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Recent insights into the molecular genetics of dementia

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Abstract

Our understanding of the molecular genetic basis of two common neurodegenerative dementias, Alzheimer's disease (AD) and frontotemporal lobar degeneration (FTLD) has greatly advanced in recent years. Progranulin mutations were identified as a major cause of FTLD and a potential susceptibility factor for other forms of dementia. In addition, through copy-number analyses of previously identified disease genes and the study of microRNA regulation in dementia, new evidence emerged to support the view that subtle variability in the expression of known disease proteins may increase the risk for sporadic forms of dementia. Finally, in late-onset AD populations, the first genome-wide association studies were performed and novel potential AD susceptibility genes reported. These exciting findings provide novel insights into the disease mechanisms underlying dementia and hold promise for the development of potential treatments.

Introduction

A brief overview of the neurodegenerative dementias

Dementia or the significant loss of intellectual abilities such as memory capacity, severe enough to interfere with social or occupational functioning, is one of world's most devastating and burdensome health conditions. It is estimated that 24.3 million people have dementia today, with 4.6 million new cases annually [1]. Moreover, as a result of our aging population, the number of people affected with dementia is expected to double every 20 years. Alzheimer's disease (AD) is by far the most frequent form of dementia comprising 50-70% of all cases and affecting 40% of individuals older than 85 years [2]. A definite diagnosis of AD requires the presence of extraneuronal β -amyloid plaques and intraneuronal neurofibrillary tangles composed of hyperphosphorylated tau in the degenerating brain [3]. Dementia is also present as a common clinical feature in a host of related neurodegenerative diseases (Table 1). Lewy-body disease (LBD) is considered the second commonest cause of dementia in patients older than 65 years [4]; however, in presenile dementia patients frontotemporal lobar degeneration (FTLD) is the second most common diagnosis after AD [5]. FTLD patients are clinically characterized by personality changes and disinhibited behavior, often combined with a gradual and progressive language dysfunction. Memory impairment is typically preserved in the early phase of disease, which distinguishes them from patients with AD. Pathologically, about 40% of FTLD patients present with neuronal and/or glial tau aggregates (FTLD-tau), whereas the majority of FTLD patients show ubiquitin-immunoreactive cytoplasmic (NCI) and intranuclear inclusions (NII) composed of hyperphosphorylated and carboxy-terminal truncated fragments of the nuclear protein TAR DNA-binding protein 43 (TDP-43), characteristic of FTLD-U. Up

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to four subtypes of FTL-D have been delineated that are based on the distribution of NCIs, dystrophic neurites and the presence of NIIs (ref). Despite well-defined clinical diagnostic criteria, the differential diagnosis of neurodegenerative dementias is often challenging. This is particularly true for AD and FTL-D which have been suggested to belong to a spectrum of neurodegenerative diseases [6].

In recent years, significant progress has been made in understanding the molecular genetics and underlying pathological substrates of AD and FTL-D, which will be the focus of the current review. For additional information on LBD and other Parkinson-related dementias we refer to recent reviews by Bonifati *et al.* [7] and Ferman and Boeve [8].

Genetic aspects of AD and FTL-D

AD is a genetically complex and heterogeneous disorder [9]. Less than 10% of AD patients have an early-onset of disease (before the age of 65 years), and about 60% of these patients are familial, with an autosomal dominant inheritance of the disease in approximately 13% of patients [10]. In autosomal dominant AD families, rare mutations in the amyloid precursor protein gene (*APP*) and the presenilin 1 (*PSEN1*) and 2 (*PSEN2*) genes have been identified [11-13]. The presenilins are components of the proteolytic γ -secretase complex that together with β -secretase (*BACE 1*) generates β -amyloid from *APP* [14]. Two major β -amyloid species of either 40 (A β 40) or 42 (A β 42) amino acids are produced with A β 40 being the more common and A β 42 being the more fibrillogenic and neurotoxic species. The identification of mutations in the genes encoding both the substrate and the key enzyme for the generation of β -amyloid has led to major advances in the characterization of the pathophysiology of AD and provides the main support for the *amyloid cascade hypothesis* which has dominated the AD field for over 15 years [15]. According to this hypothesis, the central event in AD is the imbalance between the production, maturation and clearance of β -amyloid in brain leading to neuronal degeneration and dementia (Figure 1). This original hypothesis has undergone alterations over time [16], primarily in the description of the nature of pathogenic β -amyloid species that is proposed to initiate deleterious events and cause AD, and it should be mentioned that additional disease mechanisms have also been proposed [17,18].

Aggregation of late-onset AD also occurs in families, though 75-85% of late-onset AD is sporadic [9]. The disease in these patients is expected to result from the interaction of multiple susceptibility genes and unknown environmental factors. So far, the ϵ 4 allele of the apolipoprotein E (*APOE*) gene has been the only consistently replicated genetic risk factor for late-onset AD [19]. Meta-analyses showed a 3-times increased risk to develop AD in individuals carrying one copy of the *APOE* ϵ 4 allele and a 15-times increased risk for *APOE* ϵ 4 homozygote individuals [20]. The *APOE* ϵ 4 allele further modifies the onset of disease, shifting it to an earlier age [21]. Several mechanisms have been proposed to explain *APOE* ϵ 4 detrimental effects in AD (for review, see [22]). Through its fundamental role in lipid transports, *APOE* is implicated in numerous cellular pathways linked to AD, including cholesterol redistribution, oxidative stress, neurite outgrowth, tau phosphorylation and β -amyloid clearance and aggregation. It was demonstrated that the β -amyloid plaque load is increased in *APOE* ϵ 4 allele carriers [23] and that the *APOE* ϵ 4 allele enhances fibrillar A β burden in cognitively normal older people [24].

In contrast to AD, FTL-D patients usually present first symptoms in their 50s and 60s, with a positive family history of dementia in as much as 50% of patients [5]. In the past decade, several different genes and chromosomal loci have been associated with FTL-D [25]. Mutations in the microtubule associated protein tau (*MAPT*) and progranulin (*GRN*) genes were identified as important causes of FTL-D, explaining 10-25% of familial FTL-D patients and 5-10% of all FTL-D [25-27]. Mutations in the genes encoding charged multi-vesicular body protein (*CHMP2B*) and valosin-containing protein (*VCP*) on 9p21-12 were further reported as rare

causes of FTLD [28,29]. Several families with FTLD and motor neuron disease (MND) have also shown linkage to 9p21-13 but to a gene other than *VCP*, which still awaits identification [30,31].

The detection of rare disease causing mutations in the autosomal dominant dementia genes unequivocally shaped our current understanding of the pathogenic mechanisms underlying AD and FTLD. However, despite these major advances, the cause of the disease in the majority of familial dementia patients and in the common sporadic forms largely remained unknown. Here, we review the novel insights into the molecular genetics of dementia; we discuss the identification of mutations in *GRN* as a novel cause of FTLD and a potential risk gene for other dementias, and fascinating new data suggesting that genetic variability in the expression of known disease proteins may contribute to the risk for the common sporadic forms of dementia and may cause disease in previously unexplained dementia families. We specifically discuss the importance of copy-number variants (CNVs) and the regulation of gene expression by micro RNAs (miRNAs), two novel disease mechanisms implicated in dementia. Finally, we summarize the recent progress made in the identification of genetic risk factors for AD and FTLD.

PGRN and TDP43: new players in dementia

The most exciting recent breakthrough in the search for novel causal dementia genes was undoubtedly the identification of loss-of-function mutations in *GRN* as a major cause of FTLD-U [26,27]. The identification of *GRN* mutations had long been hampered by its genomic location on chromosome 17q21, only 1.7Mb centromeric of the other major FTLD gene, *MAPT*, whose role in the development of FTLD-U was unclear. Only after exhaustive candidate-gene sequencing in 17q21-linked FTLD-U families, mutations in *GRN* could be identified.

GRN is a member of the epithelin family of growth factors and is described in the literature by various synonyms including acrogranin, epithelin precursor, proepithelin and PC cell-derived growth factor [32]. It is a secreted precursor protein composed of a signal peptide and 7.5 tandem repeats of a rare 12 cysteinyl motif [33,34]. As a result of proteolytic cleavage of GRN by extracellular proteases, a family of active ~6KDa peptides (granulins A to G and paragrulin) are formed that each contain 10-12 highly conserved cysteine residues which are folded into four stacked β -hairpins stabilized by disulphide bridges critical for biological function [35]. Both GRN and the granulins are widely expressed and have been implicated in a range of biological processes including development, wound repair, inflammation and tumorigenesis [36,37]. At present, the role of GRN and the granulins in brain remains unknown; however, recent *in vitro* findings suggest that GRN may act as a neurotrophic factor [38].

In less than three years, 66 different pathogenic *GRN* mutations have been reported in 199 families (FTD mutation database) [39]. Mutations are found in all *GRN* exons, except in the most 3' exon 13, and include different types of mutations, such as nonsense (N=14) and splice-site (N=11) mutations as well as small insertions and deletions leading to a shift in the normal reading frame (N=34) (Figure 2). All *GRN* mutations are expected to lead to the loss of 50% functional GRN, suggesting a haploinsufficiency disease mechanism [40]. Loss-of-function mutations are not typically responsible for dominantly inherited diseases, however, soon after the identification of the first *GRN* mutations, expression analyses in mutation carriers showed a 50% reduction in *GRN* transcript due to nonsense-mediated mRNA decay (NMD) as well as a 30-35% reduction of full-length GRN protein, without detectable levels of truncated GRN species [26]. The haploinsufficiency disease mechanism has since been validated by the identification of mutations directly affecting the Met1 translation initiation codon (N=3), which prevent translation of the mutant transcript, and more recently by the identification of larger

GRN deletions (N=3), including a complete heterozygous *GRN* locus deletion [41]. Finally, the p.A9D mutation located in the hydrophobic core of the signal peptide segregated with FTL-D-U in the autosomal dominant family HDDD2 [42]. This mutation showed evidence of cytoplasmic missorting with extremely low expression in cell culture along with significantly reduced levels of mutant *GRN* mRNA in brain [43].

The sequencing analyses of *GRN* in an ever-increasing number of dementia patients and controls also identified numerous missense and silent mutations with unknown pathogenicity (Figure 2). Thus far, 39 patient-specific genetic variants were observed; however, none of these segregated with disease in extended dementia families and the disease mechanism remains poorly understood. *In-vitro* analyses of missense mutations p.P248L and p.R432C demonstrated that they were expressed as immature proteins, but inefficiently transported through and partially degraded within the secretory pathway resulting in a significant reduction of secreted *GRN* [43]. An additional three missense mutations (p.C105R, p.C139R and p.C521Y) are predicted to affect highly conserved cysteine residues within the granulin peptides, which may disrupt the disulphide bonds necessary to maintain the granulin quaternary structure and function. Indeed, two independent studies detected reduced *GRN* levels in plasma of a patient and in serum of a patient and an asymptomatic carrier of the p.C139R mutation affecting granulin F [44,45]. Together it is expected that at least some missense mutations may cause disease through a (partial) loss of *GRN* function, while others may function as susceptibility alleles, potentially by making neurons more vulnerable to neurodegeneration through subtle decreases in neurotrophic support.

A limited number of studies have looked at the frequency of *GRN* mutations in other neurodegenerative diseases, including AD [39]. *GRN* loss-of-function mutations, similar to those identified in FTL-D-U, were not identified in population-based mutation screenings of AD, amyotrophic lateral sclerosis (ALS) or Parkinson disease (PD). One possible exception is the nonsense mutation, p.R535X, identified in a clinically diagnosed AD patient; however, cDNA analyses predicted that this mutation would escape NMD [46]. In contrast, numerous potentially pathogenic missense mutations were identified in AD, ALS and PD patients (Figure 2). This is an interesting observation which suggests that a partial loss of *GRN* function could also contribute to the neurodegenerative disease process in dementias other than FTL-D. In support of this hypothesis, genetic association studies showed significant association of a haplotype spanning the coding *GRN* region in a Belgian late-onset AD case-control population [47], which was partially confirmed in a Finnish AD population, although in this case the effect was male-specific [48]. In ALS, common *GRN* variants (rs9897526, rs34424835, and rs850713) and a haplotype including these variants were significantly associated with a reduction in age at onset and a shorter survival after onset of ALS in a Belgian ALS population [49]. The association of *GRN* variants and survival after disease onset was further replicated in a Dutch ALS population [49]. Additional mutation and association studies in extended AD, ALS and PD populations should shed light on the importance of *GRN* in the development of these related neurodegenerative disorders.

Soon after the discovery of *GRN* mutations, the nuclear protein TDP-43 was identified as the major disease protein in all ubiquitin-positive inclusions in FTL-D-U patients as well as in the ubiquitinated inclusions in the lower motor neurons in sporadic patients with ALS [50]. It was further shown that all *GRN* mutation carriers have a common FTL-D-U subtype, characterized by TDP-43 immunoreactive NCIs, short, thin neurites in layer II of the cortex and lentiform NIIs [51-53]. This FTL-D-U subtype is referred to as Type 1 by Mackenzie *et al.* [51] and Type 3 by Sampathu *et al.* [53]. One study provided a possible link between the loss of functional *GRN* and TDP-43 pathology, by showing that decreased *GRN* levels can induce caspase-dependent accumulation of TDP-43 fragments *in-vitro* [54]; however this finding was not confirmed by a second study using both human cell lines and zebrafish [43].

TDP-43 is a highly conserved protein, expressed by a variety of tissues and cell types including neurons, with proposed functions that include the regulation of transcription, alternative splicing and the transport and stabilization of mRNA [55]. TDP-43 has also been implicated in miRNA biogenesis [56]. Through these functions, TDP-43 may potentially be involved in the pathophysiology of FTL-D-U and ALS, as well as in other neurodegenerative dementias, including AD and Lewy-body related diseases, where TDP43-immunoreactivity was also reported in a subset of patients [57-59]. Initial mutation screenings of the *TARDBP* gene, encoding TDP-43, in FTL-D and AD populations, did not support a direct role for *TARDBP* in the etiology of dementia [60]. However, *TARDBP* missense mutations were identified as a cause of sporadic and familial ALS, supporting a direct role for TDP-43 in neurodegeneration [61,62]. More recently, the spectrum of phenotypes associated with *TARDBP* mutations expanded to include FTL-D-ALS, when two genealogically unrelated French patients carrying the p.G295S mutation were reported who each presented with clinical FTL-D, two years prior to the development of ALS symptoms [63]. Also, an Italian patient carrying p.G294V developed clinical AD three years prior to the onset of ALS [64]. These findings suggest that *TARDBP* mutations may also be a rare cause of dementia.

Finally, mutations in the *FUS/TLS* (*fused in sarcoma/translocation in liposarcoma*) gene, encoding another RNA associated protein with structural and functional similarities to TDP-43, were recently identified in patients with familial ALS. Whether mutations in *FUS/TLS* are also involved in the etiology of dementia has not yet been determined.

Copy-number alterations as a cause of dementia

In the past few years, genome-scanning array technologies and comparative DNA-sequence analyses have revealed an unexpected abundance of submicroscopic DNA rearrangements in normal individuals [65,66]. These genomic rearrangements were collectively termed copy number variations (CNVs) and defined as “DNA segments of at least 1kb in size, for which copy number differences have been observed in the comparison of two or more genomes” [67]. According to the database of genomic variants (<http://projects.tcag.ca/variation/>), almost 20,000 CNVs overlapping a total of 7,000 genes have already been discovered, illustrating that CNVs represent a substantial proportion of the total genetic variability in human populations.

The identification of duplications and triplications of the α -synuclein gene in autosomal dominant Parkinson's disease (PD) provided the first evidence for the involvement of rare CNVs in neurodegenerative diseases [68,69]. The first hint for a possible role in dementia came from the observation that presence of an additional copy of the *APP* gene on chromosome 21q21 in patients with Down syndrome, leads to overproduction of APP and deposition of A β peptide in amyloid plaques and the development of early-onset AD associated with cerebral amyloid angiopathy (CAA) [70]. In 2006, an extensive screen for *APP* copy-number mutations finally resulted in the identification of duplications at the *APP* locus in 5 families with autosomal dominant early-onset AD associated with CAA [71]. Despite variable sizes of the genomic duplications in these families, ranging from 0.58 to 6.37Mb and involving 5 to 12 genes, the phenotypes of patients were similar, without any obvious signs of Down syndrome. These results were further corroborated by another study, which demonstrated that *APP* duplication is sufficient to cause early-onset AD with CAA [72].

These data are of importance for several reasons. First, they provide further evidence that increased gene dosage can be involved in the etiology of neurodegenerative disorders caused by peptide or protein accumulation, emphasizing the importance of copy-number analyses in molecular genetic studies of dementia. Second, mechanistically, APP duplication in early-onset AD constitutes a strong support to the amyloid cascade hypothesis (Figure 1). It demonstrates

that increased *APP* gene dosage may initiate the cascade of events leading to β -amyloid plaques and neurofibrillary tangles, although it remains to be evaluated to what extent *APP* duplication leads to protein overexpression. Finally, it suggests that even a small increase in *APP* expression (<50% increase in expression), perhaps resulting from alterations in the 5' or 3' regulatory elements of *APP*, could constitute a risk factor for late-onset AD. In fact, *APP* promoter analyses in a Dutch and a Belgian early-onset AD population and an extended Belgian late-onset AD population, revealed a number of *APP* promoter mutations with an *in-vitro* 1.2-1.8-fold neuron-specific increase in *APP* transcriptional activities [73,74]. Although the association of rare *APP* promoter mutations with AD could not be confirmed in a French AD case-control population, genetic variant rs463946 located in the *APP* upstream regulatory region showed evidence of association with AD in two independent French AD case-control series [75]. Also, Lv et al. [76] demonstrated that two SNPs (rs466433 and rs364048) in linkage disequilibrium with rs463946, were associated with AD in Chinese Han populations.

In addition to *APP* duplications, a number of partial and complete gene deletions have been reported in autosomal dominant AD and FTLN. The first report described genomic deletions encompassing *PSEN1* exon 9 in patients presenting with AD associated with cotton wool plaques and spastic paraparesis [77]. More recently, deletions of *GRN* and *MAPT* genes were reported in FTLN. In *GRN*, a complete *GRN* locus deletion was described in a Belgian FTLN patient without known family history of dementia [41], followed by a second French family with two affected siblings carrying partial *GRN* deletions encompassing exons 1-11 [78]. In this family, one patient presented with FTLN-U, while the other was diagnosed with clinical PD, further illustrating the phenotypic variability associated with *GRN* mutations. Although previously hypothesized, the identification of *GRN* deletions established haploinsufficiency as the sole mechanism of *GRN* mutations necessary to initiate the disease process. This was further supported by the observation of strongly reduced *GRN* expression levels in plasma in all studied *GRN* loss-of-function mutation carriers, including a *GRN* deletion carrier [44].

Lastly, a partial *MAPT* genomic deletion encompassing exons 6 to 9 was found in an FTLN patient [79]. This genomic deletion resulted in a truncated tau protein that (i) loses its ability to bind microtubules and (ii) acquires the ability to bind microtubule-associated protein 1B (MAP-1B), another axonal MAP. Of note, complete genomic deletions of *MAPT* are not associated with dementia but were previously identified in individuals with mental retardation [80-82].

These studies demonstrate that CNVs leading to protein overexpression, haploinsufficiency or to a gene product with modified functional properties may contribute to various phenotypes and mechanisms leading to AD and FTLN. Collectively, these data highlight the importance of well-regulated expression of neurodegenerative disease proteins, not only as a cause of dominantly inherited disease in previously unexplained families but also as a risk factor for the more common sporadic forms of dementia.

Altered micro RNA regulation in dementia

Increasing evidence suggests that miRNAs may be a contributing factor in neurodegeneration and could potentially influence dementia risk. miRNAs are a class of small, endogenous, non-coding RNA molecules that serve as posttranscriptional regulators of gene expression [83]. They are thought to directly promote degradation of target mRNAs or suppress translation of corresponding protein via non-perfect base-pairing with the target mRNAs [84]. The current release of miRBase (<http://microrna.sanger.ac.uk/>), the primary repository and database resource for miRNA data maintained by the Sanger Institute, contains entries for 533 human miRNAs; however, it is predicted that there are as many as 1,000 miRNAs in the human genome that likely regulate 30% of all human transcripts. Many miRNAs are tissue-specific and/or

temporally regulated in their expression. Interestingly, compared to other organs, human brain expresses an exceptionally diverse spectrum of distinct miRNAs and at somewhat higher levels [85].

The most popular approach to identify specific miRNAs implicated in dementias has been miRNA microarray profiling in brain tissue samples derived from patients and controls. Using miRNA expression profiling in temporal cortex samples from a well characterized clinicopathological series of elderly subjects, either non-demented without AD pathology, non-demented with early AD pathology, mild cognitive impairment (MCI) with moderate AD pathology and AD, Wang *et al.* identified miR-107 to be specifically decreased early in the course of AD [86]. *In situ* hybridization further demonstrated that miR-107 is strongly expressed in neurons with a characteristic laminar expression pattern, which suggested a specific decrease of miR-107 in cortical layers with abundant AD pathology. Computational analyses predicted BACE1 mRNA as a target of miR-107 and correlative mRNA expression studies confirmed that subjects with lower miR-107 levels (generally presenting with more AD pathology) showed higher BACE1 mRNA levels. An independent miRNA profiling study by Hebert and colleagues using temporal cortex of AD cases and age-matched controls further confirmed the importance of BACE1 regulation by miRNAs and identified the miR-29a/b-1 cluster as a potential major suppressor of BACE1 protein expression [87]. The miR-29a/b-1 cluster was significantly and specifically downregulated in AD patients and correlated with increased expression of BACE1 protein in AD brain. They also showed a tight correlation of miR-29a/b-1 and BACE1 expression during brain development and in isolated primary cells. Further evidence came from Boissonneault and colleagues who showed that miR-298 and miR-328 regulate BACE1 protein expression in mouse cultured neuronal cells [88]. Finally, *in vitro* evidence suggested that brain expressed miR-106a can also directly regulate APP protein levels through translational repression of APP mRNA [89]. Together these studies provide strong support for a regulatory role of miRNAs in BACE1 expression, with possible implications for sporadic AD. Changes in miRNA expression may well contribute to the increased BACE1 expression observed in sporadic AD patients, providing a novel link between sporadic AD and the amyloid cascade hypothesis (Figure 1) [90]. Interestingly, additional BACE1 expression regulation by non-coding RNAs was recently reported when a natural antisense transcript for BACE1 was identified [91]. The levels of BACE1 antisense transcripts were elevated in AD patients and in APP transgenic mice and alterations in BACE1 antisense RNA concentrations impacted A β 40 and A β 42 production [91].

Recently the first miRNA array on pooled miRNA samples of AD cases and controls was performed, revealing yet another miRNA elevated in AD brain, miR-146a, whose expression is regulated by the transcription factor NF- κ B [92]. The authors showed that the NF- κ B sensitive miR-146a was able to downregulate complement factor H, an important repressor of the inflammatory response in the brain, suggesting a novel regulatory pathway which may contribute to inflammatory pathology in AD. Unfortunately, each miRNA profiling study in AD has thus far identified a different set of differentially expressed miRNAs, which underlines the challenge of using human brain samples with intrinsic significant biological variation. Confirmation of these findings in independent AD patient and control populations will therefore be critical.

In contrast to AD, miRNA array profiling studies have yet to be performed in other dementia populations. However, an important hint towards a possible involvement of miRNA in the development of FTLN was recently provided by a combination of genetic and biochemical studies. In a series of pathologically confirmed FTLN-U patients, it was shown that carriers homozygous for the T-allele of rs5848, located in a miR-659 binding site in the 3' untranslated region of *GRN*, have a 3.2-fold increased risk to develop FTLN-U compared to homozygous C-allele carriers [93]. *In vitro* studies confirmed that miR-659 can regulate *GRN* expression,

with miR-659 binding more efficiently to the high risk T-allele of rs5848 resulting in augmented translational inhibition of GRN. These results are consistent with the loss-of-function disease mechanism of *GRN* mutations and further emphasize the role of gene dosage effects in neurodegenerative disorders. Moreover, these findings suggest a larger contribution of *GRN* to the development of FTLT then initially anticipated. Follow-up studies in additional FTLT-U patient-control populations should now be performed to confirm these findings.

The widespread role of miRNAs in human diseases, now including dementias, has suggested that miRNAs might be viable targets for therapeutic intervention. An important future challenge associated with miRNA therapeutics for dementia will be the efficient and targeted delivery of miRNAs or antisense oligonucleotides to the brain [94].

Finally, Cogwell and colleagues discovered that miRNAs can be detected in the cerebrospinal fluid (CSF) and importantly that the expression pattern of miRNAs are altered in AD patients compared to controls [95]. This study provides initial hope that miRNAs could provide accessible biomarkers to aid clinical diagnosis in the future.

Novel genetic risk factors in dementia

Since the identification of the three autosomal dominant early-onset AD genes, *APP*, *PSEN1* and *PSEN2*, well over a decade ago, no novel causal AD genes and few AD loci have been reported [9]. This can be explained, in part, by the low prevalence of familial AD (less than 1% of the total number of AD patients have another family member with AD) and the resulting scarcity of extended AD families with sufficient power for genetic linkage studies. In recent years, the focus of AD genetic studies has therefore shifted towards association studies aimed at the identification of genetic risk factors, which has led to the identification of several potential AD risk genes.

One popular way to identify new susceptibility genes for AD has been to perform association studies in functional candidate genes, selected based on current knowledge and hypotheses of the physiopathological mechanisms underlying AD. In this view, the autosomal dominant dementia genes (*APP*, *PSEN1*, *MAPT*, *PRNP*) and genes encoding for proteins involved in APP cleavage (e.g. *BACE-1*, *nicastrin*, *presenilin enhancer-2*) or β -amyloid degradation (e.g. *insulin degrading enzyme*, *neprilysin*) have been studied. Thus far, these studies produced mostly inconsistent results and additional analyses of potential disease variants, including functional studies, will be essential to distinguish false positives from true genetic risk factors. One interesting finding is the identification of positive association of AD with a subtype of the extended H1 *MAPT* haplotype, which has been suggested to be more efficient in driving *MAPT* gene expression than the H2 haplotype [96,97]. Despite the lack of *MAPT* mutations in AD, these findings suggest that tau dysfunction may contribute to AD risk. Another important candidate gene that was recently reported to be associated with AD is *SORL1*, a neuronal sorting receptor involved in recycling of APP from the cell surface via the endocytic pathways. Two distinct clusters of intronic SNPs, one towards the 5' end and one towards the 3' end of *SORL1*, were significantly associated with late-onset AD in multiple case-control populations [98]. Since exonic functional variants could not be identified, it was hypothesized that intronic regulatory sequences within *SORL1* might govern cell type-specific or tissue-specific expression of SORL1. Lastly, novel candidate genes have also been characterized through study of expression profiles in AD brains. This strategy enabled the identification of the calcium homeostasis modulator 1 gene (*CALHM1*), located on chromosome 10q24.33, which encodes a multipass transmembrane glycoprotein that controls cytosolic Ca^{2+} concentrations and β -amyloid levels. Dreses-Werringloer et al. found that the coding p.P86L variant in *CALHM1* increases β -amyloid levels by interfering with the CALHM1-mediated Ca^{2+} permeability and was associated with late-onset AD in four case-control series [99]; however, independent

follow-up studies have not been able to confirm these findings [100-102]. For a continuously updated list of the genes most strongly associated with AD based on meta-analyses of all published studies, we refer to the AlzGene database (<http://www.alzforum.org/res/com/gen/alzgene/default.asp>)[103].

The completion of the Human Genome Project, combined with advances in high-throughput and high-density genotyping technology, has led to the emergence of genome wide association studies (GWAS). For GWAS, contrary to candidate gene studies, no a priori knowledge about the disease is needed. These studies currently represent the most promising and powerful tool to raise new susceptibility genes. Although the GWAS field is still in its relative infancy, eight GWAS have already been completed for AD and a number of novel susceptibility genes have been proposed (Table 2). However, caution is warranted when interpreting the reported findings. First, with the exception of *APOE*, none of the reported novel genes overlapped between studies. This may be explained by limited sample sizes, slight differences in study design and the stringent corrections for multiple testing inherent to these types of studies. In an attempt to overcome this last hurdle, Feulner et al. performed an initial analysis of their GWAS data only including the current top 10 candidate genes according to the AlzGene database [104]. Using this approach, all four analyzed genes that were previously identified through GWAS (*GAB2*, *PGBD1*, *PCK1* and *LMNA*) showed nominally significant association, compared to only two genes (*MAPT* and *SORL1*) identified through candidate gene studies. Second, since all GWAS studies were performed within the last two years, few independent replication studies have been reported and it is likely that some genes may not replicate. The only extensively studied gene identified by GWAS to date is *GAB2*, encoding a scaffolding protein implicated in numerous growth and differentiation signaling pathways [105]. Both positive and negative association studies for *GAB2* have been reported and a current meta-analysis of all published studies suggests a significant association of *GAB2* with AD [103]. Interestingly, some studies reported a stronger risk in those individuals who also carried an *APOE*ε4 allele [105,106]. In the future, independent replication and validation of *GAB2* and other newly identified AD candidate genes in multiple populations combined with functional genomic analyses will be essential.

In contrast to AD, candidate-gene association studies in clinical FTLN populations have produced little success in recent years. In part this may reflect the heterogeneous composition of a clinical FTLN cohort, which is predicted to include both tau-positive and FTLN-U patients as well as a subset of patients with other pathological conditions. Clinicopathological studies have suggested that as much as 15-30% of patients with a clinical diagnosis of FTD have AD at autopsy [107-109]. It is expected that future studies, focused on pathologically more homogenous FTLN subpopulations, could lead to the identification of novel genetic risk factors involved in the various pathways underlying FTLN.

Concluding Remarks

Most of our current mechanistic insight into the pathogenesis of AD and FTLN has resulted from the molecular genetic dissection of the rare autosomal dominant dementia families and the identification of mutations in *APP*, *PSEN1*, *PSEN2* and *MAPT*. The recent discovery of loss-of-function mutations in *GRN* as an important cause of FTLN-U identified yet another key player in these neurodegenerative disease processes [26,27]. Given the presumed role of *GRN* in neuronal survival and the identification of possible (partial) *GRN* loss-of-function mutations in other dementias, including AD, a wider role for *GRN* as a genetic risk factor for neurodegeneration was suggested [40]. It is the hope that future studies using *GRN* animal models will shed light on the molecular pathway by which *GRN* maintains neuronal fitness, which could lead to identification of novel therapeutic targets [110].

Despite major progress in unraveling the etiology of the rare Mendelian forms of AD and FTL, the currently known disease genes explain less than half of the familial dementia patients and only a minority of the apparently sporadic patients. However, recent interest in copy-number alterations led to the identification of genomic duplications of *APP* in AD and genomic deletions of *GRN* and *MAPT* in FTL, explaining the disease in a number of previously unresolved families [41,71]. Furthermore, a subtle change in expression of the known disease proteins was suggested to contribute to the risk for the common sporadic forms of dementia: *APP* promoter variants with an expected ~1.5-fold increase in *APP* transcriptional activities were identified in AD patients [74], while genetic variability in the 3' untranslated region of *GRN* was associated with a 3-times increased risk to develop FTL-U, as a result of partially reduced levels of GRN [93]. In addition, a new and emerging role for miRNAs in the development of AD was suggested, including a specific down-regulation in AD of miRNAs regulating BACE1, which correlated with an increase in BACE 1 expression in AD brain [86,87]. Together these findings support a much larger role for the currently known disease genes in the etiology of dementia than initially anticipated. In addition, they hold the promise that therapeutic strategies designed to target the monogenic forms of the disease will also prove effective in sporadic dementia patients.

Finally, through GWAS, a novel opportunity of identifying dementia risk genes and associated disease pathways has emerged with several potential AD risk genes already reported (Table 2). Further validation and replication in appropriately large patient populations combined with functional characterization of these genes could lead to a better understanding of the pathophysiology underlying dementia and open new therapeutic possibilities for AD and FTL.

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GLOSSARY

Nonsense-mediated decay (NMD):	Nonsense mediated decay is a eukaryotic quality control mechanism that selectively degrades mRNA species harboring premature termination (nonsense) codons to prevent the expression of truncated or erroneous proteins.
Haploinsufficiency:	Haploinsufficiency refers to a situation where an individual who is heterozygous for a certain gene mutation, is clinically affected because 50% of the level of gene function is not sufficient to assure normal function.
Cerebral amyloid angiopathy (CAA):	Cerebral amyloid angiopathy, also known as congophilic angiopathy, is a disease of small blood vessels in the brain in which deposits of β -amyloid in the blood vessel walls may lead to stroke, brain hemorrhage, or dementia. CAA is a common feature in AD patients with <i>APP</i> duplications and <i>APP</i> missense mutations in the α -secretase cleavage site.
Linkage disequilibrium:	In population genetics, the term linkage disequilibrium refers to a situation where a particular allele at one locus is found together on the same chromosome with a specific allele at a second locus - more

often than expected if the loci were segregating independently in the population.

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Figure 1. The amyloid cascade hypothesis

Autosomal dominant familial forms of Alzheimer's disease (AD) mainly result from mutations in *PSEN1* and to a smaller extent from mutations in *PSEN2* and *APP*, including *APP* duplications. *PSEN* mutations lead to aberrant γ -secretase cleavage of APP resulting in an overproduction of the more fibrillogenic A β 42 and an increased A β 42/A β 40 ratio. All missense mutations in *APP* are located at or near the three proteolytic cleavage sites of APP and depending on their location result in (i) increased production of A β 40 and A β 42, (ii) increased A β 42/A β 40 ratio, or (iii) enhanced fibrillogenic potential of mutant A β , accompanied by vascular tropism responsible for cerebral amyloid angiopathy (CAA). The essence of the amyloid cascade is that the increased production or decreased clearance of A β peptides result in the aggregation and accumulation of A β , which in a yet uncertain pathogenic form, triggers a number of downstream deleterious events, finally leading to neuronal death. Recently described *APP* duplications in AD patients also fit this model. Although it has not been finally demonstrated, it is likely that *APP* duplication leads to enhanced A β expression, which is phenotypically associated with AD and CAA in patients. Finally, this model suggests that in sporadic forms of AD slight increases in APP expression, mediated either by genetic variants in the promoter or 3' untranslated regions of *APP* or by micro RNA changes, could constitute risk factors for late-onset AD.

Figure 2. Spectrum of *GRN* mutations in dementia

Schematic representation of the genomic structure of progranulin (*GRN*) and the mRNA encoding the *GRN* protein. The dark blue numbered boxes in the genomic structure indicate non-coding exon 1 and coding exons 2-13. The light blue lettered boxes in the *GRN* protein refer to the individual granulin domains. Mutations in *GRN* were recently reported as a novel cause of dementia. Loss-of-function mutations in *GRN* result in frontotemporal lobar degeneration with TDP-43 positive inclusions (FTLD-U). A total of 66 different loss-of-function mutations scattered over all *GRN* exons except exon 13, have been reported. These mutations are indicated with vertical black lines on the *GRN* exons and listed above the exons with their cDNA numbering relative to the largest *GRN* transcript (GenBank accession number NM_002087.2). One complete and two partial *GRN* deletions have also been identified as indicated with horizontal black lines above the *GRN* genomic structure. An additional 39 patient specific mutations with unknown pathogenic significance were identified in neurodegenerative disease patients. These include 28 missense mutations, 10 silent mutations and one nonsense mutations in *GRN* exon 13. These mutations are indicated with vertical lines on the *GRN* protein relative to the individual granulin domains and listed below the *GRN* protein with the protein numbering relative to GenPept accession number NP_002078.1. Each of the mutations is color coded to indicate that the mutations were identified in patients with a clinical diagnosis of FTLD (black), FTLD and ALS (orange), ALS (yellow), AD (red), AD and FTL (green) or AD and PD (purple). Missense mutations affecting conserved cysteine residues within the granulin peptides are indicated with a hash symbol.

Table 1
Clinical and pathological features of neurodegenerative diseases associated with dementia

Neurodegenerative Disease	Main clinical features	Most affected brain regions	Characteristic pathology (major disease protein)
Alzheimer disease	Memory loss, cortical dementia (anomia, agnosia, apraxia)	Cerebral cortex, hippocampus, amygdala, basal forebrain, brainstem (locus ceruleus)	Senile plaques (β -amyloid); Neurofibrillary tangles and neuropil threads (tau)
Dementia with Lewy bodies (DLB) and Parkinson disease dementia (PDD)	Subcortical dementia, parkinsonism, recurrent visual hallucinations, sleep disorders, fluctuation	Limbic cortex, basal forebrain, amygdala, brainstem (substantia nigra, locus ceruleus)	Lewy bodies and Lewy neurites (α -synuclein); senile plaques (β -amyloid)
Frontotemporal lobar degeneration	Cortical dementia, behavior and personality changes, language dysfunction; parkinsonism or motor neuron disease in some	Frontal and temporal cortex, hippocampus, amygdala, basal ganglia (striatum)	Neuronal cytoplasmic & intranuclear inclusions and dystrophic neurites (TDP-43)
			Pick bodies (tau); ballooned neurons (neurofilament)
Progressive supranuclear palsy	Subcortical dementia, parkinsonism, vertical gaze palsy, axial dystonia, bulbar signs,	Basal ganglia (striatum & pallidum), subthalamic nucleus, brainstem (substantia nigra), cerebellum	Neurofibrillary tangles and tufted astrocytes (tau)
Corticobasal degeneration	Cortical/subcortical dementia; asymmetric rigidity, apraxia and dystonia; aphasia and frontal dementia in some	Cerebral cortex, basal ganglia (striatum & pallidum) and brainstem (substantia nigra)	Neurofibrillary tangles, neuropil threads and astrocytic plaques (tau); ballooned neurons (neurofilament)
Amyotrophic lateral sclerosis	Spasticity, muscle weakness and atrophy, bulbar signs; subcortical dementia in some	Motor cortex, brainstem and spinal cord motor neurons	Neuronal cytoplasmic, dystrophic neurites and intranuclear inclusions (TDP-43)
Huntington's disease	Chorea, psychiatric problems; subcortical dementia in some	Cerebral cortex, basal ganglia (striatum)	Intranuclear inclusions and cytoplasmic aggregates (huntingtin)
Transmissible spongiform encephalopathies	Rapidly progressive cortical dementia, myoclonus, ataxia, visual problems, psychiatric problems; insomnia	Cerebral cortex, basal ganglia, thalamus, cerebellum	Spongiform degeneration, amyloid plaques (prion)

Table 2

GWAS performed in late-onset AD

GWAS	Study Design	Sample origin	Number of samples in GWAS (patients/controls)	Genotyping Platform	Genes or SNPs most associated with late-onset AD	Additional notes	Refs
Carrasquillo <i>et al.</i>	Clinical and neuropathological case-control	USA	844/1255	Illumina HumanHap300	<i>APOE</i> , <i>PCDH1X</i>	First AD candidate gene identified on chromosome X	[111]
Beecham <i>et al.</i>	Clinical case-control	USA	492/496	Illumina HumanHap300	<i>APOE</i> , <i>FAM113B</i>	Associated SNPs were analyzed in a previous GWAS [105] using imputation data	[112]
Feulner <i>et al.</i>	Clinical case-control	Germany	491/479	Illumina HumanHap500	<i>APOE</i> ^a	Only top 10 genes from AlzGene were analyzed	[104]
Abraham <i>et al.</i>	Clinical case-control	UK	1082/1239	Illumina HumanHap300 and HumanHap240S	<i>APOE</i> , <i>LRAT</i>	DNA pooling strategy was used	[113]
Bertram <i>et al.</i>	Family based	USA	941/404	Affymetrix GeneChip Human Mapping 500K Array	<i>APOE</i> , <i>ATXN1</i> , <i>CD33</i> , rs11159647 at 14q31.2	First study using family-based method in AD field	[114]
Li <i>et al.</i>	Clinical case-control	Canada and UK	753/736	Affymetrix GeneChip Human Mapping 500K Array	<i>APOE</i> , <i>GOLM1</i> , rs9886784 at 9p24.3, rs10519262 at 15q21.2		[115]
Grupe <i>et al.</i>	Clinical case-control	USA and UK	380/396	Celera Functional SNPs	<i>APOE</i> , <i>GALP</i> , <i>TNKL1</i> , <i>PCCK1</i>	GWAS performed with functional SNPs	[116]
Coon <i>et al.</i> and Reiman <i>et al.</i>	Clinical and neuropathological case-control	USA and The Netherlands	664(446)/422(290)	Affymetrix GeneChip Human Mapping 500K Array	<i>APOE</i> , <i>GAB2</i>	<i>GAB2</i> association only identified in <i>APOE</i> ϵ ϵ carriers	[105, 117]

AD = Alzheimer's disease; *APOE* = Apolipoprotein E; *PCDH1X* = protocadherin 11, x-linked; *FAM113B* = family with sequence similarity 113, member B; *LRAT*=lecithin retinol acyltransferase; *ATXN1*= Ataxin 1, *CD33*= CD33 molecule; *GOLM1*= golgi membrane protein 1; *GALP* = galanin-like peptide precursor; *TNKL1* = non-receptor tyrosine kinase 1; *PCCK1*= phosphoenolpyruvate carboxykinase 1; *GAB2* = GRB2-associated binding protein.

^aNominally significant association was also found for *GAB2*, *PCBD1*, *PCCK1*, *LMNA*, *MAPT* and *SORL1* but p-values were not corrected for multiple testing.