Review

Convergence of genes implicated in Alzheimer’s disease on the cerebral cholesterol shuttle: APP, cholesterol, lipoproteins, and atherosclerosis

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Abstract

Polymorphic genes associated with Alzheimer’s disease (see www.polygenicpathways.co.uk) delineate a clearly defined pathway related to cerebral and peripheral cholesterol and lipoprotein homeostasis. They include all of the key components of a glia/neuron cholesterol shuttle including cholesterol binding lipoproteins APOA1, APOA4, APOC1, APOC2, APOC3, APOD, APOE and LPA, cholesterol transporters ABCA1, ABCA2, lipoprotein receptors LDLR, LRP1, LRP8 and VLDLR, and the cholesterol metabolising enzymes CYP46A1 and CH25H, whose oxysterol products activate the liver X receptor NR1H2 and are metabolised to esters by SOAT1. LIPA metabolises cholesterol esters, which are transported by the cholesteryl ester transport protein CETP. The transcription factor SREBF1 controls the expression of most enzymes of cholesterol synthesis. APP is involved in this shuttle as it metabolises cholesterol to 7-betahydroxycholesterol, a substrate of SOAT1 and HSD11B1, binds to APOE and is tethered to LRP1 via APPB1, APBB2 and APBB3 at the cytoplasmic domain and via LRPAP1 at the extracellular domain. APP cleavage products are also able to prevent cholesterol binding to APOE. BACE cleaves both APP and LRP1. Gamma-secretase (PSEN1, PSEN2, NCSTN) cleaves LRP1 and LRP8 as well as APP and their degradation products control transcription factor TFCP2, which regulates thymidylate synthase (TS) and GSK3B expression. GSK3B is known to phosphorylate the microtubule protein tau (MAPT). Dysfunction of this cascade, carved out by genes implicated in Alzheimer’s disease, may play a major role in its pathology.

Many other genes associated with Alzheimer’s disease affect cholesterol or lipoprotein function and/or have also been implicated in atherosclerosis, a feature of Alzheimer’s disease, and this duality may well explain the close links between vascular and cerebral pathology in Alzheimer’s disease. The definition of many of these genes as risk factors is highly contested. However, when polymorphic susceptibility genes belong to the same signaling pathway, the risk associated with multigenic disease is better related to the integrated effects of multiple polymorphisms of genes within the same pathway than to variants in any single gene [Wu, X., Gu, J., Grossman, H.B., Amos, C.I., Etzel, C., Huang, M., Zhang, Q., Millikan, R.E., Lerner, S., Dinney, C.P., Spitz, M.R., 2006. Bladder cancer predisposition: a multigenic approach to DNA-repair and cell-cycle-control genes. Am. J. Hum. Genet. 78, 464–479.] Thus, the fact that Alzheimer’s disease susceptibility genes converge on a clearly defined signaling network has important implications for genetic association studies.

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Keywords: Alzheimer’s disease; Atherosclerosis; Cholesterol metabolism; Lipoprotein; Cholesterol shuttle

1. Introduction

Mutations in amyloid precursor protein (APP) and the presenilins, PSEN1 and PSEN2, have been shown to be responsible for a number of familial Alzheimer’s disease cases and different apolipoprotein E (APOE 1–4) alleles strongly influences the incidence of Alzheimer’s disease. Presenilin is part of a protease complex that degrades APP. One of the functional effects of the mutations in APP, PSEN1 and PSEN2 is to increase the degradation of APP to potentially toxic neuropeptides (beta amyloid) that are a major component of amyloid plaque in the Alzheimer’s disease brain (Tanzi and Bertram, 2001). Apolipoprotein E (APOE) also interacts with APP and beta amyloid (Haas et al., 1997). Because of these relationships in key genes relating to pathology, the amyloid hypothesis of Alzheimer’s disease has now held centre stage for some time. Briefly stated, defective APP processing leads to the
generation of neurotoxic insoluble beta-amyloid peptides that accumulate in the senile plaques that characterise the disease (Hardy and Selkoe, 2002).

Recent studies have suggested that cholesterol lowering agents (3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) inhibitors or statins) (Dufouil et al., 2005; Sparks et al., 2005a; Sjogren et al., 2003; Wolozin et al., 2000; Zamrini et al., 2004) or a diet rich in fish or n − 3 unsaturated fatty acids (Morriss et al., 2003b) can reduce the incidence of Alzheimer’s disease. Negative studies with statins have also been reported (Zandi et al., 2005; Rea et al., 2005; Scott and Laake, 2001; Sparks et al., 2005b) and the results of more extensive clinical trials are eagerly awaited. Conversely, obesity is a risk factor for Alzheimer’s disease in women (Gustafson et al., 2003) and the dietary intake of saturated fatty acids increases the risk for Alzheimer’s disease (Morris et al., 2003a). Whether, and how, these factors modify the incidence of Alzheimer’s disease all require further study.

Alzheimer’s disease also has a significant vascular component. Other authors have pointed out the link between atherosclerosis and Alzheimer’s disease, which share common gene associations and risk factors and vascular problems (Cassery and Topol, 2004; de la Torre, 2004). The changes in these indices of cholesterol and lipoprotein function in Alzheimer’s disease are subtle and less evident than observed for other cholesterol/lipoprotein disorders such as atherosclerosis, cardiovascular and cerebrovascular disease. As pointed out by Pappolla et al. (2003) it is likely that larger changes in cholesterol/lipoprotein function would be fatal before Alzheimer’s disease could develop and that cholesterol/Alzheimer’s disease associations would be masked by this effect. The atherosclerosis link is supported by a close correlation between atherosclerotic markers and dementia, vascular dementia and Alzheimer’s disease (Hofman et al., 1997). In this study, including 207 Alzheimer’s disease patients, indices of peripheral atherosclerosis including carotid artery plaques, carotid artery wall thickness and a combined atherosclerosis score were all correlated with Alzheimer’s disease. A large American study (N = 3602) has also noted a link between peripheral atherosclerosis and Alzheimer’s disease (Newman et al., 2005). A similar link has been observed in the Alzheimer’s disease brain where cognitive decline is related to small vessel arteriosclerosis and cerebral amyloid angiopathy. In a neuropathological study of 137 autopsy confirmed Alzheimer’s disease cases, 92% showed cerebral arteriosclerotic changes (Tian et al., 2004). Atherosclerosis has also been observed in the circle of Willis and in large leptomeningeal vessels, and both correlate with the neuropathology and diagnosis of Alzheimer’s disease (Roher et al., 2004; Roher et al., 2003).

The mutations in APP or presenilins are observed in 30–50% of familial Alzheimer’s disease but together they account for less than 5% of all Alzheimer’s disease cases. APOE is a strong risk factor and a meta-analysis suggests that 60% of Alzheimer’s disease cases over the age of 65 years and 92% of cases below the age of 65 are related to APOE variants (Rubinsztein and Easton, 1999). Over 100 other genes have been associated with Alzheimer’s disease, in at least one study, although their role as susceptibility factors is often contested because of conflicting genetic data in different populations (see below). These genes are listed in Tables 1–8 together with a brief summary of their main effects, interactions, and relationship to APP, cholesterol, and lipoprotein function. Examples of references to positive association studies are posted at a supplementary Website at http://www.polygenic-pathways.co.uk/alzpolys.html. These website tables also provide the chromosomal location of each gene and references for linkage data implicating this region in Alzheimer’s disease, where available. A brief summary of expression and functional data is also provided. Because of the large number of references generated by so many genes, some citations in the text of this manuscript are collectively referenced by this website (Carter, 2006). All positive and negative association studies can be viewed at the Alzgene website (http://www.alzforum.org) (Bertram et al., 2005). As shown below, many of these genes (including APOE and the beta and gamma secretases) can be assembled to form most of the components of a glial/neuronal cholesterol shuttle whose function specifically relates to the control of cerebral cholesterol homeostasis. Many of these same genes are also involved in atherosclerosis. It is recognised that the multiple other properties of some of these components are also likely to play a role in the multifactorial pathology of Alzheimer’s disease (Cacabelos et al., 2005). These properties are not addressed in this review, which focuses on cholesterol and lipoprotein function to illustrate the convergence of many genes on this area. Genes associated with Alzheimer’s disease are shown in bold in the subsequent text. Gene symbols are those of the HUGO gene nomenclature committee (Wain et al., 2002).

2. Heterogeneity in association studies and reasons for including all positively associated genes

The definition of most of the genes used to create the pathways described below, as risk factors, is highly contested. APOE is accepted as an important risk factor (see above) but for the bulk of these genes, widely conflicting association data from different populations is the norm rather than the exception (see the Alzgene database for discussion and a full referencing of all positive and negative association studies (Bertram et al., 2005)). A widely held view, given the enormous problems of replicability in genetic association studies, is that many (if not most) of these report false positives because of problems in statistical power and replicability, or because of recombination effects in which a nearby linked susceptibility gene may influence the association of another with the disease (Bertram and Tanzi, 2004; Prince et al., 2001).

There is however, a precedent that provides a physiological explanation for some of the heterogeneity in association studies. Bladder cancer is also a multigenic disease involving multiple chromosomal loci and disputed susceptibility genes (Saran et al., 1996). A recent study of bladder cancer measured the association of 44 cell cycle and DNA-repair polymorphic genes, only three of which were significantly associated with...
Components of the cholesterol shuttle and cholesterol and lipoprotein related genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>General function and relationships with other gene products</th>
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<tbody>
<tr>
<td>A2M: alpha-2-macroglobulin</td>
<td>Ligand for LRP1 (Strickland et al., 1990). Binds to APOE (Krimbou et al., 1998). Binds to growth factors including BDNF (Wolf and Gonias, 1994); binds to beta amyloid and mediates degradation and transport of amyloid beta peptides via A2M associated proteases and LRP1 (Fabrici et al., 2001). Binds to lecithin cholesterol acyl transferase (LCAT) (Krimbou et al., 2001)</td>
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<tr>
<td>LPA: lipoprotein, Lp(a)</td>
<td>Binds to APOE (Bard et al., 1992). LDLR, LRP1, VLDL and gp330 lipoprotein receptors (Niemeyer et al., 1999; Reblin et al., 1997)</td>
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<tr>
<td>APOA1: apolipoprotein A1</td>
<td>Promotes cholesterol efflux from tissues to the liver for excretion, and a cofactor for lecithin cholesterol acyltransferase (LCAT), which is responsible for the formation of most plasma cholesterol esters</td>
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<tr>
<td>APOA4: apolipoprotein A4</td>
<td>Potent activator of LCAT (Emmanuel et al., 1994). Substrate for ABCA1 (Remaley et al., 2001)</td>
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<tr>
<td>APOC1: apolipoprotein C-I</td>
<td>Activates LCAT, inhibits CETP (Shachter, 2001); elevated serum cholesterol, triglycerides and free fatty acids in transgenic mice expressing hAPOC1 (Jong et al., 1998). (Shachter et al., 1996). Transported by ABCA1 (Remaley et al., 2001). Inhibits APOE mediated binding of VLDL to LRP1 and VLDL (Jong et al., 1999b)</td>
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<tr>
<td>APOC2: apolipoprotein C-II</td>
<td>Transported by ABCA1 (Remaley et al., 2001). Binds to and activates LPL (Shen et al., 2002; MacPhee et al., 2000) reduces APOE binding to LDLR (Clavey et al., 1995)</td>
</tr>
<tr>
<td>APOC3: apolipoprotein C-III</td>
<td>Transported by ABCA1 (Remaley et al., 2001). Binds to LDLR, inhibits LPL (Jong et al., 1999a)</td>
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<tr>
<td>APOD: apolipoprotein D</td>
<td>Role in Cholesterol ester exchange: binds to and activates LCAT (Holmquist, 1989). Release from astrocytes stimulated by 25-hydroxycholesterol (Patel et al., 1995)</td>
</tr>
<tr>
<td>APOE: apolipoprotein E</td>
<td>Major cholesterol carrier (Swertfeger and Hui, 2001). Ligand for LDLR (Raussens et al., 2002), LRP1 (Myklebost et al., 1989), LRP8 (Kim et al., 1996), LDLR and VLDLR (Liu et al., 2002); amyloid beta binds to APOE (Strittmatter et al., 1993)</td>
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<tr>
<td>ABCA1: ATP-binding cassette, sub-family A (ABC1), member 1</td>
<td>ABCA1 induction reduces beta and gamma secretase activity (Sun et al., 2003) and increases the secretion of amyloid beta 40 and 42 (Fukumoto et al., 2002). Transports cholesterol (Knight, 2004). Transports lipoproteins APOA-II, APOA4, APOC1, APOC2, APOC3, APOE (Remaley et al., 2001)</td>
</tr>
<tr>
<td>ABCA2: ATP-binding cassette, sub-family A (ABC2), member 2</td>
<td>Expression in CHO cells increases LDLR expression (Chen et al., 2004) Mutation in familial AD (see OMIM) (OMIM, 2004a): major component of senile plaques. APP or beta-amyloid metabolise cholesterol to the oxysterol 7-beta hydroxycholesterol (Nelson and Alkon, 2004). Beta amyloid/copper complexes have also been shown to act as a cholesterol oxidase producing 4-cholesten-3-one (Puglielli et al., 2005). Fe65 adaptor proteins (APBB1, APBB2, APBB3) bind to the intracellular domain of both APP and LRP1 (Kinoshita et al., 2003)</td>
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<tr>
<td>LDLR: low density lipoprotein receptor</td>
<td>Binds to LPA (Reblin et al., 1997), APOB, APOE (Defesche, 2004). Mediates the increase in the astrocytic expression of APOE induced by beta-amyloid (LaDu et al., 2000). AICD interacts with ARH an adaptor protein for LDLR (Noviello et al., 2003) Major role in cholesterol uptake via binding to APOE and APOE containing particles (Defesche, 2004)</td>
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<tr>
<td>LRP8 (APOER2)</td>
<td>Cleaved by gamma secretase (Kinoshita et al., 2003). Brain localised APOE receptor (Kim et al., 1996). Associated with LRPAP1 (Andersen et al., 2003)</td>
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<tr>
<td>HSPG2: heparan sulfate proteoglycan 2 (perlecan)</td>
<td>Bind to beta amyloid and promotes fibrillogenesis (Castillo et al., 1997). Binds and internalises low-density lipoproteins enriched in LPL (Fuki et al., 2000)</td>
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<tr>
<td>LRPAP1: low density lipoprotein receptor-related protein associated protein 1 (RAP)</td>
<td>Universal low density lipoprotein receptor antagonist and chaperone (LDLR, LRP1, LRP8, VLDLR) (Bu, 1998; Bu, 2001; Medhi et al., 1995). Controls APP/LRP1 association (Goto and Tanzi, 2002)</td>
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<tr>
<td>OLR1: oxidised low density lipoprotein (lectin-like) receptor 1 (LOX1; SCARE1)</td>
<td>Oxidised LDL Receptor (Yamada et al., 1998)</td>
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<td>VLDLR: very low density lipoprotein receptor</td>
<td>Binds APOE, Lipoprotein (a) (LPA), LPL, and serpin/protease complexes. Also binds to LRPAP1 (Rettenberger et al., 1999) and APOC1 (Jong et al., 1999b)</td>
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<tr>
<td>Gene</td>
<td>General function and relationships with other gene products</td>
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<tr>
<td>LPL: lipoprotein lipase</td>
<td>Bridging factor for lipoprotein receptor mediated lipoprotein uptake. Binds to LRP1 (Williams et al., 1994) and gp330 (Kounnas et al., 1993). Activated by APOC2 (Fitzharris et al., 1981). Inhibited by APOE (Jong et al., 1997). Cholesterol prevents APOC2 binding to LPL (Arimoto et al., 1998). Increases the capture of HDL cholesteryl esters in the liver via binding to hepatic sulphate proteoglycans (Rinninger et al., 1998)</td>
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<tr>
<td>CH25H: cholesterol 25 hydroxylase</td>
<td>25-hydroxycholesterol stimulates APOD secretion from astrocytes (Patel et al., 1995) and of APOE in a human astrocytoma cell line (Gueguen et al., 2001). 25-Hydroxycholesterol suppresses transcription of cholesterol synthetic enzymes and increases HMGCGR degradation (Taylor, 1992)</td>
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<tr>
<td>CYP46A1: cytochrome P450, family 46, subfamily A, polypeptide 1</td>
<td>Major cholesterol metabolising enzyme in brain (Lund et al., 2003). The CYP46 polymorphism is associated with increased brain beta-amyloid load and increased CSF levels (Papassotropoulos et al., 2003)</td>
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<tr>
<td>HSD11B1: 11-beta-hydroxysteroid dehydrogenase type 1</td>
<td>7-Beta hydroxy cholesterol, the product of cholesterol oxidation by APP is metabolised by type 1 11-hydroxysteroid dehydrogenase(s) (HSD11B1) to the corresponding keto derivative (Hult et al., 2004)</td>
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<tr>
<td>LIPA: lipase A, lysosomal acid, cholesterol esterase</td>
<td>Functions in the lysosome to catalyze the hydrolysis of cholesteryl esters and triglycerides. Mutations in this gene can result in Wolman disease and cholesteryl ester storage disease</td>
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<tr>
<td>CETP: cholesteryl ester transfer protein</td>
<td>Transfers cholesteryl esters between lipoproteins</td>
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<td>SOAT1: sterol O-acyltransferase (acyl-Coenzyme A: cholesterol acyltransferase) 1</td>
<td>Modulates Abeta production via control of cholesterol/cholesteryl ester ratio (Puglielli et al., 2001); metabolises cholesterol and o xoysterols to esters (Cases et al., 1998; Zhang et al., 2003); inhibitors reduce plasma cholesterol levels (Miyazaki et al., 2003)</td>
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<tr>
<td>PPP1R3A: protein phosphatase 1, regulatory (inhibitor) subunit 3A (glycogen and sarcoplasmic reticulum binding subunit, skeletal muscle) (PP1G; PPP1R3)</td>
<td>Dephosphorylates HMG CoA reductase? and glycogen synthase (Schelling et al., 1988)</td>
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<tr>
<td>MAPK5IP1: mitogen-activated protein kinase 8 interacting protein 1</td>
<td>Associated with cytoplasmic domain of LRP1 and VLDLR (Stockinger et al., 2000) and APP (Matsuda et al., 2001)</td>
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<tr>
<td>PIK3R3: phosphoinositide-3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)</td>
<td>Lipoprotein receptor signaling (Mineo et al., 2001). Involved in the anti-apoptotic effects of APP (Kashour et al., 2003); P38 kinase activation by insulin increases the secretion of soluble APP (Solano et al., 2000)</td>
</tr>
<tr>
<td>APBB1: amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65)</td>
<td>Linked to APP, LRP1 and LDLR (Trommsdorff et al., 1998). Repression of thymidylate synthase via interaction with TFCP2 (Bruni et al., 2002)</td>
</tr>
<tr>
<td>APBB2: amyloid beta (A4) precursor protein-binding, family B, member 2 (Fe65-like)</td>
<td>Binds to cytoplasmic domain of APP (Guenette et al., 1996). Transfection decreases expression of LRP1 (Guenette et al., 2002)</td>
</tr>
<tr>
<td>APBB3: amyloid beta (A4) precursor protein-binding, family B, member 3 (Fe65L2)</td>
<td>Repression of thymidylate synthase probably via TFCP2 (Bruni et al., 2002; Zambrano et al., 1998). Linked to LRP1 (Tanahashi and Tabira, 2002) and APP (Tanahashi and Tabira, 1999)</td>
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<tr>
<td>TFCP2: transcription factor CP2 (CP2; LSF; LBP-1C; TFCP2C)</td>
<td>Linked to LRP1 via LRPCDC effects on FE65 mediated transcription (Kinoshita et al., 2003). Linked to APP effects via APBB’s (Fe65 proteins) and AICD (Zambrano et al., 1998; Cao and Sudhof, 2004)</td>
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<tr>
<td>MTHFR: 5,10-methylenetetrahydrofolate reductase (NADPH)</td>
<td>Homocysteine stimulates HMG CoA reductase activity and cholesterol synthesis and secretion in hepatic cells and expression of HMGCRC, SREBF1 and SREBF2, FDP5 and IDL (Westermark et al., 2001; OK et al., 1998). Plasma homocysteine is transported by lipoproteins (Ventura et al., 2003); homocysteine increases the expression of LPL in macrophages (Beauchamp and Renier, 2002) and OLRI in aortic endothelial cells (Nagase et al., 2001)</td>
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<td>MTR: methionine synthase</td>
<td>Acetyl-CoA + an arylamine ⇔ CoA + an N-acetylarlamine. The endogenous substrate for NAT2 is the folate cotublate p-aminobenzoylglutamate (PABG) (Payton et al., 1999; Estrada-Rodgers et al., 1998) which is acetylated by the enzyme. PABG is derived from 7,8 dihydrolipoate, the substrate of thymidylate synthase (TS), the target of TFCP2</td>
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<tr>
<td>MTRR: 5-methyltetrahydrofolate-homocysteine methyltransferase reductase</td>
<td>Part of gamma secreatce complex (Kimberly and Wolfe, 2003; Kimberly et al., 2003) involved in degradation of APP to cytotoxic peptides. Gamma secreatce is involved in LRP1 and LRP8 processing (Kinoshita et al., 2003)</td>
</tr>
<tr>
<td>NAT2: N-acetyltransferase 2 (arylamine N-acetyltransferase)</td>
<td>Mutation in familial AD (see OMIM) (OMIM, 2004b)</td>
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<tr>
<td>PSEN1: presenilin 1</td>
<td>Part of gamma secreatce complex (Kimberly et al., 2003)</td>
</tr>
<tr>
<td>PSEN2: presenilin 2</td>
<td>Part of gamma secreatce complex (Kimberly et al., 2003)</td>
</tr>
<tr>
<td>NCSTN: nicasrin</td>
<td>Cleaves APP and LRP1 (von Arnim et al., 2005)</td>
</tr>
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</table>
| BACE1: beta-site APP-cleaving enzyme 1 | }
the disease, when assessed individually. However, in either cell cycle or DNA repair pathways, or a combination of both, an increasing number of polymorphisms, in different genes, sequentially increased the risk of developing the disease. Within the DNA repair pathway, for four or more polymorphic genes, each additional adverse allele (including those not individually associated with the disease) was associated with a 1.21-fold progressive increase in risk. In the combined DNA repair and cell cycle pathways the integrative effects of almost half of the 44 polymorphic genes influenced the eventual risk outcome. In other words, the disease was more tightly associated with the integrative effects of a number of polymorphic genes within a relevant physiological pathway, than with a variant in any particular gene (Wu et al., 2006). In addition, genes not associated with the disease, when assessed individually, are associated with the disease when these factors are taken into account.

Most of the association studies in Alzheimer’s disease relate to the effects of polymorphisms in a single gene on the risk outcome. Several other studies have shown that the risk of developing Alzheimer’s disease can be increased or modified by polymorphisms in pairs of genes. Genetic synergies and interactions in Alzheimer’s disease have been observed between the lipoprotein receptor LRP1 and the JNK kinase interacting protein, MAPK8IP1 (Helbecque et al., 2003), SERPINA3 and PSEN1 (Wang et al., 1998), and between APOE4 and a number of other polymorphic genes including CST3 (Beyer et al., 2001), LDLR (Cheng et al., 2005), LPA (Mooser et al., 2000) and MPO (Reynolds et al., 2000) inter alia. More complex interactions, akin to those observed in bladder cancer, have also been described. An American study showed that Alzheimer’s disease developing before the age of 70 is associated with combined polymorphisms in CST3, CTSD and APOE. Between the ages of 60–74, APOE, APOC1 and LDLR genotypes predicted the development of Alzheimer’s disease while APOE/LDLR genotypes predicted a later onset between ages 60–79 (Poduslo et al., 2004). In a European study (Switzerland) Alzheimer’s disease could be predicted with 74% accuracy by combined polymorphisms in APOE, SOAT1, OLR1, CYP46, LPL, LIPA, CH25H and APOA4 while PPARA, LDLR, and LRP1 were not associated with risk in this population (Papassotiropoulos et al., 2005) although each has been associated with Alzheimer’s disease in other studies. These genes are constituents of the pathways described below.

The problem of false-positives is crucial and may apply to some of the genes described below, but the problems of false-negatives that do not take into account these integrative effects

<table>
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<tr>
<th>Table 1</th>
<th>Cytokines and growth factors associated with Alzheimer’s disease</th>
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<tr>
<td>Gene</td>
<td>General function and relationships with other gene products</td>
</tr>
<tr>
<td>BACE2</td>
<td>APP processing (does not generate beta-amyloid) (Sun et al., 2005)</td>
</tr>
<tr>
<td>SREBF1: sterol regulatory element binding transcription factor 1</td>
<td>Major transcription factor controlling cholesterol synthesis and metabolism (Shimano, 2002). Controls LDLR expression (Zannis et al., 2001)</td>
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<tr>
<td>CD36: CD36 antigen (collagen type I receptor, thrombospondin receptor)</td>
<td>Scavenger receptor for oxidised lipoproteins and beta amyloid (Bamberger et al., 2003; Boullier et al., 2001)</td>
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<tr>
<th>Gene</th>
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<tr>
<td>BDNF: brain derived neurotrophic factor</td>
<td>Systemic injection lowers total serum cholesterol in mice (Tsachida et al., 2002). Binds to A2M, the ligand of LRP1 (Wolf and Gonias, 1994). Stimulates APP expression (Ruiz-Leon and Pascual, 2004; Ruiz-Leon and Pascual, 2001)</td>
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<tr>
<td>FGF1: fibroblast growth factor 1</td>
<td>Increases APOE expression and release in astrocytes (Tada et al., 2004): oxidised LDL complexes FGF1 and inhibits its activity (Ananyeva et al., 2003); Increases cholesterol synthesis in astrocytes (Ueno et al., 2002)</td>
</tr>
<tr>
<td>IL1A: interleukin alapha</td>
<td>IL1 stimulates APP expression in neural and endothelial cells (Forloni et al., 1992); oxyysterols release IL1A (Sjogren et al., 2002) IL1A increases the expression of astrocytic and microglialial HSPG2 (perlecan) in the hippocampus (Garcia et al., 1999)</td>
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<tr>
<td>IL1B: interleukin 1beta</td>
<td>Binds to A2M (Webb and Gonias, 1998). Decreases APP expression in human glioma cells (Yang et al., 1993) and affects APP processing (Dash and Moore, 1995) Insoluble amyloid beta peptides induce IL1B (Chong, 1997). Inflammatory cytokines (IL1B, TNFA) play an important role in atherosclerosis by reducing cholesterol efflux and reverse cholesterol transport (Ohashi et al., 2005)</td>
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<td>TNFA: tumor necrosis factor alpha</td>
<td>Increases APP expression in neural cells (Ohyagi and Tabira, 1993): beta-amyloid peptides induce IL6 (Chong, 1997). The APOE promoter region contains an IL6 responsive site (Lahiri, 2004) Infusion stimulates expression of most liver enzymes of cholesterol synthesis (Ruan et al., 2002). Regulates SREBF1 (Lawler et al., 1998b). Inflammatory cytokines (IL1B, TNFA) play an important role in atherosclerosis by reducing cholesterol efflux and reverse cholesterol transport (Ohashi et al., 2005)</td>
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Table 3
Nuclear receptors associated with Alzheimer’s disease

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<tr>
<th>Gene</th>
<th>General function and relationships with other gene products</th>
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<tr>
<td>AR: androgen receptor dihydrottestosterone receptor</td>
<td>Regulation of cholesterol synthetic machinery in the periphery via the control of SREBF1 and sterol regulatory element-binding protein cleavage-activating protein (SCAP) (Heemers et al., 2004, 2006). Both estrogen and androgens protect neurons from beta-amyloid toxicity via steroid receptor activation (Zhang et al., 2004b)</td>
</tr>
<tr>
<td>ESR1: estrogen receptor 1 (ER; ESR; Era; ESRA; NR3A1)</td>
<td>Estrogen response elements or control of transcription by estrogen have been observed for ABCA1 (Srividasta, 2002), APOE (Levin-Allerhand et al., 2001), LDLR, HMG-CoA reductase (Di Croce et al., 1999). An ESR1 selective ligand reduces plasma cholesterol levels in rats (Harris et al., 2002)</td>
</tr>
<tr>
<td>ESR2: estrogen receptor 2 (ER beta)</td>
<td>APOE is deposited along cerebral vessels and beta-amyloid (1-42) accumulates in cortical and limbic regions in ESR2 knockout mice (Zhang et al., 2004a)</td>
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<tr>
<td>CYP19A1: (aromatase: estrogen synthesis cf ESR1, ESR2)</td>
<td>Estrogen increases alpha-secretase activity (Hooper and Turner, 2002) and protects against beta amyloid neurotoxicity (Marin et al., 2003)</td>
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<tr>
<td>NR1H2: liver X receptor beta</td>
<td>LXR receptors positively control the transcription of many genes related to fatty acid and cholesterol function including ABCA1 and many lipoproteins including the APOE, APOC1, APOC2, APOC4 gene cluster and APOD (Mak et al., 2002; Hummasti et al., 2004; Tang et al., 2004; Liang et al., 2004)</td>
</tr>
<tr>
<td>POU2F1: pou domain class 2, transcription factor 1 (OCT-1)</td>
<td>Controls OLRL and LPL expression (Chen et al., 2006; Arca-Sedda et al., 1996).</td>
</tr>
<tr>
<td>PPARA: peroxisome proliferative activated receptor, alpha</td>
<td>Putative promoter site on BACE2 (Maloney et al., 2006). Major regulator of lipid and lipoprotein metabolism (Gierois et al., 2000). LPL generated lipoprotein metabolites are PPARA ligands (Ziouzenkova et al., 2003). In peripheral cells, activation increases expression of ABCA1 (Kok et al., 2003). Reduces SOAT1 activity (Chinetti et al., 2003). Polyunsaturated fatty acids are potent PPARA activators (Forman et al., 1997). Low levels of polyunsaturated fatty acids have been associated with an increased incidence of Alzheimer’s disease (Kyle et al., 1999; Tully et al., 2003) and fish diets rich in these compounds (n3-omega fatty acids) have been reported to reduce the incidence of Alzheimer’s disease (Morris et al., 2003b). A selective PPARA agonist increased the levels of HDL-cholesterol (‘‘good cholesterol’’) in mice</td>
</tr>
</tbody>
</table>

Table 4
Free radical defense genes associated with Alzheimer’s disease

<table>
<thead>
<tr>
<th>Gene</th>
<th>General function and relationships with other gene products</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTO1: glutathione S-transferase omega 1</td>
<td>Glutathione transferase</td>
</tr>
<tr>
<td>GSTT1: glutathione S-transferase theta 1</td>
<td>Glutathione transferase</td>
</tr>
<tr>
<td>MPO: myeloperoxidase</td>
<td>Lipoprotein oxidation, particularly APOE (Jolivalt et al., 1996). Peroxidation of beta amyloid may be involved in cross linking and fibre formation (Galeazzi et al., 1999) See below</td>
</tr>
<tr>
<td>NOS1</td>
<td>Nitric oxide is pro- or antioxidant with respect to lipoproteins, depending on oxidative status (Bloodsworth et al., 2000)</td>
</tr>
<tr>
<td>NOS3: nitric oxide synthase 3 (endothelial cell, eNOS)</td>
<td>Vitamin K binds to APOE (Lamon-Fava et al., 1998) and stimulates pregnant X receptors leading to CYP3A4 activation and LXR activation (Landes et al., 2003). Vitamin K reduces cholesterol levels (Kawashima et al., 1997)</td>
</tr>
<tr>
<td>NQO1 NAD(P)H dehydrogenase, quinone 1 (also involved in Vitamin K (phylloquinone) metabolism</td>
<td>Vitamin K binds to APOE (Lamon-Fava et al., 1998) and stimulates pregnant X receptors leading to CYP3A4 activation and LXR activation (Landes et al., 2003). Vitamin K reduces cholesterol levels (Kawashima et al., 1997)</td>
</tr>
<tr>
<td>PON1: paraoxonase 1</td>
<td>Proteins against lipoprotein oxidation (Aviram et al., 1998)</td>
</tr>
<tr>
<td>PON2: paraoxonase 2</td>
<td>Proteins against lipoprotein oxidation (Ng et al., 2001)</td>
</tr>
<tr>
<td>TF: transferrin</td>
<td>Lipoprotein oxidation by iron released from transferrin in acidic conditions (Lamb and Leake, 1994; Leake, 1997)</td>
</tr>
<tr>
<td>HFE: hemochromatosis</td>
<td>HFE is involved in iron homeostasis and competes with the binding of iron-transferrin (TF) to the transferrin receptor at neutral pH (Lebron et al., 1999)</td>
</tr>
<tr>
<td>ALDH2: aldehyde dehydrogenase 2 family (mitochondrial)</td>
<td>Lipoprotein oxidation ALDH2 polymorphisms influence the amount of lipoprotein–acetaldehyde adducts (Nagata et al., 1999)</td>
</tr>
</tbody>
</table>

are equally important. The heterogeneity in individual gene association studies may well reflect differences in the underlying oligogenic platform of other polygenic pathways in the same pathological pathway. This type of integration is likely to exist when susceptibility genes share the same function or belong to the same pathway. A logical extension of this line of argument is that the multiple genes associated with a polygenic disease might trace out this pathway. The identification of one such pathway, constructed from positively associated genes, despite the heterogeneity in individual association studies, is the aim of this review.

3. Cerebral cholesterol homeostasis and the cholesterol shuttle

The brain is the organ most enriched in cholesterol but very little cholesterol is taken up from the circulation. The predominant sites of cholesterol synthesis in the brain are
Table 5

Proteases and protease inhibitors and activators associated with Alzheimer’s disease

<table>
<thead>
<tr>
<th>Gene</th>
<th>General function and relationships with other gene products</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE:</td>
<td>Degrades amyloid beta peptide (Hu et al., 2001) ATII regulates the expression of HMGCR (Keidar et al., 1999) and activates cholesterol ester hydrolase (Cherradi et al., 2003): angiotensin II increases OLRL (Mehta and Li, 2002) and scavenger receptor (SCRAB1) expression (Cherradi et al., 2001). <strong>OLRL</strong> activation increases <strong>ACE</strong> expression (Li et al., 2003a)</td>
</tr>
<tr>
<td>BLMH:</td>
<td>Increases APP degradation (Lefterov et al., 2000)</td>
</tr>
<tr>
<td>CST3:</td>
<td>Inhibits cathepsin S which is involved in APP processing and degradation of beta amyloid (Liuzzo et al., 1999; Manger et al., 1995). Cathepsin S degrades APOAI (Lindstedt et al., 2003)</td>
</tr>
<tr>
<td>CTSD:</td>
<td>Lipoprotein and apolipoprotein degradation (Dekroon and Armati, 2001; Schneider, 1992; Tertov and Orekhov, 1997; Van Lenten and Fogelman, 1990). <strong>APP</strong> processing (Chevallier et al., 1997)</td>
</tr>
<tr>
<td>IDE:</td>
<td>Degrades beta amyloid and AICD (Edbauer et al., 2002)</td>
</tr>
<tr>
<td>IDE:</td>
<td>Beta amyloid catabolism (Evin and Weidemann, 2002). <strong>SREBP</strong> processing (Yamaguchi et al., 1999)</td>
</tr>
<tr>
<td>MME:</td>
<td>Beta amyloid catabolism (Evin and Weidemann, 2002). <strong>SREBP</strong> processing (Yamaguchi et al., 1999)</td>
</tr>
<tr>
<td>MMP3:</td>
<td>Reduces cholesterol induced HDL efflux from foam cells by degrading APOAI (Lindstedt et al., 1999)</td>
</tr>
<tr>
<td>PLAU:</td>
<td>Activates plasmin, which is involved in APP processing and beta amyloid degradation (Ledesma et al., 2000). Plasmin degrades APOAI (Lijnen and Collen, 1981) and LDL (Kruth, 2002). <strong>PLAU</strong>/plasminogen activator inhibitor complexes bind to <strong>VLDLR</strong> which mediates their endocytosis (Kasza et al., 1997)</td>
</tr>
<tr>
<td>SERPINA3:</td>
<td>Binds to beta amyloid: inhibits chymotrypsin, which degrades APP and amyloid beta (Matsubara et al., 1996). <strong>Chymotrypsin</strong> degrades APOAI (Stoffel and Niedel, 1985), <strong>APOE</strong> (Harris et al., 2003) (Clement-Collin et al., 1999) and <strong>LPL</strong> (Lookene and Bengtsson-Olivecrona, 1993). Beta-amyloid SERPINA3 complexes bind to <strong>PPARG</strong> and activate genes involved in cholesterol metabolism and transport including <strong>LDLR</strong> and <strong>HMGCR</strong> (Sun et al., 2002a,b)</td>
</tr>
</tbody>
</table>

astrocytes and oligodendrocytes and although developing neurones are able to synthesize cholesterol, it has been suggested that this capacity may be reduced in adult life (Bjorkhem and Meaney, 2004) and that neurones may instead rely on a lipoprotein-transported supply of cholesterol synthesised in astrocytes. The model proposed by Pfrieger (2003a,b) suggested that cholesterol synthesised in astrocytes is loaded onto **APOE**, and this complex exported by the cholesterol transporter **ABCA1**, and imported by neurones via lipoprotein receptors (e.g. **LRP1**). Neuronal cholesterol is metabolised to 24-hydroxycholesterol by **CYP46A1**. This oxysterol is able to freely transverse lipid membranes and it was suggested that it might act in a feedback loop to shut off astrocytic cholesterol synthesis via its potent inhibitory effects on HMG CoA reductase. 24-Hydroxy cholesterol is also a potent activator of liver X receptors alpha (NR1H3) and beta (NR1H2). In an astrocytic cell line or in primary astrocytes, 24-hydroxycholesterol increases **APOE** and **ABCA1** expression, effects accompanied by increased cholesterol efflux to **APOE**. This oxysterol also decreased the expression of the key enzymes involved in acetyl CoA shuttling and acetylcholine metabolism associated with Alzheimer’s disease

<table>
<thead>
<tr>
<th>Gene</th>
<th>General function and relationships with other gene products</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAT:</td>
<td>Synthesises acetylcholine</td>
</tr>
<tr>
<td>BCHE:</td>
<td>Metabolises acetylcholine</td>
</tr>
<tr>
<td>DLST:</td>
<td>Both cholesterol and acetyl choline are synthesised from acetyl CoA. If derived from glycolysis, this may entail a loss of Krebs cycle intermediates and problems in CoA shuttling. Within the mitochondrial Krebs cycle, two enzyme complexes, the pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase complex are able to reincorporate coenzyme A into acetylCoA and Succinyl-CoA, respectively. DLST forms a part of the alpha-ketoglutarate dehydrogenase complex which is enriched in cholinergic neurones in the brain (Calingasan et al., 1994) suggesting that it is central to the energetic problems of cholinergic neurons</td>
</tr>
<tr>
<td>GAPD:</td>
<td>Glycolytic enzyme whose activity will affect pyruvate and acetyl CoA availability. Binds to C-terminus <strong>APP</strong> (Schulze et al., 1993). Binds to <strong>POU2F1</strong> (Arca-Sedda et al., 1996). Binds to macrophage scavenger receptor (Nakamura et al., 2002)</td>
</tr>
<tr>
<td>SNCA:</td>
<td>Binds to and inhibits phospholipase D2 (Jenco et al., 1998) whose substrate Lecithin binds to <strong>APOE</strong> and <strong>APOA1</strong> (Jonas, 1984). Lecithin reacts with cholesterol to form cholesterol ester via LCAT: potential role in acetylcholine metabolism via links with lecithin</td>
</tr>
</tbody>
</table>
Table 7
Monoamine related genes associated with Alzheimer’s disease

<table>
<thead>
<tr>
<th>Gene</th>
<th>General function and relationships with other gene products</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTR6: 5-hydroxytryptamine</td>
<td>Serotonin receptor</td>
</tr>
<tr>
<td>(serotonin) receptor 6</td>
<td>Paroxetine, a 5HT uptake inhibitor increases LDL-cholesterol levels after 8 weeks treatment in Man, an effect that is attenuated on drug withdrawal (Lara et al., 2003). Fluoxetine also slightly increased HDL-cholesterol levels and lowered cholesterol levels in a clinical trial on obesity (Pedrinola et al., 1996)</td>
</tr>
<tr>
<td>SLC6A4: solute carrier</td>
<td></td>
</tr>
<tr>
<td>family 6 (neurotransmitter,</td>
<td></td>
</tr>
<tr>
<td>serotonin) member 4</td>
<td></td>
</tr>
<tr>
<td>MAOA: monoamine oxidase A</td>
<td>Regulation of cholesterol metabolism and atherogenesis by sympathomimetic amines (Kaplan and Manuck, 1994). Noradrenaline induces PPAR gamma in astrocytes and neurones (Klotz et al., 2003)</td>
</tr>
<tr>
<td>PNMT: phenylethanolamine</td>
<td></td>
</tr>
<tr>
<td>N-methyltransferase</td>
<td></td>
</tr>
<tr>
<td>ADRB1: adrenergic receptor</td>
<td>Increases APP expression (Bullido et al., 2004)</td>
</tr>
<tr>
<td>beta 1</td>
<td></td>
</tr>
<tr>
<td>GNB3: guanine nucleotide</td>
<td></td>
</tr>
<tr>
<td>binding protein (G protein),</td>
<td></td>
</tr>
<tr>
<td>beta polypeptide 3</td>
<td></td>
</tr>
</tbody>
</table>

Table 8
Miscellaneous genes associated with Alzheimer’s disease

<table>
<thead>
<tr>
<th>Gene</th>
<th>General function and relationships with other gene products</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHSG: alpha2-Heremans-Schmid</td>
<td>Involved in soft-tissue calcification (Reynolds et al., 2005)</td>
</tr>
<tr>
<td>glycoprotein</td>
<td></td>
</tr>
<tr>
<td>ARSA: arylsulfatase A</td>
<td>Generates sulfatides transported by lipoproteins (Han et al., 2003)</td>
</tr>
<tr>
<td>cerebroside sulfatase</td>
<td></td>
</tr>
<tr>
<td>CDC2: cell division cycle</td>
<td>Cell-cycle control. CDC2 also phosphorylates amyloid beta peptides whose neurotoxic effects are inhibited by CDC2 inhibition (Milton, 2001; Milton, 2002)</td>
</tr>
<tr>
<td>2 protein, p34 protein</td>
<td></td>
</tr>
<tr>
<td>kinase; cyclin-dependent</td>
<td></td>
</tr>
<tr>
<td>kinase 1 (CDK1)</td>
<td></td>
</tr>
<tr>
<td>COL11A1: collagen</td>
<td></td>
</tr>
<tr>
<td>DNMNB: dynamin binding</td>
<td></td>
</tr>
<tr>
<td>protein</td>
<td></td>
</tr>
<tr>
<td>DPYS: dihydroxyprimidase</td>
<td></td>
</tr>
<tr>
<td>GBP2: guanylate binding</td>
<td></td>
</tr>
<tr>
<td>protein 2, interferon-inducible</td>
<td></td>
</tr>
<tr>
<td>GNA11: guanine nucleotide</td>
<td></td>
</tr>
<tr>
<td>binding protein (G protein),</td>
<td></td>
</tr>
<tr>
<td>alpha 11 (Gq class)</td>
<td></td>
</tr>
<tr>
<td>GSK3B: glycogen synthase</td>
<td>GSK3B expression (tau kinase, MAPT) is controlled by TFCP2 (Lau et al., 1999) and thus by FEB5 proteins associated with APP</td>
</tr>
<tr>
<td>kinase 3 beta</td>
<td></td>
</tr>
<tr>
<td>HSPA1B: heat shock 70 kDa</td>
<td>Heat shock protein</td>
</tr>
<tr>
<td>protein 1B (HSP70-2)</td>
<td></td>
</tr>
<tr>
<td>HLA-A2: major histocompatibility</td>
<td></td>
</tr>
<tr>
<td>complex, class I, A</td>
<td></td>
</tr>
<tr>
<td>CHRNA3: nicotinic receptor</td>
<td></td>
</tr>
<tr>
<td>subunit alpha 3</td>
<td></td>
</tr>
<tr>
<td>CHRNA4: nicotinic receptor</td>
<td></td>
</tr>
<tr>
<td>subunit alpha 4</td>
<td></td>
</tr>
<tr>
<td>FCER1G: Fc fragment of IgE,</td>
<td></td>
</tr>
<tr>
<td>high affinity I, receptor for;</td>
<td></td>
</tr>
<tr>
<td>gamma polypeptide</td>
<td></td>
</tr>
<tr>
<td>ICAM1: intercellular adhesion</td>
<td></td>
</tr>
<tr>
<td>molecule 1 (CD54),</td>
<td></td>
</tr>
<tr>
<td>human rhinovirus receptor</td>
<td></td>
</tr>
<tr>
<td>LCK: lymphocyte-specific</td>
<td></td>
</tr>
<tr>
<td>protein tyrosine kinase</td>
<td></td>
</tr>
<tr>
<td>MAPT: microtubule-associated</td>
<td></td>
</tr>
<tr>
<td>protein tau</td>
<td></td>
</tr>
<tr>
<td>MCM3AP: MCM3 minichromosome</td>
<td>Initiation of DNA replication</td>
</tr>
<tr>
<td>maintenance deficient 3</td>
<td></td>
</tr>
<tr>
<td>(S. cerevisiae) associated</td>
<td></td>
</tr>
<tr>
<td>protein</td>
<td></td>
</tr>
<tr>
<td>MYH8: myosin heavy polypeptide</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Myosin</td>
</tr>
<tr>
<td>OPRS1: opioid receptor, sigma</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Sigma 1 ligand inhibits cholesterol biosynthesis at the sterol isomerase step (Labit-Le Bouteiller et al., 1998)</td>
</tr>
<tr>
<td>PRNP: prion protein</td>
<td>Binds to APOE (Baumann et al., 2000)</td>
</tr>
<tr>
<td>TFAM: transcription factor A</td>
<td>Key activator of mitochondrial transcription</td>
</tr>
<tr>
<td>mitochondrial</td>
<td></td>
</tr>
</tbody>
</table>
plasma levels reduced in late-stage Alzheimer’s disease (Papassotiropoulos et al., 2000) suggesting that cerebral cholesterol homeostasis is disrupted (Figs. 1 and 2).

4. Alzheimer’s disease susceptibility candidates converge on mechanisms of cholesterol and lipoprotein homeostasis

As shown above, almost all of the elements of the cholesterol shuttle have been implicated in Alzheimer’s disease. Many other genes related to lipoprotein and cholesterol function have also been associated with Alzheimer’s disease and together they can be assembled into a clearly defined physiological cascade, as shown in Figs. 1 and 2. This cascade may be pertinent to both cerebral and peripheral aspects of cholesterol and lipoprotein homeostasis.

For example, the apolipoproteins APOA1, APOA4, APOC1, APOC2, APOC3 and APOE are all substrates for ABCA1 and increase cholesterol and phospholipid efflux from cells transfected with this transporter (Remaley et al., 2001).
**APOD** also binds to cholesterol but its transport is less well characterised. It is closely associated with lecithin: cholesterol acyltransferase (LCAT) a cholesterol esterification enzyme. **APOD** is synthesised and secreted by astrocytes, in lipid-bound form, in response to 25-hydroxycholesterol (the product of **CH25H**) or progesterone (Patel et al., 1995). The role of the LRP2 (megalin) ligand, lipoprotein(a) (**LPA**), remains enigmatic, but elevated protein levels are a significant risk factor in atherosclerosis (Morrisett, 2000). The substrates of **ABCA2** are less well characterised but high expression in oligodendrocytes suggest a role in cholesterol/lipid transport in this cell type (Tanaka et al., 2003). Both **ABCA1** and **ABCA2** are also expressed in brain capillary endothelial cells suggesting a role in sterol/lipoprotein transport (presumably not cholesterol, see above) across the blood–brain barrier (Ohtsuki et al., 2004).

Cholesterol–lipoprotein complexes are internalised through cell surface lipoprotein receptors (**LDLR**, **LRP1**, **LRP8** and **VLDLR**) all of which bind to **LRPAP1** a universal lipoprotein receptor antagonist and chaperone also known as RAP (Bu, 1998).

Neuronally imported cholesterol is metabolised to 24-hydroxycholesterol by cholesterol 24-hydroxylase (**CYP46A1**)...
ligands (Yoshikawa et al., 2003; Whitney et al., 2002). Heparan may contribute to APOE low, low and high-density lipoproteins including sulfate proteoglycans (HSPG2) vascular endothelial cells (Mulder and Terwel, 1998). Activation of SREBF1, an effect inhibited by SOAT1, also bind and internalise very rapidly. APP or beta-amyloid have also been shown to be able to metabolise cholesterol to the oxysterols 7-beta hydroxycholesterol and 25-hydroxycholesterol activate liver X nuclear receptor generated by gamma secretase and the APP intracellular domain. Fe65 proteins inhibit the transcription of thymidylate synthase mediated by the transcription factor TFCP2 (Bruni et al., 2002). APP is physically associated with LRP1 to which it is bound via both extracellular and intracellular domains (Rebeck et al., 2001), via an LRAP1-dependent mechanism and APBB1, respectively. APP, LR1 and APBB1 form a trimeric protein complex (Pietrzik et al., 2004). APBB1 binds to a proteolytic cleavage product of APP (AICD, APP IntraCellular Domain) also known as ctf-gamma (Cao and Sudhof, 2004). In PC12 cells, or rat primary cortical neurones, AICD itself forms a complex with APBB1 and TFCP2 in the nucleus and this complex presumably activates TFCP2 as evidenced by the upregulation of one of its target genes, glycogen synthase kinase (GSK3B, also known as tau kinase). This effect was accompanied by increased phosphorylation of the microtubule protein tau (MAPT). In addition, the overexpression of APP (APPT70 or Swedish mutation) in these cells resulted in the nuclear accumulation of AICD, increased expression of GSK3B and increased tau phosphorylation (Kim et al., 2003). This cascade is thus functional in neuronal cells. GSK3B also phosphorylates and inactivates pyruvate dehydrogenase, which generates the acetyl-CoA necessary for Krebs cycle replenishment and acetylcholine synthesis via choline acetyltransferase (CHAT) (Hoshi et al., 1996) and this pathway may thus regulate two key pathological processes in Alzheimer’s disease. Interestingly, in HEK-293 and neuroglioma cells, extracellularly applied APOE, which binds to APP with nanomolar affinity (Haas et al., 1997), inhibited its gamma-secretase dependent cleavage, an effect that repressed AICD/Fe65 dependent signaling (Irizarry et al., 2004). F-spondin, which binds to the extracellular domain of APP, also modifies APP cleavage and AICD-dependent signaling (Ho and Sudhof, 2004). This genomic pathway may therefore function as a signaling cascade for an “APP receptor”. APBB2 also interacts with LRP1 and decreases its surface expression and increases its degradation (Guenette et al., 2002). APPB1 also interacts with a cytoplasmic fragment (LRPICD) of the LRP1 receptor generated by gamma secretase and the beta-secretase BACE1 (von Arnim et al., 2005). LRPICD is translocated to the nucleus and inhibits the transcriptional activity of APBB1 (Kinoshita et al., 2003). AICD also interacts with autosomal recessive hypercholesterolemia protein (ARH), which was identified as a binding partner for the cytoplasmic domain of APP in a yeast two-hybrid study (Noviello et al., 2003). This protein is an adaptor protein for the low-density lipoprotein receptor LDLR and binds to its cytoplasmic tail (He et al., 2002).

5. Lipoprotein receptor and APP signaling pathways

LRP1, LRP8 and APP all bind to the JNK kinase interacting protein MAPK8IP1 (Gotthardt et al., 2000) (Scheinfeld et al., 2002; Stockinger et al., 2000) which links these proteins to the JNK kinase signaling cascade. LRP1 (Laffont et al., 2002), LRP8 and VLDLR (Bock et al., 2003) also all signal via the phosphoinositide-3-kinase PIK3R1 (Fig. 2).

Fe65 adaptor proteins (APBB1, APBB2, APBB3) bind to the intracellular domain of both APP and LRP1 (Kinoshita et al., 2003; Dulio et al., 1998; Trommsdorff et al., 1998). Upon cleavage of either APP or LRP1, both by gamma secretase (May et al., 2003; May et al., 2002), these proteins are able to translocate to the nucleus. Fe65 proteins inhibit the transcription of thymidylate synthase mediated by the transcription factor TFCP2 (Bruni et al., 2002). APP is physically associated with LRP1 to which it is bound via both extracellular and intracellular domains (Rebeck et al., 2001), via an LRAP1-dependent mechanism and APBB1, respectively. APP, LR1 and APBB1 form a trimeric protein complex (Pietrzik et al., 2004). APBB1 binds to a proteolytic cleavage product of APP (AICD, APP IntraCellular Domain) also known as ctf-gamma (Cao and Sudhof, 2004). In PC12 cells, or rat primary cortical neurones, AICD itself forms a complex with APBB1 and TFCP2 in the nucleus and this complex presumably activates TFCP2 as evidenced by the upregulation of one of its target genes, glycogen synthase kinase (GSK3B, also known as tau kinase). This effect was accompanied by increased phosphorylation of the microtubule protein tau (MAPT). In addition, the overexpression of APP (APPT70 or Swedish mutation) in these cells resulted in the nuclear accumulation of AICD, increased expression of GSK3B and increased tau phosphorylation (Kim et al., 2003). This cascade is thus functional in neuronal cells. GSK3B also phosphorylates and inactivates pyruvate dehydrogenase, which generates the acetyl-CoA necessary for Krebs cycle replenishment and acetylcholine synthesis via choline acetyltransferase (CHAT) (Hoshi et al., 1996) and this pathway may thus regulate two key pathological processes in Alzheimer’s disease. Interestingly, in HEK-293 and neuroglioma cells, extracellularly applied APOE, which binds to APP with nanomolar affinity (Haas et al., 1997), inhibited its gamma-secretase dependent cleavage, an effect that repressed AICD/Fe65 dependent signaling (Irizarry et al., 2004). F-spondin, which binds to the extracellular domain of APP, also modifies APP cleavage and AICD-dependent signaling (Ho and Sudhof, 2004). This genomic pathway may therefore function as a signaling cascade for an “APP receptor”. APBB2 also interacts with LRP1 and decreases its surface expression and increases its degradation (Guenette et al., 2002). APPB1 also interacts with a cytoplasmic fragment (LRPICD) of the LRP1 receptor generated by gamma secretase and the beta-secretase BACE1 (von Arnim et al., 2005). LRPICD is translocated to the nucleus and inhibits the transcriptional activity of APBB1 (Kinoshita et al., 2003). AICD also interacts with autosomal recessive hypercholesterolemia protein (ARH), which was identified as a binding partner for the cytoplasmic domain of APP in a yeast two-hybrid study (Noviello et al., 2003). This protein is an adaptor protein for the low-density lipoprotein receptor LDLR and binds to its cytoplasmic tail (He et al., 2002).
lenetetrahydrofolate + thymidine monophosphate. The thymi- 
dylate synthase product 5,10-methylenetetrahydrofolate is 
metabolised to 5-methylenetetrahydrofolate by methylenetetrahy-
drofolate reductase (MTHFR). 5-Methylene tetrahydrofolate 
and homocysteine are converted to tetrahydrofolate and 
methionine by methionine synthase (MTR) an enzyme 
activated by methionine synthase reductase (MTRR). Homo-
cysteine and serine are converted to cystathionine by 
cystathionine beta synthase (CBS). Thymidylate synthase 
genotypes determine plasma folate and homocysteine con-
centrations in man. It is likely that TS and MTHFR compete 
for a limited supply of folate necessary for the remethylation 
of homocysteine to methionine (Trinh et al., 2002). Homocysteine 
potentiates the cytotoxic effects of beta-amyloid (Kruman et al., 
2003) and hyperhomocysteinemia increases the production of 
beta-amyloid peptides in female APP/PSEN1 transgenic mice 
(Pacheco-Quinto et al., 2006). Homocysteine is a neurotoxic 
NMDA receptor agonist (Kim et al., 1987) and its plasma levels 
are increased in stroke, vascular dementia and Alzheimer’s 
disease (McIlroy et al., 2002). In peripheral cells (human 
hepatocytes, vascular endothelial cells and aorta endothelium) 
homeocysteine activates the SREBP (SREBF1) pathway and 
increases the expression of genes involved in cholesterol 
synthesis (Westermark et al., 2001). Thus TFCP2 leads into a further 
pathway, again composed of Alzheimer’s disease 
susceptibility candidates, that plays an important role in 
cholesterol function. It is not known whether APBB1, AICD or 
LRP1/PCD are able to affect folate and homocysteine metabolism 
via these potential effects on TFCP2 and thymidylate synthase. 
The potential link between these pathways merits investigation 
(Fig. 2).

7. APP processing and the control of lipoprotein and 
cholesterol function

Cholesterol binds to amino acids 10–20 of beta-amyloid, a 
region that overlaps with the alpha-secretase cleavage site 
within APP. Cholesterol binding is thus able to block alpha-
secretase cleavage allowing the generation of Abeta1–40. Low 
cholesterol favours alpha secretase cleavage and the generation 
of Abeta17–40. This latter peptide was shown to inhibit the 
binding of cholesterol to APOE, while Abeta1–40 inhibited 
the binding of cholesterol to human LDL, but not to APOE. 
Abeta1–42 inhibited the binding of cholesterol to both APOE 
and LDL (Yao and Papadopoulos, 2002). Such effects may 
enable the various degradation products of APP to affect either 
the loading or release of cholesterol to and from lipoproteins. 
A recent study has shown that intracellular cholesterol levels 
are raised in mouse fibroblast cells lacking PSEN1 and PSEN2. 
Intracellular cholesterol levels were also increased in the brains 
of conditional PSEN1 knockout mice as well as in the brains of 
APP knockouts. The effects of presenilin loss could be 
restored, in vitro, by Abeta1–40 and the reduction in cholesterol 
levels induced by this peptide was related to a reduction in 
HMG CoA reductase activity (Grimm et al., 2005). A direct 
effect of Abeta1–40 on HMG CoA reductase was not measured. 
Abeta1–42 also directly activated neutral sphingomyelinase. 

This enzyme cleaves sphingomyelin to generate ceramide and 
phosphocholine. Ceramide has been shown to stimulate the 
maturation of the cholestrogenic transcription factor SREBP 
(SREBF1) in human hepatocytes, a process that is required to 
allow translocation of this transcription factor to the nucleus 
(Lawler et al., 1998a). Abeta1–42 has also been shown to 
increase ceramide and cholesterol levels in cultured hippo-
campal neurones. Both ceramide and cholesterol levels were 
reported to be increased in vulnerable regions of the 
Alzheimer’s disease brain. Ceramide has been shown to 
increase the synthesis of cholesterol in a number of peripheral 
cell types (Cutler et al., 2004). Thus multiple species of beta 
amyloid peptide may be able to influence cholesterol function 
in different ways and the differential processing of APP, 
affected by extracellular ligands (see above), may be of 
physiological significance in relation to cholesterol function.

The ability of APP to metabolise cholesterol, its association 
with LRP1, and the diverse effects of gamma-secretase 
degradation products on cholesterol function suggest that 
one of the roles of APP and its cleavage products is related to 
the control of cholesterol homeostasis and it seems reasonable 
to propose that APP is an integral component of the cerebral 
cholesterol shuttle. APP or its proteolytic enzymes are not 
restricted to the central nervous system (see Unigene 
expression data) and their potential roles in cholesterol 
homeostasis are likely to extend to the periphery. Plasma 
cholesterol levels are indeed lowered in older mice co-
expressing mutant forms of both APP and PSEN1 (Wiraths 
et al., 2006), suggesting that these mutations may also affect 
peripheral cholesterol function.

8. Lipoprotein, APP and beta amyloid proteolysis and 
export

Cathepsin D (CTSD) is involved in the degradation of 
lipoproteins and apolipoproteins including APOE (Deng et al., 
1995) and of APP (Sadik et al., 1999).

Cystatin C (CST3) inhibits cathepsin S, which degrades 
APOA1 and inhibits cholesterol efflux from mouse macro-
phages (Lindstedt et al., 2003). Cathepsin S degrades APP to 
beta-amyloid peptides and also degrades beta-amyloid peptides 
(Liu et al., 1999; Munger et al., 1995). Beta amyloid 
(Abeta1–42) is degraded by the peptidase Neprilysin (MME) 
(Iwata et al., 2000). SERPINA3 (alpha (1)-antichymotrypsin) 
inhibits chymotrypsin, which degrades APOE (Harris et al., 
2003) and lipoprotein lipase (LPL) (Lookene and Bengtsson-
Olivercrona, 1993) as well as amyloid beta peptides (Matsubara 
et al., 1996).

Urokinase-type plasminogen activator (PLAU) converts 
plasminogen to plasmin, which controls fibrinolysis but which is 
also involved in APP processing and digests amyloid beta 
peptides (Korchazhkina et al., 2002; Korchazhkina et al., 2002). 
Plasmin also degrades low-density lipoprotein (Kruth, 2002). 
PLAU/plasminogen activator inhibitor complexes are bound to 
the very low-density lipoprotein receptor (VLDLR), which 
mediates their endocytosis (Lindstedt and Kovaken, 2000). 
AICD, the gamma secretase APP product, is processed by
insulin degrading enzyme (IDE) (Edbauer et al., 2002), which also degrades amyloid beta peptides (Perez et al., 2000). Stromelysin (MMP3) degrades a number of proteins including APOA1 (Lindstedt et al., 1999) and HSPG2 (Whitelock et al., 1996).

Alpha-2macroglobulin (A2M) is a protease inhibitor and carrier protein. It binds to a number of peptides and proteins including APOE (Krimbou et al., 1998) and Beta amyloid (Du et al., 1997). A2M-beta amyloid and other A2M/peptide complexes are exported by the lipoprotein/A2M receptor, LRPI (Gonias et al., 1994).

9. Lipoprotein oxidation and removal of oxidised lipoprotein products

Myeloperoxidase (MPO) (Jolivalt et al., 1996), nitric oxide (NOS1, NOS3) (Bloodsworth et al., 2000) and iron all play an important role in lipoprotein oxidation. The iron transporter transferrin (TF) is bound to LDL and inhibits LDL oxidation (Kunitake et al., 1992). LDL oxidation is increased by releasing iron bound to transferrin under acidic conditions (Lamb and Leake, 1994). Hemochromatosis (HFE) competes with the binding of iron-transferrin (TF) to the transferrin receptor at neutral pH (Lebron et al., 1999).

The paraoxonases PON1 and PON2 both protect against cholesterol or lipoprotein oxidation and are antiatherogenic (Ng et al., 2005). Aldehydes are oxidation products of oxidized LDL and aldehyde dehydrogenase, ALDH2, is likely to be involved in the metabolism of these products as levels of LDL-aldehyde immunoreactivity in patients with liver damage vary according to the ALDH2 genotype (Nagata et al., 1999).

Oxidised lipoproteins are removed by the oxidised lipoprotein receptor OLR1 (Sawamura, 2002) and by the macrophage scavenger receptor CD36 (Nicholson et al., 2000).

10. Proteolysis of APP and lipoprotein receptors

BACE1 cleaves APP at the beta-secretase site (Vassar et al., 1999) and also cleaves LRPI resulting in the release of its intracellular domain (LRPICD) (von Armim et al., 2005). Gamma secretase, a complex of presenilin, nicastrin (NCSTN), aph-1 and pen-2 (Kimberly et al., 2003) degrades both LRPI and LRP8 (Kinoshita et al., 2003) as well as APP (Hardy and Selkoe, 2002). The ubiquitin-like protein ubiquilin-1 (UBQLN1) interacts with PSEN1, PSEN2 and NCSTN and increases presenilin protein accumulation (Massey et al., 2005; Mah et al., 2000) (Figs. 1 and 2).

11. Genes associated with both Alzheimer’s disease and atherosclerosis

As described in the introduction, Alzheimer’s disease has a significant vascular component and is associated with atherosclerotic changes in peripheral arteries leading to the brain and in cerebral vessels. Many of the genes described in the circuits above have also been associated with atherosclerosis or high cholesterol levels. These include ABCA1, ALDH2, APOA1, APOA4, APOC1, APOC2, APOC3, APOE, CD36, CETP, CST3, GSTT1, HFE, LDLR, LIPA, LPA, LPL, LRPI, MMP3, MTHFR, MPO, NOS3, OLR1, PON1, PON2 and SREBF1 (see Website Tables for references). Polymorphisms within these genes in Alzheimer’s disease are also likely to affect cholesterol and lipoprotein status in the periphery, via diverse metabolic pathways, and in so doing may well contribute to atherosclerotic pathology.

A number of other genes have also been associated with both atherosclerosis and Alzheimer’s disease. These include the estrogen synthetic enzyme aromatase (CYP19A1) and the estrogen receptors ESRI and ESRII, the peroxisome proliferator associated receptor PPARA, the cytokines IL1B, IL6, TGFB1 and TNFA as well as ACE, BCHE, GNB3, HLA-A2, HTR6 and ICAM1. A number of these gene products have multiple effects either on cholesterol homeostasis or on APP physiology, which are detailed in Tables 1–8 of the Website and only briefly discussed here.

For example, estrogen response elements or control of transcription by estrogen have been observed for ABCA1 (Srivastava, 2002), APOE (Levin-Allerhand et al., 2001) LDLR (Bruning et al., 2003) and HMG CoA reductase (Di Croce et al., 1999). Inflammatory cytokines (IL1B, TNFA) play an important role in atherosclerosis by reducing cholesterol efflux and reverse cholesterol transport (Ohashi et al., 2005). IL6 controls the expression of LCAT (lecithin: cholesterol acyltransferase) (Feister et al., 2002) a key component of reverse cholesterol transport, and increases the expression of LDLR in hepatic cells (Gierens et al., 2000). The infusion of TNFA in rats has been shown to increase the expression of most liver enzymes of cholesterol synthesis (Ruan et al., 2002). TNFA also regulates the maturation of SREBF1 (Lawler et al., 1998b). PPARA receptors are activated by fibrates, which exert multiple beneficial effects on parameters of fatty acid oxidation, vascular inflammation and atherosclerosis (Han et al., 2005). SREBF1 also controls the expression of multiple cholesterol and lipoprotein related genes (Bocher et al., 2002). Non-steroidal anti-inflammatories, whose use has been reported to reduce the incidence of Alzheimer’s disease (Veld et al., 2001), are PPARA and PPARG receptor agonists (Lehmann et al., 1997b). Interestingly, this drug class has also been shown to reduce plasma total cholesterol, triglyceride and LDL concentrations in hyperlipidaemic rats (Kourounakis et al., 2002) suggesting that their beneficial effects may be related not only to the control of inflammation but also to their effect on lipid metabolism. These factors may therefore contribute to modifications in peripheral and perhaps central lipid function.

12. Cholesterol and lipoprotein status in Alzheimer’s disease

Retrospective epidemiological studies have shown that previous mild hypercholesterolemia is an early risk factor for Alzheimer’s disease (Pappolla et al., 2003) and a history of high serum cholesterol predicts the prevalence of Alzheimer’s disease in men (Notkola et al., 1998). High serum cholesterol
levels are associated with Alzheimer’s disease in patients lacking the APOE4 allele (Evans et al., 2000). Previous high levels of plasma HDL-cholesterol are linearly associated with the number of neuritic plaques later seen at autopsy (Launer et al., 2001) and this association is reinforced in younger Alzheimer’s patients (Pappolla et al., 2003). The product of CYP46A1, 24S-hydroxycholesterol, is elevated in the plasma and CSF of early stage Alzheimer’s disease (Schonknecht et al., 2002; Papassotiropoulos et al., 2002; Papassotiropoulos et al., 2000) and the severity of Alzheimer’s disease and inheritance of the APOE4 allele are both associated with reduced plasma 24S-hydroxycholesterol/cholesterol ratios (Papassotiropoulos et al., 2000). In the CSF, APOE and cholesterol levels are slightly decreased, APOA1 concentrations are increased and LCAT activity is reduced by 50% (Demeester et al., 2000). In the Alzheimer’s disease brain, free cholesterol levels are increased in the frontal cortex and this increase correlates with disease severity (Cutler et al., 2004). Desmosterol (the immediate precursor of cholesterol) proportions are relatively high (Wender et al., 2000). The expression of HMG CoA reductase mRNA is unchanged (Yasojima et al., 2001), but the expression of CYP7B1, which metabolises cholesterol to 7α-hydroxycholesterol, is elevated in the plasma and CSF of early stages Alzheimer’s disease (Petit-Turcotte et al., 2001), and cholesterol monooxygenase (CYP11A1). Expression of the oxysterol producing enzymes CYP3A4 and CH25H was increased. 3-Hydroxy-3-methylglutaryl CoA cleaving enzyme (HMGCCL) expression was also elevated. Sterol isomerase (Ebp), sterol C5-desaturase (SCD1) sterol carrier (SCP2), oxysterol binding protein (OSBP1) and 7-dehydrocholesterol reductase (DHCR7) expression were reduced. ABCA1, ABCA2, APOBEC1, APOC1, APOC4, APOE, APOF, APOH, APOJ, APOK, APOL1, APOL2, LRP4, LRP6, LDLR and its adaptor protein ARH, LRPI0, LRPI6 and APP expression were increased. LRPI8 expression were decreased. In relation to the polymorphic genes discussed above, ABCA1, APOC1, APP, CH25H, HFE, ICAM1, IL1A, IL6, LDLR, LPL, NCSTN, SERPINA3, SREBF1, TF and TGFB1 expression were increased and ALDH2, GAPD, LRPS, MAOA, MAPK8Ip1, NQO1, PON2, SNCA and VLDLR expression were decreased.

13. Reciprocal interactions between atherosclerotic factors and APP processing

13.1. Effects of cholesterol/atherosclerosis on APP processing

Cholesterol affects APP processing in many ways. At a local level it may directly influence the activities of alpha (inhibition) (Kojro et al., 2001), beta and gamma-secretase (stimulation) (Marlow et al., 2003; Wahrle et al., 2002), but also has effects on the compartmentalisation of these enzymes that may produce different effects. For example reductions in membrane cholesterol levels increase the colocalisation of BACE1/APP in hippocampal membranes, and increases the generation of beta-amyloid by ensuring the proximity of the protease to its substrate (Abad-Rodriguez et al., 2004). Reduced central cholesterol levels, induced by sedalin (DHCR24) knockout, may increase beta secretase activity and beta-amyloid generation in vivo via this mechanism (Cramer et al., 2006). The localisation of presenilin and the processing of APP are
also affected by modifying the intracellular transport of cholesterol to the endoplasmic reticulum. Intracellular cholesterol transport inhibitors decrease the beta-secretase cleavage of APP but increase gamma-secretase cleavage and the production of beta-amyloid peptides (Runz et al., 2002). Beta-amyloid deposition is increased in the brains and cerebral vessels of ABCA1 knockout mice expressing the Swedish APP mutation (Koldamova et al., 2005). However, APP processing was not affected in this model and the effects on beta-amyloid deposition were interpreted as being due to reduced clearance. Beta-amyloid deposition is also increased in LDLR deficient APP mutant mice (TG2576) (Cao et al., 2005). The oxysterols, generated by CYP46A1, CH25H and APP, are also able to affect APP processing. For example in primary cortical neurones, 24-hydroxycholesterol, or the LXR agonist TO901317, inhibit the production and secretion of beta-amyloid (Brown et al., 2004). In a hippocampal neuronal cell line, 7-beta hydroxycholesterol (a product of APP) has been shown to inhibit the secretion of soluble APP and the activity of alpha-secretase (Nelson and Alkon, 2004). Other components of this shuttle, for example A2M, APOE and LRP1 (Moir and Tanzi, 2005) may also be involved in the control of amyloid clearance rather than processing. Whatever the mechanism, modifications in cerebral cholesterol/lipoprotein function clearly affect the fate of APP. Functional and gene expression studies suggest a disruption of cholesterol and lipoprotein function in the Alzheimer’s disease brain, although it is currently impossible to predict what effects these summated changes (or the integrative effects of diverse polymorphisms in multiple genes related to the lipid system) might have on different aspects of APP processing.

Modifications in beta-amyloid levels need not necessarily be related solely to problems in cerebral cholesterol or lipoprotein function and could well be an indirect result of the reduced cerebral perfusion induced by the atherosclerotic changes observed in Alzheimer’s disease. Transient hypoxia in cortical neurones, in vitro, produces beta amyloid deposition and tau hyperphosphorylation and many other key pathological signs of Alzheimer’s disease (Chen et al., 2003). Beta-amyloid peptides accumulate in astrocytes after transient focal ischaemia in rats (Nihashi et al., 2001). Chronic cerebral hypoperfusion in rats also increases APP mRNA expression and results in the accumulation of immunoreactive beta-amyloid and in a shift of its expression from neurones to extracellular deposits (Bennett et al., 2000; Shi et al., 2000). Dietary hypercholesterolemia also increases neuronal beta amyloid deposition in normal rabbits (Sparks et al., 1994) and beta amyloid deposition is also a feature of spontaneously hypercholesterolaemic Watanabe rabbits (Sparks et al., 2002b). Beta-amyloid deposition was not further increased in TG2576 APP mutant mice fed a high cholesterol diet although APP processing is modified, as evinced by increased levels of the c-terminal fragment, AICD, and by a reduction in the levels of the N-terminal SAPP alpha (George et al., 2004). Altered APP processing can therefore be a downstream consequence of cerebral energy failure or of problems in peripheral cholesterol function. Cerebral energy metabolism would evidently be compromised as a result of atherosclerotic lesions in the cerebral and cerebral supply vasculature.

Cerebral hypoperfusion appears to precede the onset of dementia in Alzheimer’s disease patients (Ruitenb et al., 2005; de la Torre, 2002) and modifications in cerebral metabolism can be observed before the onset of clinical symptoms or of detectable brain atrophy (Blass et al., 2002). The implication of many Alzheimer’s disease susceptibility genes in atherosclerosis may contribute to these effects.

13.2. Effects of APP processing on cholesterol/atherosclerosis

APP and its degradation products either metabolise cholesterol or influence cholesterol function as described above. Both BACE and the presenilins would be expected to modify lipoprotein function via their effects on LRP1 and LRP8 processing. In addition, the degradation products of APP are involved in the cerebrovascular pathology of Alzheimer’s disease and also in the formation of atherosclerotic plaques in peripheral atherosclerosis. Beta amyloid also prevents the adhesion of vascular smooth muscle cells to heparan sulphate proteoglycans (HSPG2) in the basement membrane, a factor likely to contribute to cerebral amyloid angiopathy in Alzheimer’s disease (Mok et al., 2006). Neuronally derived Abeta contributes massively to vascular plaque formation in TG2576 and PSAPP mutant mice (Kumar-Singh et al., 2005). APP and beta amyloid also play a role in peripheral atherosclerosis. APP is a constituent of platelets and platelet activation results in APP cleavage and the formation of beta-amyloid which has been implicated in the activation of macrophages in human atherosclerotic plaques, APP, beta-amyloid peptides and BACE are all constituents of peripheral atherosclerotic plaques (De Meyer et al., 2002; Jans et al., 2004; Skovronsky et al., 2001). In microglia and macrophages, the binding and clearance of oxidised lipoproteins by CD36 is inhibited by beta-amyloid (Kunjathoor et al., 2004) and such effects would tend to promote the accumulation of these inflammatory mediators in atherosclerotic lesions.

Thus cholesterol or atherosclerosis both affect APP processing and APP processing affects cholesterol/lipoprotein function, atherosclerosis and cerebrovascular function. Defects in various aspects of this balance might thus create a feed-forward loop due to multiple interactions between these different factors.

The effects of modified APP processing, cerebral lipid homoeostasis and peripheral atherosclerosis might thus act in synergy to promote the full features of Alzheimer’s disease pathology (Casserly and Topol, 2004). This type of synergy has indeed been observed in a transgenic atherosclerosis/Alzheimer’s disease mouse model (B6Tg2576), created from a backcross of mice expressing the human Swedish double APP mutation with a strain of mice susceptible to diet-induced atherosclerosis (C57BL/6). The resultant mice, when fed an atherogenic diet, develop aortic atherosclerotic lesions and increased cerebral amyloid deposition. The size of the aortic lesion correlated with the level of beta amyloid deposition in
the brain. Interestingly, atherosclerotic aortic lesions were also observed in the B6Tg2576 mice fed a normal diet, suggesting that the APP mutation also favoured the promotion of peripheral atherosclerosis in the absence of hypercholesterolemia (Li et al., 2003b).

13.3. Relevance to the pathology of Alzheimer’s disease

The cerebral function of the cholesterol and lipoprotein-related genes in the cholesterol shuttle pathway must presumably be to coordinate the supply of cholesterol to neurons and other cells in relation to supply and demand. Glial-derived cholesterol is believed to play a key role in synaptogenesis and synaptic function and defects in this area are likely to contribute to the modifications in synaptic function observed in Alzheimer’s disease (Goritz et al., 2005; Levi et al., 2005). Perhaps a key conclusion, derived from the assembly of this network and from the studies describing its various branches, is that APP and beta and gamma secretases also might form a part of this shuttle and that one of the key physiological functions of APP might be related to the control of cerebral cholesterol homoeostasis.

Mutations in single genes APP, PSEN1, or PSEN2 cause Alzheimer’s disease in familial cases. These mutations affect APP processing and the generation of neurotoxic beta-amyloid peptides (see http://www.alzforum.org for debates/). They are also likely to affect cholesterol and lipoprotein function and likely play a role in the cerebrovascular and atherosclerotic features of Alzheimer’s disease, all of which are functionally intertwined by the multiple interactions between the various players described above. These aspects, as well as the generation of toxic peptides, should perhaps be considered in relation to the overall pathology of both familial and late-onset Alzheimer’s disease. Both APP and the presenilins are ubiquitously expressed (see Unigene expression data) and might also be able to influence peripheral lipid metabolism. This is borne out by the fact that plasma cholesterol levels are reduced in aged APP/PSEN1 and APP/PS1ki mutant mice (Wirths et al., 2006).

In later onset Alzheimer’s disease, unrelated to mutations in these prime movers, but nevertheless producing the same plaque, tangle and vascular pathology, defects in cholesterol/ lipoprotein function, generated by diverse polymorphic genes, some situated in the same physiological pathway as APP, may well provide alternative means of affecting APP processing and beta-amyloid accumulation, leading to the same eventual outcome.

13.4. Gene selection bias and genetic considerations

Many of these genes were no doubt chosen in the original association studies because of the reported links between cholesterol and Alzheimer’s disease and this bias needs to be considered. However, the choice of genes for association studies was also influenced by previous microsatellite marker studies suggesting the presence of susceptibility genes in particular chromosomal regions. Many of these genes are located within these chromosomal hotspots,2 and their choice was not solely hypothesis driven.

The network composed of Alzheimer’s disease susceptibility candidates generates a very specific pathway, implicated in a particular aspect of lipid function, in which almost every link in a long consecutive chain is represented by one or more susceptibility candidates. Many other genes involved in cholesterol synthesis, or other areas of cholesterol metabolism, have not so far been associated with Alzheimer’s disease. At the time of gene discovery, it was not initially appreciated that APP and beta or gamma-secretase could be so closely related to cholesterol or lipoprotein function and one would not immediately associate the function of many other genes with cholesterol. Cytokines, nuclear receptors and free radical-related susceptibility genes play an important role in cerebral inflammation, oxidative stress and other aspects of cytotoxicity, properties that may be equally important in relation to AD pathology. However, their ability to also regulate cholesterol and lipoprotein function demonstrates a convergence of effect, which may be particularly important when combined with defects in genes more specifically related to cholesterol/ lipoprotein homoeostasis.

Despite the selection bias, the convergence of susceptibility genes on a common physiological process cannot be lightly dismissed, and it may indeed partly explain the variability in association studies. The overlap in function of genes belonging to a key pathological pathway may provide multiple and conditional ways of disrupting the pathway, a factor reflected by the genetic heterogeneity. The extent to which any particular gene variant can disrupt this pathway may also be expected to affect its strength of association and replicability across different studies. Important genes (e.g. APOE) and others with dual functions in atherosclerosis and neuronal pathology would exert major functional effects and relatively consistent association data, while the effects of minor genes would be more dependent upon the summative effects of additional gene variants. Analyses similar to those performed in bladder cancer (Wu et al., 2006) may help to clarify these complex genetic issues.

14. Conclusion

The common characteristics of the pathology of Alzheimer’s disease across ethnic and geographical divides suggest that disruption of a unique pathway must be responsible for its cause. One way of disrupting this pathway would be via defects in the genes coding for its components. This pathway has hitherto been considered mainly in terms of defects in APP

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2 Cholesterol or lipoprotein related (A2M, ABCA1, ABCA2, APOA1, APOA4, APOC1, APOC2, APOC3, APOE, APP, BACE1, BACE2, CH25H, CST3, CYP46A1, HFE, HSPG2, IDE, LDLR, LIPA, LPA, LRP1, MHFR, MTR, MTRR, NCSTN, NRH2, PLAU, PSEN1, PSEN2, OLR1, SERPINA3, SOAT1, TFCP2) and other (ADRB, CDC2, DLST, ESR1, Fcer1g, Gadd45a, Gadd45b, HLA-A2, HSPA1B, HTR6, ICAM1, MCM3AP, NOS3, POU2F1, Tfac, TGFb1, TNFfa) are all localised to chromosomal regions implicated in Alzheimer’s disease in linkage studies (see Website Tables for references).
processing and this concept is clearly validated and plays a key role in the pathology of Alzheimer’s disease (George-Hyslop, 2000; Hardy and Selkoe, 2002). Many of the genes described above also affect APP processing or beta amyloid degradation and export, and converge on this system as well as on lipid function (see Website) (Carter, 2006). However, the two are inextricably linked, as the group of genes converging on APP processing appear to form part of a much larger pathway specifically involved in cerebral lipid homeostasis, which in turn affects the fate of APP. Genetic association studies seem to have etched out a specific physiological process whose dysfunction may well play a key role in development of Alzheimer’s disease. In addition, a large number of Alzheimer’s disease susceptibility candidates, including APP, are involved in atherosclerosis, a feature of Alzheimer’s disease pathology, which may also participate in the disease process. Cholesterol homeostasis and lipoprotein function are disrupted in Alzheimer’s disease and their correction by drugs or diet has been suggested to influence the incidence of the disease and its development. The jury is out, concerning the potential benefits of statins, and must await the results of more extensive clinical trials. Given the potential role of atherosclerosis in Alzheimer’s disease, perhaps it is not necessary to invoke a central mechanism of action of these drugs, whose effects may in part be related to their ability to attenuate the atherosclerotic sub-pathology of Alzheimer’s disease. The wisdom of tampering with brain cholesterol metabolism has also been questioned (Sparks et al., 2002a). Indeed, HMG-CoA reductase inhibitors produce loss of neurites and neuronal cell death in vitro (Schulz et al., 2004). It is possible that the conflicting results related to the effects of statins in Alzheimer’s disease relate to this balance of beneficial and deleterious effects. It is also interesting that, despite the multiple links with atherosclerosis, late-onset Alzheimer’s disease patients have survived to a relatively old age before manifesting the disease, without developing myocardial infarction, coronary artery disease, stroke or other fatal consequences of atherosclerotic disorders. This raises an interesting question as to whether some of the Alzheimer’s disease susceptibility candidates in fact protect against these diseases of relative youth.

While many other factors controlled by these genes (inflammation, oxidative stress, and various cell death cascades) are likely to be involved in Alzheimer’s disease (Cacabelos et al., 2005), a clearer understanding of these lipid-related processes in normal brain physiology may help to understand how they might contribute to the pathology of this devastating condition.

Tables 1–8 list genes reported to be associated with Alzheimer’s disease in at least one study. Genes also associated with atherosclerosis or hypercholesterolaemia, are highlighted by a grey background. A brief summary of the role of each gene, particularly in relation to APP, cholesterol and lipoprotein function is also provided. These and other data, references to positive association studies and comments on expression and functional changes in Alzheimer’s disease are posted on a supplementary Website table at http://www.polygenicpathways.co.uk/alzpolys.html. A complete referencing of all positive and negative association studies is available at the Alzgene database (Bertram et al., 2005) (http://www.alzgene.org).

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