Recent advances in the molecular genetics of frontotemporal lobar degeneration

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Summary

The term frontotemporal lobar degeneration (FTLD) describes a spectrum of neurodegenerative disorders associated with deposition of misfolded proteins in the frontal and temporal lobes. Up to 40% of FTLD patients reports a family history of neurodegeneration, and approximately 1/3 of familial cases shows an autosomal dominant pattern of inheritance of the phenotype. Over the past two decades, several causative and susceptibility genes for FTLD have been discovered, supporting the notion that genetic factors are important contributors to the disease processes. Genetic variants in three genes, *MAPT*, *GRN* and *C9orf72*, account for about half of familial FTLD cases. In addition, rare defects in the *CHMP2B*, *VCP, TARDBP*, *SQSTM1, FUS, UBQLN, OPTN*, *TREM2, CHCHD10* and *TBK1* genes have been described. Additional genes are expected to be found in near future.
The purpose of this review is to describe recent advances in the molecular genetics of the FTLD spectrum and to discuss implications for genetic counseling.

KEY WORDS: frontotemporal dementia, frontotemporal lobar degeneration, C9orf72 gene, GRN gene, MAPT gene.

Introduction

The term frontotemporal lobar degeneration (FTLD) was coined to describe a pathology of the frontal and temporal lobes, commonly associated with cerebral deposition of misfolded proteins (Lashley et al., 2015). FTLD describes a spectrum of clinically, pathologically and genetically heterogeneous neurodegenerative disorders. Three main clinical syndromes have been related to FTLD, namely the behavioral variant of frontotemporal dementia (bvFTD), the semantic variant (SD), and progressive non-fluent aphasia (PNFA). In addition, there is a significant clinical, pathological and genetic overlap with the atypical parkinsonian syndromes, like progressive supranuclear palsy (PSP) and corticobasal syndrome (CBS), as well as amyotrophic lateral sclerosis (FTD-ALS) (Bang et al., 2015).

In the early phase of the disease, bvFTD is clinically characterized by progressive behavioral impairment and decline in executive function associated with frontal lobe-predominant atrophy, SD by a loss of object knowledge with anomia and asymmetrical atrophy of the anterior temporal lobes, and PNFA by expressive or motor speech deficits with predominantly left peri-sylvian atrophy. With the progression of the disease, a significant overlap between the different clinical syndromes may appear.

FTLD is a common cause of dementia in the general population. Once thought to be rare, FTLD is now recognized to be at least as common as Alzheimer's disease (AD) among middle-aged adults (Ratnavalli et al., 2002). In the over-65s with dementia, the incidence of FTLD ranks fourth, the top three being AD, Lewy body dementia and vascular dementia. The medical, psychological, social and economic costs of FTLD are enormous (Scalmana et al., 2013). Despite the burden associated with the disease, at present there is no FDA-approved therapy for it (Kerchner et al., 2011).

The neuropathology associated with FTLD is highly heterogeneous. From this perspective, the FTLD spectrum can be split into two major categories: FTLD-tau, where pathological inclusions contain fibrillar hyperphosphorylated tau, and FTLD-TDP, characterized by neuronal and glial inclusions immunoreactive for TDP-43. In addition, there is a small group of tau-negative and TDP-negative cases that are immunoreactive to components of the ubiquitin proteasome system (FTLD-UPS) or the fused in sarcoma protein (FTLD-FUS) (Josephs et al., 2011). In some cases, however, molecular classification is still uncertain.

Among patients with FTLD, a variable percentage, between 20 and 40%, presents a positive familyhistory. BvFTD shows the highest percentage of positive family history, around 30-50% of patients, whereas in patients with SD or PNFA the frequency is much lower. However, only 10-30% of pedigrees shows a clear autosomal dominant inheritance pattern of the phenotype (Le Ber, 2013). A recent study suggested that, even in patients with late onset, FTLD is a genetic-based disease with different inheritance modalities (Borroni et al., 2014).

The last two decades have seen tremendous advances in appreciation of the clinical assessment, genetics and molecular pathology of FTLD, thus offering hope for the development of rational therapeutic strategies. The purpose of this review is to describe recent advances in the molecular genetics of the FTLD spectrum disorders and to discuss the implications for genetic counseling.

Molecular genetics of FTLD

Recently, there has been an impressive evolution of genetic methodologies that can be used to identify genes

contributing to neurodegenerative disorders. Linkage analysis, whole exome sequencing and genomewide association studies (GWAS) have provided efficient approaches for detecting common and rare genetic variations contributing to FTLD risk. For the sake of clarity, data regarding causative genes, mainly cloned in families with autosomal dominant inheritance of the FTLD phenotype, will be discussed first. We will then review new genetic risk factors for the disease.

MAPT gene

The *MAPT* gene, linked to chromosome 17, was the first mutated gene identified, and it was discovered among families who presented with frontotemporal dementia (FTD) and parkinsonism (Hutton et al., 1998; Spillantini et al., 1998). Specifically, this gene is located on chromosome 17q21 and it consists of a non-coding exon followed by 14 coding exons. *MAPT* encodes for microtubule-associated protein tau protein (MAPT), whose main function is stabilizing and promoting assembly of microtubules (MTs), by binding to tubulin. MAPT plays also a role in modulating vesicle and organelle transport mediated by motor proteins along the MTs. Tau can be found both in the peripheral and in the central nervous system, where it is located mainly in neuron axons, astrocytes and oligodendrocytes. Alternative splicing processes of exons 2, 3 and 10 of the *MAPT* gene give rise to six main protein isoforms: three isoforms containing three amino-acid repeats (3R), and three isoforms with four repeats (4R). More specifically, the exon 10 splicing decides the number of repetions (3R or 4R), while the alternative splicing of exons 2 and 3 establishes the number of 29 amino acid N-terminal insertions (0N, 1N, 2N), which mediate tau interactions with plasma membrane (Brandt et al., 1995). It has long been known that tau protein undergoes a post-translation phosphorylation process in more than 25 sites and, notably, deposition of hyperphosphorylated insoluble tau is a common feature of several neurodegenerative disorders, collectively known as tauopathies (AD, PSP, FTD, CBS, Niemann-Pick disease) (Rademakers et al., 2004).

At present, 72 different *MAPT* gene mutations and polymorphisms have been reported, including 44 that are clearly pathogenetic (http://www.molgen.ua.ac.be), accounting for 5-20% of familial FTLD cases (frequency of mutations varies depending on the studied population, ranging from 1% in Finland to 17.8% in the Netherlands). The mutations are mainly clustered from exon 9 to 13, encoding the four MT binding domains. Silent mutations, missense mutations, in-frame single-codon deletions and intronic mutations have also been found. A substantial number of mutations are located in intron 10, leading to anomalous ratios of 3R to 4R tau (D'Souza et al., 2000). The tubulin binding affinity of the 4R isoform is three times stronger than that of the 3R isoform. The mutations thus disrupt the delicate equilibrium between cytoskeletal assembly and disassembly, altering neuronal plasticity and axonal transport across the MTs. Mutations of a second kind interfere with the tau-tubulin interaction, reducing the capacity of tau to stabilize and promote assembly of MTs, and thus promoting an increased tendency of tau to form neurotoxic aggregates. The hyperphosphorylation process also has a negative role in the tau-MT interaction. The three

most common mutations account for about 50% of the known mutations: the P301L mutation, associated mainly with bvFTD; the mutation involving the splice site in exon 10 (IVS10+16 C>T); and the N279K mutation.

The neuropathological phenotype varies substantially in morphological characteristics, severity and distribution, depending on the type of *MAPT* gene mutation. Tau deposits may be abundant in the cerebral cortex, white matter and some subcortical and brain stem nuclei. Neurofibrillary tangles (NFTs) or Pick bodies in neurons, as well as astrocytic plaques, tufted astrocytes, and coiled bodies in oligodendroglial cells in the glial pathology may be found. The location of the *MAPT* mutation influences the type of cellular involvement (i.e. neurons, astrocytes or oligodendroglia) and the type of neuronal inclusion. Mutations in exons 1 and 10 (as well as in the intron following exon 10) are associated with neuronal and glial tau deposition, while mutations in exons 9, 11, 12 and 13 lead to deposits of tau filaments, mainly in neurons. Mutations in exons 12 and 13 lead to the formation of NFTs with paired helical and straight filaments (indistinguishable from those seen in AD).

Since mutations in *MAPT* are typically associated with neuronal and glial tau deposition, the clinical picture may sometimes resemble other sporadic tauopathies such as PSP and CBS. A significant variability in age at onset of the disease (from 40 to 70 years) has been observed.

Clinically, patients show the bvFTD phenotype, including disinhibition and obsessive-compulsive behavior. Extrapyramidal signs and symptoms may also be present. Given the anterior medial temporal lobe atrophy identified in patients carrying *MAPT* mutations, an SD-like presentation associated with features of bvFTD can also be observed. Although the clinical phenotype is highly variable, early onset age, parkinsonism and oculomotor deficits should lead the clinician to suspect *MAPT* mutation in the patient. However, the lack of a clear correlation between *MAPT* gene mutations and clinical features indicates that additional genetic and/or environmental factors can produce significant phenotypic variations.

GRN gene

A major breakthrough occurred in 2006, when scientists found that the progranulin (*GRN*) gene, a protein-coding gene with 13 exons located on chromosome 17p21, was responsible for another 5-20% of familial FTLD cases and 1-5% of sporadic ones (Baker et al., 2006; Cruts et al., 2006). *GRN* encodes progranulin, an ubiquitously expressed growth factor precursor, which, together with its products, is implicated in a wide range of biological processes such as inflammation and wound repair, as well as in pathological conditions including tumorigenesis (He et al., 2003; Ahmed et al., 2007). Progranulin is predominantly expressed at the level of activated microglial cells and this finding seems to point to its regulatory role in the inflammatory response in the brain (Philips et al., 2010). Although a neuroprotective role of progranulin is universally recognized, the mechanism of this function is still under investigation (Van Damme et al., 2008; Tapia et al., 2011). Some experiments have shown that, in stressed or GRN-depleted neuronal cells, neurite outgrowth was obtainable by adding progranulin, probably due to the activation of cell survival signaling

pathways. Progranulin receptors are, at present, unknown as well. Only sortilin-1 (SORT1), already known as a receptor for other growth factors in brain, has been demonstrated to bind, endocytose and regulate Progranulin levels *in vivo* in human plasma. More recently, a direct interaction with receptors for TNF-α has also been reported. A role for progranulin in excitotoxicity and synaptic transmission has also been suggested. One most dynamic aspects of the research on *GRN* in recent years has been the study of factors, endogenous and exogenous, able to regulate the gene expression. The role of microRNAs in regulating progranulin expression is very intriguing and complex. In particular, the minor T-allele of genetic variant rs5848, located in the 3′ untranslated region of *GRN*, increases binding with a specific microRNA, miR-659, to *GRN*, thereby reducing progranulin levels (Rademakers et al., 2008). Homozygous carriers of the T-allele of rs5848 have a three-fold increased risk of developing FTLD-TDP compared with homozygous C-allele carriers.

Worldwide, 149 different *GRN* gene mutations have been described (http://www.molgen.ua.ac.be). Of these, 67 are clearly pathogenetic. Differently to *MAPT*, these substitutions are scattered all over the gene. Generally, *GRN* mutations are null mutations that lead to nonsense-mediated decay of mutant *GRN* mRNA and reduced expression of progranulin. Consequently, mutation carriers, even if asymptomatic, can be identified by measuring plasma, serum and cerebrospinal fluid progranulin concentrations (Finch et al., 2009). The most frequently observed *GRN* mutations are the R493X nonsense mutation in exon 11, and the c.-8+5 G>C (IVS0+5 G>C) substitution in a splicing site in intron 0.

Recent studies have shown, in FTLD patients carrying the *GRN* gene mutation, that the ubiquitinated neuronal inclusions contain, as a major component, the TAR DNA binding protein (TDP-43). A loss of function of progranulin leads to defective ubiquitination, resulting in accumulation of TDP-43.

Mutation penetrance is very high, with 90% of carriers manifesting symptoms by the age of 75, and the associated phenotypes are remarkably variable, both between families with different *GRN* mutations and among members of a single family. The mean age at onset is around 60 years and the mean duration of disease is about 8 years (range 3-22 years). Social withdrawal and apathy are the most common behavioral changes. About 1/3 of patients, at presentation, shows early isolated language dysfunction, suggestive of a non-fluent type. Hallucinations and delusions are frequently concomitant. Episodic memory deficits may also occur, leading to a clinical diagnosis of the amnestic variant of mild cognitive impairment or, if they occur together with parietal deficits, like dyscalculia, visuospatial dysfunction, and limb apraxia, to an erroneous diagnosis of AD. Extrapyramidal signs are frequently observed and include CBS with asymmetric parkinsonism, dystonia and limb apraxia. Amyotrophic lateral sclerosis (ALS) is only a very rare part of the clinical spectrum within *GRN* families.

C9orf72 gene

The past decade has seen an accumulation of strong evidence suggesting that the presence of a locus on chromosome 9p21 is of pivotal importance in a com-

bined FTLD-ALS phenotype. Familial clusters of FTLD and/or ALS phenotypes with autosomal dominant transmission were found to have, in common, a region of 3.7 MB containing only ten known genes. In 2011, through PCR and Southern blot analysis techniques, it was finally discovered that the disease was related to a hexanucleotide (GGGGCC) repeat located in a non-coding region of the *C9orf72* gene. This result was reached simultaneously by two independent studies, one on an FTD-ALS family designated as VSM-20 (Vancouver, San Francisco and Mayo family), the other on a Welsh family (DeJesus et al., 2011; Renton et al., 2011).

The *C9orf72* gene consists of 12 exons encoding for three different transcripts and two protein isoforms, called a and b, whose function and distribution in the structures of the brain are still under investigation. Isoform a, the longest one (481 amino acids), originates from transcripts 1 and 3, while transcript 2 codes for the shorter isoform b (222 amino acids). Inside the cell, they can be found within the neuronal cytoplasm and in the presynaptic terminals. Although the function of the C9orf72 protein is currently unclear, recent studies classify it as homologous to the DENN (differentially expressed in normal and neoplastic cells) class of proteins, which are characterized by a conserved DENN module consisting of an N-terminal longin domain, followed by the central DENN and the C-terminal alpha-helical d-DENN domains. This class of proteins works as GDP/GTP exchange factors for small GTPases of the Rab family, with a role in vesicular trafficking regulation and neuronal autophagy. In healthy subjects, the number of GGGGCC (G4C2) repeats is less than 10, although 30 is universally accepted as a non-pathological limit. By contrast, in expanded individuals, the number of repeats ranges from a minimum of 400 to several thousand.

Several studies have demonstrated that *C9orf72* pathological expansion is a major cause of both familial FTLD (12%) and ALS (22.5%), with a higher prevalence in northern Europe and, more specifically, in genetically isolated populations, for example in Sardinia and Finland. In the Finnish population, in particular, the *C9orf72* mutation reaches a prevalence of 46% of all familial ALS, 21.1% of sporadic ALS, and 29.3% of familial FTLD subjects. Another study, performed in a Flanders-Belgian cohort of FTLD, ALS and FTLD-ALS subjects, showed that, in FTLD cases, *C9orf72* expansion occurs at a frequency comparable with that of mutations in *GRN* (6 *vs* 7%). Although most of the patients included in screening studies are Caucasian, *C9orf72* expansions have also been identified in Middle Eastern, African-American and Asian patients. It is interesting to note that, regardless of clinical presentation or ethnic origin, all the patients carrying the *C9orf72* variant inherit the expansion on the same genetic background, suggesting the presence of a founder effect or, alternatively, the presence of multiple independent expansions of a fragile haplotype that predisposes to disease. Recently, the first experimental evidence has emerged on the role of *C9orf72* in the regulation of endosomal trafficking and neuronal autophagy. The expansion is located between two non-coding exons, 1a and 1b, and currently there are three main hypotheses about its influence on transcriptional products and the consequent disease mechanism. In the first, reduced synthesis of

C9orf72 isoform leads to haploinsufficiency, resulting in exocytic/endocytic membrane trafficking dysfunction. The expanded hexanucleotide resides in the core region of the promoter of transcript 1, whose reduction by at least 50% has been demonstrated in several studies. However, since in transcripts 2 and 3 the expansion is incorporated in intron 1 and produces an aberrant transcript, the second hypothesis suggests a gain-of-function mechanism. The accumulation of repeats containing GGGGCC transcripts as nuclear RNA aggregates ("foci") found in the frontal cortex and spinal cord of carriers of mutated *C9orf72* suggested RNA toxicity as a third hypothesis. Similarly to what happens in other disorders, these RNA aggregates may produce alterations in gene expression and/or alternative splicing of transcripts, or disrupt the functionality of one or more RNA binding proteins. Recently, Mori et al. (2013) demonstrated that the intronic G4C2 repeats might be aberrantly translated into dipeptides, poly-(Gly-Ala) and, to a lesser extent, poly-(Gly-Pro) and poly-(Gly-Arg) dipeptide-repeat proteins. It is also possible that all the aforementioned mechanisms, to variable extents, contribute to different clinical phenotypes.

The neuropathology associated with the *C9orf72* mutation is a combination of FTLD-TDP and ALS (Hsiung et al., 2012). Post-mortem examination usually shows TDP-43-positive inclusions in the basal ganglia, substantia nigra, hippocampus, extramotor cerebral cortex and lower motor neurons of the brainstem and spinal cord. Nevertheless, the majority of reports agrees that there is a specific pattern of neuropathological characteristics with p62-positive, TDP-43-negative inclusions. These characteristic neuronal inclusions can be found in several neuroanatomical sites, but the hippocampal pyramidal neurons and cerebellar granule cell layer seem to be the most affected (Al-Sarraj et al., 2011).

Clinically, the predominant phenotype in these patients is bvFTD associated with ALS, although the presentation may vary widely even within the same family (bvFTD or ALS or a combination of both diseases) (Murray et al., 2011; Van Langenhove et al., 2013). Considering the FTLD spectrum, the most represented subtype is bvFTD, whereas PNFA is the least frequent. Psychosis and obsessive compulsive disorders are common at the onset of the disease (Arighi et al., 2012; Galimberti et al., 2013). Memory deficits at presentation are very frequent (in up to 50-65% of cases), presenting the clinician with a challenging differential diagnosis with AD. On examination, cerebellar and extrapyramidal signs may also be present. A case showing mystical delirium, visual and auditory hallucinations at onset, in the absence of neurological symptoms and brain atrophy, has also been reported. Age at onset age ranges from 27 to 83 years, with an average of 50 years. The duration of the disease can vary, ranging from 1 to 22 years. Neuroimaging shows symmetrical frontal and/or temporal lobe atrophy, but parietal, occipital and cerebellar atrophy can also appear.

CHMP2B gene

In 2005, using a linkage analysis approach, FTLD was linked to chromosome 3 in a Danish family (FTD-3). Subsequently, a mutation was identified in the chromatin-modifying protein 2B (*CHMP2B*) gene on chromosome 3p11.2 (Van der Zee et al., 2008). This gene encodes a component of the highly conserved ESCRT-III (endosomal sorting complex required for transport III) heteromeric complex, which plays a role in the recycling or degradation of cell surface receptors, in endosomal lysosomal and autophagic degradation pathways, and spine growth. *CHMP2B* has six exons and is expressed in neurons of all major brain regions. The first identified mutation occurs in the splice acceptor site for the final *CHMP2B* exon, leading to the formation of two novel transcripts termed $CHMP2B^{Intron5}$ and $CHMP2B^{Delta10}$, which replace the final 36 amino acids with either a single valine residue ($CHMP2B^{Intron5}$) or a 29-amino-acid nonsense sequence ($CHMP2B^{Delta10}$). In 2008, a missense mutation ($CHMP2B^{Q165X}$) was identified in an FTLD family. Both mutations (accounting for less than 1% of the FTLD population) affect the C-terminus of the protein, leading to its deletion.

Histological observation shows enlarged vacuoles in the frontal cortex and parietal, temporal and occipital neurons, probably due to altered endosome lysosome fusion and autophagic mechanism impairment (Urwin et al., 2010). Cytoplasmic inclusions are negative for tau, TDP-43 and FUS, consistent with the pathological classification of FTLD-UPS. Clinically, early behavioral changes are a common onset symptom, suggesting a bvFTD, but a minority of patients have been reported to show a particular progressive aphasia characterized by a spontaneous reduction of speech, which hesitates sometimes in silence, and preserved reading and repetition skills. This picture cannot be linked to a specific diagnosis of PNFA, or SD, but is more suggestive of "dynamic aphasia". Later in the disease course, parkinsonism, dystonia, pyramidal signs and myoclonus can be observed. The age at onset is between 46 and 65 years, with an average of 58 years.

VCP gene

Mutations in valosin-containing protein (*VCP*), a 17-exon protein-coding gene located on chromosome 9p13.3, were identified in 2004 through a linkage analysis in families presenting IBMPFD, a complex clinical picture characterized by muscle weakness due to inclusion body myopathy (IBM), osteolytic lesions compatible with Paget's disease of bone (PDB), and autosomal dominant FTLD (Watts et al., 2004). Recently, *VCP* mutations have also been found in Charcot-Marie-Tooth disease type 2, in ALS and in schizophrenia. The VCP protein is a member of a family that includes putative ATP-binding proteins involved in vesicle transport and fusion, and in proteasome function. It also plays a role in dendritic spinogenesis and in several cellular events related to mitosis (including homotypic membrane fusion, and spindle pole body function) and ubiquitin-dependent protein degradation.

Today there are 18 known mutations in this gene, identified in 41 independent families, and the R155H mutation, found in exon 5, is the most frequent. The penetrance is incomplete for the three typical clinical conditions and patients may present only one clinical phenotype. All IBMPFD mutations are found in the interface site of the ATPase D1 and the N-domain of the CDC48-like protein and affect protein degradation and/or protein-mediated autophagy in the ubiquitin-proteasome

system. However, mutations in *VCP* are rare and account for less than 1% of cases of familial FTLD. Pathologically, FTLD patients carrying *VCP* mutations show several cytoplasmic and nuclear intraneuronal inclusions and dystrophic neurites, consistent with a TDP-43 proteinopathy (Snowden et al., 2012). Symptoms occur in the sixth decade of life in 25-30% of IBMPFD patients and penetrance of the three clinical conditions (IBM, PDB, FTLD) is independent of the underlying mutation. BvFTD and SD are the FLTD subtypes most frequently reported.

TARDBP gene

The identification of TDP-43-derived protein species as the major constituent of the aggregates found in patients with FILD related to ubiquitin deposition (FTLD-U) led to the identification of transactive response DNA-binding protein (*TARDBP*) gene mutations (Arai et al., 2006). The *TARDBP* gene is constituted by six transcribed exons and located on 1p36.22. The protein it encodes is TDP-43, whose major form is translated from exons 2-6, resulting in a 414-amino-acid polypeptide, a highly conserved protein, predominately nuclear localized, able to move between nucleus and cytoplasm. It forms heterogeneous nuclear ribonucleoprotein (hnRNP) complexes with numerous functions related to RNA regulation, such as the control of splicing, mRNA stability and transport, and others yet to be investigated. It acts as a transcriptional repressor that binds to chromosomally integrated TAR DNA and represses HIV-1 transcription. Recently, in animal models, TDP-43 has been shown to regulate axon growth *in vitro* and *in vivo*, a finding suggesting that the capacity of spinal motor neurons to produce and maintain an axon is compromised by TDP-43 dysregulation and that disruption of cytoskeletal integrity may play a role in the pathogenesis of both ALS and FTLD (Polymenidou et al., 2011).
Mutations in *TARDBP* have been linked to both ALS and FTLD-TDP, suggesting that TDP-43 dysfunction is mechanistic in causing disease, but while 5% of familial ALS patients have a *TARDBP* mutation, these are rarely found in FTLD and FTD associated with motor neuron disease (FTD-MND). The current list of *TARDBP* gene mutations includes over 40 different missenses, most of them residing in the C-terminal glycine-rich domain, encoded by exon 6 and involved in protein-protein interactions. Since TDP-43 is a constituent part of hnRNP, it is reasonable to hypothesize that impaired common hnRNP functions could affect certain pathways leading to neuron dysfunction and degeneration. More than 6000 RNAs are known to interact with TDP-43 but, to date, few of them have been studied as possible disease-relevant targets. Nevertheless, it remains unclear whether the main disease mechanism involved in TDP-43-associated cell death is a loss-of-function or a gain-of-function one (or both). In fact, while the identification of *TARDBP* mutations provides evidence that TDP-43 dysfunction is linked to neurodegeneration, their functional consequences are still under investigation. Current *in vivo* models show that both increased and reduced expression levels of TDP-43 can be noxious for cells, but no model has been able to fully reproduce the neuropathological and biochemical features of human TDP-43-related diseases.

The pathological form of TDP-43 accumulating in neuronal and glial inclusions in FTLD and ALS consists of abnormally ubiquitinated and hyperphosphorylated C-terminal fragments, after a redistribution of TDP-43 itself from the nucleus to the cell cytoplasm. Although, in the literature, *TARDBP* mutations have been appreciated as a cause of ALS phenotypes more than pure FTLD ones, they can give rise to the full ALS-FTLD continuum, even to early-onset pure FTLD with no evidence of ALS even in advanced FTD disease stages. Hence, *TARDBP* screening might be considered in young patients with pure neuropsychiatric symptoms and no neurodegenerative disease history in their pedigree.

SQSTM1 gene

Mutations in the sequestosome 1 (*SQSTM1*) gene were initially identified as a cause of PDB, a common disorder of bone metabolism characterized by increased tissue turnover. More recently, mutations in *SQSTM1* have been found in both sporadic and familial ALS patients (Fecto et al., 2011). Finally, in 2012, *SQSTM1* gene mutations were also found in FTLD patients (Rubino et al., 2012). *SQSTM1* is an 11-exon gene located on 5q35 and it encodes p62, a 440-amino-acid adapter protein containing several protein-protein interaction domains with multiple functions in signal transduction regulating osteoclast differentiation, activity and survival. P62 also acts as a transport factor that directs ubiquitinated proteins to the autophagic degradation or proteasome pathway, playing a key role in the formation of ubiquitin-positive protein inclusions in neurons with defective autophagy. The protein also has an important function as a scaffolding/adaptor protein in concert with TNF receptor-associated factor 6, mediating activation of NF-kB in response to upstream signals. There is growing evidence implicating p62 in neurodegeneration: *SQSTM1* knock-out mice develop memory deficits associated with accumulation of hyperphosphorylated tau and NFTs (Ramesh Babu et al., 2008). In addition, pathological studies in humans have demonstrated increased immunoreactivity for p62 in several neurodegenerative diseases such as AD disease, Lewy body dementia, FTLD, Parkinson's disease and Huntington's disease (Zatloukal et al., 2002; Geetha et al., 2012). Aggregation of TDP-43, the main protein accumulating in neuronal cells of both FTLD and ALS patients, can be significantly reduced by overexpression of p62. Detailed histopathology demonstrated that mutations in *SQSTM1* are related to widespread neuronal and glial phospho-TDP-43 pathology. Autoptic investigations have shown that FTLD or ALS carriers of *C9ORF72* expansions have a larger number of p62-positive inclusions. According to a recent description of a new role of p62 in maintaining mitochondrial integrity, a portion of p62 localizes directly within the mitochondria and stabilizes the electron transport by forming heterogeneous protein complexes (Lee and Shin, 2011). P62 interacts with several oxidizable proteins, including some components of the electron transport chain, and with chaperones and redox regulatory enzymes.
SQSTM1 gene mutations in FTLD patients have been confirmed by several studies. However, at present, no clear genotype-phenotype correlations have been demonstrated. It seems appropriate to check for bone

metabolism alterations in patients who present signs and symptoms of FTLD or ALS, whereas patients with PDB should be carefully evaluated for signs of dementia and motor neuron disease.

FUS gene

Fused in sarcoma (*FUS*) is a highly conserved, ubiquitously expressed protein-coding gene located on 16p11.2. The gene encodes for the FUS protein, a 526-amino-acid protein component of the hnRNP complex and a member of the FET protein family, which also includes Ewing's sarcoma protein (EWS) and TATA-binding protein-associated factor 15 (TAF15). These are predominantly nuclear multifunctional DNA/RNA binding proteins (like TDP-43), implicated in cellular processes that include regulation of gene expression, maintenance of genomic integrity and mRNA/microRNA processing. FUS protein has an amino-terminal region rich in QGSY residues, a glycine-rich region, an RNA recognition motif, two RGG domains, and a zinc finger motif. Of utmost importance is the prion-like domain of FUS, residing in the amino-terminal region, which plays a critical role in FUS misfolding.

Purified FUS is, in fact, extremely prone to aggregation and it aggregates more rapidly than TDP-43.

In 2009, FUS mutations were discovered to be the cause of about 3% of familial ALS cases (Kwiatkowski et al.), and shortly afterwards inclusions composed of FUS were shown to account for the bulk of remaining tau/TDP-negative FTLD cases, which include atypical FTLD-U, basophilic inclusion body disease and neuronal intermediate filament inclusion disease. In most cell types, FUS is present in both the nucleus and the cytoplasm, but in neurons there is proportionally more in the nucleus, with smaller amounts in the cytoplasm, and the expression in the glia can even be exclusively nuclear (Mackenzie et al., 2010). In FTLD, the ability of FUS to shuttle to the nucleus is impaired because of an unspecified transportin-mediated nuclear import defect; this results in cytoplasmic accumulation of the full-length protein in stress granules. Interestingly, staining for TDP-43 and FUS appears to be mutually exclusive, suggesting that they are distinct subtypes of FTLD-U. It is also noteworthy that in all FTLD-FUS subtypes TAF15, and EWS co-accumulate in FUS-positive inclusions, and a reduction in the normal nuclear staining of all three FET proteins, particularly TAF15 was also found in inclusion-bearing cells, suggesting a hypothetical involvement of all FET proteins in neurodegeneration (Neumann et al., 2011).

Disease-causing mutations, mostly located in the carboxy-terminus of the protein, seem to cause protein cytoplasmic mislocalization. This could happen through two different mechanisms: a defect in transportin, reducing the efficiency of nuclear import of all FET proteins, or certain unknown posttranslational modifications of FET proteins, decreasing their solubility. Similar to TDP-43, both a loss of functions and a gain of toxic properties via their sequestration in aggregates are plausible.

FTD-FUS should be suspected in patients with onset of symptoms before 40 years of age, with no family history of FTLD, and with atrophy of the frontoinsular and cingulate cortex and of the head of the caudate nucleus on the neuroimaging study (Josephs et al., 2010). The existence of cases of primary progressive aphasia (PPA) with FUS inclusions remains to be demonstrated.

UBQLN2 gene

Ubuiquilin 2 (*UBQLN2*) is an intronless gene on Xp11.21, discovered in 2011 as a cause of X-linked ALS and ALS/dementia (Deng et al., 2011). Ubiquilin 2 protein is a member of the ubiquilin family of proteins which are characterized by the presence of an N-terminal ubiquitin-like domain and a C-terminal ubiquitin-associated domain. These proteins physically associate with both proteasomes and ubiquitin ligases and they are thought to functionally link the ubiquitination machinery to the proteasome to mediate ubiquitinated protein degradation.

UBQLN2 mutations are rare in Central European ALS and FTLD patients, but they seem to be more frequent in the sporadic disease forms. Mutated *UBQLN2* can give rise to the full ALS-FTLD continuum. Immunohistochemistry studies reported that mutated ubiquilin 2 accumulates in neuronal inclusions in the brain and spinal cord. Importantly, a correlation of hippocampal UBQLN2 pathology with dementia in ALS cases with or without *UBQLN2* mutations has been found, suggesting that the *UBQLN2* gene could be involved in ALS-related dementia, even without *UBQLN2* mutations. In addition, ubiquilin 2 pathology has been found in association with inclusion bodies in synucleinopathies and polyglutamine diseases, leading to the hypothesis that it may be a common downstream mechanism in neurodegenerative diseases.

OPTN gene

In 2010, homozygous deletions of exon 5 of the gene encoding optineurin (*OPTN*) were described in Japanese siblings affected by ALS (Maruyama et al., 2010). *OPTN* is a 16-exon gene located on 10p13, encoding for the coiled-coil containing protein optineurin.

This gene may play a role in normal-tension glaucoma and adult-onset primary open angle glaucoma, and may also be involved in cellular morphogenesis, membrane and vesicle trafficking, and transcription activation through its interactions with the RAB8, huntingtin and transcription factor IIIA proteins. Like other proteins already described, optineurin also seems to be involved in protein degradation via autophagy.

The pathomechanism causing the disease may be different depending on the recessive and dominant nature of the underlying mutation. For example, it is thought that ALS due to a recessive mutation might show a loss of function resulting from nonsense-mediated mRNA decay of transcription; in any case, it is very difficult to confirm any hypothesis because the lack of autopsy material in these recessive families precludes extensive histopathological analyses. Intriguingly, OPTN has also been linked to PDB. OPTN is co-localized with TDP-43 in the characteristic inclusion bodies of sporadic ALS. More generally, TDP-43, FUS and OPTN are components of pathological inclusions seen in SOD1-negative familial ALS, sporadic ALS, and ALS-FTLD, but not in SOD1-linked ALS.

TREM2 gene

The triggering receptor expressed on myeloid cells 2 gene (*TREM2*) is a 5-exon encoding gene on 6p21.1 whose product is a membrane protein that forms a receptor signaling complex with the TYRO protein tyrosine kinase binding protein.

This protein is a member of the innate immune receptor TREM family, expressed on the cell surface of the monocyte-macrophage lineage including monocyte-derived dendritic cells, osteoclasts and microglia in the central nervous system. *TREM2* plays a role in the immune response and may be involved in chronic inflammation by triggering the production of constitutive inflammatory cytokines. Defects in this gene are a cause of a genetic syndrome named Nasu-Hakola disease (NHD), also known as polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy, which is characterized by early-onset progressive dementia associated with sclerosing leukoencephalopathy and systemic bone cysts (Madry et al., 2007). The early onset of dementia as well as the marked involvement of the frontal regions in NHD are features resembling FTLD. In January 2013, a study identified different homozygous mutations in *TREM2* in three Turkish probands among 44 identified with FTLD spectrum-like disease (Guerreiro et al., 2013). None of these three patients had a typical clinical presentation of NHD: they presented with behavioral changes and subsequent cognitive impairment and motor features, but without any bone cysts or bone-associated phenotypes.

To date, 14 different mutations have been identified in *TREM2.* Recently, an increased frequency of rare heterozygous *TREM2* variations was also detected in AD patients (Jonsson et al., 2013).

One rare variant, p.R47H (rs75932628), showed a strong association with late-onset AD, comparable to the association with the *APOE* ε4 allele (although it occurs with a much lower frequency). The p.R47H variant is located in *TREM2* exon 2, where several homozygous FTLD mutations were also observed. This exone encodes both the signal peptide and part of the extracellular domain containing an IgV-set domain: hypothetically, *TREM2* mutations might contribute to the neurodegenerative process leading to an altered immune response with extensive inflammation or defective microglial function or survival. Since the association of the gene with FLTD, mutations in *TREM2* have been shown to be associated with atypical signs in examined patients: epilepsy, parkinsonism, early parietal and hippocampus involvement, and corpus callosum thickness on brain MRI. This gene should be taken into account when mutations in other dementia genes are excluded.

CHCHD10 and TBK1 genes

Very recently, two new genes have been reported to be mutated in patients with FTLD: *CHCHD10* (coiled-coil-helix-coiled-coil-helix domain containing 10) is located on chromosome 22 and encodes a mitochondrial protein that is enriched at cristae junctions in the intermembrane space, while the *TBK1* (TANK1-binding kinase 1) gene, located on chromosome 12, encodes a serine/threonine kinase that plays an essential role in regulating inflammatory responses to foreign agents. Rarely, mutations in these genes have been associated with both FTLD and ALS (Zhang et al., 2015; Freischmidt et al., 2015).

Table I lists all the genes at present involved in FTLD etiopathogenesis, together with their chromosomal localizations, frequencies and suggested mechanisms of action.

Genetic risk factors

In addition to the above-mentioned genes, generally cloned in families showing autosomal dominant transmission of the FTLD spectrum, several genetic factors that are probably involved in the disease as modifier genes have also been studied. The first candidate gene studied in FTLD was the *APOE* gene, a well-known risk factor for AD. Preliminary case-control studies reported conflicting results: some Authors found a significant as-

Table I - Frequency of gene mutations and disease mechanisms in frontotemporal lobar degeneration.

Gene	Location	Gene product	Frequency in familial cases %	Disease mechanism
MAPT	17q21	Tau	7.1 – 10.5	Toxic protein
GRN	17p21	Progranulin	6.5 – 31.5	Autophagy/lysosomal pathway/inflammation
C9orf72	9p21	C9orf72 protein	12.0 – 34.5	Endosomal trafficking/toxic RNA/immune regulation
CHMP2B	3p11.2	Chromatin-modifying protein 2B	Rare	Autophagy/lysosomal pathway
VCP	9p13.3	Valosin-containing protein	Rare	Autophagy
TARDBP	1p36.22	TDP-43	Rare	DNA/RNA
SQSTM1	5q35	P62	2.0 - 3.2	Autophagy
FUS	16p11.2	Fused in sarcoma	Rare	DNA/RNA
UBQLN2	Xp11.21	Ubiquilin 2	Rare	Autophagy
OPTN	10p13	Optineurin	Rare	Autophagy
CHCHD10	22q11.23	Coiled-coil-helix-coiled-coil-helix domain containing 10	Rare	Mitochondrial function
TBK1		TANK-binding kinase 1	1.7 – 4.5	Autophagy
TREM2	6p21.1	Triggering receptor expressed on myeloid cells 2	Rare	Inflammation

sociation between the ε4 allele of the *APOE* gene and the disease risk while others were not able to confirm this association.

A recent meta-analysis showed that carriage of the ε4 allele was associated with a two-fold increased disease risk (Rubino et al., 2013). In 2010, multiple single-nucleotide polymorphisms (SNPs) mapping to a single linkage disequilibrium block on 7p21 that contains transmembrane protein 106B (*TMEM106B*) were identified as genetic risk factors for FTLD associated with TAR DNA-binding protein 43 kDa-positive inclusions (FTLD-TDP) (Giraldo et al., 2013). Multiple replication studies have been conducted in FTLD populations of different geographical origins, confirming this association.

Recently, evaluation of a role for *GRN* in sporadic FTLD has provided evidence that a single SNP in the *GRN* promoter may influence the disease risk (Galimberti et al., 2010). In addition, nitric oxide synthase (*NOS*)*3 G894T* and *NOS1 C276T* SNPs likely increase the risk of developing FTLD. Further genetic risk factors, include BCL2-associated athanogene 1 (*BAG1*), Kinesis family member 24 (*KIF24*), and defective in cullin neddylation 1 (DCN-1)-domain containing 1 (*DCUN1D1*) (Villa et al., 2009; Venturelli et al., 2010).

The first GWAS on FTLD patients was performed by Van Deerlin et al. (2010). A group of 515 individuals affected by FTLD with autopsy-proven TDP-43 inclusion pathology (FTLD-TDP) was compared with 2509 healthy controls and an association with three SNPs mapping to a single linkage disequilibrium block on 7p21 was shown. This region contains the already described *TMEM106B* gene. These findings, however, were not confirmed by replication studies performed in two clinical FTLD cohorts of British origin.

More recently, a two-stage GWAS study was performed in a large cohort of 3526 FTLD patients in comparison with 9402 healthy controls. A significant association at 6p21.3, *HLA* locus, together with a potential novel locus at 11q14, encompassing *RAB38*/*CTSC* (the transcripts of which are related to lysosomal pathway), was found for bvFTD (Ferrari et al., 2014). These findings suggest that immune system processes and possibly lysosomal and autophagy pathways are potentially involved in FTLD.

Genetic counseling

The identification of an increasing number of genes involved in FTLD is moving the field of genetic counseling in a new and challenging direction. In the majority of the FTLD patients, the clinical phenotype is related to genetically complex mechanisms, wherein many genetic variations of small effect interact to increase the risk of dementia.

A small proportion of families have an autosomal dominant family history of FTLD, caused by a mutation in one of the aforementioned genes. In approaching genetic counseling, the neurologist should obtain a detailed three-generation pedigree that captures the presence of FTLD, ALS, other dementias, parkinsonism, and also psychiatric conditions.

The pedigree should also include ages at disease onset, diagnoses and ages at death.

Medical records, including autopsy studies if available,

are essential to clarify diagnoses. In patients with autosomal dominant transmission of the disease, genetic counseling should include a discussion of the 50% risk to offspring of an expansion carrier, regardless of whether or not this expansion is *de novo*. In the remaining families, the characteristics of a genetically complex disease should be carefully explained. For the individual who is cognitively or behaviorally impaired, genetic counseling should involve a healthcare proxy, legal guardian or next of kin. Genetic counseling should take into account the uncertainties about the pathogenicity and the penetrance of some genetic mutations, the possible presence of mutations of different genes in the same individual, and the poor genotypic/phenotypic correlation in most FTLD genes.

Concluding remarks

In the last decade, several genes have been cloned in families segregating the FTLD phenotype as an autosomal dominant trait. Genetic variants in the *MAPT, PGRN* and *C9orf72* genes are present in approximately 40% of familial FTLD cases. Mutations in several additional genes (*CHMP2B*, *VCP, TARDBP, SQSTM1, FUS, UBQLN, OPTN*, *TREM2, CHCHD10* and *TBK1*), even if present with a low frequency, are of particular interest in order to elucidate the complex pathophysiological mechanisms of the disease. In addition, several genetic risk factors for FTLD have been reported. Taken together, these recent genetic findings highlight the genetic heterogeneity of the disease and suggest new therapeutic strategies.

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