic-range proteinuria was markedly reduced after the eradication of gastric *H. pylori* infection

[111]. A Chinese study did not find any difference in the *H. pylori* infection rate between MN patients and healthy controls but did find an immune reaction between kidney cells and anti-*H. pylori* antibody [112]. Occupational exposure is considered as another external factor in the development of MN. A case-control study was designed to investigate the role of occupational hydrocarbon exposure, which included 36 biopsy-proven pMN patients and 36 healthy controls and collected their lifetime hydrocarbon exposure assessed by a validated questionnaire. The study found that the exposure to hydrocarbons was significantly greater in pMN patients than in controls, which suggests a relationship between lifetime hydrocarbon exposure and the development of MN [113].

Future Perspectives on Genetic and Environmental Studies of pMN

Many discoveries on genetic and environmental risk factors of pMN have provided clues for pathogenesis research. Follow-up studies on HLA analysis will require a large-scale comprehensive sequencing effort involving the entire locus, also including noncoding segments that may regulate HLA gene expression. Such studies would be ideally performed in cohorts of diverse ancestries, including representative cohorts of Asian, European, and African ancestry. The mechanism underlying the association between long-term exposure to high levels of PM_{2.5} and the increasing risk of pMN is unknown. It is postulated that PM_{2.5} might bind transcript factors in podocytes and increase PLA2R antigen expression and consequently initiate antibody production. However, whether these links are causal or simply associated with the disease needs further investigation. Furthermore, like the gene-gene interaction that has been proven to contribute to pMN development, the environmental factors may enhance the genetic propensity for pMN in the polluted areas. To confirm this hypothesis, further explorations of gene-environment interactions are needed to calculate the proportion of patients with risk genetic as well as environmental elements, and the odds ratio for pMN with a combination of risk alleles and high-level pollution regions. A better understanding of the mechanisms and correlations between altered immune responses to environmental pathogens and initiation of the autoimmune processes could help guide the discovery of new biomarkers and stimulate more effective approaches to both prevention and therapy of the disease.

Acknowledgement

This work was supported by grants of the Natural Science Foundation of China to the Innovation Research Group (81621092), the Outstanding Young Scholar (81622009), and other programs (81330020), and the Capital of Clinical Characteristics and Applied Research Fund (Z161100000516039).

Conflict of Interest Statement

None of the authors have any competing interests.

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