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RESEARCH ARTICLE

The *TREM2* H157Y variant is associated with more severe neurodegeneration in Alzheimer's disease and altered immune-related processes

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Abstract

INTRODUCTION: Multiple *TREM2* variants are associated with an increased risk of Alzheimer's disease (AD). *TREM2* H157Y is the only variant located at the proteolytic cleavage site that enhances TREM2 protein shedding. While this variant is associated with increased AD risk predominantly in the Chinese population, its impact on AD pathology is largely unknown.

METHODS: We conducted an in-depth study integrating clinical cases, neuroimaging data, and blood proteomic data.

RESULTS: *TREM2* H157Y variant carriers with AD exhibit more severe AD pathology, more severe neurodegeneration, and more rapid clinical progression. Cognitively normal individuals carrying the variant show changes in blood proteins that are associated with neurodegeneration and inflammation. Moreover, the *TREM2* H157Y variant is associated with altered immune and vascular processes irrespective of disease state.

DISCUSSION: These findings highlight the clinical implications of the *TREM2* H157Y variant and the use of blood proteomic data to investigate the effects of genetic variants on disease-related endophenotypes.

KEYWORDS
Alzheimer's disease, blood biomarkers, clinical case study, genetic variant, neurodegeneration, peripheral immune, TREM2

Highlights

- The *TREM2* H157Y variant is associated with more rapid clinical progression of Alzheimer's disease only in the presence of the apolipoprotein E (*APOE*) ε4 allele.

Jackie Shuk Man Tsui and Danise M. Au have contributed equally to this study.

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- The *TREM2* H157Y variant is associated with neurodegeneration, irrespective of disease state.
- The *TREM2* H157Y variant is associated with altered immune and vascular processes, irrespective of disease state.
- Cognitively normal *TREM2* H157Y carriers show altered disease-associated blood proteins related to peripheral immune response.
- Blood proteomic data can be used to study the impacts of disease-associated genetic variants on disease outcomes and biological processes involved in pathogenesis.

1 | BACKGROUND

Recent studies underscore the pivotal role of TREM2 (triggering receptor expressed on myeloid cells 2) in Alzheimer's disease (AD).[1] TREM2 is a surface receptor predominantly expressed on microglia in the brain that binds anionic lipids and DNA.[2] It is essential for mediating microglial responses to AD-related pathology, including amyloid and tau.[3] TREM2 plays a protective role against amyloid pathology by promoting microglial chemotaxis and barrier formation around amyloid plaques, thereby reducing amyloid-induced toxicity to adjacent neurons.[3,4] Multiple *TREM2* variants, including R47H, R62H, D87N, and H157Y, are associated with an increased risk of AD.[5,6] However, studies on the effects of these *TREM2* variants on the age of disease onset and AD clinical presentation reported inconsistent results. For instance, some studies reported similar clinical presentation between variant carriers and non-carriers, whereas another study indicated that variant carriers tend to experience more severe cognitive impairment and psychiatric symptoms.[7–10] Moreover, few studies have examined the effects of these variants on other disease-related endophenotypes or biological processes beyond the pathological hallmarks of AD. Thus, the effects of *TREM2* variants on AD clinical presentation, AD-related endophenotypes, and pathogenesis remain unclear. In addition, there is a lack of studies on the effects of these variants on cognitively normal (CN) individuals. The findings of such studies could suggest mechanisms by which these variants predispose individuals to AD.

The *TREM2* H157Y variant is notable for its unique effects on TREM2 function and prevalence in the Chinese population. Among the most frequently studied AD-risk-associated *TREM2* variants, all except H157Y are located in the ligand-binding domain (i.e., exon 2) and are reported to decrease the ligand-binding affinity of the protein.[11,12] In contrast, H157Y is located at the proteolytic cleavage site in exon 3 of *TREM2*, which enhances protein shedding, resulting in decreased surface TREM2 levels and increased soluble TREM2 (sTREM2) levels.[13,14] This distinct property may provide valuable insights into the functions of TREM2 in AD. The most studied *TREM2* variant, R47H, is reported to increase AD risk in populations of European descent,[15–18] but several studies did not detect this variant in the Chinese population.[19–22] Despite the unique effects and prevalence of the *TREM2* H157Y variant, relatively few studies have focused on it.

Our laboratory previously identified several *TREM2* H157Y variant carriers in our in-house Hong Kong Chinese cohort and found that the variant might influence the amyloid β (Aβ) 42/40 ratio and levels of immune-associated blood proteins in plasma among patients with AD.[23] Accordingly, in the present study, we evaluated the effects of the *TREM2* H157Y variant on AD-related endophenotypes and other biological processes in both cognitively impaired and CN individuals by conducting an in-depth integrative follow-up study, utilizing clinical case studies alongside neuroimaging and blood proteomic data analysis. We found that patients with AD carrying the H157Y variant exhibited more severe AD pathology, neurodegeneration, and inflammation as indicated by neuroimaging and blood biomarkers. In particular, patients carrying the H157Y variant and the apolipoprotein E (*APOE*) ε4 allele exhibited more rapid clinical progression. Notably, CN H157Y carriers without amyloid pathology also exhibited higher levels of blood biomarkers associated with neurodegeneration. Irrespective of disease state, we found that the *TREM2* H157Y variant is associated with alterations in immune and vascular processes. Thus, our study provides further insights into the roles of TREM2 in AD pathogenesis, particularly in neurodegeneration and its interactions with *APOE* genotype, and underscores the clinical significance of the *TREM2* H157Y variant in the Chinese population. We also demonstrate the utility of blood proteomic data in investigating the effects of genetic variants on disease-related endophenotypes as well as the biological processes involved in disease pathogenesis that are altered by those variants.

2 | METHODS

2.1 | Study design

We conducted clinical case studies on the genetic variant (GV) cohort, which consisted of six unrelated families with individuals carrying a single copy of the *TREM2* H157Y variant (with the genotype GA) and their family members (with the genotypes GA and GG) (Figure 1).

Study Cohort_1 was formed to investigate the impact of the H157Y variant on AD-related endophenotypes as indicated by neuroimaging and plasma AD biomarkers. This study cohort consists of all individuals from the GV cohort and non-carrier controls who were age-, sex-, and *APOE* genotype-matched with each clinical diagnostic group. The demographic characteristics of Study Cohort_1 are summarized in Table S1.

Study Cohort_2 was formed to investigate the impact of the H157Y variant using the NULISAseq CNS Disease Panel to measure proteins related to neurodegenerative disorders. This cohort includes additional H157Y variant carriers not in the GV cohort who were subsequently identified in the database. The clinical diagnoses of this study cohort were confirmed by biomarkers of amyloid pathology. The demographic characteristics of Study Cohort_2 are summarized in Table S2.

Study Cohort_3 was formed to investigate the impact of the H157Y variant using the Olink Target series panels to measure proteins related to a broad spectrum of diseases and biological processes. As all H157Y variant carriers with available data were female, this study cohort included only female individuals. The clinical diagnoses of this study cohort were also confirmed by biomarkers of amyloid pathology. The demographic characteristics of Study Cohort_3 are summarized in Table S3.

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the literature using traditional sources (e.g., PubMed). While *TREM2* is an important AD risk gene, the impacts of *TREM2* variants on Alzheimer's disease (AD)-related endophenotypes and pathogenesis remain inconclusive, with particularly few studies on the H157Y variant.
2. **Interpretation**: The study findings indicate that the *TREM2* H157Y variant is associated with more rapid AD progression and more severe disease outcomes, emphasizing its clinical importance in the Chinese population. Notably, cognitively normal H157Y variant carriers without amyloid pathology exhibit alterations in the blood proteome that indicate increased neurodegeneration and immune-related dysregulation, suggesting biological processes that underlie the impact of the variant in AD.
3. **Future directions**: The study demonstrates the utility of blood proteomic data in investigating genetic variants in AD. Our findings also pave the way for future studies utilizing blood proteomic data and variant carrier-derived induced pluripotent stem cells to delineate the underlying processes and develop novel TREM2-targeting therapies.

2.2 | Participant recruitment and assessment

We recruited all patients with mild cognitive impairment (MCI) or AD (determined by clinical assessment) from the Specialist Outpatient Clinics of the Department of Medicine and Therapeutics at the Prince of Wales Hospital, the Department of Medicine at Queen Mary Hospital, and the Department of Medicine and Geriatrics at United Christian Hospital as part of our in-house Hong Kong Chinese cohort. We recruited all CN individuals as part of our in-house Hong Kong Chinese community cohort.

All participants underwent clinical assessment, the Hong Kong Montreal Cognitive Assessment (HK-MoCA),[24] and blood collection for plasma protein measurement. We adjusted HK-MoCA scores for education by adding one point for individuals who received 6 years of education or less. Aβ–positron emission tomography (PET) images were evaluated by a radiologist. For each study cohort, we selected individuals with appropriate demographics and for whom relevant data were available. We determined amyloid status by Aβ-PET or validated plasma biomarkers.[25] Details on the participant recruitment and assessments for each study cohort are summarized in Supplementary Method S1.

All participants or the legal guardians of participants with advanced dementia provided written informed consent for study participation and sample collection. This study was approved by the Clinical Research & Ethical Committees of Joint Chinese University of Hong Kong–New Territories Eastern Cluster for the Prince of Wales Hospital (CREC ref. no. 2015.461), the Human Research Ethics Committee of The Hong Kong University of Science and Technology (HREP-2023-0179), and the Human Participants Research Panel of The Hong Kong University of Science and Technology (CRP#180).

2.3 | Calculation of odds ratio

We calculated the odds ratio and *p*-values for the *TREM2* H157Y variant in the Hong Kong Chinese population from the in-house Hong Kong Chinese whole-genome sequencing database (demographic characteristics summarized in Table S4) using the *glm()* function in R (v4.2.2). Details on the equation used are presented in Supplementary Method S2.

2.4 | Analysis of brain imaging data

We conducted two-way multivariate analysis of covariance (MANCOVA) to determine if there was an interaction between disease stage (i.e., AD and MCI) or genetic variant (i.e., GA and GG) on the combined global measurements of brain volumes after controlling for estimated total intracranial volume and MRI scanner (i.e., Philips and Siemens). If a significant two-way interaction effect was found, we performed univariate tests for follow-up. If any follow-up analysis was statistically significant, we performed simple main effect and pairwise comparisons

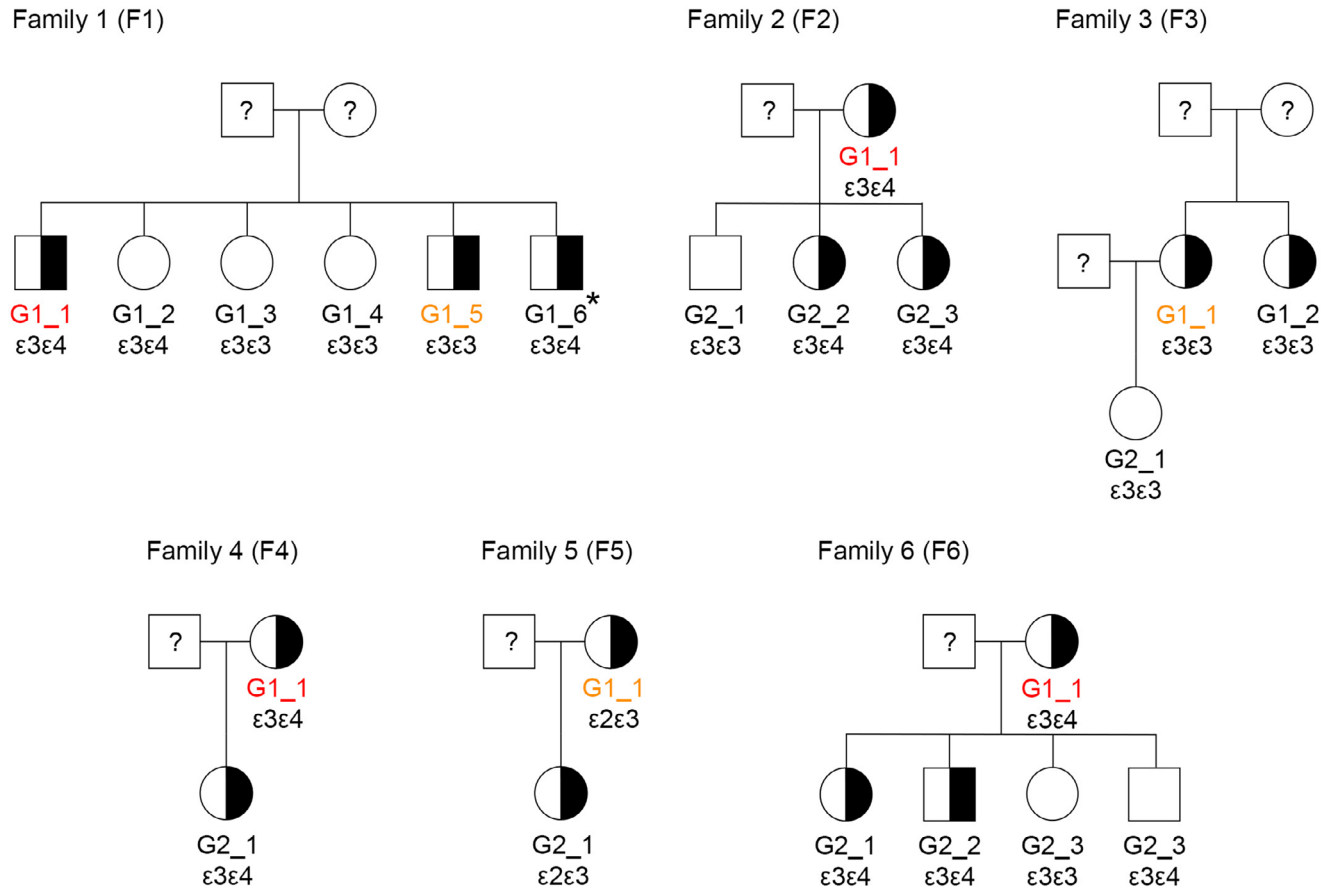

FIGURE 1 Pedigree chart of the six families (in the genetic variant cohort) with *TREM2* H157Y variant carriers. The color corresponds to their clinical diagnosis: red, orange, and black indicate AD, MCI, and CN, respectively. The second row shows the *APOE* genotype of each individual. * This individual is CN (with a MoCA score of 25) but was found to have AD pathology on neuroimaging (i.e., Aβ-positive and tau-positive on PET). Aβ, amyloid β; AD, Alzheimer's disease; *APOE*, apolipoprotein E; CN, cognitively normal; MCI, mild cognitive impairment; MoCA, Montreal Cognitive Assessment; PET, positron emission tomography; *TREM2*, triggering receptor expressed on myeloid cells 2.

as post hoc tests (i.e., Bonferroni). We performed all statistical tests in R (v4.2.2). Details on the acquisition and processing of MRI images as well as brain segmentation are presented in Supplementary Methods S3 and S4.

2.5 | Measurement of plasma proteins

We measured plasma phospho-tau 217 (pTau217) levels with an ALZpath single molecule array (Simoa) P-Tau217 V2 Assay Kit (104371) and plasma neurofilament light polypeptide (NfL) levels with a Quanterix Neurology 4-Plex E Advantage Kit (103670) using 120 µL plasma for each assay. We used the NULISAseq CNS Disease Panel to measure 127 proteins using 25 µL plasma and the Olink Target series panels to measure 1160 proteins using 20 µL plasma; the levels of assayed blood proteins are expressed in normalized protein expression units NPQ and NPX, respectively. Details on plasma extraction and protein measurements using the Quanterix, NULISAseq, and Olink kits are presented in Supplementary Methods S5 and S6, and Table S5.

2.6 | Analysis of blood proteomic data

To examine the impact of the *TREM2* H157Y variant on the levels of blood proteins measured using the NULISAseq CNS Disease Panel and Olink Target series panels, we performed linear regression analysis with the corresponding study cohort stratified by disease status. We performed linear regression analysis using the *lmrob()* function in the R robustbase package after linearization and normalization.[26] Details on the analysis are summarized in Supplementary Method S7.

2.7 | Gene Ontology, protein–protein interaction, and cell-type–specific enrichment analysis

We used the PANTHER (v19.0) online tool to perform Gene Ontology (GO) analysis on the differentially expressed proteins (DEPs) that were significantly (i.e., $p < 0.05$) associated with the *TREM2* H157Y variant.[27] We performed protein–protein interaction (PPI) analysis and functional enrichment analysis of the protein clusters by

TABLE 1 Minor allele frequencies and odds ratio of the TREM2 H157Y variant in Chinese- and European-descent populations.

	Cognitively normal			Alzheimer's disease			Total		
Ethnicity	No. of minor alleles (A)	No. of cases	MAF (%)	No. of minor alleles (A)	No. of cases	MAF (%)	MAF (%)	OR [95% CI]	*p*-value
Chinese	1	932	0.054	6	660	0.455	0.220	**9.14 [1.06–78.4]**	**0.044**
European descent[5,6]	0	504	0	1	281	0.178	0.064	N/A	N/A
	0	1610	0	3	2052	0.073	0.041		

Abbreviations: CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; N/A, not applicable.

subjecting the DEPs that were significantly associated with the *TREM2* H157Y variant to the STRING database (https://string-db.org/, v12.0). Finally, we performed cell-type-specific enrichment analysis (CSEA) using WebCSEA.[28]

2.8 | Statistical analysis and data visualization

We determined the impact of the *TREM2* H157Y variant on plasma pTau217 and NfL levels measured by Quanterix assay as well as its impact on adjusted MoCA scores by two-sample *t*-test using GraphPad Prism (v10.2.0). We performed correlation analysis using GraphPad Prism (v10.2.0). We generated all visualizations (i.e., volcano, box, line, and dot plots) using GraphPad Prism (v10.2.0), except for the PPI network, which we constructed using the STRING database (https://string-db.org/, v12.0).

3 | RESULTS

3.1 | The *TREM2* H157Y variant is associated with increased AD risk

We found that the *TREM2* H157Y variant is associated with a significantly higher risk of AD in the Hong Kong Chinese population (odds ratio [OR] = 9.13, 95% confidence interval [CI] = 1.06–78.4, $p = 0.044$; Table 1 and Table S4). The allele frequencies of the *TREM2* H157Y variant were 0.220% and 0.455% in the overall Hong Kong Chinese population and patients with AD, respectively. These findings are consistent with a previous study that reported a similar association of the H157Y variant and increased AD risk in the Chinese population.[29] Notably, the risk of AD associated with carrying a single copy of the H157Y variant is comparable to that of carrying a single copy of *APOE* ε4.[30,31]

3.2 | *TREM2* H157Y variant carriers with an *APOE* ε4 background show more rapid clinical progression

We recruited 22 individuals from six unrelated families, including 15 *TREM2* H157Y variant carriers and seven non-carriers, to form the GV cohort (Figure 1). To illustrate the role of this variant in AD, we present five cases of variant carriers: two patients with AD, two patients with MCI, and one CN individual with AD-related brain pathology. The cognitive assessment and neuroimaging findings of the five cases are summarized in Table S6.

3.2.1 | Case 1: (F1) G1_1

This male patient carries the *TREM2* H157Y variant and has an *APOE* ε3ε4 genotype. He has completed 12 years of education and lives with his wife and one of their children. He has a history of myasthenia gravis, which was controlled with medication. At age 66, he began experiencing subjective forgetfulness. Routine blood tests and brain imaging excluded other causes of cognitive decline. Two years later, he was diagnosed with AD and started on donepezil. However, cognitive decline continued, and he could not tolerate the side effects of donepezil. At age 70, 2 years after his diagnosis, the treatment was switched to rivastigmine. At that time, he remained independent in both basic and instrumental activities of daily living (ADLs) and continued to engage in leisure activities.

Less than a year later, his memory impairment began to affect his instrumental ADLs, including his ability to manage finances and navigate unfamiliar places. His family also noticed further cognitive impairment with confabulation. He was still able to navigate familiar locations and enjoyed his leisure activities with no behavioral and psychological symptoms of dementia (BPSD). He then deteriorated rapidly, becoming unable to recognize his own home and requiring occasional help with basic ADLs, such as bathing. His rivastigmine dosage was increased three times over the course of 5 months.

At 72 years old, he was noted to have BPSD with irritability. He was briefly treated with haloperidol and then switched to quetiapine. His BPSD worsened in the following year, escalating to disinhibited behavior, foul language, and conflicts with passersby on the street. By age 74, 6 years after his initial diagnosis, he was diagnosed with severe AD, with worsening BPSD precipitated by institutionalization. Rivastigmine was changed to memantine, and the dosage of quetiapine was further increased. He was also noted to have hypothyroidism and vitamin B12 deficiency, both of which were supplemented. By age 76, he was bedbound and noncommunicable. A PET—computed tomography (CT) scan done at age 76 (for research purposes) confirmed that he was amyloid-positive with significant tau accumulation. He is currently 78 years old.

3.2.2 | Case 2: (F4) G1_1

This female patient carries the *TREM2* H157Y variant and has an *APOE* ε3ε4 genotype. She has completed 6 years of education. She first experienced cognitive impairment at 80 years of age, primarily characterized by problems with immediate recall and disorientation to place along with occasional dangerous episodes where she forgot to turn off the stove. She was diagnosed with AD at age 81 and started on galantamine, which was switched to memantine and donepezil 1 year after her diagnosis. Routine blood tests and brain imaging excluded other causes of cognitive decline. She remained stable until age 83, when she developed BPSD and anxiety, leading to the addition of escitalopram. By that time, she was unable to recall her address or the number of children she had but continued to enjoy hobbies and remained largely independent in her basic and instrumental ADLs.

At 84 years old, she had recurrent admissions for subdural hematomas, precipitating delirium and worsening BPSD, which were treated with quetiapine and trazodone. Her BPSD symptoms improved after discharge, and her subdural hematomas resolved within the following year. By age 86, 5 years after her initial diagnosis, she was dysphasic and unable to recognize her daughter, and she was diagnosed with severe AD. She is currently 88 years old.

3.2.3 | Case 3: (F1) G1_5

This male patient is the younger brother of (F1) G1_1 and carries the *TREM2* H157Y variant with an *APOE* ε3ε3 genotype. He has completed 17 years of education. He began experiencing memory impairment at age 68 and was diagnosed with MCI at age 71. Routine blood tests and brain imaging excluded other causes of cognitive decline. He has memory complaints related to immediate recall but remains independent in both basic and instrumental ADLs. A PET-CT scan done at age 71 (for research purposes) confirmed that he was amyloid-positive without significant tau accumulation. He is currently 72 years old.

3.2.4 | Case 4: (F3) G1_1

This female patient carries the *TREM2* H157Y variant and has an *APOE* ε3ε3 genotype. She has no formal education and lives with her daughter. Between the ages of 68 and 70, she began to experience problems with immediate recall but remained independent in both basic and instrumental ADLs. At age 70, she was diagnosed with MCI and enrolled in a homocysteine-lowering trial for 24 months. Routine blood tests and brain imaging excluded other causes of cognitive decline. A year later, she reported a subjective improvement in memory. By the end of the trial, her condition remained mostly stable. Although she maintained her independence in ADLs, her cognitive function gradually declined over the next 3 years and sleep quality deteriorated. At age 77, she was still able to manage housework, cooking, and shopping. A PET-CT scan done at age 77 (for research purposes) indicated no significant amyloid deposition. She is currently 78 years old.

3.2.5 | Case 5: (F1) G1_6

This individual is the younger brother of both (F1) G1_1 and (F1) G1_5. He carries the *TREM2* H157Y variant and has an *APOE* ε3ε4 genotype. He has completed 12 years of education. He is being treated for hyperlipidemia but is otherwise in good health. At age 67, he was recruited for this study as a sibling and had no subjective or objective cognitive impairment. His physical examination was unremarkable. However, a PET-CT scan done at age 67 (for research purposes) indicated that he was amyloid-positive with significant tau accumulation, which is concordant with the imaging results of a patient with AD. Thus, he represents stage 1 of AD per the NIA-AA criteria.[32]

3.2.6 | The *TREM2* H157Y variant is associated with more rapid clinical progression among *APOE* ε4 carriers but does not affect the age of diagnosis

We examined whether the *TREM2* H157Y variant affects the age of diagnosis and clinical progression. We observed that the age of diagnosis of MCI (range: 66–87 years old) or AD (range: 67–83 years old) in variant carriers was not earlier than that of non-variant carriers. On the other hand, we observed that variant carriers with AD experienced rapid disease progression. Notably, there was a characteristic trend of *APOE* genotype in this cohort, in which all H157Y variant carriers with AD had a single copy of the *APOE* ε4 allele; meanwhile, non-ε4 variant carriers only reached the MCI stage (Figure 1).

Therefore, to determine if the H157Y variant affects disease progression, we compared the duration of each disease stage against a reference AD population stratified by age of onset and *APOE* genotype (Table 2 and Supplementary Method S8 on the stratification of clinical stages).[33] Patients carrying a single copy of both the H157Y variant and *APOE* ε4 (i.e., [F1] G1_1 and [F4] G1_1) exhibited quicker progression from MCI to moderate AD, although they remained in the moderate-to-severe AD stage for longer than the reference population. In contrast, patients with a single copy of the H157Y variant and a homozygous *APOE* ε3 genotype progressed at a rate similar to that of the reference AD population. For instance, (F3) G1_1 has remained in the MCI stage for 5 years, which is comparable to the estimated 4.5 years for non-carriers. Similarly, (F1) G1_5 was diagnosed with MCI 3 years after initial symptoms manifested, while his ε4-carrying family member, (F1) G1_1, was diagnosed with AD just 2 years after initial symptoms. This suggests that only *APOE* ε4 variant carriers exhibit more rapid disease progression.

3.3 | *TREM2* H157Y variant carriers with AD exhibit more severe disease-related endophenotypes

We then examined the association between the *TREM2* H157Y variant and AD-related endophenotypes, including cognitive performance, brain atrophy, and AD pathological hallmarks as indicated by plasma biomarkers, using Study Cohort_1. This study cohort includes both

TABLE 2 Clinical progression of Alzheimer's disease in *TREM2* H157Y variant carriers and estimated stage-specific duration obtained from a large-scale cohort study stratified by *APOE* genotype and age of disease onset.[33]

	Disease onset at 65 years old				Disease onset at 75 years old			
Duration, time in years [95% CI]/ (range)	Average, ε4+	H157Y carriers, ε4+ (*n* = 2)	Average, ε4−	H157Y carriers, ε4− (*n* = 1)	Average, ε4+	H157Y carriers, ε4+ (*n* = 2)	Average, ε4−	H157Y carriers, ε4− (*n* = 1)
MCI	3.3 [2.7–4.0]	**4.5 (4-5)**	4.5 [3.4–5.8]	5[a]	2.8 [2.2–3.6]	**3.5 (3-4)**	3.9 [2.9–5.1]	3[a]
Mild dementia	4.0 [3.2–4.8]		3.0 [2.2–3.8]	N/A	3.5 [2.6–4.3]		2.6 [1.8–3.4]	N/A
Moderate-to-severe dementia	4.8 [3.4–6.5]	8[b] (6–10)	4.9 [3.3–7.3]	N/A	3.7 [2.2–5.9]	4.5[c] (3–6)	3.8 [2.2–5.9]	N/A

Abbreviations: CI, confidence interval; ε4+, *APOE* ε4 carrier; ε4−, *APOE* ε4 non-carrier; MCI, mild cognitive impairment; N/A, not applicable.
[a]Individuals had not progressed to the later disease stages at the time of this study. Hence, values do not represent their final duration in that stage.
[b]Both individuals have not reached the end-stage of death. Hence, values do not represent their final duration in that stage.
[c]Only (F6) G1_1 reached the end-stage of death. Hence, values do not represent their final duration in that stage.

variant carriers (CN, *n* = 8; MCI, *n* = 3; AD, *n* = 4) and non-carriers (*n* = 15 in each clinical diagnostic group), matched for age, sex, and *APOE* genotype (Table S1).

3.3.1 | *TREM2* H157Y variant carriers with AD exhibit more severe cognitive impairment and brain volume loss

Among patients with AD, H157Y variant carriers had significantly lower MoCA scores than non-carriers ($t = 3.23$, $p = 0.005$), indicating poorer cognitive function in patients carrying the variant (Figure 2A). Magnetic resonance imaging (MRI) analysis of patients with MCI and AD (Table S7) revealed a significant interaction between the effect of the *TREM2* H157Y variant and disease stage (i.e., MCI or AD) on the volume of total gray matter ($F_{[1,30]} = 12.2$, $p = 0.001$) and cortex ($F_{[1,30]} = 11.8$, $p = 0.002$). This indicates that the impact of the variant on the changes in total gray matter and cortical volume is dependent on the disease stage of the individual. In particular, patients with AD carrying the *TREM2* H157Y variant had significantly smaller total gray matter and cortical volume than non-carriers as indicated by Bonferroni-corrected post hoc pairwise comparison (adjusted $p = 0.011$ and 0.030, respectively; Figure 2B,C). In contrast, there were no such differences between variant carriers and non-carriers with MCI. Together, these results indicate that the effect of the H157Y variant on the global brain volume is particularly relevant in the later disease stages. In addition, the H157Y variant and disease stage were significantly associated with the volume of subcortical gray matter but showed no interaction ($F_{[1,30]} = 18.0$, $p < 0.001$; $F_{[1,30]} = 40.5$, $p < 0.001$, respectively; Figure 2D). Patients with AD carrying the variant had a smaller subcortical gray matter volume than non-carriers as indicated by Bonferroni-corrected post hoc pairwise comparison (adjusted $p = 0.029$). Meanwhile, there were no differences between variant carriers and non-carriers with MCI. Further statistical analysis of smaller subdivided brain regions revealed no significant results, potentially owing to the limited sample size.

3.3.2 | *TREM2* H157Y variant carriers with AD exhibit more severe disease pathology and neurodegeneration

To investigate the impact of the *TREM2* H157Y variant on AD pathological hallmarks, we utilized the established plasma AD biomarkers pTau217 and NfL. Changes in these blood biomarkers reflect AD pathology, including Aβ deposition, tau phosphorylation, and axonal neurodegeneration.[25,34,35] We observed that among patients with AD, H157Y variant carriers had significantly higher plasma levels of pTau217 than non-carriers ($t = 2.16$, $p = 0.046$), indicating more severe AD brain pathology in variant carriers (Figure 2E). In addition, among patients with AD, variant carriers had significantly higher plasma NfL levels than non-carriers ($t = 6.06$, $p < 0.001$; Figure 2F). Notably, among patients with MCI, we observed a trend of higher plasma NfL levels in variant carriers than non-carriers ($t = 1.97$, $p = 0.066$), suggesting more severe neurodegeneration even at earlier disease stages.

We conducted a cross-sectional analysis to further examine the association between the H157Y variant and neurodegeneration without stratifying by disease stage. Accordingly, H157Y variant carriers exhibited a significantly higher rate of increase in plasma NfL levels with age than non-carriers (slope ± standard error = 1.70 ± 0.588 and 0.863 ± 0.122, respectively; $p = 0.001$), suggesting rapid neurodegeneration in variant carriers (Figure 2G).

3.4 | The *TREM2* H157Y variant is associated with biomarkers related to neurodegeneration and inflammation but not biomarkers of other central nervous system diseases

TREM2 variants are implicated in other central nervous system (CNS) diseases, including frontotemporal dementia, amyotrophic lateral sclerosis (ALS), and Parkinson's disease.[12,36] To examine whether the *TREM2* H157Y variant is associated with the pathologies of other CNS diseases, we utilized the NULISAseq CNS Disease panel to measure the plasma levels of 127 proteins associated with a broad spectrum of

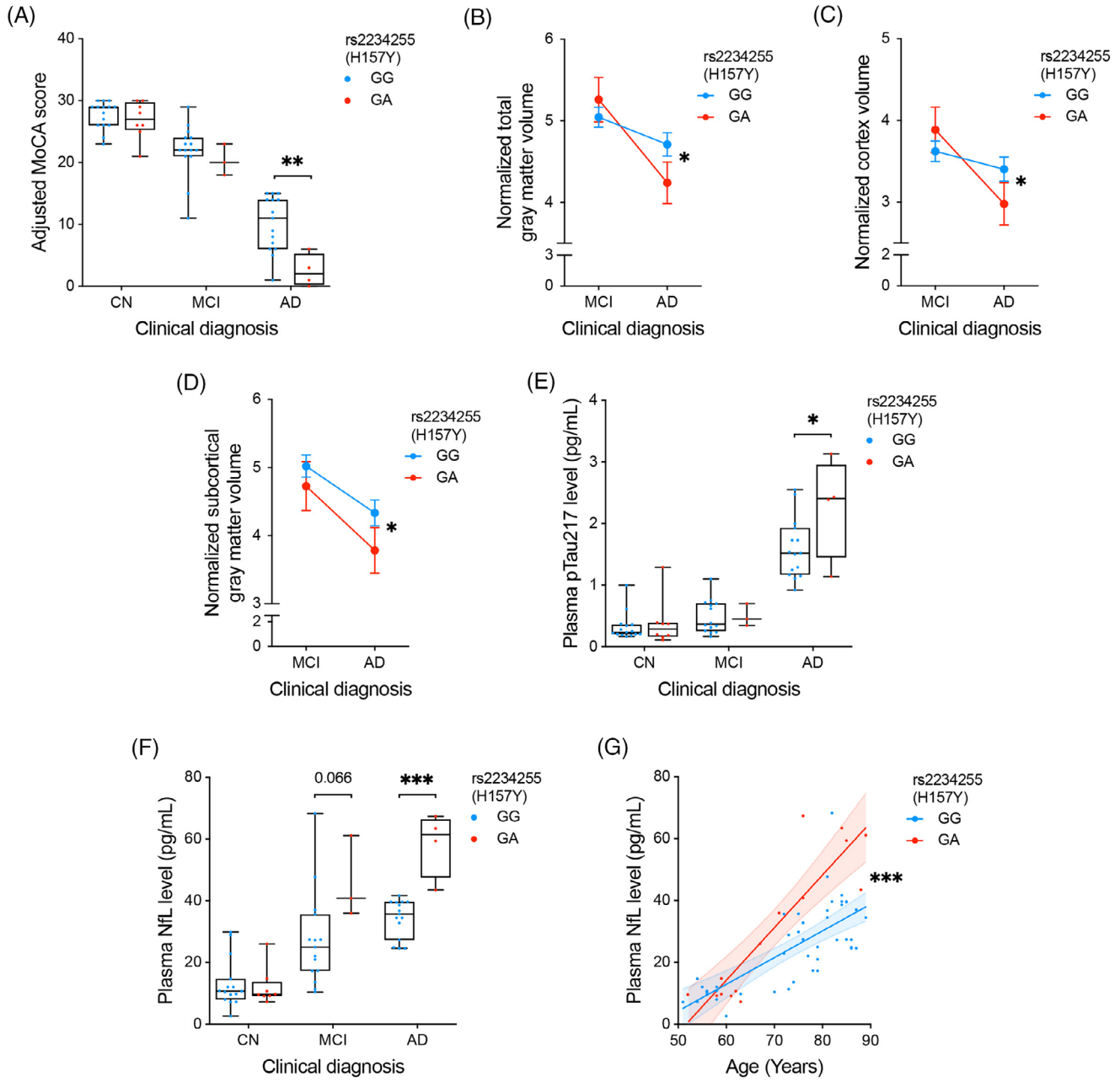

FIGURE 2 Patients with AD carrying the *TREM2* H157Y variant exhibit more severe disease-related endophenotypes. (A) Adjusted MoCA scores of *TREM2* H157Y variant carriers (GA) and non-carriers (GG) who are CN or diagnosed with MCI or AD from Study Cohort_1. Data are presented as box-and-whisker plots; whiskers indicate minimum and maximum values. Statistical analysis was performed using two-sample *t*-test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. (B–D) Normalized volumes of (B) total gray matter, (C) cortex, and (D) subcortical gray matter of *TREM2* H157Y variant carriers diagnosed with MCI and AD in Study Cohort_1. Data are presented as the normalized mean, with lower- and upper-class limits retrieved from statistical analysis (Supplementary Method S4). *Adjusted $p < 0.05$, **adjusted $p < 0.01$, ***adjusted $p < 0.001$. (E, F) Plasma levels of (E) pTau217 and (F) NfL in *TREM2* H157Y variant carriers and non-carriers who are CN or diagnosed with MCI, or AD from Study Cohort_1. Data are presented as box-and-whisker plots; whiskers indicate minimum and maximum values. Statistical analysis was performed using two-sample *t*-test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. One value of plasma pTau217 in non-carriers with AD is missing due to limited plasma volume. (G) Dot plot showing the association between plasma NfL level and age among *TREM2* H157Y variant carriers and non-carriers from Study Cohort_1. Simple linear regression analysis results are presented as regression lines. GG, slope (±standard error) = 0.863 (±0.122); GA, slope (±standard error) = 1.70 (±0.230). AD, Alzheimer's disease; CN, cognitively normal; MCI, mild cognitive impairment; MoCA, Adjusted Montreal Cognitive Assessment; NfL, neurofilament light polypeptide; *TREM2*, triggering receptor expressed on myeloid cells 2.

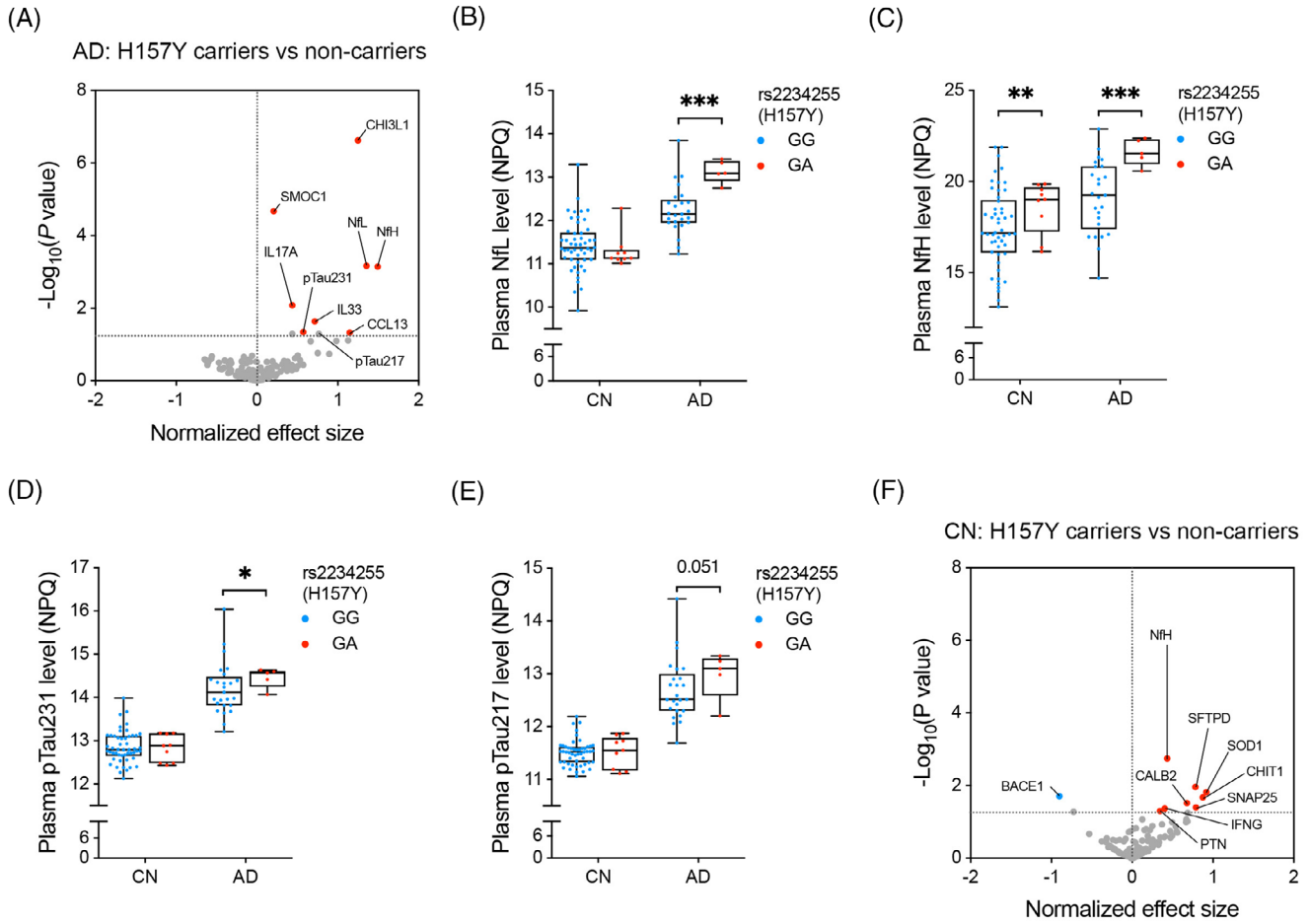

FIGURE 3 *TREM2* H157Y variant carriers exhibit dysregulation of blood proteins associated with neurodegeneration and inflammation. (A) Volcano plot showing the association of blood proteins in the NULISAseq CNS Disease panel with the *TREM2* H157Y variant among patients with AD from Study Cohort_2. Blue and red dots indicate blood proteins that are significantly ($p < 0.05$) downregulated and upregulated, respectively.(B–E) Plasma levels of (B) NfL, (C) NfH, (D) pTau231, and (E) pTau217 of *TREM2* H157Y variant carriers and non-carriers who are CN or diagnosed with AD from Study Cohort_2. Data are presented as box-and-whisker plots; whiskers indicate minimum and maximum values. Statistical analysis was performed using linear regression. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. (F) Volcano plot showing the association of blood proteins in the NULISAseq CNS Disease panel with the *TREM2* H157Y variant among CN individuals from Study Cohort_2. Blue and red dots indicate blood proteins that are significantly ($p < 0.05$) downregulated and upregulated, respectively. AD, Alzheimer's disease; CN, cognitively normal; NfH, neurofilament heavy polypeptide; NfL, neurofilament light polypeptide; *TREM2*, triggering receptor expressed on myeloid cells 2.

neurodegenerative disorders in H157Y variant carriers (CN, $n = 9$; AD, $n = 5$) and non-carriers (CN, $n = 50$; AD, $n = 25$) from Study Cohort_2 (Table S2).

Considering that all H157Y variant carriers with AD had an *APOE* ε3ε4 genotype, we only included non-carriers with an *APOE* ε3ε4 genotype as controls when analyzing the impact of the variant among patients with AD. Eight blood proteins were significantly associated ($p < 0.05$) with the *TREM2* H157Y variant among patients with AD, with all found to be upregulated in variant carriers (Figure 3A and Table S8). Four blood proteins, CHI3L1, SMOC1, NfL, and neurofilament heavy polypeptide (NfH), remained significant (false discovery rate [FDR] < 0.05) after FDR correction. Consistent with the findings in Study Cohort_1, H157Y variant carriers exhibited significantly higher levels of blood biomarkers indicating more severe neurodegeneration (i.e., NfL, NfH, and SMOC1) and AD pathology (i.e., pTau231) compared to non-carriers with AD (Figure 3B–D). Notably, NfH and NfL are both major cytoskeleton proteins specifically expressed in neurons that are upregulated in patients with AD and reflect axonal damage.[37,38] Moreover, variant carriers with AD exhibited higher levels of pTau217 with marginal significance ($p = 0.051$; Figure 3E), and higher levels of biomarkers indicating increased inflammation (i.e., CHI3L1, CCL13, IL33, and IL17A) compared to non-carriers with AD. On the other hand, blood proteins related to synuclein and synaptic disorders (e.g., synucleins, TDP43, PARK7, and HTT) were not altered in carriers compared to non-carriers among patients with AD, suggesting no significant association between the H157Y variant and the pathologies of other neurodegenerative diseases.

To explore potential mechanisms by which the *TREM2* H157Y variant predisposes carriers to AD as well as the physiological role of TREM2, we examined the impact of the H157Y variant in CN individuals without amyloid pathology. Nine blood proteins were significantly associated ($p < 0.05$) with the H157Y variant among CN individuals,

including eight upregulated and one downregulated protein (Figure 3F and Table S8). Interestingly, we also observed higher levels of NfH in CN variant carriers compared to non-carriers (Figure 3C). Similar to the impact of the H157Y variant among patients with AD, the variant was also associated with blood proteins related to neurodegeneration (i.e., NfH, SNAP25, and CALB2) and inflammation (i.e., CHIT1, SFTPD, and IFNG) among CN individuals, highlighting the role of TREM2 in modulating neuronal health and inflammation irrespective of the presence of amyloid pathology. Surprisingly, CN individuals carrying the H157Y variant showed lower levels of BACE1, the enzyme involved in the generation of Aβ, than non-carriers. Plasma BACE1 levels are reported to be elevated in patients with AD;[39] hence, the lower level observed in CN H157Y variant carriers suggests that the mechanism by which the H157Y variant increases AD risk may not be related to Aβ production. Together, these findings suggest that, in addition to effects through amyloid and tau pathology, the H157Y variant has major impacts on inflammation and neurodegeneration even in the absence of amyloid pathology.

Interestingly, we did not observe any association between the *TREM2* H157Y variant and plasma TREM2 levels regardless of whether we analyzed AD and CN carriers separately (adjusting for age and sex) or pooled them together (adjusting for age, sex, diagnosis, and *APOE* genotype) (Figure S1). The *TREM2* H157Y variant is reported to be associated with increased sTREM2 levels in cellular models and *TREM2* H157Y variant-bearing mice.[13,14,40] The lack of association in the present study may be due to different mechanisms regulating sTREM2 levels in an endogenous system compared to an isolated cell model as well as in mice compared to humans.

3.5 | *TREM2* H157Y variant carriers exhibit an altered blood proteomic profile associated with immune and vascular processes

Given the association of the *TREM2* H157Y variant with various inflammation-related proteins and the roles of TREM2 in cancers and metabolic diseases, we utilized Olink Target series panels to measure the levels of 1160 unique proteins associated with various types of human diseases and biological processes.[41–44] Our group previously demonstrated that the Olink Target series panels can capture various biological processes altered in patients with MCI and AD, including inflammation, innate immune function, metabolic function, and vascular function.[45,46] Therefore, we compared the blood protein levels between H157Y variant carriers (CN, $n = 6$; AD, $n = 3$) and non-carriers (CN, $n = 16$; AD, $n = 22$) from Study Cohort_3 (Table S3 and Figure S2).

3.5.1 | Patients with AD carrying the *TREM2* H157Y variant exhibit alterations in vascular, bone, and immune processes

By comparing the blood protein levels between *TREM2* H157Y variant carriers and non-carriers with the same *APOE* genotype among patients with AD, we identified 150 proteins significantly associated ($p < 0.05$) with the H157Y variant, including 73 downregulated and 77 upregulated proteins (Figure 4A and Table S9). Among the DEPs, 20 of them remained significantly associated with the H157Y variant after FDR correction, with LRP1 being the most downregulated blood protein. LRP1 binds Aβ and is associated with amyloid clearance from the brain, with lower levels observed in the plasma of patients with AD.[47] Among the 20 proteins, the downregulated blood proteins SOD2 and PRDX6 are involved in the detoxification of reactive oxygen species, while the upregulated blood proteins (i.e., GBP2, LAMP3, JAM_A, YES1, and STK4) are involved in immune system processes. To better understand the impact of the variant, we performed GO enrichment analysis with the 150 DEPs, which revealed that the H157Y variant is associated with altered cellular functions (i.e., migration, adhesion, and proliferation), immune system processes, and vasculature development (Figure 4B). PPI analysis supports the alterations of these processes in H157Y variant carriers, showing the upregulation of proteins involved in immune system processes, particularly those related to the regulation of lymphocyte activation (i.e., GAL, BANK1, YES1, LAT, FGR, and SRC) and VEGF receptor activity (i.e., ANGPT2, KDR, and PDGFC) (Figure 4C). PPI analysis further indicated the upregulation of a protein cluster related to ossification (i.e., MRC2, PTN, SFRP1, and CCN4), which is a process involved in bone formation, and downregulation of protein clusters related to extracellular matrix binding (i.e., TNXB, NCAN, THBS2, LTBP2, and DCN), apoptosis (i.e., BID, PSME1, SMPD1, CASP8, and BIRC2), and response to external stimuli, including antimicrobial peptides (i.e., LCN2, DEFA1B, and REG3A). In addition, we performed CSEA to examine the cellular sources of the H157Y variant-associated DEPs. CSEA revealed significant enrichment in peripheral cells (i.e., dendritic, stromal, and T cells) involved in immune response (Figure S3).

3.5.2 | Cognitively normal individuals carrying the *TREM2* H157Y variant exhibit alterations in inflammatory response and peripheral immune cell functions

We subsequently examined the effect of the *TREM2* H157Y variant among CN individuals who do not exhibit amyloid pathology. We identified 96 proteins significantly associated ($p < 0.05$) with the H157Y variant, including 33 downregulated and 63 upregulated proteins (Figure 4D and Table S9). Among the DEPs, only GGA1, USO1, and ACAN remained significant after FDR correction. GGA1, a protein that interacts with BACE1 and is implicated in the generation of Aβ, was significantly ($FDR < 0.05$) upregulated in H157Y variant carriers irrespective of disease state. Indeed, the impacts of the H157Y variant in patients with AD and CN individuals are positively correlated ($r = 0.267$, $p < 0.001$), with 19 blood proteins significantly associated ($p < 0.05$) with the variant in both states (Figure S4). The 19 overlapping DEPs highlight the impact of the H157Y variant on immune-related processes (i.e., BIRC2, LXN, REG3A, GBP2, and CD244) and vascular functions (i.e., ECE1, SFRP1, and KDR).

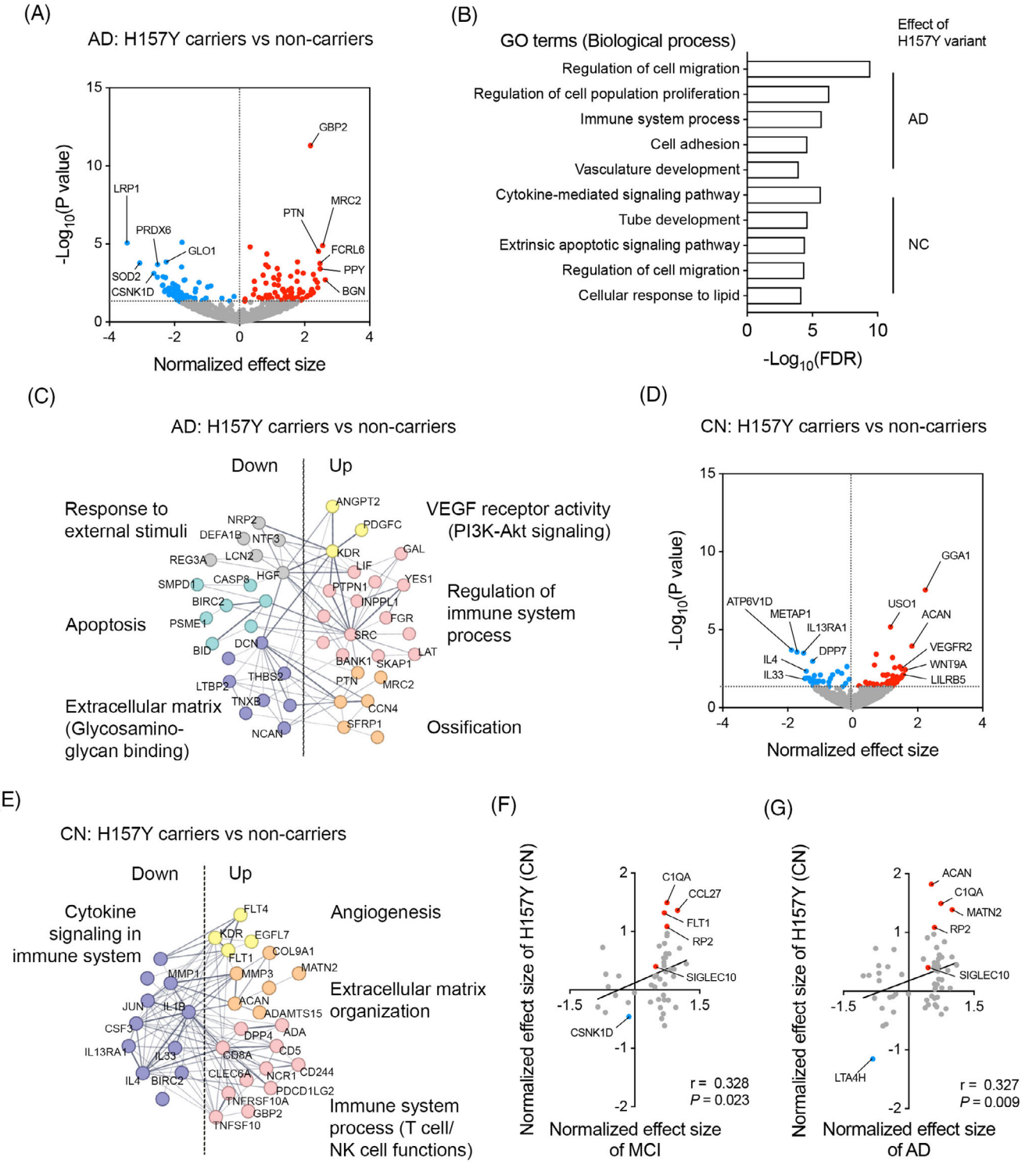

FIGURE 4 *TREM2* H157Y variant carriers exhibit alterations in blood proteins associated with immune and vascular processes, and cognitively normal variant carriers exhibit dysregulation of disease-related blood proteins. (A) Volcano plot showing the association of blood proteins in the Olink Target series panels with the *TREM2* H157Y variant among patients with AD from Study Cohort_3. Blue and red dots indicate blood proteins that are significantly ($p < 0.05$) downregulated and upregulated, respectively. (B) Bar chart showing the top five GO biological processes altered in *TREM2* H157Y variant carriers among patients with AD and CN individuals. (C) PPI network of blood proteins associated with the *TREM2* H157Y variant among patients with AD. The top three downregulated and upregulated protein clusters are displayed. The functional enrichment of each cluster was retrieved from the STRING database (v12.0). (D) Volcano plot showing the association of blood proteins in the Olink Target series panels with the *TREM2* H157Y variant among CN individuals from Study Cohort_3. Blue and red dots indicate blood proteins that are significantly ($p < 0.05$) downregulated and upregulated, respectively. (E) PPI network of blood proteins associated with the *TREM2* H157Y variant among CN individuals. The top three upregulated protein clusters and the major downregulated protein cluster are displayed. The

GO enrichment analysis of the 96 DEPs highlighted the impact of the H157Y variant on cytokine-mediated signaling pathways, tube development, and cellular response to lipids (Figure 4B). PPI analysis further demonstrated alterations in immune-related processes, particularly the downregulation of cytokine signaling (i.e., IL33, IL4, CSF3, and IL1B) as well as upregulation of proteins related to T cell activation (i.e., DPP4, PDCD1LG2, ADA, and CD5) and natural killer (NK) cell-mediated cytotoxicity (i.e., CD244, TNFSF10, TNFRSF10A, and NCR1) (Figure 4E). CSEA revealed significant enrichment of H157Y variant-associated DEPs in macrophages, which is the cell type that predominantly expresses TREM2 in the peripheral system (Figure S3).

3.5.3 | Cognitively normal individuals carrying the *TREM2* H157Y variant exhibit alterations in disease-associated blood proteins related to peripheral immune response

As the *TREM2* H157Y variant is associated with a higher risk of AD, we examined whether CN variant carriers exhibit disease-related alterations in the blood proteome. We used the aforementioned analysis of variant carriers and non-carriers among CN individuals to identify variant effect-associated proteins. By comparing the blood proteome of non-carriers with MCI or AD to that of CN non-carriers, we identified 48 and 62 blood proteins significantly associated with MCI and AD, respectively. The effect of the H157Y variant was positively correlated with MCI ($r = 0.328$, $p = 0.023$; Figure 4F) and AD ($r = 0.328$, $p = 0.009$; Figure 4G). This suggests that CN variant carriers exhibit a trend of blood proteome dysregulation that is associated with AD pathogenic processes. In particular, we identified nine proteins significantly associated with the H157Y variant among CN individuals and patients with MCI (i.e., C1QA, RP2, SIGLEC10, CCL27, CSNK1D, and FLT1) or AD (i.e., C1QA, RP2, SIGLEC10, ACAN, MATN2, and LTA4H) (Figure S5). C1QA, RP2, and SIGLEC10 were altered across all groups. These proteins are enriched in peripheral immune cells (i.e., macrophages and monocytes), which are involved in the innate immune response.[48,49] Together, these findings highlight the role of the *TREM2* H157Y variant in immune-related processes and suggest a mechanism by which the variant increases AD risk.

4 | DISCUSSION

Here, we present an in-depth study investigating the impacts of the *TREM2* H157Y variant on AD clinical progression, AD-related endophenotypes, and other biological processes. We observed that the H157Y variant is associated with more rapid clinical progression in the presence of the *APOE* ε4 allele. Compared to non-carriers, H157Y variant carriers with AD exhibited more severe AD pathology, neurodegeneration, and inflammation as indicated by blood biomarkers and neuroimaging results. These individuals also showed changes in their blood proteomic profile that indicate altered immune and vascular processes. Notably, compared to CN non-carriers, CN H157Y variant carriers exhibited alterations in immune-related processes, particularly those involved in inflammation and lymphocyte activation, suggesting that the alteration of potential biological processes in carriers underlies the increased risk of AD.

Previous studies on the impacts of other *TREM2* variants (i.e., R47H and T96K) on AD clinical presentation and progression report more rapid cognitive decline but not earlier age of diagnosis.[9,50] In our study cohort, we also observed more rapid clinical progression in *TREM2* H157Y variant carriers but only in those with an *APOE* ε4 background. This suggests an interaction between the *TREM2* H157Y variant and *APOE* genotype in the modulation of AD-related pathology. APOE is an endogenous ligand for TREM2, and both proteins are involved in mediating microglial response against amyloid pathology.[51–53] Nevertheless, further study is needed to elucidate the mechanisms underlying their interaction. Such studies are necessary for predicting and understanding the responses of patients with different *APOE* genotypes to future TREM2-targeting therapies. Moreover, CN H157Y variant carriers with an *APOE* ε4 background, such as (F1) G1_6, who is already in stage 1 of AD, may also benefit from close monitoring and early intervention.

In addition to the impact of the H157Y variant on clinical progression, variant carriers with AD exhibited more severe AD-related outcomes, particularly neurodegeneration. Patients with AD carrying the H157Y variant exhibited significantly higher plasma levels of NfL across two different study cohorts using two different measurement platforms. Notably, other studies report no association between other AD-risk-associated *TREM2* variants (i.e., R47H and T96K) with plasma and cerebrospinal fluid (CSF) NfL levels, suggesting a distinct impact of the H157Y variant.[50,54] Interestingly, markers of neurodegeneration are also seen in CN H157Y variant carriers compared to non-carriers, specifically higher levels of SNAP25, CALB2, and NfH, indicating more severe neurodegeneration than that observed in normal aging. For instance, SNAP25 is reported to be increased in the plasma and CSF of patients with AD, indicating synapse degeneration.[55,56] The increase in neurodegeneration-related proteins in CN H157Y variant carriers without amyloid pathology suggests an amyloid-independent role of TREM2 in neurodegeneration. This may explain why *TREM2* variants are implicated in multiple neurodegenerative diseases. Altogether, these findings may provide valuable insights regarding the timing of monitoring and types of interventions offered at early disease stages.

functional enrichment of each cluster was retrieved from the STRING database (v12.0). (F, G) Correlation analysis of the effects of (F) MCI and (G) AD on the blood proteomic profile and that of the H157Y variant on the blood proteomic profile among CN individuals. Blue and red dots indicate downregulated and upregulated blood proteins, respectively, that are significantly associated ($p < 0.05$) with the *TREM2* H157Y variant in CN individuals. AD, Alzheimer's disease; CN, cognitively normal; GO, Gene Ontology; MCI, mild cognitive impairment; PPI, protein–protein interaction; *TREM2*, triggering receptor expressed on myeloid cells 2.

Additionally, TREM2 has been implicated in the inflammatory responses of different physiological and pathological contexts.[57–59,43] We observed increased inflammation (i.e., upregulation of blood levels of CHI3L1, CCL13, IL33, and IL17A) in H157Y variant carriers compared to non-carriers among patients with AD. Higher plasma levels of CHI3L1, a glial and inflammation marker, are reported to be associated with poorer cognitive function and a higher risk of dementia.[60] IL17A, also upregulated in variant carriers with AD, is a pro-inflammatory cytokine that is elevated in the CSF and plasma of patients with various neurological diseases; it is also associated with glial cell activation and T-cell infiltration, suggesting the potential involvement of the peripheral immune system in the inflammation process.[61] Infiltration of peripheral immune cells and their crosstalk with the central immune system have been reported in the context of AD.[62,63] We found that the blood proteins differentially expressed in variant carriers with AD are enriched in peripheral immune cells (i.e., dendritic cells and T cells). In particular, we observed upregulation of proteins associated with leukocyte activation and cell migration as well as downregulation of proteins associated with blood vessel morphogenesis and cell adhesion, suggesting altered trafficking of immune cells. Specifically, SMOC1, which is found to be co-localized with amyloid plaques and cerebral amyloid angiopathy, is consistently upregulated in the plasma and CSF of patients with AD and also reflects cerebovascular changes in AD.[64] We also observed a similar trend of dysregulation of biological processes in CN H157Y variant carriers compared to non-carriers. The differentially expressed blood proteins in CN H157Y variant carriers were significantly enriched in macrophages and are related to multiple immune-related processes, including inflammation, T-cell activation, and NK cell-mediated cytotoxicity. TREM2-expressing macrophages have been reported to suppress the anti-tumor activity of NK and T cells.[42,65] Thus, these results suggest a role of the *TREM2* H157Y variant in modulating macrophage functions and their interactions with various peripheral immune cells. We also observed alterations in endothelial cell function, with the upregulation of PTN and all soluble forms of VEGF receptor subtypes (i.e., KDR, FLT4, and FLT1), which are involved in the regulation of angiogenesis and lymphangiogenesis. Among these variant-associated proteins, FLT1 and C1QA are also altered in non-variant-carrying patients with MCI or AD, indicating that these changes in immune and endothelial functions may have implications in disease pathogenesis. *FLT1*-expressing endothelial cells contribute to angiogenesis and immune response in the context of AD.[66] Meanwhile, C1QA is a complement protein that was recently found to bind to TREM2 and prevent complement-mediated removal of synapses by microglia.[67] Altogether, these findings suggest that the involvement of TREM2 in the immune system contributes to the pathogenic process of AD related to the *TREM2* H157Y variant.

In summary, our study advances the current understanding on the role of TREM2 in AD and lays the groundwork for further studies of the mechanisms by which the *TREM2* H157Y variant modulates TREM2 functions in neurodegeneration as well as its interaction with the *APOE* genotypes using molecular models, such as brain cell models from patient-derived induced pluripotent stem cells (iPSCs). In addition, this study highlights the clinical implications of the *TREM2* H157Y variant, as carriers exhibit more rapid cognitive decline in the early stages of AD, suggesting that they may benefit from early disease-modifying interventions. Furthermore, we demonstrated the utility of blood proteomic profiling in investigating the impacts of genetic variants on disease outcomes and the biological processes involved in the pathogenesis of AD. Accordingly, longitudinal studies tracking the clinical progression of variant carriers alongside changes in the blood proteomic profile may facilitate the identification of markers for early diagnosis and disease monitoring.

4.1 | Study limitations

Although the small size of the study cohort limits statistical power, this is unavoidable due to the nature of rare genetic variants. CSF samples were not collected because this is not commonly accepted among patients in the region. Moreover, the limited follow-up duration prevents extensive analysis of disease progression, especially on the transition from normal cognition to MCI or AD. Due to the lack of local data on disease stage duration in non-variant carriers with MCI or AD, we used a non-Chinese reference population to compare the disease stage duration between variant carriers and non-carriers. In addition, the clinical data of this cohort were not standardized, as clinical history prior to recruitment was collected retrospectively from existing clinical notes and interviews. Notably, clinical guidelines in our locality have shifted from the MMSE to the MoCA for both clinical and practical reasons. The recent coronavirus disease 2019 (COVID-19) pandemic also severely affected follow-up for many elderly patients, resulting in large gaps in clinical history. Furthermore, the timing of diagnosis was partially affected by the subjective evaluation of patients' cognitive function (by patients themselves or their family members) before they sought medical attention. Thus, the MCI stage may be longer than that reported by the patients.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. Author disclosures are available in the supporting information.

CONSENT STATEMENT

All participants or the legal guardians of participants with advanced dementia provided written informed consent for study participation and sample collection.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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