1998; Savkur et al., 2001; Kimura et al., 2005; Lin et al., 2006). Lin and colleagues report that alternative transcripts observed in myotonic dystrophy are all fetal isoforms (Lin et al., 2006). Muscleblind normally translocates from cytoplasm to nucleus in the postnatal period to induce adult-type splicings, and lack of muscleblind in nucleus due to sequestration to RNA foci recapitulates fetal splicing patterns.

5.2 Alzheimer's disease (AD) and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17)

AD is the most common neurodegenerative disease representing dementia. It is characterized by intracellular neurofibrillary tangles (NFTs) and extracellular amyloid plaques. NFTs are composed of aggregates of the hyperphosphorylated tau protein encoded by *MAPT*. The amyloid plaques are composed of amyloid β peptide (Aβ) that originates from enzymatic cleavage of the amyloid precursor protein (*APP*) by β-secretase followed by γ-secretase (LaFerla et al., 2007). The γ-secretase is an enzyme complex composed of presenilin-1 (*PS1*) or presenilin-2 (*PS2*), as well as nicastrin, anterior pharynx defective (APH-1), and presenilin enhancer 2 (PEN-2) (Takasugi et al., 2003). Autosomal dominant forms of AD constitutes ~5% of AD and are caused by mutations in *APP*, *PS1*, or *PS2* (Bertram and Tanzi, 2008).

Although the pathomechanisms underlying sporadic AD remain mostly unknown, *PS2* exon 5 is exclusively skipped in brains of sporadic AD, which is mediated by overexpression of a splicing *trans*-factor, HMGA1a (Sato et al., 1999; Manabe et al., 2003). As hypoxia induces the overexpression of HMGA1a, the upregulation of HMGA1a in sporadic AD may or may not represent an agonal state of AD, in which respiratory insufficiency possibly associated with pneumonia frequently becomes the cause of death.

Mutations in *MAPT* are not observed in AD, but are present in FTDP-17. *MAPT* exon 10 is alternatively spliced in normal brain. N279K, K280del, and L284L mutations on exon 10 provoke aberrant splicing of exon 10 by disrupting or enhancing exonic splicing *cis*-elements, and cause FTDP-17 (D'Souza et al., 1999) **(Fig. 7)**. The splicing *trans*-factors for these *cis*-elements are also identified (Jiang et al., 2004; Kondo et al., 2004).

5.3 Facioscapulohumeral muscular dystrophy (FSHD)

FSHD is the third most common hereditary muscular dystrophy after Duchenne muscular dystrophy and myotonic dystrophy. As its name represents, the disease predominantly affects the face, the scapulae, and the proximal arm muscles. In FSHD, the number of a 3.3-kb repeat in the subtelomeric region of 4q (4q35), designated *D4Z4*, are abnormally reduced (Wijmenga et al., 1992). Loss of *D4Z4* causes upregulation of *FRG1* located upstream of *D4Z4* (Gabellini et al., 2002). FRG1 is a splicing *trans*-factor, and its overexpression causes aberrant splicing of *TNNT3* encoding the troponin T type 3 of fast skeletal muscle and *MTMR1* encoding the myotubularin-related protein 1 (Gabellini et al., 2006). The reported splicing aberrations in FSHD, however, have not been confirmed by us (unpublished data) or by the other groups (personal communications).

5.4 Fragile X-associated tremor/ataxia syndrome (FXTAS)

Fragile X mental retardation syndrome is caused by abnormal expansion of a CGG repeat in the 5' untranslated region of *FMR1*, which culminates in hypermethylation of *FMR1* and silences its expression (Kremer et al., 1991). On the other hand, moderate expansion of the CGG repeat in *FMR1* causes FXTAS, which is

characterized by intention tremor, parkinsonism, cognitive decline, and neuropathy (Hagerman and Hagerman, 2004). In FXTAS, CGG-binding proteins including hnRNP A2 and muscleblind are excessively bound to the expanded CGG repeats of *FMR1* and are depleted from the cellular pool (Iwahashi et al., 2006), which results in the loss their functions in other regulatory processes (Jacquemont et al., 2007).

5.5 Prader-Willi syndrome (PWS)

PWS is an autosomal dominant disorder characterized by obesity, muscular hypotonia and weakness, mental retardation, short stature, hypogonadotropic hypogonadism, and small distal extremities. The proximal long arm of chromosome 15 (15q11-q13) is normally imprinted in order to achieve parent-specific monoallelic gene expressions. Some genes in this region are expressed only from the maternal allele, and some others are only from the paternal allele. Lack of a functional paternal copy of 15q11-13 causes PWS, whereas lack of a functional maternal copy of *UBE3A* in the same region results in Angelman syndrome (Horsthemke and Wagstaff, 2008). PWS is caused by a deletion of the paternal 15q11-q13 or by maternal uniparental disomy 15.

A snoRNA HBII-52 is located in the defective region of PWS. HBII-52 binds to an ESS in exon Vb of *HTR2C* encoding the serotonin receptor 2C, and its disruption in PWS causes aberrant splicing of *HTR2C* and potentially accounts for dysfunctional serotonergic system in PWS (Kishore and Stamm, 2006).

5.6 Rett syndrome

Rett syndrome is a neurodevelopmental disorder in females, which is characterized by loss of speech, stereotypical movements of hands, microcephaly,

seizures, and mental retardation. Rett syndrome is caused by a mutation in *MECP2* encoding the metyl-CpG-binding protein 2 (Amir et al., 1999). MeCP2 binds to a splicing *trans*-factor YB-1 and the abnormal regulation of YB-1 causes aberrant splicing of its target genes (Young et al., 2005).

5.7 Spinocerebellar ataxia type 8 (SCA8)

SCA8 is caused by an abnormal expansion of CTA/CTG repeats in the protein-noncoding *ATXN8OS*, which represents the *ATXN8* opposite strand (Ikeda et al., 2008). Expanded CUG repeats on the *ATXN8OS* transcript potentially bind to and sequestrate CUG-binding proteins, as we observe in myotonic dystrophy (Mutsuddi and Rebay, 2005). In addition, *ATXN8* on the opposite strand of *ATXN8OS* encodes the Kelch-like 1, and the expanded CAG repeats on *ATXN8* give rise to a polyglutamine tract that forms a cytotoxic aggregate in neuronal cells (Moseley et al., 2006). Furthermore, expression of *ATXN8OS* is colocalized with that of *ATXN8* (Chen et al., 2008). *ATXN8OS* thus potentially serves as an antisense RNA for *ATXN8*, and the abnormal CTA/CTG expansion in *ATXN8OS* may dysregulate the expression of *ATXN8* **(Fig. 8)**.

5.8 Paraneoplastic neurological disorders (PND)

In PND, tumors outside of the nervous system excrete humoral factors such as hormones and cytokines, or provoke an immune response against specific molecules expressed in tumors, and cause a wide range of neurological symptoms. In paraneoplastic opsoclonus myoclonus ataxia (POMA), autoantibodies are raised against the Nova family of neuron-specific splicing *trans*-factor (Jensen et al., 2000; Ule et al., 2003; Ule et al., 2006; Licatalosi et al., 2008). In paraneoplastic

encephalomyelitis and sensory neuropathy (PEN/SN or Hu syndrome), autoantibodies recognize the Hu family of RNA-binding protein (Szabo et al., 1991), a human homolog of the Drosophila splicing *trans*-factor Elav (Koushika et al., 2000; Soller and White, 2003). In both disorders, autoantibodies downregulate the splicing *trans*-factors and cause aberrant splicing in neuronal cells.

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Legends for Figures

Fig. 1. Representative splicing *cis*-elements and *trans*-factors. Tissue-specific and developmental stage-specific expressions of splicing *trans*-factors including SR proteins and hnRNP A1 enable precise regulations of alternative splicing. ISE and ISS have similar activities as ESE and ESS, but are omitted from the figure.

Fig. 2. U1 snRNA recognizes three nucleotides at the 3' end of an exon and six nucleotides at the 5' end of an intron

Fig. 3. Human consensus BPS. **(A)** Pictogram and **(B)** WebLogo presentations of BPS. Position 0 represents the branch point. **(C)** Representative sequences and positions of splicing *cis*-elements.

Fig. 4. *CHRNA1* carries a 75-nt exon P3A. Its inclusion generates a non-functional alpha subunit of the acetylcholine receptor. hnRNP H and PTB silence recognition of exon P3A and induce its skipping. The IVS3-8G>A mutation identified in a patient with congenital myasthenic syndrome weakens the binding of hnRNP H and causes inclusion of exon P3A. Tannic acid facilitates the expression of PTB and partially ameliorates aberrant splicing due to IVS3-8G>A.

Fig. 5. NASRE. Wild-type *CHRNE* generates the normally spliced transcript (a) and the exon 6-skipped transcript (b), because exon 6 carries weak splicing signals. The exon-skipped transcript carries a premature termination codon (PTC) and is degraded by NMD. A 7-nt deletion (arrowhead) in exon 7 generates a PTC in the normally

spliced transcript (c) and is degraded by NMD. The deletion resumes the open reading frame from the exon 6-skipped transcript, and the transcript escapes NMD (d).

Fig. 6. In DM1, expanded CUG repeats in the 3' UTR of DMPK sequestrate muscleblind and upregulates CUG-binding protein. Dysregulation of these splicing *trans*-factors cause aberrant splicing of their inherent target genes. Four representative target genes are indicated.

Fig. 7. Mutations on *MAPT* exon 10 cause excessive skipping (N279K and L284L) or inclusion (K280del) of exon 10.

Fig. 8. Expanded CTG on *ATXN8OS* exerts three toxic effects on the bidirectional transcripts.

Footnote for NMD in Section 4.2

Nonsense-mediated mRNA decay (NMD). NMD is a quality-assurance mechanism that degrades mRNAs harboring a premature termination codon (PTC) (Chang et al., 2007). Proteins translated from mRNAs harboring PTCs potentially have dominant-negative or deleterious activities. In pre-mRNA splicing, an exon-junction complex (EJC) is deposited 20-24 nucleotides upstream of each exon-exon junction. Ribosomes remove EJCs, but, in the presence of a PTC, EJCs stay on the transcript and trigger the NMD pathway in the cytoplasm.

Figure 1

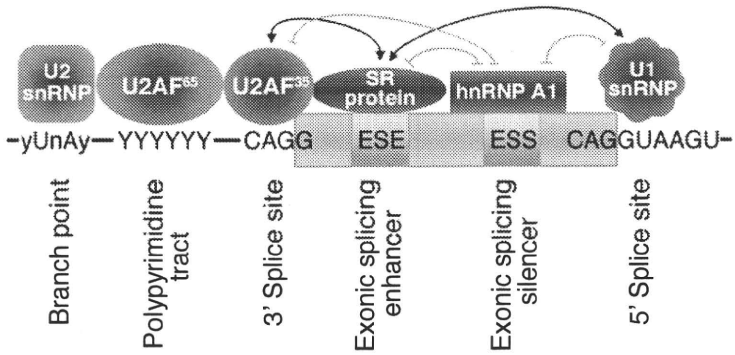
U2 snRNP
U2AF65
U2AF35
SR protein
hnRNP A1
U1 snRNP
-yUnAy—YYYYYY—CAGG
ESE
ESS
CAGGUAAGU-
Branch point
Polypyrimidine tract
3' Splice site
Exonic splicing enhancer
Exonic splicing silencer
5' Splice site

Figure 2