What is giving the phenotype in neurodegeneration:

Age
Genetics
Environment

Figure 1
This scheme depicts the risk spectrum predisposing to common diseases as one continuum, using AD as an example. The continuum extends from the most extreme genetic form ("Mendelian genes"; green) to cases influenced by genetic susceptibility factors ("Genetic risk factors"; orange), until reaching into a less well-defined area of cases that may be caused by genes of lesser penetrance/lower effect size and/or altogether nongenetic factors ("Nongenetic risk factors"; gray). Established Mendelian genes (APP, PSEN1, and PSEN2) or genetic risk factors (APOE-ε4) are represented by shaded boxes and represent the most obvious candidates of AD genetics; the width of these boxes approximately represents the relative contribution to the overall risk for disease. Black boxes indicate still-elusive disease genes/risk factors ("?"). Colored arrows indicate possible gene-gene and gene-environment interaction patterns: yellow arrows represent previously suggested interactions (e.g., between PSEN1 and APOE-ε4). Note that some interactions (red arrows) as well as the number of elusive genes are entirely hypothetical and are depicted for didactic purposes only.
Classical strategy to find disease genes:

Figure 2
Flow chart of current strategies used to identify novel disease genes. This scheme outlines strategies for identifying mutations and/or polymorphisms causing or predisposing to disease. Candidate genes are chosen based on genetic linkage data and/or known or hypothesized pathobiological relevance to disease mechanisms. This procedure is referred to as the “candidate gene approach.” An alternative and inherently similar strategy is based on the detection of formerly unknown genes/proteins according to genetic linkage data and is referred to as “positional cloning.” Dashed lines indicate “shortcuts” allowing the definition of a novel disease gene based on the genetic evidence alone, e.g., APOE-ε4 in AD, of which the precise functional consequences remain unknown despite an established genetic role. Note that there are examples of genes/mutations with reduced penetrance or minor risk effects (red boxes) within bona fide disease genes (e.g., certain mutations in PSEN1 in AD).

Bertram and Tanzi, JCI, 2005
Genome-wide association studies (GWAS) are an approach that involves rapidly scanning markers across the complete sets of DNA, or genomes, of many people to find genetic variations associated with a particular disease.

DNA from patients and controls is then purified from the blood or cells, placed on tiny chips and scanned on automated machines for selected markers of genetic variation, which are called single nucleotide polymorphisms, or SNPs.

If certain SNPs are found to be significantly more frequent in people with the disease compared to people without disease, the variations are said to be "associated" with the disease and serve as powerful pointers to the region of the human genome where the disease-causing problem resides.
These approaches have led to a tremendous increase in the number of genes associated with the pathology: 126 genes identified so far that explain 2/3 of fALS and 5-10% of ALS.
The problem of data storage and handling of disease genes and genomes:

- Every human DNA contains more than 3 billion bases. After sequencing, AT BEST it will occupy approximately 300 Megabytes. How do you integrate and compare data from several hundred patients and controls?
Sequencing DNA will not be enough because humans have approximately 20,000 genes but make more than 100,000 proteins.

**Figure 1 | Sources of errors in eukaryotic protein synthesis.** Errors arise at many stages, from the transcription of genetic information to the folding and post-translational modification of the finished polypeptide.
But also for the general society:

Getting married?

You need to know...

Genetic disorders are often more common in specific community groups.

Tay-Sachs disease is more common in descendants of Ashkenazi Jews.

A free screening program at Wolper Jewish Hospital tests for a number of genetically inherited diseases including:

<table>
<thead>
<tr>
<th>Disease</th>
<th>Carrier rate in Ashkenazi Jews</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tay-Sachs</td>
<td>1 in 27 people</td>
</tr>
<tr>
<td>Cystic Fibrosis</td>
<td>1 in 30 people</td>
</tr>
<tr>
<td>Canavan Disease</td>
<td>1 in 40 people</td>
</tr>
<tr>
<td>Fanconi Anaemia</td>
<td>1 in 100 people</td>
</tr>
<tr>
<td>Familial Dysautonomia</td>
<td>1 in 40 people</td>
</tr>
</tbody>
</table>

Testing is also available for Bloom Syndrome, Congenital Deafness, Gaucher Disease, Niemann-Pick Disease and Fragile X at a standard cost.

“RTF® test helped us live with more confidence and less fear”

Are You Ready To Fight Disease Before It Starts?

If any of your family members have had:

- Alzheimer’s Disease
- Rheumatoid Arthritis
- Cancer (Breast Cancer, Colon Cancer, Lung Cancer, Pancreatic Cancer)

then you may be at increased risk too.

Medical science has shown that we inherit many diseases from our ancestors. If someone in your family has been diagnosed with one of the above hereditary medical conditions, you too may be at increased risk. Early detection, along with proactive medical care, is proven to help reduce risk and save lives.

Ask your doctor about RTF® genetic testing because understanding your risk is the first step to reducing it. RTF® analysis - a test that uses a simple painless custom patch from your cheek - can help you understand your personal risk for developing any of these serious medical conditions. Expert risk assessment, along with a discussion of testing and medical options, is your chance to begin fighting a serious disease before it starts. After RTF® analysis, you and your doctor can discuss effective choices and steps you can take to ensure your own health.

Are You Ready To Fight Disease Before It Starts?

Talk to your doctor or call today for a free educational brochure.

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www.wolper.com.au
Interestingly, when one of these genes is mutated or its expression is altered there are only a few key proteins that accumulate in the brain of patients:

- Tau, TDP-43, FUS/TLS
- Alpha-synuclein
- Huntigtin
- TDP-43, FUS/TLS

Bertram and Tanzi, JCI, 2005
The identification of these proteins has very profound implications for finding the cure to diseases:

- TDP-43, FUS/TLS, Tau, Prp, Alpha synuclein, etc.
  - Use as a biomarker
  - Model pathological features
  - Characterization of the role played in neuronal functions
  - Screen for mutations
    - Monitor disease onset/progression
    - Develop new and more specific therapeutic strategies
    - Clinical counselling
Two major gray areas are present in our understanding of the ALS/FTD spectrum:

Adapted from Hardy and Rogaeva, 2013
Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease. It is characterized by the progressive loss of upper and lower motor neurons from the spinal cord, brain stem, and motor cortex, leading to muscle weakness and eventual respiratory failure.

Approximately 5–10% of ALS cases are familial with the remaining 90% being sporadic, indicating that both genetic and environmental factors contribute to risk.

97% of patients display a common phenotype in disease-affected tissues, namely the deposition of the TAR-DNA binding protein (TDP-43).
What is TDP-43?

RNA binding proteins play an essential role in neuronal cells

- Dendrites
- Nucleus
- Cell body
- Axon terminals
- Axon
- Myelin sheath
- Schwann’s cells

Pre-mRNA splicing, mRNA stability and translation
- TDP43
- FUS
- PTBP2 (nPTB)
- TAF15
- hnRNPA1
- hnRNPA2/B1
- EWS
- NOVA1/2
- SMN
- RBFOX3
- HuD, HuC, HuD

RNA transport
- TDP43
- FUS
- TAF15
- hnRNPA2/B1
- EWS
- SMN
- ATXN2

Synaptic Vescicle
- FMRP
- PARK7

De Conti et al., WIREs RNA
414 amino acid nuclear protein

Ubiquitously expressed DNA-/RNA-binding protein

Encoded by the TARDBP gene on chromosome 1

Family of \textit{hnRNPs}

Mostly nuclear (although up to \( \sim 30\% \) of TDP-43 protein can be found in the cytoplasm)

Aggregation-prone protein involved in many neurodegenerative disorders, generally referred to as TDP-43 proteinopathies (Alzheimer's and Parkinson disease, ALS, FTLD)
Structural approaches
The main distinguishing feature of TDP-43 is its ability to bind RNA in a sequence-specific manner:

Lukavsky et al., 2013
Novel $\beta$-turn configuration: new amyloid structure. We predict that pathological mutations would destabilize this structure, possibly favoring smaller toxic oligomers.
Functional approaches
In collaboration with the Neurology group we have started a search for hnRNP modifiers of TDP-43 pathology:

**Loss-of-function fly disease model**
- Flies expressing 50% normal TDP-43 levels (haploinsufficient)
- Flies expressing 1-2% normal TDP-43 levels (hypomorphic)

**Gain-of-function fly disease model**
- Flies overexpressing wild-type TDP-43 in eye (hypomorphic)

**Crossing with fly strains that can be silenced for all major hnRNPs conserved between flies and humans:**

<table>
<thead>
<tr>
<th>Human gene</th>
<th>Fly gene symbol</th>
<th>ID RNA</th>
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<tbody>
<tr>
<td>hnRNP U</td>
<td>hCG30122</td>
<td>156984KK</td>
</tr>
<tr>
<td>hnRNP K</td>
<td>hCG13423</td>
<td>150571KK</td>
</tr>
<tr>
<td>hnRNP D</td>
<td>hCG16001</td>
<td>32265GD</td>
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<td>hCG2458</td>
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<td>hCG5946</td>
<td>27792GD</td>
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<td>hCG17038</td>
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<td>RNP10018K</td>
<td>10018K</td>
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</tr>
</tbody>
</table>

**Validation of functional relationship in Human neuronal cells**

**RNA sequencing analysis of promising transcripts**

**Validate the effect of individual transcript to modulate TDP43-associated pathology**
In addition to a small group of phenotype enhancers we were able to find a group of strong suppressors (Hrb27c, CG42458, Glo and Syp) which silencing, rescued almost completely TBPH phenotype (Fig. 1 E-H, M) and a group of mild suppressors (Herb87F, Sm, Heph and Rump) that recovered only partially TBPH defects (Fig. I-L, M).
Multi-disease approaches
Niemann Pick C disease [NPCD-MIM 257220; MIM607625] is an autosomal recessive lysosomal storage disorder due to mutations in \( \text{NPC1} \) (95% of patients) or \( \text{NPC2} \) genes, encoding two proteins involved in the intracellular trafficking of cholesterol and other lipids.

The deficiency of either of them leads to the accumulation of endocytosed unesterified cholesterol, gangliosides, and other lipids within the lysosome/late endosome compartment.

The clinical presentation of the disease is extremely variable and the age at onset ranges from the perinatal period to adulthood. The disease is typically characterised by visceral and neurological signs and symptoms that follow a completely independent clinical course. Neuropathological features include megaloneurite formation, extensive growth of ectopic dendrites, formation of neurofibrillary tangles, neuroinflammation and neuroaxonal dystrophy. As the disease progresses, neuronal death becomes prominent, affecting particularly Purkinje cells of the cerebellum.
In collaboration with Andrea dardis/Bruno Bembi (Centro Malattie Rare Ospedale di Udine, Italy), Sonia Canterini and Maria Teresa Fiorenza (Rome University) and Bernardino Ghetti/Kathy Newell of the Indianapolis Alzheimer Centre, USA, we were able to investigate the presence/expression of TDP-43 in three model systems:

- Mouse model of disease NPC(-1)
- MASC model cell system from four different NPC patients
- Human autopic tissue from a NPC patient

- Analysis of TDP-43 distribution in mice brain
- Analysis of TDP-43 expression
- Analysis of TDP-43 cellular localisation
- Analysis of TDP-43 functional targets
- Response to NPC approved drugs
- Analysis of TDP-43 distribution in patients

- Identification of TDP43-specific targets that functionally correlate with pathology

- Small-molecule or antisense oligo-based approaches to correct RNA expression defects
Reduction of TDP43 immunohistochemistry of cerebellum of WT and \textit{Npc1}\textsuperscript{−/−} mice at PN11 and especially at PN80. Arrows showed a significant reduction of TDP-43 expression in \textit{Npc1}\textsuperscript{−/−} compared to wild-type mice in Purkinje cells (PCs).
MASC derived neuronal cell lines: Intracellular localization of TDP-43 (labeled in green) in neuron-like cells obtained from wt (A) and NPC patients (B). Neuronal differentiation was confirmed by immunofluorescence anti-NeuN (labeled in red) (C-D). In the NPC cells TDP-43 is also phosphorylated like in the ALS pathology.
In the 61 year-old proband's brain, Purkinje cells show cytoplasmic staining but lack nuclear staining (A). Dentate nucleus neurons show nuclear staining (B). In brain sections from 60 year-old (C) and 89 year-old (D) control subjects, Purkinje cells demonstrate nuclear staining with variable degrees of cytoplasmic staining. (polyclonal TDP43, 1:1000, Proteintech; original magnifications x400)
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Sonia Canterini and Maria Teresa Fiorenza (Rome University)
Bernardino Ghetti and Kathy Newell (Indiana and Kansas Alzheimer Disease Centers, USA)
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