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INPLASY

CYP2D6 genetic variation and clinical response to donepezil and galantamine in Alzheimer's disease: a systematic review and meta-analysis

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ADMINISTRATIVE INFORMATION

Support - No support.

Review Stage at time of this submission - The review has not yet started.

Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 4 September 2026 and was last updated on 4 September 2026.

INTRODUCTION

Review question / Objective The primary objective of this study is to qualitatively and quantitatively summarise the current evidence on the association between CYP2D6 gene polymorphisms, their predicted metabolic phenotypes, and the clinical response to acetylcholinesterase inhibitors (AChEIs) that are primarily metabolised via the CYP2D6 pathway (specifically donepezil and galantamine) in patients diagnosed with Alzheimer's disease (AD). In particular, we aim to determine whether genetic variations in the CYP2D6 pathway and functional changes driven by drug–drug interactions (phenoconversion) can serve as reliable biomarkers for predicting both cognitive improvement and the risk of therapeutic failure with these specific agents.

Rationale Acetylcholinesterase inhibitors (AChEIs) remain the gold standard for the symptomatic management of Alzheimer's disease (AD); however, their clinical efficacy shows considerable inter-individual variability, with response rates ranging from 20% to 60%. This variability is largely driven by the specific pharmacokinetic profiles of the agents employed. Critically, whereas drugs such as rivastigmine undergo non-cytochrome-mediated hydrolysis, donepezil and galantamine are major substrates of the hepatic CYP2D6 enzyme. Consequently, genetic variations that alter CYP2D6 enzymatic activity are likely to be primary drivers of treatment outcomes for these specific agents.
A meta-analysis published in 2016 examined the role of CYP2D6 variants in donepezil efficacy but was constrained by the limited number of primary reports and the lack of representative data from diverse ethnic populations available at that time. Since then, the evidence base has grown, yet findings remain inconclusive for broad clinical implementation. Furthermore, prior research has largely failed to account for the impact of "phenoconversion" – a clinical phenomenon in which a patient's functional metabolic capacity does not match their genetic profile because of

drug–drug interactions. In AD management, the common use of concomitant medications that act as CYP2D6 inhibitors (such as SSRIs or certain antipsychotics) can effectively turn a genetic "normal metaboliser" into a functional "poor metaboliser".

Given that Alzheimer's patients are typically elderly and subject to complex polypharmacy regimens, an updated and more robust meta-analysis is now essential. This study will specifically focus on AChEIs that are CYP2D6 substrates, integrating a decade of new evidence to clarify the association between CYP2D6 status and treatment outcomes. By evaluating these polymorphisms through the lens of contemporary real-world clinical practice and explicitly addressing the gap in phenoconversion data, this review aims to establish whether CYP2D6 can serve as a clinically useful pharmacogenetic biomarker for personalised AD therapy.

Condition being studied The condition under study is Alzheimer's disease (AD), a progressive and irreversible neurodegenerative disorder that represents the most common cause of dementia in the elderly population worldwide. Clinically, the disease is characterized by a gradual and relentless decline in cognitive functions — particularly short-term memory, language, orientation, and executive functions — associated with behavioral symptoms and a progressive loss of independence in activities of daily living. Pathophysiologically, AD is defined by the extracellular accumulation of amyloid-beta plaques and the intracellular formation of neurofibrillary tangles of hyperphosphorylated tau protein, leading to synaptic damage, neuroinflammation, and neuronal death, severely affecting cerebral cholinergic systems.

Standard symptomatic pharmacological treatment relies on acetylcholinesterase inhibitors (AChEIs), such as donepezil and galantamine, aimed at prolonging acetylcholine activity in the synaptic cleft. However, the clinical efficacy of these agents exhibits pronounced inter-individual variability, with response rates ranging from 20% to 60%. This review specifically focuses on the therapeutic response (in terms of cognitive improvement or stability) and safety (incidence of cholinergic adverse events) in patients with Alzheimer's disease treated with AChEIs metabolized via the CYP2D6 pathway, evaluating how this response is influenced by individual CYP2D6 genetic variation and phenoconversion driven by concomitant medications.

METHODS

Search strategy A comprehensive systematic search will be conducted across different databases, including PubMed, Web of Science, Scopus, and OpenGrey. The search strategy presented below is specifically formatted for PubMed; it will be subsequently adapted to match the unique syntax and technical requirements of each respective database: (donepezil OR galantamine OR Aricept OR Reminyl OR "cholinesterase inhibitor" OR "cholinesterase inhibitors" OR AChEI OR AChEIs) AND (CYP2D6 OR 2D6 OR "Cytochrome P450 2D6") AND (Alzheimer OR Dementia) AND (polymorphism OR SNP* OR variant* OR genotype* OR allele* OR pharmacogenet* OR pharmacogenom*)

The retrieved studies will be read in full to assess their suitability for inclusion in the meta-analysis. To avoid double-counting participants, if two or more studies are found to share part of the same patient population or describe overlapping cohorts, only the most comprehensive study or the one with the largest sample size will be included. A manual search to identify additional primary studies not retrieved in the initial literature search will be carried out by checking the reference lists of the identified articles.

Participant or population The target population consists of adult patients diagnosed with Alzheimer's disease (AD) according to standardised clinical criteria (e.g., NIA-AA or DSM-IV/V). Eligible participants are individuals receiving pharmacological treatment with acetylcholinesterase inhibitors (AChEIs) that are substrates of the CYP2D6 enzyme, primarily donepezil or galantamine. Studies involving patients treated with other AChEIs that do not follow this metabolic pathway (e.g., rivastigmine) will be excluded from the primary analysis to ensure the pharmacokinetic relevance of the genetic data.

Intervention The "intervention" or exposure under investigation is the presence of specific CYP2D6 genetic variants. The analysis will be conducted in two distinct stages.

In the first stage, we will perform a genotype-specific analysis focusing on individual high-frequency Single Nucleotide Polymorphisms (SNPs). This stage will specifically evaluate the impact of the *10 (rs1065852) variant, prevalent in East Asian populations, and the *4 (rs3892097) and *2 (rs1080985) variants, which are more common in Caucasian cohorts. This approach aims to identify variant-specific drivers of drug response

and resolve inconsistencies observed in previous meta-analyses.
In the second stage, we will perform a phenotype-based analysis, grouping patients into functional metabolic categories—such as Poor Metabolisers (PM), Intermediate Metabolisers (IM), Extensive Metabolisers (EM), and Ultrarapid Metabolisers (UM). This analysis will be based exclusively on the metabolic phenotypes as explicitly reported by the authors of the included studies.

Comparator The primary comparison will be made against the wild-type reference genotype (e.g. *1/*1) or the Extensive Metaboliser (EM) phenotype, which represents the baseline enzymatic activity for drug clearance.

Study designs to be included This review will include randomised controlled trials (RCTs), prospective and retrospective observational cohort studies, and case–control studies that report original data on genetic variants and clinical outcomes.

Eligibility criteria Studies will be selected based on the following inclusion criteria: 1) participants must be adult patients diagnosed with Alzheimer's Disease (AD) receiving treatment with AChEIs that are established substrates of the CYP2D6 enzyme (specifically donepezil or galantamine); 2) the study must report original data on CYP2D6 genotypes, diplotypes, or metabolic phenotypes (e.g., PM, IM, EM, UM); 3) outcome measures must include either dichotomous clinical response data (e.g., number of "responders" vs. "non-responders" according to standardized scales) or continuous data (e.g., mean changes and standard deviations in cognitive scores such as MMSE or ADAS-Cog from baseline to follow-up). The exclusion criteria are as follows:1) pre-clinical, in vitro, or animal studies; 2) narrative reviews, systematic reviews without original data, editorials, or case reports; 3) studies involving patients treated exclusively with AChEIs that are not metabolised via the CYP2D6 pathway (e.g., rivastigmine); 4) Studies not available in English. Studies where genotype data are insufficient to calculate the effect size will be included in the systematic review but excluded from the quantitative analysis.

Information sources Relevant studies will be identified using a multi-modal search strategy involving the following sources: 1) major high-impact databases, specifically PubMed, Scopus, and Web of Science, to identify peer-reviewed primary studies; 2) OpenGrey, to minimise publication bias by identifying unpublished reports (grey literature); 3) manual screening of the reference lists of all eligible studies, as well as relevant systematic and narrative reviews identified during the search, to retrieve additional primary sources.

Main outcome(s) The primary efficacy outcomes will be analysed using both dichotomous and continuous variables to ensure a robust evaluation. The dichotomous outcome will be defined as the clinical response rate, with "responders" identified according to study-specific thresholds of cognitive stability or improvement (for example, a change in MMSE score ≥ 0 or a significant decrease in ADAS-Cog score). The continuous outcome will be the mean change from baseline to the final follow-up in standardised cognitive assessment scores, primarily the Mini-Mental State Examination (MMSE) and the Alzheimer's Disease Assessment Scale – Cognitive Subscale (ADAS-Cog). This dual approach allows evaluation of both the magnitude of the effect and the clinical probability of success.

Additional outcome(s) Secondary outcomes will include measures of safety and pharmacokinetic validation to support and contextualise the clinical efficacy findings. We will narratively and quantitatively describe the incidence of cholinergic adverse events, such as nausea, vomiting and diarrhoea, to determine whether a patient's CYP2D6 metabolic status – including the functional impact of phenoconversion – correlates with drug-related toxicity. This is particularly relevant for Poor Metabolisers (PMs), or those functionally converted to PM status, who may experience higher systemic exposure.
Additionally, where data are available, we will evaluate the steady-state plasma concentrations (Cpss) of donepezil or galantamine in relation to different CYP2D6 genotypes and predicted metabolic phenotypes. This pharmacokinetic analysis will serve as a crucial physiological bridge, confirming whether the observed clinical differences in treatment response or safety are directly driven by altered drug metabolism and varying systemic exposure to these specific agents.

Data management Two investigators (M.G., S.T.) will independently screen titles, abstracts, and full texts, with a third reviewer (F.F) resolving disagreements. To prevent double-counting of participants, overlapping cohorts will be identified by cross-checking demographics and study periods; only the most comprehensive study will be included.
Two reviewers (S.C., F.F.) will independently extract data from each included study, resolving any discrepancies through consultation with a third

reviewer (S.T.). Data extraction will follow a standardised form capturing author, year, location, ethnicity (to account for CYP2D6 allele variations), sample size, and baseline AD severity (mean MMSE or ADAS-Cog scores). Intervention details will include the specific substrate (donepezil or galantamine), dosage, and treatment duration. Genetic data will encompass CYP2D6 polymorphisms, assessment of Hardy Weinberg equilibrium (HWE) for each SNP, diplotypes, and predicted phenotypes. Crucially, we will systematically extract data regarding the potential for phenoconversion. For each study, we will record: (i) whether the use of concomitant CYP2D6 inhibitors (e.g., SSRIs such as fluoxetine or paroxetine, and certain antipsychotics) was an explicit exclusion criterion; (ii) if not excluded, whether the study provided a detailed list or the prevalence of such medications among participants; and (iii) whether the authors performed any adjustment of the predicted metabolic phenotype (e.g., calculating a phenoconversion-adjusted Activity Score) or conducted sensitivity analyses to account for drug–drug interactions.

Dichotomous outcomes (responders/non-responders) and continuous outcomes (mean change and standard deviation in cognitive scores) will be extracted. For continuous outcomes, separate genotypes will be pooled into functional metabolic categories (e.g. “Reduced Function”) using validated statistical tools (e.g. https://www.statstodo.com/CombineMeansSDs.php). If studies report outcomes as medians or ranges, the mean and standard deviation will be estimated using validated methods (PMID: 25524443) or online calculators (https://www.math.hkbu.edu.hk/~tongt/papers/median2mean.html).

Quality assessment / Risk of bias analysis The methodological quality of the included studies will be independently assessed by two researchers (M.G. and S.T.). For observational and cohort studies, we will utilize the Newcastle-Ottawa Scale (NOS), which evaluates participant selection, group comparability, and outcome assessment. For any randomized trials, the Cochrane Risk of Bias 2 (RoB 2) tool will be employed. Disagreements between the primary reviewers will be resolved through a consensus-based discussion with a third reviewer (F.F.).

Strategy of data synthesis For dichotomous outcomes, we will calculate the pooled odds ratio (OR) with 95% confidence intervals (CI) to estimate the likelihood of clinical response (responders vs non-responders) across different genotypes and phenotypes. For continuous data, we will calculate the mean difference (MD) when the same cognitive scale (e.g. MMSE) is used across studies, or the standardised mean difference (SMD) when multiple scales (e.g. MMSE and ADAS-Cog) are combined.

Due to the anticipated clinical and methodological heterogeneity in AD severity, ethnic backgrounds, and treatment duration, we will use a random-effects model (DerSimonian–Laird method) as the primary statistical framework. This model is more conservative and accounts for variation in the true effect size across different study settings. Statistical heterogeneity between studies will be quantified using the I^2 statistic and Cochrane’s Q test, with $I^2 > 50\%$ considered indicative of substantial heterogeneity. Meta-analyses for primary and secondary outcomes will be conducted only when data from at least three independent studies are available for each specific comparison. If fewer than three studies are identified for a given outcome or subgroup, the results will be presented solely as a qualitative narrative synthesis.

To assess potential publication bias, we will use a combination of graphical and statistical methods. Publication bias will be assessed visually using funnel plots and statistically using Egger’s regression test, provided that at least 10 studies are included in the meta-analysis. If evidence of asymmetry is detected, the trim-and-fill method will be applied to estimate the number of missing studies and the adjusted effect size.

Subgroup analysis To investigate potential sources of clinical heterogeneity and provide a more nuanced understanding of genetic effects, several subgroup analyses are planned. First, we will stratify the data by pharmacological agent, specifically comparing patients who received donepezil exclusively with those treated with galantamine or mixed cohorts of CYP2D6 substrates. This analysis is crucial to isolate the genetic impact on the most clinically used drug within this metabolic pathway and to determine whether the association is drug-specific or a class effect among CYP2D6 substrates. Second, we will stratify by ethnicity (Asian vs Caucasian) to account for well-documented differences in allele frequencies, such as the prevalence of the reduced-function *10 variant in Asian populations compared with the no-function *4 variant in Caucasians. Third, leveraging the data recorded on concomitant medications, we will perform a subgroup analysis to evaluate the impact of phenoconversion. Studies will be stratified into two categories: (i) 'clean' studies, which strictly excluded patients taking potent or moderate CYP2D6 inhibitors (e.g., SSRIs, antipsychotics); and (ii) 'real-world' studies, which included patients

on complex polypharmacy or did not account for these drug–drug interactions. This comparison will allow us to determine whether the inclusion of phenoconverted patients (genotypic 'normal metabolisers' who become functional 'poor metabolisers') masks or confounds the association between CYP2D6 genotypes and clinical response. Finally, if data permit, we will analyse subgroups based on Alzheimer's disease severity at baseline (mild vs moderate) to evaluate whether the predictive value of the CYP2D6 genotype remains consistent or diminishes as the disease progresses and neurodegeneration becomes more advanced.

Sensitivity analysis To ensure the robustness and stability of the pooled estimates, a leave-one-out sensitivity analysis will be conducted by iteratively excluding one study at a time and recalculating the overall effect size and heterogeneity. This process is essential to determine whether the final results are disproportionately influenced by a single study or whether the observed statistical heterogeneity is driven by specific reports. Additionally, we may perform sensitivity analyses based on methodological quality, for instance by restricting the meta-analysis to studies with a high-quality score (e.g. Newcastle-Ottawa Scale score ≥ 7). This approach will assess whether the findings remain consistent when only the most methodologically rigorous evidence is considered, thereby strengthening the clinical reliability of the pharmacogenetic associations identified.

Language restriction The systematic review will be restricted to articles published in English. This limitation is based on practical resource constraints regarding professional medical translation services and the necessity to ensure maximum.

Country(ies) involved Italy.

Keywords CYP2D6; Donepezil; AChEIs; Alzheimer's Disease; Pharmacogenomics; Phenoconversion; Meta-analysis.

Dissemination plans The findings of this systematic review and meta-analysis will be disseminated through the following channels: 1) submission for presentation (either as an oral communication or a poster) at major national and international congresses, and relevant neurological and pharmacological meetings; 2) publication of the final results in a high-impact international scientific journal specialising in pharmacogenetics, clinical pharmacology, or neurology; 3) prioritisation of open-access publication, where possible, to ensure that the data are accessible to a global audience of clinicians, researchers, and healthcare policy-makers.

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