The Genetics of Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive, age-dependent neurodegenerative disorder of motor neurons. It causes paralysis, bulbar dysfunction and respiratory failure and is invariably fatal within 2-5 years of onset. Riluzole, the only disease-modifying agent used in ALS, has only a modest effect on survival. Treatment is essentially supportive and palliative and ~1200 people die of ALS annually in the UK. ALS is challenging to study as it is mostly sporadic, rapidly progressive and clinically heterogeneous. Significantly, a family history of ALS is seen in 5-10% of cases (FALS), usually with autosomal dominant inheritance. FALS is essentially indistinguishable from the more common sporadic ALS (SALS). Identification of FALS genes offers a direct approach to elucidating common mechanisms of disease in ALS and potentially identifying therapeutic targets.

Classical Mendelian inheritance in FALS kindreds is complicated by a variable age of onset, phenotypic heterogeneity and incomplete penetrance. There is also growing evidence that SALS has a genetic basis. Environmental factors interacting with genetic variants of small effect may predispose to SALS and may also explain the heterogeneity of FALS. ALS is a complex genetic disease with sporadic and familial ALS existing at opposite ends of a genetic spectrum. Ten forms of FALS linked to separate genetic loci have been classified, six of which cause typical ALS (Table 1). Two loci linked to ALS from frontotemporal dementia (ALS-FTD) have also been characterised. ALS and FTD are recognised to be part of a clinicopathological spectrum. Up to half of ALS patients may have clinical features of FTD, and both conditions demonstrate characteristic pathological inclusions containing ubiquitinated TAR DNA-binding protein (TDP-43).

In this review we will discuss the major genetic causes of ALS, starting with genes identified by linkage analysis of FALS kindreds. The most significant genes associated with sporadic disease will also be highlighted.

FALS genes causing typical ALS

ALS1- SOD1

Autosomal dominant FALS was first linked to chromosome 21q22 and mutations in Cu/Zn superoxide dismutase (SOD1), an antioxidant enzyme, were subsequently identified. Over 120 SOD1 mutations in all five exons affecting all functional domains are recognised. SOD1 mutations are the commonest cause of FALS accounting for ~20% of cases. The pathogenicity of mutant SOD1 is not fully understood but is probably due to a toxic gain of function affecting many cellular processes, including mitochondrial function and axonal transport. SOD1 mutations are also seen in 1-7% of SALS cases.

ALS6-FUS

Linkage of ALS to chromosome 16p (ALS6) was originally described in several families in 2003. The mean age of onset is ~45 years with disease duration ~33 months and lower motor neuron predominance. Mutations in fusion (FUS) have recently been found in these kindreds. FUS has roles in gene transcription and RNA processing. Although a predominantly nuclear protein, mutations result in cytoplasmic sequestration. FUS mutations cluster at the C-terminus and may account for as many as ~7% of FALS cases, though more studies are needed to accurately determine their frequency. FUS mutations have not been found in SALS cases.

ALS10-TARDBP

Mutations in TARDBP, which encodes TDP-43, have been found in FALS and SALS cases. This demonstrates a mechanistic role in neurodegeneration for TDP-43, the hallmark protein of ALS. Around 30 TARDBP mutations, mostly C-terminally, have been described by various groups in ALS (Table 2). The mean age of disease onset is ~55 years with survival ~54 months. There is little evidence of cognitive dysfunction, which is surprising given that TDP-43 inclusions are also a hallmark of FTD. TARDBP mutations account for ~3% of FALS and ~1% of SALS, though these values vary between populations.

Genes causing rare ALS variants

ALS2-Atxin

ALS2 is a rare, recessively inherited, juvenile-onset disease characterised by slowly progressive spasticity beginning in the lower limbs and spreading to the upper limbs and bulbar musculature. Truncation mutations in the ALS2 gene (coding for atxin) were found in Tunisian and Arab kindreds. Atxin has roles in cellular trafficking and the cytoskeleton. Atxin also protects neurons against mutant SOD1-mediated toxicity and promote neurite outgrowth. Mutations are thought to result in loss of function.

ALS4-Senataxin

ALS4 is a rare, non-fatal, autosomal dominant, juvenile-onset distal hereditary motor neuropathy characterised by limb weakness, muscle wasting and pyramidal involvement. Bulbar and respiratory muscles are spared. Missense mutations in

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Christopher E Shaw

Christopher E Shaw is Professor of Neurology and Neurogenetics, at the Institute of Psychiatry, King’s College London. He is also an Honorary Consultant Neurologist at King’s College and Guy’s Hospitals. His early training in General Medicine and Neurology was conducted in New Zealand. He came to the UK in 1992 on a Wellcome Trust Fellowship to study Neurogenetics at Cambridge. His clinical and research focus is the genetic and molecular basis of motor neuron disease (MND). He has run a clinic for patients with MND at King’s College Hospital and another for people with a variety of inherited neurological disorders at Guy’s Hospital since 1995. He is Head of the Department of Clinical Neurosciences and Director of the Medical Research Council Centre for Neurodegeneration Research.
### Table 1 Genes and loci linked with ALS

<table>
<thead>
<tr>
<th>Disorder</th>
<th>OMIM</th>
<th>Locus</th>
<th>Gene (protein) function</th>
<th>Inheritance</th>
<th>Onset</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Typical ALS</strong></td>
<td></td>
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<td></td>
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<tr>
<td>ALS1</td>
<td>105400</td>
<td>2q22.1</td>
<td>SOD1 (Cu/Zn superoxide dismutase 1) Converts superoxide to water or hydrogen peroxide</td>
<td>Dominant</td>
<td>Adult</td>
<td>Siddique et al., 1991; Rosen et al., 1993</td>
</tr>
<tr>
<td>ALS3</td>
<td>606640</td>
<td>18q21</td>
<td>?</td>
<td>Dominant</td>
<td>Adult</td>
<td>Hand et al., 2002</td>
</tr>
<tr>
<td>ALS6</td>
<td>608030</td>
<td>16q12</td>
<td>TLS/FUS (TLS/FUS) Gene transcription, RNA processing</td>
<td>Dominant</td>
<td>Adult</td>
<td>Ruddy et al., 2003; Sapp et al., 2003; Vance et al., 2009</td>
</tr>
<tr>
<td>ALS7</td>
<td>608031</td>
<td>20pter-p13</td>
<td>?</td>
<td>Dominant</td>
<td>Adult</td>
<td>Sapp et al., 2003</td>
</tr>
<tr>
<td>ALS9</td>
<td>611895</td>
<td>14q11</td>
<td>ANG (Angiogenin) Angiogenesis</td>
<td>Dominant</td>
<td>Adult</td>
<td>Greenway et. al., 2006; Wu et. al., 2007; Gellera et. al., 2008</td>
</tr>
<tr>
<td>ALS10</td>
<td>612069</td>
<td>1p36.2</td>
<td>TARDBP (TDP-43) DNA-RNA binding, splicing, transcriptional regulation</td>
<td>Dominant</td>
<td>Adult</td>
<td>Sreedharan et. al., 2008; Kabashi et. al., 2008</td>
</tr>
<tr>
<td><strong>ALS with frontotemporal dementia</strong></td>
<td></td>
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<td></td>
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<tr>
<td>ALS-FTD1</td>
<td>105550</td>
<td>9q21-22</td>
<td>?</td>
<td>Dominant</td>
<td>Adult ALS with FTD</td>
<td>Hosler et al., 2000; Ostojic et al., 2003</td>
</tr>
<tr>
<td>ALS-FTD2</td>
<td>611454</td>
<td>9q21-13</td>
<td>?</td>
<td>Dominant</td>
<td>Adult ALS with FTD</td>
<td>Vance et al., 2006; Morita et al., 2006; Valdmanis et al., 2007</td>
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<tr>
<td><strong>Atypical ALS</strong></td>
<td></td>
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<tr>
<td>ALS2</td>
<td>205100</td>
<td>2q33</td>
<td>ALS2 (ALS2/Alsin) Endosomal dynamics, Guanine exchange factor for Rab5 and Rac1, Neuronal survival factor</td>
<td>Recessive</td>
<td>Juvenile – predominantly UMN (ALS, infantile-onset ascending HSP)</td>
<td>Hadano et al., 2001; Hentati et al., 1994; Yamanaka et al., 2006; Yang et al., 2001</td>
</tr>
<tr>
<td>ALS4</td>
<td>602433</td>
<td>9q34</td>
<td>SETX (Senataxin) Putative DNA/RNA helicase, RNA metabolism</td>
<td>Dominant</td>
<td>Juvenile – recessive mutations Cause ataxia-oculomotor apraxia type 2</td>
<td>Chance et al., 1998; Chen et al., 2004</td>
</tr>
<tr>
<td>ALS5</td>
<td>602099</td>
<td>15q15.1-21.1</td>
<td>?</td>
<td>Recessive</td>
<td>Juvenile</td>
<td>Hentati et al., 1998</td>
</tr>
<tr>
<td>ALS8</td>
<td>608627</td>
<td>20q13.3</td>
<td>VAPB (VAMP associated membrane protein B) Endosomal trafficking, calcium metabolism</td>
<td>Dominant</td>
<td>Adult – causes slowly progressive SMA phenotype, tremor or typical ALS</td>
<td>Nishimura et al., 2004; Nishimura et al., 2004b</td>
</tr>
</tbody>
</table>

**SETX** (coding for senataxin) have been found in three Caucasian kindreds. The function of senataxin is unknown, but it is notable that recessive **SETX** mutations (mostly truncations) cause ataxia-oculomotor apraxia 2 (AOA2). This suggests that a toxic gain of senataxin function may be responsible for ALS4, while loss of function may lead to AOA2.

**ALS8-VAPB**

Following linkage of a large Portuguese Brazilian kindred with dominantly inherited atypical ALS to chromosome 20q13.3 (ALS8), a mutation in the **VAMP/synaptobrevin-associated membrane protein B gene (VAPB)** was identified. The same mutation was found in seven more Brazilian families with an ancient common founder. Three distinct phenotypes are seen: late-onset SMA, typical ALS and slowly-progressive ALS with tremor. VAPB can associate with microtubules and is implicated in axonal and intracellular transport, and may also be important as a motor neuronal survival factor. Mutant VAPB may have excitotoxic properties.

**Dynactin**

A large kindred with a slowly progressive ALS-like syndrome was linked to chromosome 2p13.16. The phenotype was predominantly lower motor neuron, involving the limbs and face and causing vocal cord paresis. A mutation of the axonal motor protein dynactin was identified, and further mutations found in one SALS, two FALS cases and one ALS-FTD kindred. Mutant dynactin mutations have not been formally classified as an ALS subtype.

**SALS genes: candidate approaches**

The search for SALS genes has frequently involved a candidate-gene approach. This has demonstrated a role for FALS genes, most notably **SOD1** and **TARDBP** in a minority of sporadic cases. Numerous candidate-gene studies in ALS have produced conflicting results. The most significant of candidates are discussed below.

**VEGF** (OMIM 192240)

Vascular endothelial growth factor (VEGF) was identified as a candidate for ALS on the
basis of a mouse model displaying motor neuron degeneration following deletion of the hypoxia response element of the VEGF promoter. However, VEGF mutations have not been found in ALS cases and association studies have generated conflicting results.40-42,43

ALS9-ANG

Angiogenin is functionally similar to VEGF. Significant association between SALS and the angiogenin gene (ANG) was identified in Irish and Scottish populations.44 Coding mutations were subsequently found in sporadic and familial cases and shown to impair the angiogenic properties of angiogenin.45,46 Although ANG mutations are a rare cause of FALS, they are classified as ALS9.

Paraoxonase (OMIM 168820, 602447)

Paraoxonases have antioxidant and detoxifying roles.47,48 Their candidacy in ALS stems from evidence that chemical exposure may increase the risk of ALS.49,50 Functional polymorphisms have been found in ALS59,60 and are associated with abnormal NF assembly in vitro and marked peripherin aggregation in anterior horn cells in vivo. These data suggest that peripherin variants may play a small role in the pathogenesis of ALS.

SMN (OMIM 600354, 601627)

Spinal muscular atrophy (SMA) is an autosomal recessive lower motor neuron disorder of neonates and children usually caused by deletion of the Survival Motor Neuron 1 (SMN1) gene. Two copies of SMN exist at the chromosome 5 locus in humans, with deletions in SMN1 causing disease. SMN2 is only partially functional due to splice variation, but an increase in SMN2 copy number can ameliorate the severity of SMA. SMA may result from a loss of motor neuron-specific functions of SMN, including processing and transport of RNAs.44,45 Although studies have not demonstrated a direct role for SMN1 variants in ALS, copy number analysis demonstrates that ALS patients may have reduced SMN protein levels, or deletions of SMN2, although reports are conflicting.46,47,48,49

SALS genes: Genome-wide association studies

Unlike candidate approaches, genome-wide association (GWA) studies can be used to identify susceptibility genes without making assumptions about the likely disease mechanisms. They have the potential to identify new mechanistic pathways. Early GWA studies did not identify polymorphisms linked to ALS, probably because they were underpowered, reporting on only a few hundred ALS individuals and controls. Evidence from other so-called complex diseases such as Type II diabetes suggests that ~2-3000 cases and controls are required to generate reliable results, when one accounts for the stringent corrections required for multiple analyses on the same data set. More recent GWA studies in ALS have been large-scale studies analyzing thousands of cases and controls using high-density mapping techniques such as DNA microarrays. These approaches have led to the identification of four significant genetic associations in ALS: FLJ10986, the inositol 1,4,5-triphosphate receptor 2 gene, ITPR2, the dipeptidyl peptidase 6 gene, DPP6 and the elongator protein 3 gene, ELP3.

Table 2 TARDBP genetic screens in ALS

<table>
<thead>
<tr>
<th>Reference</th>
<th>Index FALS screened (mutations)</th>
<th>SALS screened (mutations)</th>
<th>Mutation Frequency (FALS, SALS)</th>
<th>Ethnic origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seetharaman et al., 2008</td>
<td>154 (1)</td>
<td>372 (2)</td>
<td>0.65%, 0.54%</td>
<td>UK &amp; Australian Caucasian</td>
</tr>
<tr>
<td>Gitcho et al., 2008</td>
<td>8 (1)</td>
<td>0</td>
<td>12.5%, -</td>
<td>European</td>
</tr>
<tr>
<td>Kabashi et al., 2008</td>
<td>80 (3)</td>
<td>120 (6)</td>
<td>3.75%, 5%</td>
<td>France/Quebec</td>
</tr>
<tr>
<td>Van Deerlin et al., 2008</td>
<td>65 (2)</td>
<td>86 (0)</td>
<td>3%, 0%</td>
<td>Eastern Europe, China</td>
</tr>
<tr>
<td>Yokoseki et al., 2008</td>
<td>16 (1)</td>
<td>112 typed for mutation only (0)</td>
<td>6.25%, -</td>
<td>Japan</td>
</tr>
<tr>
<td>Kuhnelein et al., 2008</td>
<td>31 (2)</td>
<td>134 (0)</td>
<td>6.25%, 0%</td>
<td>German</td>
</tr>
<tr>
<td>Rutherford et al., 2008</td>
<td>92 (3)</td>
<td>24 (0)</td>
<td>3.26%, 0%</td>
<td>Caucasian</td>
</tr>
<tr>
<td>Daou et al., 2008</td>
<td>0</td>
<td>285 (6)</td>
<td>- 2.1%</td>
<td>French</td>
</tr>
<tr>
<td>Lammas et al., 2009</td>
<td>20 (1)</td>
<td>0</td>
<td>5%, -</td>
<td>Belgian</td>
</tr>
<tr>
<td>Corradino et al., 2009</td>
<td>125 (6)</td>
<td>541 (12)</td>
<td>4.8%, 2.2%</td>
<td>Italian</td>
</tr>
<tr>
<td>Giselinck et al., 2008</td>
<td>0</td>
<td>237 (0)</td>
<td>- 0%</td>
<td>Belgian</td>
</tr>
<tr>
<td>Guerreiro et al., 2008</td>
<td>0</td>
<td>297 (0)</td>
<td>0%, 0%</td>
<td>Caucasian &amp; African</td>
</tr>
</tbody>
</table>
Gene tic links between ALS and FTD

A genetic link between ALS and FTD is strongly suggested by linkage of families displaying inheritance of both ALS and FTD to loci on chromosome 5p15 and 14q22-24 (Table 1). No mutations have been identified as yet. There is little evidence that pure FTD genes (PGRN, MAPT and CHMP2B) are a significant cause of ALS.

Conclusions

The identification of SOD1 mutations in 20% of FALS kindreds and the use of SOD1 models of disease have enhanced our knowledge of motor neuron degeneration, but therapeutic developments have been disappointing. Several new genes have recently been identified, notably TARDBP, FUS and ANG, and suggest that there may be a role in RNA-processing abnormalities in ALS. ELP3 variants add further weight to this hypothesis. SOD1 screening has been available as a clinical test for some years. TARDBP and FUS screening should prove cost effective as most mutations cluster within a single exon. The identification of gene mutations in the remaining ~75% of FALS cases, and the characterisation of genes that contribute to SALS will add further pieces to the jigsaw puzzle that is ALS.

REFERENCES

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