Association of B cell profile and receptor repertoire with the progression of Alzheimer's disease

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Article

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Association of B cell profile and receptor repertoire with the progression of Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is the most common type of dementia. Numerous reports have revealed that peripheral immune systems are linked to neuropathology; however, little is known about the contribution of B lymphocytes in AD. For this longitudinal study, one hundred-thirty-three participants were included in both baseline and follow-up 2nd year. Next, we performed next-generation sequencing (NGS)-based B cell receptor (BCR) repertoire profiling followed by pair-wise overlap analysis. The longitudinal change in the B lymphocytes population was associated with increased cerebral amyloid deposition. Furthermore, patients with AD shared highly similar class-switched BCR sequences with identical isotypes despite the high somatic hypermutation rate of BCR sequences. These commonalities of BCR repertoires of the patients with AD show the possibility of immunological stimuli by amyloid precursor protein (APP). Thus, we provide evidence for both quantitative and qualitative changes in B lymphocytes during AD pathogenesis. The genetic information of patients from the NGS-based BCR repertoire profiling can lead to the development of immune-based therapeutics and treatments for AD.

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Abbreviations: AD = Alzheimer's disease; APP = amyloid precursor protein; BCR = B cell receptor; NGS = next-generation sequencing; PBMC = peripheral blood mononuclear cells; PiB = Pittsburgh compound B; SUVR = standardized uptake value ratio

Introduction

Recent researches have shown that Alzheimer's disease (AD) phenotypes are no longer limited to the changes in the brain. There are increasing shreds of evidence that the peripheral immune systems are closely linked to AD pathology, especially critical roles of immune cells in the pathogenesis of AD ^{1, 2}. So far, however, there has been little discussion about the contribution of blood lymphocytes in AD. Only several papers have revealed the possible association of blood lymphocytes with the decline in cognitive function or accumulation of cerebral amyloid deposition in AD, but these studies have been conducted as a cross-sectional study using lymphocyte population data or failed to correlate specific lymphocyte characteristics with the pathology of AD ^{3, 4, 5}. Besides, although the latest technologies such as B cell receptor (BCR) or T cell receptor (TCR) next-generation sequencing (NGS) for their repertoire profiling have been developed ^{6, 7, 8}, few cases have been applied to the study for AD ⁹. Especially, no single study exists related to BCR repertoire analysis in AD. Therefore, this study aims to identify which type of lymphocytes is directly linked with brain amyloid deposition and discover characteristics of BCR repertoires shared among the patients with AD. We found that the population change in B lymphocyte over 2 years was significantly associated with the increase in cerebral A β deposition. Furthermore, NGS for B cell receptor followed by pair-wise overlap analysis revealed that the commonalities of BCR repertoires within AD patients but not within normal participants. We also suggest the possibility that the overlapped BCR sequences might be related to the immunological reaction to amyloid precursor protein (APP). This novel approach through both the longitudinal analysis and BCR repertoire profiling to AD provides a perspective of understanding the link between peripheral immune systems and neuropathology in the brain of AD patients.

Results

The longitudinal study revealed B lymphocyte population is associated with brain amyloid deposition in AD

Fig. 1A shows the overall strategy for flow cytometry analysis (**Supplementary Fig. 1**) followed by the longitudinal analysis for B lymphocyte population changes. For longitudinal

analyses, we sorted participants into three groups: PiB decrease group (PiB^{Dec}), PiB stable group (PiB^{Stable}), PiB increase group (PiB^{Inc}) based on the changes in PiB-PET SUVR for 2 years (**Fig. 1A, Table 1**). Stepwise multiple regression analysis showed that the population change in B lymphocyte over 2 years (ΔB) was significantly associated with the increase in brain A β (change of PiB standardized uptake value ratio; ΔPiB), but the changes in other lymphocytes (ΔT , ΔNK) were not (**Fig. 1B**). That was the decisive reason for us to start focusing on the profiling of B cells. Interestingly, PiB^{Inc} had a higher portion of participants with an increasing number of B lymphocytes (**Fig. 1C**), regardless of the baseline status of A β accumulation (**Fig. S2**). Furthermore, partial correlation and ANOVA post-hoc analysis showed that ΔB was positively correlated with ΔPiB (**Fig. 2A, left**) and ΔB quartile four (Q4) had the highest ΔPiB value ($Q1 < Q2 < Q3 < Q4$; **Fig. 2A, right**). Moreover, PiB^{Inc} had higher ΔB than PiB^{Dec} and PiB^{Stable} (**Fig. 2B**). Additional ROC curve analysis showed that ΔB significantly increases the discrimination power for the PiB increase as a variable for the logistic regression analysis (**Fig. 2C**). The combination of ApoE and ΔB variable had higher sensitivity (70.6%) and specificity (72.7%) with a 0.754 area under curve (AUC) than ApoE alone (0.710 AUC with 55.9% sensitivity and 76.8% specificity) (**Fig. 2D**). To do further validation, we classified participants depending on the ΔB quartiles (BQ; $BQ1 < BQ2 < BQ3 < BQ4$), and compare their ΔPiB . As a result, BQ4 and BQ2-3 groups had higher ΔPiB than BQ1 (**Fig. 2E**). These results led us to focus on B lymphocyte profiling for the following studies.

Pair-wised overlap analysis followed by NGS-based BCR repertoire analysis

For deep profiling of B lymphocytes in the BCR sequence level, we randomly selected representative 13 participants (3 Normal, 10 AD) with even distribution of apolipoprotein E allele frequencies (2 $\epsilon 3/\epsilon 3$ and 1 $\epsilon 3/\epsilon 4$ for Normal; 3 $\epsilon 3/\epsilon 3$, 4 $\epsilon 3/\epsilon 4$, 3 $\epsilon 4/\epsilon 4$ for AD), ages (73.6 ± 1.9 for Normal, 68.6 ± 2.2 for AD), and the number of total lymphocytes (30.7 ± 3.4 for Normal, 31.6 ± 2.6 for AD) that can interfere in the analysis for B lymphocyte^{4, 10, 11} (**Fig. 3A**). The cerebral amyloid deposition (standardized uptake value ratio, SUVR), mini-mental state exam (MMSE) cognitive score, plasma t-tau, and beta-amyloid 42/40 ratio were significantly different between normal and AD, but not age (**Fig. 3B-3C**). Next, we generated BCR repertoire data of these samples. In the whole BCR repertoire level, we discovered no distinctive traits shared among the AD samples, probably due to the chronic characteristics of

the diseases compared to non-chronic characteristics of diseases such as auto-immune or infectious diseases (**Supplementary Fig. 3 to 6**)¹². Thus, to discover characteristics shared among the AD samples, we focused our analysis on a subset of the whole BCR repertoire overlapped among the samples. Due to the limited number of BCR sequences shared among more than two patients, we conducted the overlap analysis in a pairwise manner. Overlapped BCR sequences were categorized into two groups, Normal&X (comparison between Normal and Normal or Normal and AD) and AD&AD (comparison between AD and AD) (**Fig. 3A, 3D left**), according to overlap pattern. We hypothesized that overlap between Normal and AD has an identical pattern with that between Normal and Normal because the two groups of samples do not share common immunological stimuli. Thus, we defined the two comparisons collectively as Normal&X and compared it with AD&AD. Interestingly, AD&AD had more numbers of the overlapped BCR sequences and lineages compared to Normal&X (**Fig. 3D, right**). However, in divergence and isotype composition, the two groups showed no significant difference (**Fig. 3E-3F**).

Pair-wised overlap analysis with highly similar overlapped BCR sequences revealed the commonalities of its repertoires within AD patients

To exclude coincidently defined as the overlapped BCR sequences and investigate overlapped BCR sequences in a stricter aspect, we calculated inter-sample full amino acid (AA) distance (shorter distance means higher similarity) of the overlapped BCR sequences¹³. We defined the overlapped BCR sequences by VJ gene usage (related to the mechanism of somatic recombination)¹⁴ and complementarity-determining region 3 (CDR3; the most variable portion of Ig molecules)¹⁵ AA sequence homology (> 70%) as previously reported^{13, 16}. Also, due to the limited resolution of the used criteria, BCR sequences with distinctive somatic hypermutation (SHM; a process diversifying BCRs through point-mutations into Ig genes) patterns¹⁷ were included in the overlapped BCR sequences. Thus, we quantified the degree of SHM sharing by defining the inter-sample full AA distance as the Hamming distance calculated in full AA level between the overlapped BCR sequences from the different samples¹⁸. AD&AD showed a shorter inter-sample full AA distance compared to Normal&X (**Fig. 4A, left**).

Then, the sub-population of the overlapped BCR sequences with small inter-sample full AA distance (< 5) was extracted and defined as highly similar overlapped BCR sequences. These highly similar overlapped BCR sequences can be generated in two ways. First, for naïve B lymphocytes, gene-rearrangement could generate coincidentally similar CDR3 with some bias inherent in the process. These sequences would have a very small number of SHMs and be in IgM isotype^{6, 19}. Second, for activated B lymphocytes, induction of somatic mutations by antigens can produce converged BCR sequences^{20, 21}. In this case, the sequences have high divergence values and are class-switched to isotypes other than IgM. These highly similar overlapped BCR sequences of AD&AD showed distinctive characteristics in the aspect of divergence and isotype composition compared to those of Normal&X. Among the sequences, sequences with a high divergence despite a small inter-sample full AA distance were only detected in AD&AD, which means mutation induction in the same direction resulting in identical residues for binding occurred only in AD&AD (**Fig. 4A, right**). Also, the number of Ig counts were significantly increased in AD&AD group (**Fig. 4B**). Furthermore, most of these sequences with high divergence values were class-switched to the isotypes other than IgM (**Fig. 4C**). A total of 579 sequences and 108 lineages was class-switched among the highly similar overlapped BCR sequences. Among 108 lineages, 92 lineages contained more than one class-switched BCR sequence in both repertoires with a distinctive VJ gene utilization compared to whole class-switched repertoires of the samples (**Fig. 4D** and **Supplementary Table 2-3**). Strikingly, in 91 out of 92 lineages, BCR sequences were represented with identical isotypes in two different repertoires, which means it is feasible for these sequences to result in the same effector functions. In one lineage with different isotypes, the sequences emerged as IgA1 and IgA2, respectively (**Fig. 4E**). These identical characteristics of the highly similar and class-switched overlapped sequences were also reproduced in a phylogenetic analysis by confirming that the sequences from different samples were clustered into the same sub-family with identical isotypes (**Supplementary Fig. 7**). We concluded there is a possible response to the stimulation from the same antigen^{22, 23}. In other words, the commonalities of BCR repertoires in the patients with AD implied the possibility of immunological stimuli by common antigens.

Estimation of target proteins through in silico mapping analysis

To estimate the target proteins of the overlapped BCR sequences, we conducted *in silico* mapping of the overlapped BCR sequences to the BCR sequences, of which 3D structure and

binding targets were defined. From the IMGT 3D structure database, we could confirm 39 unique BCR sequences and their 3D structure known to bind to AD-related proteins²⁴ (**Fig. 5**). Then, we mapped the 39 BCR sequences binding to the AD-related proteins to the overlapped BCR sequences identified in our analysis while increasing the allowed number of CDR3 amino acid mismatches during the mapping (**Fig. 5A**). For most of the AD-related protein-binding antibodies, which showed no significant match with the overlapped BCR sequences, there was no difference in mapping patterns between AD&AD and Normal&X. However, AD&AD and Normal&X showed different mapping patterns for two APP-binding antibodies with the simple CDR3 amino acids sequence of GDY (**Fig. 5B-5C, Supplementary Fig. 8-9**). A total of 82 overlapped BCR sequences with the perfectly matched CDR3 amino acid sequences were detected in AD&AD compared to the case of Normal&X where seven BCR sequences were detected. Also, class-switched overlapped BCR sequences were only detected in AD&AD (40 out of the 82 overlapped BCR sequences, 48.78%). This implies the possibility that these overlapped BCR sequences having the CDR3 amino acid sequence of GDY could be related to the immunological reaction to amyloid precursor protein (APP). **Fig. 6** shows a graphical summary of this study.

Discussion

This is the first investigation into the association of B cell and its receptor repertoire with AD. We provide clear evidence for both quantitative and qualitative changes of B cell during the progression of AD pathology. Although there have been several reports suggests that the links between the population of B lymphocyte and pathology of AD or clinical dementia symptoms, these researches just relied on the cross-sectional study and the results were quite controversial. For example, one prospective cohort study including 105 participants showed no differences in the percentage of B lymphocytes per total PBMC in the patients with AD compared to healthy controls but identified an increased number of B lymphocytes producing autoantibodies against beta-amyloid in AD²⁵. On the other hand, other cross-sectional studies found a decrease in B lymphocytes as well as T lymphocytes in the patients with AD^{4, 26, 27}. Even though this discrepancy has been questioned continuously, many studies entertain the concept that lymphocytic abnormality is accompanied by dementia, suggesting that the population of lymphocytes could be a biomarker for AD²⁸. A recent study identified the specific intracellular signaling markers, PLC γ 2 in stimulated PBMC subsets from AD patients through single cell quantification and machine learning model²⁹, however, the

relevance of specific cell type among PBMC to AD pathology has been still uncertain, especially B cell population. In the present study, our quantitative analyses revealed that the more increase in the number of B lymphocytes, the more brain A β deposition occurred. The difference from previous studies is that we investigated the direct relationship with the changes in brain beta-amyloid through our longitudinal analyses. We thought this approach could lead to additional findings that would be difficult to identify in the cross-sectional study, and we revealed the longitudinal association of B lymphocyte with the pathology of AD.

Through our longitudinal study, we realized that it is worth trying to closely monitor the characteristics of B lymphocytes themselves within AD patients. Furthermore, since many of the latest technologies such as unbiased next-generation TCR/BCR repertoire analysis or single-cell sequencing have been developed, we thought it would be interesting to apply these technologies to our cohorts. Thus, we performed NGS-based BCR repertoire analysis for the deep profiling of BCR repertoire, and consequently, our analyses for B lymphocytes in the BCR sequence level identified the commonalities of BCR repertoires within AD patients implying the possibility of immunological stimuli by the common antigens such as plasma beta-amyloid or tau proteins ^{22, 23}. Moreover, we found the possibility that the overlapped BCR sequences matched with the CDR3 amino acid sequence of GDY could be related to the immunological reaction to APP, which is known as the precursor molecule of beta-amyloid.

Our work has some limitations and suggestions for future study. First, the short length of CDR3 and the limited diversity the region could contribute to the prevalence of these BCR sequences. Stringent experimental methods have to be followed to prove the relevance of the mapped BCR sequences with the disease. Second, future works should concentrate to find out the cause of this phenomenon. The other concern is that we performed the BCR repertoire analysis with only 13 participants. A validation study with a larger sample size is needed to confirm these data. Alternatively, further research through the isolated human B lymphocyte culture or induced- pluripotent cell (iPSC) culture could help us find specific antigens stimulating B lymphocytes.

Our approach through both the longitudinal analysis and BCR repertoire profiling to AD suggest that the immunological response of peripheral B lymphocyte is clearly associated with neuropathology in the brain of AD patients. Furthermore, the genetic information of BCR obtained from this study using next-generation repertoire analysis techniques can lead to the development of various immune-based therapeutics and treatments for AD.

Methods

Recruitment of participants

Total 133 participants were included, and they are sorted into three groups for our longitudinal analysis: Pittsburgh compound B-positron emission tomography (PiB-PET; brain PET-imaging for the cerebral amyloid deposition) standardized uptake value ratio (SUVR) decrease group for 2 years (PiB^{Dec} , $n = 33$), PiB stable group ($\text{PiB}^{\text{Stable}}$, $n = 66$), and PiB increase group (PiB^{Inc} , $n = 34$) (**Table 1**). All of the subjects underwent brain imaging such as structural MRI and PiB-PET as well as psychological or clinical assessments according to the expert's guideline. The assessments corresponded to neuropsychological test based on the Korea Brain Aging Study for the Early diagnosis and prediction of AD (KBASE) protocol including neuropsychological battery (including Mini-Mental State Examination; MMSE), the Consortium to Establish a Registry for Alzheimer's Disease Assessment Packet (CERAD) and KBASE clinical assessments protocol (CERAD-K) ³⁰. MMSE z-score was calculated by the normalization of MMSE scores with the appropriate correction for age, gender, and education levels.

Neuroimaging

PiB-PET imaging and MR imaging were conducted using a simultaneous 3.0 T PET-MR scanner (Biograph mMR scanner; Siemens, Washington DC, USA) as previously reported manner ³¹. 555 MBq of ^{11}C -PiB were injected intravenously and PiB-PET emission scan was acquired and the images were analyzed ^{23, 32, 33, 34, 35}. Prominent ^{11}C -PiB retention in lateral temporal areas and brain regions has been described ³⁶ and the regions of interest (ROIs) were reported ³⁷. A standardized uptake value ratio (SUVR) of each ROI was obtained by normalizing the all voxels mean value of each ROI by the mean values of cerebellar uptake. Participants were divided as PiB-positive (PiB+) or PiB-negative (PiB-) by SUVR value based on the criteria 1.4 in at least one of the four ROIs ^{32, 34}.

Blood sampling, isolation of PBMCs and their storage

All blood samples were collected in the morning, using K2 EDTA tubes (BD Vacutainer Systems, Plymouth, UK) after overnight fasting. The EDTA tubes were immediately centrifuged and plasma supernatants were removed. The remaining blood was mixed with

PBS and transferred into Ficoll-Hypaque solution for isolation of peripheral blood mononuclear cells (PBMC) using gradient through repeating centrifugation and PBS washing. The collected PBMCs were immediately cryopreserved by using cell freezing buffer (90% fetal bovine serum + 10% DMSO) and stored for one week in the Mr.Frosty Freezing Container (Thermo Scientific; Waltham, MA, USA). After a week, the vials moved to the LN2 tank and stored at -192°C. The detailed information of used reagents for this study was summarized in **Table S1**.

Flow cytometry

Cryopreserved PBMCs have recovered in RPMI 1640 media and then used for flow cytometry (FACS) analysis. After the recovery process, HBSS based FACS buffer containing 2% FBS and 0.05% sodium-azide were used. For lymphocyte immunophenotyping, after excluding the pan-myeloid marker (CD33) positive population, remaining lymphocyte populations were identified as pan-T cell marker (CD3), pan-B cell marker (CD19), and pan-NK marker (CD16) positive populations, respectively. Used fluorescence conjugated antibody information are as indicated in the text: APC anti-human CD33 antibody (1:100, clone WM53), FITC anti-human CD3 antibody (1:100, clone OKT3), PE anti-human CD19 antibody (1:100, clone HIB19), and APC/Cy7 anti-human CD16 antibody (1:100, clone Ts1/18). For immunostaining, cells were passed through a 35 µm nylon mesh strainer, blocked for 15min on ice with Human TruStain FcX (5 µl/million cells in 100ul staining volume, BioLegend), and stained for 30min on ice with antibody mixture. DAPI staining (0.4 µg/ml) was formed to identify dead cells. Prepared cells were analyzed on a Fortessa X-20 (BD Biosciences) and the data were analyzed using FlowJo software (Tree Star). The detailed information of used reagents for this study was summarized in **Supplementary Table 1**.

Next-Generation Sequencing (NGS) library preparation

Cryopreserved CD19⁺ B lymphocytes were thawed and total RNA was extracted using the TRIzol Plus RNA Purification Kit (Life Technologies). 1 µg of total RNA was used as input for library preparation. Reverse transcription was performed according to the manufacturer's instructions using SuperScript IV reverse transcriptase (Life Technologies) and primers for five immunoglobulin heavy chain isotypes containing UMI (unique molecular identifiers) barcode consists of 14 random nt and partial Illumina adapters (**Supplementary Table 2**). Primer annealing was carried out at 72°C for 3 min then immediately placed on ice for 2 min.

The first-strand cDNA was purified using AmPure XP beads (Beckman Coulter) at a 1:1.8 ratio, then second-strand cDNA was synthesized using KAPA HiFi HotStart DNA polymerase (Kappa Bioscience) and a pool of 6 immunoglobulin heavy chain variable region-specific primers (**Supplementary Table 3**) (98°C for 4 min, 52°C for 1 min, 72°C for 5 min)^{19, 38}. Double-stranded cDNA was purified using AmPure XP beads (Beckman Coulter) at a 1:1 ratio, then amplified with KAPA HiFi HotStart DNA polymerase (Kappa Bioscience) using two primers containing Illumina adapters and index sequences (95°C for 3 min, 25 cycles of 95°C for 30 s, 65°C for 30 s, 72°C for 1 min, and 72°C for 5 min). Final NGS libraries were purified using AmPure XP beads (Beckman Coulter) at a 1:1 ratio, then submitted for a quality control procedure on TapeStation 2200 (Agilent Technologies). Libraries with a single peak of correct sequence length were subjected to NGS analysis using the MiSeq platforms (Illumina, Inc.).

Consensus sequence extraction from raw NGS reads

The raw NGS reads were q-filtered by the q20p95 condition, which means 95% nucleotides of the reads have to have higher Phred scores than 20. On average, 83.51% of the reads were passed the q-filtering condition. Then, the primer regions used in the experiments were extracted from the reads while allowing one mismatch (substitution, insertion, or deletion). Based on the location of the primer sequences, UMI sequences were extracted and the reads were clustered according to the UMI sequences. The reads in the same UMI clusters were aligned using a multiple sequence alignment tool, the Clustal Omega 1.2.4^{39, 40}. By using the nucleotides frequency information of the alignment results, the consensus sequence of the UMI clusters was extracted, then the read-count of the sequences was re-defined as the number of unique UMI sequences.

Isotype annotation and functionality filtering of the consensus sequences

The constant region of the consensus sequences was used for isotype annotation. The constant region sequences were recognized in a location-based manner, then the sequences were aligned to an in-house constant gene database constructed based on the IMGT (the international immunogenetics information system) database⁴¹. The isotype of the consensus sequences was determined by using the alignment results. Then, the V/D/J genes and

CDR1/2/3 regions of the sequences were extracted and annotated by the IgBLAST 1.8.0⁴². After the annotation, non-functional consensus sequences were filtered-out by the following condition. 1. Shorter sequence length than 250bp, 2. Existence of stop-codon or frame-shift in full AA sequences, 3. Annotation failure in more or equal to one of the CDR1/2/3 regions.

Divergence from germline V gene segments calculation

As the first step of the divergence calculation, the BCR sequences were aligned to germline variable gene segments by using IgBLAST⁴². Based on the IgBLAST alignment results, the aligned length of the sequences to the gene segments and the number of mutations within the aligned region were calculated. We defined the divergence value as the number of mutations divided by the aligned length as reported previously⁶

Pair-wise overlap analysis and inter-sample full AA distance calculation

Pair-wise overlap analysis was conducted at the BCR lineage level. To define BCR lineages, a previously reported hierarchical clustering method was used¹⁸. BCR lineages satisfying the following conditions from two different repertoire data were merged and defined as overlapped BCR lineages. 1. Same V/J gene usage, 2. Same CDR3 sequence length, 3. CDR3 AA sequence homology more than 0.7. In the above condition, CDR3 AA sequence homology was set as one minus CDR3 AA sequence discrepancy, which was calculated by minimum Hamming distance in CDR3 AA sequence between all the combinations of BCR sequences from two different BCR lineages divided by CDR3 AA sequence length. BCR sequences of the overlapped BCR lineages were defined as overlapped BCR sequences. To define inter-sample full AA distance, BCR sequences in the same overlapped BCR lineages were used. First, the full AA sequence of BCR sequences in the same overlapped BCR lineages was trimmed and aligned. Then, Hamming distance in the full AA sequence between all the combinations of BCR sequences from two different samples was calculated. Among the calculated Hamming distance values, the minimum value was set as an inter-sample full AA distance value of the overlapped BCR lineage.

In silico mapping of the overlapped BCR sequences to antibodies binding to AD-related proteins

First, we defined V_H sequences of the antibodies binding to AD-related proteins using the IMGT 3D structure database, which led to 39 unique V_H sequences⁴³. Then, we mapped the identified V_H sequences to the overlapped BCR sequences of our analysis based on the identity of CDR3 amino acid sequences. The overlapped BCR sequences having the same CDR3 length as that of the V_H sequence of antibodies binding to AD-related proteins were identified, and CDR3 mismatch was defined as the Hamming distance between their CDR3 amino acid sequences. The CDR3 amino acid mismatch threshold was defined as the number of allowed mismatches divided by the length of CDR3 amino acid sequences. The number of mapped V_H sequences was normalized by the number of V_H sequences with the same CDR3 length and the normalized values were defined as the mapping efficiency. This means that when the CDR3 amino acid mismatch threshold has a value of 1.0, mapping efficiency can only have a value of 0.0 or 1.0.

Statistical analyses

All statistical analyses were performed by GraphPad Prism 8 (San Diego, CA, USA) or Medcalc Software (Acaciaaan, Ostend, Belgium). Categorical data were analyzed using the chi-square test. Independent *t*-test and ANOVA post-hoc test were used for the comparison of numerical data. The outliers were excluded by the Grubb's test. The association between the values was determined by partial correlation analyses, with the correction for the several covariates. Multiple regression analysis was also performed. To test the discrimination power of variables, logistic regression followed by receiver operating characteristic (ROC) curve analysis was conducted. For comparison of numerical values used in the pair-wise overlap analysis, the Wilcoxon rank-sum test was conducted after the Shapiro Wilk test.

Ethical approval

This study was approved under the recommendations of the Institutional Review Board (IRB) of the Seoul National University Hospital (SNUH), South Korea. Written informed consent was applied to all the participants, corresponding to the Declaration of Helsinki. Protocols and manuals were also approved by the IRB of the SNUH.

Reporting Summary

Available soon.

Data availability

The sequencing data from this study are available at NCBI under SRA accession number PRJNA667860 (<https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA667860>). Also, all other data are available upon reasonable request.

Code availability

Non-applicable.

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Author contributions

J-C.P, J.N, S.J, S-H.H, S.K, and I.M-J conceptualized this study. J-C.P, J.N, K.K, H.C. D.L, D.Y, and A.C.L carried out experiments. J-C.P, J.N, S.J, H.L, and D.Y analyzed statistics. J.N, Y.L, and H.L analyzed the NGS data. J.C and D.Y.L provided the resources. J-C.P, J.N, S.J, and S-H.H wrote the original draft. S.K and I.M-J reviewed and edited the manuscript.

Completing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at online.

Figures and Tables

Figure 1. The strategy for longitudinal analysis (A) Timeline of the longitudinal study ($n = 133$). Brain imaging and isolation of peripheral mononuclear cell (PBMC) were performed at both baseline (BL) and 2nd year follow-up (FL) time-point. Classification of the PBMCs by the FACS analysis ($CD3^+$ for T lymphocytes, $CD19^+$ for B lymphocytes, and $CD16^+$ for NK lymphocytes). Participants were classified into three groups for the in-depth analyses (PiB increase group, PiB^{Inc} , getting worse for 2 years; PiB stable group, PiB^{Stable} , none the worse for 2 years; PiB possible decrease group, PiB^{Dec} , relatively getting better for 2 years). **(B)** Step-wise multiple regression analysis between the delta (Δ) lymphocytes (B, T, NK lymphocytes) and Δ global PiB deposition (standardized uptake value ratio; SUVR). Age and gender were also included as covariates. Only B lymphocyte is selected by the stepwise method. **(C)** B lymphocyte changes between baseline and follow-up 2nd year. PiB^{Inc} group has higher portion of B cell increased- participants than PiB^{Dec} and PiB^{Stable} group (Chi-trend test, $^{##}p < 0.01$; Chi-squared test, $^{**}p < 0.01$).

Figure 2. Longitudinal analysis and receiver operation curve (ROC) analysis for B lymphocyte profiling (A) Partial correlation analysis between Δ global PiB SUVR and Δ B lymphocytes ($***p < 0.001$, left) and ANOVA with post-hoc test between the quartiles of Δ B lymphocytes ($Q1 < Q2 < Q3 < Q4$) and Δ global PiB SUVR. (B) PiB^{Inc} group significantly showed less decrease in B cell population than PiB^{Dec} group for 2 years ($**p < 0.01$, ANOVA with Tukey's post-hoc test). (C-D) Biomarkers of B cell showed better discrimination power to PiB-progression when combined with APOE genotyping. $*p < 0.05$ by continuous net reclassification index (NRI). Interactive dot diagram showed sensitivity/specificity/cut-off (p_i values by Youden index, > 0.2706 , > 0.2516) values for PiB increase- ROC curves. (E) Participants were re-classified according to their quartiles of Δ B lymphocytes ($BQ1 < BQ2 < BQ3 < BQ4$) and divided into three groups (BQ1, BQ2-3, BQ4). Delta PiB-PET values were significantly increased in BQ2-3 and BQ4 than BQ1 ($***p < 0.001$; ANOVA with Tukey's post-hoc test). Two outliers were excluded by Grubb's test.

Figure 3. NGS-based BCR repertoire profiling followed by pair-wise BCR overlap analysis (A) B cell receptor (BCR) repertoire analysis (n = 13). Total of 13 participants (10 AD patients and 3 cognitively normals) were used for the BCR repertoire analysis. Overlapped BCR sequences were defined by pair-wise overlap analysis on BCR repertoire of the samples, which was followed by comparative analysis between Normal&X (comparison between Normal and Normal or Normal and AD; blue bidirectional arrow of the upper panel) and AD&AD (comparison between AD and AD; red bidirectional arrow of the upper panel) groups. **(B)** Characterization of samples subjected to BCR repertoire analysis. *p < 0.05 and **p < 0.01, independent *t*-test. **(C)** The changes in the levels of plasma t-tau and beta-amyloid 42/40 ratio. *p < 0.05, independent *t*-test. **(D)** The number of overlapped BCR sequences resulted from pair-wise overlap analysis. AD&AD had more overlapped BCR sequences than Normal&X. **p < 0.001; Wilcoxon rank-sum test. **(E)** Divergence of the overlapped BCR sequences. **(F)** Isotype composition of the overlapped BCR sequences.

Figure 4. NGS-based BCR repertoire profiling followed by pair-wise BCR overlap analysis (high similar overlapped sequences) (A) Inter-sample full amino acid (AA) distance of the overlapped BCR sequences. The averaged hamming distance between BCR sequences, which were overlapped and derived from different samples, was calculated using full AA sequences. This distance was named as inter-sample full AA distance. Among the overlapped BCR sequences, sequences with inter-sample full AA distance value smaller than 5 were defined as ‘highly similar overlapped BCR sequence’. Significantly increased divergence of highly similar overlapped BCR sequences in AD&AD group. **** $p < 0.0001$; Wilcoxon rank-sum test. (B) The number of Ig counts between Normal&X vs AD&AD. **** $p < 0.0001$; Man-Whitney test. (C) Isotype composition of the highly similar overlapped BCR sequences. Despite of a small inter-sample full AA distance, the sequences with a high divergence were only shown in AD&AD. (D) VJ gene usage of the class-switched and highly similar overlapped BCR sequences. (E) Isotype identity of the class-switched and highly similar overlapped BCR sequences. Matches in isotypes in different samples of the class-switched and highly similar overlapped BCR sequences were quantified. If the isotypes of sequences were identical in different two samples, they were counted as “identical”. And, if not, they were counted as "different". The isotype of the sequences was also represented by colors following the color coding used in (C).

Figure 5. In silico mapping of the overlapped BCR sequences to antibodies binding to AD-related genes (A) Experimental flow chart for *In silico* mapping analysis. The Immunogenetics information system (IMGT) database was used for the generation of antibody sequences (unique V_H sequences). **(B)** The mapping results from the comparison between the whole or highly shared V_H sequences and the unique V_H sequences. The APP-binding antibodies showed different mapping patterns between AD&AD and Normal&X. **(C)** The information of APP-specific V_H sequences from the IMGT database.

Figure 6. A graphical summary of this study From the quantitative and longitudinal analysis, we found that the population change in B lymphocyte over two years is associated with the increase in cerebral beta-amyloid deposition in AD. Furthermore, from the qualitative BCR repertoire analysis and *in-silico* mapping results, we identified that the commonalities of BCR repertoires within AD patients (Ig counts, Ig isotype composition, divergence) and suggested the possibility of the immunological reaction to amyloid precursor protein (APP). NGS, next-generation sequencing; Ab, antibody; Aβ, beta-amyloid; APP, amyloid precursor protein; Ig, immunoglobulin.

Table 1. Demographic information and details of ROC curve analysis

Characteristics	PiB ^{Dec} (33)	PiB ^{Stable} (66)	PiB ^{Inc} (34)	P-value
Gender, M/F (n)	14/19	30/36	15/19	P = 0.94
Age, years, mean $\pm$ SEM	73.97 $\pm$ 1.46	67.51 $\pm$ 0.83	72.79 $\pm$ 1.11	< 0.001*
Education, mean $\pm$ SEM	11.67 $\pm$ 0.73	11.27 $\pm$ 0.63	11.52 $\pm$ 0.95	P = 0.93
MMSE score, mean $\pm$ SEM	23.97 $\pm$ 0.82	25.83 $\pm$ 0.47	22.12 $\pm$ 0.85	< 0.001*
CN/MCI/Dementia	18/10/5	53/9/4	15/8/11	< 0.001 [†]
Δ PiB, mean $\pm$ SEM	-0.14 $\pm$ 0.02	0.02 $\pm$ 0.01	0.29 $\pm$ 0.04	< 0.001*
Δ B, mean $\pm$ SEM	-11.20 $\pm$ 2.01	-7.31 $\pm$ 1.06	-4.48 $\pm$ 1.36	< 0.05*
ApoE4 positivity, ϵ 4+/N (%)	14/33 (42.4%)	11/66 (15.7%)	14/34 (41.2%)	< 0.01 [†]

Abbreviations: CN, cognitively normal; MCI, mild cognitive impairment; ADD, Alzheimer's disease dementia; PiB, Pittsburgh compound B; SEM, Standard Error of Mean; n, number of participants; MMSE, Mini-Mental State Examination; ApoE, Apolipoprotein E; SUVR, standardized uptake value ratio; N, total number of participants. Δ PiB, the changes in cerebral amyloid deposition for 2 years; Δ B, the changes in B lymphocyte cell numbers for 2 years.

- *, significance by *t*-test; [†], significance by chi-squared test.

Supplementary information

Title: Association of B cell profile and receptor repertoire with the progression of Alzheimer's disease

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Supplementary information

Supplementary Fig. 1: The gating strategy for analysis; PBMC T, B, and NK lymphocytes population

Supplementary Fig. 2: The increase in B cell in PiB^{Inc} group

Supplementary Fig. 3: Isotype composition of BCR repertoire of the samples

Supplementary Fig. 4: Divergence of BCR repertoire of the samples

Supplementary Fig. 5: VJ gene usage of the whole BCR repertoire of the samples

Supplementary Fig. 6: VJ gene usage of the class-switched BCR repertoire of the samples

Supplementary Fig. 7: Phylogenetic analysis for the representative BCR lineages composed of the highly similar overlapped BCR sequences

Supplementary Fig. 8: In silico mapping results of 39 BCR sequences (whole sequences overlapped) binding to AD-related proteins

Supplementary Fig. 9: In silico mapping results of 39 BCR sequences (highly similar shared) binding to AD-related proteins

Supplementary Table 1: Reagents

Supplementary Table 2: Statistics for BCR repertoire NGS data

Supplementary Table 3: Class-switched and highly similar overlapped BCR lineages

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29 **Supplementary Fig. 1. The gating strategy for analysis; PBMC T, B, and NK lymphocytes**
 30 **population** Singlets were selected by forward scatter and side scatter. After excluding DAPI
 31 positive dead cells and pan-myeloid marker (CD33) positive population, remaining populations
 32 were identified as pan-T cell marker (CD3), pan-B cell marker (CD19), and pan-NK marker
 33 (CD16) positive populations.

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Supplementary Fig. 2. The increase in B cell in PiB^{Inc} group (A-B) PiB^{Inc} group has a higher portion of B cell increased- participants than PiB^{Dec} and PiB^{Stable} group both in PiB⁻ and PiB⁺. (C) Chi-squared test (#p < 0.10, *p < 0.05, **p < 0.01).

Supplementary Fig. 3. Isotype composition of BCR repertoire of the samples Isotype composition of BCR repertoire was calculated for each sample. Values were calculated in the number of unique BCR sequences.

Supplementary Fig. 4. Divergence of BCR repertoire of the samples Divergence values of the BCR sequences were calculated by each isotype. Isotypes were allocated on the x-axis according to the sequential order of class-switching events. Sample names were represented on the left top of the violin plots.

Supplementary Fig. 5-1. VJ gene usage of the whole BCR repertoire of the samples (Normal #1 to Normal #3) VJ gene usage of BCR repertoire of the sample was calculated in the number of unique BCR sequences and displayed as a heatmap. To minimize the effect of outliers, a threshold value of 0.05 was used. Sample names were represented on the left top of the heatmaps.

Supplementary Fig. 5-2. VJ gene usage of the whole BCR repertoire of the samples (AD #1 to AD #5) VJ gene usage of BCR repertoire of the sample was calculated in the number of unique BCR sequences and displayed as a heatmap. To minimize the effect of outliers, a threshold value of 0.05 for visualization was used. Sample names were represented on the left top of the heatmaps.

Supplementary Fig. 5-3. VJ gene usage of the whole BCR repertoire of the samples (AD #6 to AD #10) VJ gene usage of BCR repertoire of the sample was calculated in the number of unique BCR sequences and displayed as a heatmap. To minimize the effect of outliers, a threshold value of 0.05 was used. Sample names were represented on the left top of the heatmaps.

Supplementary Fig. 6-1. VJ gene usage of the class-switched BCR repertoire of the samples (Normal #1 to Normal #3) VJ gene usage of the class-switched BCR repertoire of the sample was calculated in the number of unique BCR sequences and displayed as a heatmap. The class-switched BCR repertoire means the collection of BCR sequences with isotypes other than IgM or IgD. Values were calculated within the defined class-switched population. To minimize the effect of outliers, a threshold value of 0.05 was used. Sample names were represented on the left top of the heatmaps.

Supplementary Fig. 6-2. VJ gene usage of the class-switched BCR repertoire of the samples (AD #1 to AD #5) VJ gene usage of the class-switched BCR repertoire of the sample was calculated in the number of unique BCR sequences and displayed as a heatmap. The class-switched BCR repertoire means the collection of BCR sequences with isotypes other than IgM or IgD. Values were calculated within the defined class-switched population. To minimize the effect of outliers, a threshold value of 0.05 was used. Sample names were represented on the left top of the heatmaps.

Supplementary Fig. 6-3. VJ gene usage of the class-switched BCR repertoire of the samples (AD #6 to AD #10) VJ gene usage of the class-switched BCR repertoire of the sample was calculated in the number of unique BCR sequences and displayed as a heatmap. The class-switched BCR repertoire means the collection of BCR sequences with isotypes other than IgM or IgD. Values were calculated within the defined class-switched population. To minimize the effect of outliers, a threshold value of 0.05 was used. Sample names were represented on the left top of the heatmaps.

A

B

Supplementary Fig. 7. Phylogenetic analysis for the representative BCR lineages composed of the highly similar overlapped BCR sequences Phylogenetic analysis was conducted for the representative BCR lineages composed of the highly similar overlapped BCR sequences. The BCR lineages were categorized into two groups, (A) IgM-major lineages and Class-switched lineages. The ID of BCR lineages and size of them were represented on the top of the phylogenetic trees. Isotypes of the sequences were denoted in the parenthesis of the sequence names. The green scale bars on the right bottom of the trees represent the inter-sequence distance calculated by the IgPhyML phylogenetic tree construction tool.

Supplementary Fig. 8-1. In silico mapping results of 39 BCR sequences (whole sequences overlapped) binding to AD-related proteins (related to Fig. 4).

Supplementary Fig. 8-2. In silico mapping results of 39 BCR sequences (whole sequences overlapped) binding to AD-related proteins (related to Fig. 4).

Supplementary Fig. 9-1. In silico mapping results of 39 BCR sequences (highly similar shared) binding to AD-related proteins (related to Fig. 4).

Supplementary Fig. 9-2. In silico mapping results of 39 BCR sequences (highly similar shared) binding to AD-related proteins (related to Fig. 4).

Supplementary Table 1. Reagents

Reagent	Supplier	Catalog number	etc.
BD K2 EDTA tubes	BD Bioscience	368861	
Mr. Frosty Freezing Container	Thermo Scientific	5100-0001	
FITC anti-human CD3 antibody	BioLegend	317306	Dilution, 1:100; Clone, OKT3
PE anti-human CD19 antibody	BioLegend	302208	Dilution, 1:100; Clone, HIB19
APC/Cy7 anti-human CD16 antibody	BioLegend	302018	Dilution, 1:100; Clone, 3G8
APC anti-human CD33 antibody	BioLegend	303408	Dilution, 1:100; Clone, WM53
Human TruStain FcX (Fc Receptor blocking solution)	BioLegend	422302	5 µm/million cells in 100 µl staining volume
RPMI1640 media	Gibco	11875-093	
FBS	Gibco	26140079	
HBSS media	WELGENE	LB003-02	
DAPI reagent	Sigma Aldrich	D9542	400 ng/ml
FACS tube	BD Bioscience	352052	
FACS tube strainer	BD Bioscience	352235	335 µm nylon mesh

134 **Supplementary Table 2. Statistics for BCR repertoire NGS data**

Identifier	UMI-processed read	Unique consensus sequence #	B cell lineage #
N01	292,035	37,048	30,112
N02	184,553	40,905	33,765
N03	266,446	50,090	43,203
AD01	160,743	51,240	46,161
AD02	465,866	265,763	255,041
AD03	319,869	87,427	78,126
AD04	548,559	129,448	106,345
AD05	319,839	112,895	104,610
AD06	345,679	103,515	94,390
AD07	683,273	309,252	279,808
AD08	738,650	246,287	205,783
AD09	382,745	83,242	61,505
AD10	456,861	135,060	96,408

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137 **Supplementary Table 3. Class-switched and highly similar overlapped BCR lineages**
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CDR3 AA	V gene	J gene	Sequence #	Divergence	Readcount	Isotype	Sample
QGVFGGDHEGPGDFFD	IGHV3-23	IGHJ4	1	0.17+-0.0	1.0+-0.0	G3	AD10
QGVFGGDHEGPGDFFD	IGHV3-23	IGHJ4	90	0.17+-0.01	33.99+-303.02	G3	AD07
AERRGTVRGVINHYHNFNGMEV	IGHV3-13	IGHJ6	1	0.16+-0.0	1.0+-0.0	A1	AD10
AERRGTVRGVINHSYYHNFNGMDV	IGHV3-13	IGHJ6	6	0.16+-0.0	17.0+-11.65	A1	AD07
QRRKIPVALPGSLATFDM	IGHV1-46	IGHJ3	1	0.13+-0.0	1.0+-0.0	A2	AD10
QRRKIPVALPGSLATFDM	IGHV1-46	IGHJ3	5	0.13+-0.0	29.8+-57.6	A2	AD07
HKMGTRSFYD	IGHV4-59	IGHJ4	1	0.09+-0.0	1.0+-0.0	A2	AD10
HKMGTRSFYD	IGHV4-59	IGHJ4	3	0.09+-0.0	41.33+-57.04	A2	AD07
GGSSWYIGRD	IGHV1-69	IGHJ4	1	0.05+-0.0	1.0+-0.0	G1	AD10
GGSSWYIGRD	IGHV1-69	IGHJ4	2	0.05+-0.0	16.0+-15.0	G1	AD07
LGMAARIIDY	IGHV4-31	IGHJ4	1	0.15+-0.0	1.0+-0.0	G1	AD10
LGMAARIIDY	IGHV4-31	IGHJ4	2	0.15+-0.0	86.0+-85.0	G1	AD07
RGNSAGWYYFDE	IGHV3-23	IGHJ4	1	0.12+-0.0	1.0+-0.0	A1	AD10
RGNSAGWYYFDE	IGHV3-23	IGHJ4	1	0.12+-0.0	9.0+-0.0	A1	AD07
DGCSSTWERNGFHP	IGHV4-39	IGHJ5	1	0.11+-0.0	1.0+-0.0	A1	AD10
DGCSSTWERNGFHP	IGHV4-39	IGHJ5	1	0.1+-0.0	16.0+-0.0	A1	AD07
EGYGYVLGIDY	IGHV4-4	IGHJ4	1	0.07+-0.0	1.0+-0.0	A1	AD10
EGYGYVLGIDY	IGHV4-4	IGHJ4	1	0.07+-0.0	13.0+-0.0	A1	AD07
DLDDSLEGGAMGV	IGHV3-7	IGHJ6	30	0.04+-0.0	74.33+-394.36	G1	AD06
DLDDSLEGGAMGV	IGHV3-7	IGHJ6	1	0.04+-0.0	3.0+-0.0	G1	AD04
GGWGDLSPDY	IGHV1-18	IGHJ4	1	0.09+-0.0	1.0+-0.0	G2	AD04
GGWGDLSPDY	IGHV1-18	IGHJ4	14	0.08+-0.01	9.79+-8.11	G2 A2	AD06
HWGSEHYDYSFDS	IGHV3-23	IGHJ4	9	0.11+-0.02	67.56+-187.89	G2	AD06
HWGSEHYDYSFDS	IGHV3-23	IGHJ4	1	0.11+-0.0	1.0+-0.0	G2	AD04
DFRWAFDL	IGHV6-1	IGHJ3	1	0.14+-0.0	1.0+-0.0	A2	AD04
DFRWAFDL	IGHV6-1	IGHJ3	11	0.14+-0.01	28.91+-33.3	G2 A2	AD06
ERQIWGASDY	IGHV3-23	IGHJ4	2	0.13+-0.01	1.0+-0.0	G1	AD04
ERQIWGASDY	IGHV3-23	IGHJ4	7	0.13+-0.01	19.29+-30.83	G1	AD06
DAFGRADS	IGHV3-74	IGHJ4	2	0.13+-0.0	1.0+-0.0	G2	AD04
DAFGRADS	IGHV3-74	IGHJ4	8	0.14+-0.0	4.88+-10.25	G2 A2	AD06
GWDEGYFYFYYMDV	IGHV3-23	IGHJ6	1	0.04+-0.0	1.0+-0.0	G2	AD04
GWDEGYFYFYYMDV	IGHV3-23	IGHJ6	5	0.05+-0.0	34.8+-67.6	G2	AD06
GSHISGTTKVLFDY	IGHV3-74	IGHJ4	1	0.02+-0.0	1.0+-0.0	G2	AD04
GSHISGTTKVLFDY	IGHV3-74	IGHJ4	4	0.02+-0.0	42.5+-71.88	G2	AD06
HGLYGGNGYHWHG	IGHV3-23	IGHJ4	1	0.1+-0.0	1.0+-0.0	G2	AD04
HGLYGGNGYHWHG	IGHV3-23	IGHJ4	4	0.1+-0.0	47.75+-80.97	G2	AD06
GSWVADI	IGHV3-7	IGHJ3	1	0.07+-0.0	1.0+-0.0	G2	AD04
GSWVADI	IGHV3-7	IGHJ3	4	0.08+-0.0	27.5+-45.9	G2	AD06
NTGRGTGLERPWDWFD	IGHV3-7	IGHJ5	1	0.09+-0.0	1.0+-0.0	G2	AD04
NTGRGTGLERPWDWFD	IGHV3-7	IGHJ5	4	0.09+-0.0	8.0+-9.46	G2	AD06
ALEYARGSNFYFGLDV	IGHV4-34	IGHJ6	1	0.12+-0.0	1.0+-0.0	A1	AD04
ALEYARGSNFYFGLDV	IGHV4-34	IGHJ6	3	0.12+-0.0	6.33+-7.54	A1	AD06
GRVLVRGSGYFDS	IGHV4-34	IGHJ4	1	0.13+-0.0	1.0+-0.0	G1	AD04
GRVLVRGSGYFDS	IGHV4-34	IGHJ4	3	0.13+-0.0	47.33+-65.53	G1	AD06
DDNYGTFTYYYGMDV	IGHV3-30	IGHJ6	1	0.13+-0.0	1.0+-0.0	A2	AD04
DDNYGTFTYYYGMDV	IGHV3-30	IGHJ6	3	0.13+-0.0	10.0+-12.03	A2	AD06
RNSSGPWAFDI	IGHV3-23	IGHJ3	1	0.09+-0.0	1.0+-0.0	A2	AD04
RNSSGPWAFDI	IGHV3-23	IGHJ3	2	0.1+-0.0	25.0+-24.0	A2	AD06
GGLIALDY	IGHV3-49	IGHJ4	1	0.06+-0.0	1.0+-0.0	G2	AD04
GGLIALDY	IGHV3-49	IGHJ4	3	0.06+-0.0	26.33+-35.83	G2	AD06
GINGYFDTSGSFYD	IGHV1-69	IGHJ4	1	0.09+-0.0	1.0+-0.0	G1	AD04
GINGYFDTSGSFYD	IGHV1-69	IGHJ4	3	0.09+-0.0	18.33+-24.51	G1	AD06
GLGYSSSS	IGHV3-74	IGHJ5	1	0.12+-0.0	1.0+-0.0	G2	AD04
GLGYSSSS	IGHV3-74	IGHJ5	4	0.11+-0.01	33.75+-56.72	G2 A2	AD06
GGDPDS	IGHV4-4	IGHJ5	1	0.05+-0.0	1.0+-0.0	A1	AD04
GGDPDS	IGHV4-4	IGHJ5	3	0.05+-0.0	8.67+-10.14	A1 A2	AD06
GEDSGSYIIDY	IGHV1-3	IGHJ4	1	0.1+-0.0	1.0+-0.0	G2	AD04
GEDSGSYIIDY	IGHV1-3	IGHJ4	2	0.1+-0.0	14.5+-13.5	G2	AD06
GKVRGVIKDY	IGHV4-4	IGHJ4	1	0.03+-0.0	1.0+-0.0	G2	AD04
GKVRGVIKDY	IGHV4-4	IGHJ4	2	0.03+-0.0	92.0+-91.0	G2	AD06
EYNDVVGSHLNHYMDV	IGHV1-8	IGHJ6	1	0.14+-0.0	1.0+-0.0	G1	AD04
EYNDVVGSHLNHYMDV	IGHV1-8	IGHJ6	2	0.14+-0.0	15.0+-14.0	G1	AD06
GYHDSGSGYRGL	IGHV4-4	IGHJ4	1	0.06+-0.0	1.0+-0.0	G1	AD04
GYHDSGSGYRGL	IGHV4-4	IGHJ4	2	0.06+-0.0	31.5+-30.5	G1	AD06
EKVNSGTFDY	IGHV1-3	IGHJ4	1	0.11+-0.0	1.0+-0.0	A1	AD04
EKVNSGTFDY	IGHV1-3	IGHJ4	1	0.11+-0.0	4.0+-0.0	A1	AD06
DGGMLHQNYRNV	IGHV3-23	IGHJ6	1	0.1+-0.0	1.0+-0.0	G2	AD04
DGGMLHQNYRNV	IGHV3-23	IGHJ6	2	0.11+-0.0	20.0+-19.0	G2	AD06
VLWNAGRLFDY	IGHV1-18	IGHJ4	1	0.06+-0.0	1.0+-0.0	A2	AD04
VLWNAGRLFDY	IGHV1-18	IGHJ4	2	0.06+-0.0	29.0+-28.0	A2	AD06
GGGYQLPPRGMDV	IGHV3-30	IGHJ6	1	0.0+-0.0	1.0+-0.0	G1	AD04
GGGYQLPPRGMDV	IGHV3-30	IGHJ6	2	0.0+-0.0	3.0+-0.0	G1	AD06
AGGFYGDILY	IGHV1-46	IGHJ4	2	0.19+-0.0	2.5+-1.5	A2	AD06
AGGFYGDILY	IGHV1-46	IGHJ4	1	0.19+-0.0	1.0+-0.0	A2	AD04
DPPQSGYSLDC	IGHV3-33	IGHJ4	1	0.05+-0.0	1.0+-0.0	A2	AD04
DPPQSGYSLDC	IGHV3-33	IGHJ4	2	0.05+-0.0	26.5+-1.5	A2	AD06

EMYSSSGTFTWYFAMDV	IGHV4-4	IGHJ6	1	0.09+-0.0	1.0+-0.0	G2	AD04
EMYSSSGTFTWYFAMDV	IGHV4-4	IGHJ6	2	0.09+-0.0	38.0+-37.0	G2	AD06
EYYYDSRGFKPTGFDS	IGHV3-30	IGHJ4	1	0.07+-0.0	1.0+-0.0	G2	AD04
EYYYDSRGFKPTGFDS	IGHV3-30	IGHJ4	2	0.07+-0.0	59.0+-58.0	G2	AD06
ESLHGSGSYIRAFDI	IGHV3-21	IGHJ3	1	0.03+-0.0	1.0+-0.0	G1	AD04
ESLHGSGSYIRAFDI	IGHV3-21	IGHJ3	2	0.04+-0.0	1.5+-0.5	G1	AD06
KGQGGLAHFDY	IGHV2-5	IGHJ4	1	0.04+-0.0	1.0+-0.0	G2	AD04
KGQGGLAHFDY	IGHV2-5	IGHJ4	2	0.04+-0.0	29.5+-28.5	G2	AD06
NPQARAASINWYFEL	IGHV3-30	IGHJ2	1	0.07+-0.0	1.0+-0.0	G2	AD04
NPQARAASINWYFEL	IGHV3-30	IGHJ2	2	0.07+-0.0	44.5+-43.5	G2	AD06
HTDHHVVVLGFSTVAAFDM	IGHV3-49	IGHJ3	1	0.16+-0.0	1.0+-0.0	G2	AD04
HTDHHVVVLGFSTVAAFDM	IGHV3-49	IGHJ3	2	0.16+-0.0	3.0+-1.0	G2	AD06
GPVVVGPLDP	IGHV3-13	IGHJ5	1	0.09+-0.0	1.0+-0.0	G2	AD04
GPVVVGPLDP	IGHV3-13	IGHJ5	2	0.1+-0.0	54.5+-53.5	G2	AD06
SQLLIGSGGWELDS	IGHV3-30-3	IGHJ5	1	0.16+-0.0	1.0+-0.0	A2	AD04
SQLLIGSGGWELDS	IGHV3-30-3	IGHJ5	2	0.16+-0.0	6.5+-1.5	A2	AD06
AKYTYSPGIHFDY	IGHV4-59	IGHJ4	1	0.07+-0.0	1.0+-0.0	G1	AD04
AKYTYSPGIHFDY	IGHV4-59	IGHJ4	1	0.07+-0.0	31.0+-0.0	G1	AD06
MTVGNWFDP	IGHV4-39	IGHJ5	1	0.1+-0.0	1.0+-0.0	A2	AD04
MTVGNWFDP	IGHV4-39	IGHJ5	1	0.1+-0.0	14.0+-0.0	A2	AD06
DQAARVPWYFDH	IGHV1-18	IGHJ4	1	0.1+-0.0	1.0+-0.0	A1	AD04
DQAARVPWYFDH	IGHV1-18	IGHJ4	1	0.1+-0.0	8.0+-0.0	A1	AD06
APKGAGGSY	IGHV3-7	IGHJ4	1	0.04+-0.0	1.0+-0.0	G2	AD04
APKGAGGSY	IGHV3-7	IGHJ4	1	0.04+-0.0	47.0+-0.0	G2	AD06
ASARTYTAYGVALSHMDV	IGHV3-23	IGHJ6	1	0.14+-0.0	1.0+-0.0	A1	AD04
ASARTYTAYGVALSHMDV	IGHV3-23	IGHJ6	1	0.14+-0.0	13.0+-0.0	A1	AD06
SPYYASGGSTFFEY	IGHV3-23	IGHJ4	1	0.13+-0.0	1.0+-0.0	A2	AD04
SPYYASGGSTFFEY	IGHV3-23	IGHJ4	1	0.13+-0.0	2.0+-0.0	A2	AD06
DLPVGFSSAY	IGHV1-46	IGHJ4	1	0.15+-0.0	1.0+-0.0	G2	AD04
DLPVGFSSAY	IGHV1-46	IGHJ4	1	0.15+-0.0	5.0+-0.0	G2	AD06
FEGMESSAYYFDS	IGHV3-23	IGHJ4	1	0.09+-0.0	1.0+-0.0	G2	AD04
FEGMESSAYYFDS	IGHV3-23	IGHJ4	1	0.09+-0.0	46.0+-0.0	G2	AD06
QRAAGYYYYYMDV	IGHV3-23	IGHJ6	1	0.0+-0.0	1.0+-0.0	G1	AD04
QRAAGYYYYYMDV	IGHV3-23	IGHJ6	2	0.0+-0.0	31.0+-0.0	G1	AD06
DRAGRKAPRSLYFDY	IGHV3-11	IGHJ4	1	0.07+-0.0	1.0+-0.0	G1	AD04
DRAGRKAPRSLYFDY	IGHV3-11	IGHJ4	1	0.07+-0.0	51.0+-0.0	G1	AD06
DRMFFDS	IGHV3-23	IGHJ4	1	0.15+-0.0	1.0+-0.0	G1	AD04
DRMFFDS	IGHV3-23	IGHJ4	1	0.15+-0.0	23.0+-0.0	G1	AD06
HRGPPQEWPPKYWFDP	IGHV4-39	IGHJ5	1	0.13+-0.0	1.0+-0.0	A1	AD04
HRGPPQEWPPKYWFDP	IGHV4-39	IGHJ5	1	0.13+-0.0	4.0+-0.0	A1	AD06
HLRRYTYGYFDF	IGHV4-39	IGHJ4	1	0.09+-0.0	1.0+-0.0	A1	AD04
HLRRYTYGYFDF	IGHV4-39	IGHJ4	1	0.09+-0.0	10.0+-0.0	A1	AD06
DGAAYVHYHYGMDV	IGHV3-49	IGHJ6	1	0.03+-0.0	1.0+-0.0	G2	AD04
DGAAYVHYHYGMDV	IGHV3-49	IGHJ6	1	0.03+-0.0	36.0+-0.0	G2	AD06
SLVAVQLPHYYYAMDV	IGHV3-53	IGHJ6	1	0.1+-0.0	1.0+-0.0	G1	AD04
SLVAVQLPHYYYAMDV	IGHV3-53	IGHJ6	1	0.1+-0.0	52.0+-0.0	G1	AD06
GQDQYKTGNY	IGHV4-34	IGHJ4	1	0.09+-0.0	1.0+-0.0	A2	AD04
GQDQYKTGNY	IGHV4-34	IGHJ4	1	0.09+-0.0	17.0+-0.0	A2	AD06
SWTATSGYCNWFDS	IGHV2-5	IGHJ5	1	0.08+-0.0	1.0+-0.0	A2	AD04
SWTATSGYCNWFDS	IGHV2-5	IGHJ5	1	0.08+-0.0	3.0+-0.0	A2	AD06
GLFCIDGDCFSSWFDS	IGHV3-48	IGHJ5	1	0.19+-0.0	1.0+-0.0	A1	AD04
GLFCIDGDCFSSWFDS	IGHV3-48	IGHJ5	1	0.19+-0.0	5.0+-0.0	A1	AD06
ALFGPGTSTFDY	IGHV1-24	IGHJ4	1	0.08+-0.0	1.0+-0.0	A2	AD04
ALFGPGTSTFDY	IGHV1-24	IGHJ4	1	0.08+-0.0	10.0+-0.0	A2	AD06
EYFGVADH	IGHV3-49	IGHJ4	1	0.11+-0.0	1.0+-0.0	G2	AD04
EYFGVADH	IGHV3-49	IGHJ4	1	0.11+-0.0	15.0+-0.0	G2	AD06
VPRAFSAPYYYYGMDV	IGHV1-46	IGHJ6	1	0.05+-0.0	1.0+-0.0	G2	AD04
VPRAFSAPYYYYGMDV	IGHV1-46	IGHJ6	1	0.05+-0.0	31.0+-0.0	G2	AD06
WNSGSCSSF	IGHV3-49	IGHJ4	1	0.08+-0.0	1.0+-0.0	G2	AD04
WNSGSCSSF	IGHV3-49	IGHJ4	1	0.08+-0.0	31.0+-0.0	G2	AD06
DWEYHLQD	IGHV4-4	IGHJ4	1	0.06+-0.0	1.0+-0.0	G2	AD04
DWEYHLQD	IGHV4-4	IGHJ4	1	0.06+-0.0	36.0+-0.0	G2	AD06
DSTRAYSHDYLRGWFDP	IGHV3-23	IGHJ5	1	0.12+-0.0	1.0+-0.0	G1	AD04
DSTRAYSHDYLRGWFDP	IGHV3-23	IGHJ5	1	0.12+-0.0	2.0+-0.0	G1	AD06
EHCGGGGCYSLDY	IGHV3-74	IGHJ4	1	0.03+-0.0	1.0+-0.0	A2	AD04
EHCGGGGCYSLDY	IGHV3-74	IGHJ4	1	0.03+-0.0	15.0+-0.0	A2	AD06
YSSEHFYGLDV	IGHV1-69	IGHJ6	1	0.05+-0.0	1.0+-0.0	G1	AD04
YSSEHFYGLDV	IGHV1-69	IGHJ6	1	0.05+-0.0	6.0+-0.0	G1	AD06
GGHYFGADV	IGHV1-3	IGHJ6	1	0.07+-0.0	1.0+-0.0	A2	AD04
GGHYFGADV	IGHV1-3	IGHJ6	1	0.07+-0.0	4.0+-0.0	A2	AD06
HTGSSLRIDY	IGHV1-69	IGHJ4	1	0.11+-0.0	1.0+-0.0	G1	AD04
HTGSSLRIDY	IGHV1-69	IGHJ4	1	0.11+-0.0	17.0+-0.0	G1	AD06
GHLVDFWSGPLFDY	IGHV1-24	IGHJ4	1	0.05+-0.0	1.0+-0.0	G1	AD04
GHLVDFWSGPLFDY	IGHV1-24	IGHJ4	1	0.05+-0.0	34.0+-0.0	G1	AD06
LRYCSMTRCYRVDY	IGHV1-24	IGHJ4	1	0.15+-0.0	1.0+-0.0	G2	AD04
LRYCSMTRCYRVDY	IGHV1-24	IGHJ4	1	0.15+-0.0	4.0+-0.0	G2	AD06
DLSGEASLDGR	IGHV3-72	IGHJ3	1	0.11+-0.0	1.0+-0.0	G2	AD04
DLSGEASLDGR	IGHV3-72	IGHJ3	1	0.11+-0.0	46.0+-0.0	G2	AD06
LDYSHGSTYSVPPV	IGHV3-64	IGHJ6	1	0.11+-0.0	1.0+-0.0	G1	AD04
LDYSHGSTYSVPPV	IGHV3-64	IGHJ6	1	0.11+-0.0	29.0+-0.0	G1	AD06
QVLGEADYSDSVGSYYVDYFDS	IGHV4-39	IGHJ4	1	0.09+-0.0	1.0+-0.0	G1	AD04

QVLGEADYSDSVGSYYVDYFDS	IGHV4-39	IGHJ4	1	0.09+-0.0	54.0+-0.0	G1	AD06
DGVPSSSSPLMDV	IGHV7-4-1	IGHJ6	1	0.01+-0.0	1.0+-0.0	A2	AD04
DGVPSSSSPLMDV	IGHV7-4-1	IGHJ6	1	0.01+-0.0	7.0+-0.0	A2	AD06
EGDYGGNIYFQH	IGHV1-18	IGHJ1	1	0.14+-0.0	1.0+-0.0	A2	AD04
EGDYGGNIYFQH	IGHV1-18	IGHJ1	1	0.14+-0.0	15.0+-0.0	A2	AD06
FDM	IGHV3-7	IGHJ3	1	0.13+-0.0	1.0+-0.0	A2	AD10
FDM	IGHV3-7	IGHJ3	1	0.13+-0.0	2.0+-0.0	A2	AD09
DRRFGELSVSTMDV	IGHV3-74	IGHJ6	1	0.11+-0.0	1.0+-0.0	G2	AD09
DRRFGELSVSTMDV	IGHV3-74	IGHJ6	11	0.11+-0.01	1.55+-0.78	G2 A2	AD08
SWYRLESPKFDF	IGHV4-34	IGHJ4	1	0.16+-0.0	1.0+-0.0	A1	AD04
SWYRLESPKFDF	IGHV4-34	IGHJ4	144	0.16+-0.02	80.28+-822.47	A1	AD03
VSRMEARDY	IGHV3-7	IGHJ4	1	0.07+-0.0	1.0+-0.0	G2	AD04
VSRMEARDY	IGHV3-7	IGHJ4	2	0.08+-0.0	4.5+-3.5	G2	AD03
RAGGHSSGYHYFFDK	IGHV4-59	IGHJ4	1	0.12+-0.0	1.0+-0.0	A2	AD04
RAGGHSSGYHYFFDK	IGHV4-59	IGHJ4	2	0.11+-0.01	36.0+-21.0	A2	AD03
DLATLEWLLSN	IGHV3-23	IGHJ4	1	0.09+-0.0	1.0+-0.0	G1	AD04
DLATLEWLLSN	IGHV3-23	IGHJ4	1	0.09+-0.0	20.0+-0.0	G1	AD03
SADYYHYNYNGMDV	IGHV3-33	IGHJ6	1	0.13+-0.0	1.0+-0.0	A1	AD01
SADYYHYNYNGMDV	IGHV3-33	IGHJ6	5	0.13+-0.0	9.2+-16.4	A1	AD05
GGYGLADEFDY	IGHV4-4	IGHJ4	1	0.08+-0.0	1.0+-0.0	A1	AD01
GGYGLADEFDY	IGHV4-4	IGHJ4	2	0.08+-0.0	8.5+-7.5	A1	AD05
HAGHLGLVLDI	IGHV3-7	IGHJ3	1	0.1+-0.0	1.0+-0.0	G2	AD01
HAGHLGLVLDI	IGHV3-7	IGHJ3	2	0.09+-0.0	19.0+-18.0	G2	AD05
RYCSGGSCYSGIDY	IGHV3-11	IGHJ4	1	0.01+-0.0	1.0+-0.0	A2	AD09
RYCSGGSCYSGIDY	IGHV3-11	IGHJ4	1	0.0+-0.0	12.0+-0.0	A2	AD04
DGGRVVGNLPGY	IGHV3-7	IGHJ4	1	0.03+-0.0	1.0+-0.0	A1	AD04
DGGRVVGNLPGY	IGHV3-7	IGHJ4	1	0.07+-0.0	9.0+-0.0	A2	AD06

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Figures

Figure 1

The strategy for longitudinal analysis (A) Timeline of the longitudinal study (n = 133). Brain imaging and isolation of peripheral mononuclear cell (PBMC) were performed at both baseline (BL) and 2nd year follow-up (FL) time-point. Classification of the PBMCs by the FACS analysis (CD3+ for T lymphocytes, CD19+ for B lymphocytes, and CD16+ for NK lymphocytes). Participants were classified into three groups for the in-depth analyses (PiB increase group, PiBInc, getting worse for 2 years; PiB stable group, PiBStable, none the worse for 2 years; PiB possible decrease group, PiBDec, relatively getting better for 2 years). (B) Step-wise multiple regression analysis between the delta (Δ) lymphocytes (B, T, NK lymphocytes) and Δ global PiB deposition (standardized uptake value ratio; SUVR). Age and gender were also included as covariates. Only B lymphocyte is selected by the stepwise method. (C) B lymphocyte changes between baseline and follow-up 2nd year. PiBInc group has higher portion of B cell increased-participants than PiBDec and PiBStable group (Chi-trend test, $##p < 0.01$; Chi-squared test, $**p < 0.01$).

Figure 2

Longitudinal analysis and receiver operation curve (ROC) analysis for B lymphocyte profiling (A) Partial correlation analysis between Δ global PiB SUVR and Δ B lymphocytes ($***p < 0.001$, left) and ANOVA with post-hoc test between the quartiles of Δ B lymphocytes ($Q1 < Q2 < Q3 < Q4$) and Δ global PiB SUVR. (B) PiB-Inc group significantly showed less decrease in B cell population than PiB-Dec group for 2 years ($**p < 0.01$, ANOVA with Tukey's post-hoc test). (C-D) Biomarkers of B cell showed better discrimination power to PiB-progression when combined with APOE genotyping. $*p < 0.05$ by continuous net reclassification index (NRI). Interactive dot diagram showed sensitivity/specificity/cut-off (pi values by Youden index, > 0.2706 , > 0.2516) values for PiB increase-ROC curves. (E) Participants were re-classified according to their quartiles of Δ B lymphocytes ($BQ1 < BQ2 < BQ3 < BQ4$) and divided into three groups (BQ1, BQ2-3, BQ4). Delta PiB-PET values were significantly increased in BQ2-3 and BQ4 than BQ1 ($***p < 0.001$; ANOVA with Tukey's post-hoc test). Two outliers were excluded by Grubb's test.

Figure 3

NGS-based BCR repertoire profiling followed by pair-wise BCR overlap analysis (A) B cell receptor (BCR) repertoire analysis (n = 13). Total of 13 participants (10 AD patients and 3 cognitively normals) were used for the BCR repertoire analysis. Overlapped BCR sequences were defined by pair-wise overlap analysis on BCR repertoire of the samples, which was followed by comparative analysis between Normal&X (comparison between Normal and Normal or Normal and AD; blue bidirectional arrow of the upper panel) and AD&AD (comparison between AD and AD; red bidirectional arrow of the upper panel) groups. (B) Characterization of samples subjected to BCR repertoire analysis. * $p < 0.05$ and ** $p < 0.01$, independent t-test. (C) The changes in the levels of plasma t-tau and beta-amyloid 42/40 ratio. * $p < 0.05$, independent t-test. (D) The number of overlapped BCR sequences resulted from pair-wise overlap analysis. AD&AD had more overlapped BCR sequences than Normal&X. ** $p < 0.001$; Wilcoxon rank-sum test. (E) Divergence of the overlapped BCR sequences. (F) Isotype composition of the overlapped BCR sequences.

Figure 4

NGS-based BCR repertoire profiling followed by pair-wise BCR overlap analysis (high similar overlapped sequences) (A) Inter-sample full amino acid (AA) distance of the overlapped BCR sequences. The averaged hamming distance between BCR sequences, which were overlapped and derived from different samples, was calculated using full AA sequences. This distance was named as inter-sample full AA distance. Among the overlapped BCR sequences, sequences with inter-sample full AA distance value smaller than 5 were defined as 'highly similar overlapped BCR sequence'. Significantly increased divergence of highly similar overlapped BCR sequences in AD&AD group. **** $p < 0.0001$; Wilcoxon rank-sum test. (B) The number of Ig counts between Normal&X vs AD&AD. **** $p < 0.0001$; Man-Whitney test. (C) Isotype composition of the highly similar overlapped BCR sequences. Despite of a small inter-sample full AA distance, the sequences with a high divergence were only shown in AD&AD. (D) VJ gene usage of the class-switched and highly similar overlapped BCR sequences. (E) Isotype identity of the class-switched and highly similar overlapped BCR sequences. Matches in isotypes in different samples of the class-switched and highly similar overlapped BCR sequences were quantified. If the isotypes of

sequences were identical in different two samples, they were counted as “identical”. And, if not, they were counted as “different”. The isotype of the sequences was also represented by colors following the color coding used in (C).

Figure 5

In silico mapping of the overlapped BCR sequences to antibodies binding to AD-related genes (A)

Experimental flow chart for In silico mapping analysis. The Immunogenetics information system (IMGT)

database was used for the generation of antibody sequences (unique VH sequences). (B) The mapping results from the comparison between the whole or highly shared VH sequences and the unique VH sequences. The APP-binding antibodies showed different mapping patterns between AD&AD and Normal&X. (C) The information of APP-specific VH sequences from the IMGT database.

Figure 6

A graphical summary of this study From the quantitative and longitudinal analysis, we found that the population change in B lymphocyte over two years is associated with the increase in cerebral beta-amyloid deposition in AD. Furthermore, from the qualitative BCR repertoire analysis and in-silico mapping results, we identified that the commonalities of BCR repertoires within AD patients (Ig counts, Ig isotype composition, divergence) and suggested the possibility of the immunological reaction to amyloid precursor protein (APP). NGS, next-generation sequencing; Ab, antibody; A β , beta-amyloid; APP, amyloid precursor protein; Ig, immunoglobulin.