



Association Between Clonal Hematopoiesis of Indeterminate Potential and Brain β -Amyloid Deposition in Korean Patients With Cognitive Impairment

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Few studies have focused on the association between clonal hematopoiesis of indeterminate potential (CHIP) and β -amyloid (A β) deposition in the brain, which causes Alzheimer's disease. We aimed to investigate the potential role of CHIP in brain A β deposition in Korean patients. We enrolled 58 Korean patients over 50 yrs of age with cognitive impairment who underwent brain A β positron emission tomography. We explored CHIP in their peripheral blood using deep-targeted next-generation sequencing. Irrespective of the presence or absence of brain A β deposition, mutations in *DNMT3A* and the C:G > T:A single-nucleotide variants were identified as the primary characteristics, which reflect aged hematopoiesis in the study population. Multivariate logistic regression revealed that the presence of CHIP was not associated with brain A β deposition. As both CHIP and brain A β deposition are associated with aging, further research is required to elucidate their possible interplay.

Key Words: Alzheimer's disease, β -Amyloid protein, Clonal hematopoiesis of indeterminate potential, Next-generation sequencing, Positron emission tomography

Received: February 16, 2024

Revision received: March 12, 2024

Accepted: May 17, 2024

Published online: May 28, 2024

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Clonal hematopoiesis of indeterminate potential (CHIP) is characterized by the presence of somatic mutations in genes associated with hematologic malignancies in hematopoietic stem and progenitor cells (HSPCs) with a variant allele frequency (VAF) of $\geq 2\%$ in the absence of overt hematologic malignancy or cytopenia [1]. The prevalence of these mutations, predominantly in *DNMT3A*, *TET2*, and *ASXL1*, increases with age [2]. Age-related clonal hematopoiesis is associated with an increased risk of hematologic cancer and cardiovascular disease, including coronary artery disease and ischemic stroke [2, 3]. In addition, recent studies have identified associations between CHIP and other

diseases, including aortic valve stenosis [4, 5], chronic ischemic heart failure [6], chronic obstructive pulmonary disease [7, 8], and rheumatoid arthritis [9]. Notably, Bouzid, *et al.* [10] observed that individuals with CHIP had a reduced risk of developing Alzheimer's disease (AD) over time, which also attenuated the effect of the *APOE* $\epsilon 4$ allele. Furthermore, they found a negative association between CHIP and AD neuropathological characteristics, including brain β -amyloid (A β) deposition and tau neurofibrillary tangles, as detected in autopsies.

In Korea, brain A β deposition in patients with cognitive impairment is often examined using amyloid positron emission tomog-

raphy (PET) to evaluate AD or other causes of cognitive impairment. A positive amyloid PET scan does not necessarily confirm AD but indicates the presence of significant levels of $A\beta$, the hallmark pathology of AD, and suggests that AD is likely the cause of cognitive impairment in the patient [11-13]. Conversely, a negative amyloid PET reduces the likelihood that cognitive impairment is due to AD [11-13]. The previous study demonstrated the direct association between AD and CHIP; however, no study has investigated the association between these two factors using amyloid PET results. Therefore, we aimed to investigate the association between CHIP and brain $A\beta$ deposition in the Korean population.

Fifty-eight patients with cognitive impairment were enrolled. All patients underwent ^{18}F -labeled florbetaben amyloid brain PET-computed tomography (CT) using a Gemini TF 16 scanner (Philips Medical Systems, Cleveland, OH, USA), which was visually assessed based on regional cortical tracer uptake and brain amyloid plaque load (BAPL) scores [14]. BAPL scores of 2 and 3 indicate $A\beta$ -positivity, whereas a score of 1 indicates $A\beta$ -negativity. Ultimately, 43 patients who tested $A\beta$ -positive and 15 who tested $A\beta$ -negative were included in the study. Subjective cognitive decline (SCD), mild cognitive impairment (MCI), and dementia (i.e., major cognitive impairment) were diagnosed based on the criteria of the Subjective Cognitive Decline Initiative Working Group [15], Petersen's criteria [16], and the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [17], respectively. Age, cognitive status, and brain $A\beta$ deposition were determined at the time of blood draw. All patients provided written informed consent. The study was reviewed and approved by the Institutional Review Board (IRB) of Chung-Ang University College of Medicine, Seoul, Korea (IRB No. 2207-012-514).

Peripheral blood DNA was used for *APOE* genotyping and next-generation sequencing (NGS). *APOE* genotyping was performed

using real-time PCR analysis. PCRs were run using a Real-Q ApoE genotyping kit (BioSewoom, Seoul, Korea) on an ABI 7500 Real-time PCR System (Applied Biosystems, Waltham, MA, USA). CHIP was detected by deep-targeted NGS with a mean depth of 1,576 on an Ion Torrent system (Thermo Fisher, Waltham, MA, USA) using a panel of 61 hematological malignancy-related genes (Supplemental Data Table S1). As CHIP variants, single-nucleotide variants (SNVs) and small insertion-deletion variants with a VAF of $\geq 2\%$ were filtered using an in-house filtering strategy (detailed in Supplemental Methods).

Student's *t*-test and the Mann-Whitney test were used to compare continuous variables between groups. The chi-squared test, chi-squared test for trend, and Fisher's exact test were used to compare categorical variables between groups. Multivariate logistic regression analysis was performed to evaluate the risk of brain $A\beta$ deposition, and odds ratios (ORs) were calculated with 95% confidence intervals (CIs). Statistical analyses were conducted using R version 4.2.3 (The R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism version 9.3.1 (GraphPad, San Diego, CA, USA). $P < 0.05$ was considered statistically significant.

The demographics of the study population are presented in Table 1. The $A\beta$ -positive group ($N = 43$) tended to be more severely cognitively impaired (chi-squared test for trend, $P = 0.011$) and comprised more *APOE* $\epsilon 4$ carriers (60.5% vs. 20.0%, $P = 0.015$) than the $A\beta$ -negative group ($N = 15$).

Of the 58 patients, nine patients (15.6%) harbored 11 CHIP variants in *CBL*, *DNMT3A*, *JAK2*, *KMT2A*, *PRPF8*, and *TET2* (Table 2). As all patients included in this study showed complete blood counts within reference ranges, the possibility of undiagnosed blood cancer was ruled out. Seven patients (16.3%) were $A\beta$ -positive, and two patients (13.2%) were $A\beta$ -negative. The prevalence of CHIP in this study was similar to that in previous studies detecting CHIP in Korean individuals based on a VAF cut-

Table 1. Demographics of the study population

Variable	$A\beta$ -positive ($N = 43$)	$A\beta$ -negative ($N = 15$)	<i>P</i>
Age, yrs, median (range)	69 (56-86)	70 (51-80)	0.756
Female sex, <i>N</i> (%)	27 (62.8)	9 (60.0)	>0.999
Clinical diagnosis, <i>N</i> (%)			0.011
Subjective cognitive decline	4 (9.3)	6 (40.0)	0.014
Mild cognitive impairment	13 (30.2)	4 (26.7)	>0.999
Dementia	26 (60.5)	5 (33.3)	0.082
<i>APOE</i> $\epsilon 4$ carrier, <i>N</i> (%)	26 (60.5)	3 (20.0)	0.015

Abbreviation: $A\beta$, β -amyloid.

Table 2. CHIP variants observed in our study population

A β group	Sex/age, yrs	Cognitive status	Gene and reference sequence	Coding DNA sequence and amino acid change	VAF (%) / total depth	COSMIC	
						No.	Hematologic malignancy case count*
A β -positive	F/58	Dementia	<i>DNMT3A</i> NM_022552	c.1015-1G>A	4.5/1,549	None	None
	F/69	Dementia	<i>DNMT3A</i> NM_022552	c.2206C>T (p.Arg736Cys)	4.1/1,941	COSM231560	18
	M/72	MCI	<i>PRPF8</i> NM_006445	c.6427C>T (p.Arg2143Cys)	8.5/1,735	None	None
	F/74	Dementia	<i>TET2</i> NM_001127208	c.1588C>T (p.Gln530Ter)	4.6/1,602	COSM4383816	3
	F/77	SCD	<i>DNMT3A</i> NM_022552	c.1040T>A (p.Leu347Gln)	3.5/1,764	None	None
			<i>JAK2</i> NM_004972	c.1849G>T (p.Val617Phe)	22.6/1,683	COSM12600	42,776
	F/79	Dementia	<i>DNMT3A</i> NM_022552	c.2478+1G>A	4.2/851	COSM4766079	1
	F/80	MCI	<i>CBL</i> NM_005188	c.1253T>C (p.Phe418Ser)	3.3/2,000	COSM34070	8
A β -negative	M/73	Dementia	<i>KMT2A</i> NM_001197104	c.2329T>G (p.Ser777Ala)	3.3/1,997	None	None
	F/80	MCI	<i>DNMT3A</i> NM_022552	c.1851+1G>A	4.4/1,477	COSM5878805	1
				c.2311C>T (p.Arg771Ter)	3.2/1,811	COSM231563	15

*Counts in the hematopoietic and lymphoid tissue category in COSMIC.

Abbreviations: A β , β -amyloid; VAF, variant allele frequency; CHIP, Clonal hematopoiesis of indeterminate potential; COSMIC, Catalogue of Somatic Mutations in Cancer; F, female; M, male; MCI, mild cognitive impairment; SCD, subjective cognitive decline.

off of 2% (6.5%–21%) [4, 18]. However, a previous study that utilized a VAF cut-off of 1% reported a higher prevalence of CHIP among Korean individuals (41%) [19]. The differences in CHIP prevalence may be attributed to not only the variant filtering strategy used but also heterogeneity in sample size, the sequencing method used, and the genes analyzed. Among the 11 CHIP variants, seven are reported in the Catalogue of Somatic Mutations in Cancer, and their occurrence in hematologic malignancy cases is variable (Table 2).

The prevalence of CHIP did not significantly increase with age, which is likely attributable to the uneven distribution of patients in age subgroups (Fig. 1A). *DNMT3A* mutations were the most commonly observed in both A β -positive and A β -negative groups (Fig. 1B), in line with findings in previous Korean reference studies [2, 18, 19]. Regarding the variant types, A β -positive patients tended to exhibit a higher prevalence of missense variants (62.5%) than of nonsense and splice site variants (33.3% each), although the difference was not statically significant ($P=0.546$, Fig. 1C). All CHIP variants detected were SNVs (Table 2), with the most frequent base changes being C:G>T:A in both A β -positive and A β -negative groups (Fig. 1D), which is consistent with findings by Savola, *et al.* [9], who studied CHIP in patients with rheumatoid arthritis. When DNA is replicated, the most frequent type of mutation that occurs involves C>T transversion, which is notably characteristic of mutations associated with aging [20]. Among the patients with CHIP, VAFs were not significantly differ-

ent between the A β -positive and A β -negative groups (Fig. 1E; median VAF, 4.45% vs. 3.83%, $P=0.361$). Finally, to evaluate the risk of brain A β deposition, multivariate logistic regression analysis was performed using age, sex, presence of CHIP, and presence of the *APOE* ϵ 4 allele as variables (Fig. 1F). Although CHIP was not associated with brain A β deposition (OR 0.85, 95% CI 0.13–7.29, $P=0.686$), the *APOE* ϵ 4 allele emerged as an independent risk factor for brain A β deposition (OR 6.58, 95% CI 1.70–33.80, $P=0.011$). Collectively, our results indicate that the characteristic patterns of CHIP in older patients with cognitive impairment reflect the aging of the hematopoietic system, characterized by C>T transitions and mutations in *DNMT3A*, a representative epigenetic modifier.

Our study had some limitations. First, this was a preliminary study investigating the relationship between CHIP and brain A β deposition; therefore, a power calculation for the sample size was not performed. Consequently, the statistical power may have been insufficient to detect the influence of CHIP on brain A β deposition. Second, the small sample size did not allow analysis of the association of CHIP with brain A β deposition according to gene, mutation type, and clone size. Third, the association between CHIP and brain A β deposition was studied cross-sectionally, and temporal causality was not evaluated.

In summary, older Korean patients with cognitive impairment had CHIP with typical age-related patterns, irrespective of the presence or absence of brain A β deposition, but CHIP was not

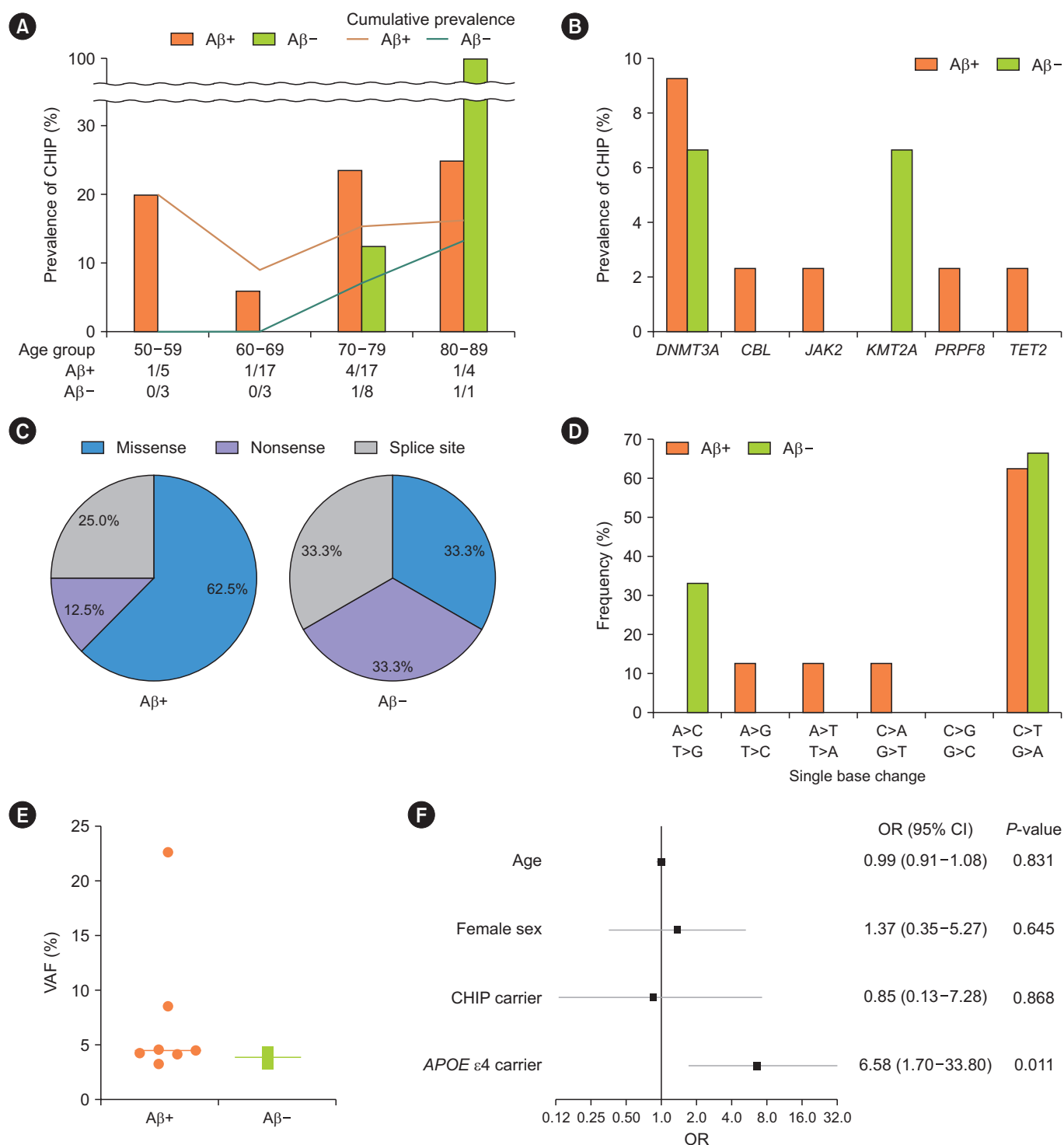


Fig. 1. Characteristics of CHIP variants in the A β -positive (N=43) and A β -negative (N=15) groups and forest plot of multivariate regression for the risk of brain A β deposition. (A) CHIP prevalence according to age. Bars indicate CHIP prevalence in different age groups. The line graph represents the cumulative prevalence of CHIP across the age groups. The absolute number of patients with CHIP and the total number of patients in each age group are displayed at the base of the graph. (B) CHIP prevalence according to gene. (C) Proportions of variant types in CHIP variants. (D) Percentages of different SNVs in CHIP variants. (E) VAFs of CHIP variants detected in the A β -positive and A β -negative groups. (F) Forest plot of multivariate logistic regression for the risk of brain A β deposition. Covariates included age, sex, presence of CHIP, and presence of the APOE ϵ 4 allele. Abbreviations: A β , β -amyloid; CHIP, clonal hematopoiesis of indeterminate potential; CI, confidence interval; OR, odds ratio; SNV, single-nucleotide variant; VAF, variant allele frequency.

found to be correlated with brain A β deposition. To our knowledge, this study was the first to investigate the relationship between CHIP and brain A β deposition in Korean patients using amyloid PET. However, our findings are preliminary, and a larger patient cohort is required to draw conclusive evidence. The protective effect of CHIP against AD may result from the migration of marrow-derived mutant cells into brain microglia by enhancing microglial phagocytosis [10]. Further research is required to understand the functional implications of CHIP on brain A β deposition, particularly in older individuals, as both CHIP and brain A β deposition are age-related phenomena.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via <https://doi.org/10.3343/alm.2024.0086>

ACKNOWLEDGEMENTS

None.

AUTHOR CONTRIBUTIONS

Kim HR contributed to the study conception and design, and reviewed and revised the manuscript. Yun J was responsible for investigating clinical data, analyzing the results, performing statistical analysis, and drafting the manuscript. Youn YC assisted in collecting clinical samples. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST

None declared.

RESEARCH FUNDING

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korea Government (MSIT) (NRF-2017R1A2B4011631 and 2021R1A2C2013359).

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