doi:10.1093/brain/awx198 BRAIN 2017: 140; 3081–3104 | 3081

BRAIN
A JOURNAL OF NEUROLOGY

REVIEW ARTICLE

Progranulin: a new avenue towards the understanding and treatment of neurodegenerative disease

Babykumari P. Chitramuthu, Hugh P. J. Bennett and Andrew Bateman

Progranulin, a secreted glycoprotein, is encoded in humans by the single *GRN* gene. Progranulin consists of seven and a half, tandemly repeated, non-identical copies of the 12 cysteine granulin motif. Many cellular processes and diseases are associated with this unique pleiotropic factor that include, but are not limited to, embryogenesis, tumorigenesis, inflammation, wound repair, neurodegeneration and lysosome function. Haploinsufficiency caused by autosomal dominant mutations within the *GRN* gene leads to frontotemporal lobar degeneration, a progressive neuronal atrophy that presents in patients as frontotemporal dementia. Frontotemporal dementia is an early onset form of dementia, distinct from Alzheimer's disease. The *GRN*-related form of frontotemporal lobar dementia is a proteinopathy characterized by the appearance of neuronal inclusions containing ubiquitinated and fragmented TDP-43 (encoded by *TARDBP*). The neurotrophic and neuro-immunomodulatory properties of progranulin have recently been reported but are still not well understood. Gene delivery of *GRN* in experimental models of Alzheimer's- and Parkinson's-like diseases inhibits phenotype progression. Here we review what is currently known concerning the molecular function and mechanism of action of progranulin in normal physiological and pathophysiological conditions in both *in vitro* and *in vivo* models. The potential therapeutic applications of progranulin in treating neurodegenerative diseases are highlighted.

Endocrine Research Laboratory, Royal Victoria Hospital, and McGill University Health Centre Research Institute, Centre for Translational Biology, Platform in Metabolic Disorders and Complications, 1001 Decarie Boulevard, QC, Canada, H4A 3J1

Correspondence to: Hugh P. J. Bennett,
E-mail: hugh.bennett@mcgill.ca

Keywords: progranulin; frontotemporal lobar degeneration; TDP-43; FTLD-U; neurodegeneration

Abbreviations: ALS = amyotrophic lateral sclerosis; FTD = frontotemporal dementia; FTLD = frontotemporal lobar degeneration; NCL = neuronal ceroid lipofuscinosis

Introduction

With the ageing human population, neurodegenerative disease has become a growing and currently intractable health challenge (Finès, 2015). Progranulin, a secreted glycoprotein, maintains cerebrocortical viability. It has been identified as an important factor controlling neural function while also suppressing neuroinflammation and playing roles in neuronal lysosome function (Toh *et al.*, 2011). Loss of one allele of *GRN*, the gene encoding progranulin, leads to the development of frontotemporal dementia (FTD) (Baker *et al.*, 2006; Cruts *et al.*, 2006). This is the second most common form of dementia in people under the age of 65 (Arvanitakis, 2010). Loss of both alleles leads to the development of neuronal ceroid lipofuscinosis (NCL) (Smith *et al.*, 2012), a lysosome storage disease. Importantly, in addition to its link to the development of frontotemporal lobar degeneration (FTLD), progranulin is protective in a range of animal models with disrupted neuronal growth and developmental processes. For example,

Received November 11, 2016. Revised June 15, 2017. Accepted June 26, 2017. Advance Access publication August 18, 2017

progranulin overexpression reverses the impaired development of primary motor neurons in zebrafish models of motor neuron disease including spinal muscular atrophy caused by reduced expression of survival motor neuron 1 (*smn1*) (Chitramuthu *et al.*, 2010) and amyotrophic lateral sclerosis (ALS) (Laird *et al.*, 2010) due to exogenous expression of mutant TAR DNA binding protein-43 (TDP43). Progranulin has a neuroprotective role in a *Caenorhabditis elegans* model of Huntington's disease and in immortalized striatal neurons carrying pathological variations of the polyglutamine expansion of the huntingtin protein (Tauffenberger *et al.*, 2013). In mice, the lentiviral delivery of *Grn* to the brain halts the development of drug-induced Parkinson's-like phenotypes (Van Kampen *et al.*, 2014) and genetically-induced Alzheimer's disease-like phenotypes (Minami *et al.*, 2014). These observations indicate that progranulin has a broad spectrum of potential therapeutic utility in several presently incurable neurological disorders including both those with or without associated TDP-43 proteinopathy. To exploit this potential, we must fully understand the mode of action of progranulin. This review focuses on the roles of progranulin in the CNS and neuronal diseases.

Progranulin structural organization and processing

Progranulin (Fig. 1) is the sole mammalian representative of a gene family the evolutionary origins of which can be traced to single celled organisms (Palfree *et al.*, 2015). Human progranulin is a glycoprotein of ~75–80 kDa that contains seven and a half granulin modules namely p (a granulin half domain), G, F, B, A, C, D, and E (Bhandari *et al.*, 1992; Plowman *et al.*, 1992) (Fig. 1). Solution nuclear magnetic resonance analysis of granulin-1 isolated from the spleen of a teleost fish the carp (Hrabal *et al.*, 1996), and individual human granulin modules (Tolkatchev *et al.*, 2008) revealed a unique conformation for the granulin motif as a parallel stack of beta-hairpins in the form of a left-handed helix held together with six disulphide bridges (Hrabal *et al.*, 1996; Tolkatchev *et al.*, 2008). Progranulin can be subject to protease cleavages that release individual granulin modules in the form of polypeptides of ~6 kDa called granulins (Bateman *et al.*, 1990), sometimes also known as epithelins (Shoyab *et al.*, 1990).

Both intact progranulin and its constituent granulin peptides are biologically active although often with opposing actions. For example, progranulin plays anti-inflammatory roles (Zhu *et al.*, 2002; Jian *et al.*, 2013) whereas granulin peptides have pro-inflammatory functions (Zhu *et al.*, 2002; Jian *et al.*, 2013). With respect to cell growth, intact progranulin is mitogenic whereas the different granulin polypeptides may have growth promoting or growth inhibiting activities on, for example, breast cancer cells, although they usually act with lower potency than intact progranulin. In the periphery, neutrophil proteases including elastases and proteinase-3 cleave progranulin into individual granulin peptides (Zhu *et al.*, 2002). The balance between undigested progranulin and its proteolytically derived granulin peptides is controlled at least in part by the secretory leucocyte protease inhibitor (SLPI) (Zhu *et al.*, 2002), which binds to progranulin, thereby preventing proteolysis by elastase and other neutrophil proteases (Fig. 1). The functional interplay between progranulin and proteases

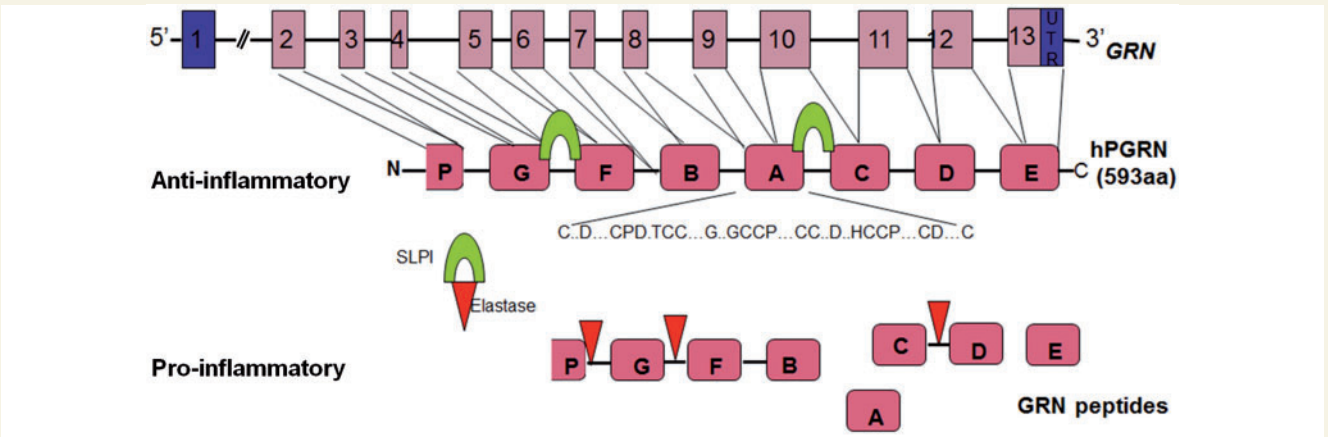

Figure 1 Diagrammatic representation of the human *GRN* gene, progranulin protein and its proteolytic digestion pathway to granulin peptides. (*Top*) The exonic structure of the *GRN* gene (dark blue for UTRs). (*Middle*) The protein structure with lettered boxes that represent individual granulin modules. The thin lines descending from the gene structure indicate which exons contribute to which protein modules (Bhandari and Bateman, 1992). SLPI binds at sequences between granulin modules to protect full length progranulin from proteolysis by elastase and other proteases. Intact progranulin blunts the inflammatory reaction and has growth factor-like activity. SLPI therefore promotes the anti-inflammatory and growth factor properties of progranulin. The *bottom* panel illustrates the cleavage of progranulin into granulin peptides some of which have proinflammatory and anti-proliferative activities (Zhu *et al.*, 2002).

has been confirmed in a study of compound murine knockouts of neutrophil elastase (*Elane*) and proteinase-3 (*Prtn3*) (Kessenbrock *et al.*, 2008). This concept is further strengthened by a prior study showing increased inflammation in mice lacking *Slpi* (Ashcroft *et al.*, 2000).

Progranulin expression and function in the healthy brain

GRN mRNA expression has been reported in the developing CNS and peripheral nervous system (dorsal root ganglia and sympathetic ganglia) (Daniel *et al.*, 2003) although another study found little evidence of early CNS *Grn* expression in neuronal progenitors (Petkau *et al.*, 2010). In the adult mouse, progranulin is found in specific neurons in Purkinje cells, pyramidal cells of the hippocampus, and some cerebral cortical neurons, motor neurons in the brain and spinal cord and in microglia (Daniel *et al.*, 2000; Moisse *et al.*, 2009; Ryan *et al.*, 2009; Petkau *et al.*, 2010; De Muynck and Van Damme, 2011) as well as neural stem cells (Lu *et al.*, 2013).

In vitro studies have implicated progranulin in normal neuron biology. It promotes neurite extension (Van Damme *et al.*, 2008; Ryan *et al.*, 2009; Gao *et al.*, 2010; Gass *et al.*, 2012), neuronal cell survival (Van Damme *et al.*, 2008; Ryan *et al.*, 2009; Gao *et al.*, 2010; Guo *et al.*, 2010; Kleinberger *et al.*, 2010; Xu *et al.*, 2011) and differentiation (Gao *et al.*, 2010; Raitano *et al.*, 2015). The major research focus has been on progranulin and its neuropathology. In contrast, its physiological roles in the brain and nervous system are not well understood. Progranulin reduces neuropathic pain (Lim *et al.*, 2012; Altmann *et al.*, 2016*a*) and stimulates regrowth following nerve injury (Altmann *et al.*, 2016*b*). It is a developmentally regulated sex steroid-responsive factor involved in masculinization of the hypothalamus (Suzuki *et al.*, 2009) and it may regulate hypothalamic control of feeding behaviour (Kim *et al.*, 2011).

Progranulin in brain diseases

Progranulin has many roles in brain diseases either through loss of one *GRN* allele (FTLD), both allelles (NCL) or, when overexpressed, by contributing to the development of brain tumours (Fig. 2).

GRN and frontotemporal lobar degeneration clinical pathology

A detailed review of clinical and diagnostic features of *GRN*-related FTD can be found elsewhere (Hsiung and Feldman, 2013). FTLD is a general term used for a group of disorders that are clinically, pathologically and genetically heterogeneous, and is characterized by the degeneration of the frontal and temporal lobes, areas that control planning, judgement, emotion, speech and some types of movement. About 15–22 in 100 000 people are estimated to suffer from FTLD with no apparent differences in disease incidence reported between males and females (Onyike and Diehl-Schmid, 2013). It often has a relatively early onset, around age 45 to 65 years (Onyike and Diehl-Schmid, 2013). The clinical pathology of FTLD includes: (i) behavioural variant FTD (bvFTD) characterized by progressive decline in behaviour and executive function; (ii) progressive non-fluent aphasia (PNFA) characterized by deficits in expressive and motor speech; and (iii) semantic dementia (Stagi *et al.*, 2014) characterized by loss of semantic memory (reviewed in Premi *et al.*, 2012). Patients with FTD sometimes develop clinical signs that overlap with motor neuron disease/amyotrophic lateral sclerosis (FTD-ALS), progressive supranuclear palsy and corticobasal syndrome (CBS) associated with Parkinson's disease while 50% of patients with ALS show cognitive deficits (Lomen-Hoerth *et al.*, 2002; Seltman and Matthews, 2012). *GRN* mutations are generally associated with bvFTD, PNFA and CBS but not semantic dementia or FTD-ALS (Le Ber *et al.*, 2007; Schymick *et al.*, 2007; Whitwell *et al.*, 2007; Del Bo *et al.*, 2011). Given that *GRN* mutations result in decreased production of progranulin, its measurement in serum or CSF has proven a very reliable biomarker to distinguish between FTLD caused by *GRN* mutation and other FTLDs and can discern whether an at-risk family member is likely to carry a *GRN* mutation long before any clinical symptoms may appear (Ghidoni *et al.*, 2008; Carecchio *et al.*, 2009; Finch *et al.*, 2009; Sleegers *et al.*, 2009; Schofield *et al.*, 2010; Almeida *et al.*, 2014).

About 30–50% of FTLD cases are heritable (Onyike and Diehl-Schmid, 2013) with most cases being due to mutations within the three genes *GRN*, *MAPT* (microtubule-associated protein tau), and a GGGGCC hexanucleotide repeat expansion in *C9orf72* (chromosome 9 open reading frame 72) (reviewed in Sieben *et al.*, 2012). In addition, FTD is less commonly associated with mutations in *TARDBP* (TAR DNA binding protein, encoding TDP-43) (Borroni *et al.*, 2009; Caroppo *et al.*, 2016), *FUS* (fused in sarcoma) (Van Langenhove *et al.*, 2010), *CHMP2B* (charged multivesicular body protein 2B) (Skibinski *et al.*, 2005; Holm *et al.*, 2007), *VCP* (valosin containing protein) (Watts *et al.*, 2004), *SQSTM1* (sequestome 1/p62) (Le Ber *et al.*, 2013; Kovacs *et al.*, 2016), *UBQLN2* (ubiquilin 2) (Deng *et al.*, 2011; Vengoechea *et al.*, 2013), *TBK1* (TANK binding kinase 1) and *OPTN* (optineurin) (Pottier *et al.*, 2015). Unlike *GRN*, *C9orf72* and *UBQLN2* are often associated with FTLD and ALS while most mutations of *TARDBP*, *FUS*, *TBK1* and *OPTN* are associated with ALS and only rarely with pure FTLD (Morris *et al.*, 2012).

Frequencies of *GRN* mutations in FTD cohorts in different parts of the world vary significantly. For example, the frequency of *GRN* mutations in Italy was 15.6%, in Japan it was 1.6% and in Brazil was 9.6% (Benussi *et al.*, 2010;

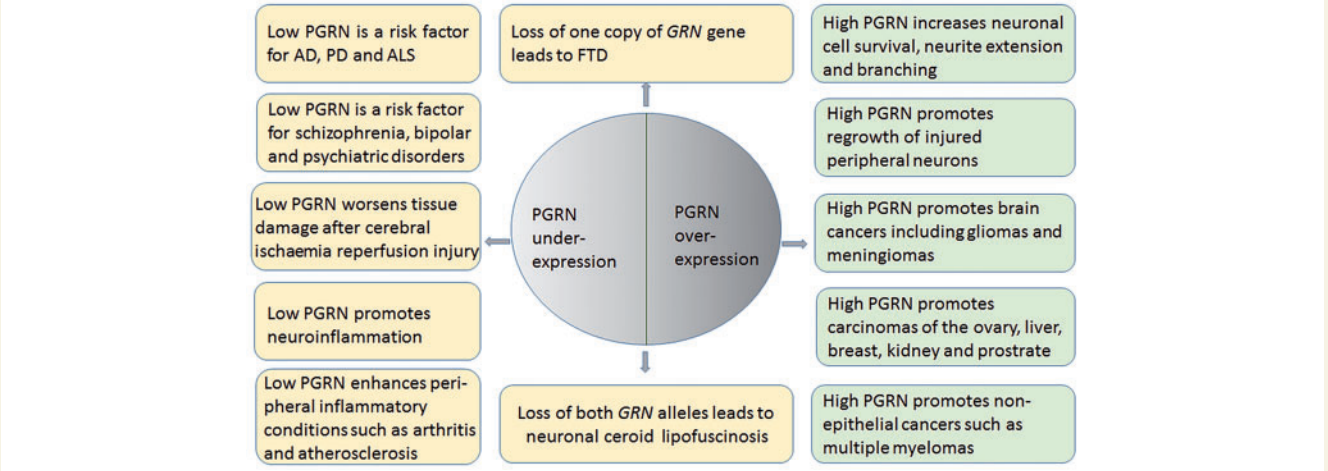

Figure 2 Both progranulin deficiency and its excess are associated with disease. Low levels of progranulin (PGRN) expression lead to, or are risk factors for, multiple neurodegenerative and immune modulatory diseases (yellow boxes), while high levels of expression are either neurotrophic or may lead to various types of cancers (green boxes). For details see the main text. Comparable immunomodulatory and proliferative conditions result from depleted or elevated progranulin acting on non-neuronal cells (Toh *et al.*, 2011). AD = Alzheimer's disease; PD = Parkinson's disease.

Ogaki *et al.*, 2013; Takada *et al.*, 2016). C9ORF72 hexanucleotide repeat expansion is the most common underlying genetic cause in patients with ALS or FTD with or without ALS (Cruts *et al.*, 2015; Rohrer *et al.*, 2015*a*).

Neuroimaging and neuropathology of *GRN*-related frontotemporal dementia

Over and above the extensive loss of frontotemporal tissue, each of the major FTLD genes results in distinct patterns of brain atrophy as revealed by neuroimaging studies, with *GRN* mutations often resulting in a more temporo-parietal atrophy than that resulting from *MAPT* or *C9orf72* mutations (Whitwell *et al.*, 2009, 2012; Rohrer *et al.*, 2010). Atrophy due to *GRN* mutations, but not *MAPT* or *C9orf72*, is typically asymmetric (Ghetti *et al.*, 2008; Whitwell *et al.*, 2013) with both left and right asymmetry observed even in the same family (Beck *et al.*, 2008). The underlying cause for this asymmetry is unclear. Comparing the effects of *GRN*, *MAPT* and *C9orf72* mutations by longitudinal MRI of symptomatic individuals revealed that whole brain and regional rates of volume loss were most rapid in patents with *GRN* mutations (Whitwell *et al.*, 2015). In presymptomatic individuals *GRN* mutation carriers showed structural differences first in the insula 15 years before expected clinical onset. There follows a further period until 5 years before the expected clinical onset, during which atrophy develops in the temporal and parietal lobes and then in the striatum (Rohrer *et al.*, 2015*b*). Regional hypometabolism and decreased cerebral blood flow also occur early and before major structural changes or clinical symptoms are detectable (Jacova *et al.*, 2013; Caroppo *et al.*, 2015; Dopper *et al.*, 2016). Changes in white matter tracts and functional connectivity predate the onset of clinical symptoms in some instances by more than 10 years. (Borroni *et al.*, 2008; Pievani *et al.*, 2014; Premi *et al.*, 2014). Brains from *GRN*-related FTD often show greater white matter lesions than other forms of FTD (Caroppo *et al.*, 2014). Functional imaging in patients with *GRN*-related FTD show a significant decrease in connectivity between the parietal lobe and temporal pole region whereas in FTD without *GRN* mutations changes in functional connectivity have a more frontal orientation (Premi *et al.*, 2013). The specific loss of connectivity pathways in *GRN*-related FTD is accompanied by potentially compensatory increases in connectivity of other pathways (Borroni *et al.*, 2012; Premi *et al.*, 2013). The ability to detect structural changes in grey and white matter, decreases in blood flow, and functional changes in connectivity in the brains of *GRN* mutation carriers years before the onset of clinical symptoms makes imaging strategies very useful as biomarkers to follow both disease progression and to monitor response to novel disease-modifying therapies during clinical trials.

Spontaneous and inherited FTLDs are characterized by the presence of cellular inclusions. Most of these are of either tau protein (FTLD-tau), associated in familial FTLD with *MAPT* mutations, or ubiquitinated fragments of TDP-43 (FTLD-TDP) associated with *GRN* and *C9orf72* mutations. In FTLD-FUS inclusions occur containing FET proteins [FET stands for the RNA-binding proteins FUS, Ewing's sarcoma (EWS) and TATA-box binding protein associated factor 15 (TAF15)]. In neuronal intermediate filament inclusion disease, a subset of FTLD-FUS, (Cairns *et al.*, 2004) inclusions of type IV neuronal intermediate filament also occur (Mackenzie and Neumann, 2016) (Fig. 3). TDP-43 pathology is found in other

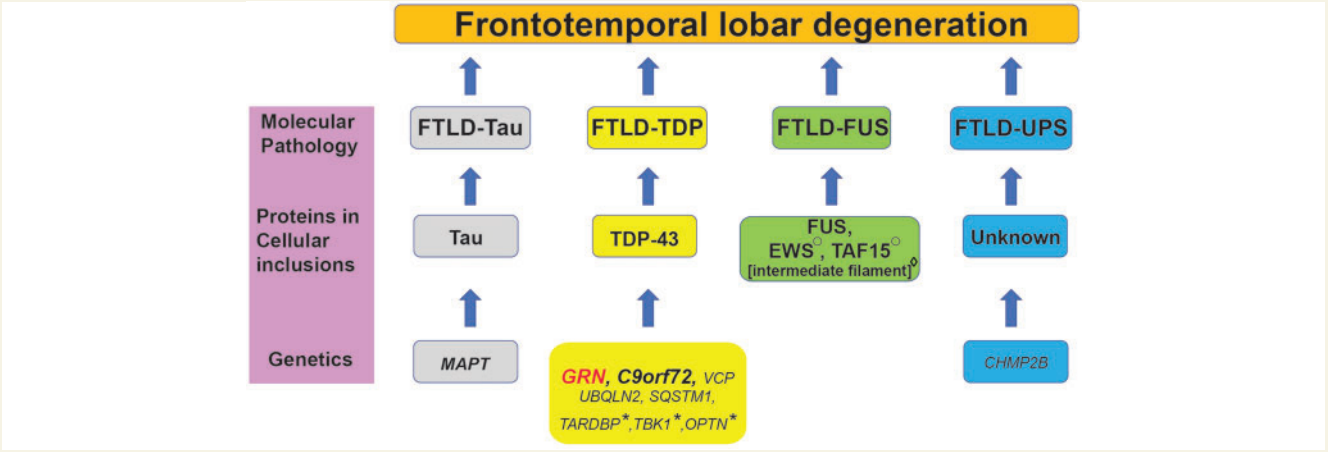

Figure 3 Classification of heritable FTLD molecular pathology. The figure represents a series of genetic and molecular cascades leading to FTD. *GRN* mutations result in FTLD-TDP43, as do several other genes suggestive of convergent, but not yet fully understood, disease mechanisms. Other FTLD molecular pathologies exhibit tau inclusions (FTLD-MAPT), or ubiquitin positive inclusions immunoreactive for FET proteins, FUS, EWS, TAF15 (FTLD-FUS) (indicated by open circles), which also, in the case of neuronal intermediate filament inclusion disease, display neuronal intermediate filament inclusions (indicated by a diamond). While mutation of FUS can lead to neurodegeneration in ALS, no comparable FUS mutations leading to FTLD-FUS molecular pathologies have been identified. Mutations of CHMP2B result in FTLD-UPS, with ubiquitin inclusion pathology that lacks either TDP-43 or FET proteins. Rare FTLD genes are in smaller font; asterisks represent genes the mutation of which generally cause ALS and only infrequently causes pure FTLD. The scheme is based on Mackenzie and Neumann (2016).

neurodegenerative diseases including ALS (Arai *et al.*, 2006; Neumann *et al.*, 2006) and sometimes Alzheimer's disease (Wilson *et al.*, 2011). Four subcategories of FTLD-TDP, types A–D, are recognized on the basis of neuropathology (Mackenzie *et al.*, 2011). *GRN* mutations result in FTLD-TDP type A, characterized by short dystrophic neurites, ubiquitin-positive- neuronal cytoplasmic inclusions concentrated in neocortical layer 2 and often though less abundantly lentiform neuronal intranuclear inclusions (NII) in regions that include the neocortex and striatum (Mackenzie *et al.*, 2006; Josephs *et al.*, 2007; Armstrong and Cairns, 2011; Armstrong, 2014). Mutation of *VCP* causes extensive NII formation (Forman *et al.*, 2006) and it has been suggested that a convergent neuropathology, possibly centred on the proteasomal clearance of proteins that generates the NIIs in response to both *GRN* and *VCP* mutations (Mackenzie *et al.*, 2006). Representative micrographs of TDP-43 inclusions in *GRN*-related FTD can be found in Mackenzie *et al.* (2006) and Mackenzie and Neumann (2016).

Genetics of *GRN*-related frontotemporal dementia

Known *GRN* mutations are collated in www.molgen.ua.ac.be/FTDmutations/. In most cases *GRN*-related FTD is caused by mutation of a single allele of *GRN* resulting in a premature stop codon that triggers nonsense-mediated decay of *GRN* mRNA and concomitant loss of 50% of progranulin protein levels (Baker *et al.*, 2006; Cruts *et al.*, 2006). This may occur due to nonsense mutations, small insertions or deletions that result in frameshift introduction of stop codons, larger deletions or mutations of splice acceptor sites (Yu *et al.*, 2010; Chen-Plotkin *et al.*, 2011). There are no obvious regional 'hot-spots' of mutation. Locus deletion mutants (Gijselinck *et al.*, 2008; Rovelet-Lecrux *et al.*, 2008) that lack all or most of the *GRN* gene confirm that it is indeed the loss of a single *GRN* allele and not the acquired toxicity of mutant progranulin proteins that causes the FTLD phenotype. Several missense mutations of *GRN* have been reported. These transcripts are not cleared by nonsense-mediated decay but since the *GRN* gene tolerates a number of benign polymorphisms (Guerreiro *et al.*, 2010) it is sometimes difficult to distinguish pathological *GRN* missense mutations from non-pathological variations. Missense mutations in the initiator methionine codon, or its removal by splicing out of exon 1, prevent *GRN* mRNA translation (Baker *et al.*, 2006; Cruts *et al.*, 2006; Gass *et al.*, 2006; Le Ber *et al.*, 2008). The pathogenicity of the missense mutation progranulin A9D is well documented (Mukherjee *et al.*, 2006; Spina *et al.*, 2007; Chen-Plotkin *et al.*, 2011; Cannon *et al.*, 2013). A charged aspartic acid residue takes the place of alanine in the hydrophobic core of the signal peptide, preventing passage of progranulin through the secretory pathway (Shankaran *et al.*, 2008; Finch *et al.*, 2009). As with the haploinsufficient *GRN* null mutations, the net effect is to reduce secreted progranulin levels by ~50%. Additional factors may also contribute to the pathology of this mutation since, by mislocating to the cytoplasm (Shankaran *et al.*, 2008), progranulin A9D can associate with stress granules and may initiate harmful actions independently of its exclusion from the secretory pathway (Li *et al.*, 2017). Other missense mutations, such as progranulin P248L in granulin domain B, and R432C between granulin domains C and D,

reduce progranulin secretion, but to a lesser extent than for the A9D signal peptide mutants (Shankaran *et al.*, 2008). Missense mutations, C139R (Brouwers *et al.*, 2008; Bernardi *et al.*, 2009; Wang *et al.*, 2010*a*; Antonell *et al.*, 2012; Gass *et al.*, 2012), in the F domain and C521Y (Wang *et al.*, 2010*a*) in the E granulin domain do not alter progranulin secretion (Brouwers *et al.*, 2008) or do so to a lesser degree than null mutations (Finch *et al.*, 2009). However, progranulin P248L, R432C, C139R and C521Y are all functionally impaired in the ability to stimulate neurite outgrowth *in vitro* (Wang *et al.*, 2010*a*; Gass *et al.*, 2012). Their reduced functionality and, in some cases, reduced secretion may confer a milder and less penetrant phenotype than the null mutations.

Genetic risk factors for *GRN*-related frontotemporal dementia

There is a wide clinical heterogeneity and disparity in the age of onset of FTD even among closely related family members who carry the same *GRN* mutation (Bruni *et al.*, 2007; Le Ber *et al.*, 2007; Rademakers *et al.*, 2007; Beck *et al.*, 2008; Benussi *et al.*, 2015). This has spurred the search for genetic modifiers that might account for this variability. Genome-wide association studies identified a SNP (rs646776), linked to the gene *SORT1*, that influences serum levels of progranulin (Carrasquillo *et al.*, 2010). *SORT1* encodes sortilin 1, a transmembrane protein that had been found to modulate secreted levels of progranulin through endocytosis (Hu *et al.*, 2010). Sortilin is a regulator of lysosomal enzyme trafficking and binds to multiple neurotrophic factors in addition to progranulin including neurotensin, pro-NGF (Nykjaer *et al.*, 2004) and other members of the neurotrophin family (Bartkowska *et al.*, 2010). Similarly, genome-wide association studies revealed that another lysosome trafficking protein, prosaposin, also regulates progranulin levels in the circulation (Nicholson *et al.*, 2016). Progranulin binds to prosaposin (Zhou *et al.*, 2015*b*). Single nucleotide polymorphisms (SNPs) that are associated with the gene *transmembrane protein 106B* (*TMEM106B*) were found to be risk factors for FTLD-TDP through a mechanism resulting in increased *TMEM106B* expression (Van Deerlin *et al.*, 2010; Finch *et al.*, 2011). Importantly, the risk is highly significant for *GRN*-mutation carriers (Van Deerlin *et al.*, 2010; Finch *et al.*, 2011; van der Zee *et al.*, 2011). For example, the risk allelle of rs1990622 (TMEM106B) is associated with a mean decrease in the age of onset of FTD of 13 years in *GRN*-mutation carriers (Cruchaga *et al.*, 2011), and is linked to decreases in plasma progranulin levels observed both in the carriers and in healthy older adults (Van Deerlin *et al.*, 2010; Cruchaga *et al.*, 2011; Finch *et al.*, 2011). As with sortilin and prosaposin, TMEM106B may act on progranulin levels through lysosomal pathways since TMEM106B, a type 2 transmembrane glycoprotein (Lang *et al.*, 2012), plays a role in regulating lysosome size, motility and the stress response (Stagi *et al.*, 2014). Over-expression of TMEM106B in neuronal cell lines reduces lysosomal breakdown of progranulin (Brady *et al.*, 2013).

While the apolipoprotein E (*APOE* ε4) allele is a major genetic risk factor in the development of Alzheimer's disease, it has the opposite effect in *GRN*-related FTD, delaying disease onset by a mean of ~6 years (Gass *et al.*, 2006; Beck *et al.*, 2008). The biological rationale behind the different effects of APOE in Alzheimer's disease and *GRN*-related FTD is unclear. The actin-binding protein filamin-c is highly upregulated in the frontal cortices of FTD patients with TDP-43 inclusions especially those with *GRN* mutations compared to *C9orf72* tract expansions and *VCP* mutations (Janssens *et al.*, 2015). The *MAPT* haplotype has no major effect on the age of disease onset in the case of *GRN*-related FTLD (Gass *et al.*, 2006; Rademakers *et al.*, 2007). However, greater phosphorylation and intraneuronal accumulation of tau protein was noted in P301L *Mapt*/*Grn* hemizygotic mice compared to P301L *Mapt* mice, suggesting a possible mechanism through which progranulin levels might interact with tau pathologies in, for example, Alzheimer's disease (Hosokawa *et al.*, 2015).

Multiple miRNAs may contribute to the pathogenesis of FTLD due to progranulin deficiency. For example, genetic variability in a microRNA binding site near the 3' untranslated region (3'-UTR) (rs5848) of *GRN*, which contributes to translational inhibition may lead to reduced progranulin levels (Rademakers *et al.*, 2008; Hsiung *et al.*, 2011). MiR-29b and miR-107 directly regulate progranulin levels (Jiao *et al.*, 2010; Wang *et al.*, 2010*b*; Gascon and Gao, 2014). Three members of the miR-132 cluster that are downregulated in FTD brains with TDP-43 inclusions were associated with upregulation of *TMEM106B*, a gene that modifies progranulin action as noted earlier (Chen-Plotkin *et al.*, 2012). *GRN* expression is, in addition, regulated epigenetically by promoter methylation. When compared to age-matched control brains, patients with sporadic FTD exhibit hypermethylation of functionally critical CpG units within the *GRN* promoter (Banzhaf-Strathmann *et al.*, 2013; Galimberti *et al.*, 2013).

Taken together these observations reveal a complex network of genetic factors that act to influence progranulin protein levels or mRNA expression. Alterations in the activity of any one of these factors may influence the progression of *GRN*-related FTD.

Genetic variability of *GRN* in neurodegenerative conditions other than frontotemporal lobar degeneration

The influence of genetic variation of *GRN* as risk factors in a range of neurological diseases has been investigated. Some studies found either no association of *GRN* variants with Parkinson's disease or Alzheimer's disease or only revealed variants that are predicted to be non-pathological

(Nuytemans *et al.*, 2008; Yu *et al.*, 2010; Karch *et al.*, 2013). However, other research did detect significant contribution of *GRN* genetic variability to Alzheimer's disease (Brouwers *et al.*, 2007, 2008; Kelley *et al.*, 2010; Perry *et al.*, 2013; Redaelli *et al.*, 2017) and hippocampal sclerosis (Dickson *et al.*, 2010). In addition, *GRN* variability is reported in schizophrenia (Momeni *et al.*, 2010), bipolar disorder (Galimberti *et al.*, 2014) and multiple sclerosis (Fenoglio *et al.*, 2010), although many of these results are based on relatively small sample sizes.

Homozygous mutation of *GRN* and lysosome storage disease

While heterozygous mutations of *GRN* result in FTLD, homozygous *GRN* mutations lead to a different disease associated with lysosomal dysfunction, namely late onset NCL (Smith *et al.*, 2012; Almeida *et al.*, 2016). NCL is a lysosomal storage disease that is caused by mutations in at least 14 different genes and is characterized by the appearance of deposits of the autofluorescent storage material lipofuscin. NCL caused by loss of progranulin is classified as NCL 11. These results strongly suggest a role for progranulin in lysosome function.

Progranulin and brain tumours

Just as a deficit in progranulin levels leads to disease, proliferative brain disorders are associated with excessive expression of progranulin. Thus, it is often highly expressed in gliomas (Liau *et al.*, 2000). Progranulin plays a role in astrocytoma progression and is a prognostic biomarker for glioblastoma, with its overexpression predicting decreased survival (Wang *et al.*, 2012). Progranulin is overexpressed in tumours of patients with glioblastoma multiforme and is associated with tumorigenicity and temozolomide resistance (Bandey *et al.*, 2015). This is caused by upregulation of cancer stemness genes by progranulin (Bandey *et al.*, 2015). Progranulin is, moreover, implicated in the growth of intracranial meningiomas (Kim *et al.*, 2010). Although relatively little is known about progranulin in brain cancers, its ability to promote cancer more generally is amply confirmed by a wealth of studies demonstrating tumorigenic activity in many other types of cancer. These include carcinomas of the ovary (Jones *et al.*, 2003; Kamrava *et al.*, 2005; Liu *et al.*, 2007; Cuevas-Antonio *et al.*, 2010), endometrium (Jones *et al.*, 2006), liver (Cheung *et al.*, 2004; Ho *et al.*, 2008), breast (Lu and Serrero, 2001; Leerkes *et al.*, 2002; Egland *et al.*, 2003; Serrero and Ioffe, 2003; Tangkeangsirisin and Serrero, 2004; Elkabets *et al.*, 2011), kidney (Donald *et al.*, 2001), prostate (Monami *et al.*, 2009), adrenal cortex (He *et al.*, 2002) and bladder (Monami *et al.*, 2006) as well as non-epithelial cancers such as multiple myelomas (Wang *et al.*, 2003) and leiomyosarcomas (Matsumura *et al.*, 2006). In addition to tumour growth stimulation, progranulin promotes cancer cell migration, invasiveness, anchorage independence and chemoresistance (He and Bateman, 1999; He *et al.*, 2002; Tangkeangsirisin and Serrero, 2004; Monami *et al.*, 2006, 2009; Cheung *et al.*, 2011). Progranulin expression alone does not transform primary human cells. However, if the primary cells are first immortalized by co-expression of the SV40 T-antigen and telomerase, the further expression of *GRN* transforms them from a precancerous to a highly tumorigenic state (Matsumura *et al.*, 2006; Miyanishi *et al.*, 2007). It is anticipated that a similar relationship may hold with respect to progranulin in the transition from precancerous but non-transformed cells to cancerous cells in the brain.

Progranulin and neuroinflammation

Microglial progranulin expression is upregulated after traumatic brain injury (Tanaka *et al.*, 2013*a*), toxin-induced injury (Martens *et al.*, 2012), spinal cord injury (Naphade *et al.*, 2010; Byrnes *et al.*, 2011) and sciatic axotomy (Moisse *et al.*, 2009) suggesting roles for progranulin in the neuroinflammatory reaction to brain injury. Activated microglia with strong progranulin expression are observed in affected brain regions of *GRN*-related FTLD patients (Chen-Plotkin *et al.*, 2010), in ALS (Malaspina *et al.*, 2001; Irwin *et al.*, 2009; Philips *et al.*, 2010), Alzheimer's disease (Gliebus *et al.*, 2009; Pereson *et al.*, 2009), Lewy body-associated dementia (Revuelta *et al.*, 2008) and multiple sclerosis (Vercellino *et al.*, 2011). Interestingly, neuronal progranulin expression either did not change (Petkau *et al.*, 2010) or actually decreased (Moisse *et al.*, 2009) following injury to nerve cells.

Broadly, progranulin is anti-inflammatory whereas the granulin peptides are pro-inflammatory (Zhu *et al.*, 2002). The ability of progranulin to modulate inflammation in the periphery is well supported by results from $Grn^{-/-}$ knockout mice, which show normal immune development but a highly over-active inflammatory response to, for example, bacterial endotoxin (Yin *et al.*, 2010*a*). In general, progranulin appears to maintain inflammation within physiologically appropriate limits, and in its absence, a wide range of non-neuronal conditions with an inflammatory component exhibit a more severe phenotype (Liu, 2011; Wu and Siegel, 2011; Liu and Bosch, 2012; Miller *et al.*, 2013). There is strong evidence for a comparable influence of progranulin on neuroinflammation (Yin *et al.*, 2010*a*; Martens *et al.*, 2012) and as will be discussed below, there is growing evidence from animal models that disruption of neuroimmunomodulatory actions

of progranulin are important in the development of FTLD-related phenotypes.

In vitro and *in vivo* models to study *GRN* function

In vitro models of *GRN* function

Several *in vitro* models have been developed to investigate molecular mechanisms by which progranulin deficiency leads to neurodegeneration. These models demonstrated the ability of progranulin to promote neuronal survival, neurite outgrowth and neuronal morphology (Van Damme *et al.*, 2008; Ryan *et al.*, 2009; Gao *et al.*, 2010; Kleinberger *et al.*, 2010). In primary murine hippocampal cultures, for example, progranulin deficiency decreased gross neural connectivity while enhancing transmission at individual synapses (Tapia *et al.*, 2011), possibly reflecting a compensatory mechanism resulting from a reduced number of synapses. A similar increase in the number of synaptic vesicles per synapse was also observed in tissues prepared from progranulin-associated FTLD patients (Tapia *et al.*, 2011). A member of the granulin peptide family, granulin-E, enhanced cell survival in cortical neurons and spinal cord motor neurons (Van Damme *et al.*, 2008; Gass *et al.*, 2012; De Muynck *et al.*, 2013), suggesting that both full-length progranulin and granulin peptides may possess neurotrophic functions. In contrast, other research suggests that granulin peptides may worsen TDP-43 pathologies (Salazar *et al.*, 2015). In a motor neuron-like cell line, stable overexpression of progranulin promoted cell survival for 2 months in the absence of serum in the culture medium whereas the control cells did not survive without serum beyond 2 weeks (Ryan *et al.*, 2009). The increased survival was associated with an inhibition of apoptosis. The powerful anti-apoptotic action of progranulin in neurons is amply confirmed by RNA silencing of progranulin expression in neuron cell cultures or cell lines and in cultured cortical neurons derived from *Grn* knockout mice, where, relative to control cells, caspase 3 activity is elevated in response to apoptotic stimuli (Zhang *et al.*, 2007; Gao *et al.*, 2010; Guo *et al.*, 2010). This may promote formation of carboxyl-terminal fragments (CTFs) of TDP-43 (Zhang *et al.*, 2007), although in cultured cortical neurons derived from $Grn^{-/-}$ mice the TDP-43 CTFs were reported to form both through caspase-dependent and independent pathways (Kleinberger *et al.*, 2010). Together these studies support the involvement of progranulin in neuronal survival through an anti-apoptotic pathway and that removal of progranulin regulation results in TDP-43 cleavage. It should be noted, however, that the influence of progranulin on caspase degradation of TDP-43 was not observed by others (Dormann *et al.*, 2009). In addition, amino-terminal sequencing indicated that the TDP-43 CTFs observed upon caspase cleavage are different from those isolated from the brains of FTLD patients (Igaz *et al.*, 2009). The mislocalization of TDP-43 from the nucleus to the cytoplasm is characteristic of FTLD. Neurons differentiated from induced pluripotent stem cells (iPSCs) obtained from FTLD patients (Almeida *et al.*, 2012) or murine cortical cells depleted of progranulin by RNA silencing (Gao *et al.*, 2010) exhibited increased redistribution of TDP-43 to the cytoplasm from the nucleus. In the striatal cells from huntingtin (*Htt*) knock-in mice, progranulin protects against cell death whereas TDP-43 or FUS promotes cell death. Progranulin counteracts the effects of TDP-43 but not FUS in these cells (Tauffenberger *et al.*, 2013), supporting the concept of that progranulin is neuroprotective against TDP-43 abnormalities.

In vitro models utilizing progranulin depletion have provided some understanding of the biological functions of progranulin but cannot recapitulate all the pathologies associated with FTLD. To further characterize the roles of progranulin in FTLD pathology and widen understanding of the underlying mechanism of action of progranulin *in vivo*, various progranulin deficient animal models have been developed.

Worm models

Approximately 20% fewer progeny are observed in *pgrn-1*-deficient *C. elegans* mutants compared to wild-type (Kao *et al.*, 2011). These *pgrn-1* deficient mutants showed increased clearance of apoptotic nerve cells suggesting that the precocious removal of nerve cells prevents the damaged or injured cells from recovering (Kao *et al.*, 2011). In a *C. elegans* model of neuronal TDP-43 proteinopathy, complete loss of the *pgrn-1* gene, equivalent to NCL in humans, did not alter the severity of TDP-43 toxicity, but the loss of one copy of the *pgrn-1* gene, as in FTLD, worsened TDP-43 toxicity. In addition, this study demonstrated a novel pathogenic role for granulin peptides in FTLD-TDP as motor impairments were significantly greater in a TDP-43 proteinopathy model co-expressing *C. elegans* granulin peptides than animals expressing either TDP-43 or granulin alone (Salazar *et al.*, 2015). Supporting this interpretation, the corresponding accumulation of granulin fragments in the diseased brain regions in human neurodegenerative disease subjects was also observed (Salazar *et al.*, 2015). In *C. elegans*, loss-of-function mutations of TDP-43 or FUS reduced behavioural defects and neurodegeneration caused by *Htt* exon-1 encoding an expanded polyglutamine tract implying roles for TDP-43 and FUS in huntingtin polyglutamine pathology. Progranulin attenuates the severity of this phenotype for TDP-43 but not FUS suggesting that TDP-43 acts upstream of progranulin (Tauffenberger *et al.*, 2013).

Zebrafish models

Unlike mammals that have only a single *GRN* gene, fish often have several distinct *grn* genes. Of the four *grn* genes

in zebrafish, *grna* (encoding zfPGRN-A) is the true orthologue of the single human *GRN* gene (Cadieux *et al.*, 2005). Morpholino-based knockdown of zfPGRN-A resulted in truncated and inappropriate premature branching of developing primary motor neurons. In addition, overexpression of zfPGRN-A or human progranulin rescued truncation defects caused by the knockdown of zfPGRN-A (Chitramuthu *et al.*, 2010). The knockdown of zfPGRN-A causes greater reduction of motor neuron axonal length than the knockdown of zfPGRN-B (Laird *et al.*, 2010). Over-expressing progranulin rescued the axonopathy induced either by mutant TDP-43 or by a reduction in the expression of wild-type TDP-43 (Laird *et al.*, 2010; Chitramuthu *et al.*, 2017) as well as by the reduced expression of the *smn1* causative gene for spinal muscular atrophy (Chitramuthu *et al.*, 2010). Another study reported that the knockdown of zfPGRN-A reduced the number of myogenic progenitor cells and impaired muscle growth (Li *et al.*, 2013), although a different study observed no such neuronal and muscle defects in a zinc finger nuclease (ZFN) derived $grna^{-/-}$ *or* $grnb^{-/-}$ models (Solchenberger *et al.*, 2015). The absence of neuronal and muscle defects in *grn* knockout models suggest the activation of compensatory mechanisms upon the complete loss of the *grna* and *grnb* gene (Solchenberger *et al.*, 2015) compared to knockdown models. Other examples of genetic compensation in zebrafish gene knockout studies have been reported (Rossi *et al.*, 2015). Nerve cell independent roles of zfPGRN-A have been proposed in the control of liver size, hepatic proliferation and MET signalling in liver morphogenesis in zebrafish (Li *et al.*, 2010). There is no apparent NCL phenotype observed in $grna^{-/-}/grnb^{-/-}$ double mutants in zebrafish (Solchenberger *et al.*, 2015) but the short life-span of zebrafish may preclude the appearance of the NCL phenotype.

Mouse models: neuropathology, behaviour and neuroinflammation

Targeted overexpression of progranulin in peripheral and central neurons of mice promoted markedly faster axonal regrowth, reformation of neuromuscular junctions, reduction in recruitment of microglia to the site of injury and an improvement of motor functions after sciatic nerve injury compared to control mice (Altmann *et al.*, 2016*b*). Correspondingly, $Grn^{-/-}$ mice show significantly poorer recovery and greater inflammation than control mice (Altmann *et al.*, 2016*b*). Clearly, therefore, the specific neuronal over production of progranulin promotes neuronal recovery after injury.

Progranulin deficiency in mouse models produces abnormal neuronal and behavioural phenotypes. Abnormalities of $Grn^{-/-}$ mice include age-dependent neuronal ubiquitin accumulation, tissue vacuolation, microgliosis and astrogliosis, and selective neuronal loss (Ahmed *et al.*, 2010; Yin *et al.*, 2010*a*, *b*; Ghoshal *et al.*, 2012; Petkau *et al.*, 2012; Wils *et al.*, 2012). Some reports have shown the presence of TDP-43 inclusions in the brains of $Grn^{-/-}$ mice (Yin *et al.*, 2010*a*) while others observed little or no TDP-43 pathology (Ahmed *et al.*, 2010). Patients carrying *GRN* mutations exhibit retinal thinning before the onset of dementia, and a similar retinal phenotype has been reported in $Grn^{-/-}$ mice associated with the loss of nuclear TDP-43 and cellular depletion of the GTPase, Ran (Ward *et al.*, 2014). Behavioural abnormalities of *Grn*-deficient mice include increased depression, disinhibition-like behaviour, together with deficiency in social recognition and spatial learning and memory, of which the most common behavioural phenotype is reduced social interaction (Yin *et al.*, 2010*b*; Ghoshal *et al.*, 2012; Petkau *et al.*, 2012). The behavioural phenotypes may be more severe in male animals (Kayasuga *et al.*, 2007) possibly due to gender-specific differences in synaptic connectivity and impaired synaptic plasticity (Petkau *et al.*, 2012). The assessment of spatial memory functions by Morris water maze was less consistent between mouse models with reports ranging from reduced spatial memory (Yin *et al.*, 2010*b*) to no difference in spatial memory (Petkau *et al.*, 2012). Defects in emotional behavioural and social recognition, synaptic transmission, and neuronal morphology occur before obvious signs of neuroinflammation (Yin *et al.*, 2010*b*) or major neuropathological changes (Petkau *et al.*, 2012).

The absence of progranulin resulted in an apparent accelerated ageing of the brain as revealed by the significant increase with age in ubiquitin positive granular cytoplasmic staining and, importantly (Ahmed *et al.*, 2010; Wils *et al.*, 2012) extensive lipofuscinosis in the *Grn* knockout mice compared to age-matched wild-type control animals (Ahmed *et al.*, 2010). This is similar to observations in patients with *GRN*-related NCL (Smith *et al.*, 2012) and may suggest that the *Grn* knockout mice are better models of NCL-11 than they are of FTLD. Clearly, NCL-11 and *GRN*-dependent FTLD are intimately related, but it remains uncertain at present to what extent they form a phenotypic continuum or are dissociable disease entities each with their own distinctive properties. With this caveat the relative paucity of strong FTLD-like phenotypes in the $Grn^{-/-}$ homozygotes, in particular with respect to the presence of ubiquitin TDP inclusions, may reflect the slow onset of disease in human patients, or suggest that additional factors, not yet well understood, are necessary to manifest a more robust FTLD-like phenotype. Interestingly it has been shown that the progranulin levels decrease with age in the cortex, hippocampus, and hypothalamus of wild-type mice (Matsuwaki *et al.*, 2011) suggesting that progranulin depletion may play a role in age-related decline in brain function.

Progranulin is highly upregulated in activated macrophages and microglia (Moisse *et al.*, 2009; Philips *et al.*, 2010; Tanaka *et al.*, 2013*a*, *b*; Zhu *et al.*, 2013). In general, in *Grn* knockout mice there is increased expression of proinflammatory cytokines and exacerbated microglial activation and astrogliosis (Yin *et al.*, 2010*a*, *b*; Ghoshal *et al.*, 2012). Macrophages and microglia from $Grn^{-/-}$

mice are cytotoxic to hippocampal cells *in vitro* (Yin *et al.*, 2010*a*), and show greater rates of phagocytotic apoptosis (Kao *et al.*, 2011). Progranulin deficiency increased microgliosis and the loss of dopaminergic neurons after toxin-induced injury caused by 1-methyl-4-(2'-methylphenyl)-1,2,3,6-tetrahydrophine (MPTP), a drug that specifically ablates dopaminergic neurons (Martens *et al.*, 2012). This was reproduced in mice in which *Grn* was conditionally knocked out only in microglia and macrophages strongly supporting the hypothesis that the susceptibility of $Grn^{-/-}$ mice to neurodegenerative processes results from a disruption in normal neuroinflammatory regulation (Martens *et al.*, 2012). Reducing microglial expression of progranulin in Alzheimer's disease mouse models increased their plaque burden and worsened cognitive deficits, demonstrating an essential role for microglial-derived progranulin in the clearance of aberrant amyloid-β deposits (Minami *et al.*, 2014). Progranulin deficiency leads to progressive upregulation of lysosomal and innate immunity genes, increased production of complement proteins and enhanced synaptic pruning by microglia in an age-dependent manner (Lui *et al.*, 2016). Indeed, in progranulin-deficient mice it has been proposed that aberrant microglial activation by complement proteins and the resultant increased synaptic pruning is a major cause, rather than a consequence, of neurodegeneration (Lui *et al.*, 2016).

Taken together these results support a role for progranulin in the CNS as a regulator of microglial activity, reducing their deleterious effects on neurons while enhancing their ability to remove amyloid plaques. Not all studies, however, are fully consistent with this conclusion. For example, both *in vitro* studies and lentiviral delivery of progranulin into mouse brains suggest that progranulin may be a chemoattractant for microglia (Pickford *et al.*, 2011; Zhu *et al.*, 2013). In pilocarpine-induced epilepsy in mice, while progranulin was not required for microglial activation, it had potentiating activity (Zhu *et al.*, 2013). Depleting microglia of *Grn* mRNA by siRNA *in vitro* lowered rather than increased inflammatory cytokine production, leading to the conclusion that there is a stimulatory role for progranulin in microglial cytokine production (Suh *et al.*, 2012). Strongly pro-inflammatory stimuli such as lipopolysaccharide and polyIC (polyriboinosinic-polyribocytidilic acid) treatment inhibit microglial progranulin secretion *in vitro* (Suh *et al.*, 2012). In astroglia, however, which normally produce very little progranulin, polyIC has the opposite effect in that it stimulates progranulin secretion (Suh *et al.*, 2012). It is likely, therefore, that the inflammatory regulation of progranulin production in the CNS is cell-type specific.

Almost all studies that showed a strong neuroinflammatory phenotype were conducted in homozygous $Grn^{-/-}$ mice but in humans the loss of only a single allele of *GRN* is sufficient to cause FTLD. Rather mild phenotypes accrue in heterozygotic $Grn^{+/-}$ mice. Indeed, recent work suggests that the behavioural phenotypes of homozygous and heterozygous *Grn* knockout animals are distinct (Arrant *et al.*, 2016). Abnormalities of social and emotional behaviour, as well as changes in brain structure, particularly in the amygdala, occur in $Grn^{+/-}$ mice (Filiano *et al.*, 2013) but this is independent of neuroinflammation or gliosis. Thus, structural and behavioural phenotypes can emerge in heterozygous $Grn^{+/-}$ mice, even in the absence of extensive microglial activation. In MPTP intoxicated mice (Martens *et al.*, 2012) the loss of dopaminergic neurons in the substantia nigra follows an approximately linear relationship with respect to *Grn* allele number, falling by 66% in $Grn^{-/-}$ mice, by 50% in $Grn^{+/-}$ mice and 37% in wild-type mice. In contrast, excessive microglial neuroinflammation following MPTP treatment is markedly inflected with respect to *Grn* allele number, demonstrating a very strong 2.84-fold increase in the $Grn^{-/-}$ mice versus wild-type but a much smaller 1.33-fold increase in $Grn^{+/-}$ mice (Martens *et al.*, 2012). While the role of microglia in neuronal death in $Grn^{-/-}$ mice is well established, a question remains as to whether the rather marginal increase in the levels of microglia in the substantia nigra found in $Grn^{+/-}$ mice is sufficient by itself to cause the degree of dopaminergic neuronal loss that was observed. Given that: (i) behavioural phenotypes in $Grn^{+/-}$ mice can be dissociated from inflammation; (ii) progranulin influences neuronal survival *in vitro* independently of inflammatory stimuli; and (iii) that progranulin has actions in non-inflammatory *C. elegans* and zebrafish models (see above), it is reasonable to suppose that progranulin has a double-edged role in combating neurodegenerative disease, both by preventing the onset of a hostile neuroinflammatory microenvironment and supporting the survival of neurons in the face of injurious challenges.

Little is known about progranulin and neuroinflammation in humans with FTD and therefore how well the neuroinflammatory phenotypes of $Grn^{-/-}$ mice reproduce clinical FTD. Levels of IL6, but not TNF-α, increase in the serum and CSF of carriers of *GRN* mutations once they become symptomatic (Bossu *et al.*, 2011). Postmortem brains of patients with FTLD-TDP43 caused by *GRN* mutations showed a clear, but limited increase in microglial number in the grey matter of the frontal cortex when compared to FTLD-TDP43 brains without *GRN* mutations. Microglial numbers were graded at 1.7 in *GRN* mutation carriers compared to 1.4 in FTD affected brains with normal *GRN* on a four-point scale of 0 to 3 (Chen-Plotkin *et al.*, 2010). In *GRN*-related FTD, brain regions that were relatively unaffected showed a clear reduction in progranulin protein levels, whereas in the frontal and temporal cortices, no statistically significant decreases in progranulin were observed when compared to control samples, although a trend towards decreased progranulin can be discerned (Chen-Plotkin *et al.*, 2010). Moreover, *GRN* mRNA was higher in the frontal cortex of *GRN* mutation carriers than it was in controls, a result clearly at variance with expectations based on *GRN* haploinsufficiency (Chen-Plotkin *et al.*, 2010). The 'extra' *GRN* mRNA in frontal and temporal lobes was attributed to its production by

microglia using the remaining functional *GRN* allele. Clearly, therefore, in the later stages of disease there is little if any deficit in microglial production of progranulin in regions of the brain with the most neuronal loss. Whether the microglial compensation of progranulin production occurs throughout disease progression is not known.

Mechanisms of action of progranulin

Progranulin interacts with many cellular processes, including lysosome function and autophagy, cellular localization of TDP-43, inflammation, cell survival, cell morphology, and cell signalling. Its actions appear to be mediated by binding to a range of different cell membrane proteins (Figs 4 and 5). Understanding how these diverse activities integrate at the cellular level will be essential to understand how progranulin deficiency causes neurodegeneration.

Progranulin and lysosomes

It is increasingly clear that protein degradation is compromised in FTLD (Gotzl *et al.*, 2016). Progranulin is involved in lysosome function and disruption of this function may be critical in the aetiology of *GRN*-related FTD. Homozygous loss of *GRN* in humans (Smith *et al.*, 2012; Almeida *et al.*, 2016) and mice (Ahmed *et al.*, 2010) results in a profound disruption of lysosome function. The existence of ubiquitinated TDP-43 aggregates in *GRN*-related FTD implies a failure in protein clearance pathways. Furthermore, it is noteworthy that the genetic modifiers of *GRN*-related FTD, sortilin, TMEM106B and prosaposin all play roles in lysosomal function (Carrasquillo *et al.*, 2010; Hu *et al.*, 2010; Van Deerlin *et al.*, 2010; Finch *et al.*, 2011; Lang *et al.*, 2012; Brady *et al.*, 2013; Stagi *et al.*, 2014; Zhou *et al.*, 2015*b*). In addition to binding progranulin (see above), sortilin is implicated in lysosomal trafficking of the acid sphingomyelinase and sphingolipid activator proteins (SAPs), which are necessary for the degradation of glycosphingolipids within lysosomes (Saftig and Klumperman, 2009). Progranulin binds to prosaposin (Zhou *et al.*, 2015*b*), which is the precursor for SAPs suggesting a possible role for progranulin in this pathway (Fig. 4). Progranulin deficiency results in an increase in TMEM106B in ageing mouse brains, while progranulin deficiency in neuronal TMEM106B transgenic mice significantly worsens lipofuscinosis (Zhou *et al.*, 2017). Interestingly, in humans, gene variants of *TMEM106B* and *GRN* are both associated with accelerated ageing in human brains (Rhinn and Abeliovich, 2017) suggesting a role for the progranulin/TMEMB106B pathway not only in FTD but also in healthy brain ageing. Autophagy is impaired in progranulin depleted neurons whereas in neuron-specific progranulin transgenic mice progranulin was found to interact with autophagy-related proteins, and proteins of the lysosome and endocytosis pathway (Altmann *et al.*, 2016*a*). This is consistent with reports from non-neuronal cells that also identified roles for progranulin in autophagy (Tian *et al.*, 2016). As the clearance of TDP-43 aggregates is mediated by autophagy (Scotter *et al.*, 2014), the depletion of progranulin in *GRN*-related FTD and the resultant impairment of autophagy may therefore favour the accumulation of TDP-43 aggregates, although this has yet to be formally proven. In a further link with the vesicular trafficking pathways, progranulin is involved in exosome signalling. Exosomes, which are secreted vesicles that are derived from endosomes, mediate important aspects of cell-cell communication. Not only is progranulin part of the cargo in human fibroblast exosomes but also, when progranulin content is depleted due to *GRN* mutation, the pattern of global exosome protein secretion was markedly altered (Benussi *et al.*, 2016) supporting a role for progranulin in regulating exosome formation or secretion. Transcription factor EB (TFEB) has a central role in controlling lysosome biogenesis and when it is overexpressed in HeLa cells results in increased transcription of *GRN* mRNA (Belcastro *et al.*, 2011). The induction of lysosomes in HeLa cells by sucrose treatment elevated progranulin expression and correspondingly, the overexpression of progranulin resulted in increased lysosome size caused by a factor, most likely progranulin, secreted into the medium (Belcastro *et al.*, 2011). The brains of $Grn^{-/-}$ mice show enhanced expression of many lysosomal genes, as well as a higher proportion of microglia with nuclear TFEB, implying greater activation of the lysosome biogenesis pathways (Tanaka *et al.*, 2013*b*). When lysosome activity is sufficient to meet the needs of the cell, TFEB is retained in the cytosol through its phosphorylation by mammalian target of rapamycin (mTORC1) situated on the lysosomal membrane (Roczniak-Ferguson *et al.*, 2012). Loss of progranulin in $Grn^{-/-}$ mice appears to interfere with this process, releasing TFEB for translocation to the nucleus (Tanaka *et al.*, 2013*b*). Very high progranulin levels are found associated with amyloid plaques in Alzheimer's disease (Pereson *et al.*, 2009). The plaque-associated progranulin was found to be enriched in microglia in one study (Pereson *et al.*, 2009), but in another study, it was associated with a subset of protease-depleted axonal lysosomes (Gowrishankar *et al.*, 2015) reinforcing a probable connection between progranulin and lysosomal function.

TDP-43 and progranulin

Given that haploinsufficiency of *GRN* leads to TDP-43 proteinopathy, an appreciation of possible mechanisms that connect TDP-43 to neurodegeneration is clearly needed to understand the roles of progranulin in neuropathology. TDP-43 is an RNA-binding protein that is concentrated in the nucleus in healthy cells and consists of an N-terminal nuclear localization signal, two RNA

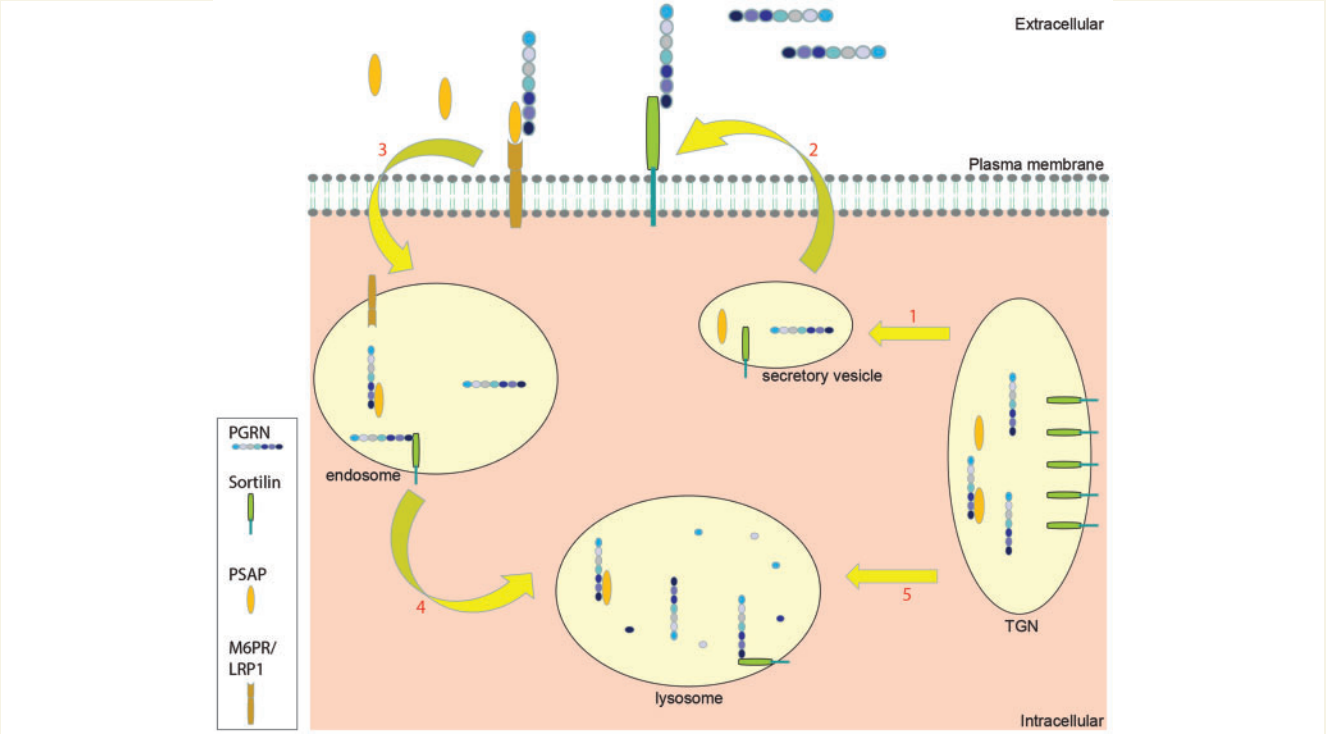

Figure 4 Progranulin binds to sortilin and prosaposin. The binding of progranulin (PGRN) to sortilin and prosaposin regulates extracellular levels of progranulin through endocytosis. (1) Sortilin, prosaposin (PSAP) and progranulin are packaged in the trans-Golgi network (TGN). (2) Progranulin and prosaposin are secreted and sortilin is inserted into the cell membrane. (3) Binding of progranulin to sortilin facilitates the uptake of progranulin/sortilin to endosomes. Progranulin may also associate with prosaposin and be carried to endosomes as a passenger with prosaposin binding to the mannose-6-phosphate receptor (M6P). The MR6P-mediated internalization of prosaposin/progranulin requires in addition lipoprotein receptor-related protein 1 (LRP1). (4) Progranulin is transferred to lysosomes, where it may be broken down by proteolysis to granulin peptides (small coloured dots). (5) In addition, sortilin and prosaposin may carry progranulin into lysosomes directly from the TGN (Hu *et al.*, 2010; Zhou *et al.*, 2015*b*).

recognition motifs and an extended glycine-rich carboxyl-terminal domain where pathogenic mutations cluster (reviewed in Cohen *et al.*, 2011). TDP43 plays roles in transcriptional regulation of RNA, RNA splicing, miRNA regulation, mRNA stabilization and mRNA transport (reviewed in Hanson *et al.*, 2012). Disruption of TDP-43-mediated RNA processing would, therefore, be likely to profoundly disrupt neuronal function, and there is considerable evidence for such loss of function mechanisms in TDP-43 neuropathology (Vanden Broeck *et al.*, 2014). Simply increasing TDP-43 levels in neuronal cells, however, results in a neurotoxic response (Suzuki and Matsuoka, 2011, 2012; Yamashita *et al.*, 2014; Suzuki *et al.*, 2015), which is consistent with a gain of toxic function and TDP-43 neurotoxicity may be, in fact, a complex interaction between both loss of function and gain of toxicity mechanisms (Cascella *et al.*, 2016). While disturbances of RNA processing are likely to be important in FTLD-TDP, whether progranulin interacts with these processes is not well understood. Depletion of progranulin in neurons results in mislocalization of TDP-43 from the nucleus to the cytoplasm (Gao *et al.*, 2010; Almeida *et al.*, 2012). The nuclear import of TDP-43 is regulated by the GTPase Ran, the levels of which are in turn regulated by TDP-43 (Ward *et al.*, 2014). In neurons that are deficient in progranulin, increasing the expression of Ran elevates nuclear TDP-43 levels and enhances cell survival (Ward *et al.*, 2014) suggesting a functional linkage between progranulin depletion, Ran and the mislocation of TDP-43.

Progranulin signalling pathways

Progranulin activates both ERK1/2 (extracellular regulated kinase 1/2) and Akt signalling in many cell types (Zhou *et al.*, 1993; Zanocco-Marani *et al.*, 1999; Lu and Serrero, 2001; He *et al.*, 2002; He and Bateman, 2003; Kamrava *et al.*, 2005; Monami *et al.*, 2006; Guerra *et al.*, 2007) including neurons (Gao *et al.*, 2010; Xu *et al.*, 2011). Neurons treated with progranulin showed enhanced phosphorylation of the serine/threonine kinase Akt and glycogen synthase kinase-3β (GSK-3β), a substrate of Akt, with subsequent inactivation of GSK-3β (Gao *et al.*, 2010; Nedachi *et al.*, 2011). Reduction of baseline levels of phospho-Akt were observed in $Grn^{-/-}$ neurons (Kleinberger *et al.*, 2010) and FTLD-derived iPSCs (Almeida *et al.*, 2012) while knockdown of GSK-3β

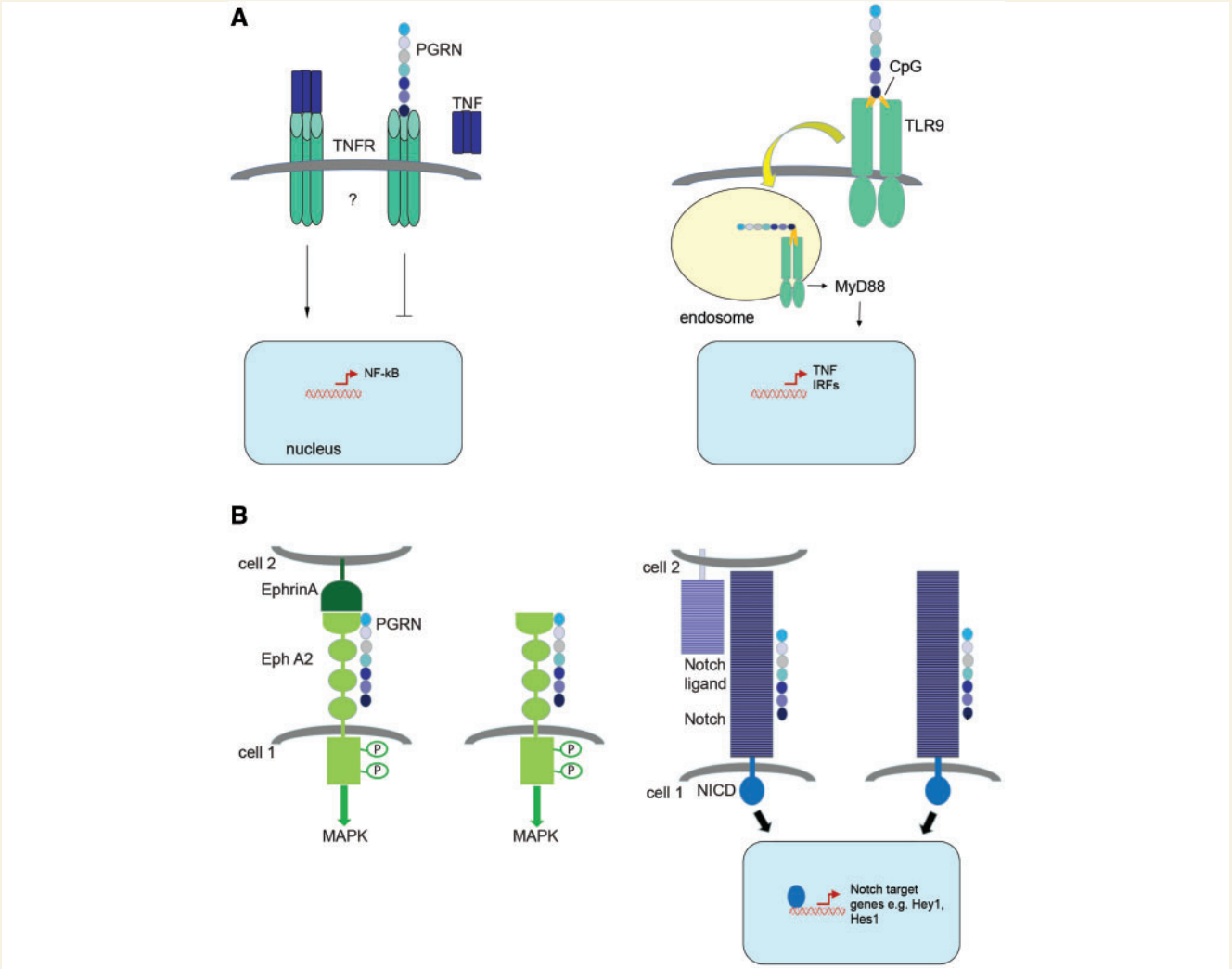

Figure 5 Progranulin binds to multiple cell surface receptors. (**A**) Progranulin (PGRN) may influence inflammatory signalling by interfering with TNF binding to TNF-receptors (Tang *et al.*, 2011). The arrow indicates activation and the blunt-ended line indicates inhibition. The question mark indicates that not all research groups detect progranulin binding to the TNFR (Chen *et al.*, 2013; Etemadi *et al.*, 2013). Progranulin is, in addition, a cofactor for CpG binding to toll-like receptor 9 either on cell membranes or in endocytotic vesicles (Park *et al.*, 2011). (**B**) Progranulin may influence non-inflammatory cell signalling through EphA receptors (Neill *et al.*, 2016) or by notch signalling (Altmann *et al.*, 2016*b*). It is not yet clear whether progranulin forms a complex with the receptor and its typical ligand (ephrin or notch-ligand) or acts alone with these receptors. The bent red arrow indicates gene expression. CpG = unmethylated CpG; Eph A2 = Ephrin type-A receptor 2; Hes1 = hairy and enhancer of split-1; Hey1 = hairy/enhancer-of-split related with YRPW motif protein 1; IRFs = interferon-regulatory factors; MAPK = mitogen-activated protein kinase; MyD88 = myeloid differentiation primary response gene 88; NF-κB = nuclear factor kappa-lightchain- enhancer of activated B cells; NICD = notchintracellular domain; TLR9 = Toll-like receptor 9; TNF = tumour necrosis factor; TNFR = TNF receptors.

completely abolished the neurotrophic effect of progranulin (Gao *et al.*, 2010). Akt plays a role in pro-survival signalling pathways and transduces several functions including cell growth, apoptosis and survival among its many other activities (Song *et al.*, 2005). These results suggest a role for progranulin in mediating the Akt/GSK-3β pathway in neurite outgrowth and neuronal survival. In non-neuronal cells, progranulin signalling is associated with the activation of FAK (focal adhesion kinase) (He *et al.*, 2002; Monami *et al.*, 2006). It is unknown whether progranulin similarly influences FAK activity in neurons.

There is increasing evidence that progranulin interacts with Wnt signalling. Thus, a functional genomic study in a human neural progenitor model of progranulin-deficiency and in FTD brains, identified apparently greater Wnt signalling as a result of progranulin deficiency (Rosen *et al.*, 2011). Wnt1 and progranulin expression are regulated reciprocally with progranulin increasing Wnt1 levels and *vice versa* (Wexler *et al.*, 2011). In lymphocytes from individuals carrying a loss-of-function *GRN* mutation, activation of non-canonical Wnt5a signalling was detected (Alquezar *et al.*, 2014). Aberrant activation of the Wnt signalling

pathway was also observed upon neuronal differentiation using iPSCs generated from patients with FTLD carrying a *GRN* null mutation (Raitano *et al.*, 2015). Both canonical and non-canonical Wnt signalling cascades were over-activated following *GRN* knockdown in SH-SY5Y neuroblastoma cells (de la Encarnacion *et al.*, 2016). As Wnt signalling may improve neuronal survival it is likely that its enhancement in *GRN*-related FTD acts as a protective adaptation to progranulin depletion (Rosen *et al.*, 2011; Wexler *et al.*, 2011).

Progranulin receptors and binding proteins

No biologically active ligand functions in isolation but acts through its interaction with binding partners. With the exception of sortilin (Hu *et al.*, 2010), little is known about progranulin binding proteins in the CNS. Notwithstanding this limitation, progranulin has been reported to bind to several proteins in the periphery although in many cases how some of these contribute to progranulin action remains to be fully determined. Progranulin binds to SLPI (Zhu *et al.*, 2002), as outlined earlier, and to several members of the matrix metalloproteinase family namely MMP2, MMP9, MMP13 and MMP14 and MMP17 (Liu *et al.*, 2007; Butler *et al.*, 2008; Suh *et al.*, 2012), and ADAMPTS-7 (a disintegrin and metalloproteinase with thrombospondin motifs 7) (Bai *et al.*, 2009). In addition, it binds to the extracellular matrix proteins perlecan (Gonzalez *et al.*, 2003) and cartilage oligomeric matrix protein (Xu *et al.*, 2007). The association of progranulin with these extracellular matrix proteins may regulate its activity (Gonzalez *et al.*, 2003; Xu *et al.*, 2007; Bai *et al.*, 2009).

Membrane proteins that bind to progranulin (Figs 4 and 5) include sortilin (Hu *et al.*, 2010), the tumour necrosis factor receptor (TNFR) (Tang *et al.*, 2011), the toll-like receptor 9 (TLR9) (Park *et al.*, 2011), Dlk (Baladron *et al.*, 2002), EphA2 (Chitramuthu and Bateman, 2016; Neill *et al.*, 2016) and Notch receptors (Altmann *et al.*, 2016*b*). As noted above, sortilin regulates extracellular levels of progranulin (Carrasquillo *et al.*, 2010; Hu *et al.*, 2010). Progranulin binds to the beta-propeller region of sortilin through its carboxyl-terminal tail (Zheng *et al.*, 2011). Progranulin is reported to act as a competitive antagonist of TNF-α binding to the tumour necrosis factor type 1 and type 2 receptors (TNFR-I, TNFR-II) (Tang *et al.*, 2011). While this is consistent with the proposed role for progranulin in neuroinflammation, progranulin binding to TNFR has been challenged by other studies that found no progranulin binding to TNFRs (Chen *et al.*, 2013; Etemadi *et al.*, 2013). In addition, progranulin binds to both CpG ODNs and TLR9 acting as a soluble cofactor for TLR9 signalling (Park *et al.*, 2011) thereby providing a further route through which progranulin might regulate inflammation and neuroinflammation (Aravalli *et al.*, 2008; Scholtzova *et al.*, 2014).

Proteomic analysis of the prefontal cortex of mice engineered to specifically overexpress progranulin in neurons suggested a role for progranulin in Notch signalling (Altmann *et al.*, 2016*b*). Progranulin binds to all four Notch receptors through the Notch extracellular domain (Altmann *et al.*, 2016*b*). Others identified the tyrosine receptor kinase EphA2, an ephrin receptor, as a likely signalling partner for progranulin, although in this case in epithelial and endothelial cells (Neill *et al.*, 2016). It is noteworthy that both Notch and Ephrin signalling classically involve intimate cell-to-cell contact between a membrane-expressed ligand on one cell and the receptor on an immediately adjacent cell. Whether progranulin enters into the Notch-ligand/Notch or ephrin/EphA2 coupling, or instead binds Notch and EphA2 independently of their conventional partners is unknown (Chitramuthu and Bateman, 2016). Defining the roles of these receptors in the neurobiology of progranulin is clearly an important goal.

Progranulin as the basis of potential therapeutics

Increasing the baseline expression of progranulin in *GRN*-related FTD is a promising avenue for treatment, but, given the combined cytoprotective and immunomodulatory activities of progranulin, its use in other chronic neurodegenerative conditions or acute brain injury is also being explored. This is summarized in Fig. 6 and compared with similar strategies for the therapeutic use of progranulin in non-neuronal conditions.

Small molecule disease modifiers of *GRN*-related frontotemporal dementia

Current pharmacological treatment of FTD and related diseases are limited to providing relief for the behavioural, cognitive and motor symptoms of the disease (reviewed in Riedl *et al.*, 2014). There are no available therapies to stop or slow progression of the underlying neurodegeneration associated with FTLD (Riedl *et al.*, 2014). Of several recent or ongoing trials for potential FTD disease-modifying drugs listed in ClinicalTrails.gov (Tsai and Boxer, 2016) only two are targeted towards increasing brain progranulin levels or secretion, namely a phase 2 trial with the histone deacetylase (HDAC) inhibitor FRM-0334 (ClinicalTrials.gov Identifier: NCT02149160) and a phase 1 trial with the calcium channel blocker nimopidine, (ClinicalTrials.gov Identifier: NCT01835665) (Tsai and Boxer, 2016). Results have not yet been reported from either trial.

HDAC inhibitors serve as drugs to treat cancer (Wang *et al.*, 2005; Mottamal *et al.*, 2015). The HDAC inhibitor suberoylanilide hydroxamic acid (SAHA), also called vorinostat, was identified by chemical library screening to

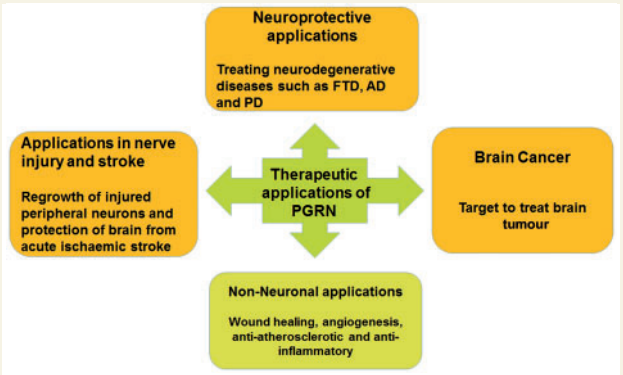

Figure 6 Potential therapeutic applications of progranulin. Progranulin may potentially be used to treat multiple disease conditions including neurodegenerative diseases such as FTD, Alzheimer's disease (AD) and Parkinson's disease (PD) (Capell *et al.*, 2011; Cenik *et al.*, 2011; Van Kampen *et al.*, 2014; Minami *et al.*, 2015) acute brain injury (Tao *et al.*, 2012; Egashira *et al.*, 2013; Jackman *et al.*, 2013; Kanazawa *et al.*, 2015; Zhao and Bateman, 2015; Altmann *et al.*, 2016*b*; Xie *et al.*, 2016), Targeted depletion of progranulin levels may be beneficial in treating gliomas (Bandey *et al.*, 2015). Progranulin has been suggested as a therapeutic target for many peripheral conditions particularly those with an important inflammatory component (He *et al.*, 2003; Sfikakis and Tsokos, 2011; Guo *et al.*, 2012; Jian *et al.*, 2013; Choi *et al.*, 2014; Huang *et al.*, 2015; Zhou *et al.*, 2015*a*).

increase progranulin levels at both mRNA and protein levels and to reconstitute close to normal levels of progranulin in human *GRN* haploinsufficient cells in culture (Cenik *et al.*, 2011). SAHA does not easily cross the blood–brain barrier, but FRM-0334 does, stimulating investigation of its use as a potential progranulin-raising therapy (Tsai and Boxer, 2016).

In an attempt to increase progranulin bioavailability, compounds were screened to determine if they might inhibit proteolytic degradation of progranulin (Capell *et al.*, 2011). While inhibitors of lysosomal proteases or inhibition of the ubiquitin proteasome system or inhibition of the neutrophil elastase, which cleaves progranulin into granulin peptides, had no effect on the amount of intracellular or secreted progranulin, the alkalizing reagent bafilomycinA1 (BafA1) significantly increased both intracellular as well as secreted progranulin levels (Capell *et al.*, 2011). Since vacuolar ATPase was identified as a target for BafA1, the properties of inhibitors of vacuolar ATPase such as concanamycin A, archazolid B and apicularen A were investigated and found to increase progranulin levels using both neuronal and non-neuronal cells including cultured primary cells derived from human patients with *GRN* loss-of-function mutations (Capell *et al.*, 2011). Amiodarone was one of the agents found to increase progranulin secretion through this mechanism, although preliminary trials of amiodarone treatment in patients did not demonstrate a clear therapeutic result (Alberici *et al.*, 2014).

Other groups have attempted to block the sortilin-progranulin axis with the goal of elevating progranulin levels (Lee *et al.*, 2014). Sortilin peptide antagonists based structurally upon the carboxyl-terminal region of progranulin inhibit sortilin-mediated progranulin endocytosis and were found to restore extracellular progranulin in mammalian cell lines including patient-derived cell models as did a small molecule organic compound, 1-[2-(2-tert-butyl-5-methylphenoxy)-ethyl]-3-methylpiperidine, that specifically lowered sortilin levels (Lee *et al.*, 2014). Prompted by the role of progranulin in lysosome biology, a cell-based screen of autophagy/lysosome modulators for molecules that regulate *GRN* promoter activity identified trehalose, an mTOR-independent activator of autophagy (Holler *et al.*, 2016). Trehalose enhanced progranulin expression in iPSC-derived human neurons carrying a *GRN* mutation as well as in the brains of *Grn* haploinsufficient mice (Holler *et al.*, 2016). Trehalose has postulated therapeutic benefit in several neurodegenerative diaseases (Emanuele, 2014; Giorgetti *et al.*, 2015; Li *et al.*, 2015; Tanji *et al.*, 2015; Tien *et al.*, 2016) and is therefore a very promising lead in the search for new drugs to manage *GRN*-related FTD. Additional therapeutic targets are suggested by the discovery that receptor-interacting protein kinase 1 (RIPK1) regulates progranulin levels (Mason *et al.*, 2017). The strong inflammatory response to *GRN* depletion suggests that therapies that blunt overactive neuroinflammation may have potential benefit (Minami *et al.*, 2015).

GRN gene therapy in animal models of frontotemporal lobar degeneration, Parkinson's and Alzheimer's diseases

Gene therapy may be an alternate strategy to increase brain progranulin levels. Viral delivery of progranulin to the medial prefrontal cortex of $Grn^{+/-}$ mice restored normal social dominance behaviour and normalized lysosomal abnormalities supporting a therapeutic potential for targeted progranulin treatment in FTD (Arrant *et al.*, 2017). Other animal models have focused on neurological diseases other than FTD. In a model of Parkinson's disease in mice, the lentiviral delivery of the *GRN* gene protected nigrostriatal neurons against the toxic effects of pretreatment with MPTP with the preservation of both dopamine content and locomotor function, as well as reduced expression of markers of inflammation and apoptosis (Van Kampen *et al.*, 2014). Another study demonstrated that lentiviral delivery of progranulin in a murine genetic model of Alzheimer's disease reduced plaque load, preserved hippocampal neurons and lowered memory deficits (Minami *et al.*, 2015). These results strongly suggest possible therapeutic avenues based on progranulin in many neurodegenerative pathologies in addition to FTD.

Progranulin, a potential therapy in nerve injury and stroke

In addition to chronic neurodegenerative conditions, progranulin may have therapeutic properties in traumatic brain damage and peripheral nerve injury. Progranulin promotes the regrowth of injured sciatic nerves, suggesting that it may have benefits in the regeneration of damaged peripheral nerves (Altmann *et al.*, 2016*b*). Several studies using transgenic *Grn* mice or *Grn* knockout mice support a protective role for progranulin in stroke models (Tao *et al.*, 2012; Egashira *et al.*, 2013; Jackman *et al.*, 2013; Kanazawa *et al.*, 2015). Lentiviral delivery of progranulin (Tao *et al.*, 2012), or intracerebroventricular delivery of recombinant progranulin 2 h following ischaemia (Egashira *et al.*, 2013) or intraperitoneal injection of progranulin (Kanazawa *et al.*, 2015) reduced infarct size, oedema, and mortality and enhanced neurological functions. Several mechanisms have been proposed for the protective action of progranulin in stroke models, including reduced reperfusion inflammation, cytoprotection of neurons, and effects on the integrity of the blood–brain barrier (Egashira *et al.*, 2013; Jackman *et al.*, 2013; Kanazawa *et al.*, 2015). Together these results suggest that progranulin has potential as an acute therapeutic treatment to improve stroke recovery.

Conclusion

Progranulin is a secreted protein whose reduced expression causes neurodegeneration. It has a multiplicity of targets regulating neuroinflammation, neuronal survival and neuronal cell structure. While much remains to discover with respect to its mode of action, an increased sensitivity of neurons to injury coupled to a more aggressive neuroinflammatory response is likely to account for the slow but relentless atrophy of key regions of the brain brought on by haploinsufficiency of *GRN*. That the complete deletion of the *GRN* results in lysosomal defects implies a hitherto unexpected but vital role for progranulin in the endocytosis-lysosomal pathway. Progranulin expression patterns, its molecular function and mechanism of action both in *in vitro* and *in vivo* models demonstrate the complexity of its effects. The potential therapeutic applications of progranulin in treating neurodegenerative diseases are enormous. Integrating the many facets of progranulin action, its roles as a neuroinflammatory modulator and a neurotrophic protein, as an extracellular regulatory signal and a protein involved in lysosomal function will be essential if the potential therapeutic applications of progranulin in treating various diseases are to be realized.

Funding

Research conducted by H.P.J.B. and A.B. was funded by Canadian Institute of Health Research, Natural Science and Engineering Research Council Canada, Canadian Cancer Society Research Institute and the Department of Medicine McGill University. B.C. was supported by Natural Science and Engineering Research Council Canada Alexander Graham Bell Award, The Fonds de recherche du Québec, the Department of Medicine, McGill University and MUHC-RI Fellowship awards. A.B. and H.P.J.B. are currently supported by Weston Brain Institute.

References

Ahmed Z, Sheng H, Xu YF, Lin WL, Innes AE, Gass J, et al. Accelerated lipofuscinosis and ubiquitination in granulin knockout mice suggest a role for progranulin in successful aging. Am J Pathol 2010; 177: 311–24.

Alberici A, Archetti S, Pilotto A, Premi E, Cosseddu M, Bianchetti A, et al. Results from a pilot study on amiodarone administration in monogenic frontotemporal dementia with granulin mutation. Neurol Sci 2014; 35: 1215–19.

Almeida MR, Baldeiras I, Ribeiro MH, Santiago B, Machado C, Massano J, et al. Progranulin peripheral levels as a screening tool for the identification of subjects with progranulin mutations in a Portuguese cohort. Neurodegener Dis 2014; 13: 214–23.

Almeida MR, Macario MC, Ramos L, Baldeiras I, Ribeiro MH, Santana I. Portuguese family with the co-occurrence of frontotemporal lobar degeneration and neuronal ceroid lipofuscinosis phenotypes due to progranulin gene mutation. Neurobiol Aging 2016; 41: 200.e1–5.

Almeida S, Zhang Z, Coppola G, Mao W, Futai K, Karydas A, et al. Induced pluripotent stem cell models of progranulin-deficient frontotemporal dementia uncover specific reversible neuronal defects. Cell Rep 2012; 2: 789–98.

Alquezar C, Esteras N, de la Encarnacion A, Alzualde A, Moreno F, Lopez de Munain A, et al. PGRN haploinsufficiency increased Wnt5a signaling in peripheral cells from frontotemporal lobar degeneration-progranulin mutation carriers. Neurobiol Aging 2014; 35: 886–98.

Altmann C, Hardt S, Fischer C, Heidler J, Lim HY, Haussler A, et al. Progranulin overexpression in sensory neurons attenuates neuropathic pain in mice: role of autophagy. Neurobiol Dis 2016a; 96: 294–311.

Altmann C, Vasic V, Hardt S, Heidler J, Haussler A, Wittig I, et al. Progranulin promotes peripheral nerve regeneration and reinnervation: role of notch signaling. Mol Neurodegener 2016b; 11: 69.

Antonell A, Gil S, Sanchez-Valle R, Balasa M, Bosch B, Prat MC, et al. Serum progranulin levels in patients with frontotemporal lobar degeneration and Alzheimer's disease: detection of GRN mutations in a Spanish cohort. J Alzheimers Dis 2012; 31: 581–91.

Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun 2006; 351: 602–11.

Aravalli RN, Hu S, Lokensgard JR. Inhibition of toll-like receptor signaling in primary murine microglia. J Neuroimmune Pharmacol 2008; 3: 5–11.

Armstrong RA. Cortical degeneration in frontotemporal lobar degeneration with TDP-43 proteinopathy caused by progranulin gene mutation. Int J Neurosci 2014; 124: 894–903.

Armstrong RA, Cairns NJ. A morphometric study of the spatial patterns of TDP-43 immunoreactive neuronal inclusions in frontotemporal lobar degeneration (FTLD) with progranulin (GRN) mutation. Histol Histopathol 2011; 26: 185–90.

Arrant AE, Filiano AJ, Unger DE, Young AH, Roberson ED. Restoring neuronal progranulin reverses deficits in a mouse model of frontotemporal dementia. Brain 2017; 140: 1447–65.

Arrant AE, Filiano AJ, Warmus BA, Hall AM, Roberson ED. Progranulin haploinsufficiency causes biphasic social dominance abnormalities in the tube test. Genes Brain Behav 2016; 15: 588–603.

Arvanitakis Z. Update on frontotemporal dementia. Neurologist 2010; 16: 16–22.

Ashcroft GS, Lei K, Jin W, Longenecker G, Kulkarni AB, Greenwell-Wild T, et al. Secretory leukocyte protease inhibitor mediates non-redundant functions necessary for normal wound healing. Nat Med 2000; 6: 1147–53.

Bai XH, Wang DW, Kong L, Zhang Y, Luan Y, Kobayashi T, et al. ADAMTS-7, a direct target of PTHrP, adversely regulates endochondral bone growth by associating with and inactivating GEP growth factor. Mol Cell Biol 2009; 29: 4201–19.

Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature 2006; 442: 916–19.

Baladron V, Ruiz-Hidalgo MJ, Bonvini E, Gubina E, Notario V, Laborda J. The EGF-like homeotic protein dlk affects cell growth and interacts with growth-modulating molecules in the yeast two-hybrid system. Biochem Biophys Res Commun 2002; 291: 193–204.

Bandey I, Chiou SH, Huang AP, Tsai JC, Tu PH. Progranulin promotes temozolomide resistance of glioblastoma by orchestrating DNA repair and tumor stemness. Oncogene 2015; 34: 1853–64.

Banzhaf-Strathmann J, Claus R, Mucke O, Rentzsch K, van der Zee J, Engelborghs S, et al. Promoter DNA methylation regulates progranulin expression and is altered in FTLD. Acta Neuropathol Commun 2013; 1: 16.

Bartkowska K, Turlejski K, Djavadian RL. Neurotrophins and their receptors in early development of the mammalian nervous system. Acta Neurobiol Exp 2010; 70: 454–67.

Bateman A, Belcourt D, Bennett H, Lazure C, Solomon S. Granulins, a novel class of peptide from leukocytes. Biochem Biophys Res Commun 1990; 173: 1161–8.

Beck J, Rohrer JD, Campbell T, Isaacs A, Morrison KE, Goodall EF, et al. A distinct clinical, neuropsychological and radiological phenotype is associated with progranulin gene mutations in a large UK series. Brain 2008; 131 (Pt 3): 706–20.

Belcastro V, Siciliano V, Gregoretti F, Mithbaokar P, Dharmalingam G, Berlingieri S, et al. Transcriptional gene network inference from a massive dataset elucidates transcriptome organization and gene function. Nucleic Acids Res 2011; 39: 8677–88.

Benussi A, Padovani A, Borroni B. Phenotypic heterogeneity of monogenic frontotemporal dementia. Front Aging Neurosci 2015; 7: 171.

Benussi L, Ciani M, Tonoli E, Morbin M, Palamara L, Albani D, et al. Loss of exosomes in progranulin-associated frontotemporal dementia. Neurobiol Aging 2016; 40: 41–9.

Benussi L, Ghidoni R, Binetti G. Progranulin mutations are a common cause of FTLD in Northern Italy. Alzheimer Dis Assoc Disord 2010; 24: 308–9.

Bernardi L, Tomaino C, Anfossi M, Gallo M, Geracitano S, Costanzo A, et al. Novel PSEN1 and PGRN mutations in early-onset familial frontotemporal dementia. Neurobiol Aging 2009; 30: 1825–33.

Bhandari V, Bateman A. Structure and chromosomal location of the human granulin gene. Biochem Biophys Res Commun 1992; 188: 57–63.

Bhandari V, Palfree RG, Bateman A. Isolation and sequence of the granulin precursor cDNA from human bone marrow reveals tandem cysteine-rich granulin domains. Proc Natl Acad Sci USA 1992; 89: 1715–19.

Borroni B, Alberici A, Cercignani M, Premi E, Serra L, Cerini C, et al. Granulin mutation drives brain damage and reorganization from preclinical to symptomatic FTLD. Neurobiol Aging 2012; 33: 2506–20.

Borroni B, Alberici A, Premi E, Archetti S, Garibotto V, Agosti C, et al. Brain magnetic resonance imaging structural changes in a pedigree of asymptomatic progranulin mutation carriers. Rejuvenation Res 2008; 11: 585–95.

Borroni B, Bonvicini C, Alberici A, Buratti E, Agosti C, Archetti S, et al. Mutation within TARDBP leads to frontotemporal dementia without motor neuron disease. Hum Mutat 2009; 30: E974–83.

Bossu P, Salani F, Alberici A, Archetti S, Bellelli G, Galimberti D, et al. Loss of function mutations in the progranulin gene are related to pro-inflammatory cytokine dysregulation in frontotemporal lobar degeneration patients. J Neuroinflammation 2011; 8: 65.

Brady OA, Zheng Y, Murphy K, Huang M, Hu F. The frontotemporal lobar degeneration risk factor, TMEM106B, regulates lysosomal morphology and function. Hum Mol Genet 2013; 22: 685–95.

Brouwers N, Nuytemans K, van der Zee J, Gijselinck I, Engelborghs S, Theuns J, et al. Alzheimer and Parkinson diagnoses in progranulin null mutation carriers in an extended founder family. Arch Neurol 2007; 64: 1436–46.

Brouwers N, Sleegers K, Engelborghs S, Maurer-Stroh S, Gijselinck I, van der Zee J, et al. Genetic variability in progranulin contributes to risk for clinically diagnosed Alzheimer disease. Neurology 2008; 71: 656–64.

Bruni AC, Momeni P, Bernardi L, Tomaino C, Frangipane F, Elder J, et al. Heterogeneity within a large kindred with frontotemporal dementia: a novel progranulin mutation. Neurology 2007; 69: 140–7.

Butler GS, Dean RA, Tam EM, Overall CM. Pharmacoproteomics of a metalloproteinase hydroxamate inhibitor in breast cancer cells: dynamics of membrane type 1 matrix metalloproteinase-mediated membrane protein shedding. Mol Cell Biol 2008; 28: 4896–914.

Byrnes KR, Washington PM, Knoblach SM, Hoffman E, Faden AI. Delayed inflammatory mRNA and protein expression after spinal cord injury. J Neuroinflammation 2011; 8: 130.

Cadieux B, Chitramuthu BP, Baranowski D, Bennett HP. The zebrafish progranulin gene family and antisense transcripts. BMC Genomics 2005; 6: 156.

Cairns NJ, Grossman M, Arnold SE, Burn DJ, Jaros E, Perry RH, et al. Clinical and neuropathologic variation in neuronal intermediate filament inclusion disease. Neurology 2004; 63: 1376–84.

Cannon A, Fujioka S, Rutherford NJ, Ferman TJ, Broderick DF, Boylan KB, et al. Clinicopathologic variability of the GRN A9D mutation, including amyotrophic lateral sclerosis. Neurology 2013; 80: 1771–7.

Capell A, Liebscher S, Fellerer K, Brouwers N, Willem M, Lammich S, et al. Rescue of progranulin deficiency associated with frontotemporal lobar degeneration by alkalizing reagents and inhibition of vacuolar ATPase. J Neurosci 2011; 31: 1885–94.

Carecchio M, Fenoglio C, De Riz M, Guidi I, Comi C, Cortini F, et al. Progranulin plasma levels as potential biomarker for the identification of GRN deletion carriers. A case with atypical onset as clinical amnestic mild cognitive impairment converted to Alzheimer's disease. J Neurol Sci 2009; 287: 291–3.

Caroppo P, Camuzat A, Guillot-Noel L, Thomas-Anterion C, Couratier P, Wong TH, et al. Defining the spectrum of frontotemporal dementias associated with TARDBP mutations. Neurol Genet 2016; 2: e80.

Caroppo P, Habert MO, Durrleman S, Funkiewiez A, Perlbarg V, Hahn V, et al. Lateral temporal lobe: an early imaging marker of the presymptomatic GRN disease? J Alzheimers Dis 2015; 47: 751–9.

Caroppo P, Le Ber I, Camuzat A, Clot F, Naccache L, Lamari F, et al. Extensive white matter involvement in patients with frontotemporal lobar degeneration: think progranulin. JAMA Neurol 2014; 71: 1562–6.

Carrasquillo MM, Nicholson AM, Finch N, Gibbs JR, Baker M, Rutherford NJ, et al. Genome-wide screen identifies rs646776 near sortilin as a regulator of progranulin levels in human plasma. Am J Hum Genet 2010; 87: 890–7.

Cascella R, Capitini C, Fani G, Dobson CM, Cecchi C, Chiti F. Quantification of the relative contributions of loss-of-function and

gain-of-function mechanisms in TAR DNA-binding protein 43 (TDP-43) proteinopathies. J Biol Chem 2016; 291: 19437–48.
Cenik B, Sephton CF, Dewey CM, Xian X, Wei S, Yu K, et al. Suberoylanilide hydroxamic acid (vorinostat) up-regulates progranulin transcription: rational therapeutic approach to frontotemporal dementia. J Biol Chem 2011; 286: 16101–8.
Chen X, Chang J, Deng Q, Xu J, Nguyen TA, Martens LH, et al. Progranulin does not bind tumor necrosis factor (TNF) receptors and is not a direct regulator of TNF-dependent signaling or bioactivity in immune or neuronal cells. J Neurosci 2013; 33: 9202–13.
Chen-Plotkin AS, Martinez-Lage M, Sleiman PM, Hu W, Greene R, Wood EM, et al. Genetic and clinical features of progranulin-associated frontotemporal lobar degeneration. Arch Neurol 2011; 68: 488–97.
Chen-Plotkin AS, Unger TL, Gallagher MD, Bill E, Kwong LK, Volpicelli-Daley L, et al. TMEM106B, the risk gene for frontotemporal dementia, is regulated by the microRNA-132/212 cluster and affects progranulin pathways. J Neurosci 2012; 32: 11213–27.
Chen-Plotkin AS, Xiao J, Geser F, Martinez-Lage M, Grossman M, Unger T, et al. Brain progranulin expression in GRN-associated frontotemporal lobar degeneration. Acta Neuropathol 2010; 119: 111–22.
Cheung ST, Cheung PF, Cheng CK, Wong NC, Fan ST. Granulin-epithelin precursor and ATP-dependent binding cassette (ABC)B5 regulate liver cancer cell chemoresistance. Gastroenterology 2011; 140: 344–55.
Cheung ST, Wong SY, Leung KL, Chen X, So S, Ng IO, et al. Granulin-epithelin precursor overexpression promotes growth and invasion of hepatocellular carcinoma. Clin Cancer Res 2004; 10: 7629–36.
Chitramuthu B, Bateman A. Progranulin and the receptor tyrosine kinase EphA2, partners in crime? J Cell Biol 2016; 215: 603–5.
Chitramuthu BP, Baranowski DC, Kay DG, Bateman A, Bennett HP. Progranulin modulates zebrafish motoneuron development *in vivo* and rescues truncation defects associated with knockdown of survival motor neuron 1. Mol Neurodegener 2010; 5: 41.
Chitramuthu BP, Kay DG, Bateman A, Bennett HP. Neurotrophic effects of progranulin *In Vivo* in reversing motor neuron defects caused by over or under expression of TDP-43 or FUS. PLoS One 2017; 12: e0174784.
Choi KM, Hwang SY, Hong HC, Choi HY, Yoo HJ, Youn BS, et al. Implications of C1q/TNF-related protein-3 (CTRP-3) and progranulin in patients with acute coronary syndrome and stable angina pectoris. Cardiovasc Diabetol 2014; 13: 14.
Cohen TJ, Lee VM, Trojanowski JQ. TDP-43 functions and pathogenic mechanisms implicated in TDP-43 proteinopathies. Trends Mol Med 2011; 17: 659–67.
Cruchaga C, Graff C, Chiang HH, Wang J, Hinrichs AL, Spiegel N, et al. Association of TMEM106B gene polymorphism with age at onset in granulin mutation carriers and plasma granulin protein levels. Arch Neurol 2011; 68: 581–6.
Cruts M, Engelborghs S, van der Zee J, Van Broeckhoven C. C9orf72-Related amyotrophic lateral sclerosis and frontotemporal dementia. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, et al, editors. GeneReviews(R). Seattle, WA: University of Washington, 2015.
Cruts M, Gijselinck I, van der Zee J, Engelborghs S, Wils H, Pirici D, et al. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. Nature 2006; 442: 920–4.
Cuevas-Antonio R, Cancino C, Arechavaleta-Velasco F, Andrade A, Barron L, Estrada I, et al. Expression of progranulin (Acrogranin/PCDGF/Granulin-Epithelin Precursor) in benign and malignant ovarian tumors and activation of MAPK signaling in ovarian cancer cell line. Cancer Invest 2010; 28: 452–8.
Daniel R, Daniels E, He Z, Bateman A. Progranulin (acrogranin/PC cell-derived growth factor/granulin-epithelin precursor) is expressed in the placenta, epidermis, microvasculature, and brain during murine development. Dev Dyn 2003; 227: 593–9.
Daniel R, He Z, Carmichael KP, Halper J, Bateman A. Cellular localization of gene expression for progranulin. J Histochem Cytochem 2000; 48: 999–1009.
de la Encarnacion A, Alquezar C, Martin-Requero A. Increased Wnt signaling and reduced viability in a neuronal model of progranulin-deficient frontotemporal lobar degeneration. Mol Neurobiol 2016; 53: 7107–18.
De Muynck L, Herdewyn S, Beel S, Scheveneels W, Van Den Bosch L, Robberecht W, et al. The neurotrophic properties of progranulin depend on the granulin E domain but do not require sortilin binding. Neurobiol Aging 2013; 34: 2541–7.
De Muynck L, Van Damme P. Cellular effects of progranulin in health and disease. J Mol Neurosci 2011; 45: 549–60.
Del Bo R, Corti S, Santoro D, Ghione I, Fenoglio C, Ghezzi S, et al. No major progranulin genetic variability contribution to disease etiopathogenesis in an ALS Italian cohort. Neurobiol Aging 2011; 32: 1157–8.
Deng HX, Chen W, Hong ST, Boycott KM, Gorrie GH, Siddique N, et al. Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. Nature 2011; 477: 211–15.
Dickson DW, Baker M, Rademakers R. Common variant in GRN is a genetic risk factor for hippocampal sclerosis in the elderly. Neurodegener Dis 2010; 7: 170–4.
Donald CD, Laddu A, Chandham P, Lim SD, Cohen C, Amin M, et al. Expression of progranulin and the epithelin/granulin precursor acrogranin correlates with neoplastic state in renal epithelium. Anticancer Res 2001; 21: 3739–42.
Dopper EG, Chalos V, Ghariq E, den Heijer T, Hafkemeijer A, Jiskoot LC, et al. Cerebral blood flow in presymptomatic MAPT and GRN mutation carriers: a longitudinal arterial spin labeling study. Neuroimage Clin 2016; 12: 460–5.
Dormann D, Capell A, Carlson AM, Shankaran SS, Rodde R, Neumann M, et al. Proteolytic processing of TAR DNA binding protein-43 by caspases produces C-terminal fragments with disease defining properties independent of progranulin. J Neurochem 2009; 110: 1082–94.
Egashira Y, Suzuki Y, Azuma Y, Takagi T, Mishiro K, Sugitani S, et al. The growth factor progranulin attenuates neuronal injury induced by cerebral ischemia-reperfusion through the suppression of neutrophil recruitment. J Neuroinflammation 2013; 10: 105.
Egland KA, Vincent JJ, Strausberg R, Lee B, Pastan I. Discovery of the breast cancer gene BASE using a molecular approach to enrich for genes encoding membrane and secreted proteins. Proc Natl Acad Sci USA 2003; 100: 1099–104.
Elkabets M, Gifford AM, Scheel C, Nilsson B, Reinhardt F, Bray MA, et al. Human tumors instigate granulin-expressing hematopoietic cells that promote malignancy by activating stromal fibroblasts in mice. J Clin Invest 2011; 121: 784–99.
Emanuele E. Can trehalose prevent neurodegeneration? Insights from experimental studies. Curr Drug Targets 2014; 15: 551–7.
Etemadi N, Webb A, Bankovacki A, Silke J, Nachbur U. Progranulin does not inhibit TNF and lymphotoxin-alpha signalling through TNF receptor 1. Immunol Cell Biol 2013; 91: 661–4.
Fenoglio C, Scalabrini D, Esposito F, Comi C, Cavalla P, De Riz M, et al. Progranulin gene variability increases the risk for primary progressive multiple sclerosis in males. Genes Immun 2010; 11: 497–503.
Filiano AJ, Martens LH, Young AH, Warmus BA, Zhou P, Diaz-Ramirez G, et al. Dissociation of frontotemporal dementia-related deficits and neuroinflammation in progranulin haploinsufficient mice. J Neurosci 2013; 33: 5352–61.
Finch N, Baker M, Crook R, Swanson K, Kuntz K, Surtees R, et al. Plasma progranulin levels predict progranulin mutation status in frontotemporal dementia patients and asymptomatic family members. Brain 2009; 132 (Pt 3): 583–91.

Finch N, Carrasquillo MM, Baker M, Rutherford NJ, Coppola G, Dejesus-Hernandez M, et al. TMEM106B regulates progranulin levels and the penetrance of FTLD in GRN mutation carriers. Neurology 2011; 76: 467–74.

Finès P. Estimating costs of health care for neurological conditions in Canada in 2031. Statistics Canada Report, 2015. Ottawa: Statistics Canada Health Analysis Division; Available from: https://www.cahspr.ca/en/presentation/5574e17837dee87418501956

Forman MS, Mackenzie IR, Cairns NJ, Swanson E, Boyer PJ, Drachman DA, et al. Novel ubiquitin neuropathology in frontotemporal dementia with valosin-containing protein gene mutations. J Neuropathol Exp Neurol 2006; 65: 571–81.

Galimberti D, D'Addario C, Dell'osso B, Fenoglio C, Marcone A, Cerami C, et al. Progranulin gene (GRN) promoter methylation is increased in patients with sporadic frontotemporal lobar degeneration. Neurol Sci 2013; 34: 899–903.

Galimberti D, Prunas C, Paoli RA, Dell'Osso B, Fenoglio C, Villa C, et al. Progranulin gene variability influences the risk for bipolar I disorder, but not bipolar II disorder. Bipolar Disord 2014; 16: 769–72.

Gao X, Joselin AP, Wang L, Kar A, Ray P, Bateman A, et al. Progranulin promotes neurite outgrowth and neuronal differentiation by regulating GSK-3beta. Protein Cell 2010; 1: 552–62.

Gascon E, Gao FB. The emerging roles of microRNAs in the pathogenesis of frontotemporal dementia-amyotrophic lateral sclerosis (FTD-ALS) spectrum disorders. Journal of neurogenetics 2014; 28: 30–40.

Gass J, Cannon A, Mackenzie IR, Boeve B, Baker M, Adamson J, et al. Mutations in progranulin are a major cause of ubiquitin-positive frontotemporal lobar degeneration. Hum Mol Genet 2006; 15: 2988–3001.

Gass J, Lee WC, Cook C, Finch N, Stetler C, Jansen-West K, et al. Progranulin regulates neuronal outgrowth independent of sortilin. Mol Neurodegener 2012; 7: 33.

Ghetti B, Spina S, Murrell JR, Huey ED, Pietrini P, Sweeney B, et al. *In vivo* and postmortem clinicoanatomical correlations in frontotemporal dementia and parkinsonism linked to chromosome 17. Neurodegener Dis 2008; 5: 215–17.

Ghidoni R, Benussi L, Glionna M, Franzoni M, Binetti G. Low plasma progranulin levels predict progranulin mutations in frontotemporal lobar degeneration. Neurology 2008; 71: 1235–9.

Ghoshal N, Dearborn JT, Wozniak DF, Cairns NJ. Core features of frontotemporal dementia recapitulated in progranulin knockout mice. Neurobiol Dis 2012; 45: 395–408.

Gijselinck I, van der Zee J, Engelborghs S, Goossens D, Peeters K, Mattheijssens M, et al. Progranulin locus deletion in frontotemporal dementia. Hum Mutat 2008; 29: 53–8.

Giorgetti E, Rusmini P, Crippa V, Cristofani R, Boncoraglio A, Cicardi ME, et al. Synergic prodegradative activity of bicalutamide and trehalose on the mutant androgen receptor responsible for spinal and bulbar muscular atrophy. Hum Mol Genet 2015; 24: 64–75.

Gliebus G, Rosso A, Lippa CF. Progranulin and beta-amyloid distribution: a case report of the brain from preclinical PS-1 mutation carrier. Am J Alzheimers Dis Other Demen 2009; 24: 456–60.

Gonzalez EM, Mongiat M, Slater SJ, Baffa R, Iozzo RV. A novel interaction between perlecan protein core and progranulin: potential effects on tumor growth. J Biol Chem 2003; 278: 38113–16.

Gotzl JK, Lang CM, Haass C, Capell A. Impaired protein degradation in FTLD and related disorders. Ageing Res Rev 2016; 32: 122–39.

Gowrishankar S, Yuan P, Wu Y, Schrag M, Paradise S, Grutzendler J, et al. Massive accumulation of luminal protease-deficient axonal lysosomes at Alzheimer's disease amyloid plaques. Proc Natl Acad Sci USA 2015; 112: E3699–708.

Guerra RR, Kriazhev L, Hernandez-Blazquez FJ, Bateman A. Progranulin is a stress-response factor in fibroblasts subjected to hypoxia and acidosis. Growth Factors 2007; 25: 280–5.

Guerreiro RJ, Washecka N, Hardy J, Singleton A. A thorough assessment of benign genetic variability in GRN and MAPT. Hum Mutat 2010; 31: E1126–40.

Guo A, Tapia L, Bamji SX, Cynader MS, Jia W. Progranulin deficiency leads to enhanced cell vulnerability and TDP-43 translocation in primary neuronal cultures. Brain Res 2010; 1366: 1–8.

Guo Z, Li Q, Han Y, Liang Y, Xu Z, Ren T. Prevention of LPS-induced acute lung injury in mice by progranulin. Mediators Inflamm 2012; 2012: 540794.

Hanson KA, Kim SH, Tibbetts RS. RNA-binding proteins in neurodegenerative disease: TDP-43 and beyond. Wiley Interdiscip Rev RNA 2012; 3: 265–85.

He Z, Bateman A. Progranulin gene expression regulates epithelial cell growth and promotes tumor growth *in vivo*. Cancer Res 1999; 59: 3222–9.

He Z, Bateman A. Progranulin (granulin-epithelin precursor, PC-cell-derived growth factor, acrogranin) mediates tissue repair and tumorigenesis. J Mol Med 2003; 81: 600–12.

He Z, Ismail A, Kriazhev L, Sadvakassova G, Bateman A. Progranulin (PC-cell-derived growth factor/acrogranin) regulates invasion and cell survival. Cancer Res 2002; 62: 5590–6.

He Z, Ong CH, Halper J, Bateman A. Progranulin is a mediator of the wound response. Nat Med 2003; 9: 225–9.

Ho JC, Ip YC, Cheung ST, Lee YT, Chan KF, Wong SY, et al. Granulin-epithelin precursor as a therapeutic target for hepatocellular carcinoma. Hepatology 2008; 47: 1524–32.

Holler CJ, Taylor G, McEachin ZT, Deng Q, Watkins WJ, Hudson K, et al. Trehalose upregulates progranulin expression in human and mouse models of GRN haploinsufficiency: a novel therapeutic lead to treat frontotemporal dementia. Mol Neurodegener 2016; 11: 46.

Holm IE, Englund E, Mackenzie IR, Johannsen P, Isaacs AM. A reassessment of the neuropathology of frontotemporal dementia linked to chromosome 3. J Neuropathol Exp Neurol 2007; 66: 884–91.

Hosokawa M, Arai T, Masuda-Suzukake M, Kondo H, Matsuwaki T, Nishihara M, et al. Progranulin reduction is associated with increased tau phosphorylation in P301L tau transgenic mice. J Neuropathol Exp Neurol 2015; 74: 158–65.

Hrabal R, Chen Z, James S, Bennett HP, Ni F. The hairpin stack fold, a novel protein architecture for a new family of protein growth factors. Nat Struct Biol 1996; 3: 747–52.

Hsiung GY, Fok A, Feldman HH, Rademakers R, Mackenzie IR. rs5848 polymorphism and serum progranulin level. J Neurol Sci 2011; 300: 28–32.

Hsiung GYR, Feldman HH. GRN-related frontotemporal dementia. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, et al, editors. GeneReviews(R). Seattle, WA: University of Washington, 2013.

Hu F, Padukkavidana T, Vaegter CB, Brady OA, Zheng Y, Mackenzie IR, et al. Sortilin-mediated endocytosis determines levels of the frontotemporal dementia protein, progranulin. Neuron 2010; 68: 654–67.

Huang K, Chen A, Zhang X, Song Z, Xu H, Cao J, et al. Progranulin is preferentially expressed in patients with psoriasis vulgaris and protects mice from psoriasis-like skin inflammation. Immunology 2015; 145: 279–87.

Igaz LM, Kwong LK, Chen-Plotkin A, Winton MJ, Unger TL, Xu Y, et al. Expression of TDP-43 C-terminal fragments *in Vitro* recapitulates pathological features of TDP-43 proteinopathies. J Biol Chem 2009; 284: 8516–24.

Irwin D, Lippa CF, Rosso A. Progranulin (PGRN) expression in ALS: an immunohistochemical study. J Neurol Sci 2009; 276: 9–13.

Jackman K, Kahles T, Lane D, Garcia-Bonilla L, Abe T, Capone C, et al. Progranulin deficiency promotes post-ischemic blood-brain barrier disruption. J Neurosci 2013; 33: 19579–89.

Jacova C, Hsiung GY, Tawankanjanachot I, Dinelle K, McCormick S, Gonzalez M, et al. Anterior brain glucose hypometabolism predates dementia in progranulin mutation carriers. Neurology 2013; 81: 1322–31.

Janssens J, Philtjens S, Kleinberger G, Van Mossevelde S, van der Zee J, Cacace R, et al. Investigating the role of filamin C in Belgian patients with frontotemporal dementia linked to GRN deficiency in FTLD-TDP brains. Acta Neuropathol Commun 2015; 3: 68.

Jian J, Konopka J, Liu C. Insights into the role of progranulin in immunity, infection, and inflammation. J Leukoc Biol 2013; 93: 199–208.

Jiao J, Herl LD, Farese RV, Gao FB. MicroRNA-29b regulates the expression level of human progranulin, a secreted glycoprotein implicated in frontotemporal dementia. PLoS One 2010; 5: e10551.

Jones MB, Houwink AP, Freeman BK, Greenwood TM, Lafky JM, Lingle WL, et al. The granulin-epithelin precursor is a steroid-regulated growth factor in endometrial cancer. J Soc Gynecol Investig 2006; 13: 304–11.

Jones MB, Spooner M, Kohn EC. The granulin-epithelin precursor: a putative new growth factor for ovarian cancer. Gynecol Oncol 2003; 88 (1 Pt 2): S136–9.

Josephs KA, Ahmed Z, Katsuse O, Parisi JF, Boeve BF, Knopman DS, et al. Neuropathologic features of frontotemporal lobar degeneration with ubiquitin-positive inclusions with progranulin gene (PGRN) mutations. J Neuropathol Exp Neurol 2007; 66: 142–51.

Kamrava M, Simpkins F, Alejandro E, Michener C, Meltzer E, Kohn EC. Lysophosphatidic acid and endothelin-induced proliferation of ovarian cancer cell lines is mitigated by neutralization of granulin-epithelin precursor (GEP), a prosurvival factor for ovarian cancer. Oncogene 2005; 24: 7084–93.

Kanazawa M, Kawamura K, Takahashi T, Miura M, Tanaka Y, Koyama M, et al. Multiple therapeutic effects of progranulin on experimental acute ischaemic stroke. Brain 2015; 138 (Pt 7): 1932–48.

Kao AW, Eisenhut RJ, Martens LH, Nakamura A, Huang A, Bagley JA, et al. A neurodegenerative disease mutation that accelerates the clearance of apoptotic cells. Proc Natl Acad Sci USA 2011; 108: 4441–6.

Karch CM, Jeng AT, Skorupa T, Cruchaga C, Goate AM. Novel progranulin variants do not disrupt progranulin secretion and cleavage. Neurobiol Aging 2013; 34: 2538–40.

Kayasuga Y, Chiba S, Suzuki M, Kikusui T, Matsuwaki T, Yamanouchi K, et al. Alteration of behavioural phenotype in mice by targeted disruption of the progranulin gene. Behav Brain Res 2007; 185: 110–18.

Kelley BJ, Haidar W, Boeve BF, Baker M, Shiung M, Knopman DS, et al. Alzheimer disease-like phenotype associated with the c.154delA mutation in progranulin. Arch Neurol 2010; 67: 171–7.

Kessenbrock K, Frohlich L, Sixt M, Lammermann T, Pfister H, Bateman A, et al. Proteinase 3 and neutrophil elastase enhance inflammation in mice by inactivating antiinflammatory progranulin. J Clin Invest 2008; 118: 2438–47.

Kim CH, Cheong JH, Kim JM. Correlation of granulin expression in intracranial meningiomas to clinical parameters. Exp Ther Med 2010; 1: 493–6.

Kim HK, Shin MS, Youn BS, Namkoong C, Gil SY, Kang GM, et al. Involvement of progranulin in hypothalamic glucose sensing and feeding regulation. Endocrinology 2011; 152: 4672–82.

Kleinberger G, Wils H, Ponsaerts P, Joris G, Timmermans JP, Van Broeckhoven C, et al. Increased caspase activation and decreased TDP-43 solubility in progranulin knockout cortical cultures. J Neurochem 2010; 115: 735–47.

Kovacs GG, van der Zee J, Hort J, Kristoferitsch W, Leitha T, Hoftberger R, et al. Clinicopathological description of two cases with SQSTM1 gene mutation associated with frontotemporal dementia. Neuropathology 2016; 36: 27–38.

Laird AS, Van Hoecke A, De Muynck L, Timmers M, Van den Bosch L, Van Damme P, et al. Progranulin is neurotrophic *in vivo* and protects against a mutant TDP-43 induced axonopathy. PLoS One 2010; 5: e13368.

Lang CM, Fellerer K, Schwenk BM, Kuhn PH, Kremmer E, Edbauer D, et al. Membrane orientation and subcellular localization of transmembrane protein 106B (TMEM106B), a major risk factor for frontotemporal lobar degeneration. J Biol Chem 2012; 287: 19355–65.

Le Ber I, Camuzat A, Guerreiro R, Bouya-Ahmed K, Bras J, Nicolas G, et al. SQSTM1 mutations in French patients with frontotemporal dementia or frontotemporal dementia with amyotrophic lateral sclerosis. JAMA Neurol 2013; 70: 1403–10.

Le Ber I, Camuzat A, Hannequin D, Pasquier F, Guedj E, Rovelet-Lecrux A, et al. Phenotype variability in progranulin mutation carriers: a clinical, neuropsychological, imaging and genetic study. Brain 2008; 131 (Pt 3): 732–46.

Le Ber I, van der Zee J, Hannequin D, Gijselinck I, Campion D, Puel M, et al. Progranulin null mutations in both sporadic and familial frontotemporal dementia. Hum Mutat 2007; 28: 846–55.

Lee WC, Almeida S, Prudencio M, Caulfield TR, Zhang YJ, Tay WM, et al. Targeted manipulation of the sortilin-progranulin axis rescues progranulin haploinsufficiency. Hum Mol Genet 2014; 23: 1467–78.

Leerkes MR, Caballero OL, Mackay A, Torloni H, O'Hare MJ, Simpson AJ, et al. In silico comparison of the transcriptome derived from purified normal breast cells and breast tumor cell lines reveals candidate upregulated genes in breast tumor cells. Genomics 2002; 79: 257–65.

Li S, Chen Y, Sun D, Bai R, Gao X, Yang Y, et al. Angiogenin prevents progranulin A9D mutation-induced neuronal-like cell apoptosis through cleaving tRNAs into tiRNAs. Mol Neurobiol 2017.

Li Y, Guo Y, Wang X, Yu X, Duan W, Hong K, et al. Trehalose decreases mutant SOD1 expression and alleviates motor deficiency in early but not end-stage amyotrophic lateral sclerosis in a SOD1-G93A mouse model. Neuroscience 2015; 298: 12–25.

Li YH, Chen HY, Li YW, Wu SY, Wangta L, Lin GH, et al. Progranulin regulates zebrafish muscle growth and regeneration through maintaining the pool of myogenic progenitor cells. Sci Rep 2013; 3: 1176.

Li YH, Chen MH, Gong HY, Hu SY, Li YW, Lin GH, et al. Progranulin A-mediated MET signaling is essential for liver morphogenesis in zebrafish. J Biol Chem 2010; 285: 41001–9.

Liau LM, Lallone RL, Seitz RS, Buznikov A, Gregg JP, Kornblum HI, et al. Identification of a human glioma-associated growth factor gene, granulin, using differential immuno-absorption. Cancer Res 2000; 60: 1353–60.

Lim HY, Albuquerque B, Haussler A, Myrczek T, Ding A, Tegeder I. Progranulin contributes to endogenous mechanisms of pain defense after nerve injury in mice. J Cell Mol Med 2012; 16: 708–21.

Liu CJ. Progranulin: a promising therapeutic target for rheumatoid arthritis. FEBS Lett 2011; 585: 3675–80.

Liu CJ, Bosch X. Progranulin: a growth factor, a novel TNFR ligand and a drug target. Pharmacol Ther 2012; 133: 124–32.

Liu Y, Xi L, Liao G, Wang W, Tian X, Wang B, et al. Inhibition of PC cell-derived growth factor (PCDGF)/granulin-epithelin precursor (GEP) decreased cell proliferation and invasion through downregulation of cyclin D and CDK4 and inactivation of MMP-2. BMC Cancer 2007; 7: 22.

Lomen-Hoerth C, Anderson T, Miller B. The overlap of amyotrophic lateral sclerosis and frontotemporal dementia. Neurology 2002; 59: 1077–9.

Lu L, Luo L, Lu Y, Chen L, Xu J, Guo K. Progranulin expression in neural stem cells and their differentiated cell lineages: an immunocytochemical study. Mol Med Rep 2013; 8: 1359–64.

Lu R, Serrero G. Mediation of estrogen mitogenic effect in human breast cancer MCF-7 cells by PC-cell-derived growth factor (PCDGF/granulin precursor). Proc Natl Acad Sci USA 2001; 98: 142–7.

Lui H, Zhang J, Makinson SR, Cahill MK, Kelley KW, Huang HY, et al. Progranulin deficiency promotes circuit-specific synaptic pruning by microglia via complement activation. Cell 2016; 165: 921–35.

Mackenzie IR, Baker M, Pickering-Brown S, Hsiung GY, Lindholm C, Dwosh E, et al. The neuropathology of frontotemporal lobar degeneration caused by mutations in the progranulin gene. Brain 2006; 129 (Pt 11): 3081–90.

Mackenzie IR, Neumann M. Molecular neuropathology of frontotemporal dementia: insights into disease mechanisms from postmortem studies. J Neurochem 2016; 138 (Suppl 1): 54–70.

Mackenzie IR, Neumann M, Baborie A, Sampathu DM, Du Plessis D, Jaros E, et al. A harmonized classification system for FTLD-TDP pathology. Acta Neuropathol 2011; 122: 111–13.

Malaspina A, Kaushik N, de Belleroche J. Differential expression of 14 genes in amyotrophic lateral sclerosis spinal cord detected using gridded cDNA arrays. J Neurochem 2001; 77: 132–45.

Martens LH, Zhang J, Barmada SJ, Zhou P, Kamiya S, Sun B, et al. Progranulin deficiency promotes neuroinflammation and neuron loss following toxin-induced injury. J Clin Invest 2012; 122: 3955–9.

Mason AR, Elia LP, Finkbeiner S. The receptor-interacting serine/threonine protein kinase 1 (RIPK1) regulates progranulin levels. J Biol Chem 2017; 292: 3262–72.

Matsumura N, Mandai M, Miyanishi M, Fukuhara K, Baba T, Higuchi T, et al. Oncogenic property of acrogranin in human uterine leiomyosarcoma: direct evidence of genetic contribution in *in vivo* tumorigenesis. Clin Cancer Res 2006; 12: 1402–11.

Matsuwaki T, Asakura R, Suzuki M, Yamanouchi K, Nishihara M. Age-dependent changes in progranulin expression in the mouse brain. J Reprod Dev 2011; 57: 113–19.

Miller ZA, Rankin KP, Graff-Radford NR, Takada LT, Sturm VE, Cleveland CM, et al. TDP-43 frontotemporal lobar degeneration and autoimmune disease. J Neurol Neurosurg Psychiatry 2013; 84: 956–62.

Minami SS, Min SW, Krabbe G, Wang C, Zhou Y, Asgarov R, et al. Progranulin protects against amyloid beta deposition and toxicity in Alzheimer's disease mouse models. Nat Med 2014; 20: 1157–64.

Minami SS, Shen V, Le D, Krabbe G, Asgarov R, Perez-Celajes L, et al. Reducing inflammation and rescuing FTD-related behavioral deficits in progranulin-deficient mice with alpha7 nicotinic acetylcholine receptor agonists. Biochem Pharmacol 2015; 97: 454–62.

Miyanishi M, Mandai M, Matsumura N, Yamaguchi K, Hamanishi J, Higuchi T, et al. Immortalized ovarian surface epithelial cells acquire tumorigenicity by Acrogranin gene overexpression. Oncol Rep 2007; 17: 329–33.

Moisse K, Volkening K, Leystra-Lantz C, Welch I, Hill T, Strong MJ. Divergent patterns of cytosolic TDP-43 and neuronal progranulin expression following axotomy: implications for TDP-43 in the physiological response to neuronal injury. Brain Res 2009; 1249: 202–11.

Momeni P, DeTucci K, Straub RE, Weinberger DR, Davies P, Grafman J, et al. Progranulin (GRN) in two siblings of a Latino family and in other patients with schizophrenia. Neurocase 2010; 16: 273–9.

Monami G, Emiliozzi V, Bitto A, Lovat F, Xu SQ, Goldoni S, et al. Proepithelin regulates prostate cancer cell biology by promoting cell growth, migration, and anchorage-independent growth. Am J Pathol 2009; 174: 1037–47.

Monami G, Gonzalez EM, Hellman M, Gomella LG, Baffa R, Iozzo RV, et al. Proepithelin promotes migration and invasion of 5637 bladder cancer cells through the activation of ERK1/2 and the formation of a paxillin/FAK/ERK complex. Cancer Res 2006; 66: 7103–10.

Morris HR, Waite AJ, Williams NM, Neal JW, Blake DJ. Recent advances in the genetics of the ALS-FTLD complex. Curr Neurol Neurosci Rep 2012; 12: 243–50.

Mottamal M, Zheng S, Huang TL, Wang G. Histone deacetylase inhibitors in clinical studies as templates for new anticancer agents. Molecules 2015; 20: 3898–941.

Mukherjee O, Pastor P, Cairns NJ, Chakraverty S, Kauwe JS, Shears S, et al. HDDD2 is a familial frontotemporal lobar degeneration with ubiquitin-positive, tau-negative inclusions caused by a missense mutation in the signal peptide of progranulin. Ann Neurol 2006; 60: 314–22.

Naphade SB, Kigerl KA, Jakeman LB, Kostyk SK, Popovich PG, Kuret J. Progranulin expression is upregulated after spinal contusion in mice. Acta Neuropathol 2010; 119: 123–33.

Nedachi T, Kawai T, Matsuwaki T, Yamanouchi K, Nishihara M. Progranulin enhances neural progenitor cell proliferation through glycogen synthase kinase 3beta phosphorylation. Neuroscience 2011; 185: 106–15.

Neill T, Buraschi S, Goyal A, Sharpe C, Natkanski E, Schaefer L, et al. EphA2 is a functional receptor for the growth factor progranulin. J Cell Biol 2016; 215: 687–703.

Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006; 314: 130–3.

Nicholson AM, Finch NA, Almeida M, Perkerson RB, van Blitterswijk M, Wojtas A, et al. Prosaposin is a regulator of progranulin levels and oligomerization. Nat Commun 2016; 7: 11992.

Nuytemans K, Pals P, Sleegers K, Engelborghs S, Corsmit E, Peeters K, et al. Progranulin variability has no major role in Parkinson disease genetic etiology. Neurology 2008; 71: 1147–51.

Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P, Nielsen MS, et al. Sortilin is essential for proNGF-induced neuronal cell death. Nature 2004; 427: 843–8.

Ogaki K, Li Y, Takanashi M, Ishikawa K, Kobayashi T, Nonaka T, et al. Analyses of the MAPT, PGRN, and C9orf72 mutations in Japanese patients with FTLD, PSP, and CBS. Parkinsonism Relat Disord 2013; 19: 15–20.

Onyike CU, Diehl-Schmid J. The epidemiology of frontotemporal dementia. Int Rev Psychiatry 2013; 25: 130–7.

Palfree RG, Bennett HP, Bateman A. The evolution of the secreted regulatory protein progranulin. PLoS One 2015; 10: e0133749.

Park B, Buti L, Lee S, Matsuwaki T, Spooner E, Brinkmann MM, et al. Granulin is a soluble cofactor for toll-like receptor 9 signaling. Immunity 2011; 34: 505–13.

Pereson S, Wils H, Kleinberger G, McGowan E, Vandewoestyne M, Van Broeck B, et al. Progranulin expression correlates with dense-core amyloid plaque burden in Alzheimer disease mouse models. J Pathol 2009; 219: 173–81.

Perry DC, Lehmann M, Yokoyama JS, Karydas A, Lee JJ, Coppola G, et al. Progranulin mutations as risk factors for Alzheimer disease. JAMA Neurol 2013; 70: 774–8.

Petkau TL, Neal SJ, Milnerwood A, Mew A, Hill AM, Orban P, et al. Synaptic dysfunction in progranulin-deficient mice. Neurobiol Dis 2012; 45: 711–22.

Petkau TL, Neal SJ, Orban PC, MacDonald JL, Hill AM, Lu G, et al. Progranulin expression in the developing and adult murine brain. J Comp Neurol 2010; 518: 3931–47.

Philips T, De Muynck L, Thu HN, Weynants B, Vanacker P, Dhondt J, et al. Microglial upregulation of progranulin as a marker of motor neuron degeneration. J Neuropathol Exp Neurol 2010; 69: 1191–200.

Pickford F, Marcus J, Camargo LM, Xiao Q, Graham D, Mo JR, et al. Progranulin is a chemoattractant for microglia and stimulates their endocytic activity. Am J Pathol 2011; 178: 284–95.

Pievani M, Paternico D, Benussi L, Binetti G, Orlandini A, Cobelli M, et al. Pattern of structural and functional brain abnormalities in asymptomatic granulin mutation carriers. Alzheimers Dement 2014; 10 (Suppl 5): S354–63.e1.

Plowman GD, Green JM, Neubauer MG, Buckley SD, McDonald VL, Todaro GJ, et al. The epithelin precursor encodes two proteins with opposing activities on epithelial cell growth. J Biol Chem 1992; 267: 13073–8.

Pottier C, Bieniek KF, Finch N, van de Vorst M, Baker M, Perkersen R, et al. Whole-genome sequencing reveals important role for TBK1 and OPTN mutations in frontotemporal lobar degeneration without motor neuron disease. Acta Neuropathol 2015; 130: 77–92.

Premi E, Cauda F, Gasparotti R, Diano M, Archetti S, Padovani A, et al. Multimodal FMRI resting-state functional connectivity in granulin mutations: the case of fronto-parietal dementia. PLoS One 2014; 9: e106500.

Premi E, Grassi M, Gazzina S, Paghera B, Pepe D, Archetti S, et al. The neuroimaging signature of frontotemporal lobar degeneration associated with Granulin mutations: an effective connectivity study. J Nucl Med 2013; 54: 1066–71.

Premi E, Padovani A, Borroni B. Frontotemporal lobar degeneration. Adv Exp Med Biol 2012; 724: 114–27.

Rademakers R, Baker M, Gass J, Adamson J, Huey ED, Momeni P, et al. Phenotypic variability associated with progranulin haploinsufficiency in patients with the common 1477C–>T (Arg493X) mutation: an international initiative. Lancet Neurol 2007; 6: 857–68.

Rademakers R, Eriksen JL, Baker M, Robinson T, Ahmed Z, Lincoln SJ, et al. Common variation in the miR-659 binding-site of GRN is a major risk factor for TDP43-positive frontotemporal dementia. Hum Mol Genet 2008; 17: 3631–42.

Raitano S, Ordovas L, De Muynck L, Guo W, Espuny-Camacho I, Geraerts M, et al. Restoration of progranulin expression rescues cortical neuron generation in an induced pluripotent stem cell model of frontotemporal dementia. Stem Cell Reports 2015; 4: 16–24.

Redaelli V, Rossi G, Maderna E, Kovacs GG, Piccoli E, Caroppo P, et al. Alzheimer neuropathology without frontotemporal lobar degeneration hallmarks (TAR DNA-binding protein 43 inclusions) in missense progranulin mutation Cys139Arg. Brain Pathol 2017. doi: 10.1111/bpa.12480.

Revuelta GJ, Rosso A, Lippa CF. Association between progranulin and beta-amyloid in dementia with Lewy bodies. Am J Alzheimers Dis Other Demen 2008; 23: 488–93.

Rhinn H, Abeliovich A. Differential aging analysis in human cerebral cortex identifies variants in TMEM106B and GRN that regulate aging phenotypes. Cell Syst 2017; 4: 404–15.e5.

Riedl L, Mackenzie IR, Forstl H, Kurz A, Diehl-Schmid J. Frontotemporal lobar degeneration: current perspectives. Neuropsychiatr Dis Treat 2014; 10: 297–310.

Roczniak-Ferguson A, Petit CS, Froehlich F, Qian S, Ky J, Angarola B, et al. The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis. Sci Signal 2012; 5: ra42.

Rohrer JD, Isaacs AM, Mizielinska S, Mead S, Lashley T, Wray S, et al. C9orf72 expansions in frontotemporal dementia and amyotrophic lateral sclerosis. Lancet Neurol 2015a; 14: 291–301.

Rohrer JD, Nicholas JM, Cash DM, van Swieten J, Dopper E, Jiskoot L, et al. Presymptomatic cognitive and neuroanatomical changes in genetic frontotemporal dementia in the Genetic Frontotemporal dementia Initiative (GENFI) study: a cross-sectional analysis. Lancet Neurol 2015b; 14: 253–62.

Rohrer JD, Ridgway GR, Modat M, Ourselin S, Mead S, Fox NC, et al. Distinct profiles of brain atrophy in frontotemporal lobar degeneration caused by progranulin and tau mutations. Neuroimage 2010; 53: 1070–6.

Rosen EY, Wexler EM, Versano R, Coppola G, Gao F, Winden KD, et al. Functional genomic analyses identify pathways dysregulated by progranulin deficiency, implicating Wnt signaling. Neuron 2011; 71: 1030–42.

Rossi A, Kontarakis Z, Gerri C, Nolte H, Holper S, Kruger M, et al. Genetic compensation induced by deleterious mutations but not gene knockdowns. Nature 2015; 524: 230–3.

Rovelet-Lecrux A, Deramecourt V, Legallic S, Maurage CA, Le Ber I, Brice A, et al. Deletion of the progranulin gene in patients with frontotemporal lobar degeneration or Parkinson disease. Neurobiol Dis 2008; 31: 41–5.

Ryan CL, Baranowski DC, Chitramuthu BP, Malik S, Li Z, Cao M, et al. Progranulin is expressed within motor neurons and promotes neuronal cell survival. BMC Neurosci 2009; 10: 130.

Saftig P, Klumperman J. Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. Nat Rev Mol Cell Biol 2009; 10: 623–35.

Salazar DA, Butler VJ, Argouarch AR, Hsu TY, Mason A, Nakamura A, et al. The progranulin cleavage products, granulins, exacerbate TDP-43 toxicity and increase TDP-43 levels. J Neurosci 2015; 35: 9315–28.

Schofield EC, Halliday GM, Kwok J, Loy C, Double KL, Hodges JR. Low serum progranulin predicts the presence of mutations: a prospective study. J Alzheimers Dis 2010; 22: 981–4.

Scholtzova H, Chianchiano P, Pan J, Sun Y, Goni F, Mehta PD, et al. Amyloid beta and Tau Alzheimer's disease related pathology is reduced by Toll-like receptor 9 stimulation. Acta Neuropathol Commun 2014; 2: 101.

Schymick JC, Yang Y, Andersen PM, Vonsattel JP, Greenway M, Momeni P, et al. Progranulin mutations and amyotrophic lateral sclerosis or amyotrophic lateral sclerosis-frontotemporal dementia phenotypes. J Neurol Neurosurg Psychiatry 2007; 78: 754–6.

Scotter EL, Vance C, Nishimura AL, Lee YB, Chen HJ, Urwin H, et al. Differential roles of the ubiquitin proteasome system and autophagy in the clearance of soluble and aggregated TDP-43 species. J Cell Sci 2014; 127 (Pt 6): 1263–78.

Seltman RE, Matthews BR. Frontotemporal lobar degeneration: epidemiology, pathology, diagnosis and management. CNS Drugs 2012; 26: 841–70.

Serrero G, Ioffe OB. Expression of PC-cell-derived growth factor in benign and malignant human breast epithelium. Hum Pathol 2003; 34: 1148–54.

Sfikakis PP, Tsokos GC. Towards the next generation of anti-TNF drugs. Clin Immunol 2011; 141: 231–5.

Shankaran SS, Capell A, Hruscha AT, Fellerer K, Neumann M, Schmid B, et al. Missense mutations in the progranulin gene linked to frontotemporal lobar degeneration with ubiquitin-immunoreactive inclusions reduce progranulin production and secretion. J Biol Chem 2008; 283: 1744–53.

Shoyab M, McDonald VL, Byles C, Todaro GJ, Plowman GD. Epithelins 1 and 2: isolation and characterization of two cysteine-rich growth-modulating proteins. Proc Natl Acad Sci USA 1990; 87: 7912–16.

Sieben A, Van Langenhove T, Engelborghs S, Martin JJ, Boon P, Cras P, et al. The genetics and neuropathology of frontotemporal lobar degeneration. Acta Neuropathol 2012; 124: 353–72.

Skibinski G, Parkinson NJ, Brown JM, Chakrabarti L, Lloyd SL, Hummerich H, et al. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. Nat Genet 2005; 37: 806–8.

Sleegers K, Brouwers N, Van Damme P, Engelborghs S, Gijselinck I, van der Zee J, et al. Serum biomarker for progranulin-associated frontotemporal lobar degeneration. Ann Neurol 2009; 65: 603–9.

Smith KR, Damiano J, Franceschetti S, Carpenter S, Canafoglia L, Morbin M, et al. Strikingly different clinicopathological phenotypes determined by progranulin-mutation dosage. Am J Hum Genet 2012; 90: 1102–7.

Solchenberger B, Russell C, Kremmer E, Haass C, Schmid B. Granulin knock out zebrafish lack frontotemporal lobar degeneration and neuronal ceroid lipofuscinosis pathology. PLoS One 2015; 10: e0118956.

Song G, Ouyang G, Bao S. The activation of Akt/PKB signaling pathway and cell survival. J Cell Mol Med 2005; 9: 59–71.

Spina S, Murrell JR, Huey ED, Wassermann EM, Pietrini P, Grafman J, et al. Corticobasal syndrome associated with the A9D progranulin mutation. J Neuropathol Exp Neurol 2007; 66: 892–900.

Stagi M, Klein ZA, Gould TJ, Bewersdorf J, Strittmatter SM. Lysosome size, motility and stress response regulated by fronto-temporal dementia modifier TMEM106B. Mol Cell Neurosci 2014; 61: 226–40.

Suh HS, Choi N, Tarassishin L, Lee SC. Regulation of progranulin expression in human microglia and proteolysis of progranulin by

matrix metalloproteinase-12 (MMP-12). PLoS One 2012; 7: e35115.
Suzuki H, Matsuoka M. Amyotrophic lateral sclerosis-linked mutant VAPB enhances TDP-43-induced motor neuronal toxicity. J Neurochem 2011; 119: 1099–107.
Suzuki H, Matsuoka M. TDP-43 toxicity is mediated by the unfolded protein response-unrelated induction of C/EBP homologous protein expression. J Neurosci Res 2012; 90: 641–7.
Suzuki H, Shibagaki Y, Hattori S, Matsuoka M. Nuclear TDP-43 causes neuronal toxicity by escaping from the inhibitory regulation by hnRNPs. Hum Mol Genet 2015; 24: 1513–27.
Suzuki M, Lee HC, Kayasuga Y, Chiba S, Nedachi T, Matsuwaki T, et al. Roles of progranulin in sexual differentiation of the developing brain and adult neurogenesis. J Reprod Dev 2009; 55: 351–5.
Takada LT, Bahia VS, Guimaraes HC, Costa TV, Vale TC, Rodriguez RD, et al. GRN and MAPT mutations in 2 frontotemporal dementia research centers in Brazil. Alzheimer Dis Assoc Disord 2016; 30: 310–17.
Tanaka Y, Matsuwaki T, Yamanouchi K, Nishihara M. Exacerbated inflammatory responses related to activated microglia after traumatic brain injury in progranulin-deficient mice. Neuroscience 2013a; 231: 49–60.
Tanaka Y, Matsuwaki T, Yamanouchi K, Nishihara M. Increased lysosomal biogenesis in activated microglia and exacerbated neuronal damage after traumatic brain injury in progranulin-deficient mice. Neuroscience 2013b; 250: 8–19.
Tang W, Lu Y, Tian QY, Zhang Y, Guo FJ, Liu GY, et al. The growth factor progranulin binds to TNF receptors and is therapeutic against inflammatory arthritis in mice. Science 2011; 332: 478–84.
Tangkeangsirisin W, Serrero G. PC cell-derived growth factor (PCDGF/GP88, progranulin) stimulates migration, invasiveness and VEGF expression in breast cancer cells. Carcinogenesis 2004; 25: 1587–92.
Tanji K, Miki Y, Maruyama A, Mimura J, Matsumiya T, Mori F, et al. Trehalose intake induces chaperone molecules along with autophagy in a mouse model of Lewy body disease. Biochem Biophys Res Commun 2015; 465: 746–52.
Tao J, Ji F, Wang F, Liu B, Zhu Y. Neuroprotective effects of progranulin in ischemic mice. Brain Res 2012; 1436: 130–6.
Tapia L, Milnerwood A, Guo A, Mills F, Yoshida E, Vasuta C, et al. Progranulin deficiency decreases gross neural connectivity but enhances transmission at individual synapses. J Neurosci 2011; 31: 11126–32.
Tauffenberger A, Chitramuthu BP, Bateman A, Bennett HP, Parker JA. Reduction of polyglutamine toxicity by TDP-43, FUS and progranulin in Huntington's disease models. Hum Mol Genet 2013; 22: 782–94.
Tian R, Li Y, Yao X. PGRN suppresses inflammation and promotes autophagy in keratinocytes through the Wnt/beta-catenin signaling pathway. Inflammation 2016; 39: 1387–94.
Tien NT, Karaca I, Tamboli IY, Walter J. Trehalose alters subcellular trafficking and the metabolism of the Alzheimer-associated amyloid precursor protein. J Biol Chem 2016; 291: 10528–40.
Toh H, Chitramuthu BP, Bennett HP, Bateman A. Structure, function, and mechanism of progranulin; the brain and beyond. J Mol Neurosci 2011; 45: 538–48.
Tolkatchev D, Malik S, Vinogradova A, Wang P, Chen Z, Xu P, et al. Structure dissection of human progranulin identifies well-folded granulin/epithelin modules with unique functional activities. Protein Sci 2008; 17: 711–24.
Tsai RM, Boxer AL. Therapy and clinical trials in frontotemporal dementia: past, present, and future. J Neurochem 2016; 138 (Suppl 1): 211–21.
Van Damme P, Van Hoecke A, Lambrechts D, Vanacker P, Bogaert E, van Swieten J, et al. Progranulin functions as a neurotrophic factor to regulate neurite outgrowth and enhance neuronal survival. J Cell Biol 2008; 181: 37–41.
Van Deerlin VM, Sleiman PM, Martinez-Lage M, Chen-Plotkin A, Wang LS, Graff-Radford NR, et al. Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. Nat Genet 2010; 42: 234–9.
van der Zee J, Van Langenhove T, Kleinberger G, Sleegers K, Engelborghs S, Vandenberghe R, et al. TMEM106B is associated with frontotemporal lobar degeneration in a clinically diagnosed patient cohort. Brain 2011; 134 (Pt 3): 808–15.
Van Kampen JM, Baranowski D, Kay DG. Progranulin gene delivery protects dopaminergic neurons in a mouse model of Parkinson's disease. PLoS One 2014; 9: e97032.
Van Langenhove T, van der Zee J, Sleegers K, Engelborghs S, Vandenberghe R, Gijselinck I, et al. Genetic contribution of FUS to frontotemporal lobar degeneration. Neurology 2010; 74: 366–71.
Vanden Broeck L, Callaerts P, Dermaut B. TDP-43-mediated neurodegeneration: towards a loss-of-function hypothesis? Trends Mol Med 2014; 20: 66–71.
Vengoechea J, David MP, Yaghi SR, Carpenter L, Rudnicki SA. Clinical variability and female penetrance in X-linked familial FTD/ALS caused by a P506S mutation in UBQLN2. Amyotroph Lateral Scler Frontotemporal Degener 2013; 14: 615–19.
Vercellino M, Grifoni S, Romagnolo A, Masera S, Mattioda A, Trebini C, et al. Progranulin expression in brain tissue and cerebrospinal fluid levels in multiple sclerosis. Mult Scler 2011; 17: 1194–201.
Wang DF, Helquist P, Wiech NL, Wiest O. Toward selective histone deacetylase inhibitor design: homology modeling, docking studies, and molecular dynamics simulations of human class I histone deacetylases. J Med Chem 2005; 48: 6936–47.
Wang J, Van Damme P, Cruchaga C, Gitcho MA, Vidal JM, Seijo-Martinez M, et al. Pathogenic cysteine mutations affect progranulin function and production of mature granulins. J Neurochem 2010a; 112: 1305–15.
Wang M, Li G, Yin J, Lin T, Zhang J. Progranulin overexpression predicts overall survival in patients with glioblastoma. Med Oncol 2012; 29: 2423–31.
Wang W, Hayashi J, Kim WE, Serrero G. PC cell-derived growth factor (granulin precursor) expression and action in human multiple myeloma. Clin Cancer Res 2003; 9: 2221–8.
Wang WX, Wilfred BR, Madathil SK, Tang G, Hu Y, Dimayuga J, et al. miR-107 regulates granulin/progranulin with implications for traumatic brain injury and neurodegenerative disease. Am J Pathol 2010b; 177: 334–45.
Ward ME, Taubes A, Chen R, Miller BL, Sephton CF, Gelfand JM, et al. Early retinal neurodegeneration and impaired Ran-mediated nuclear import of TDP-43 in progranulin-deficient FTLD. J Exp Med 2014; 211: 1937–45.
Watts GD, Wymer J, Kovach MJ, Mehta SG, Mumm S, Darvish D, et al. Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. Nat Genet 2004; 36: 377–81.
Wexler EM, Rosen E, Lu D, Osborn GE, Martin E, Raybould H, et al. Genome-wide analysis of a Wnt1-regulated transcriptional network implicates neurodegenerative pathways. Sci Signal 2011; 4: ra65.
Whitwell JL, Boeve BF, Weigand SD, Senjem ML, Gunter JL, Baker MC, et al. Brain atrophy over time in genetic and sporadic frontotemporal dementia: a study of 198 serial magnetic resonance images. Eur J Neurol 2015; 22: 745–52.
Whitwell JL, Jack CR Jr, Baker M, Rademakers R, Adamson J, Boeve BF, et al. Voxel-based morphometry in frontotemporal lobar degeneration with ubiquitin-positive inclusions with and without progranulin mutations. Arch Neurol 2007; 64: 371–6.
Whitwell JL, Jack CR Jr, Boeve BF, Senjem ML, Baker M, Rademakers R, et al. Voxel-based morphometry patterns of atrophy in FTLD with mutations in MAPT or PGRN. Neurology 2009; 72: 813–20.

Whitwell JL, Weigand SD, Boeve BF, Senjem ML, Gunter JL, DeJesus-Hernandez M, et al. Neuroimaging signatures of frontotemporal dementia genetics: C9ORF72, tau, progranulin and sporadics. Brain 2012; 135 (Pt 3): 794–806.

Whitwell JL, Xu J, Mandrekar J, Boeve BF, Knopman DS, Parisi JE, et al. Frontal asymmetry in behavioral variant frontotemporal dementia: clinicoimaging and pathogenetic correlates. Neurobiol Aging 2013; 34: 636–9.

Wils H, Kleinberger G, Pereson S, Janssens J, Capell A, Van Dam D, et al. Cellular ageing, increased mortality and FTLD-TDP-associated neuropathology in progranulin knockout mice. J Pathol 2012; 228: 67–76.

Wilson AC, Dugger BN, Dickson DW, Wang DS. TDP-43 in aging and Alzheimer's disease—a review. Int J Clin Exp Pathol 2011; 4: 147–55.

Wu H, Siegel RM. Progranulin resolves inflammation. Science 2011; 332: 427–8.

Xie S, Lu L, Liu L, Bi G, Zheng L. Progranulin and short-term outcome in patients with acute ischaemic stroke. Eur J Neurol 2016; 23: 648–55.

Xu J, Xilouri M, Bruban J, Shioi J, Shao Z, Papazoglou I, et al. Extracellular progranulin protects cortical neurons from toxic insults by activating survival signaling. Neurobiol Aging 2011; 32: 2326.e5–16.

Xu K, Zhang Y, Ilalov K, Carlson CS, Feng JQ, Di Cesare PE, et al. Cartilage oligomeric matrix protein associates with granulin-epithelin precursor (GEP) and potentiates GEP-stimulated chondrocyte proliferation. J Biol Chem 2007; 282: 11347–55.

Yamashita M, Nonaka T, Hirai S, Miwa A, Okado H, Arai T, et al. Distinct pathways leading to TDP-43-induced cellular dysfunctions. Hum Mol Genet 2014; 23: 4345–56.

Yin F, Banerjee R, Thomas B, Zhou P, Qian L, Jia T, et al. Exaggerated inflammation, impaired host defense, and neuropathology in progranulin-deficient mice. J Exp Med 2010a; 207: 117–28.

Yin F, Dumont M, Banerjee R, Ma Y, Li H, Lin MT, et al. Behavioral deficits and progressive neuropathology in progranulin-deficient mice: a mouse model of frontotemporal dementia. FASEB J 2010b; 24: 4639–47.

Yu CE, Bird TD, Bekris LM, Montine TJ, Leverenz JB, Steinbart E, et al. The spectrum of mutations in progranulin: a collaborative study screening 545 cases of neurodegeneration. Arch Neurol 2010; 67: 161–70.

Zanocco-Marani T, Bateman A, Romano G, Valentinis B, He ZH, Baserga R. Biological activities and signaling pathways of the granulin/epithelin precursor. Cancer Res 1999; 59: 5331–40.

Zhang YJ, Xu YF, Dickey CA, Buratti E, Baralle F, Bailey R, et al. Progranulin mediates caspase-dependent cleavage of TAR DNA binding protein-43. J Neurosci 2007; 27: 10530–4.

Zhao C, Bateman A. Progranulin protects against the tissue damage of acute ischaemic stroke. Brain 2015; 138 (Pt 7): 1770–3.

Zheng Y, Brady OA, Meng PS, Mao Y, Hu F. C-terminus of progranulin interacts with the beta-propeller region of sortilin to regulate progranulin trafficking. PLoS One 2011; 6: e21023.

Zhou J, Gao G, Crabb JW, Serrero G. Purification of an autocrine growth factor homologous with mouse epithelin precursor from a highly tumorigenic cell line. J Biol Chem 1993; 268: 10863–9.

Zhou M, Tang W, Fu Y, Xu X, Wang Z, Lu Y, et al. Progranulin protects against renal ischemia/reperfusion injury in mice. Kidney Int 2015a; 87: 918–29.

Zhou X, Sun L, Bastos de Oliveira F, Qi X, Brown WJ, Smolka MB, et al. Prosaposin facilitates sortilin-independent lysosomal trafficking of progranulin. J Cell Biol 2015b; 210: 991–1002.

Zhou X, Sun L, Brady OA, Murphy KA, Hu F. Elevated TMEM106B levels exaggerate lipofuscin accumulation and lysosomal dysfunction in aged mice with progranulin deficiency. Acta Neuropathol Commun 2017; 5: 9.

Zhu J, Nathan C, Jin W, Sim D, Ashcroft GS, Wahl SM, et al. Conversion of proepithelin to epithelins: roles of SLPI and elastase in host defense and wound repair. Cell 2002; 111: 867–78.

Zhu S, Tai C, Petkau TL, Zhang S, Liao C, Dong Z, et al. Progranulin promotes activation of microglia/macrophage after pilocarpine-induced status epilepticus. Brain Res 2013; 1530: 54–65.