

# Investigating a Haptoglobin (HP) structural variant as a potential modifier of *APOE* allele risks on Alzheimer Disease

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## ABSTRACT

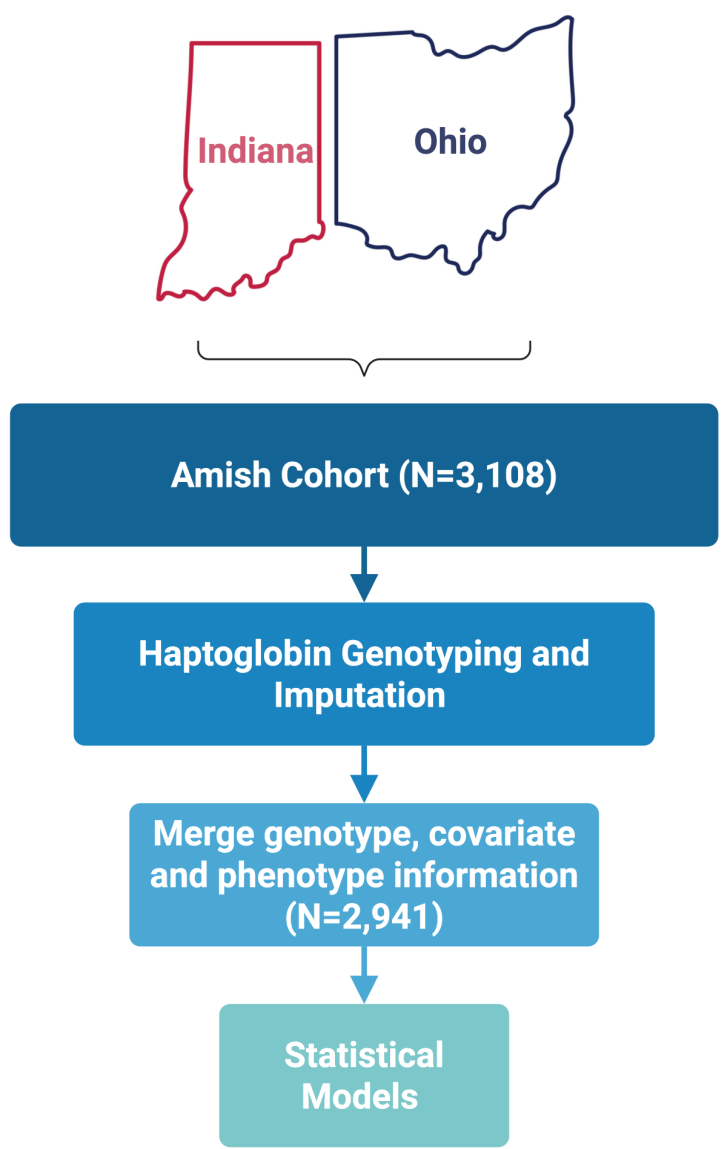
We investigated whether a structural variant in the haptoglobin (HP) gene modifies the effect of *APOE* alleles on Alzheimer's disease (AD) risk in the genetically homogeneous Midwestern Amish. Among 3,108 participants, survival analyses show that the *HP2* allele attenuates *APOE*  $\epsilon 4$  associated AD risk, with extended Cox time-dependent models showing the log-hazard for each HP-*APOE* genotype combination changes over time. These findings support HP as a potential effect modifier of *APOE*.

## INTRODUCTION

- Alleles of apolipoprotein E (*APOE*) gene ( $\epsilon 2$ ,  $\epsilon 3$ ,  $\epsilon 4$ ) are the strongest genetic risk factor of AD<sup>1</sup>
- Structural variant in *HP* gene produces two alleles, *HP1* and *HP2*<sup>2,3</sup>
- *HP1* and *HP2* confer differential binding and protection on *APOE*, acting as an antioxidant<sup>4</sup>
- Prior work (Bai et al. 2023) showed *HP2*  $\uparrow$  AD risk in  $\epsilon 4$  carriers and  $\downarrow$  AD risk in  $\epsilon 2$  carriers<sup>5</sup>

## MATERIALS and METHODS

The Collaborative Amish Aging and Memory Project (CAAMP) has collected family history, clinical and neuropsychological data, and blood samples on Amish individuals from Ohio and Indiana. *HP* genotypes were available for 2,941 individuals after using a *HP* specific reference panel<sup>5</sup>. A ssociation analyses compared AD cases (N = 218) and cognitively unimpaired controls (N = 1008). Mixed-effects logistic and frail Cox proportional hazards regression models accounting for familial structure were conducted to evaluate *HP-APOE* interaction effects on AD risk and age of onset.



## RESULTS

Logistic regression models reproduced the general patterns reported by Bai et al. (2023), though predicted risk magnitudes differed in the Amish cohort, while stratified logistic models were uninformative. In survival analyses, *APOE*-stratified Cox models accounting for relatedness suggested attenuated  $\epsilon 4$ -associated AD hazard and later onset among *HP2* carriers, and extended Cox models indicated that HP-*APOE* effects on AD hazard vary with age.

| Variable                | Control      | Case         |
|-------------------------|--------------|--------------|
| N                       | 1008         | 218          |
| HP Genotype             |              |              |
| HP1/HP1                 | 201 (19.9%)  | 32 (14.7%)   |
| HP1/HP2                 | 450 (44.6%)  | 92 (42.2%)   |
| HP2/HP2                 | 357 (35.4%)  | 94 (43.1%)   |
| <i>APOE</i> Genotype    |              |              |
| $\epsilon 2/\epsilon 2$ | 0 (0%)       | 0 (0%)       |
| $\epsilon 2/\epsilon 3$ | 95 (9.4%)    | 13 (6.0%)    |
| $\epsilon 2/\epsilon 4$ | 20 (2.0%)    | 2 (0.9%)     |
| $\epsilon 3/\epsilon 3$ | 709 (70.3%)  | 105 (48.6%)  |
| $\epsilon 3/\epsilon 4$ | 178 (17.7%)  | 77 (35.3%)   |
| $\epsilon 4/\epsilon 4$ | 6 (0.6%)     | 20 (9.2%)    |
| Sex                     |              |              |
| Female                  | 623 (61.8%)  | 140 (64.2%)  |
| Male                    | 385 (38.2%)  | 78 (35.8%)   |
| Age [mean(SD)]          | 79.10 [7.80] | 85.02 [6.65] |
| Site                    |              |              |
| Site (OH)               | 593 (58.8)   | 89 (40.8%)   |
| Site (IN)               | 415 (41.2)   | 129 (59.2%)  |

Table 1: Study Population Characteristics

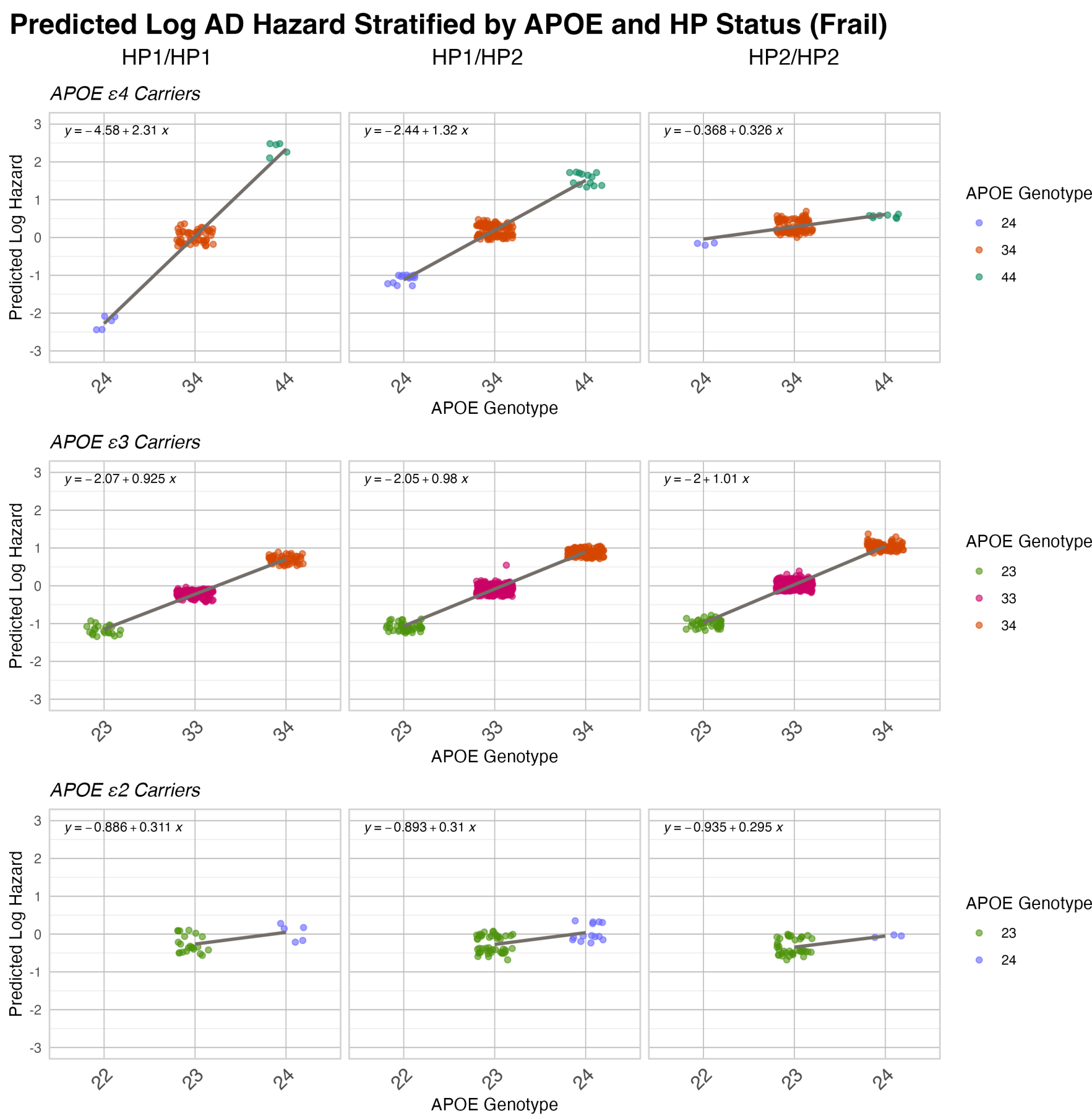


Figure 1: Effect of apolipoprotein E (*APOE*) and haptoglobin (*HP*) genotype on AD age of onset. Jitter plot and trend lines show fitted effect log hazard estimates and standard errors from frail Cox proportional hazards regression model.

| Frail Cox Proportional Hazards Model by APOE Subgroup |                   |               |         |                    |              |         |                   |               |         |
|-------------------------------------------------------|-------------------|---------------|---------|--------------------|--------------|---------|-------------------|---------------|---------|
| Variable                                              | APOE ε2 (N = 130) |               |         | APOE ε3 (N = 1178) |              |         | APOE ε4 (N = 303) |               |         |
|                                                       | HR                | 95% CI        | P-value | HR                 | 95% CI       | P-value | HR                | 95% CI        | P-value |
| HP2 count                                             | 0.81              | (0.03, 25.5)  | 0.905   | 1.08               | (0.63, 1.87) | 0.772   | 3.07              | (0.9, 10.46)  | 0.074   |
| APOE remaining                                        | 1.19              | (0.04, 38.91) | 0.924   | 2.55               | (1.46, 4.46) | 0.001   | 10.03             | (2.23, 45.06) | 0.003   |
| Sex: Female                                           | 0.66              | (0.19, 2.3)   | 0.513   | 0.92               | (0.67, 1.26) | 0.600   | 1.06              | (0.64, 1.75)  | 0.830   |
| PC1                                                   | 1.09              | (0.61, 1.93)  | 0.773   | 0.95               | (0.8, 1.12)  | 0.543   | 0.92              | (0.6, 1.43)   | 0.715   |
| siteOH                                                | 1.00              | (0.29, 3.44)  | 0.999   | 1.21               | (0.84, 1.76) | 0.310   | 1.41              | (0.74, 2.68)  | 0.303   |
| frailty(gpfamid)                                      |                   |               | 0.954   |                    |              | 0.087   |                   |               | 0.028   |
| HP2 count:APOE remaining                              | 1.16              | (0.05, 25.5)  | 0.925   | 1.05               | (0.72, 1.55) | 0.788   | 0.38              | (0.13, 1.14)  | 0.083   |

Table 2: Hazard Ratio (HR) estimates from the Cox proportional hazards regression model, stratified by *APOE* allele. *APOE* remaining represents effect of remaining *APOE* allele given the genotype and is encoded in additive, ascending order for the alleles (0 =  $\epsilon 2$ , 1 =  $\epsilon 3$ , 2 =  $\epsilon 4$ ).

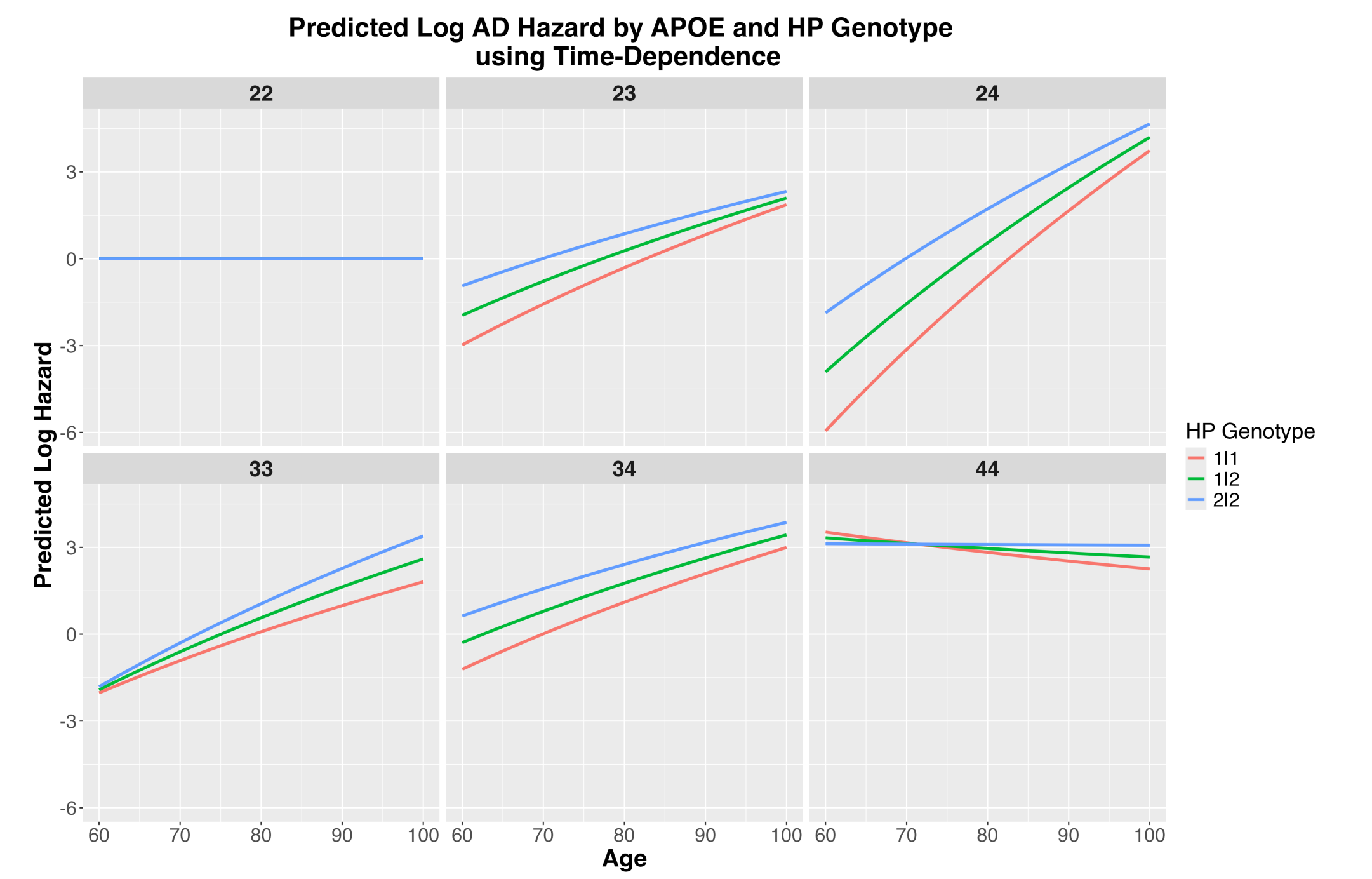


Figure 2: Predicted log-hazard of AD by *APOE* and *HP* genotype from an extended frail Cox proportional hazards regression model .

## CONCLUSIONS

Our findings support *HP*'s role as a potential effect modifier of *APOE* in a homogeneous Amish cohort, with increasing *HP2* dosage among *APOE*  $\epsilon 4$  carriers associated with attenuated AD risk. This is in contrast to Bai et al. 2023 who reported that *HP2* increased AD risk in *APOE*  $\epsilon 4$  carriers. Together, these findings suggest heterogeneity in *HP-APOE* effects and support the plausibility of *APOE* risk modification by *HP*, though interaction estimates may be model or population-dependent. Translationally, *HP* supplementation may represent a potential genotype-tailored therapeutic strategy.

## REFERENCES/ACKNOWLEDGEMENTS

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