Supplementary Material

Vaccination Against Pneumonia May Provide Genotype-Specific Protection Against Alzheimer's Disease (Ukraintseva et al., JAD, 2023)

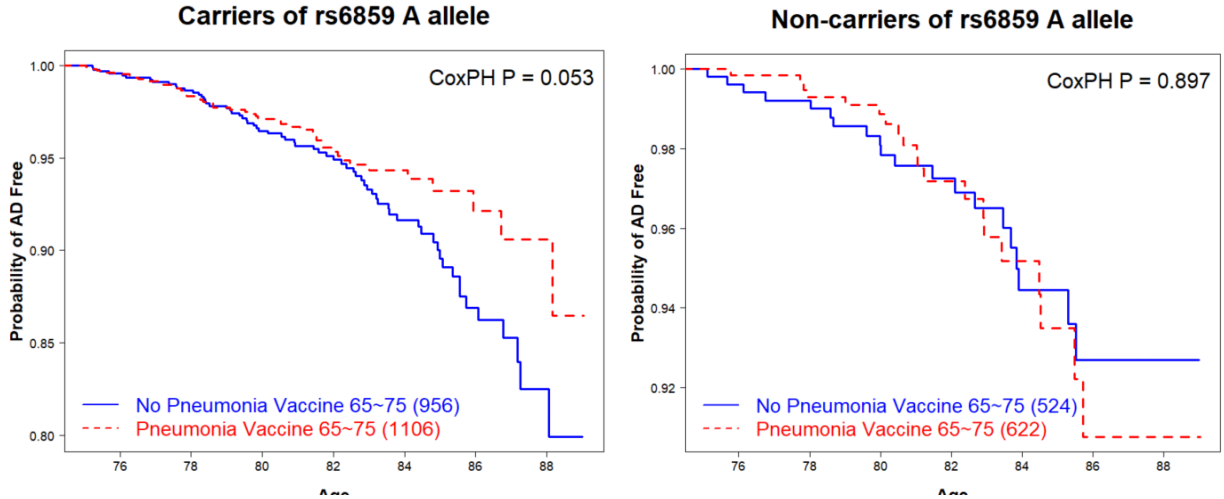
Data

Version 7 of Cardiovascular Health Study (CHS) data provided by the Database of Genotypes and Phenotypes (dbGaP) resource (dbGaP study accession # phs000287) of the National Center for Biotechnology Information (NCBI) was used in this study [1]. All study participants were 65 years or older, and recruited from Forsyth County, NC; Sacramento County, CA; Washington County, MD; and Pittsburgh, PA. 5,201 participants were enrolled in 1989-1990, as original cohort, and additional 687, predominantly African-American, participants were enrolled in 1992-1993. Annual clinical examinations were performed through 1999, and twice-yearly telephone contacts were done for another 20 years (2000-present), with an additional clinic examination in 2005–2006. Genetic information was retrieved from dbGaP CHS sub-study, the Candidate Gene Association Resource (CARe), data (dbGaP accession # phs000377). The CARe data were genotyped on the Illumina ITMAT-Broad-CARe (IBC) chip [2] covering ~49K SNPs in ~2,100 candidate health-related genes.

Among 5,599 (95.09%) CHS participants who consented to health/medical/biomedical study purposes, 5,146 (91.91%) had a lifespan longer than 75 years. Of those, all who had available information about AD, vaccinations, and covariates, were included in the study (see also Table 1 in main text). The proportion of people excluded due to mortality before the age 75 did not differ between the pneumococcal polysaccharide vaccine (PPSV23) exposure subgroups (p=0.45), and between the rs6859 genotype strata (p=0.81). Discharge summary and diagnoses for all hospitalizations were obtained. For each hospitalization, there were up to ten ICD-9-CM discharge codes. The ICD-9-CM code 331.0 was used to define the occurrence of AD. CHS participants who did not have this code at discharge were considered AD free. Patients with AD onset before age 75 were excluded from the analysis sample. The AD diagnosis was based on occurrence of at least one record containing the ICD-9-CM code 331.0. As we discussed in our earlier publications [3, 4], definitions based on occurrence of two distinct claims might provide somewhat more reliable definitions, however, the sample size is a limiting factor. We did not

consider other dementias or mild cognitive impairment in this analysis. Individual histories of pneumonia vaccination and flu shots between ages 65 and 75 were obtained from CHS questionnaires for respective years. Information about history of vaccination against pneumonia (PPSV23), and information about flu shots in the past year, were available from year 2 through year 11.

Kaplan-Meier survival trajectories of the probability of staying AD-free at ages 75 and older, following vaccination against pneumonia between ages 65-75 in carriers and non-carriers of rs6859 A allele are shown in Supplementary Figure 1. This figure graphically illustrates the genotype-specific effect of vaccination against pneumonia on AD onset. The maximum follow-up time after the age 75 was about 29 years, with average follow-up about 9 years. P-value on the graphs was calculated from the Cox proportional hazards (CoxPH) model, using R package *survival*. In that model, we used age at onset of AD, or age at last follow up (if AD did not occur), as time variable; AD onset at age ≥75 years (coded as 1), or no AD onset at age ≥75 years (coded as 0) as event variable; and adjustments for sex, race, and birth cohort, to avoid potential confounding. The proportionality of hazards assumption was checked using R package *survival*, function cox.zph(), which used Schoenfeld residuals against the transformed time. The trajectories on Supplementary Figure 1 additionally support the association of pneumonia vaccination with AD onset in carriers of the rs6859 A allele, but not in non-carriers.



A **B**

Supplementary Figure 1. Trajectories of probability of staying AD-free at ages 75 and older, following vaccination against pneumonia between ages 65 and 75 in two CHS subsamples: A) rs6859 A allele carriers; B) rs6859 A allele non-carriers.

Sensitivity analysis.

We also computed E-value estimates to quantify the robustness of estimates to unmeasured confounding for those estimates that showed marginal significance. We used the R-package *EValue* (function evalues.OR() with option "rare=FALSE" to adjust for non-rare outcomes). For carriers of rs6859, E-values were 1.74 for pneumonia vaccine, and 1.23 for total number of pneumonia and flu shots. In unstratified analysis, E-values were 1.22 for number of flu shots, and 1.20 for total number of pneumonia and flu shots. The estimated E-values suggest that the observed association of pneumonia vaccine with AD is likely to be robust to residual confounding, as the residual confounding needs to be large enough to rule out this association. Meanwhile, for all other E-values computed, the observed associations were more likely to be influenced by an unaccounted confounding variable.

REFERENCES

[1] dbGaP: Cardiovascular Health Study (CHS) Cohort: an NHLBI-funded observational study of risk factors for cardiovascular disease in adults 65 years or older. https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000287.v7.p1

[2] Musunuru K, Lettre G, Young T, Farlow DN, Pirruccello JP, Ejebe KG, Keating BJ, Yang Q, Chen MH, Lapchyk N, Crenshaw A, Ziaugra L, Rachupka A, Benjamin EJ, Cupples LA, Fornage M, Fox ER, Heckbert SR, Hirschhorn JN, Newton-Cheh C, Nizzari MM, Paltoo DN, Papanicolaou GJ, Patel SR, Psaty BM, Rader DJ, Redline S, Rich SS, Rotter JI, Taylor HA Jr, Tracy RP, Vasan RS, Wilson JG, Kathiresan S, Fabsitz RR, Boerwinkle E, Gabriel SB; NHLBI Candidate Gene Association Resource (2010) Candidate gene association resource (CARe): design, methods, and proof of concept. *Circ Cardiovasc Genet* **3**, 267-275.

[3] Akushevich I, Kravchenko J, Ukraintseva S, Arbeev K, Yashin AI (2012) Age patterns of incidence of geriatric disease in the U.S. elderly population: Medicare-based analysis. *J Am Geriatr Soc* **60**, 323-327.

[4] Akushevich I, Yashkin AP, Kravchenko J, Ukraintseva S, Stallard E, Yashin AI (2018) Time trends in the prevalence of neurocognitive disorders and cognitive impairment in the United States: the effects of disease severity and improved ascertainment. *J Alzheimers Dis* **64**, 137-148.

ADDITIONAL LINKS

https://support.sas.com/software/94/
https://www.cdc.gov/pneumococcal/vaccination.html

The Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines

This study followed STROBE guidelines, as shown in the following Checklist:

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	p.2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	p.2
Introduction			
Background/ rationale	2	Explain the scientific background and rationale for the investigation being reported	p.3
Objectives	3	State specific objectives, including any prespecified hypotheses	p.3
Methods			
Study design	4	Present key elements of study design early in the paper	p.3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	p.3 and Supplementary Material
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	p.3 and Supplementary Material
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	p.3-4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	p.3-4 and Supplementary Material
Bias	9	Describe any efforts to address potential sources of bias	p.3-4
Study size	10	Explain how the study size was arrived at	p.6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	p.3-4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	p.4
		(*b*) Describe any methods used to examine subgroups and interactions	p.4
		(*c*) Explain how missing data were addressed	p.4
		(*d*) If applicable, explain how loss to follow-up was addressed	n/a
		(*e*) Describe any sensitivity analyses	n/a
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed	n/a
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) Summarize follow-up time (e.g., average and total amount)	Table 1
Outcome data	15*	Report numbers of outcome events or summary measures over time	Table 2

Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
		(b) Report category boundaries when continuous variables were categorized	Table 2
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	n/a
Discussion			
Key results	18	Summarize key results with reference to study objectives	p.5
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	p.5
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	p.5
Generalizability	21	Discuss the generalizability (external validity) of the study results	p.5
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	p.5-6

Cuschieri S (2019) The STROBE guidelines. *Saudi J Anaesth* **13**(Suppl 1), S31-S34.