Patient-derived skin fibroblasts as a model to study frontotemporal lobar degeneration

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Frontotemporal lobar degeneration (FTLD) is one of the most common causes of early-onset dementia in patients under the age of 65 years. It is a clinically, genetically, and neuropathologically heterogeneous group of neurodegenerative syndromes, causing atrophy in the temporal and frontal lobes of the brain. This is accompanied by progressive cognitive dysfunction, behavioral changes, difficulties in understanding or producing speech, and often also neuropsychiatric symptoms (Haapasalo and Remes, 2015). Moreover, the clinical, genetic, and neuropathological features of FTLD may overlap with those of amyotrophic lateral sclerosis (ALS). Motor dysfunction is commonly present in FTLD patients and a portion of ALS patients experience frontal and temporal lobe dysfunction (Smith et al., 2019). In addition to the sporadic forms of FTLD, which are not linked to any known mutations, approximately half of the FTLD cases are caused by different mutations in several genes, including GRN (granulin), MAPT (microtubule-associated protein tau), VCP (valosin-containing protein), TARDBP (TAR DNA-binding protein 43 kDa; TDP-43), FUS (fused in sarcoma) and C9orf72 (chromosome 9 open reading frame 72). Of these, the GGGGCC hexanucleotide repeat expansion in the C9orf72 gene (C9-HRE) is the most common genetic cause of both FTLD and ALS (Haapasalo and Remes, 2015). The expansion length can typically vary from hundreds to thousands of repeats in affected individuals.

The prevalence of FTLD is 15 to 22 per 100,000 adults with significant variation in different geographical areas. Although FTLD is less common than Alzheimer’s disease, the most common dementia-causing neurodegenerative disease, the socioeconomic burden it creates is substantial. One factor contributing to this may be the earlier onset of the disease at an age where patients and their caregivers are often still active in work life and the society.

As of now, no treatments exist to halt the progression of FTLD nor are there specific diagnostic or prognostic biomarkers available. Therefore, well-characterized disease models that recapitulate the pathogenic pathways and functional changes observed in the brain tissue of FTLD patients are needed. Today, induced pluripotent stem cell (iPSC) technology provides excellent opportunities to generate human disease models from clinically and genetically well-defined patients. However, while e.g., iPSC-derived neurons present an important option for disease modeling, their production and maintenance are expensive and laborious, which can be an obstacle for their use in certain purposes, such as in screenings of a large number of drug candidates. Therefore, other patient-derived models might offer alternative options for such studies. One choice is utilizing patient-derived fibroblasts, which are easily obtained from skin biopsy samples and commonly used as starting material to generate the iPSCs.

Pathological hallmarks and impaired mitochondrial function in FTLD and ALS patients: Depending on the causative mutation, different pathological hallmarks can be observed in the cytoplasm or nuclei of neuronal and glial cells of FTLD patients. The formation of cytoplasmic TDP-43 and phosphorylated TDP-43 aggregates is a common pathology found in patients with mutations in the GRN, MAPT, FUS, and C9orf72 genes. Also, accumulation of sequestosome 1 (p62/SQSTM1, hereafter p62) is a typical neuropathological change detected in patients carrying mutations in different genes, including C9orf72 (Haapasalo and Remes, 2015). Besides these common hallmarks, other protein pathologies are specific to certain mutations, such as the tau aggregates in the cytoplasm caused by mutations in the MAPT gene and the cytoplasmic inclusions of FUS protein in patients with mutations in the FUS gene. In the neurons of C9-HRE carriers, the formation of aggregates of the dipeptide repeat-containing (DPR) proteins (poly-GP(Glycine-Proline), poly-GA(Glycine-Alanine), poly-GR(Glycine-Arginine), poly-PA(Proline-Alanine), and poly-PR(Proline-Arginine)), generated from the expanded repeat through repeat-associated non-AUG (RAN) translation, are specific pathological hallmarks (Haapasalo and Remes, 2015).

In addition to these neuropathological hallmarks, mitochondrial dysfunction has been observed in several neurodegenerative diseases, including FTLD and ALS. Structural alterations and aggregation of mitochondria were among the first pathological changes detected in the motor neurons of ALS patients. Furthermore, reduced activity of complex I, II, III, and IV of the electron transport chain (ETC) has been observed in post-mortem spinal cord of sporadic ALS patients (Wiedemann et al., 2002). Several of the above-mentioned mutations have been linked to mitochondrial dysfunction, including the C9-HRE. Mitochondrial dysfunction can be directly caused by the expression of DPR proteins but also as a result of impaired autophagy/mitophagy, which hinders mitochondrial quality control (Smith et al., 2019). The Poly-GR DPR protein has been shown to bind to mitochondrial ribosomal proteins needed for the translation of subunits of several mitochondrial respiratory chain complexes, leading to impaired mitochondrial function, increased oxidative stress, and DNA damage in iPSC-derived motor neurons from FTLD and ALS patients carrying the C9-HRE (Lopez-Gonzalez et al., 2016).

Pathological hallmarks and cellular dysfunction in non-neuronal cells: While most research has focused on neurons and glial cells, some of the pathological hallmarks can also be found in other cell types. Accumulation of RNA foci and protein aggregates containing the Poly-GR DPR protein and increased cytoplasmic aggregation of phosphorylated TDP-43 have been described in iPSC-derived skeletal myocytes from ALS patients carrying the C9-HRE. In addition, these cells show changes in the expression of several genes related to mitochondrial function, suggesting that skeletal myocytes might also exhibit impaired...
mitochondrial function (Lynch et al., 2019). Accumulation of RNA foci has also been described in the fibroblasts and lymphoblasts derived from ALS patients carrying the C9-HRE (Lagier-Tourenne et al., 2013) as have increased levels of p62 protein and number of p62-positive foci in ALS/FTLD patient-derived fibroblasts (Aoki et al., 2017).

Furthermore, mitochondrial alterations, including mitochondrial fragmentation, aggregation, and deformation or loss of mitochondrial cristae have not only been observed in several in vivo and in vitro models of ALS with different genetic backgrounds but also in the fibroblasts of ALS and FTLD patients carrying the C9-HRE (Smith et al., 2019). Mitochondrial dysfunction, associated with reduced activity of different complexes of the ETC, can also be observed in fibroblasts with different mutation carriers, including the C9-HRE (Smith et al., 2019).

**Patient-derived fibroblasts as a model system:** Based on the above-mentioned results, FTLD-patient derived fibroblasts appear to have potential as an easy and fast established, cost-effective disease model recapitulating at least some of the pathological hallmarks and dysfunction observed in neurons. Thus, in our recent study, we obtained skin punch biopsies from FTLD patients with and without the C9-HRE and established fibroblast cultures from them. Primary fibroblasts become available for experiments within a few weeks of culturing the biopsies, as depicted in Figure 1A. We then conducted a study examining several cell pathological hallmarks as well as proteasomal, autophagosomal, and mitochondrial function in these patient-derived fibroblasts (Leskelä et al., 2021). As expected, the fibroblasts from the C9-HRE carriers, but not from the non-carriers, were found to display nuclear RNA foci but no DPR proteins, which is in line with previous publications.

We did not observe any TDP-43-related pathologies, such as increased cytoplasmic localization, aggregate formation, or increased phosphorylation, in the FTLD patient fibroblasts. Discrepancies in the extent of TDP-43 pathology in fibroblasts have been previously described and could be related to differences in culture conditions and subsequent cellular stress that might render the cells more susceptible to TDP-43 mislocalization (Riancho et al., 2020). However, an increase in the size and number of p62-positive puncta was evident in the fibroblasts from both C9-HRE-carriers and non-carriers as compared to those from healthy controls, as seen in Figure 1B. Basal and induced autophagy and function of the ubiquitin-proteasome system were unaltered in FTLD patient-derived fibroblasts when compared to healthy controls (Leskelä et al., 2021), suggesting that the increase in p62-positive puncta might be related to other mechanisms besides impaired protein degradation, such as increased transcription.

In addition to the pathological hallmarks and protein degradation pathways, we also assessed mitochondrial function. As shown in Figure 1C, our results indicate that some mitochondrial functions, in particular basal and maximal respiration, and respiration linked to adenosine triphosphate (ATP) production, were significantly impaired in the FTLD patient-derived fibroblasts from both C9-HRE carriers and non-carriers compared to healthy controls (Leskelä et al., 2021).

**Usability of patient-derived fibroblasts in identifying potential biomarkers and therapeutic targets:** Our findings demonstrated that FTLD patient-derived fibroblasts, regardless of whether they carry the C9-HRE expansion or not, show unchanged proteasomal and autophagic function, but significantly impaired mitochondrial function and increased accumulation of p62-positive puncta when compared to fibroblasts from healthy controls (Leskelä et al., 2021). A previous study by Aoki et al. (2017) also found that ALS/FTLD patient-derived fibroblasts displayed increased levels of p62 protein and a number of p62-positive foci. These findings altogether suggest that patient-derived fibroblasts could be beneficial in the study of potential biomarkers or therapeutic candidates. Further investigations are needed to assess whether the accumulation of p62 is specific for FTLD patient-derived fibroblasts or can also be observed in other neurodegenerative diseases. If it is specific to FTLD patients, it might be employed as a potential biomarker for these patients in the future, but this needs further confirmation.

The impaired mitochondrial function, on the other hand, might be an attractive
therapeutic target as mitochondrial dysfunction appears to be a common denominator found in neurons and fibroblasts of FTLD and ALS patients with different disease-causing mutations (Smith et al., 2019).

**Pathological hallmarks in non-neuronal cells might contribute to the disease phenotype:**

The presence of some of the pathological hallmarks in non-neuronal cells, such as fibroblasts, could also imply that pathological changes and cellular dysfunctions are not limited to the brain, suggesting that other cell types may contribute to the disease phenotype. In accordance with the finding that iPSC-derived myocytes express some of the pathological hallmarks, biopsies from skeletal muscles of ALS patients carrying the C9-HRE have been reported to express poly-GA and poly-GP DPR proteins and also phosphorylated TDP-43 inclusions, which co-localized with p62 (Cykowski et al., 2019). Also, skin changes have been described in ALS patients, such as low collagen and increased insulin-like growth factor-I levels. A study of molecular networks in iPSC-derived motor neurons of ALS patients with the C9-HRE showed down-regulation of several genes for collagens, as well as genes related to the synthesis, assembly, and crosslinking of collagen fibrils (Paré and Gros-Louis, 2017), showing show that the C9-HRE can lead to reduced expression of collagen. This might suggest skin involvement, especially in C9-HRE carriers, given that diseases linked to the C9-HRE have been reported to express poly-GA and poly-GP DPR proteins and also poly-GA and poly-GP (Gros-Louis et al., 2014).

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**Conclusion:** In conclusion, recent research, including ours, has indicated that FTLD patient-derived skin fibroblasts display some of the key neuropathological hallmarks typically present in neurons as well as disturbances in energy metabolism. Therefore, depending on the research question, fibroblasts might provide a more affordable and easily accessible and maintainable patient-derived model when compared to e.g., iPSC-derived neurons in deciphering the underlying molecular mechanisms of the disease. Furthermore, patient fibroblasts might be suitable for biomarker discovery and early stages of drug screening targeted at specific pathologies or cellular functions, such as mitochondrial dysfunction.

**References**

Aoki Y, Manzano R, Lee Y, Dafinca R, Aoki M, Douglas AGL, Varela MA, Sathyaprakash C, Scaber J, Barbagallo P, Vador P, Mager I, Ezzat K, Turner MR, Ito N, Gasco S, Ohtbayashi N, El Andaloussi S, Takeda S, Fukuda M, et al. (2017) C9orf72 and RAB7L1 regulate vesicle trafficking in amyotrophic lateral sclerosis and frontotemporal dementia. Brain 140:887-897.

Cykowski MD, Dickson DW, Powell SZ, Arumanyagam AS, Rivera AL, Appel SH (2019) Diptides repeat (DPR) pathology in the skeletal muscle of ALS patients with C9orf72 repeat expansion. Acta Neuropathol 138:667-670.

Yang D, Petrucelli L, Miller BL, Almeida S, Gao F (2016) Poly(GR) in C9ORF72-related ALS/FTD compromises mitochondrial function and increases oxidative stress and DNA damage in iPSC-derived motor neurons. Neuron 19:383-391.

Lynch E, Semrad T, Belsito VS, FitzGibbons C, Reilly M, Hayakawa K, Suzuki M (2019) C9ORF72-related cellular pathology in skeletal myocytes derived from ALS patient-induced pluripotent stem cells. Dis Model Mech 12:dmn039552.

Paré B, Gros-Louis F (2017) Potential skin involvement in ALS: Revisiting Charcot’s observation-A review of skin abnormalities in ALS. Rev Neurosci 28:551-572.

Riancho J, Arrozarena S, López de Múnain A (2020) Dermic-derived fibroblasts for the study of amyotrophic lateral sclerosis. Neural Regen Res 15:2043-2044.

Smith EH, Shaw PJ, De Vos KJ (2019) The role of mitochondria in amyotrophic lateral sclerosis. Neurosci Lett 710:132933.

Ling SC, Zhu Q, Polymenidou M, Drenner K, Artates JW, McAlonis-Downes M, Markmiller S, Hutt KR, et al. (2013) Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration. Proc Natl Acad Sci U S A 110:E4530-E4539.

Leskelä S, Hoffmann D, Rostański H, Huber N, Wittthahn R, Hartkainen P, Korhonen V, Leinonen V, Hiltunen M, Solje E, Remes AM, Haapasalo A (2021) FTLD patient-derived fibroblasts show defective mitochondrial function and accumulation of p62. Mol Neurobiol 58:5438-5458.

Lopez-Gonzalez R, Lu Y, Gendron TF, Karydas A, Tran H, Yang D, Petrucci L, Miller BL, Almeida S, Gao F (2016) Poly(GR) in C9ORF72-related ALS/FTD compromises mitochondrial function and increases oxidative stress and DNA damage in iPSC-derived motor neurons. Neuron 19:383-391.

Lynch E, Semrad T, Belsito VS, FitzGibbons C, Reilly M, Hayakawa K, Suzuki M (2019) C9ORF72-related cellular pathology in skeletal myocytes derived from ALS patient-induced pluripotent stem cells. Dis Model Mech 12:dmn039552.

Paré B, Gros-Louis F (2017) Potential skin involvement in ALS: Revisiting Charcot’s observation-A review of skin abnormalities in ALS. Rev Neurosci 28:551-572.

Riancho J, Arrozarena S, López de Múnain A (2020) Dermic-derived fibroblasts for the study of amyotrophic lateral sclerosis. Neural Regen Res 15:2043-2044.

Smith EH, Shaw PJ, De Vos KJ (2019) The role of mitochondria in amyotrophic lateral sclerosis. Neurosci Lett 710:132933.

Waite AJ, Bäumer D, East S, Neal J, Morris HR, Ansource O, Blake DJ (2014) Reduced C9orf72 protein levels in frontal cortex of amyotrophic lateral sclerosis and frontotemporal degeneration brain with the C9ORF72 hexanucleotide repeat expansion. Neurobiol Aging 35:1779.e5-1779.e13.

Wiedemann FR, Manfredi G, Maiuri C, Flint Beal M, Schon EA (2002) Mitochondrial DNA and respiratory chain function and DNA damage in iPSC-derived motor neurons. Neurosci Lett 35:1779.e5-1779.e13.

Yang D, Petrucelli L, Miller BL, Almeida S, Gao F (2016) Poly(GR) in C9ORF72-related ALS/FTD compromises mitochondrial function and increases oxidative stress and DNA damage in iPSC-derived motor neurons. Neuron 19:383-391.