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Pharmacogenomics of Alzheimer's Disease: Genetic Determinants of Phenotypic Variation and Therapeutic Outcome

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ABSTRACT

Alzheimer's disease (AD) is a polygenic/complex disorder in which genomic, epigenomic, cerebrovascular, metabolic and environmental factors converge to define a progressive neurodegenerative phenotype. Conventional anti-dementia drugs are not cost-effective, and pharmacological breakthroughs have not been achieved for the past 10 years. Major determinants of therapeutic outcome in Alzheimer's disease include age- and sex-related factors, pathogenic phenotype, concomitant disorders, treatment modality and polypharmacy, and pharmacogenetics. Different categories of genes are potentially involved in the pharmacogenetic network responsible for drug efficacy and safety. Pathogenic, mechanistic, metabolic, transporter, and pleiotropic genes represent the major genetic determinants of response to treatment in AD. In pharmacogenetic studies, *APOE-4* carriers are the worst responders and *APOE-3* carriers are the best responders to conventional treatments. Patients harboring a large (L) number of poly T repeats in intron 6 of the *TOMM40* gene (L/L or S/L genotypes) in haplotypes associated with *APOE-4* are the worst responders and patients with short (S) *TOMM40* poly T variants (S/S genotype), and to a lesser extent S/VL and VL/VL carriers, in haplotypes with *APOE-3* are the best responders to treatment. Only 25% of the Caucasian population are extensive metabolizers for trigenic haplotypes integrating *CYP2D6-CYP2C19-CYP2C9* variants. Patients harboring CYP-related poor (PM) and/or ultra-rapid (UM) geno-phenotypes display more irregular profiles in drug metabolism than extensive (EM) or intermediate (IM) metabolizers. Among 111 pentagenic (*APOE-APOB-APOC3-CETP-LPL*) haplotypes associated with lipid metabolism, carriers of the H26 haplotype (23-TT-CG-AG-CC) exhibit the lowest cholesterol levels and patients with the H104 haplotype (44-CC-CC-AA-CC) are severely hypercholesterolemic. Epigenetic aberrations (DNA methylation, histone modifications, miRNA dysregulation) in genes configuring the pharmacoepigenetic cascade also influence the response/resistance to drugs. Consequently, novel strategies in drug development, either preventive or therapeutic, for AD should take into consideration these pharmacogenetic determinants for treatment optimization.

Keywords: Alzheimer's disease, anti-dementia drugs, APOE, Atorvastatin, Cholesterol, Epigenetics, CYP haplotypes, LipoEsar, Pharmacogenomics, Pharmacoepigenomics.

INTRODUCTION

Alzheimer's disease (AD) is a major problem of health in developed countries and the most prevalent form of dementia, representing the 6th cause of death in the USA with and age-adjusted death rate of 25.4 per 100,000. Genomic, epigenomic, cerebrovascular, metabolic and environmental factors are potentially involved in the pathogenesis of AD. The age- and sex-related syndromic profile of AD reflects, at least, a tetravalent phenotype: (i) a neuropathological component (classic hallmarks: senile plaques, neurofibrillary tangles, neuritic desarborization, neuronal loss); (ii) a

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neurobehavioral component: cognitive deterioration, behavioral changes, functional decline; (iii) an age-related biological component (direct-, indirect-, and un-related biochemical, hematological and metabolic phenotypes); and (iv) gender-related phenotypes [1-3]. According to this heterogeneous, complex clinical picture, the therapeutic intervention in dementia is polymodal in order to modify the expression of all these complex phenotypes. AD patients present concomitant disorders including hypertension (20-30%), overweightness or obesity (20-40%), diabetes (20-25%), hypercholesterolemia (>40%), hypertriglyceridemia (20%); excess of urea (>80%), creatinine (6%) and uric acid (5%); alterations in transaminases (ASAT, ALAT, GGT) (>15%), alkaline phosphatase (14%), bilirubin (17%), and ions (>10%); deficits of iron (5%), ferritin (3%), folate (5%), and vitamin B_{12} (4%); thyroid dysfunction (5-7%), and reduced levels of RBC (3%), HCT (33%), and Hb (35%)[4]. Cardiovascular disorders (>40%), atherosclerosis (>60%), and different modalities of cerebrovascular damage (>60%) are also frequent among patients with AD. Most of these biochemical, hematological and metabolic anomalies exhibit gender differences and may contribute to accelerate the dementia process. The pharmacological treatment of these concomitant pathologies adds complexity and risks to the multifactorial therapeutic intervention in patients with dementia. Of major relevance is the treatment of diabetes, hypertension, dyslipidemia, and cardiovascular, cerebrovascular and neuropsychiatric disorders. The chronic treatment of these illnesses increases the risk of drug interactions and toxicity, aggravating the clinical condition of the demented patient. In this context, the incorporation of pharmacogenetic protocols into clinical practice is fundamental to minimize drug-drug interactions and ADRs, and to optimize the global therapeutic outcome, avoiding deleterious effects on mental function and cognition.

Major determinants of therapeutic outcome in AD include age- and sex-related factors, pathogenic phenotype, concomitant disorders, treatment modality and polypharmacy, and pharmacogenetics. Different categories of genes are potentially involved in the pharmacogenetic network responsible for drug efficacy and safety. Pathogenic, mechanistic, metabolic, transporter, and pleiotropic genes represent the major genetic determinants of response to treatment in AD [5,6]. By-products of these genes are integrated in transcriptomic, proteomic and metabolic networks which are disrupted in AD and represent potential targets for therapeutic intervention [6,7] (**Figure 1**).

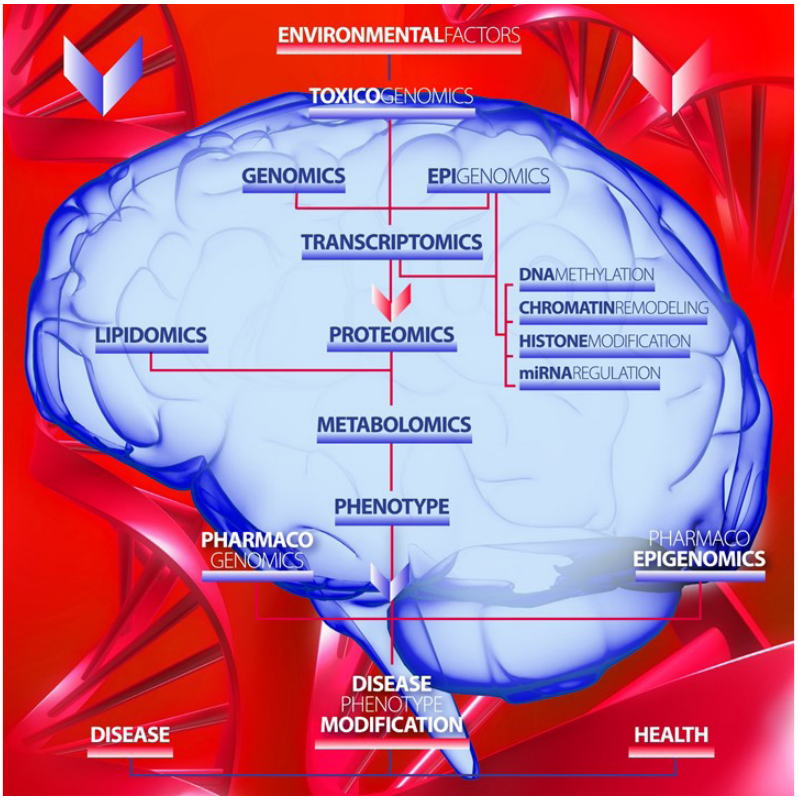


Figure 1. Pathogenic mechanisms and potential intervention targets for disease phenotype modification in Alzheimer's disease.

TREATMENTS

AD patients may take 6-12 different drugs/day for the treatment of dementia-related symptoms, including memory deterioration (conventional anti-dementia drugs, neuroprotectants), behavioral changes (antidepressants, neuroleptics, sedatives, hypnotics), and functional decline, or for the treatment of concomitant pathologies (epilepsy, cardiovascular and cerebrovascular disorders, parkinsonism, hypertension, dyslipidemia, anemia, arthrosis, etc). Over 20% of dementia patients are current users of cardiovascular drugs. A high throughput screening study assessed 1600 FDA-approved drugs for their ability to modulate Aβ activity; 559 drugs of the 1600 had no effect on APP processing or were toxic to neurons at the concentration tested, while 800 drugs could reduce Aβ content by over 10% in primary neurons derived from Tg2576 mice, among which, 184 drugs were able to reduce Aβ content by more than 30%; 241 drugs could potentially promote Aβ accumulation, including 26 drugs that could increase the level of Aβ by over 30% [8].The co-administration of several drugs may cause side-effects and adverse drug reactions in over 60% of AD patients, who in 2-10% of the cases require hospitalization. The prevalence of potentially inappropriate medication (PIM) is around 50% in some European cohorts. Cerebral vasodilators are the most widely used class of PIM, accounting for 24.0% of all prescriptions, followed by atropinic drugs and long half-life benzodiazepines. Atropinic drugs were associated with cholinesterase inhibitors in 16% of patients. In over 20% of the patients, behavioral deterioration and psychomotor function can be severely altered by polypharmacy [9]. The principal causes of these iatrogenic effects are the inappropriate combination of drugs, and the genomic background of the patient, responsible for his/her pharmacogenomic outcome.

During the past 10 years, over 1,000 different compounds have been studied as potential candidate drugs for the treatment of AD [6,7,10,11]. About 50% of these substances are novel molecules obtained from natural sources [6,7]. The candidate compounds can be classified according to their pharmacological properties and/or the AD-related pathogenic cascade to which they are addressed to halt disease progression. In addition to the FDA-approved drugs since 1993 (tacrine, donepezil, rivastigmine, galantamine, memantine) (**Table 1**), most candidate strategies fall into 6 major categories: (i) novel cholinesterase inhibitors and neurotransmitter regulators, (ii) anti-Aβ treatments (APP regulators, Aβ breakers, active and passive immunotherapy with vaccines and antibodies, β- and γ-secretase inhibitors or modulators), (iii) anti-tau treatments, (iv) pleiotropic products (most of them of natural origin), (v) epigenetic intervention, and (vi) combination therapies [6,7,10,12].

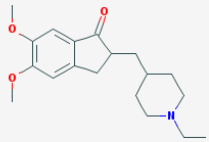
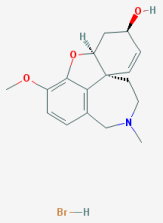
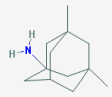
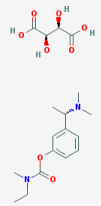
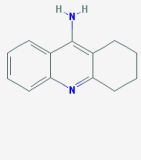
During the 2002-2012 period, 413 AD trials were performed (124 Phase 1 trials, 206 Phase 2 trials, and 83 Phase 3 trials) (78% sponsored by pharmaceutical companies). Registered trials addressed symptomatic agents (36.6%), disease-modifying small molecules (35.1%) and disease-modifying immunotherapies (18%), with a very high attrition rate (overall success rate: 0.4%; failure: 99.6%)[13]. During the past 15 years no new drugs have been approved for the treatment of AD and the available drugs are not cost-effective [14]. Therefore, the pharmacogenetics of AD is very limited, circumscribed to cholinesterase inhibitors and memantine (**Table 1**), remaining stuck in a primitive stage of underdevelopment due to the lack of novel therapeutic options. Although many studies on the pharmacogenetics of AD have been published since the early 2000's [15,16], many of them are redundant and contradictory, focusing mainly on the *APOE* gene and, to a lesser extent, on some *CYP* family genes and other minor genes [17]. In this context, several considerations are pertinent regarding further steps to be followed in order to achieve a more mature profile of AD pharmacogenomics: (i) a better characterization of the roles played in drug efficacy and safety by genes involved in the pharmacogenomic network is necessary; (ii) since most genes are under the influence of the epigenetic machinery, pharmacoepigenomics is becoming an attractive field which deserves special attention; (iii) drug-drug interactions represent a problematic issue in over 80% of AD patients (most patients require a multifactorial treatment with different drugs); (iv) since the neurodegenerative process underlying AD neuropathology starts 20-30 years before the onset of the disease, novel therapeutics should be addressed to prevent premature neuronal death; (v) specific biomarkers for AD are necessary in 3 different contexts: predictive markers before disease onset, early diagnosis in initial stages, and drug monitoring (in both preventive and/or therapeutic strategies); and (vi) physicians should be aware of the usefulness of pharmacogenomics to prescribe more accurately, avoid adverse reactions and optimize the limited therapeutic resources available for the treatment of dementia [12,18].

PHARMACOGENOMICS

Pharmacogenomics accounts for 60-90% variability in pharmacokinetics and pharmacodynamics. The modest effect (and toxicity) of current AD drugs (**Table 1**) is in part due to their pharmacogenomic profile, since over 70% of AD patients are deficient metabolizers[3,6,10]. The genes involved in the pharmacogenomic response to drugs in dementia fall into five major categories:

(i) Genes associated with disease pathogenesis: Mendelian mutations affect genes directly linked to AD, including >30 mutations in the amyloid beta precursor protein (*APP*) gene (21q21) (*AD1*); >160 mutations in the presenilin 1 (*PSEN1*) gene (14q24.3)(*AD3*); and >10 mutations in the presenilin 2 (*PSEN2*) gene (1q31-q42) (*AD4*)[19-23]. *PSEN1* and *PSEN2* are important determinants of γ-secretase activity responsible for

Table 1. Pharmacological properties and pharmacogenomics of conventional anti-dementia drugs.

Drug	Properties	Pharmacogenetics
	**Name**: **Donepezil hydrochloride,** Aricept, 120011-70-3, Donepezil HCl, BNAG, E-2020, E2020 **IUPAC Name:** 2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one;hydrochloride **Molecular Formula:** $C_{24}H_{30}ClNO_3$ **Molecular Weight:** 415.9529 g/mol **Category:** Cholinesterase inhibitor **Mechanism:** Centrally active, reversible acetylcholinesterase inhibitor; increases the acetylcholine available for synaptic transmission in the CNS **Effect**: Nootropic agent, cholinesterase inhibitor, parasympathomimetic effect	**Pathogenic genes:** *APOE, CHAT* **Mechanistic genes:** *CHAT, ACHE, BCHE* **Drug metabolism-related genes:** - **Substrate:** *CYP2D6* (major), *CYP3A4* (major), *UGTs ACHE* - **Inhibitor:** *ACHE, BCHE* **Transporter genes:** *ABCB1*
	**Name**: **Galantamine hydrobromide**, Galanthamine hydrobromide**,** 1953-04-4, Nivalin, Razadyne, UNII-MJ4PTD2VVW, Nivaline **IUPAC Name:** (1S,12S,14R)-9-methoxy-4-methyl-11-oxa-4-azatetracyclo[8.6.1.0^{1,12}.0^{6,17}]heptadeca-6,8,10(17),15-tetraen-14-ol **Molecular Formula:** $C_{17}H_{22}BrNO_3$ **Molecular Weight:** 368.26548 g/mol **Category**: Cholinesterase inhibitor **Mechanism**: Reversible and competitive acetylcholinesterase inhibition leading to an increased concentration of acetylcholine at cholinergic synapses; modulates nicotinic acetylcholine receptor; may increase glutamate and serotonin levels **Effect:** Nootropic agent, cholinesterase inhibitor, parasympathomimetic effect	**Pathogenic genes**: *APOE, APP* **Mechanistic genes**: *ACHE, BCHE, CHRNA4, CHRNA7, CHRNB2* **Drug metabolism-related genes:** - **Substrate**: *CYP2D6* (major), *CYP3A4* (major), *UGT1A1* - **Inhibitor**: *ACHE, BCHE*
	**Name: Memantine Hydrochloride**, 41100-52-1, Namenda, Memantine HCL, Axura, 3,5-Dimethyl-1-adamantanamine hydrochloride, 3,5-dimethyladamantan-1-amine hydrochloride **IUPAC Name:** 3,5-dimethyladamantan-1-amine;hydrochloride **Molecular Formula:** $C_{12}H_{22}ClN$ **Molecular Weight:** 215.76278 g/mol **Category:** N-Methyl-D-Aspartate receptor antagonist **Mechanism:** Binds preferentially to NMDA receptor-operated cation channels; may act by blocking actions of glutamate, mediated in part by NMDA receptors **Effect:** Dopamine agent, antiparkinson agent, excitatory amino acid antagonist, antidyskinetic	**Pathogenic genes:** *APOE, MAPT, PSEN1* **Mechanistic genes**: *CHRFAM7A, DLGAP1, FOS, GRIN2A, GRIN2B, GRIN3A, HOMER1, HTR3A* **Drug metabolism-related genes:** **-Inhibitor**: *CYP1A2* (weak), *CYP2A6* (weak), *CYP2B6* (strong), *CYP2C9* (weak), *CYP2C19* (weak), *CYP2D6* (strong), *CYP2E1* (weak), *CYP3A4* (weak), *NR1I2* **Transporter genes:** *NR1I2* **Pleiotropic genes:** *APOE, MAPT, MT-TK, PSEN1*
	**Name**: **Rivastigmine tartrate**, 129101-54-8, SDZ-ENA 713, Rivastigmine hydrogentartrate, Rivastigmine Hydrogen Tartrate, ENA 713, ENA-713 **IUPAC Name:** (2R,3R)-2,3-dihydroxybutanedioic acid;[3-[(1S)-1-(dimethylamino)ethyl]phenyl] N-ethyl-N-methylcarbamate **Molecular Formula:** $C_{18}H_{28}N_2O_8$ **Molecular Weight:** 400.42352 g/mol **Category:** Cholinesterase inhibitor **Mechanism:** Increases acetylcholine in CNS through reversible inhibition of its hydrolysis by cholinesterase **Effect:** Neuroprotective agent, cholinesterase inhibitor, cholinergic agent	**Pathogenic genes:** *APOE, APP, CHAT* **Mechanistic genes:** *ACHE, BCHE, CHAT, CHRNA4, CHRNB2* **Drug metabolism-related genes:** **-Inhibitor:** *ACHE, BCHE* **Pleiotropic genes:** *APOE, MAPT*
	**Name: Tacrine Hydrochloride,** Tacrine HCl, 1684-40-8, Hydroaminacrine, tacrine.HCl, 9-AMINO-1,2,3,4-TETRAHYDROACRIDINE HYDROCHLORIDE, Tenakrin **IUPAC Name:** 1,2,3,4-tetrahydroacridin-9-amine;hydrochloride **Molecular Formula:** $C_{13}H_{15}ClN_2$ **Molecular Weight:** 234.7246 g/mol **Category:** Cholinesterase inhibitor **Mechanism:** Elevates acetylcholine in cerebral cortex by slowing degradation of acetylcholine **Effect:** Nootropic agent, cholinesterase inhibitor, Parasympathomimetic effect	**Pathogenic genes:** *APOE* **Mechanistic genes:** *ACHE, BCHE, CHRNA4, CHRNB2* **Drug metabolism-related genes:** **-Substrate**: *CYP1A2 (major), CYP2D6 (minor), CYP3A4 (major)* **-Inhibitor**: *ACHE, BCHE, CYP1A2 (weak)* **Transporter genes:** *SCN1A* **Pleiotropic genes**: *APOE, CES1, GSTM1, GSTT1, LEPR, MTHFR*

ABCB1: ATP binding cassette subfamily B member 1; ***ACHE*****:** Acetylcholinesterase (Yt blood group); ***APOE*****:** Apolipoprotein E; ***APP*****:** Amyloid beta precursor protein; ***BCHE*****:** butyrylcholinesterase; ***CES1*****:** Carboxylesterase 1; ***CHAT*****:** Choline O-acetyltransferase; ***CHRFAM7A*****:** CHRNA7 (exons 5-10) and FAM7A (exons A-E) fusion; ***CHRNA4*****:** Cholinergic receptor nicotinic alpha 4 subunit; ***CHRNA7*****:** Cholinergic receptor nicotinic alpha 7 subunit; ***CHRNB2:*** Cholinergic receptor nicotinic beta 2 subunit; ***CYP1A2*****:** Cytochrome P450, family 1, subfamily A, polypeptide 2; ***CYP2A6*****:** Cytochrome P450, family 2, subfamily A, polypeptide 6; ***CYP2B6*****:** Cytochrome P450, family 2, subfamily B, polypeptide 6; ***CYP2C9*****:** Cytochrome P450, family 2, subfamily C, polypeptide 9; ***CYP2C19*****:** Cytochrome P450, family 2, subfamily C, polypeptide 19; ***CYP2D6*****:** Cytochrome P450, family 2, subfamily D, polypeptide 6; ***CYP2E1*****:** Cytochrome P450, family 2, subfamily E, polypeptide 1; ***CYP3A4*****:** Cytochrome P450, family 3, subfamily A, polypeptide 4; ***DLGAP1*****:** Discs large homolog associated protein 1; ***FOS*****:** FBJ murine osteosarcoma viral oncogene homolog; ***GRIN2A*****:** glutamate ionotropic receptor NMDA type subunit 2A; ***GRIN2B*****:** glutamate ionotropic receptor NMDA type subunit 2B; ***GRIN3A*****:** glutamate ionotropic receptor NMDA type subunit 3A; ***GSTM1*****:** Glutathione S-transferase mu 1**; *GSTT1*:** Glutathione S-transferase theta 1; ***HOMER1*****:** Homer homolog 1 (Drosophila); ***HTR3A*****:** 5-Hydroxytryptamine receptor 3A; ***LEPR*****:** Leptin receptor; ***MAPT*****:** Microtubule associated protein tau; ***MT-TK*****:** thymidine kinase 2, mitochondrial; ***MTHFR*****:** Methylenetetrahydrofolate reductase (NAD(P)H); ***NR1/2*****:** Nuclear receptor 1/2; ***PSEN1*****:** Presenilin 1; ***SCN1A*****:** Sodium voltage-gated channel alpha subunit 1; ***UGT1A1*****:** UDP glucuronosyltransferase 1 family, polypeptide A1; ***UGTs*****:** UDP glucuronosyltransferases.

Source: Cacabelos et al (Ref. 10)

proteolytic cleavage of APP and NOTCH receptor proteins. Mendelian mutations are very rare in AD (1:1000). Mutations in exons 16 and 17 of the *APP* gene appear with a frequency of 0.30% and 0.78%, respectively, in AD patients. Likewise, *PSEN1*, *PSEN2*, and microtubule-associated protein Tau (*MAPT*) (17q21.1) mutations are present in less than 2% of the cases. Mutations in these genes confer specific phenotypic profiles to patients with dementia: amyloidogenic pathology associated with *APP*, *PSEN1* and *PSEN2* mutations and tauopathy associated with *MAPT* mutations representing the two major pathogenic hypotheses for AD [19-25].

Multiple polymorphic risk variants can increase neuronal vulnerability to premature death. There are at least 695 genes potentially associated with AD, of which the top ten are: *APOE* (19q13.2), *BIN1* (2q14), *CLU* (8p21-p12), *ABCA7* (19p13.3), *CR1* (1q32), *PICALM* (11q14), *MS4A6A* (11q12.1), *CD33* (19q13.3), *MS4A4E* (11q12.2), and *CD2AP* (6p12)[10,23]. Potentially defective genes associated with AD represent about 1.39% (35,252.69 Kb) of the human genome, which is integrated by 36,505 genes (3,095,677.41 Kb). The highest number of AD-related defective genes concentrate on chromosomes 10 (5.41%; 7,337.83 Kb), 21 (4.76%; 2,289.15 Kb), 7 (1.62%; 2,584.26 Kb), 2 (1.56%; 3,799.67 Kb), 19 (1.45%; 854.54 Kb), 9 (1.42%; 2,010.62 Kb), 15 (1.23%; 1,264.4 Kb), 17 (1.19%; 970.16 Kb), 12 (1.17%; 1,559.9 Kb), and 6 (1.15%; 1,968.22 Kb)[6]. Among susceptibility genes, the apolipoprotein E (*APOE*) gene (*AD2*) is the most prevalent as a risk factor for AD, especially in those subjects harboring the *APOE-4* allele, whereas carriers of the *APOE-2* allele might be protected against dementia[19]. Polymorphic variants in other genes (GRB-associated binding protein 2 (*GAB2*), *TLR9* rs187084 variant homozygote GG, *LRRK2* R1628P variant) might also be protective[6]. Ten novel private pathogenic copy number variations (CNVs) in 10 early-onset familial Alzheimer's disease (EO-FAD) families overlapping a set of genes (*A2BP1*, *ABAT*, *CDH2*, *CRMP1*, *DMRT1*, *EPHA5*, *EPHA6*, *ERMP1*, *EVC*, *EVC2*, *FLJ35024* and *VLDLR*) have also been identified[26].

(ii) Genes associated with the mechanism of action of drugs (enzymes, receptors, transmitters, messengers).

(iii) Genes associated with drug metabolism: (a) phase I reaction enzymes: alcohol dehydrogenases (*ADH1-7*), aldehyde dehydrogenases (*ALDH1-9*), aldo-keto reductases (*AKR1A-D*), amine oxidases (*MAOA, MAOB, SMOX*), carbonyl reductases (*CBR1-4*), cytidine deaminase (*CDA*), cytochrome P450 family (*CYP1-51, POR, TBXAS1*), cytochrome b5 reductase (*CYB5R3*), dihydropirimidine dehydrogenase (DPYD), esterases (*AADAC, CEL, CES1, CES1P1, CES2, CES3, CES5A, ESD, GZMA, GZMB, PON1, PON2, PON3, UCHL1, UCHL3*), epoxidases (*EPHX1-2*), flavin-containing monooxygenases (*FMO1-6*), glutathione reductase/peroxidases (*GPX1-7, GSR*), short-chain dehydrogenases/reductases (*DHRS1-13, DHRSX, HSD11B1, HSD17B10, HSD17B11, HSD17B14*), superoxide dismutases (*SOD1-2*), and xanthine dehydrogenase (*XDH*); and (b): phase II reaction enzymes: amino acid transferases (*AGXT, BAAT, CCBL1*), dehydrogenases (*NQO1-2, XDH*), esterases (*CES1-5*), glucuronosyl transferases (*UGT1-8*), glutathione transferases (*GSTA1-5, GSTK1, GSTM1-5, GSTO1-2, GSTP1, GSTT1-2, GSTZ1, GSTCD, MGST1-3, PTGES*), methyl transferases (*AS3MT, ASMT, COMT, GNMT, GAMT, HNMT, INMT, NNMT, PNMT, TPMT*), N-acetyl transferases (*ACSL1-4, ACSM1, ACSM2B, ACSM3, AANAT, GLYAT, NAA20, NAT1-2, SAT1*), thioltransferase (*GLRX*), and sulfotransferases (*CHST2-13, GAL3ST1, SULT1A1-3, SULT1B1, SULT1C1-4, SULT1E1, SULT2A1, SULT2B1, SULT4A1, SULT6B1, CHST1*).

(iv) Genes associated with drug transporters: In humans there are 49 *ABC* transporter genes and the multidrug resistance associated proteins (MRP1/ABCC1, MRP2/ABCC2, MRP3/ABCC3, MRP4/ABCC4, MRP5/ABCC5, MRP6/ABCC6, MRP7/ABCC10, MRP8/ABCC11 and MRP9/ABCC12) which belong to the ABCC family integrated by 13 members. Other genes encoding transporter proteins are genes of the solute carrier superfamily (SLC) and solute carrier organic (SLCO) transporter family, responsible for the transport of multiple endogenous and exogenous compounds.

(v) Pleiotropic genes involved in multifaceted cascades and metabolic reactions[5,6,27].

All these genes are under the influence of the epigenetic machinery conditioning their expression and the efficiency of their drug-metabolizing products (enzymes, transporters) [28-30].

GENETIC DETERMINANTS OF THE PHARMACOGENETIC OUTCOME WITH CONVENTIONAL NEUROPROTECTANTS AND ACETYL CHOLINESTERASE INHIBITORS

Although the *APP, PSEN1, PSEN2* and *MAPT* genes are considered major pathogenic genes for AD and classic tauopathies[23], mutations in these genes represent less than 5% of the AD population and, consequently, their influence on AD pharmacogenetics associated with conventional anti-dementia drugs is quantitatively negligible; not so in the case of immunotherapy addressing Aβ deposition. Most anti-AD vaccines (active and passive immunization) are based on transgenic models with *APP, PSEN1* and *PSEN2* mutants[31,32]. In general, most pharmacogenetic studies in AD have been performed with susceptibility genes (*APOE*) and metabolic genes (CYPs)[6,10].

APOE-TOMM40

To date, the most influential gene in AD pharmacogenetics is the *APOE* gene[2,6,10,27,33]. *APOE* is a pleiotropic gene with multifaceted activities in physiological and pathological conditions, and the presence of the *APOE*-4 allele is determinant in AD pathogenesis [19]. *APOE-4* may influence AD pathology by interacting with APP metabolism and Aβ accumulation, enhancing hyperphosphorylation of tau protein and neurofibrillary tangle formation, reducing choline acetyltransferase activity, increasing oxidative processes, modifying inflammation-related neuroimmunotrophic activity and glial activation, altering lipid metabolism, lipid transport and membrane biosynthesis in sprouting and synaptic remodeling, and inducing neuronal apoptosis and premature neuronal death[5,19]. Multiple studies over the past two decades have demonstrated that *APOE* variants may affect the therapeutic response to anti-dementia drugs[3,5,6,10,15,16,19,27,33-35]. At least 20 major phenotypic features illustrate the biological disadvantage of *APOE-4* homozygotes and the potential consequences that these patients may experience when they receive pharmacological treatment for AD and/or concomitant pathologies[5,6,15,19,33,34,38].

In over 100 clinical trials for dementia, *APOE* has been used as the only gene of reference for the pharmacogenomics of AD. Several studies indicate that the presence of the *APOE-4* allele differentially affects the quality and extent of drug responsiveness in AD patients treated with cholinergic enhancers, neuroprotective compounds, endogenous nucleotides, immunotrophins, neurotrophic factors, combination therapies and other drug categories[1,5,6,35-40]; however, controversial results are frequently found due to methodological problems, study design, and patient recruitment in clinical trials. The major conclusion in most studies is that *APOE-4* carriers are the worst responders to conventional treatments[5,6,10]. When *APOE* and *CYP2D6* genotypes are integrated in biogenic clusters and the *APOE*+*CYP2D6*-related therapeutic response to a combination therapy is analyzed in AD patients, it becomes clear that the presence of the *APOE-4/4* genotype is able to convert pure *CYP2D6*1/*1* extensive metabolizers into full poor responders to conventional treatments, indicating the existence of a powerful influence of the *APOE-4* homozygous genotype on the drug-metabolizing capacity of pure *CYP2D6* extensive metabolizers[3]. In addition, a clear accumulation of *APOE-4/4* genotypes is observed among CYP2D6 poor and ultra-rapid metabolizers[3].

Adjacent to the *APOE* locus (19q13.2) and in linkage disequilibrium with *APOE* is the *TOMM40* gene. A poly T repeat in an intronic polymorphism (rs10524523) (intron 6) in the *TOMM40* gene, which encodes an outer mitochondrial membrane translocase involved in the transport of Aβ and other proteins into mitochondria, has been implicated in AD[41-54]. *APOE-TOMM40* genotypes have been shown to modify disease risk and age at onset of symptoms[42-47,55]. The rs4420638 at the *TOMM40/APOE/APOC1* gene locus is associated with longevity[56,57]. The *APOE-TOMM40* genomic region is associated with cognitive aging[58] and with pathological cognitive decline[59]. There are 3 allele groups for rs10524523 ('523'), based on the number of 'T'-residues: 'Short' (S, T ≤ 19), 'Long' (L, 20 ≤ T ≤ 29) and 'Very Long' (VL, T ≥ 30)[49]. Longer lengths of rs10524523 are associated with a higher risk for late-onset AD (LOAD) [43-47]. Intronic poly T (rs10524523) within this region affects expression of the *APOE* and *TOMM40* genes in the brain of patients with LOAD[60]. The 523 VL poly T shows higher expression than the S poly T, indicating that the 523 locus may contribute to LOAD susceptibility by modulating the expression of *TOMM40* and/or *APOE* transcription[60]. S/VL and VL/VL are the only *TOMM40* poly T genotypes which interact with all major *APOE* genotypes; in contrast, the *APOE-4/4-TOMM40-L/L* association is unique, representing approximately 30% of *APOE-4/4* carriers[61] (**Figure 2**). The first pharmacogenetic study of the *APOE-TOMM40* region in AD patients receiving a multifactorial treatment revealed that: (i) *APOE-4* carriers are the worst responders (**Figures 3-4**) and *APOE-3* carriers are the best responders to conventional treatments (**Figures 3-4**); (ii) *TOMM40 poly T-S/S* carriers are the best responders (**Figures 3-4**), VL/VL and S/VL carriers are intermediate responders, and L/L carriers are the worst responders to treatment (**Figures 3-4**); (iii) patients harboring a large (L) number of poly T repeats in intron 6 of the *TOMM40* gene (L/L or S/L genotypes) in haplotypes associated with *APOE-4* are the worst responders to treatment; (iv) patients with short (S) *TOMM40* poly T variants (S/S genotype), and to a lesser extent S/VL and VL/VL carriers, in haplotypes with *APOE-3* are the best responders to treatment; and (v) in 100% of the cases, the L/L genotype is exclusively associated with the *APOE-4/4* genotype (**Figure 2**), and this haplotype (4/4-L/L) is probably responsible for early onset of the disease, a faster cognitive decline, and a poor response to different treatments[4,61].

Other recent pharmacogenetic studies with pathogenic or mechanistic genes indicate that the response to AChEIs is associated with 2 SNPs in the intronic region of *CHAT* rs2177370 and rs3793790[62]. The *CHRNA7* T allele (rs6494223) also associates with a better response to AChEIs and there is further confirmation that *APOE-4* carriers are the worst responders to conventional AChEIs[63].

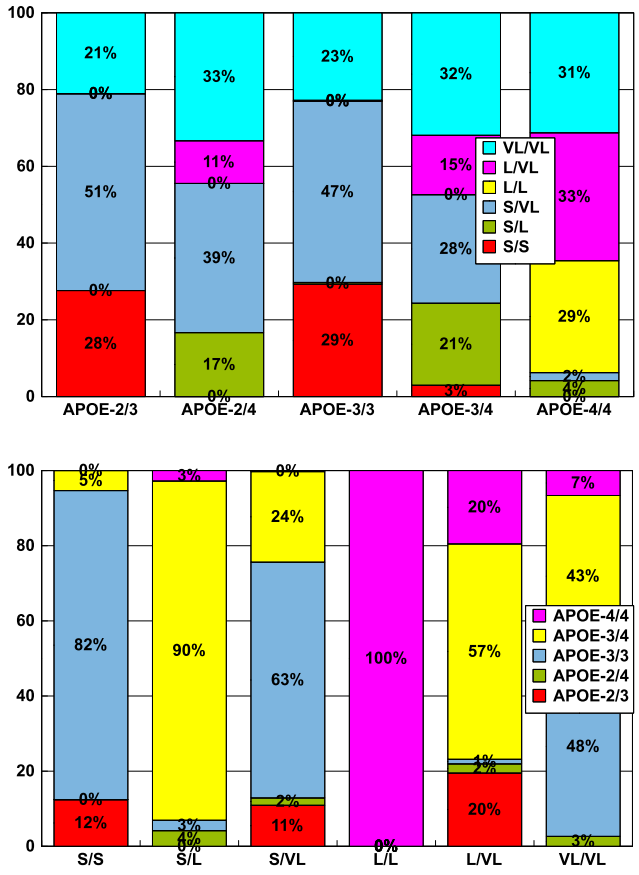


Figure 2. Distribution and frequency of *TOMM40* S/S, S/L, L/L, L/VL and VL/VL variants in carriers of *APOE* genotypes (upper panel) and of *APOE* variants in carriers of TOMM40 poly-T genotypes (lower panel). (Adapted from Cacabelos et al [61])

CYPs

Over 70% of AD patients are deficient metabolizers for the *CYP2D6/2C19/2C9* trigenic cluster; and for the *CYP2D6/2C19/2C9/3A4* tetragenic cluster, more than 80% of the patients exhibit a deficient metabolizer geno-phenotype[3]. These four *CYP* genes encode enzymes responsible for the metabolism of 60-80% of drugs of current use, showing ontogenic-, age-, sex-, circadian- and ethnic-related differences[5,6,33,64]. According to the database of the World Guide for Drug Use and Pharmacogenomics[38], 982 drugs are CYP2D6-related: 371 drugs are substrates, over 300 drugs are inhibitors, and 18 drugs are CYP2D6 inducers. Over 600 drugs are *CYP2C9*-related, 311 acting as substrates (177 are major substrates, 134 are minor substrates), 375 as inhibitors (92 weak, 181 moderate, and 102 strong inhibitors), and 41 as inducers of the CYP2C9 enzyme[38]. Nearly 500 drugs are CYP2C19-related, 281 acting as substrates (151 are major substrates, 130 are minor substrates), 263 as inhibitors (72 weak, 127 moderate, and 64 strong inhibitors), and 23 as inducers of the CYP2C19 enzyme[38]. The CYP3A4/5 enzyme metabolizes over 1900 drugs, 1033 acting as substrates (897 are major substrates, 136 are minor substrates), 696 as inhibitors (118 weak, 437 moderate, and 141 strong inhibitors), and 241 as inducers of the CYP3A4 enzyme[38].

The distribution and frequency of CYP2D6 genotypes are very similar in the general population (GP)(N=3232) and in AD (N=1289), with the exception of the CYP2D6-*3/*4 genotype ($p<0.05$) which is absent in AD samples (**Figure 5**). In the GP, CYP2D6 extensive metabolizers (EMs) account for 58.85%, whereas intermediate metabolizers (IMs) account for 31.11%, poor metabolizers (PMs) 4.49%, and ultra-rapid metabolizers (UMs) 5.55%[6,10] (**Figure 5**). In AD, EMs, IMs, PMs, and UMs are 57.54%, 31.01%, 5.49%, and 5.96%, respectively. There is an accumulation of AD-related genes of risk in PMs and UMs. EMs and IMs are the best responders, and PMs and UMs are the worst responders to a combination therapy with AChEIs, neuroprotectants, and vasoactive substances. The pharmacogenetic response in AD appears to be dependent upon the networking activity of genes involved in drug metabolism and genes involved in AD pathogenesis[5,6,19,33,34,65-67]. By phenotypes, in the GP, CYP2C9-PMs represent 4.82%, IMs 33.83%, and EMs 61.35%. In AD, PMs, IMs, and EMs are 4.76%, 34.87%, and 60.37%, respectively[6,38] (**Figure 6**). The frequencies of the *CYP2C19* geno-phenotypes in the GP are: *CYP2C19*-EMs 74.11%, *CYP2C19*-IMs 24.43%, and *CYP2C19*-PMs 1.46% (**Figure 7**). EMs, IMs, and PMs account for 75.41%, 23.56%, and 1.03%, respectively, in AD[6,38] (**Figure 7**) Concerning *CYP3A4/5* polymorphisms in AD, 83.84% of the cases are EMs (*CYP3A5*3/*3*), 14.62% are IMs (*CYP3A5*1/*3*), and

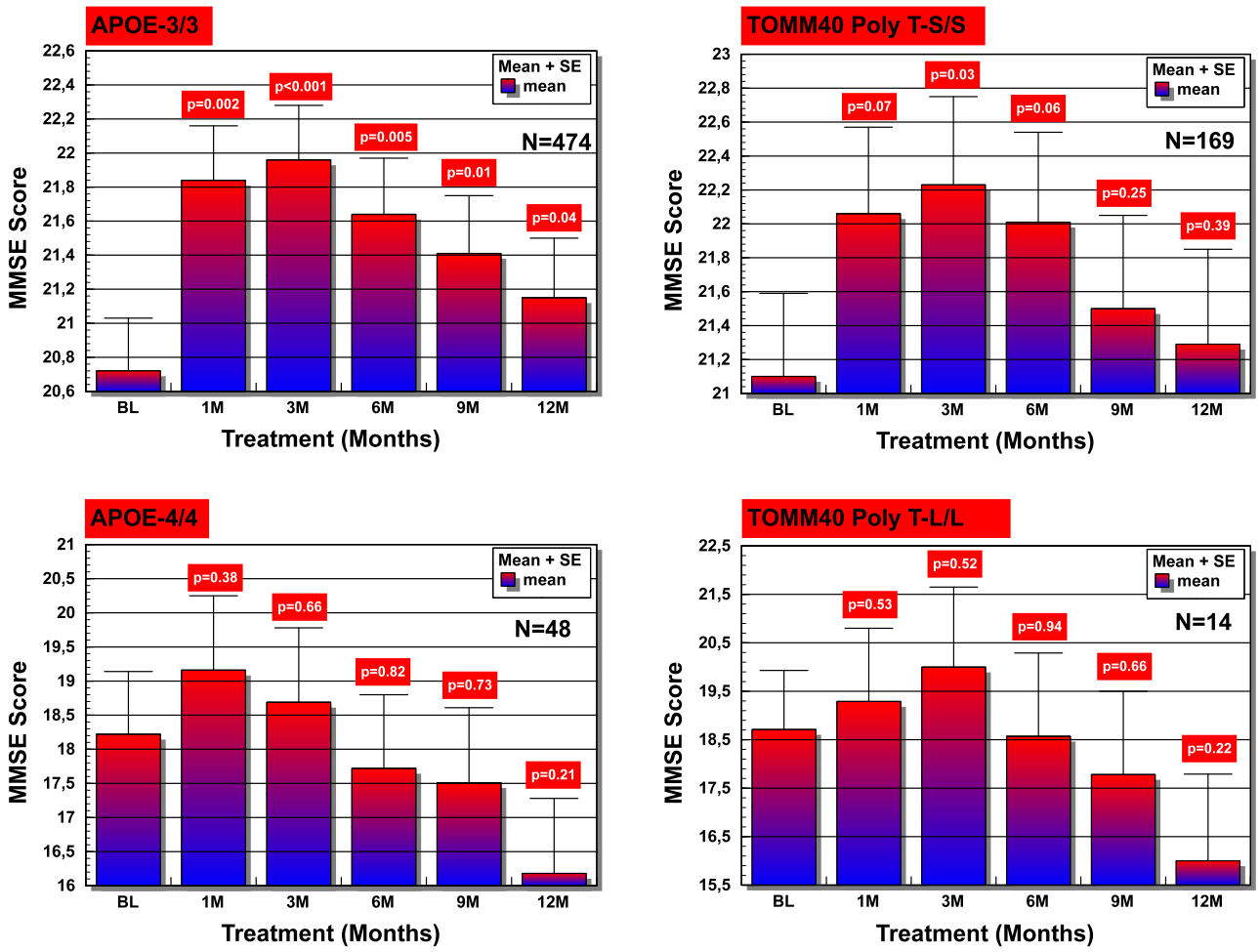


Figure 3. *APOE*- and *TOMM40*-related therapeutic response to a multifactorial treatment in patients with Alzheimer's disease.
(Adapted from Cacabelos et al [61])

1.54% are RMs (*CYP3A5*1/*1*)[6](**Figure 8**), whereas in the GP, EMs, IMs and RMs represent 82.17%, 16.48%, and 1.35%, respectively (**Figure 8**).

Tetragenic haplotypes integrating *CYP2D6*, *CYP2C9*, *CYP2C19* and *CYP3A4*/5 variants yield 156 genotypes (**Figure 9**). The most frequent haplotype is H3 (1/1-1/1-1/1-3/3)(20.87%), representing full extensive metabolizers, and only 17 haplotypes exhibit a frequency higher than 1% in the Spanish population (**Figure 10**). In addition to H3, the most frequent haplotypes (>2%) are H55 (1/4-1/1-1/1-1/3) (8.41%), H26 (1/1-1/2-1/1-3/3)(8.07%), H4 (1/1-1/1-1/2-3/3)(8.07%), H58 (1/4-1/1-1/2-3/3)(3.99%), H72 (1/4-1/2-1/1-3/3)(3.82%), H2 (1/1-1/1-1/1-1/3)(3.74%), H9 (1/1-1/1-1/3-3/3)(3.57%), and H38 (1xN/1-1/1-1/1-3/3)(2.46%) (**Figure 10**). This indicates that in the Spanish GP about 80% of the population is deficient for the biotransformation of current drugs which are metabolized via CYP2D6-2C9-2C19-3A4 enzymes.

Most anti-dementia drugs are metabolized via CYP enzymes. Donepezil is a major substrate of CYP2D6, CYP3A4, ACHE, and UGTs, inhibits ACHE and BCHE, and is transported by ABCB1[2,6,17,33,34,38,65,67-69] (**Table 1**). *CYP2D6* variants affect donepezil efficacy and safety in AD[2,6,17,33,34,38,65,66,69]. The common variant rs1080985 of *CYP2D6* is associated with poor response to donepezil[70,71]. A higher frequency of mutated *CYP2D6* allele **2A* was found in responder than in non-responder patients (75.38% vs 43.48%)[72]. In an Italian study, 67% of patients were responders and 33%

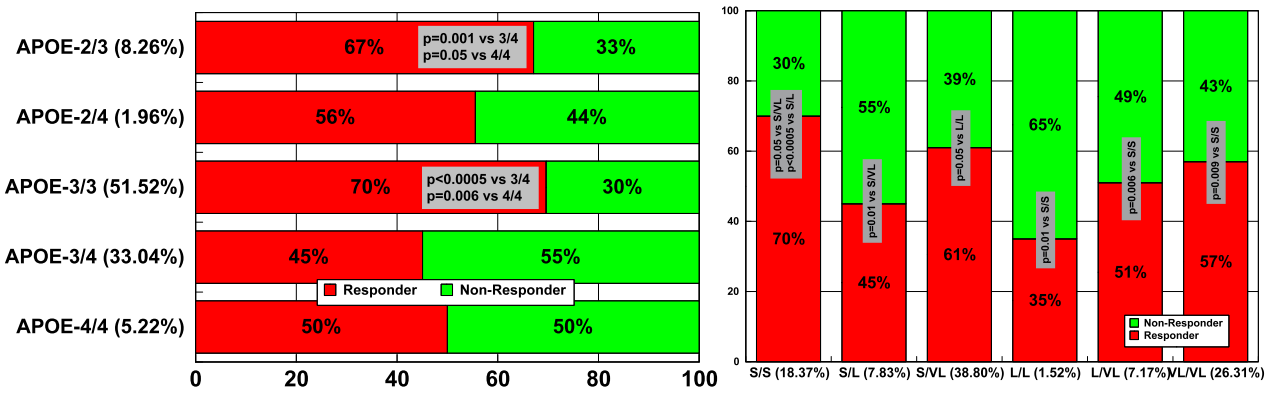


Figure 4. APOE- and TOMM40-related response rate to a multifactorial treatment in patients with Alzheimer's disease.
(Adapted from Cacabelos et al [61])

were non-responders to donepezil treatment, with abnormal enzymes accumulating in responders[73]. Chinese AD patients with the mutant allele *CYP2D6*10* may respond better (58% responders) to donepezil than those with wild allele *CYP2D6*1*[74]. In contrast, other studies revealed that CYP2D6-PMs and UMs tend to be poor responders to conventional doses of donepezil as compared to EMs and IMs[2,6,17,33,34,38,69,75-77].

In Italian patients, no association was found between *CYP3A4* or *CYP3A5* genotypes and plasma donepezil concentrations, or between genotypes and clinical response. The most common *ABCB1* haplotypes were 1236C/2677G/3435C (46%) and 1236T/2677T/3435T (41%), and patients homozygous for the T/T/T haplotype had lower plasma donepezil concentration-to-dose ratios and better clinical response than patients with other genotypes[78]. In Brazilian patients treated with AChEIs

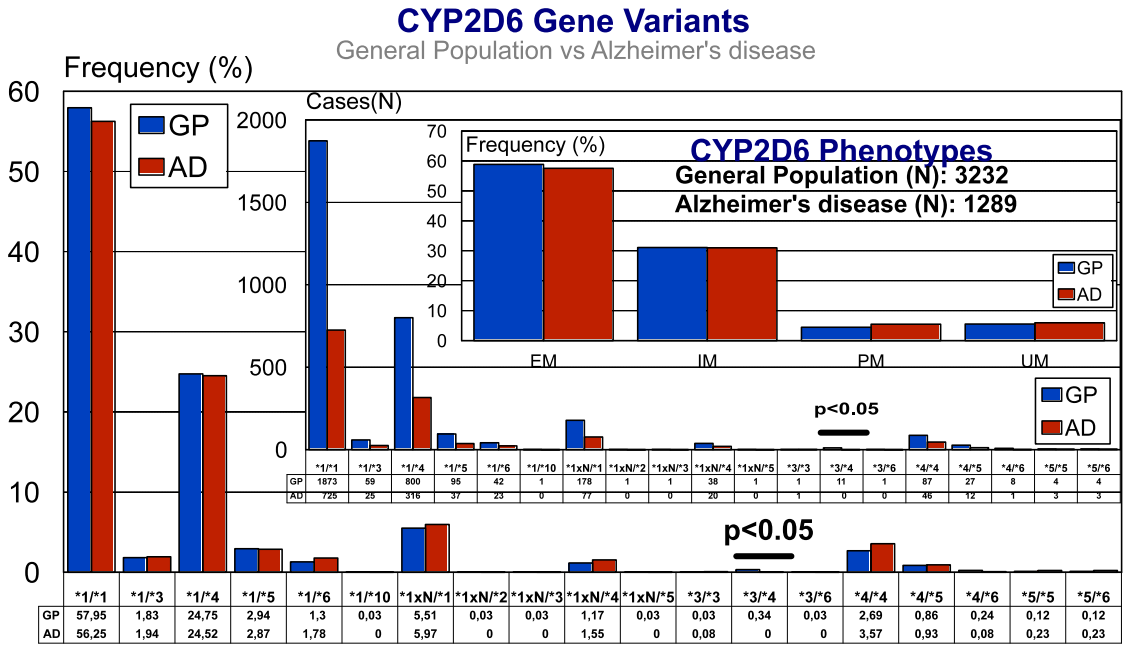


Figure 5. Distribution and frequency of *CYP2D6* gene variants and extensive (EM), intermediate (IM), poor (PM) and ultra-rapid metabolizers (UM) in the Spanish general population vs Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

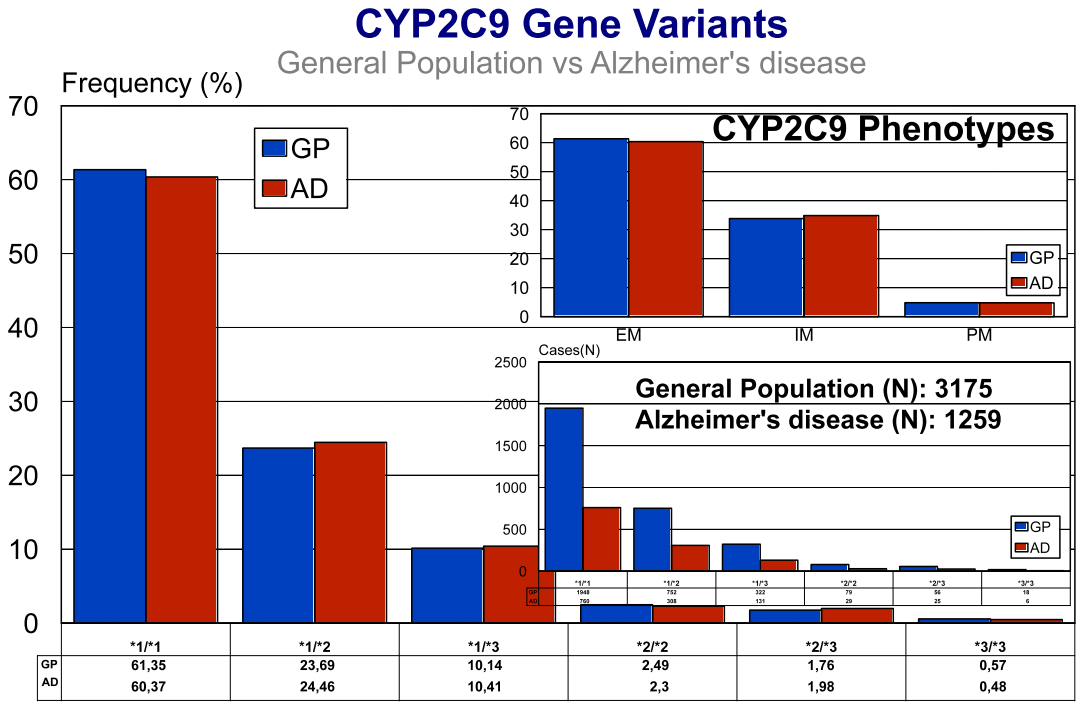


Figure 6. Distribution and frequency of *CYP2C9* gene variants and extensive (EM), intermediate (IM), and poor metabolizers (PM) in the Spanish general population vs Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

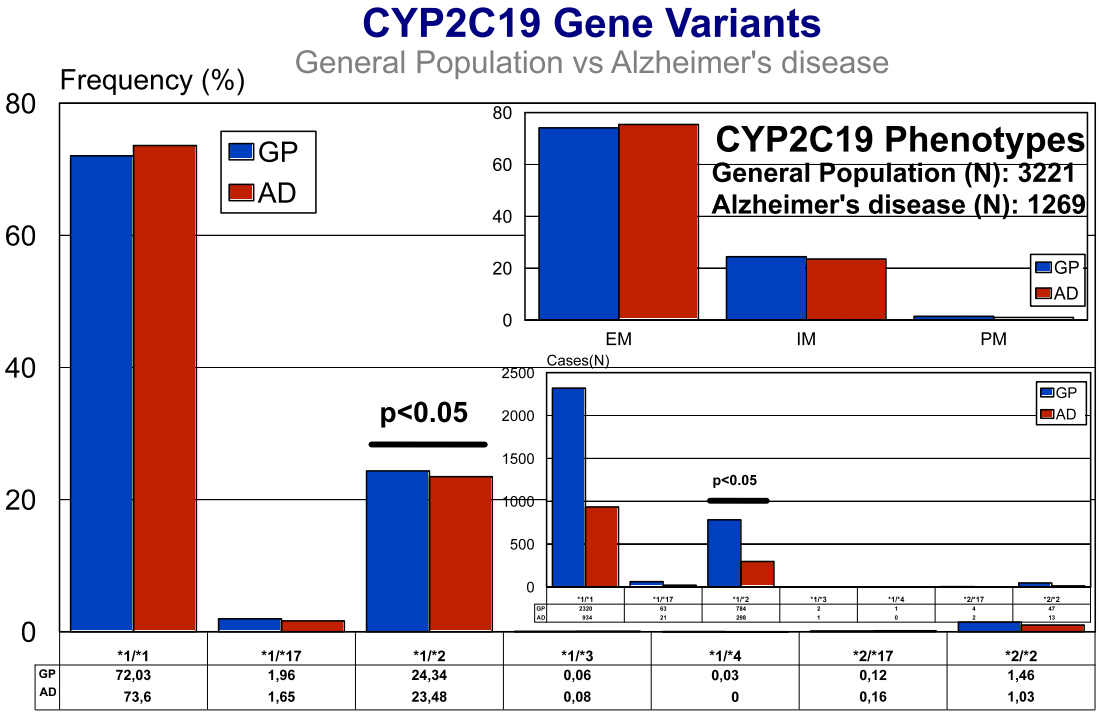


Figure 7. Distribution and frequency of *CYP2C19* gene variants and extensive (EM), intermediate (IM), and poor metabolizers (PM) in the Spanish general population vs Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

the response rate was 27.8%, with no apparent effect of APOE and/or CYP2D6 polymorphic variants[79].

The effects of galantamine are potentially influenced by *APOE*, *APP*, *ACHE*, *BCHE*, *CHRNA4*, *CHRNA7*, *CHRNB2* variants. This drug is a major substrate of CYP2D6, CYP3A4, and UGT1A1, and an inhibitor of ACHE and BCHE[38,68,69,80-82] (**Table 1**). Major metabolic pathways are glucuronidation, O-demethylation, N-demethylation, N-oxidation, and epimerization[83]. Galantamine is extensively metabolized by the enzymes CYP2D6 and CYP3A and is a substrate of the P-gp. *CYP2D6* variants are determinant for galantamine pharmacokinetics. CYP2D6-PMs exhibit higher dose-adjusted galantamine plasma concentrations than heterozygous and homozygous CYP2D6-EMs[84]; however, these pharmacokinetic changes might not substantially affect pharmacodynamics[85]. The co-administration of galantamine with paroxetine (a CYP2D6 strong inhibitor), ketoconazole (a CYP3A4 strong inhibitor) and erythromycin increases its bioavailability[86,87]. Interaction with foods and nutritional components may alter galantamine bioavailability and therapeutic effects[88].

APOE, *APP*, *CHAT*, *ACHE*, *BCHE*, *CHRNA4*, *CHRNB2* and *MAPT* variants may affect rivastigmine pharmacokinetics and pharmacodynamics, but CYP enzymes are not involved in the metabolism of rivastigmine[38,68,69,86,89]. UGT2B7-PMs show higher rivastigmine levels with a poor response to treatment[90].

ACHE, ABCB4, *BCHE*, *CHRNA4*, *CHRNB2*, *APOE*, *MTHFR*, *CES1*, *LEPR*, *GSTM1* and *GSTT1* variants may affect the therapeutic and toxic effects of tacrine (the first AChEI introduced in 1993 and stopped years later due to hepatotoxicity). Tacrine is a major substrate of CYP1A2 and CYP3A4, a minor substrate of CYP2D6, and is transported via SCN1A and ABCB4. Tacrine is an inhibitor of ACHE, BCHE, and CYP1A2[38]. Both tacrine and some tacrine-hybrids may cause an induction of *CYP1A1, 2B1* and *3A2* expression[91]. Tacrine is associated with transaminase elevation in up to 50% of patients. The mechanism of tacrine-induced liver damage is influenced by genetic factors. The strongest association was found between alanine aminotransferase levels and three *ABCB4* SNPs[92].

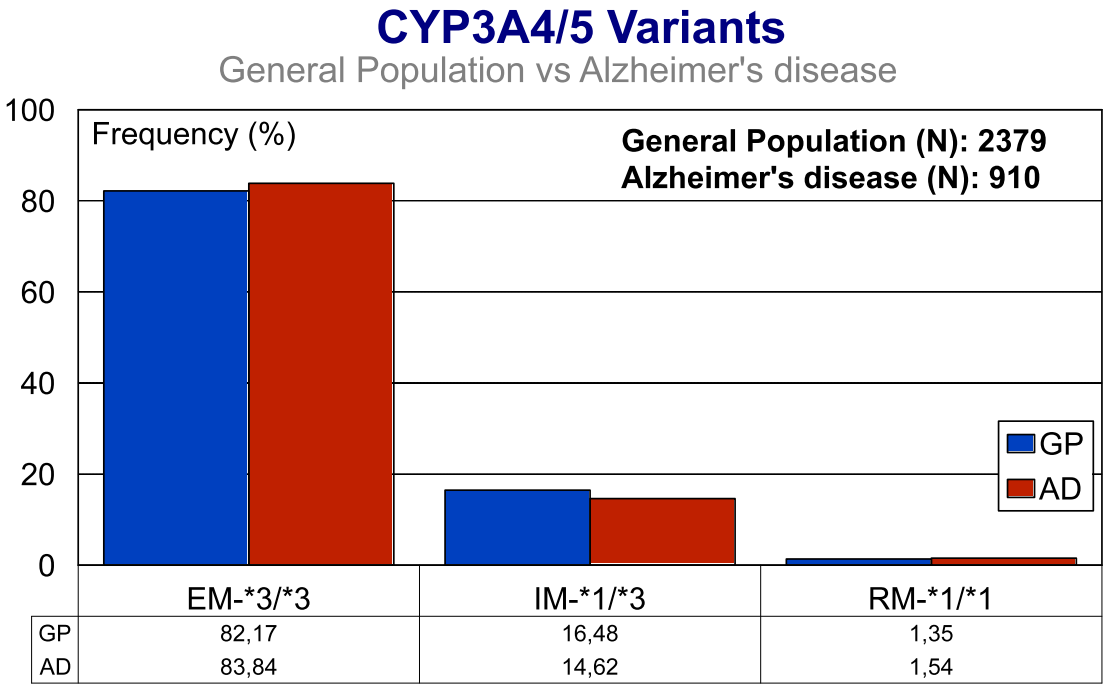


Figure 8. Distribution and frequency of *CYP3A4/5* gene variants and extensive (EM), intermediate (IM), and rapid metabolizers (RM) in the Spanish general population vs Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

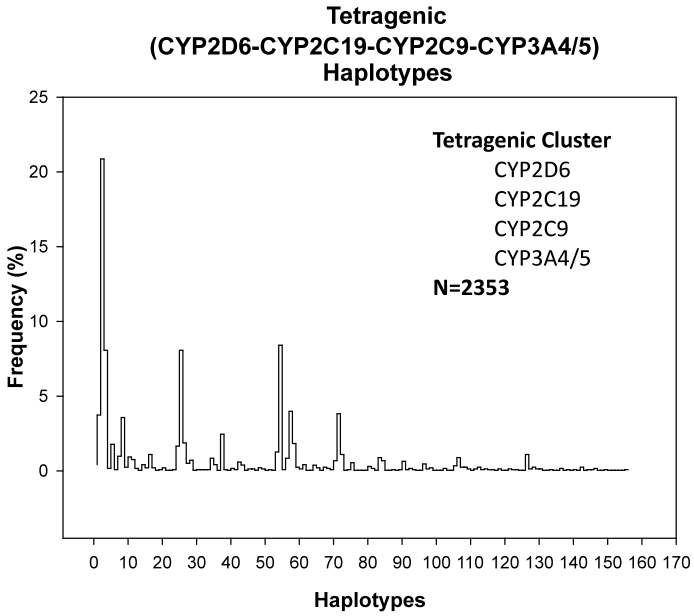


Figure 9. Tetragenic (*CYP2D6-CYP2C19-CYP2C9-CYP3A4/5*) haplotypes in the Spanish population.
Source: R. Cacabelos. CIBE DataBase, 2016.

Memantine is an N-Methyl-D-Aspartate (NMDA) receptor antagonist which binds preferentially to NMDA receptor-operated cation channels; it may act by blocking actions of glutamate, mediated in part by NMDA receptors, and is also an antagonist of GRIN2A, GRIN2B, GRIN3A, HTR3A and CHRFAM7A. Several pathogenic (*APOE*, *PSEN1*, *MAPT*) and mechanistic gene variants (*GRIN2A*, *GRIN2B*, *GRIN3A*, *HTR3A*, *CHRFAM7A, c-Fos, Homer1b* and *PSD-95*) may influence its therapeutic effects. Memantine is a strong inhibitor of CYP2B6 and CYP2D6, and a weak inhibitor of CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2E1, and CYP3A4[38,69,93]. In human liver microsomes (HLM), memantine inhibits CYP2B6 and CYP2D6 activities, decreases CYP2A6 and CYP2C19 activities, and has no effect on CYP1A2, CYP2E1, CYP2C9, or CYP3A4 activities[94]. The co-administration of memantine with CYP2B6 substrates elicits a 65% decrease in its metabolism. In clinical studies, *NR1I2* rs1523130 was identified as the unique significant genetic covariate for memantine clearance, with carriers of the *NR1I2* rs1523130 CT/TT genotypes presenting a 16% slower memantine elimination than carriers of the CC genotype[95].

TRANSPORTERS

Polymorphic variants in genes encoding transporter proteins may affect drug metabolism, brain penetrance and accessibility to neuronal/glial targets, and drug resistance[96-98]. Of special importance in AD are the ABC and SLC family genes[98]. ABC genes (*ABCB1*, *ABCC1*, *ABCG2*), and other genes of this family encode proteins which are essential for drug metabolism and transport. Mutations in ABC transporters influence pathogenesis and therapeutics of brain disorders[98,99]. The multidrug efflux transporters (P-gp1/MDR1, multidrug-resistance associated protein 4 (MRP4), breast cancer resistance protein (BCRP)), are located on endothelial cells lining brain vasculature and play important roles in limiting movement of substances into and enhancing their efflux from the brain.

ABCB1 is one of the most important drug transporters in the brain. Over 1270 drugs have been reported to be associated with the Abcb1 transporter protein (P-gp), of which 490 are substrates, 618 are inhibitors, 182 are inducers, and 269 additional compounds which belong to different pharmacological categories of products with potential Abcb1 interaction[38]. The *ABCB1* gene has 116 polymorphic sites in Caucasians and 127 in African-Americans, with a minor allele frequency greater than 5%. Common variants are 1236C>T, 2677G>A/T and 3435C>T, and the *ABCB1*13* haplotype involves the 1236, 2677 and 3435 (TTT) SNPs and 3 intronic SNPs (in intron 9, 13, and 14)[38]. The *ABCB1* C1236T, G2677T/A and C3435T SNPs influence blood-brain barrier (BBB) P-glycoprotein function. AD patients with one or more T in C1236T, G2677T and C3435T have significantly higher binding potential values than patients without a T. Genetic variations in *ABCB1* might contribute to the progression of Aβ deposition in the brain[100] and some *ABCB1* SNPs (C1236T in exon 12, G2677T/A in exon 21 and C3435T in exon 26) and inferred haplotypes might represent novel biomarkers of AD[101]. ABCB1 directly transports Aβ from the brain into the blood circulation, whereas the cholesterol transporter ABCA1 neutralizes Aβ aggregation capacity in an APOE-dependent manner, facilitating subsequent Aβ elimination from the brain[102]. Some *ABCB1* variants are frequent in AD cases over 65 years of age and among females. This association of *ABCB1* 2677G>T (rs2032582) is more pronounced in *APOE4*-negative cases[100].

Some other ABCs have shown potential association with AD[98,103]. The G allele of the *ABCA7* rs115550680 SNP is associated with AD in Europeans. The effect size for the SNP in *ABCA7* was comparable with that of the *APOE ε4*-determining SNP rs429358[104]. ABCG2 is involved in Aβ transport and is up-regulated in AD brains. The *ABCG2* gene (C421A; rs2231142) (*ABCG2* C/C genotype) is associated with AD and the *ABCG2* C/C genotype and the *APOE ε4*

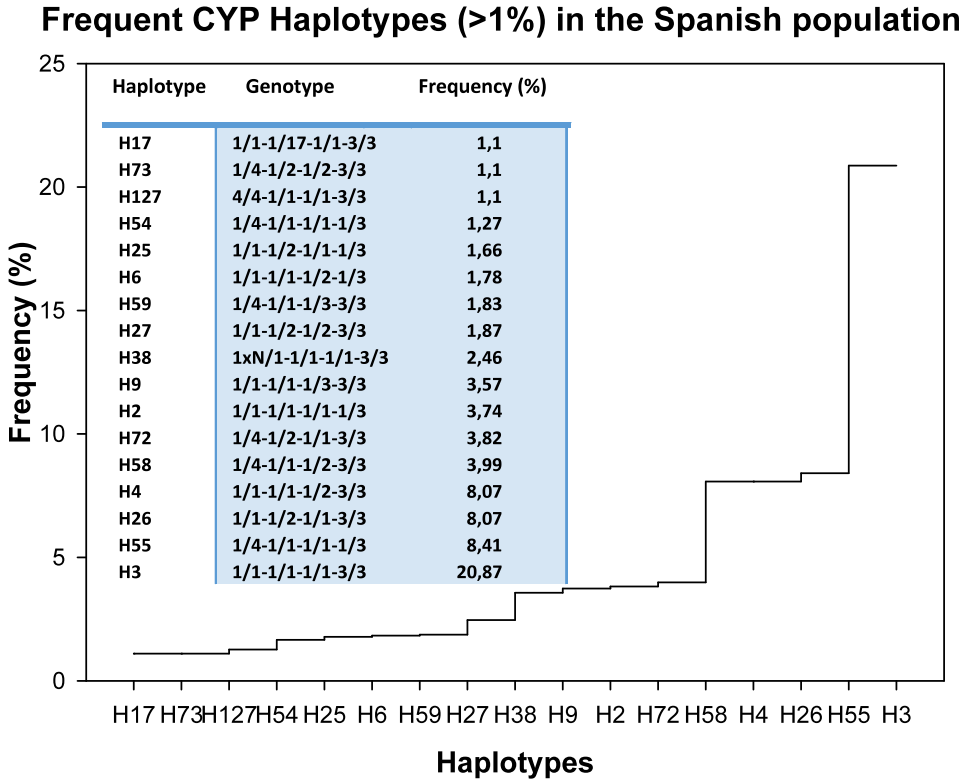


Figure 10. Fequent tetragenic *CYP* haplotypes (>1%) in the Spanish population.
Source: R. Cacabelos. CIBE DataBase, 2016.

allele may exert an interactive effect on AD risk[105]. Also of importance for AD pharmacogenomics are transporters encoded by genes of the solute carrier superfamily (*SLC*) and solute carrier organic (*SLCO*) transporter family, responsible for the transport of multiple endogenous and exogenous compounds, including folate (*SLC19A1*), urea (*SLC14A1-2*), monoamines (*SLC29A4*, *SLC22A3*), aminoacids (*SLC1A5*, *SLC3A1*, *SLC7A3*, *SLC7A9*, *SLC38A1*, *4-5*, *7*, *SLC43A2*, *SLC45A1*), nucleotides (*SLC29A2-3*), fatty acids (*SLC27A1-6*), neurotransmitters (*SLC6A2* (noradrenaline transporter), *SLC6A3* (dopamine transporter), *SLC6A4* (serotonin transporter, SERT), *SLC6A5-6*, *9*, *11*, *12*, *14-19*), glutamate (*SLC1A6-7*), and others[98,106]. Some organic anion transporters (OAT), which belong to the solute carrier (SLC) 22A family, are also expressed at the BBB, and regulate the excretion of endogenous and exogenous organic anions and cations[107]. The transport of amino acids and di- and tripeptides is mediated by a number of different transporter families, and the bulk of oligopeptide transport is attributable to the activity of members of the SLC15A superfamily (SLC15A1-2, SLC15A2, SLC15A3-4). ABC and SLC transporters expressed at the BBB may cooperate to regulate the passage of different molecules into the brain[6,10,27,108].

GENETIC DETERMINANTS ASSOCIATED WITH LIPID METABOLISM AND CHOLESTEROL RESPONSE TO HYPOLIPEMIC DRUGS IN HYPERCHOLESTEROLEMIC PATIENTS WITH AD

Among hundreds of genes potentially involved in AD pathogenesis and concomitant disorders (cardiovascular and cerebrovascular disorders, hypercholesterolemia), at least 4 categories of genes deserve special attention: (i) genes associated with lipid metabolism: *APOB* (OMIM 107730; rs693 [7545C>T]; risk SNP 7545T)(participates in the atherogenic process in cooperation with VLDL, IDL and LDL); *APOEC3* (OMIM 107720; rs5128 [3175G>C, S1/S2]; risk SNP 3175G (S2))(associated with triglyceride levels; inhibits the activity of lipoprotein lipase and hepatic lipase); *APOE* (OMIM 107741; rs429358/rs7412 [112T>C/158T>C, *E2*, *E3*, *E4*]; risk SNP 112C/158C (*E4*)) (encodes apolipoprotein E, involved in the catabolism of triglyceride-rich lipoproteins and cholesterol homeostasis); *CETP* (OMIM 118470; rs708272 [+279G>A, B1/B2]; risk

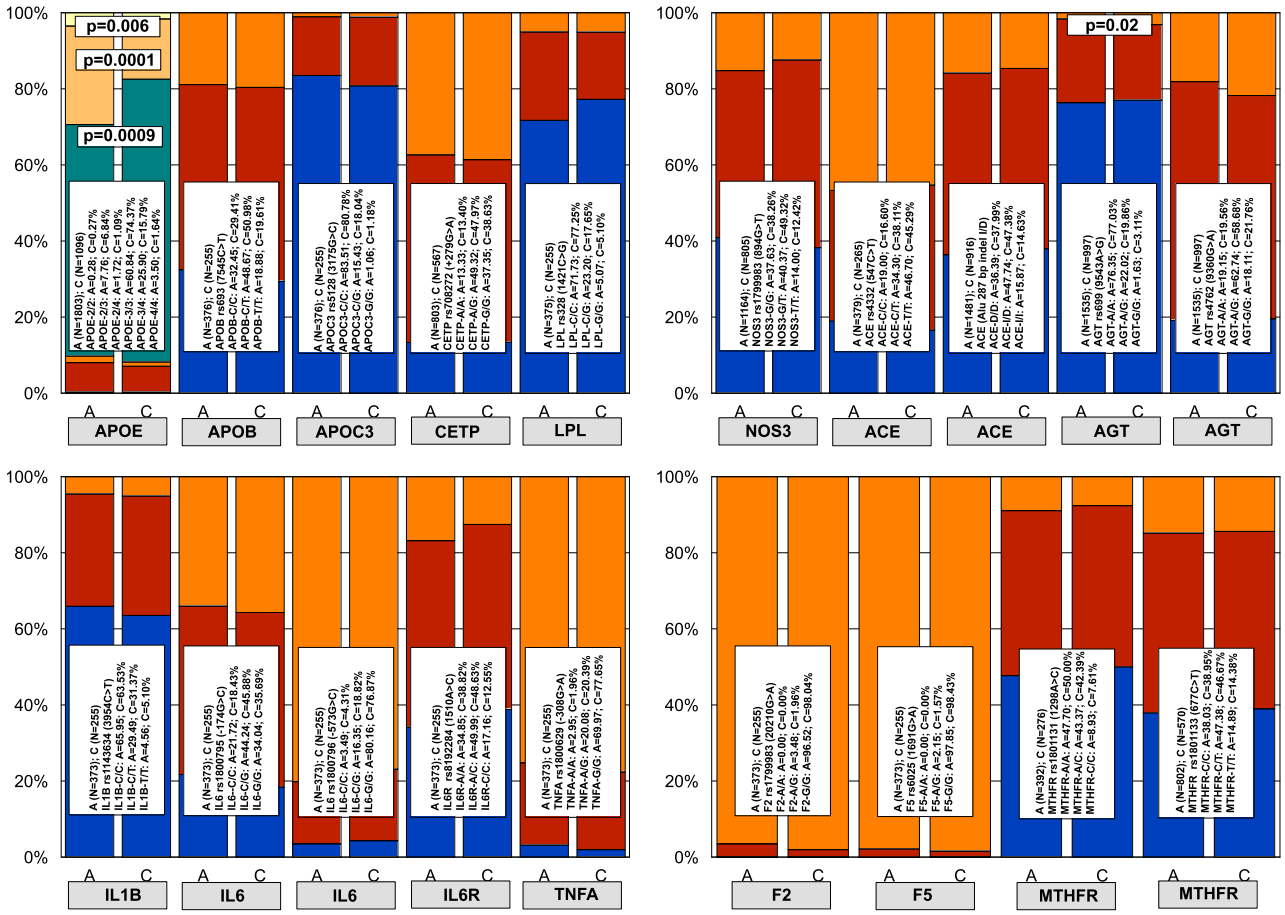


Figure 11. Distribution and frequency of the *APOE, APOB, APOC3, CETP, LPL, NOS3, ACE, AGT, IL1B, IL6, IL6R, TNFA, F2, F5* and *MTHFR* genes in the general population and in patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

SNP +279G (B1))(contributes to eliminate cholesterol from tissues via reverse cholesterol transport); and *LPL* (OMIM 609708; rs328 [1421C>G, S474X]; protective SNP 1421G) (hydrolyzes triglycerides which are part of VLDL and chylomicrons and removes lipoproteins from circulation) [28,109-112]; (ii) genes associated with endothelial function and hypertension: *NOS3* (OMIM 163729; rs1799983 [894G>T]; risk SNP 894T)(encodes nitric oxide synthase 3 which synthesizes nitric oxide (NO) from the amino acid arginine); *ACE* (OMIM 106189; rs4332 [547C>T]; risk SNP 547T)(hydrolyzes angiotensin I to angiotensin II, a potent vasopressor and aldosterone-stimulating peptide, and inactivates bradykinin, a potent vasodilator); and *AGT* (OMIM 1906150; rs699 [9543A>G, T174M]; risk SNP 174M; rs4762 [9360G>A, M235T]; risk SNP 235T)(encodes angiotensinogen, which is converted into angiotensin I by renin)[38,113-116]; (iii) genes associated with immune function and inflammation: *IL1B* (OMIM 147720; rs1143634 [3954C>T]; risk SNP 3954T)(encodes interleukin-1β, which is involved in the modulation of the inflammatory reaction in thrombus formation); *IL6* (OMIM 147620; rs1800795 [-174G>C]; risk SNP -174C; rs1800796 [-573G>C]; risk SNP -573C)(encodes interleukin-6, a pleiotropic cytokine involved in the regulation of the acute phase reaction, immune response, hematopoiesis, and platelet production); *IL6R* (OMIM 147880; rs8192284 [1510A>G]; risk SNP 1510C)(encodes a subunit of the IL6 receptor complex); and *TNFA* (OMIM 191160; rs1800629 [-308G>A]; risk SNP -308A)(encodes tumor necrosis factor, a proinflammatory cytokine that influences lipid metabolism, coagulation,

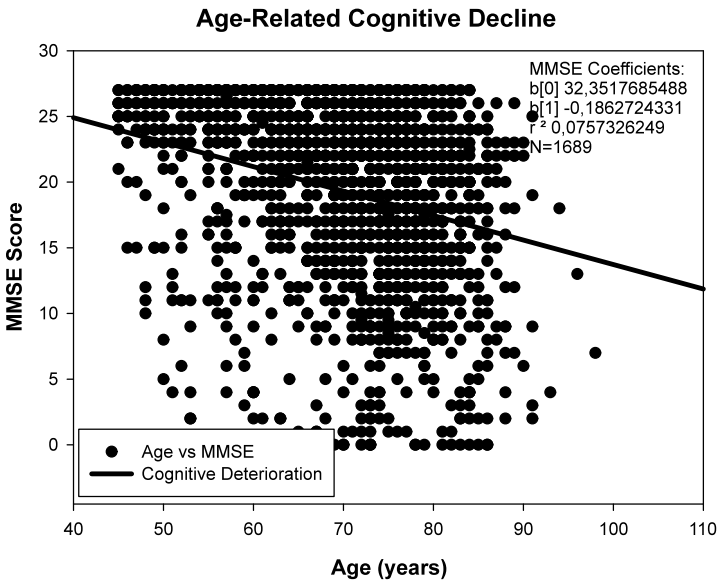


Figure 12. Age-related cognitive decline in patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

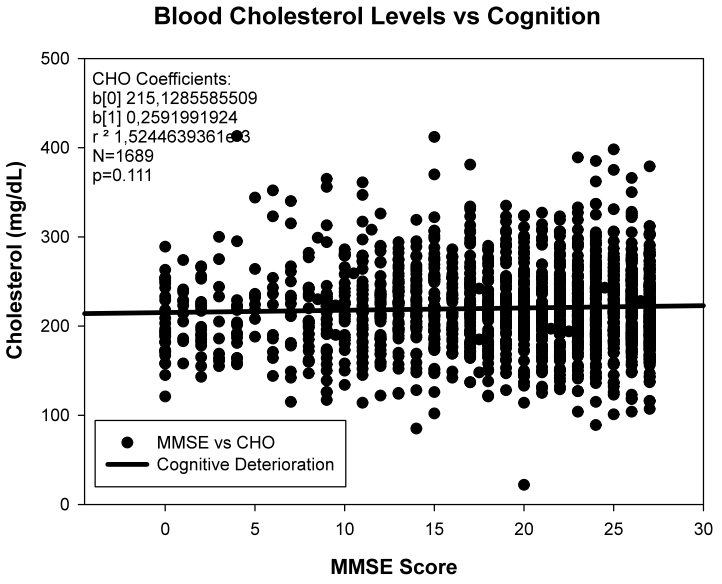


Figure 13. Blood total colesterol levels vs cognitive performance (MMSE Score) in patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

insulin resistance and endothelial function)[38,117-127]; and (iv) genes associated with thrombosis and coagulation: *F2* (OMIM 17693; rs1799983 [20210G>A]; risk SNP 2021A)(encodes Coagulation Factor 2 (Prothrombin), involved in blood clotting); *F5* (OMIM 227400; rs6025 [1691G>A]; risk SNP 1691A)(encodes Factor V Leiden, an important factor involved in blood coagulation); and *MTHFR* (OMIM 607093; rs1801133 [677C>T]; risk factor 677T; rs1801131 [1298A>C]; risk SNP 1298A)(encodes methylenetetrahydrofolate reductase, an enzyme that catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for the re-methylation of homocysteine to methionine)[38,128-132].

Although differences in genotype distribution and frequencies of all these genes between patients with AD and control subjects are negligible, except in the case of *APOE*[133] (**Figure 11**) some of them may influence the pharmacogenetic outcome in the treatment of major risk factors for dementia, such as hypercholesterolemia, cardiovascular disorders and hypertension[133-137]. Furthermore, many of these genes interact in pathogenic cascades contributing to alter brain cholesterol and Aβ metabolism, subsequently accelerating neuronal death in AD.

PHARMACOGENETICS OF HYPERCHOLESTEROLEMIA IN ALZHEIMER'S DISEASE

Alterations in cholesterol (CHO) metabolism are involved in AD pathogenesis and over 40% of AD patients are hypercholesterolemic. Cognitive deterioration shows a clear age-dependent profile (**Figure 12**), with an average decline on 3-5 points/year (MMSE score); however, total CHO levels do not appear to affect mental deterioration in AD (**Figure 13**). Blood lipid levels also show a moderate age-dependent profile (**Figure 14**). In the GP, CHO levels tend to increase with age reaching a plateau at 60-70 years of age, declining thereafter; however, CHO levels in AD tend to diminish in an age-related fashion (**Figure 15**).

In a group of AD patients (N=920) recruited for pharmacogenomic studies treated with a multifactorial therapy for one year[61], we evaluated the effects of Sardilipin (E-SAR-94010; LipoEsar®)(500 mg/day)(nutraceutical with lipid-lowering effects and anti-atherosclerotic and neuroprotective properties, Patent ID: P9602566)[6,38,138] (whole group), and atorvastatin (10 mg/day)(patients with hypercholesterolemia >220 mg/dL)(43.48%)(first month of treatment) on lipid metabolism (total-cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides) according to the *APOE* and *CYP* genotypes of the patients. From these

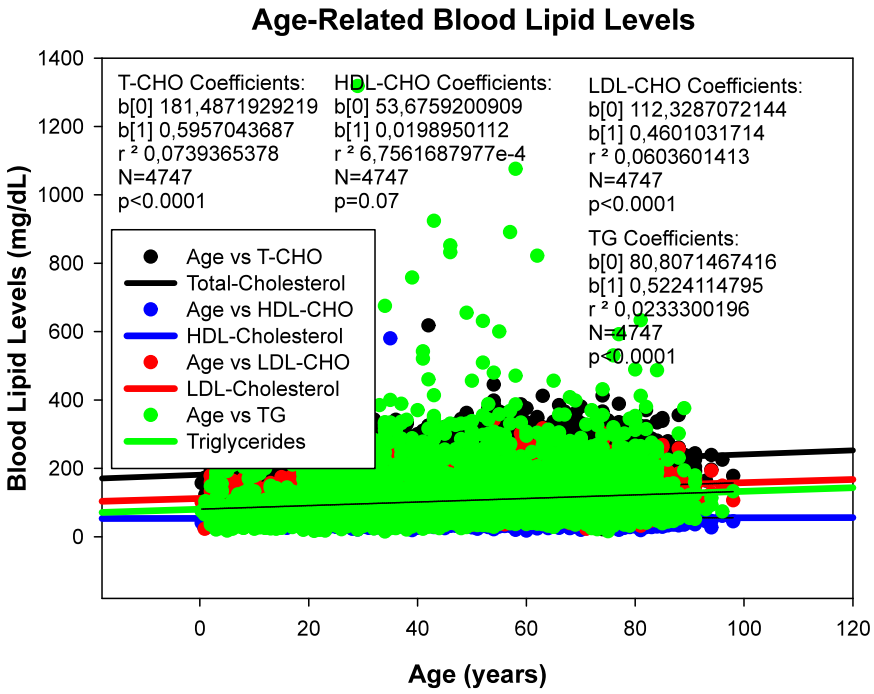


Figure 14. Age-related blood lipid levels in the general population.
Source: R. Cacabelos. CIBE DataBase, 2016.

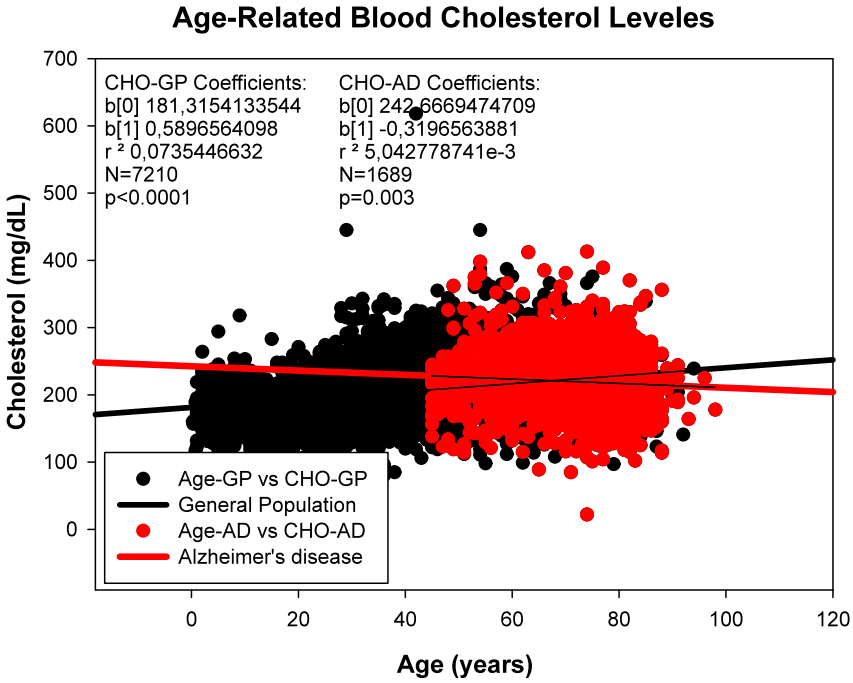


Figure 15. Age-related blood colesterol levels in the general population and in patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

studies we obtained interesting results which enable us to infer some conclusions with important repercussions on the pharmacogenetics of AD: (i) Body Mass Index (BMI) is not affected by total cholesterol (T-CHO) or LDL-CHO; however, there is a clear positive correlation between BMI and triglyceride (TG) levels and an inverse correlation between BMI and HDL-CHO. (ii) Liver transaminase activity is important for lipid metabolism. ASAT, ALAT and GGT exhibit different correlation patterns in relation to lipid levels. ASAT shows an inverse correlation with T-CHO and LDL-CHO; ALAT and GGT activities increase in parallel with TG levels, and tend to show an inverse correlation with HDL-CHO. (iii) Hypercholesterolemic females and males with AD show a similar response to the combination of Atorvastatin+LipoEsar, but more females (60%) are hypercholesterolemic than males (<20%). (iv) CHO levels are APOE-dependent. *APOE-4/4* carriers exhibit the highest CHO levels. *APOE-2/3*, *APOE-3/4* and *APOE-4/4* carriers experience a gradual age-dependent decrease in CHO levels. (v) The therapeutic response of CHO to Atorvastatin+LipoEsar is *APOE*-dependent. *APOE-3/3* and *APOE-3/4* carriers are the best responders and *APOE-2/4* and *APOE-4/4* carriers are the worst responders. (vi) Basal CHO levels are similar in CYP2D6-EMs, IMs, PMs and UMs. CYP2D6-EMs and IMs show a significant decrease in CHO levels in response to Atorvastatin+LipoEsar, whereas PMs and UMs exhibit a poorer CHO-lowering effect. (vii) Basal CHO levels are higher in CYP2C9-IMs than in EMs. CYP2C9-EMs and IMs effectively respond to Atorvastatin+LipoEsar, with a significant reduction in CHO levels, and *CYP2C9*-PMs do not respond. (viii) Basal CHO levels are non-significantly higher in CYP2C19-EMs and IMs than in PMs. CYP2C19-EMs and IMs significantly respond to Atorvastatin+LipoEsar, and PMs do not show any effect. (ix) CYP3A4/5-EMs show a significant decrease in CHO levels after one month of treatment with Atorvastatin+LipoEsar. This response is similar for LDL-CHO in EMs and IMs. In hypercholesterolemic patients, over 80% of EMs respond to 20 mg of Atorvastatin + 500 mg of LipoEsar, with an almost complete normalization of CHO levels. The effect in IMs is spectacular, with over 90% of the patients experiencing a drastic reduction in CHO levels, 50% of them entering into a condition of iatrogenic hypocholesterolemia; and 60% of RMs do not respond at all.

In a larger study with 1345 hypercholesterolemic AD patients (CHO>220 mg/dL)(**Figure 16**) we investigated the pharmacogenetics of cholesterol response to the hypolipemic compounds Atorvastatin+LipoEsar for one month. In the whole sample, the response rate (RR) was 78.95% responders (CHO<baseline levels) and 21.04% non-responders (CHO≥baseline levels). APOE-related basal

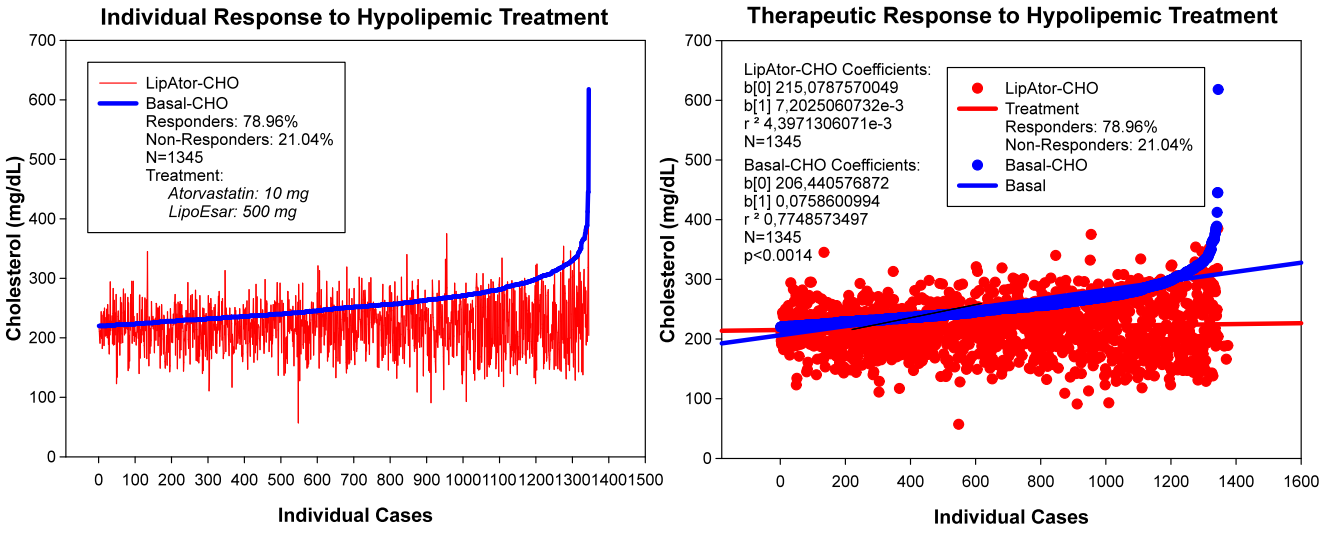


Figure 16. Individual profile of cholesterol response to a combination treatment (Atorvastatin + LipoEsar) in patients with Alzheimer's disease (upper panel) and correlation analysis (basal cholesterol levels vs treatment) of the response of cholesterol to a combination treatment in patients with Alzheimer's disease (lower panel).
Source: R. Cacabelos. CIBE DataBase, 2016.

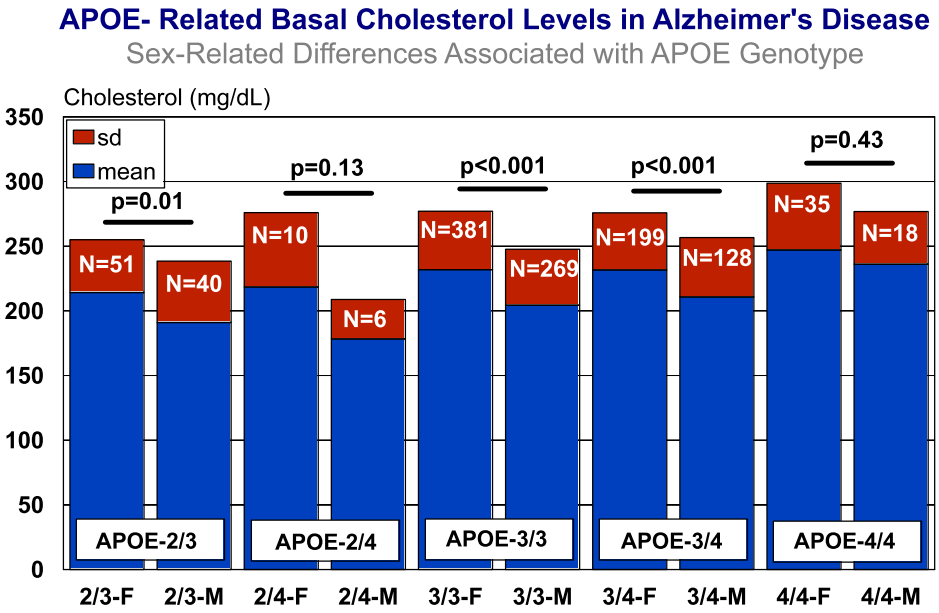


Figure 17. *APOE*- and sex-related basal colesterol levels in patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

CHO levels are significantly different, with females showing higher CHO levels than males (**Figure 17**); however, females and male responded similarly to the hypolipemic treatment (**Figure 18**). The stratification of patients according to their *APOE, APOB, APOC3, CETP* and *LPL* genotypes showed no genotype-related differences at basal CHO levels, except in the case of APOE carriers where the highest baseline levels of CHO were found in APOE-4/4 carriers and the lowest levels in APOE-2/2 carriers (**Figure 19**), in addition to a clear age-related profile (**Figure 19**).

In a selected group of 933 AD patients we constructed a pentagenic haplotype integrating all possible variants of the *APOE+APOB+EPOC3+CETP+LPL* genes and identified 111 haplotypes (H)(**Figure 20**) with differential basal CHO levels (**Figure 21**). About 75% of these haplotypes in the AD population have a frequency below 1%, 10% have a frequency between 1% and 2%, 8% have a frequency between 2% and 5%, and only 4% of the haplotypes are present in more than 5% of AD patients (**Figure 20**). The haplotypes most frequently found are *H55* (33-CT-CC-AG-CC)(8.79%), *H58* (33-CT-CC-GG-CC) and *H37* (33-CC-CC-AG-CC)(7.07%). Haplotypes *H104* (44-CC-CC-AA-CC)(0.11%), *H110* (44-TT-CC-AG-CG)(0.11%) and *H98* (34-TT-CC-AA-CG) (0.11%) showed the highest CHO levels, and the lowest levels corresponded to haplotypes *H26* (23-TT-CG-AG-CC) (0.11%), *H8* (23-CC-CG-AG-CC)(0.21%), *H50* (33-CC-GG-AG-CC)(0.21%), and *H63* (33-CT-CG-AA-GG)(0.11%) (**Figure 21**).

Basal CHO levels tend to be higher in AD patients as compared to GP levels (**Figures 22-23**). APOE-related blood total CHO profiles are qualitatively distinct among carriers of different APOE genotypes (**Figure 24**). The results of *APOE*-related cholesterol response to hypolipemic treatment in hypercholesterolemic AD patients revealed that in absolute terms all *APOE* variants respond similarly (RR>70%) to treatment with a significant reduction in

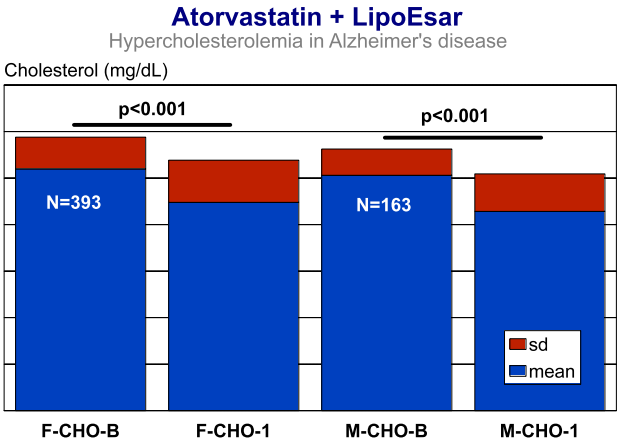


Figure 18. Sex-related colesterol response to Atorvastatin and LipoEsar in patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

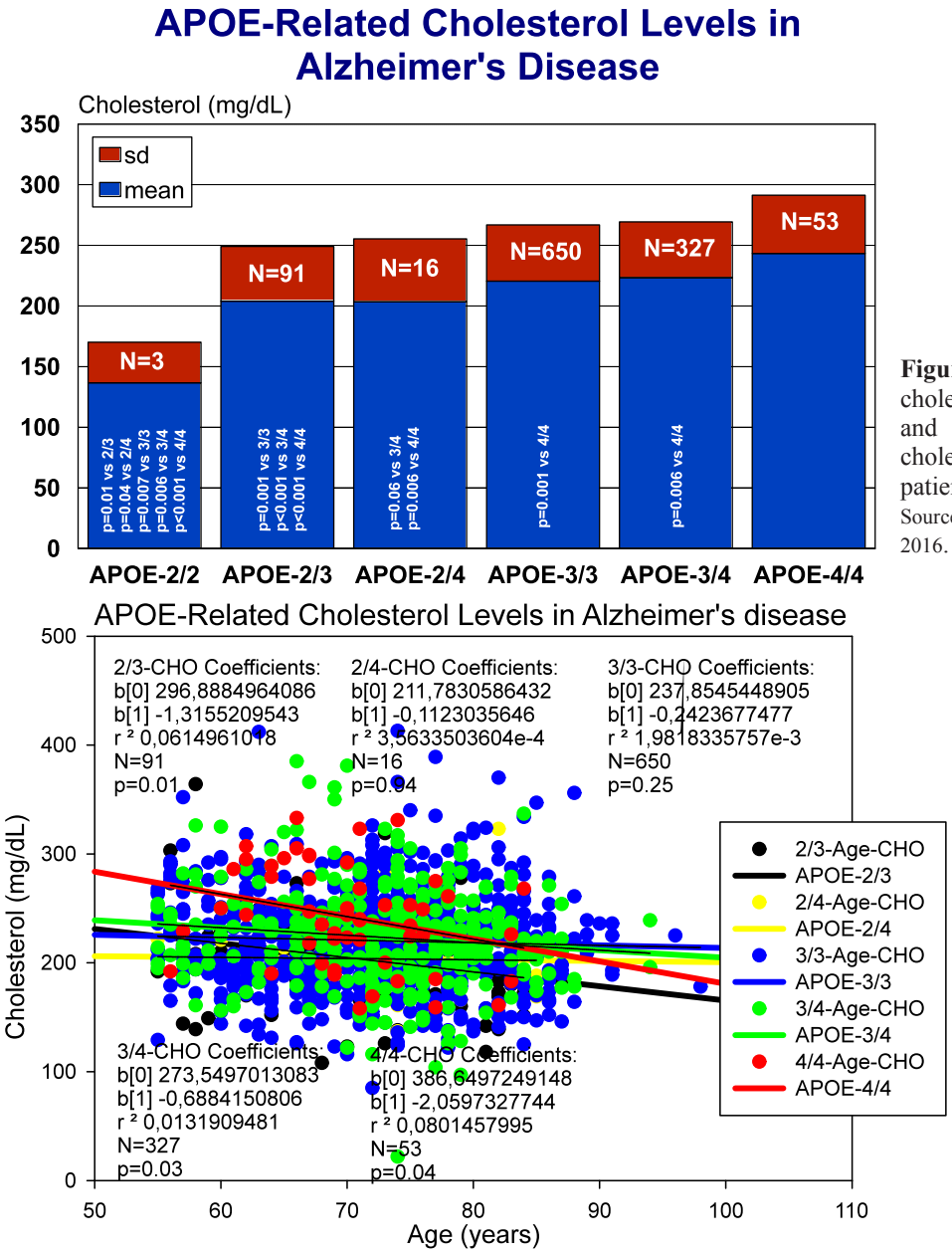


Figure 19. *APOE*-related basal cholesterol levels (upper panel) and *APOE*- vs age-related basal cholesterol levels (lower panel) in patients with Alzheimer's disease. Source: R. Cacabelos. CIBE DataBase, 2016.

CHO levels (p<0.001)(**Figures 25-26**); however, genotype-related correlation analysis case-by-case (**Figure 27**) and comparative correlation analyses of *APOE* variants (**Figure 28**) show a clear differential *APOE*-related pattern of CHO response to treatment.

Carriers of *APOB*-C/C, *APOB*-C/T and *APOB*-T/T variants exhibit a similar response (RR>80%), with a significant decrease in CHO levels after treatment (**Figure 29**) and almost identical efficiency in comparative analyses (**Figure 30**). *APOC3*-C/C, *APOC3*-C/G and *APOC3*-G/G carriers also respond similarly (p<0.001)(RR>80%)(**Figure 31**), with a differential comparative profile (**Figure 32**). *CETP*-A/A, *CETP*-A/G and *CETP*-G/G carriers show an identical response (p<0.001; RR>80%)(**Figure 33**), with insignificant variability in comparative studies (**Figure 34**). The same therapeutic response is observed in *LPL*-C/C, *LPL*-C/G and *LPL*-G/G carriers (p<0.001; RR>80%)(**Figure 35**), though in this case *LPL*-C/C carriers are the best responders, *LPL*-C/G carriers are intermediate responders, and *LPL*-G/G carriers are the most heterogeneous responders (**Figure 36**).

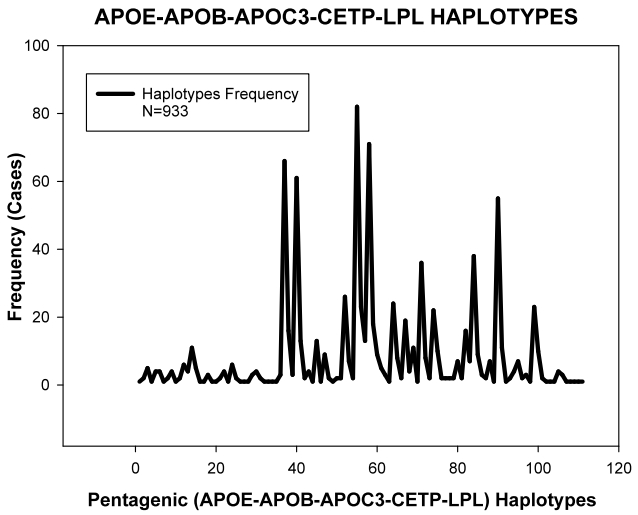


Figure 20. Distribution and frequency of pentagenic haplotypes integrating *APOE, APOB, APOC3, CETP* and *LPL* genotypes in patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

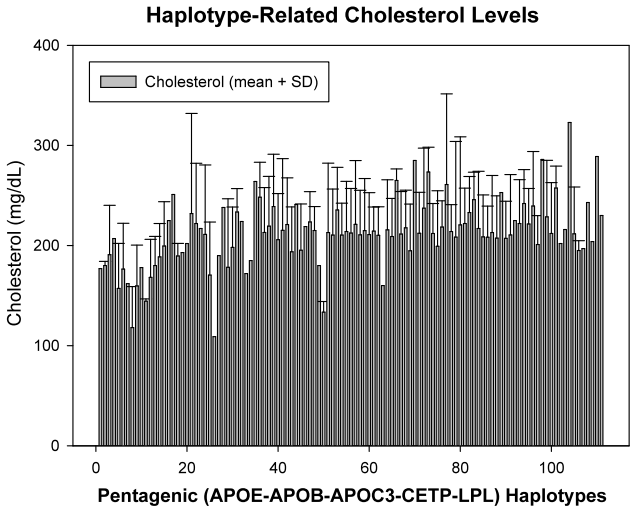


Figure 21. Pentagenic haplotype-related basal cholesterol levels in patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

CYP haplotype-related blood total CHO levels are very heterogeneous (**Figure 37**), but absolute values of total CHO among the most frequent haplotypes are almost identical (**Figure 38**). The histograms of frequency associated with CHO levels are qualitatively different among carriers of different CYP variants (**Figures 39-40**). Basal CHO levels are higher in AD patients harboring the CYP2D6-*1/*1 ($p<0.05$) and *1xN/*1 genotypes ($p<0.003$) than in the corresponding GP genotypes (**Figure 41**), but no differences have been found according to the EM, IM, PM or UM condition (**Figure 42**). The therapeutic response according to SNPs of metabolic genes (*CYP2D6, CYP2C9, CYP2C19, CYP3A4/4*) in hypercholesterolemic patients is variable and geno-phenotype-dependent. Although all CYP2D6 variants exhibit a positive response to treatment, significant differences have only been detected in 2D6-*1/*1 ($p<0.001$), 2D6-*1/*4 ($p<0.001$) and 2D6-*1/*6 carriers ($p<0.05$)(**Figure 43**). In absolute values, CYP2D6 extensive (EM), intermediate (IM), poor (PM) and ultra-rapid (UM) metabolizers behave in a similar manner with a significant reduction in CHO levels ($p<0.001$)(**Figure 44**); however, the RR is different in EMs (81%), IMs (78%), PMs (84%), and UMs (90%)(**Figure 44**), indicating a variable efficiency of CYP2D6 enzymes (**Figures 45-46**). The comparative analysis indicates that carriers of mutant enzymes (PMs>UMs), with limitations in drug metabolism, display a more efficient response to hypolipemic treatment (**Figures 45-46**).

No differences are present in basal CHO levels between the GP and AD patients (**Figures 47-48**). CYP2C9-EMs, IMs and PMs (**Figure 49**) show a similar response ($p<0.001$), with lower RR (75%) in PMs as compared with EMs (81%) and IMs (82%), and a clear differential comparative profile (**Figure 50**).

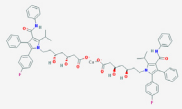
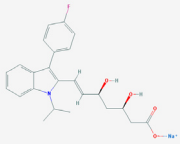
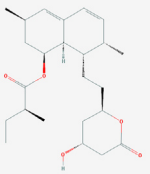
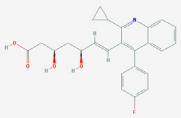
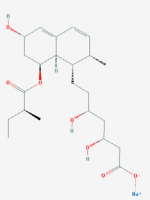
AD cases harboring the CYP2C19-*1/*2 genotype (**Figure 51**), corresponding to CYP2C19-IMs (**Figure 52**), exhibit higher basal CHO levels ($p<0.05$) than their homologous in the GP (**Figures 51-52**).

The CHO response among CYP2C19-EMs, IMs, PMs and UMs is more variable, with PMs showing a deficient response (RR=84%)(**Figure 56**) in comparison to EMs ($p<0.001$; RR=81%), IMs ($p<0.001$; RR=78%), and UMs ($p<0.001$; RR=90%)(**Figure 53**), and a clearly different behavioral profile, especially in PMs and UMs (**Figures 54-55**).

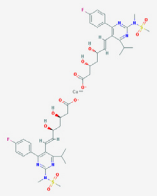
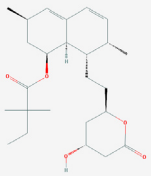
CYP3A4/5 geno-phenotypes in AD and GP show similar basal CHO levels (**Figure 56**). CYP3A4/5-RMs respond poorly to hypolipemic treatment, with the worst RR (66%), whereas CYP3A4/5-EMs and IMs exhibit an excellent response ($p<0.001$; RR>80%)(**Figures 57-58**).

Most of these effects can, in part, be explained on a pharmacogenetic basis. It is obvious that a simple stratification of patients according to single genotypes is of poor value for

Table 2. Pharmacological properties and pharmacogenetics of statins

HMG CoA reductase inhibitors		
Drug	**Properties**	**Pharmacogenetics**
	**Name: ATORVASTATIN CALCIUM**; Lipitor; 134523-03-8; Tahor; Sortis; CI-981 **Molecular Formula:** $C_{66}H_{68}CaF_2N_4O_{10}$ **Molecular Weight:** 1155.341726 g/mol **Mechanism**: Inhibits HMG-CoA reductase, resulting in a compensatory increase in the expression of LDL receptors on hepatocyte membranes and a stimulation of LDL catabolism. **Effect:** Anticholesteremic Agent; HMG-CoA Reductase Inhibitor; Apolipoprotein B reduction; Triglyceride reduction; Anti- atherosclerotic; Heart-health effects.	**Pathogenic genes:** *ABCA1, ACE, APOA1, APOA5, APOB, APOC3, APOE, CETP, FGB, GNB3, LDLR, LIPC, MMP3, MTTP, NOS3, PON1* **Mechanistic genes:** *ABCB1, ABCC1, APOA1, APOA5, APOB, APOC3, APOE, CRP, CYP11B2, HMGCR, IL10, IL6, LDLR, MMP3, PON1, TNF* **Metabolic genes:** **Substrate**: *CYP2C8, CYP3A4* (major), *CYP3A5* **Inhibitor**: *ABCB1, CYP2B6* (moderate), *CYP2C8, CYP2C9* (moderate), *CYP2C19* (strong), *CYP2D6* (moderate), *CYP3A4* (moderate), *HMGCR* **Inducer**: *CYP2B6, CYP7A1* **Transporter genes:** *ABCA1, ABCB1, ABCB11, ABCC1, ABCC2, ABCC3, ABCG2, SLCO1B1, SLCO1B3* **Pleiotropic genes:** *APOA1, APOE, CRP, CYP11B2, ESR1, FGB, GNB3, HTR3B, IL6, IL10, ITGB3, MMP3, NOS3, TNF, USP5*
	**Name: FLUVASTATIN SODIUM,** Lescol; Fluvastatin; Fluvastatin sodium salt; 93957-55-2; Sri-62320 **Molecular Formula:** $C_{24}H_{25}FNNaO_4$ **Molecular Weight:** 433.447772 g/mol **Mechanism**: Acts by competitively inhibiting HMGCR, the enzyme that catalyzes reduction of HMG-CoA to mevalonate. HDL is increased while total, LDL and VLDL cholesterols, apolipoprotein B, and plasma triglycerides are decreased. **Effect:** Anticholesteremic Agent; HMG-CoA Reductase Inhibitor; Heart-health effects; Antineoplastic activity; Immune response modulation	**Pathogenic genes**: *ABCA1, ACE, APOA1, APOA5, APOB, APOC3, APOE, CETP, CYP7A1, LDLR, LIPC, LPL, NOS3, PPARD, PON1* **Mechanistic genes:** *APOA1, APOB, HMGCR, LPL, PON1* **Metabolic genes:** **Substrate**: *CYP1A1* (minor), *CYP2C8* (minor), CYP2C9 (major), *CYP2D6* (minor), *CYP3A4* (minor), *UGT1A3* **Inhibitor**: *CYP1A2* (weak), *CYP2C8* (weak), *CYP2C9,* (moderate), *CYP2C19* (moderate), *CYP2D6* (moderate), *CYP3A4* (moderate), *HMGCR* **Inducer**: *CYP2B6, CYP3A4,* **Transporter genes:** *ABCA1, ABCB1, ABCB11, ABCC2, ABCG2, SLC15A1, SLC22A8, SLCO1B1, SLCO1B3, SLCO2B1* **Pleiotropic genes:** *ACE, APOA1, APOE, NOS3, NR1I2, NR1I3, PPARD, USP5*
	**Name: LOVASTATIN;** Mevinolin; Mevacor; 75330-75-5; Monacolin K; Altoprev **Molecular Formula:** $C_{24}H_{36}O_5$ **Molecular Weight:** 404.53964 g/mol **Mechanism**: Acts by competitively inhibiting HMG-CoA reductase, enzyme which catalyzes rate-limiting step in cholesterol biosynthesis. **Effect:** Anticholesteremic Agent; HMG-CoA Reductase Inhibitor; Heart-health effects; Antineoplastic activity.	**Pathogenic genes:** *ABCA1, APOA1, APOA5, APOB, APOC3, CETP, LDLR, LIPC, LPL* **Mechanistic genes:** *APOA1, APOB, CETP, HMGCR, LDLR* **Metabolic genes:** **Substrate**: *CYP3A4* (major), *CYP3A5, UGT1A3.* **Inhibitor**: *ABCB1, CYP2C8, CYP2C9* (weak), *CYP2C19* (strong), *CYP2D6* (weak), *CYP3A4* (weak), *HMGCR, KCNH2* **Inducer**: *CYP2B6, CYP7A1* **Transporter genes:** *ABCA1, ABCB1, ABCB11, ABCC2, ABCG2, KCNH2, SLCO1B1, SLCO1B3* **Pleiotropic genes:** *TP53, USP5*
	**Name: PITAVASTATIN;** Itavastatin; NK 104; Livalo; 147511-69-1; Nisvastatin **Molecular Formula:** $C_{25}H_{24}FNO_4$ **Molecular Weight:** 421.460763 g/mol **Mechanism**: Works to control the synthesis of cholesterol via competitive inhibition of the liver enzyme, HMG-CoA reductase. As a result, a compensatory increase in LDL-receptor expression can be observed which facilitates an increase LDL catabolism. **Effect:** Anticholesteremic Agent; HMG-CoA Reductase Inhibitor; Heart-health effects.	**Pathogenic genes:** *APOB, LDLR* **Mechanistic genes:** *APOB, HMGC, LDLR, PPARG, PON1, VCAM1* **Metabolic genes:** **Substrate:** *ABCB1, CYP2C8* (minor), *CYP2C9* (minor), *CYP3A4* (minor), *SLCO1B1, UGT1A3, UGT2B7.* **Inhibitor**: *HMGCR* **Transporter genes:** *ABCB1, ABCG2, SLCO1B1, SLCO2B1* **Pleiotropic genes:** *PPARG, VCAM1*
	**Name: PRAVASTATIN SODIUM,** Mevalotin; Elisor; 81131-70-6; Lipostat; Pravachol **Molecular Formula:** $C_{23}H_{35}NaO_7$ **Molecular Weight:** 446.509569 g/mol **Mechanism**: A competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. **Effect:** Anticholesteremic Agent; HMG-CoA Reductase Inhibitor; Heart-health effects; Immune response modulation; MHC II suppression.	**Pathogenic genes:** *ABCA1, ACE, APOA1, APOA5, APOB, APOC3, APOE, CETP, CYP7A1, FGB, LDLR, LIPC, LPL, NOS3* **Mechanistic genes**: *APOA1, APOB, APOC3, APOE, CRP, HMGCR, LDLR, IL1B, IL6, IL10, MMP2, NOS3* **Metabolic genes:** **Substrate:** *ABCB1, ABCC2, CYP3A4* (minor), *SLCO1B1, SLCO2B1, UGT1A3* **Inhibitor:** *ABCB1, CYP2C8*, *CYP2C9* (weak), *CYP2C19* (weak), *CYP2D6* (weak), *CYP3A4* (weak), *HMGCR* **Inducer:** *CYP1A1, CYP2B6, CYP2E1* **Transporter genes:** *ABCA1, ABCB1, ABCB11. ABCG2, SLC22A8, SLCO1A2, SLCO1B1, SLCO1B3, SLCO2B1* **Pleiotropic genes:** *ACE, ALDH1A1, APOE, CBS, FGB, HTR3B, IL6, IL10, ITGB3, LEP, MTHFR, MMP2, MMP3, NOS3, TP53, USP5*

HMG CoA reductase inhibitors

Drug	Properties	Pharmacogenetics
	**Name: ROSUVASTATIN CALCIUM,** Crestor; Rosuvastatin hemicalcium; 147098-20-2; ZD 4522; Rosuvastatin Calcium Salt **Molecular Formula:** $C_{44}H_{54}CaF_2N_6O_{12}S_2$ **Molecular Weight:** 1001.137366 g/mol **Mechanism:** Inhibitor of HMG-CoA reductase, rate-limiting enzyme in cholesterol synthesis. This results in compensatory increase in expression of LDL receptors on hepatocyte membranes and stimulation of LDL catabolism. **Effect:** Anticholesteremic Agent; HMG-CoA Reductase Inhibitor; Heart-health effects; Antineoplastic activity.	**Pathogenic genes:** *ABCA1, ACE, APOA1, APOA5, APOB, APOC3, APOE, CETP CYP7A1, FGB, LDLR, LIPC, LPL, NOS3* **Mechanistic genes:** *APOA1, APOB, CETP, FGB, HMGCR, LDLR, LPL, NOS3* **Metabolic genes:** **Substrate:** *ABCB1, ABCC1, ABCC4, ABCG2, CYP2C9* (minor), *CYP2C19, CYP3A4* (minor), *SLC10A1, SLCO1A2, SLCO1B1, SLCO1B3, SLCO2B1, UGT1A3* **Inhibitor:** *CYP3A4, CYP3A5, HMGCR, SLCO1B1* **Inducer:** *CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4* **Transporter genes:** *ABCA1, ABCB1, ABCB11, ABCG2, SLC10A1, SLC22A8, SLCO1A2, SLCO1B1, SLCO1B3, SLCO2B1* **Pleiotropic genes:** *ACE, APOE, FGB, ITGB3, NOS3, TCF20, USP5*
	**Name: SIMVASTATIN,** Zocor; 79902-63-9; Synvinolin; MK-733; Sinvacor **Molecular Formula:** $C_{25}H_{38}O_5$ **Molecular Weight:** 418.56622 g/mol **Mechanism**: Prodrug requiring hydrolysis in vivo for activity. Inhibits HMG-CoA reductase, causing subsequent reduction in hepatic cholesterol synthesis. Reduces serum concentrations of total cholesterol, LDL-C, Apo B, and triglycerides **Effect:** Anticholesteremic Agent; HMG-CoA Reductase Inhibitor; Heart-health effects; Anti-inflammatory activity	**Pathogenic genes:** *ABCA1, APOA1, APOA5, APOB, APOC3, APOE, CETP, CYP7A1, FGB, GNB3, LIPC, LDLR, LPL, NOS3* **Mechanistic genes:** *ABCA1, APOA1, APOB, APOE, CETP, HMGCR, IL6, LDLR, LPL, VCAM1* **Metabolic genes:** **Substrate:** *ABCB1, CYP2C8* (minor), *CYP2C9,* (minor), *CYP2C19* (minor), *CYP2D6, CYP3A4* (major), *CYP3A5 ,POR, SLCO1B1, UGT1A3* **Inhibitor:** *CYP2C8* (weak), *CYP2C9* (weak), *CYP2C19* (strong), *CYP2D6* (weak), *CYP3A4* (moderate), *HMGCR* **Inducer:** *CYP2B6* **Transporter genes:** *ABCA1, ABCB1, ABCB11, ABCC2, ABCC3, ABCG2, SLCO1B1, SLCO1B3* **Pleiotropic genes:** *APOE, F2, FGB, GNB3, NOS3, PRNP, TNF, VCAM1, USP5*

ABCA1*:* ATP binding cassette subfamily A member 1; ***ABCB1****:* ATP binding cassette subfamily B member 1; ***ABCB11****:* ATP binding cassette subfamily B member 11; ***ABCC1****:* ATP binding cassette subfamily C member 1; ***ABCC2****:* ATP binding cassette subfamily C member 2; ***ABCC3****:* ATP binding cassette subfamily C member 3; ***ABCC4***: ATP binding cassette subfamily C member 4; ***ABCG2****:* ATP binding cassette subfamily G member 2; ***ACE***: angiotensin I converting enzyme; ***ALDH1A1***: aldehyde dehydrogenase 1 family member A1; ***APOA1****:* apolipoprotein A-I; ***APOA5****:* apolipoprotein A-V; ***APOB****:* apolipoprotein B; ***APOC3****:* apolipoprotein C-III; ***APOE****:* apolipoprotein E; ***CBS***: cystathionine-beta-synthase; ***CETP****:* cholesteryl ester transfer protein, plasma; ***CRP****:* C-reactive protein, pentraxin-related; ***CYP1A1***: cytochrome P450 family 1 subfamily A member 1; ***CYP1A2***: cytochrome P450 family 1 subfamily A member 2; ***CYP2B6***: cytochrome P450 family 2 subfamily B member 6; ***CYP2C19***: cytochrome P450 family 2 subfamily C member 19; ***CYP2C8***: cytochrome P450 family 2 subfamily C member 8; ***CYP2C9***: cytochrome P450 family 2 subfamily C member 9; ***CYP2D6***: cytochrome P450 family 2 subfamily D member 6; ***CYP2E1***: cytochrome P450 family 2 subfamily E member 1; ***CYP3A4***: cytochrome P450 family 3 subfamily A member 4; ***CYP3A5***: cytochrome P450 family 3 subfamily A member 4; ***CYP7A1***: cytochrome P450 family 7 subfamily A member 1; ***CYP11B2****:* cytochrome P450 family 11 subfamily B member 2; ***ESR1****:* estrogen receptor 1; ***F2***: coagulation factor II, thrombin; ***FGB***: fibrinogen beta chain; ***GNB3****:* guanine nucleotide binding protein (G protein), beta polypeptide 3; **HMG-CoA**: 3-hydroxy-3-methylglutaryl coenzyme A; ***HMGCR****:* 3-hydroxy-3-methylglutaryl-CoA reductase; ***HTR3B****:* 5-hydroxytryptamine (serotonin) receptor 3B, ionotropic*;* ***IL1B****:* interleukin 1 beta*;* ***IL6****:* interleukin 6; ***IL10****:* interleukin 10; ***ITGB3****:* integrin subunit beta 3; ***KCNH2***: potassium voltage-gated channel subfamily H member 2; ***LDLR****:* low density lipoprotein receptor; ***LEP***: leptin; ***LIPC****:* lipase C, hepatic; ***LPL***: lipoprotein lipase; ***MMP2***: matrix metallopeptidase 2; ***MMP3****:* matrix metallopeptidase 3; ***MTHFR***: methylenetetrahydrofolate reductase (NAD(P)H); ***MTTP****:* microsomal triglyceride transfer protein; ***NOS3****:* nitric oxide synthase 3; ***NR1I2***: nuclear receptor subfamily 1 group I member 2; ***NR1I3***: nuclear receptor subfamily 1 group I member 3; ***PON1****:* paraoxonase 1; ***POR***: P450 (cytochrome) oxidoreductase; ***PPARD***: peroxisome proliferator activated receptor delta; ***PPARG***: peroxisome proliferator activated receptor gamma; ***PRNP***: prion protein; ***SLC10A1***: solute carrier family 10 (sodium/bile acid cotransporter), member 1; ***SLC15A1***: solute carrier family 15 (oligopeptide transporter), member 1; ***SLC22A8****:* solute carrier family 22 (organic anion transporter), member 8; ***SLCO1A2***: solute carrier organic anion transporter family member 1A2; ***SLCO1B1****:* solute carrier organic anion transporter family member 1B1; ***SLCO1B3:*** solute carrier organic anion transporter family member 1B3; ***SLCO2B1***: solute carrier organic anion transporter family member 2B1; ***TCF20***: transcription factor 20 (AR1); ***TNF****:* tumor necrosis factor; ***TP53****:* tumor protein p53*;* ***UGT1A3****:* UDP glucuronosyltransferase 1 family, polypeptide A3; ***UGT2B7***: UDP glucuronosyltransferase 2 family, polypeptide B7; ***USP5***: ubiquitin specific peptidase 5 (isopeptidase T); ***VCAM1****:* vascular cell adhesion molecule 1.

a fine interpretation of pharmacogenetic results; however, the integration of gene clusters associated with specific phenotypes yields informative haplotypes with potential utility in pharmacogenetic studies. It is likely that thousands of genes are involved in CHO metabolism, and probably not a single gene plays an absolute dominant role over the others; however, some genes exert a powerful effect on other congeners associated with a specific pathogenic cascade (e.g. *APOE* in AD) or a pharmacogenetic pathway (e.g. *APOE* vs *CYPs* in AD treatment with donepezil)[3,6,17,33,66,67]. Several pathogenic (*ACE, APOA1, APOA5, APOB, APOC3, APOE, CETP, FGB, GNB3, LIPC, MMP3, MTTP, NOS3, PON*) and mechanistic genes (*ABCB1, ABCC1, APOA1, APOA5, APOB, APOC3, APOE, CRP, CYP11B2, HMGCR, IL10, IL6, LDLR, MMP3, PON1, TNF*) are potentially influenced by atorvastatin. This statin is a major substrate of CYP2C8 and CYP3A4/5; it is a strong inhibitor of CYP2C19, a moderate inhibitor of ABCB1, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and HMGCR, and an inducer of CYP2B6 and CYP7A1. Atorvastatin is transported by ABCA1, ABCB1, ABCB11, ABCC1, ABCC2, ABCC3, ABCG2, SLCO1B1 and SLCO1B3 proteins, and interacts with the products of various pleiotropic genes (*APOA1, APOE, CRP, CYP11B2, ESR1, GNB3, HTR3B, IL6, IL10, ITGB3, MMP3, TNF, USP5)*[38] (**Table 2**). The lipid-lowering effects and the anti-atherosclerotic properties of LipoEsar are *APOE*-dependent, with *APOE-3* carriers acting as the best responders and *APOE-4* carriers behaving as the worst responders[5,6]. Sex-related changes in cholesterol response to statins have been reported in carriers of the HMGCR-AA genotype at rs3846662, who have higher levels of total and LDL-cholesterol. The percentage reduction in LDL-cholesterol upon statin treatment is decreased in women with the AA genotype compared with women without it. In hypercholesterolemic patients, HMGCR alternative splicing may explain 22-55% of the variance in statin response[139]. The powerful effect of Atorvastatin in CYP3A4/5-IMs is the result of a poor metabolization of Atorvastatin by mutant CYP3A4/5 enzymes, since Atorvastatin is a major substrate of CYP3A4/5. In contrast, the lack of effect in CYP3A4/5-RMs results from a rapid destruction of the drug in the liver mediated by excessive CYP3A4/5 enzymatic activity. Therefore, the dose of statins should be adjusted to the metabolizing condition of each patient to optimize the lipid-lowering effects of statins and to avoid toxicity[38]. Furthermore, the co-administration of the nutraceutical LipoEsar enhances the hypolipemic effect of Atorvastatin and facilitates a dose reduction of the statin by 50%, minimizing potential ADRs in susceptible patients.

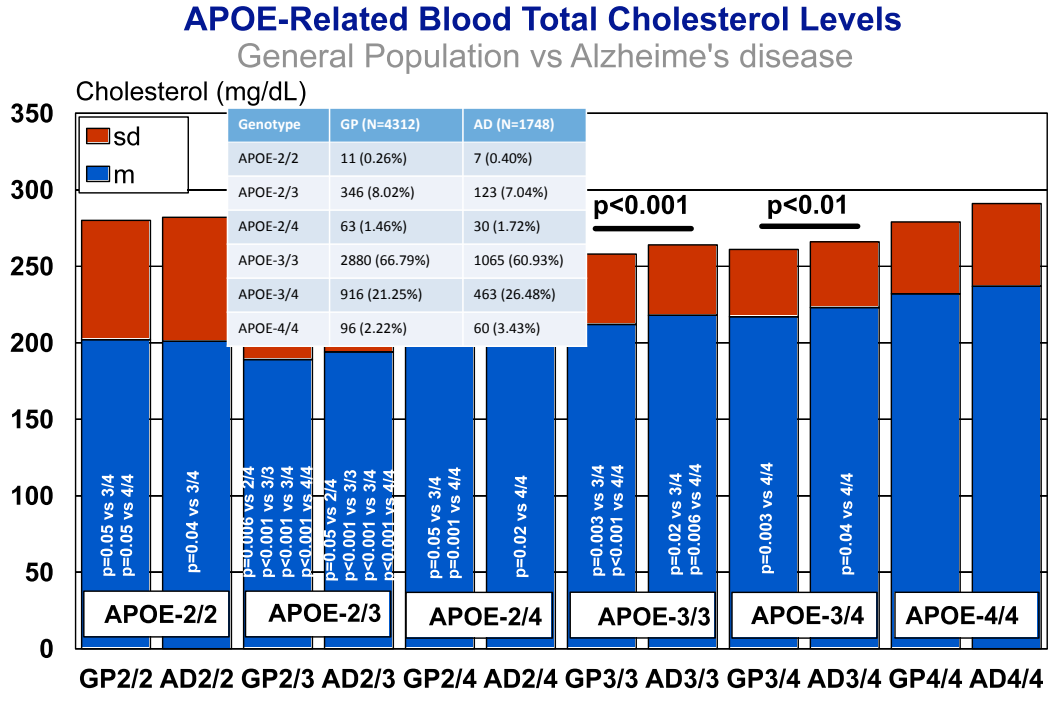


Figure 22. *APOE*-related blood cholesterol levels in the general population and in patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

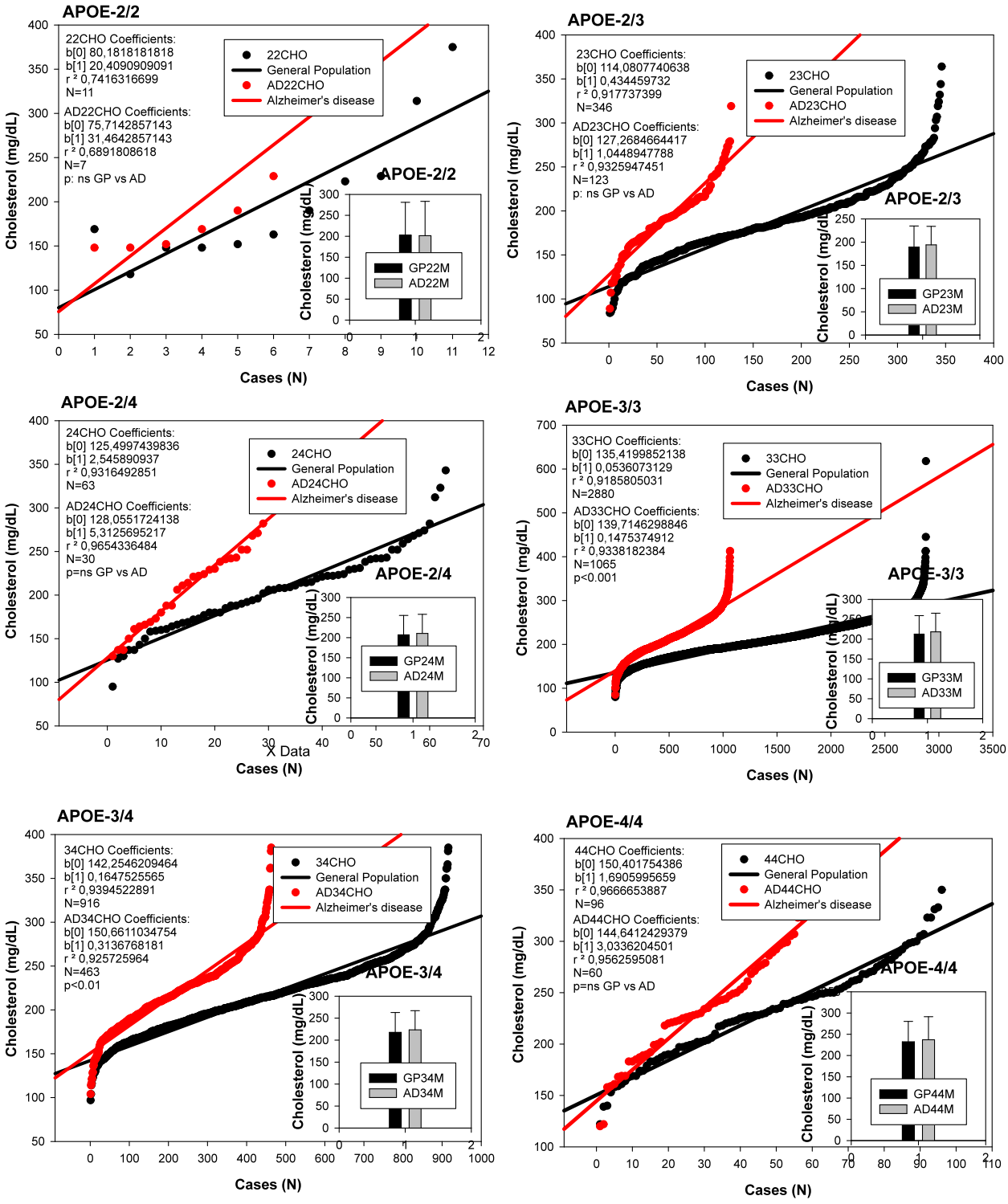


Figure 23. *APOE*-related comparative profile of cholesterol phenotypes in the general population and in patients with Alzheimer's disease.

Source: R. Cacabelos. CIBE DataBase, 2016.

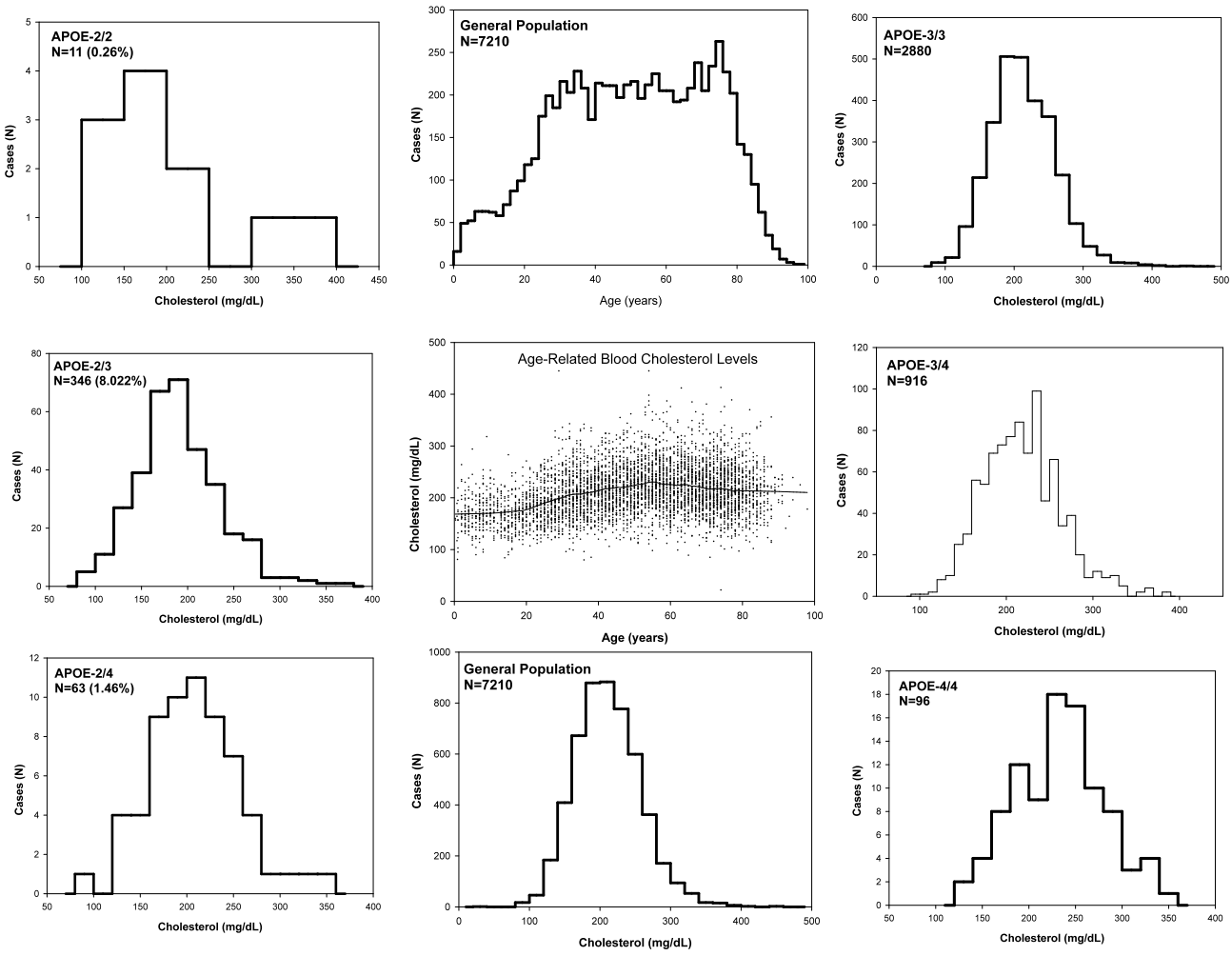


Figure 24. Histogram of frequencies of age-related cholesterol levels (central panel) and *APOE*-related histogram of frequencies of cholesterol levels in the general population (lateral panels).
Source: R. Cacabelos. CIBE DataBase, 2016.

PHARMACOEPIGENOMICS

Pharmacogenetics alone does not predict all phenotypic variation in drug response[27]. The genes involved in the pharmacogenomic network are under the regulatory control of the epigenetic machinery (DNA methylation, histone modifications, miRNA regulation), this configuring the novel pharmacoepigenomic apparatus[27]. Epigenetics involves heritable alterations of gene expression, chromatin organization, and microRNA (miRNA) regulation without changes in DNA sequence. Classical epigenetic mechanisms, including DNA methylation and histone modifications, and regulation by microRNAs (miRNAs), are among the major regulatory elements that control metabolic pathways at the molecular level, with epigenetic modifications regulating gene expression transcriptionally and miRNAs suppressing gene expression post-transcriptionally[140]. Methylation varies spatially across the genome with a majority of the methylated sites mapping to intragenic regions[141]. About 70% of CpG dinucleotides within the human genome are methylated. Not only nuclear DNA (nDNA), but also mitochondrial DNA (mtDNA) may be subjected to epigenetic modifications related to disease development, environmental exposure, drug treatment and aging.

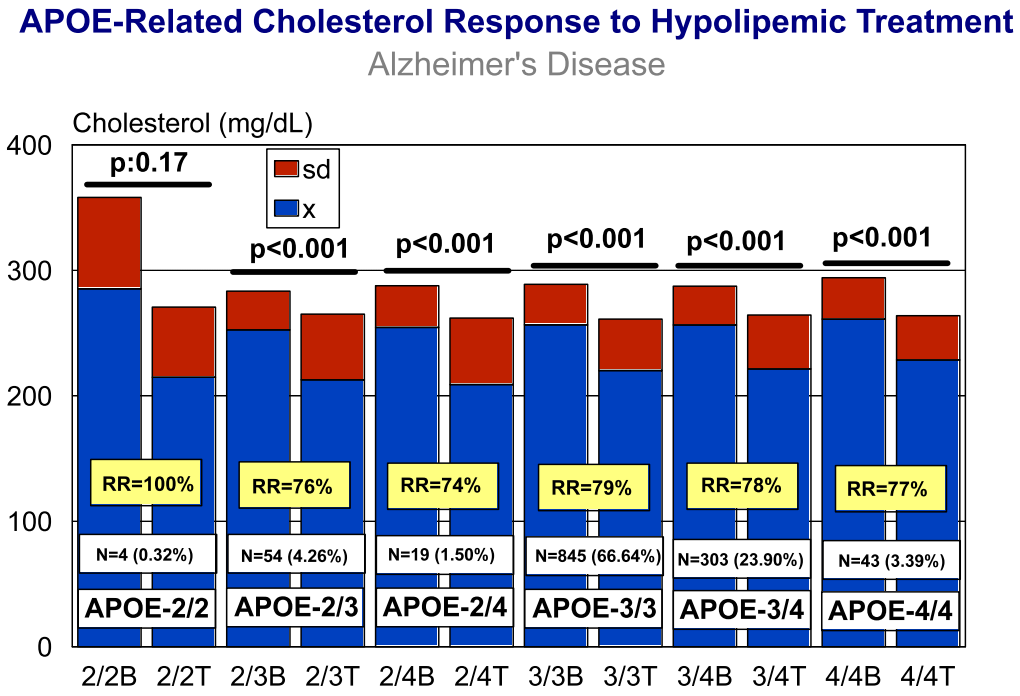


Figure 25. *APOE*-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

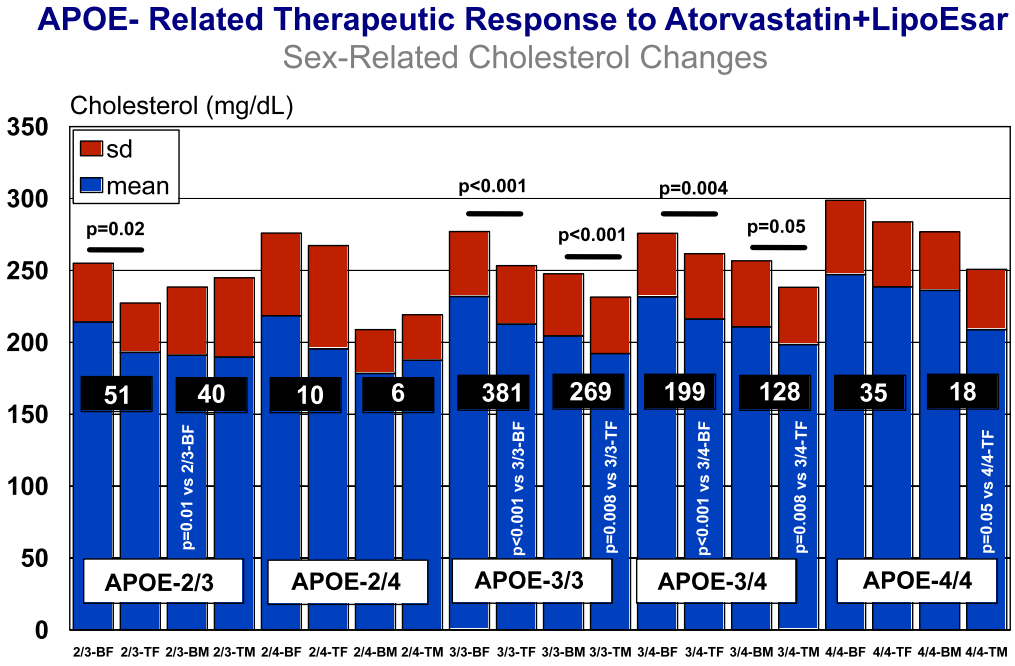


Figure 26. *APOE*- and sex-related cholesterol response to a combination treatment with Atorvastatin and LipoEsar in patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

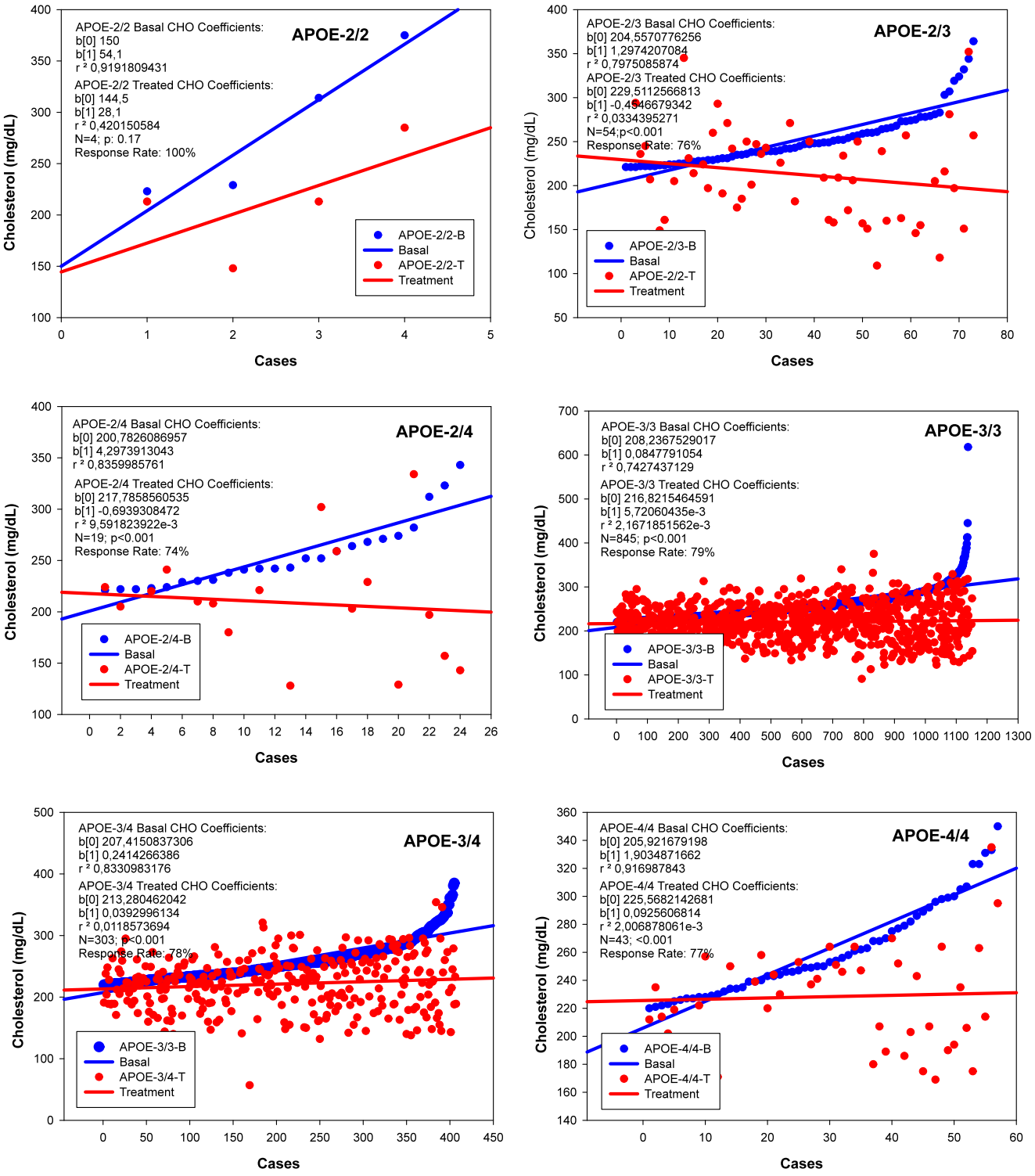


Figure 27. *APOE*-related individual response of cholesterol to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease.

Source: R. Cacabelos. CIBE DataBase, 2016.

Table 3. Epigenetic modifications in pathogenic genes associated with Alzheimer's disease

Pathogenic gene	Locus	Promoter lenght (bp)	3' UTR length (bp)	Defective protein	DNA Methylation / mRNA expression	Histone modifications/ chromatine remodeling	non-coding RNAs	Refs
APOE	19q13.2	996	…	apolipoprotein E	Hypomethylated / Upregulated mRNA	Reduced H3 acetylation Decreased SIRT1 Increased HDAC6 levels Increased HDAC2 levels	**Linked to AD** miR-34a, miR-34b/c, miR-107, miR-124, miR-125b, miR-137 **Epigenetically regulated** let-7, miR-9, miR-132/212, miR146a, miR-148a, miR-184, miR-200, miR-200c/141	142,143,158-165
BIN1	2q14	1076	642	bridging integrator 1	Upregulated mRNA			23,166,167
CLU	8p21-p12	1094	1399	clusterin	Upregulated mRNA			168
ABCA7	19p13.3	967	…	ATP binding cassette subfamily A member 7	Hypomethylated / Upregulated mRNA			154,169
CR1	1q32	966	2579	complement component 3b/4b receptor 1	Upregulated mRNA			23
CD33	19q13.3	1190	387	CD33 molecule	Upregulated mRNA			170,171
APP	21q21.3	1086	1176	amyloid beta (A4) precursor protein	Hypomethylated / Upregulated mRNA			5,29,142,143,160 171-173
PSEN1	14q24.3	929	1198	presenilin 1	Hypomethylated / Upregulated mRNA			29,142,143,173
MAPT (TAU)	17q21.1	1094	…	microtubule-associated protein tau	Hypermethylated / Downregulated mRNA			29,142,160
BACE1	11q23.2-q23.3	987	3994	beta secretase 1	Hypomethylated / Upregulated mRNA			23
SORL1	11q23.2-q24.2	996	…	sortilin-related receptor	Downregulated mRNA			29,142,143,160, 173,174

Table 4. Epigenetic modifications in genes associated with lipid metabolism, vascular risk factors, and inflammation

Pathogenic gene	Locus	Promoter lenght (bp)	3' UTR length (bp)	Defective protein	Epìgenetic modifications	Pathology	Refs
APOB	2p24.1	--	394	apolipoprotein B	miR-30 family miR-122 Hypermethylated Upregulated mRNA expression	Hepatocarcinoma cell line Fibrosis in hepatitis B infection Aberrant birth weight Risk for Alzheimer's disease	175-179
APOC3	11q23.3	960	278	apolipoprotein CIII	Hypermethylation Down-regulated mRNA expression	Stroke, Atherosclerosis Risk for Alzheimer's disease	179
APOE	19q13.2	996	--	apolipoprotein E	Hypomethylated Overexpressed mRNA Up-regulated mRNA expression	Alzheimer's disease Ischemia in mice's heart Dementia	142,143, 158-165
CETP	16q21	982	299	cholesteryl ester transfer protein plasma	Hypermethylated	Low HDL / vascular risk	180
LPL	8p22	1197	497	lipoprotein lipase	Hypermethylated	Low HDL / vascular risk	180
NOS3	7q36	975	2200	nitric oxide synthase	Histone acetylation (foetal stage) mRNA upregulation (foetal stage) Hypermethylation / mRNA downregulation	Adverse intrauterine environment Adverse intrauterine environment Vascular disorders / hypertension	181-185
ACE	17q23.3	1070	299	angiotensin I converting enzyme	Hypermethylated Hypomethylated Hypomethylated	Major depression Increased systolic blood pressure Low birth weight	186,187
AGT	1q42.2	803	698	angiotensinogen	Upregulated mRNA expression Histone acetylation	Hypertension Hypertension	188
IL1B	2q14	964	668	interleukin 1 beta	Hypomethylated	Alzheimer's disease	189
IL6	7p21	1214	500	interleukin 6	Hypomethylated Hypomethylated Hypomethylated Upregulated mRNA expression	Alzheimer's disease Rheumatoid arthritis Lupus erythematous Hypertension	190,191
TNFA	6p21.33	1028	907	tumor necrosis factor	Hypomethylated Hypomethylated Downregulated mRNA expression	Alzheimer's disease Parkinson's disease Oxidative stress in T cells	189,192, 193
MTHFR	1p26.32	959	--	methylenetetrahydrofolate	Hypermethylated Hypermethylated Hypermethylated	Alzheimer's disease Non-Hodgking Lymphoma Recurrent spontaneous abortion	29,142,160, 194,196

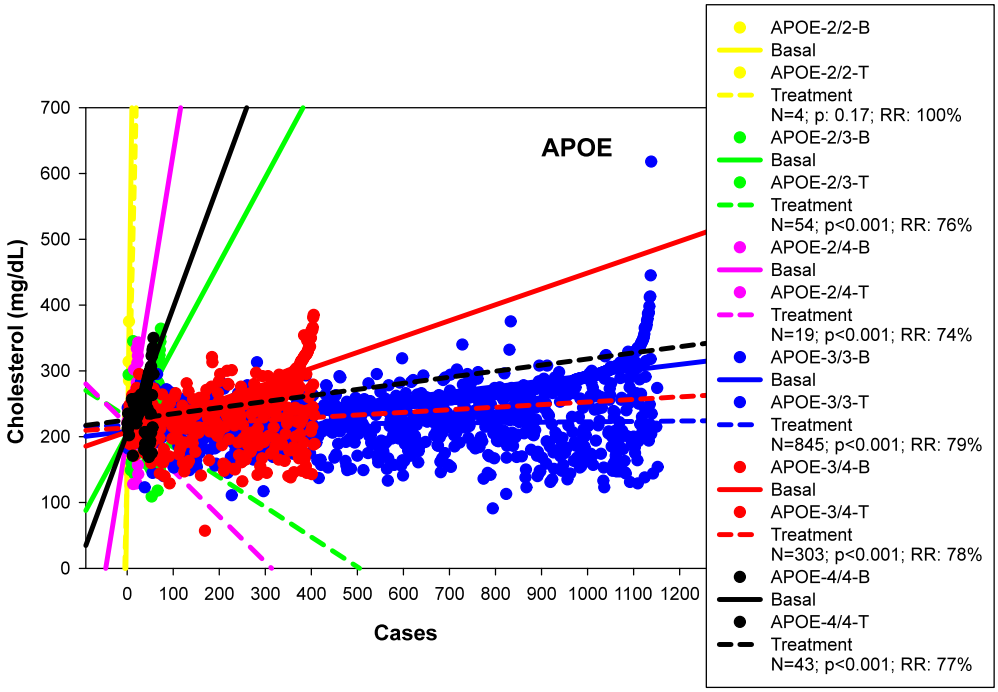


Figure 28. Comparative analysis of *APOE* variant-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease. Source: R. Cacabelos. CIBE DataBase, 2016.

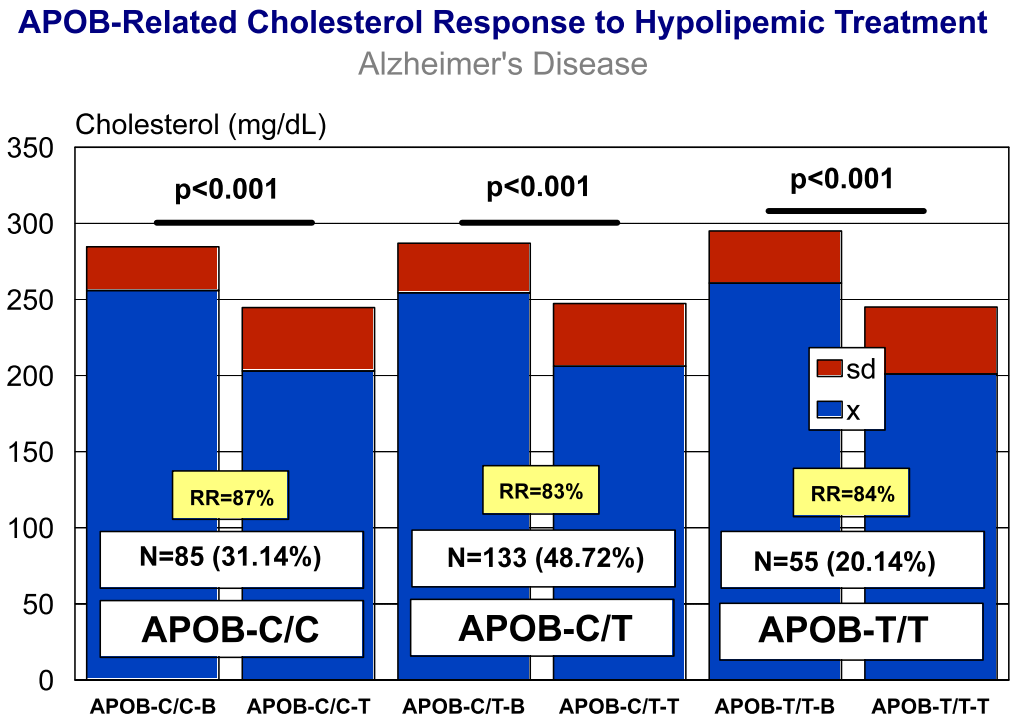


Figure 29. *APOB*-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease. Source: R. Cacabelos. CIBE DataBase, 2016.

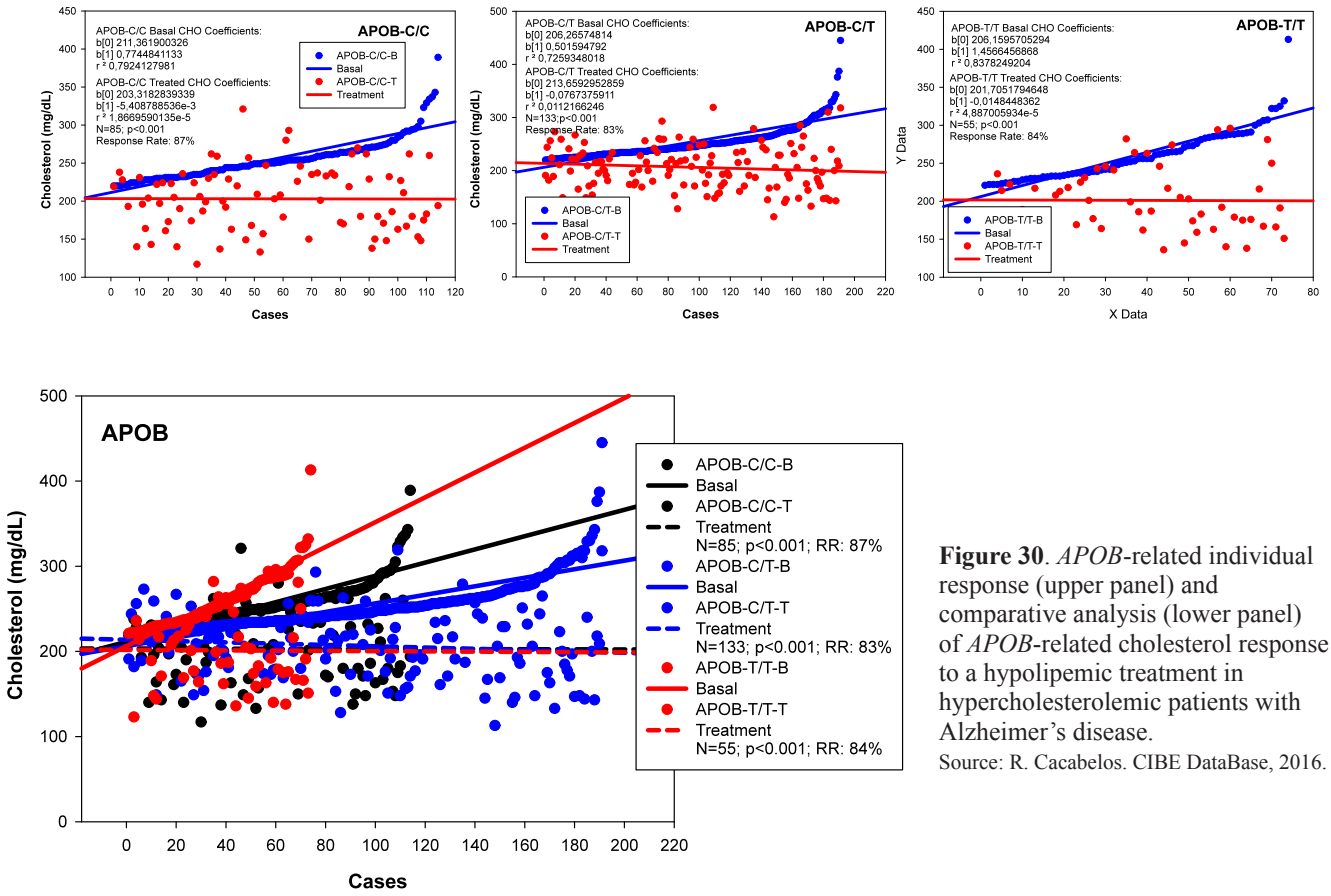


Figure 30. *APOB*-related individual response (upper panel) and comparative analysis (lower panel) of *APOB*-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

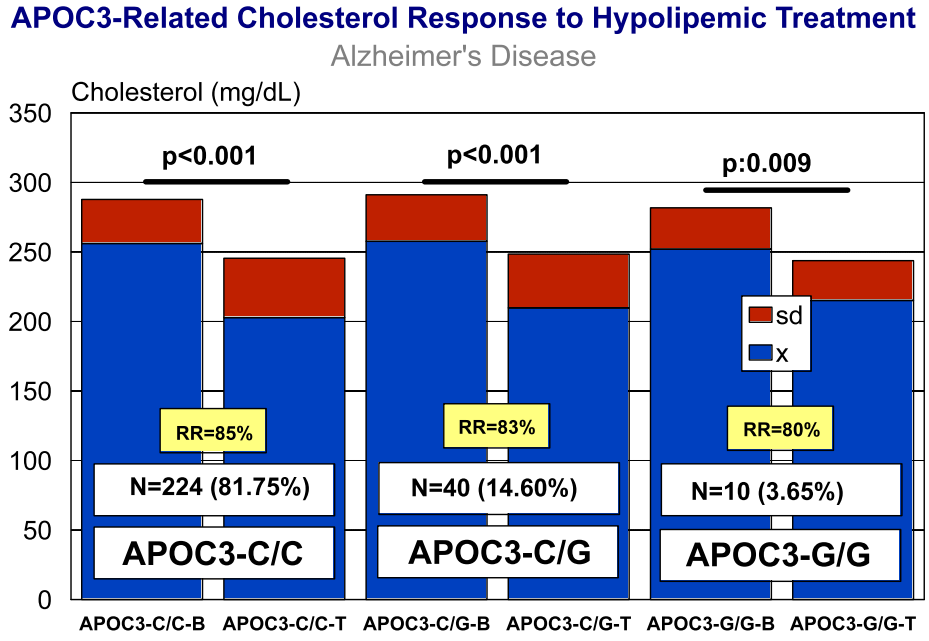
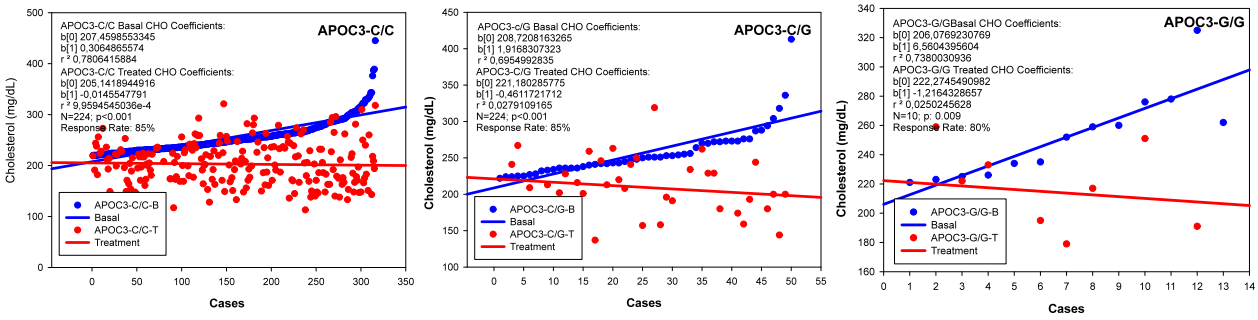


Figure 31. *APOC3*-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.



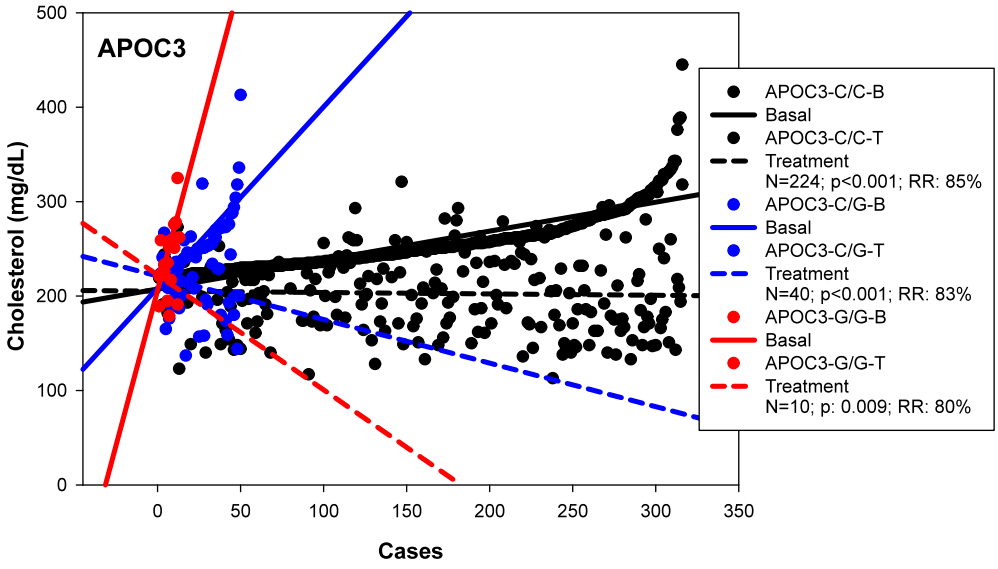


Figure 32. *APOC3*-related individual response (upper panel) and comparative analysis (lower panel) of *APOC3*-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

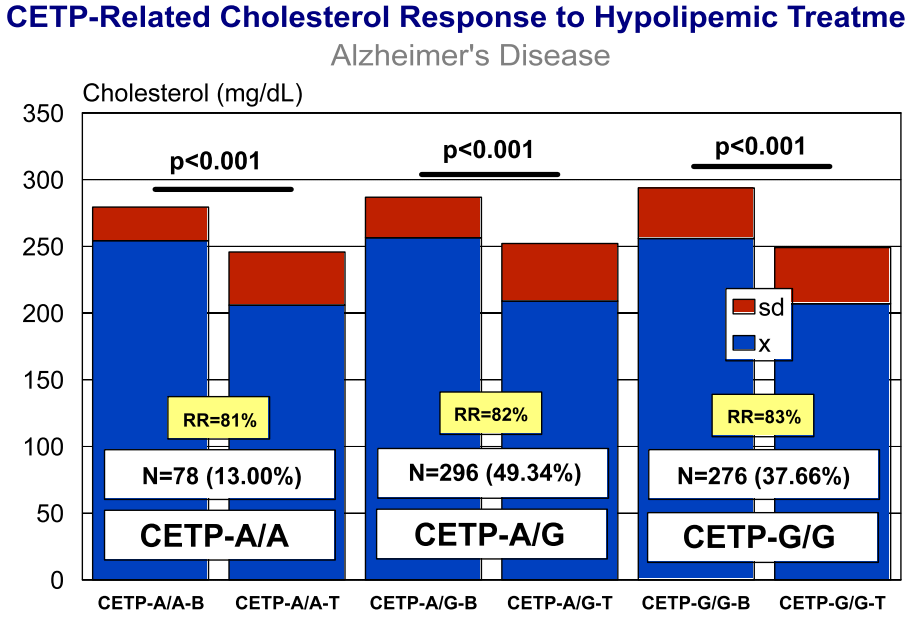
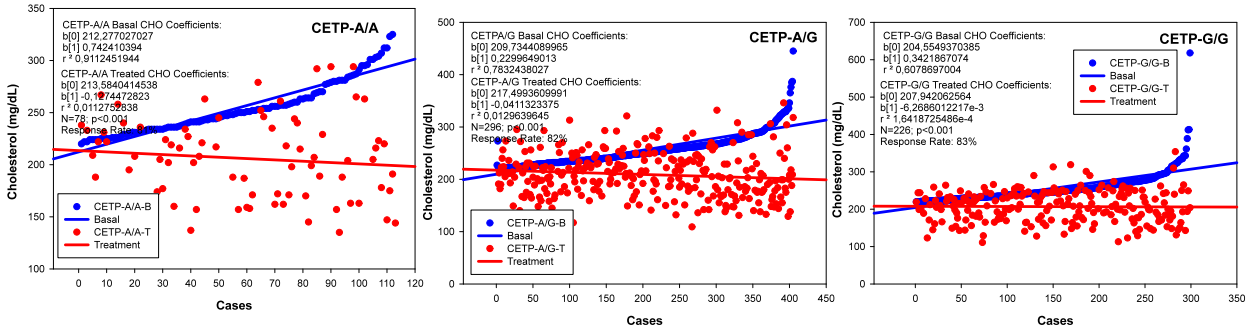


Figure 33. *CETP*-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.



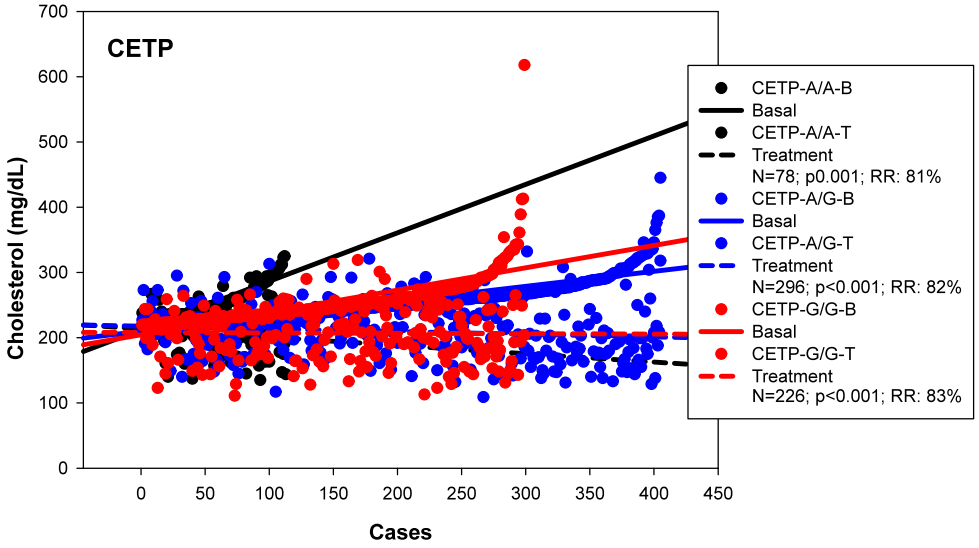


Figure 34. *CETP*-related individual response (upper panel) and comparative analysis (lower panel) of *CETP*-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease. Source: R. Cacabelos. CIBE DataBase, 2016.

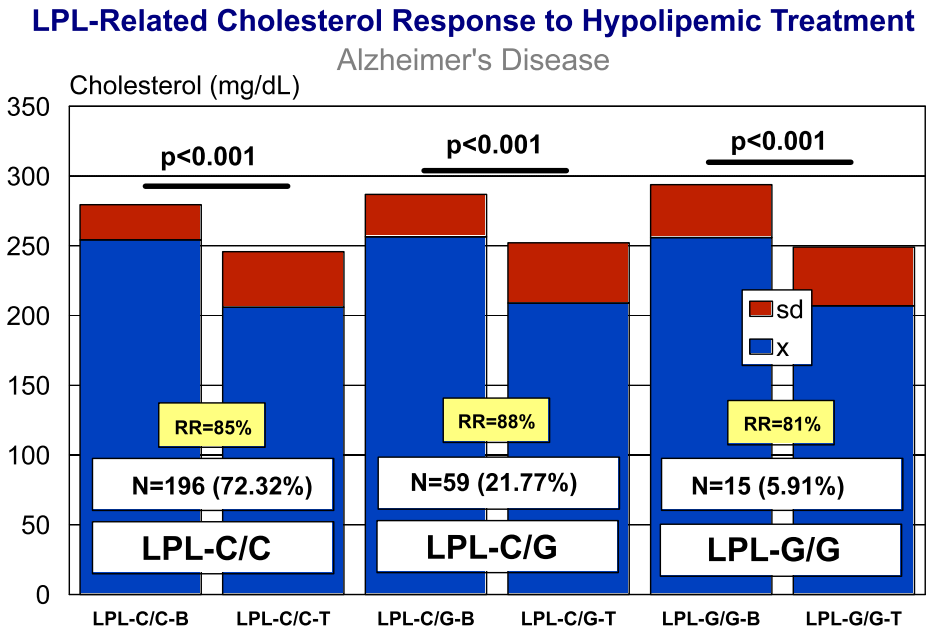


Figure 35. *LPL*-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease. Source: R. Cacabelos. CIBE DataBase, 2016.

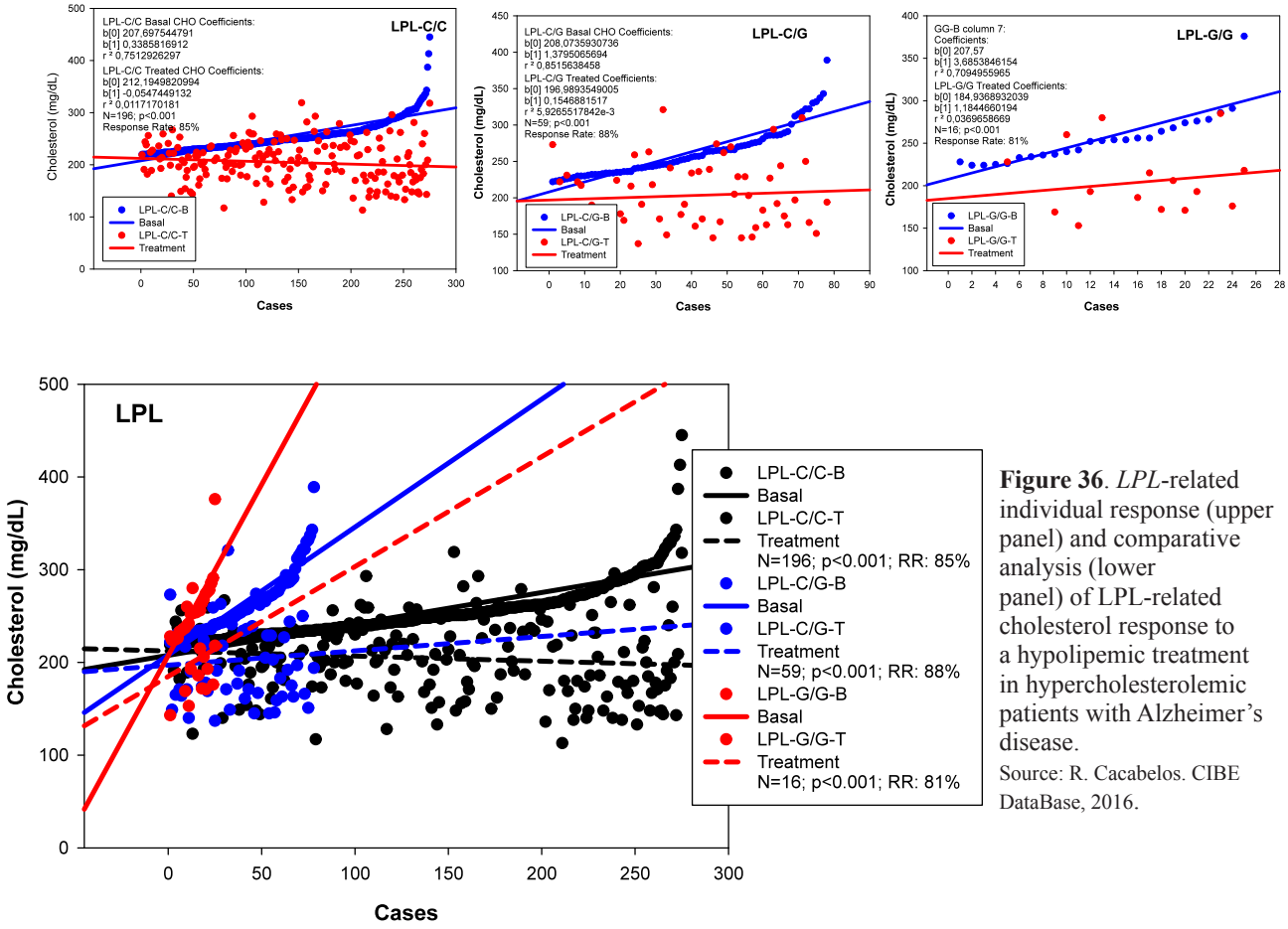


Figure 36. *LPL*-related individual response (upper panel) and comparative analysis (lower panel) of LPL-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

Several pathogenic genes (**Table 3**) and many other AD-related susceptibility genes with direct or indirect influence on the AD phenotype (i.e. genes associated with vascular risk factors and lipid metabolism)(**Table 4**) contain methylated CpG sites which exhibit alterations in DNA methylation[142,143]. Different modalities of histone aberrations are present in AD[27-29,142,144,145]. Alterations in epigenetically-regulated miRNAs may contribute to the abnormal expression of pathogenic genes in AD[146,147]. Several lncRNAs are dysregulated in AD (*Sox2OT, 1810014B01Rik, BC200, BACE1-AS, NAT-Rad18, 17A, GDNFOS*)[147]. Examples of miRNAs directly linked to AD pathogenesis include miR-34a (1p36.22), miR-34b/c (11q23.1), miR-107 (10q23.31), miR-124 (8p23.1/8p12.3/20q13.33), miR-125b (11q24.1/21q21.1), and miR-137 (1p21.3); and examples of epigenetically-regulated miRNAs with targets linked to AD pathogenesis are let-7b (22q13.1), miR-9 (1q22/5q14.3/15q26.1), miR-132/212 (17p13.3), miR-146a (5q34), miR-148a (7p15.2), miR-184 (15q25.1), and miR-200 (miR-200b/200a/429, 1p36.33; miR-200c/141, 12p13.31) [146]. AD-related SNPs interfere with miRNA gene regulation and affect AD susceptibility. The significant interactions include target SNPs present in seven genes related to AD prognosis with the miRNAs- miR-214, -23a & -23b, -486-3p, -30e*, -143, -128, -27a & -27b, -324-5p and -422a. The dysregulated miRNA network contributes to the aberrant gene expression in AD[148-150].

Epigenetic regulation is also responsible for the tissue-specific expression of genes involved in pharmacogenetic processes, and epigenetics plays a key role in the development of drug efficacy, safety and resistance. Epigenetic changes affect CYP expression, major transporter function, and nuclear receptor interactions[151-154]. Variable methylation patterns have been detected in genes encoding phase I-III enzymes (**Table 5**). Although this is a still poorly explored field, epigenetic regulation of genes encoding drug-metabolizing enzymes (*CYP1A1*, *1A2*, *1B1*, *1A6*, *2A13*, *2B6*, *2C8*, *2C9*,

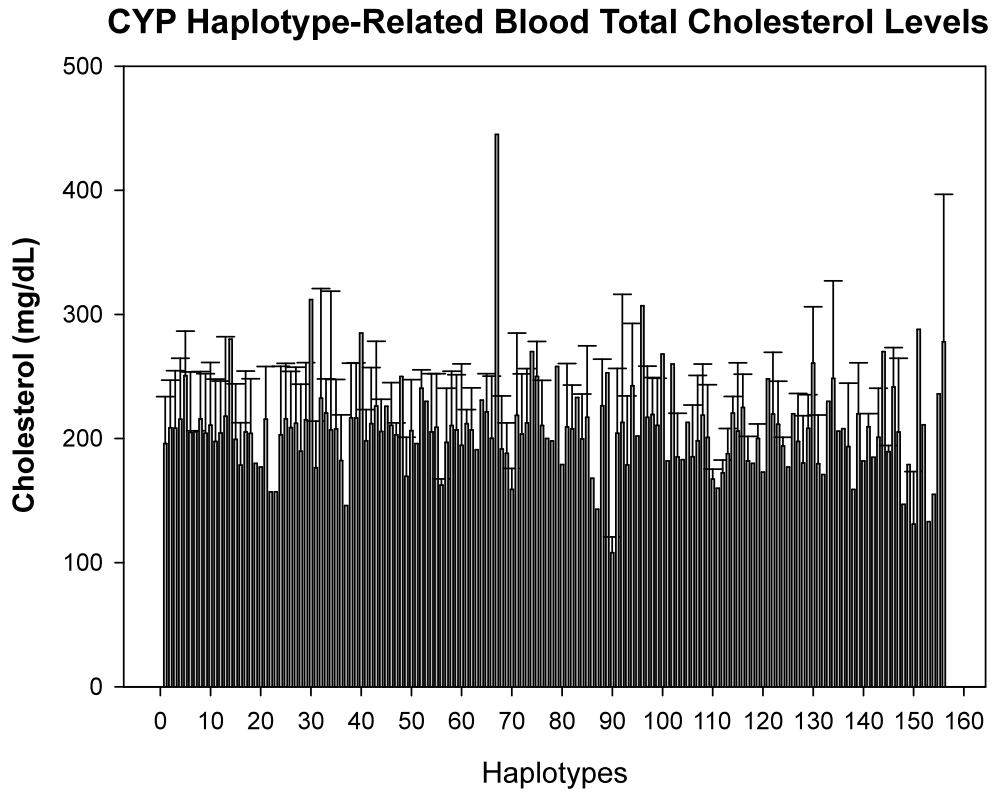


Figure 37. *CYP* haplotype-related blood total cholesterol levels.
Source: R. Cacabelos. CIBE DataBase, 2016.

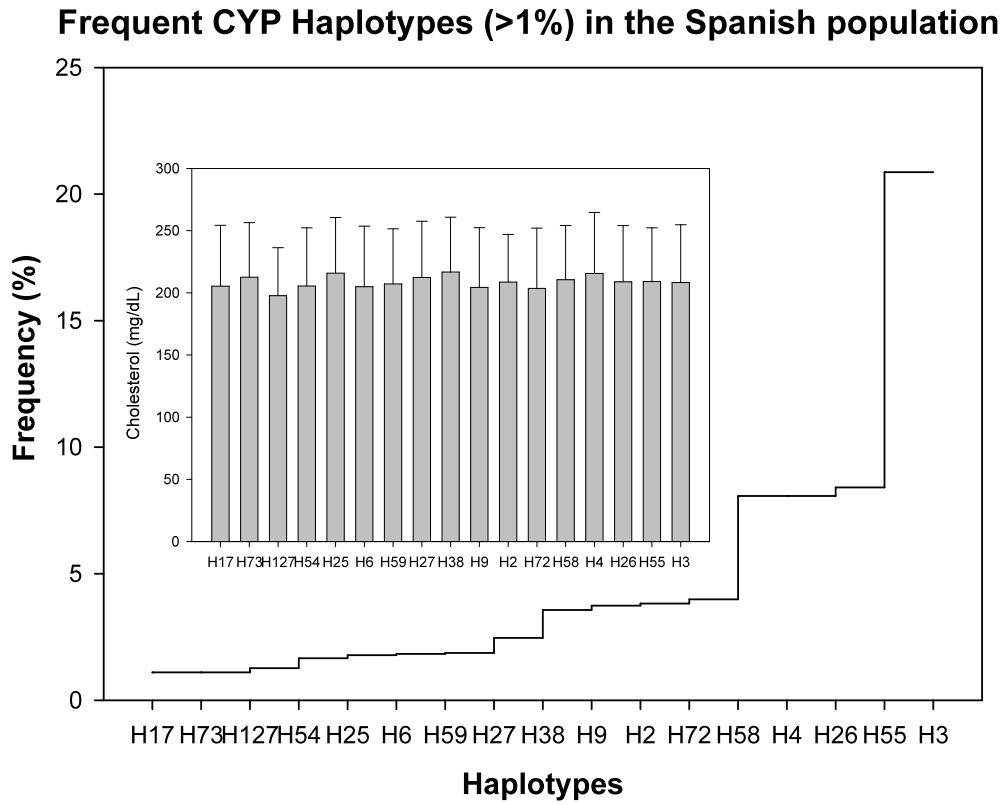


Figure 38. Blood cholesterol levels in subjects harboring the most frequent (>1%) *CYP* haplotypes in the Spanish population.
Source: R. Cacabelos. CIBE DataBase, 2016.

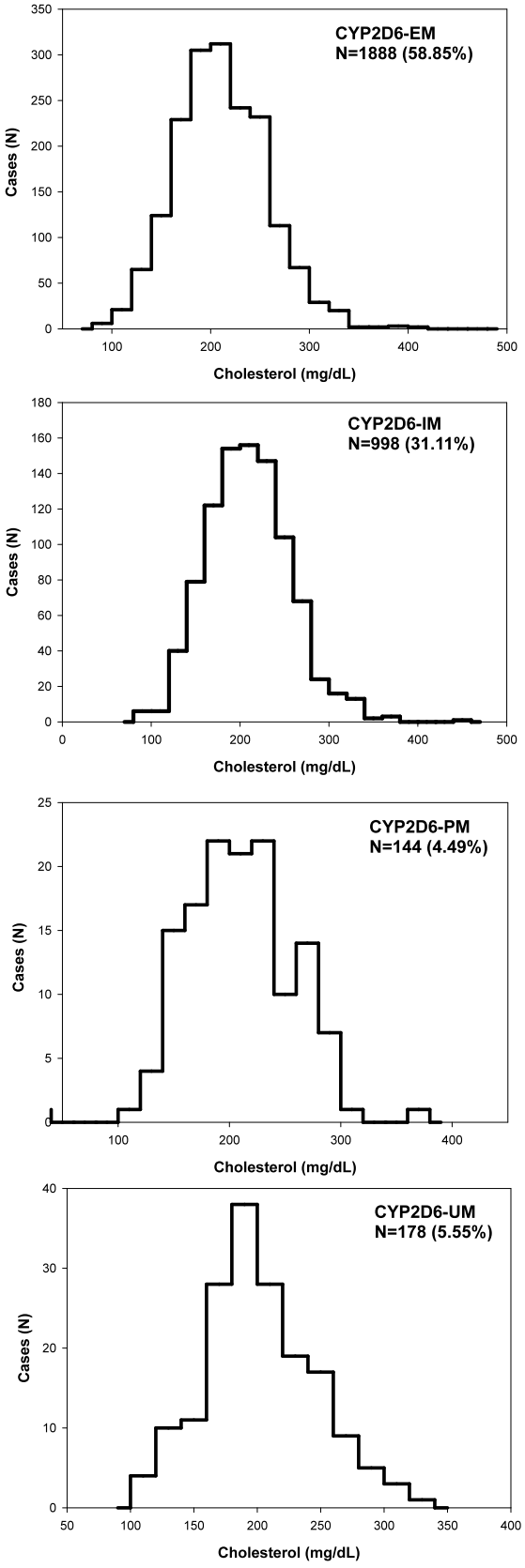


Figure 39. *CYP2D6*-related histogram of frequencies of cholesterol levels in the Spanish population.
Source: R. Cacabelos. CIBE DataBase, 2016.

2C18, *2C19*, *2D6*, *2E1*, *2J2*, *2F1*, *2R1*, *2S1*, *2W1*, *3A4*, *3A5*, *3A7*, *3A43*, *UGT1*, *GSTP1*), drug transporters (*ABCB1*/*MDR1*/*P-gp*, *ABCC1*/*MRP1*, *ABCC11*/*MRP8*, *ABCG2*/*BCRP*, *SLC19A1*, *SLC22A8*), and nuclear receptors (*RARB2*, *ESR1*, *NR1I2*, *HNF41*) has been documented in pioneering studies of pharmacoepigenetics[27,151-154].

Epigenetic modifications are also associated with drug resistance[27,153,155]. The acquisition of drug resistance is tightly regulated by post-transcriptional regulators such as RNA-binding proteins (RBPs) and miRNAs, which change the stability and translation of mRNA-encoding factors involved in cell survival, proliferation, epithelial-mesenchymal transition, and drug metabolism[153]. In the complex cascade of pharmacoepigenetic events, the epigenetic factory may act as a promiscuous, redundant security system in which several miRNAs target genes encoding epigenetic regulators. For example, miR-29, -29c, -370, and -450A target DNMT3A, and miR-29, -148, and -29b target DNMT3B, inducing hypomethylation and expression of tumor suppressor genes; let-7a, miR-26a, -101, -138, and -124 target EZH2, decreasing histone methylation and increasing expression of tumor suppressor genes; miR-449 and -874 target *HDAC1*, inducing growth arrest by decreasing histone acetylation; miR-1 and -155 target *HDAC4*, promoting myogenesis and impairing transcriptional activity of B-cell lymphoma 6 (*BCL6*); miR-627 and -155 target JMJD1A, decreasing histone demethylation and hypoxic gene expression; miR-132 and -483-5p target *MECP2*, promoting demethylation and cell differentiation[156]. Furthermore, epigenetic drugs reverse epigenetic changes in gene expression and might open new avenues in AD therapeutics[29,30,145,157].

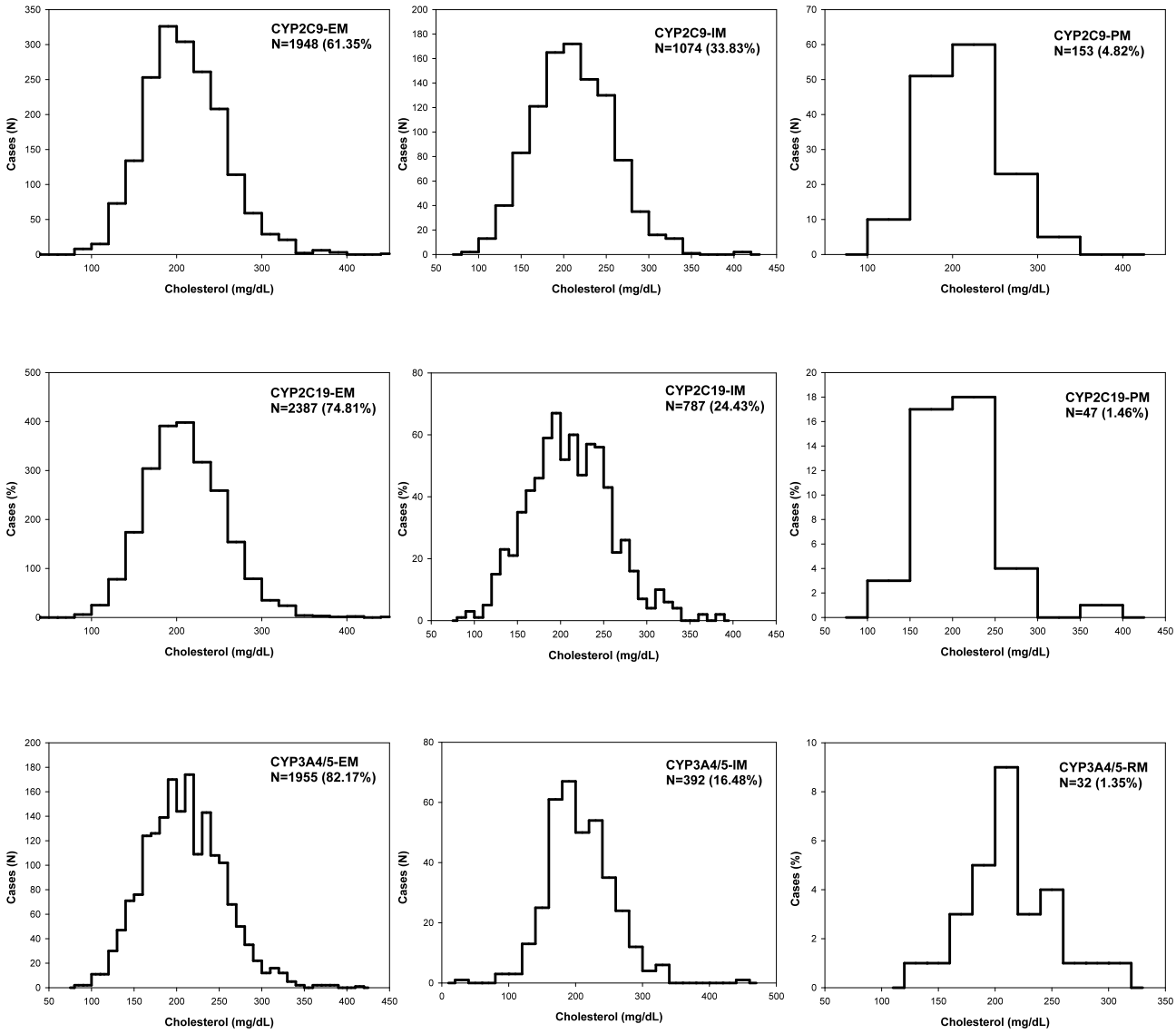


Figure 40. *CYP2C9*-, *CYP2C19*-,and *CYP3A4/5*-related histogram of frequencies of cholesterol levels in the Spanish population.

Source: R. Cacabelos. CIBE DataBase, 2016.

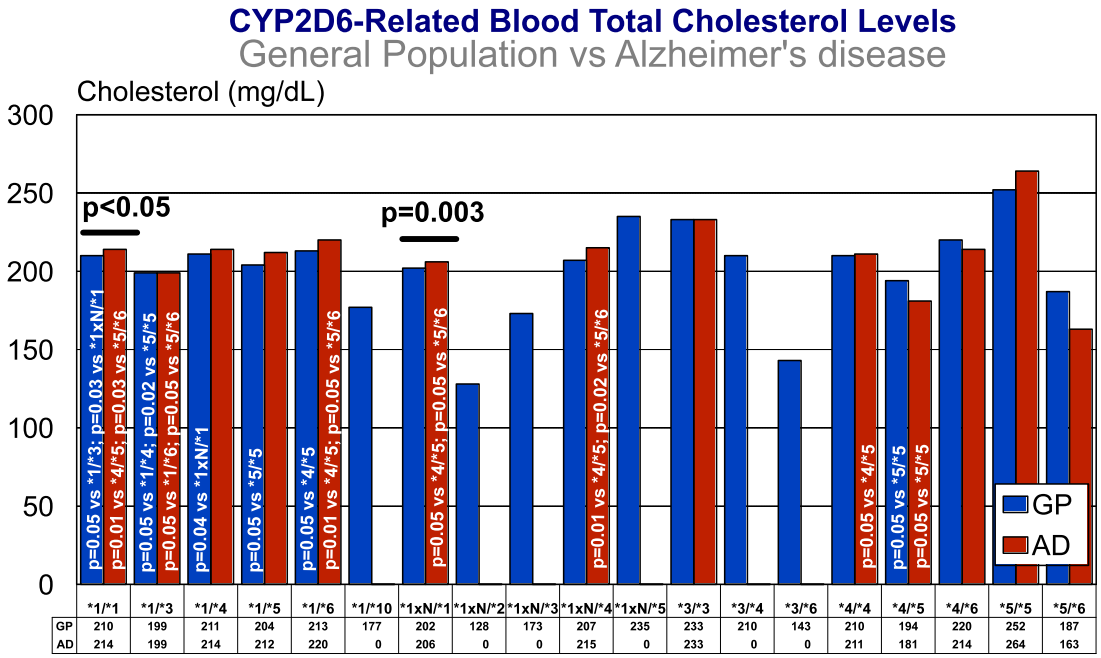


Figure 41. *CYP2D6*-related blood total cholesterol levels in the general population and in patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

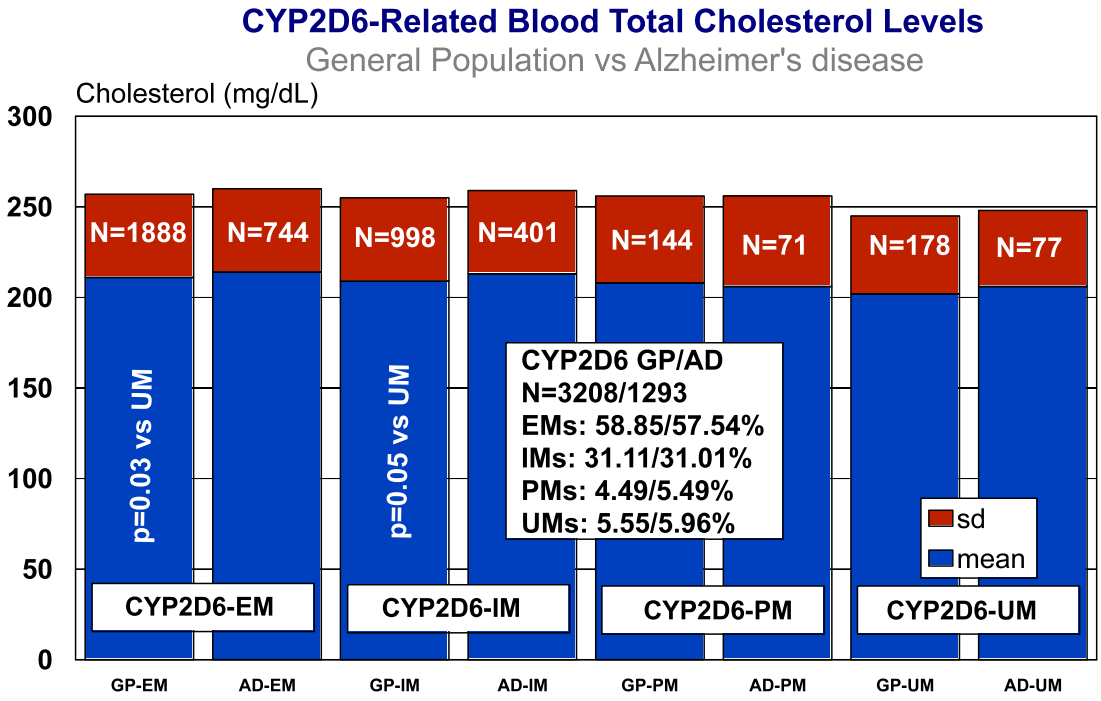


Figure 42. *CYP2D6*-EM-, IM-, PM- and UM-related blood total cholesterol levels in the general population and in patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

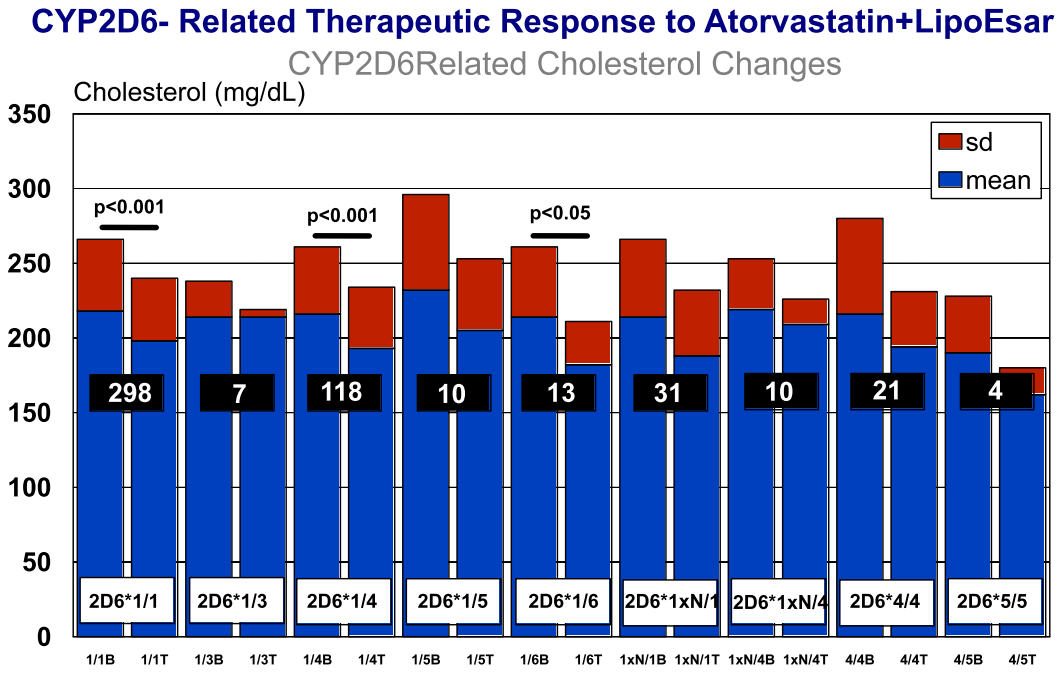


Figure 43. *CYP2D6* genotype-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

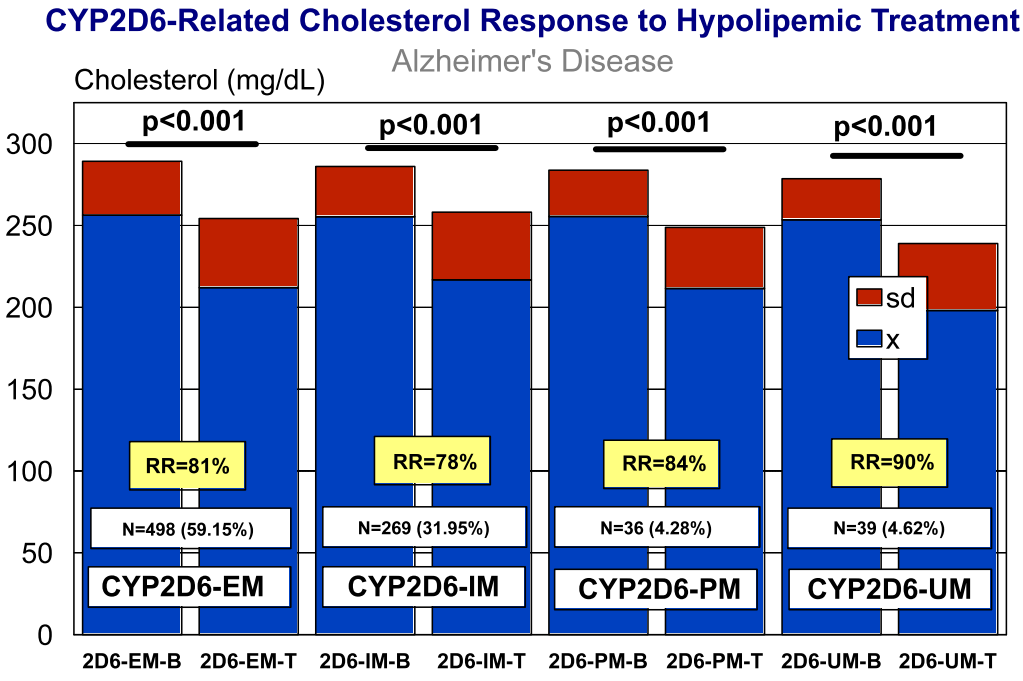


Figure 44. *CYP2D6*-EM-, IM-, PM- and UM-related response of cholesterol to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

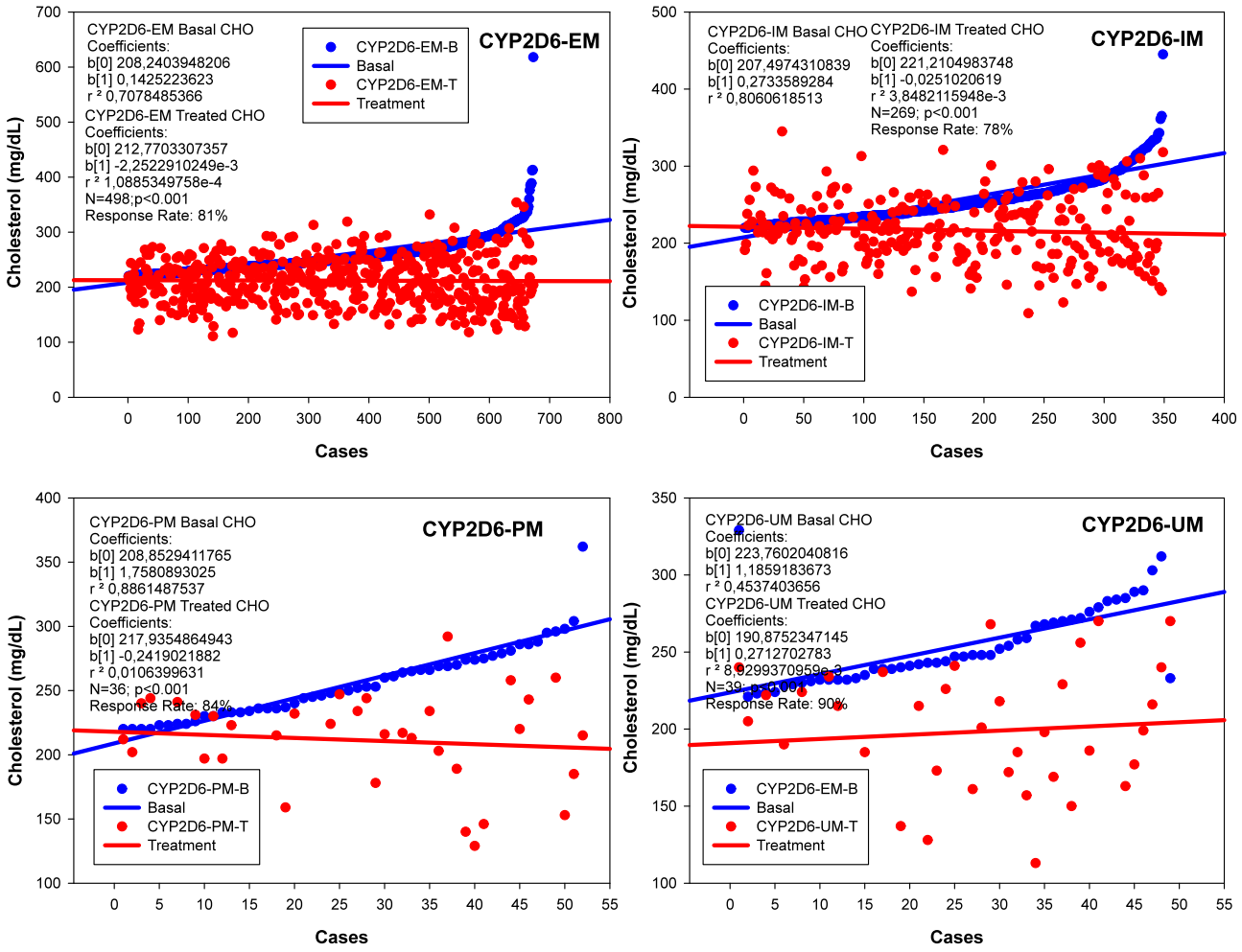


Figure 45. *CYP2D6*-EM-, IM-, PM- and UM-related individual response of cholesterol to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

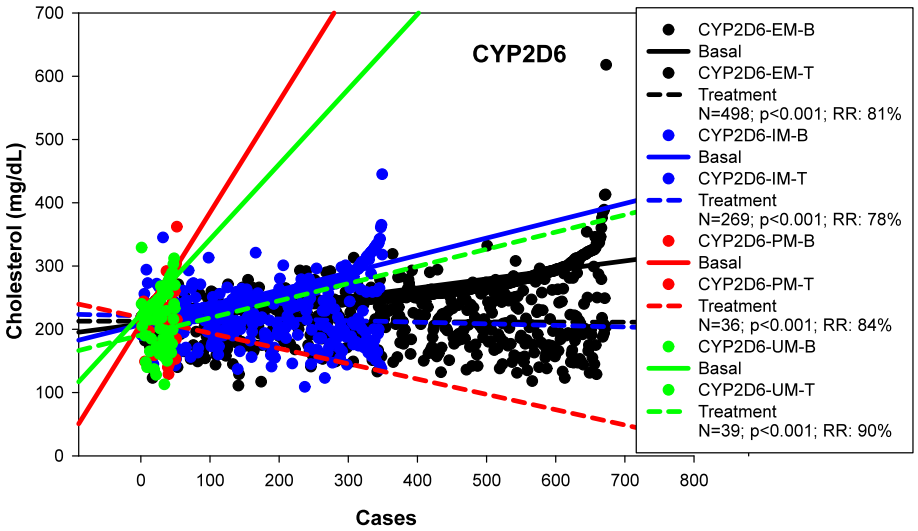


Figure 46. Comparative analysis of *CYP2D6* geno-phenotype-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

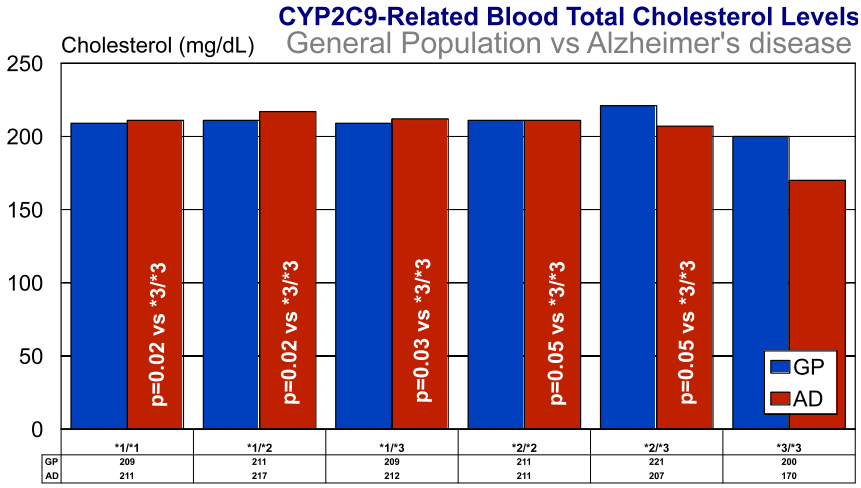


Figure 47. *CYP2C9* genotype-related blood total cholesterol levels in the general population and in patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

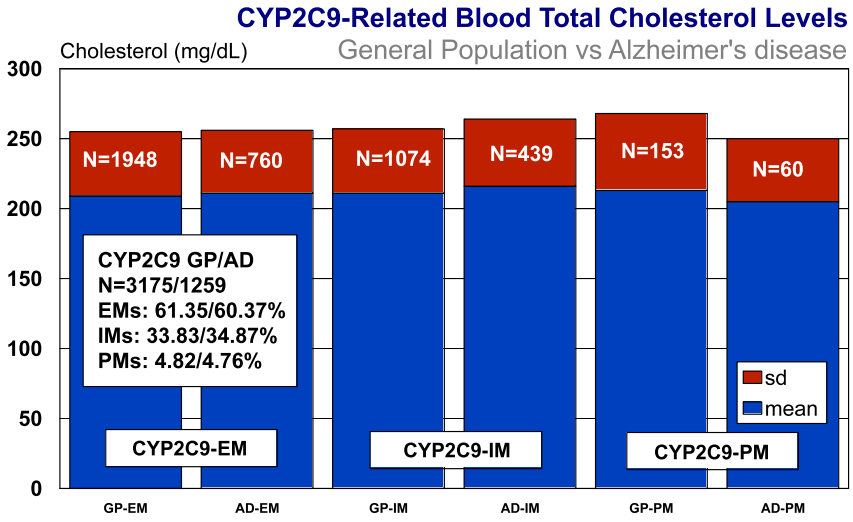


Figure 48. CYP2C9-EM-, IM- and PM-related blood total cholesterol levels in the general population and in patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

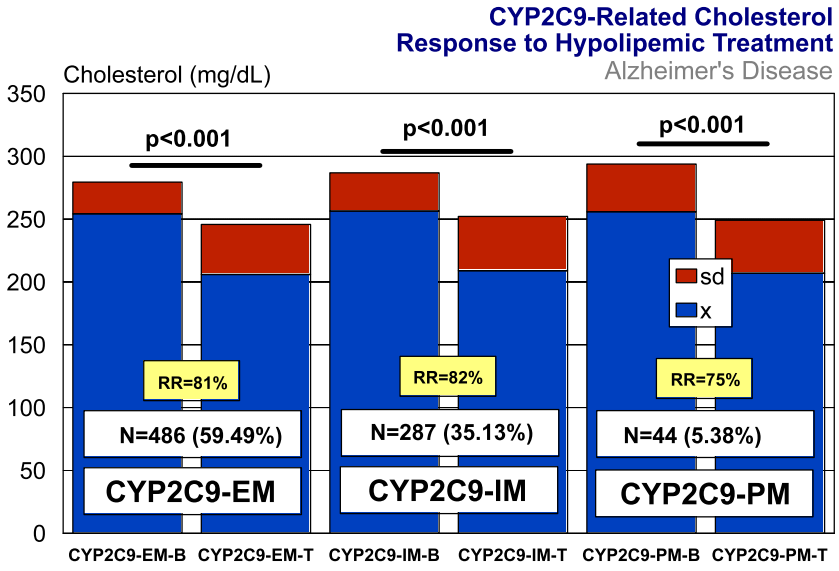


Figure 49. *CYP2C9*-EM-, IM- and PM-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

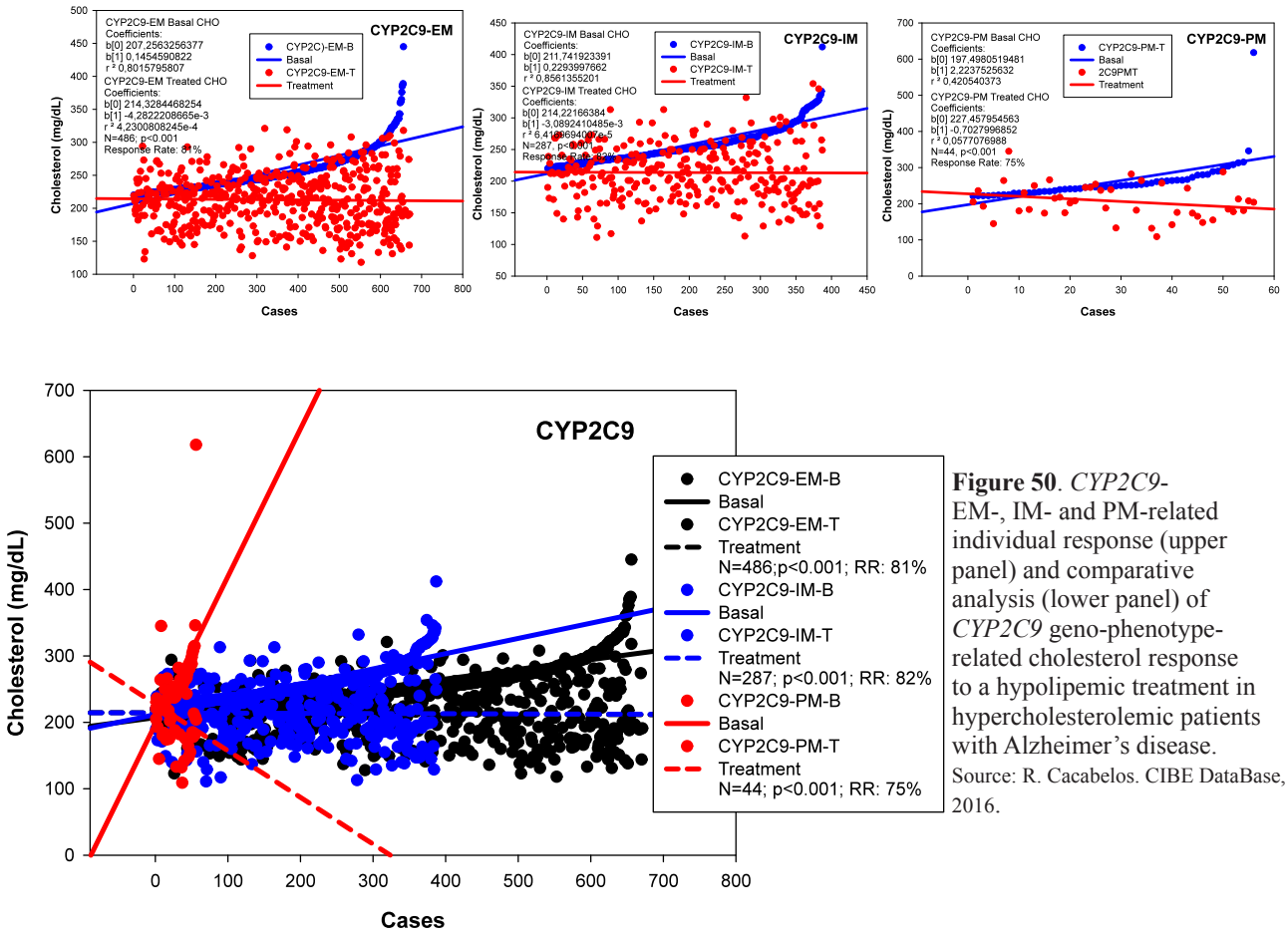


Figure 50. *CYP2C9*-EM-, IM- and PM-related individual response (upper panel) and comparative analysis (lower panel) of *CYP2C9* geno-phenotype-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

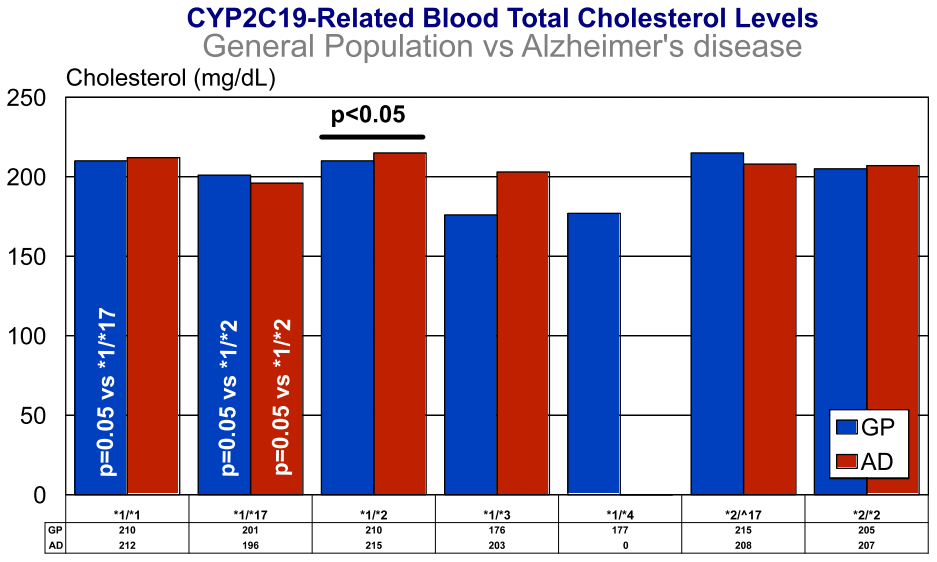


Figure 51. *CYP2C19* genotype-related blood total cholesterol levels in the general population and in patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

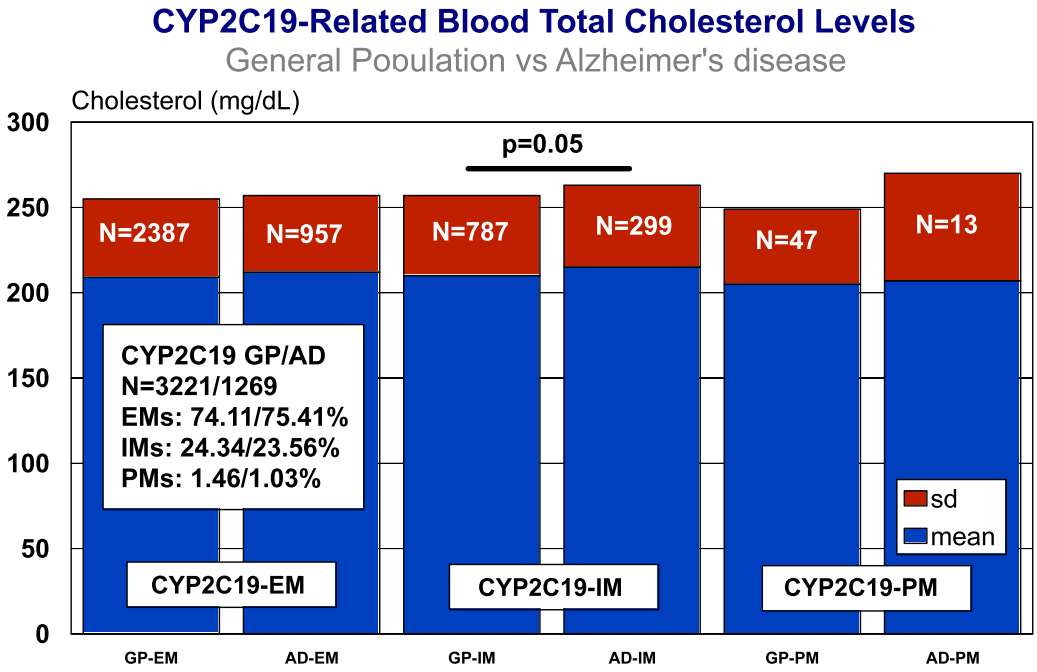


Figure 52. *CYP2C19*-EM-, IM- and PM-related blood total cholesterol levels in the general population and in patients with Alzheimer's disease.

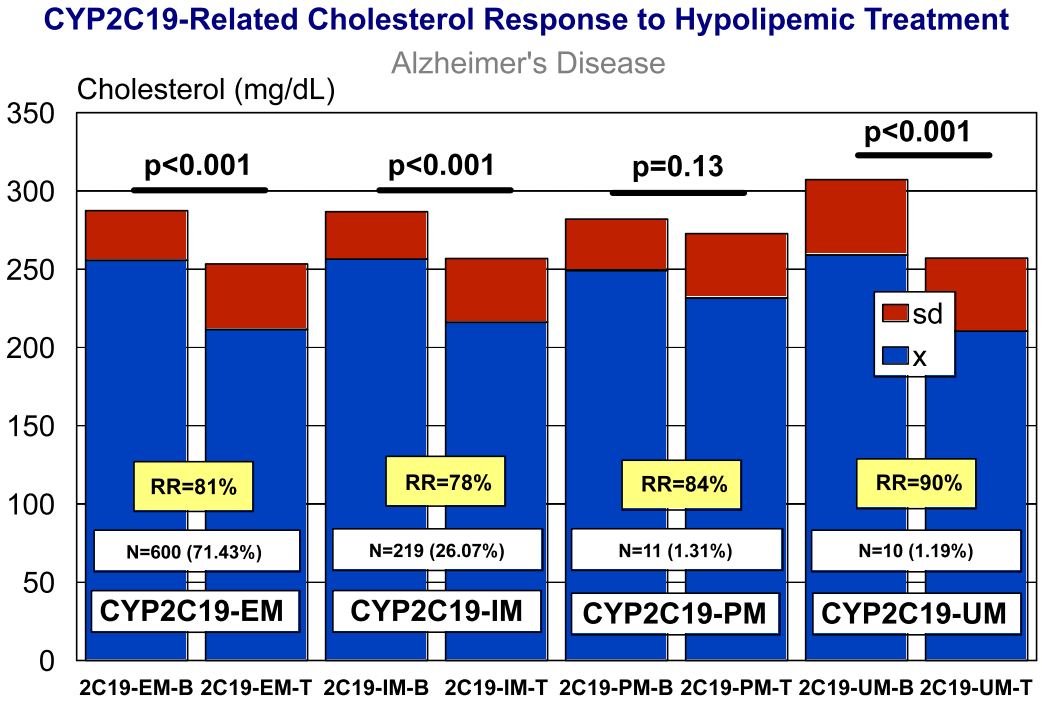


Figure 53. *CYP2C19*-EM-, IM-, PM- and UM-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

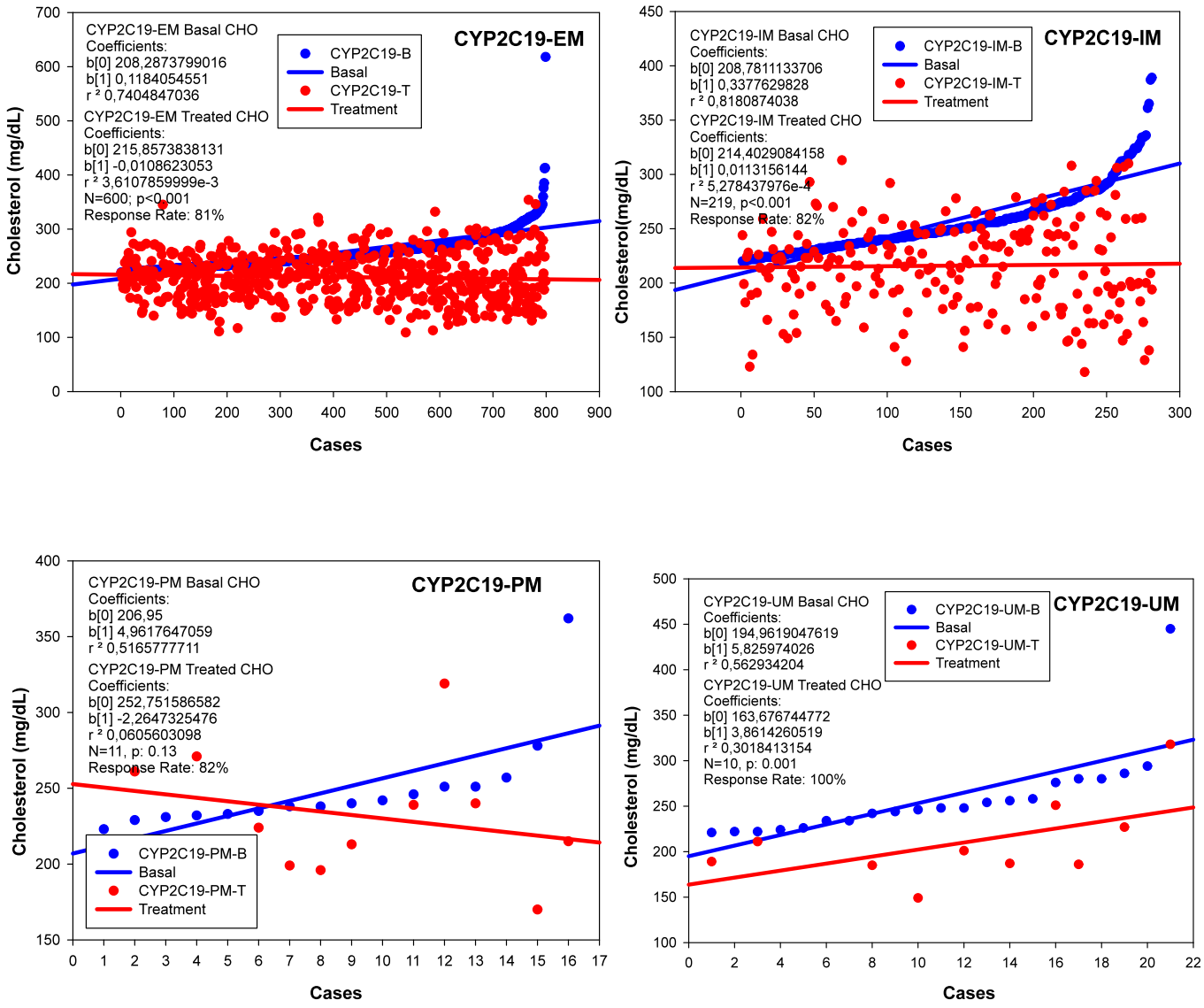


Figure 54. *CYP2C19*-EM-, IM-, PM- and UM-related individual response of cholesterol to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

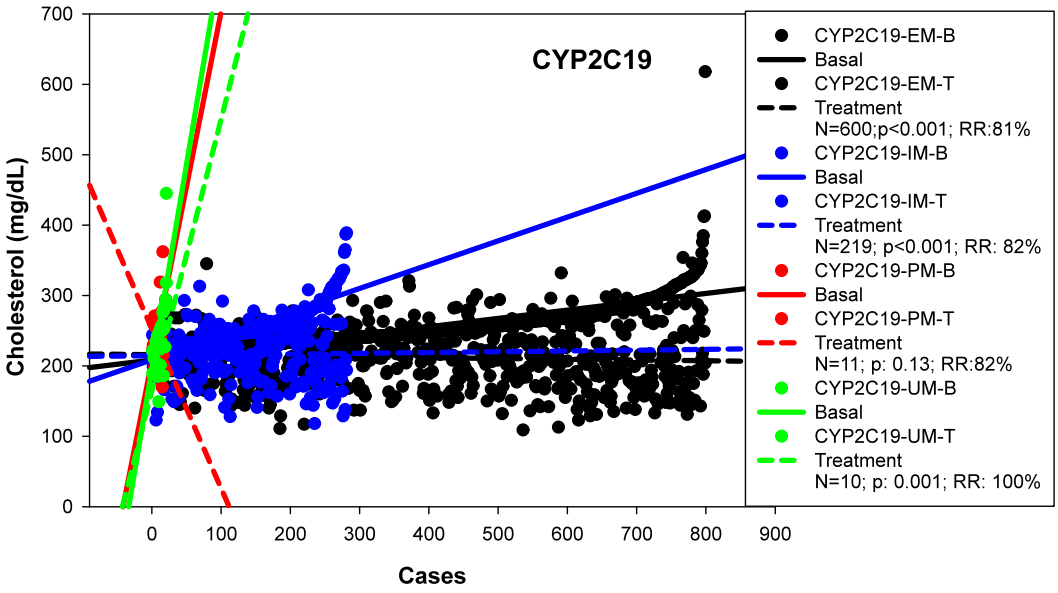


Figure 55. Comparative analysis of *CYP2C19* geno-phenotype-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

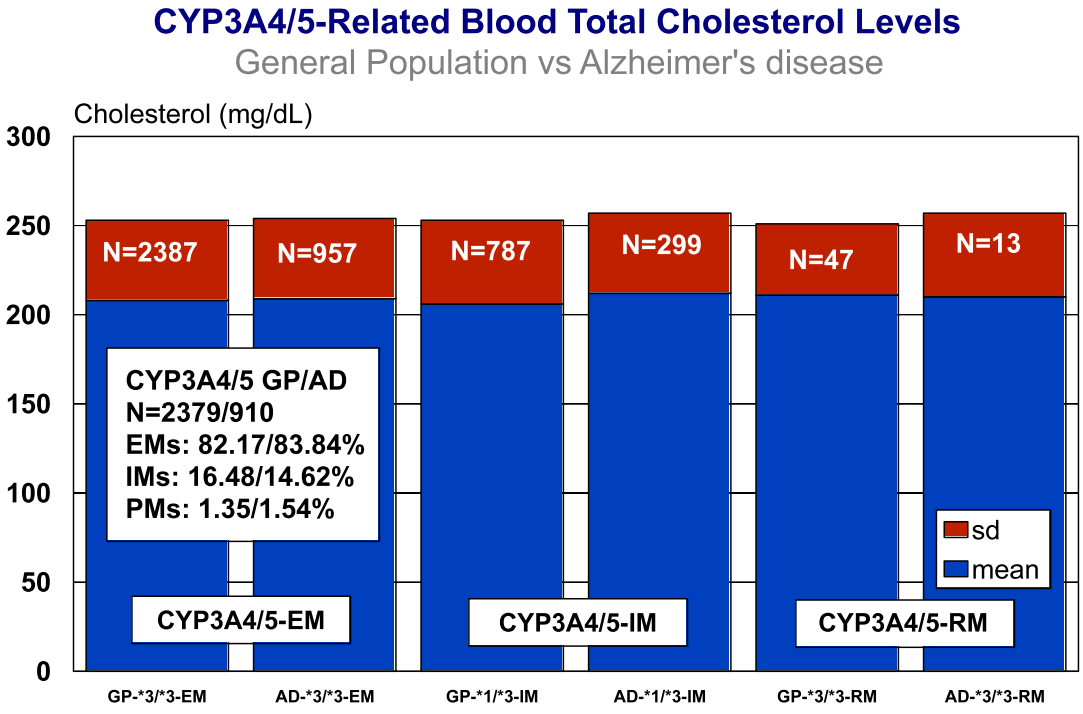


Figure 56. *CYP3A4/5* geno-phenotype-related blood total cholesterol levels in the general population and in patients with Alzheimer's disease.
Source: R. Cacabelos. CIBE DataBase, 2016.

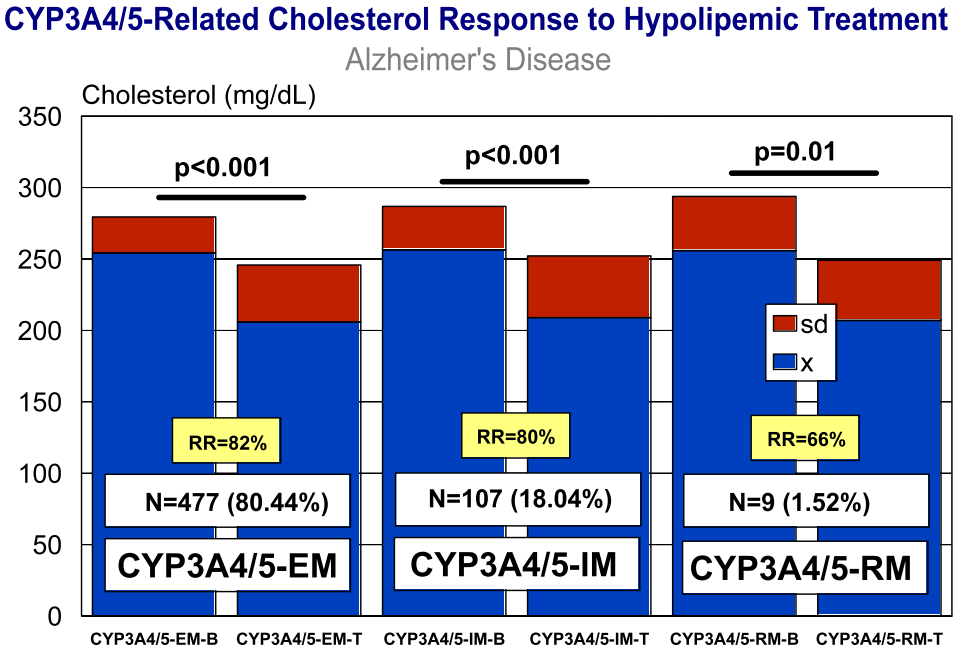


Figure 57. *CYP3A4/5* geno-phenotype-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease. Source: R. Cacabelos. CIBE DataBase, 2016.

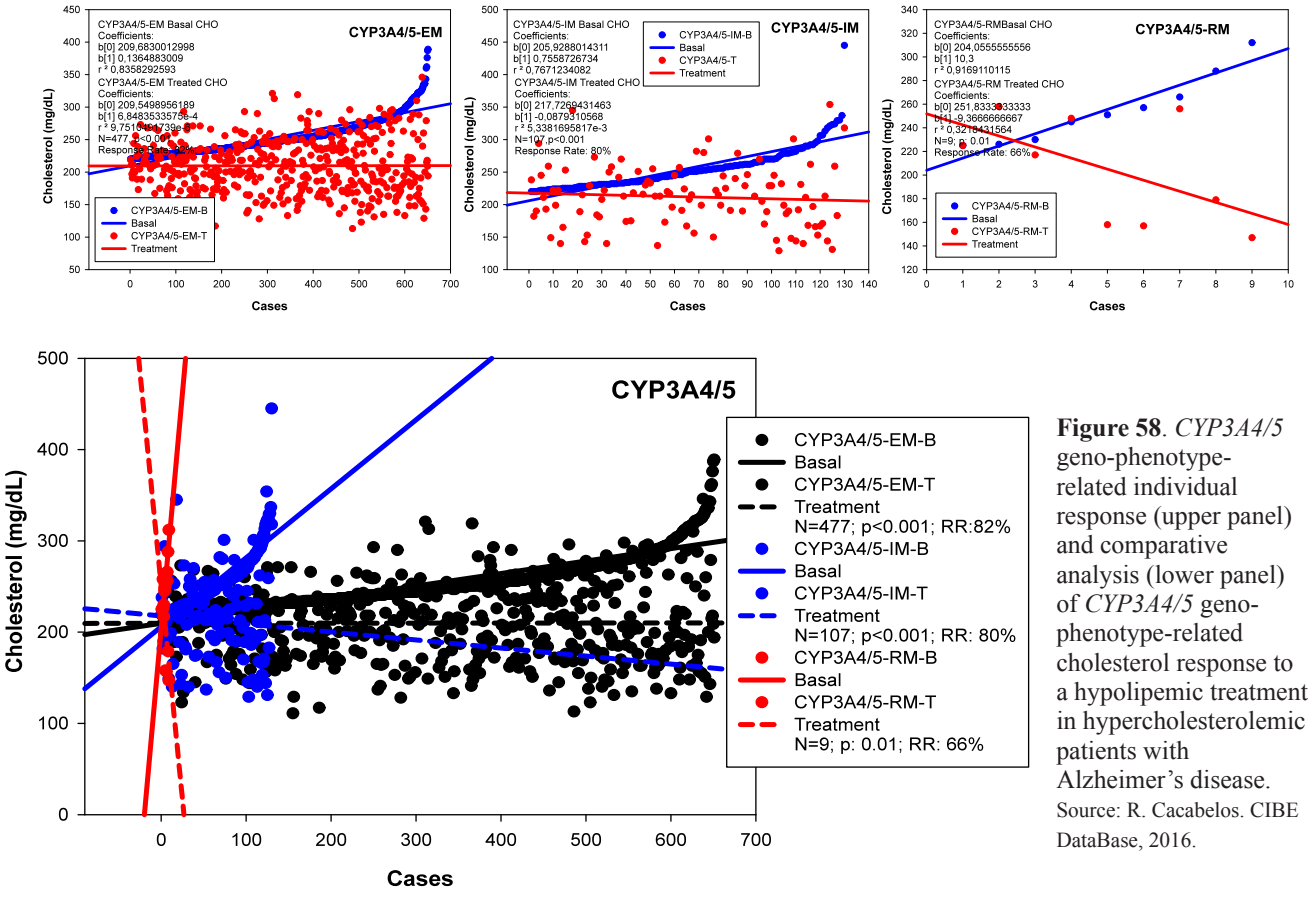


Figure 58. *CYP3A4/5* geno-phenotype-related individual response (upper panel) and comparative analysis (lower panel) of *CYP3A4/5* geno-phenotype-related cholesterol response to a hypolipemic treatment in hypercholesterolemic patients with Alzheimer's disease. Source: R. Cacabelos. CIBE DataBase, 2016.

Table 5. Methylation patterns in genes encoding Phase I and Phase II drug metabolizing enzymes and transporters

Category	Gene	Locus	Promoter length (bp)	Pathology	Methylation	References
Phase I Drug Metabolism Genes	*ALDH1A2*	15q21.3	982	prostate cancer	Hypermethylated	154,197,198
	CYP1A1	15q24.1	1200	head and neck cancer prostate cancer fetal growth restriction (toxics) smoking-related	Hypermethylated Hypermethylated Hypomethylated Hypomethylated	197-199 152,154,197,199,200 154,199,201 29,154,199,202 152,154,199,203,204
	CYP1B1	2p22.2	1193	colorectal cancer prostate cancer hepatoma cell lines breast cancer	Hypermethylated Hypomethylated Hypermethylated Hypermethylated	29,151,154,199,202,205 152,154,197,200,201,206 29,154,199,205,207,208 29,154,199,205,209
	CYP24A1	20q13	945	vitamin D deficiency tumor-derived endothelial cells	Hypermethylated Hypermethylated	29,154,197,199,205, 210,211
	CYP27B1	12q14.1	917	breast cancer choriocarcinoma lymphoma and leukemia	Hypermethylated Hypermethylated Hypermethylated	20,154,199,205,212,213 29,154,199,205,214 29,154,199,205,215
	CYP2A13	19q13.2	928	head and neck cancer	Hypermethylated	154,197,198
	CYP2C19	10q24	1048	Drug resistance	Hypermethylated	29,154,199,205
	CYP2E1	10q26.3	918	Parkinson's disease toluene exposure	Hypomethylated Hypomethylated	29,154,197,199,205,216 154,202,216,217

CONCLUSIONS

1. AD is a complex disorder with a tetravalent phenotype (neuropathological, neurobehavioral, age-related, and gender-related components).
2. Major determinants of therapeutic outcome in AD include age- and sex-related factors, pathogenic phenotype, concomitant disorders, treatment modality and polypharmacy, and pharmacogenetics.
3. Different categories of genes are potentially involved in the pharmacogenetic network responsible for drug efficacy and safety.
4. Pathogenic, mechanistic, metabolic, transporter, and pleiotropic genes represent the major genetic determinants of response to treatment in AD.
5. The genes involved in the pharmacogenomic network are under the regulatory control of the epigenetic machinery (DNA methylation, histone modifications, miRNA regulation), this configuring the novel pharmacoepigenomic apparatus and constituting a novel source of potential therapeutic targets.
6. By-products of these genes are integrated in transcriptomic, proteomic and metabolic networks which are disrupted in AD and represent potential targets for therapeutic intervention.
7. In pharmacogenetic studies with conventional anti-dementia drugs and combination treatments, *APOE-4* carriers are the worst responders and *APOE-3* carriers are the best responders; patients harboring a large (L) number of poly T repeats in intron 6 of the *TOMM40* gene (L/L or S/L genotypes) in haplotypes associated with *APOE-4* are the worst responders to treatment; patients with short (S) *TOMM40* poly T variants (S/S genotype) in haplotypes with *APOE-3* are the best responders to treatment; and *CYP2D6* and *ABCB1* variants may influence the therapeutic response to conventional treatments.
8. Over 80% of AD patients are daily consumers of different treatments for concomitant disorders. Only 20% of the Caucasian population are extensive metabolizers for the tetragenic haplotype integrated by *CYP2D6, CYP2C9, CYP2C19* and CYP3A4/5 variants.
9. Tetragenic haplotypes integrating CYP2D6, CYP2C9, CYP2C19 and CYP3A4/5 variants yield 156 genotypes. The most frequent haplotype is H3 (1/1-1/1-1/1-3/3)(20.87%), representing full extensive metabolizers, and only 17 haplotypes exhibit a frequency higher than 1% in the Spanish population.
10. AD patients exhibit at least 111 pentagenic (*APOE-APOB-APOC3-CETP-LPL*) haplotypes associated with cholesterol levels. The highest levels of cholesterol are present in carriers of the haplotype *H104* (44-CC-CC-AA-CC) and the lowest levels of cholesterol are detected in carriers of the haplotype *H26* (23-TT-CG-AG-CC).
11. The response of cholesterol to specific hypolipemic treatments in hypercholesterolemic AD patients is highly efficient in over 70% of the cases and associates with CHO-related haplotypes and drug-specific *CYP* metabolizer geno-phenotypes.
12. Further considerations for a mature profile of AD pharmacogenomics include the following: (i) a better characterization of the roles played in drug efficacy and safety by genes involved in the pharmacogenomic network is necessary; (ii) since most genes are under the influence of the epigenetic machinery, pharmacoepigenomics is becoming an attractive field which deserves special attention; (iii) drug-drug interactions represent a problematic issue in over 80% of AD patients; (iv) since the neurodegenerative process underlying AD neuropathology starts 20-30 years before the onset of the disease, novel therapeutics should be addressed to prevent premature neuronal death; (v) specific biomarkers for AD are necessary in 3 different contexts: predictive markers before disease onset, early diagnosis in initial stages, and drug monitoring (in both preventive and/or therapeutic strategies); and (vi) physicians should be aware of the usefulness of pharmacogenomics to prescribe more accurately, avoid adverse reactions and optimize the limited therapeutic resources available for the treatment of dementia.

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