Decreased N-TAF1 expression in X-linked dystonia-parkinsonism patient-specific neural stem cells

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ABSTRACT

X-linked dystonia-parkinsonism (XDP) is a hereditary neurodegenerative disorder involving a progressive loss of striatal medium spiny neurons. The mechanisms underlying neurodegeneration are not known, in part because there have been few cellular models available for studying the disease. The XDP haplotype consists of multiple sequence variations in a region of the X chromosome containing \textit{TAF1}, a large gene with at least 38 exons, and a multiple transcript system (MTS) composed of five unconventional exons. A previous study identified an XDP-specific insertion of a SINE-VNTR-Alu (SVA)-type retrotransposon in intron 32 of \textit{TAF1}, as well as a neural-specific \textit{TAF1} isoform, N-TAF1, which showed decreased expression in post-mortem XDP brain compared with control tissue. Here, we generated XDP patient and control fibroblasts and induced pluripotent stem cells (iPSCs) in order to further probe cellular defects associated with this disease. As initial validation of the model, we compared expression of \textit{TAF1} and MTS transcripts in XDP versus control fibroblasts and iPSC-derived neural stem cells (NSCs). Compared with control cells, XDP fibroblasts exhibited decreased expression of \textit{TAF1} transcript fragments derived from exons 32-36, a region spanning the SVA insertion site. N-TAF1, which incorporates an alternative exon (exon 34'), was not expressed in fibroblasts, but was detectable in iPSC-differentiated NSCs at levels that were threefold lower in XDP cells than in controls. These results support the previous findings that N-TAF1 expression is impaired in XDP, but additionally indicate that this aberrant transcription might occur in neural cells at relatively early stages of development that precede neurodegeneration.

KEY WORDS: X-linked dystonia-parkinsonism, Induced pluripotent stem cells, TAF1

INTRODUCTION

The dystonias are a diverse collection of movement disorders arising from different etiologies that lead to characteristic patterns of involuntary muscle contractions and twisted postures (Albanese et al., 2013a). Most cases in which dystonia is the primary clinical manifestation seem to have a genetic predisposition, with over 25 gene loci linked to different forms of the disease (Lohmann and Klein, 2013). Among the many subtypes of dystonia are numerous examples that combine clinical features of Parkinson’s disease (PD), such as (1) DOPA-responsive dystonia, caused by variations in genes encoding dopamine biosynthetic enzymes (\textit{GCH1}, \textit{TH}, \textit{SR}; Lee and Jeon, 2014); (2) rapid-onset dystonia-parkinsonism (RDP), caused by variations in a subunit of the sodium/potassium ATPase (\textit{ATP1A3}; Geyer and Bressman, 2011); (3) DYT16 dystonia, associated with variations in the PKR regulatory protein, PACT (\textit{PRKRA}; Zech et al., 2014; Camargos et al., 2012); and (4) other degenerative disorders such as Wilson’s disease and neurodegeneration with brain iron accumulation (NBIA; Schneider et al., 2009; Schneider and Bhatia, 2010). Yet in no form of hereditary dystonia is a connection to PD more apparent than in X-linked dystonia-parkinsonism (XDP, DYT3, OMIM #3142590), a progressive neurodegenerative disease endemic to the island of Panay, Philippines (Lee et al., 2011). XDP follows an unusual clinical course, consisting primarily of dystonic symptoms at early disease stages that shift over time towards a more parkinsonian phenotype (Lee et al., 2002, 2011). The underlying disease mechanisms and pathogenic substrates are not clear, but neuropathological studies indicate that XDP involves a progressive loss of medium spiny neurons in the striatum (Waters et al., 1993; Goto et al., 2005) and decreased numbers of neural progenitor cells within the subventricular zone (Goto et al., 2013).

Previous genetic linkage (Wilhelmsen et al., 1991; Kupke et al., 1992; Graeber and Müller, 1992) and association (Müller et al., 1993; Goto et al., 2005) and decreased numbers of neural progenitor cells within the subventricular zone (Goto et al., 2013). Sequecing of this region revealed seven XDP-specific sequence variants: five disease-specific single nucleotide changes (designated DSC1, 2, 3, 10 and 12), a 48-bp deletion, and a SINE-VNTR-Alu (SVA)-type retrotransposon insertion (Nolte et al., 2003; Makino et al., 2007). Only one variant, DSC3, falls within a potential coding region, introducing a single nucleotide substitution in one of the MTS exons (Nolte et al., 2003; Makino et al., 2007). The other variants are localized either within TAF1 introns or the intergenic region containing the MTS that is 3’ to exon 38 of TAF1. To date it has not been possible to exclude any of these variants as pathogenic, as extensive genotyping of large numbers of XDP individuals and ethnically matched control subjects has detected all markers in essentially all cases; the few exceptions,
which were negative for all markers, were considered likely phenocopies (Domingo et al., 2015).

Of the five known genes within this interval, TAF1 has arguably received the most attention as the one likely to be responsible for XDP pathogenesis. TAF1 is a large gene with at least 38 exons that encodes a transcription factor, TATA-binding protein-associated factor-1 (TAF1), which is part of the transcription factor IID (TFIID) complex involved in RNA polymerase II-mediated transcription (Ruppert et al., 1993; Thomas and Chiang, 2006). The hypothesis that TAF1 might be the culprit in XDP has been based in part on the relationship between the XDP sequence variants and its genomic structure and transcripts. Three of the haplotype markers (DSC10, DSC12 and the SVA) alter sequences within TAF1 introns, and RNAs derived from MTS exons can reportedly splice to various TAF1 transcripts (Nolte et al., 2003; Herzfeld et al., 2007; Makino et al., 2007). In addition, a previous study identified a neural-specific TAF1 isoform, N-TAF1, which was significantly downregulated in XDP caudate relative to control brain tissue (Makino et al., 2007). N-TAF1 contains an alternative exon, exon 34', of only six nucleotides, which adds an alanine and lysine to the encoded protein (Makino et al., 2007; Sako et al., 2011; Jambaldorj et al., 2012). It has been suggested that the decreased expression of N-TAF1 in XDP might be the result of the 2.6 kb SVA insertion within intron 32 of TAF1 (Makino et al., 2007). However, it is not clear how this insertion acts to interfere with transcription of N-TAF1.

In this study we generated XDP and matched control induced pluripotent stem cell (iPSC) lines that can be differentiated into neural cells in order to establish a model system for probing cellular defects associated with the disease. Towards that end, we derived fibroblasts from confirmed XDP individuals and unaffected family members as matched control subjects, reprogrammed these cells to iPSCs, and then re-differentiated iPSCs to generate XDP and control neural stem cells (NSCs). As a first application of this model, we assayed expression of TAF1 and MTS-derived transcripts in both the iPSC-derived NSCs as well as the parent fibroblast lines. These analyses revealed genotypic differences in the expression of multiple TAF1 transcripts in fibroblasts and decreased expression of N-TAF1 in XDP versus control NSCs. These findings are consistent with the previous report of an XDP-related defect in N-TAF1 expression (Makino et al., 2007), indicating that the patient iPSC-derived neural cells recapitulate a phenotype detected in patient post-mortem brain tissue. In addition, the present results further suggest that this defect might not be solely associated with advanced disease stages but might instead occur in XDP neural cells at early stages that precede the development of neuronal cell death.

**RESULTS**

**Clinical characteristics and genotype of XDP individuals**

Of the five XDP individuals that participated in this study, only one (32517; Table 1) was examined prior to undergoing deep brain stimulation (DBS). At the time of fibroblast derivation, this subject presented with segmental dystonia affecting the jaw and larynx. Dystonic symptoms subsequently worsened and generalized to other muscle groups over the course of 1 year, at which point the individual underwent bilateral pallidal DBS. The other four subjects had similar clinical histories, involving initial onset of dystonic symptoms in various muscle groups. At the time of skin biopsy, these subjects exhibited variable degrees of dystonia, ranging from generalized symptoms affecting the left hand, torso and left leg in one subject (34363) to focal neck dystonia in another (33109). Bradykinesia without rigidity was a common feature among subjects. All control family members showed no neurological abnormalities upon examination.

Genotypes of affected individuals and control family members were determined by PCR for six of the seven reported haplotype markers (Nolte et al., 2003; Makino et al., 2007). The five single nucleotide substitutions (DSC1, 2, 3, 10 and 12) were evaluated by PCR amplification followed by Sanger sequencing of the products. The affected subjects showed a consistent pattern, with the same nucleotide substitutions at each DSC that had been previously associated with XDP (Table 2). The control individuals all had wild-type sequences at each site. The 2.6 kB SVA retrotransposon was detected by long-range PCR amplification using primers flanking the insertion site. A single PCR product was detected in both XDP and control samples, which differed in size by ~2.6 kB (0.6 kb in control versus 3.2 kb in XDP samples), consistent with the size of the SVA (Fig. 1A). There were no genotypic differences noted in growth rate or cell division among the fibroblast lines. As expected, fibroblasts from the older donors, 33808 (XDP) and 33809 (control), had decreased doubling times in culture compared with the lines derived from younger subjects. However, most of the lines grew at comparable rates, and all fibroblast experiments were performed at equivalent early passages.

| Status   | Subject | Sex | Age at onset (years) | Age at exam (years) | Initial presentation | Presentation at skin biopsy⁴ | Affected body regions⁴  |
|----------|---------|-----|----------------------|---------------------|----------------------|-----------------------------|--------------------------|
| XDP      | 32517   | M   | 32                   | 35                  | Dystonia: jaw        | Segmental                   | Jaw, larynx              |
|          | 33109   | M   | 58                   | 72                  | Dystonia: right upper extremity | Focal                      | Neck                     |
|          | 33363   | M   | 38                   | 44                  | Dystonia: tongue     | Multifocal                 | Jaw, tongue, left arm     |
|          | 33808   | M   | 48                   | 80                  | Dystonia: eyes       | Multifocal                 | Jaw, pelvis, torso        |
|          | 34363   | M   | 40                   | 57                  | Dystonia: torso, right/left lower extremities | Generalized                | Left hand, torso, left foot |
| Control  | 32643   | M   | –                    | 70                  | –                    | Unaffected                  | –                        |
|          | 33113   | M   | –                    | 42                  | –                    | Unaffected                  | –                        |
|          | 33114   | M   | –                    | 34                  | –                    | Unaffected                  | –                        |
|          | 33362   | M   | –                    | 18                  | –                    | Unaffected                  | –                        |
|          | 33809   | F   | –                    | 77                  | –                    | Unaffected                  | –                        |

⁴Denotes extent of dystonia and muscle groups affected.
F, female; M, male.
We compared expression of transcripts derived from *TAF1* and the putative MTS exons in XDP versus control fibroblasts using a panel of previously described primers (Makino et al., 2007) interrogating 21 mRNAs (Fig. 1B,C). Of the 21 transcripts assayed, seven were undetectable in both XDP and control fibroblast samples. The panel included six primer sets targeting transcripts that reportedly incorporate MTS sequences, either alone (MTS-2/3, MTS-3/4, MTS-V4) or spliced to exons of *TAF1* (MTS-32′/34′, MTS-37/1, MTS-37/3) (Makino et al., 2007). None of these mRNAs was expressed in fibroblasts. In addition, primer set TA14-391, which was previously shown to be specific to the neural TAF1 isoform, N-TAF1, did not detect a product in fibroblasts.

Among the remaining 14 mRNAs tested, five showed differential expression distinguishing XDP from control samples at \( P < 0.05 \) (Fig. 1B). Three primer sets (TAF1-3′, TA14-385N and TAF1-3′N) amplified transcript fragments derived from exons 32-36 that were significantly decreased in XDP fibroblasts compared with control cells. These exons flank the SVA insertion in intron 32 and span the region containing the alternative exon, 34′, from which the neural isoform, N-TAF1, is generated. Other primers within the panel also targeted fragments derived from these exons; although levels of their respective products were also lower in XDP cells relative to controls, the differences did not achieve statistical significance. Two other transcript fragments showed significant differences in expression in XDP versus control cells: one fragment (amplified by TA02-334) derived from exons 4-5 had lower levels in XDP cells, and another (from TA09-693) incorporating sequences from exons 19-21 had higher expression in XDP cells than in controls. In addition to *TAF1* transcripts, we assayed expression of three genes also contained within the XDP genomic region (*CXCR3, ACRC* and *OGT*) but none showed differential expression in XDP versus control fibroblasts (Fig. S1).

### Table 2. Genotype of XDP single-nucleotide haplotype markers

| Subject | DSC12 | DSC10 | DSC1 | DSC3 | DSC2 |
|---------|-------|-------|------|------|------|
| 32517   | G     | T     | A    | T    | A    |
| 33109   | G     | T     | A    | T    | A    |
| 33363   | G     | T     | A    | T    | A    |
| 33808   | G     | T     | A    | T    | A    |
| 34363   | G     | T     | A    | T    | A    |
| 32643   | T     | C     | T    | C    | G    |
| 33113   | T     | C     | T    | C    | G    |
| 33114   | T     | C     | T    | C    | G    |
| 33362   | T     | C     | T    | C    | G    |
| 33809   | T     | C     | T    | C    | G    |

DSC, disease-specific sequence change.

### Analysis of TAF1 and MTS transcripts in fibroblasts

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In other genes, SVA insertions have been shown to generate aberrant transcripts consisting of retrotransposon sequences spliced to RNA derived from the surrounding exons (Hancks and Kazazian, 2012). To probe for such exonization of the SVA in TAF1, we designed two additional primer sets flanking the insertion (Table S1) and performed qRT-PCR on RNA from XDP and control fibroblasts. Because aberrant transcripts might potentially be targeted for nonsense-mediated decay (NMD), fibroblasts were cultured in a specific NMD inhibitor, NM22114 (Martin et al., 2014), prior to RNA isolation and reverse transcription. Gel electrophoresis of the PCR products showed bands of equivalent sizes in XDP and control samples (Fig. S2), suggesting that SVA sequences were not incorporated into these particular transcript fragments.

The observation that N-TAF1 was not detectable in fibroblast RNA seems consistent with its previously documented pattern of neural tissue-specific expression (Makino et al., 2007; Sako et al., 2011; Jambaldorj et al., 2012). As additional validation, we used the TA14-391 primer set to assay N-TAF1 transcript levels in RNA obtained from SH-SY5Y human neuroblastoma cells, as well as human brain. Moderate N-TAF1 transcript expression was detected in SH-SY5Y cells, which increased slightly in cells differentiated in all-trans-retinoic acid (ATRA) for 72 h (Table 3). N-TAF1 expression in human brain was considerably higher, representing a more than 60-fold increase above the levels detected in undifferentiated SH-SY5Y cells.

**Generation of iPSCs and neural conversion**

Given previous data specifically implicating N-TAF1 in XDP pathogenesis (Makino et al., 2007), a major objective of this study was to generate XDP cell lines in which expression of this variant could be further examined. Towards that end, we reprogrammed five XDP and four control fibroblast lines to iPSCs that could be re-differentiated into neural cells. Reprogramming was accomplished using non-integrating Sendai virus. Control fibroblast line 33809 exhibited slow growth in culture and typically underwent senescence at lower passages than the other cell lines, making it difficult to expand sufficiently for viral inoculation. Given that fibroblast growth rate can greatly influence reprogramming efficiency (Hanna et al., 2010), this cell line was not reprogrammed. All of the XDP fibroblasts and three of the control lines (33113, 33114 and 33362) generated at least two iPSC clones each that exhibited significant upregulation of pluripotency-related genes, Dmhn3b, hTERT, Nanog, Oct4, Rex1 and Sox2, compared with the parent fibroblast lines, as measured by qRT-PCR (Fig. 2A). Clones also expressed alkaline phosphatase and were immunopositive for standard iPSC lines, as well as human brain. Moderate N-TAF1 transcript expression was detected in SH-SY5Y cells, which increased slightly in cells differentiated in all-trans-retinoic acid (ATRA) for 72 h (Table 3). N-TAF1 expression in human brain was considerably higher, representing a more than 60-fold increase above the levels detected in undifferentiated SH-SY5Y cells.

| Table 3. N-TAF1 mRNA levels in fibroblasts, SH-SY5Y cells and human brain |
|-------------------------------------|-------------------|-------------------|
| RNA source                         | FC vs fibroblasts | FC vs SH-SY5Y     |
| Fibroblasts                         | N                 | –                 |
| SH-SY5Y                             | 34.62             | 1.00              |
| SH-SY5Y+ATRA                        | 48.73             | 1.41              |
| Human brain                         | 2267.15           | 65.50             |

FC, fold change; N, not detected; ATRA, all-trans-retinoic acid.

To quantify functional pluripotency of each iPSC line, embryoid bodies (EBs) were generated in culture from all clones and assayed for expression of germ layer marker genes using the Taqman hPSC Scorecard Assay (Bock et al., 2011; Tsankov et al., 2015). As expected, the differentiated EBs exhibited little to no expression of genes involved in self-renewal but were positive for multiple markers of each lineage (Fig. 3A). There was some variability in expression of individual genes across the various clones, but the overall pattern was largely consistent and revealed no genotypic differences in pluripotency. Mesoderm-related genes seemed to be more highly expressed than markers of endoderm and ectoderm, but the observed signatures suggest that all clones retain the capacity to differentiate towards all three lineages. In parallel to the EB analysis, selected iPSC clones were also injected into mice to evaluate pluripotency in vivo. All clones tested formed teratomas containing cells representative of the three germ layers, based on morphology visible by Hematoxylin and Eosin (H&E) staining (Fig. 3B-G). The iPSC lines had comparable morphologies (Fig. 4A) and growth behavior in culture, except for clone 33109-2G, which exhibited a high rate of spontaneous differentiation that interfered with propagation and prevented neural conversion. This behavior seemed to be a clone-specific property, as the companion line, 33109-2B, did not differ in its growth characteristics from the other iPSC lines. All iPSC clones have been deposited at the repository at WiCell (Madison, WI, USA; www.wicell.org) and are publicly available.

To generate NSCs, XDP and control iPSCs were cultured in PSC Neural Induction Medium for seven days, at which point they were re-plated on Gelrex-coated culture dishes and propagated in Neural Expansion Medium. For most of the iPSC lines, the neural induction protocol generated cells with characteristic NSC-like morphology (Fig. 4B), although two clones (32517-A and 32643-C) consistently detached during induction and could not be converted to NSCs. At the initial plating (immediately after seven days of induction), NSC cultures occasionally contained clusters of cells resembling small iPSC colonies, potentially suggesting residual pluripotent cells. After 1-2 passages in culture, these cells were no longer apparent, and immunostaining with an antibody for Oct3/4 detected no positive cells (data not shown). By passage 3 post-induction, XDP and control cultures typically consisted predominantly of cells immunopositive for nestin, musashi and Sox1 (Fig. 4C-E). For a quantitative assessment of neural conversion, RNA from all NSC lines, as well as the parent iPSC clones, was assayed by qRT-PCR using a panel of primers interrogating 22 genes known to be expressed in neural stem cells (Fig. 4F). Expression of all neural marker genes was low and/or non-detectable in iPSC clones and increased in NSCs with only few exceptions. Control iPSC clone 33362-D exhibited high expression of APBB1 and CDK5RAP2, unlike the other iPSC clones, whereas XDP NSC line 33363-C showed low expression of DLL and EGF; in contrast to the other NSC lines. Yet despite some variability in expression level of individual genes among the cell lines, assessment of the full panel of markers revealed no patterns to indicate any genotypic differences in neural conversion between XDP and control cells. Furthermore, no NSC clone differed consistently from all other lines across the entire panel that might suggest aberrant differentiation. Based on these data, the final set of NSCs used for analysis of TAF1 transcript expression consisted of 7 XDP clones derived from five patients versus six control clones from three individuals.

**Analysis of TAF1 and MTS transcripts in NSCs**

We used the panel of primers interrogating TAF1 and MTS-derived mRNAs to compare transcriptional patterns in XDP and control
NSCs. RNA was isolated from all NSC lines at passage 4 post-induction. As in the fibroblasts, the NSC lines did not seem to express any of the MTS-derived RNAs (Fig. 5A). Unlike in fibroblasts, the N-TAF1 variant was expressed in NSCs at levels that were on average ∼threefold lower in XDP versus control clones (P<0.05; Fig. 5A). Although multiple TAF1 fragments seemed to be differentially expressed in XDP versus control fibroblasts (Fig. 1B), N-TAF1 was the only transcript in this panel that distinguished XDP from control NSCs. N-TAF1 contains an alternative exon, 34′ (Fig. 1C), consisting of only six nucleotides that encode two additional residues, an alanine and a lysine, which become positions 1646-1647, respectively, of a 1847-aa protein (Fig. 5B) (Makino et al., 2007). The insertion is C-terminal to two tandem bromodomains, which mediate histone interactions (Jacobson et al., 2000), and immediately adjacent to a threonine, T1643, which is a potential phosphorylation site (Hornbeck et al., 2012). These three residues, T1643, A1646 and K1647, fall within a short cluster of prolines that could potentially form a polyproline helix, although it is difficult to accurately predict such motifs in silico. The other notable feature of this segment is the preponderance of aspartate and glutamate residues that seem to make up an acidic tail. Aside from these limited observations, the protein sequence itself does not reveal potential function(s) of the N-TAF1-unique residues, and because the insertion falls within an intrinsically disordered region of the protein, structural analyses of this segment have not been possible (Jacobson et al., 2000).

**DISCUSSION**

XDP was first described by Lee et al. (1976) as torsion dystonia of Panay, with 19 of 23 cases coming from the province of Capiz in the Philippines. Since that pioneering report, the natural history of XDP has been documented in detail, facilitated by the formation of an XDP Study Group and an XDP Registry (Lee et al., 2011). As of January 2010, this registry consisted of 505 cases corresponding to an approximate prevalence of 5.74 cases per 100,000 individuals in Panay (1:4000 men specifically in Capiz), or 0.31:100,000 within the entire Philippine population (Lee et al., 2011). The clinical profiles of these cases, as well as ones from other centers, consist of an adult-onset movement disorder that typically begins as focal dystonia that generalizes over time and is combined with or replaced by parkinsonian features (Lee et al., 1991, 2002, 2011; Evidente et al., 2002a). In a small percentage of documented cases, parkinsonian symptoms appear as the initial clinical findings (Lee et al., 2002; Evidente et al., 2002b), but in most individuals characterized to date, disease onset involved focal dystonia of either the lower extremities, craniofacial musculature (e.g. oromandibular dystonia, blepharospasm) or upper extremities (Lee et al., 2002). The vast majority of affected individuals are males, but there are reports of symptomatic females resulting from homozygosity of the disease allele, skewed X-inactivation or Turner’s syndrome (Lee et al., 2001; Evidente et al., 2004; Westenberger et al., 2013; Domingo et al., 2014, 2015). Among affected individuals, disease progression is variable, but the most frequent clinical picture involves spread of dystonic symptoms during the first decade following diagnosis, combined with the emergence of parkinsonian symptoms that predominate by 15 years after the initial onset (Lee et al., 2002).

The XDP subjects investigated here appeared consistent with previous reports in terms of clinical presentations and positive responses to pallidal DBS, which has been documented by multiple groups (Evidente et al., 2007; Wadia et al., 2010; Oyama et al., 2010; Aguilar et al., 2011). Another consistent finding was the combined presence of multiple haplotype markers. The most recent effort to narrow the XDP genomic region involved genotyping of 163 males with clinical diagnoses of XDP and 473 Filipino controls, of which 452 had ancestry specifically to Panay (Domingo et al., 2015). The seven known disease-related variants (five DSCs, a 48-bp deletion and the SVA; Nolte et al., 2003; Makino et al., 2007) were found in all but five of the affected individuals; the latter group, thought to be phenocopies, were negative for all markers, similar to the control subjects (Domingo et al., 2015). In this study, we genotyped six of the seven sequence variants and observed a similar ‘all or none’ distribution in affected versus unaffected family members. This inability to rule out any of the seven genomic variants has complicated efforts to understand XDP pathogenesis.
That these sequence variants cluster around TAF1 and the intergenic MTS has focused most hypothesized disease models on their respective gene products, but crucial questions remain unanswered. With respect to the MTS, it is noteworthy that it contains the only XDP sequence variant, DSC3, which alters a potential coding exon (Nolte et al., 2003; Herzfeld et al., 2007). Furthermore, ectopic expression of wild-type or mutated (DSC3) MTS cDNAs in control cell lines resulted in different transcriptional profiles, suggesting

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**Fig. 3. Pluripotency analyses.** (A) Trilineage potential of each XDP and iPSC clone determined by Taqman Scorecard Assay analysis of RNA from embryoid bodies differentiated in culture from each cell line. Heatmaps depicting expression of 94 marker genes related to self-renewal, primitive mesendoderm, mature ectoderm, mesoderm and endoderm. Values represent mean fold changes (FC) for each gene relative to Scorecard reference pluripotent cell lines, calculated by PSC Scorecard analysis software. Red, high expression; blue, low expression. (B-G) Representative XDP (33363-C; upper panel) and control (33362-C; lower panel) teratomas produced in mice following cell implantation. Arrows denote ectodermal neuroepithelium (B) and hair follicle cells (E); mesodermal cartilage (C,F); endodermal mucous gland (D) and gut epithelium (G). Scale bars: 100 μm.
that the DSC3 substitution could have functional effects (Herzfeld et al., 2013). However, very little is known about the normal distribution of endogenous MTS transcripts, the type(s) of protein(s) that might be generated from these mRNAs, and what role, if any, they might play within neural structures thought to be affected in XDP.

TAF1, by contrast, has been extensively characterized as part of the general transcriptional machinery. The human gene was cloned in the effort to define co-activators that associate with the TATA-binding protein (TBP) within the general TFIID complex (Ruppert et al., 1993). It proved identical to a previously discovered cell cycle-related gene, CCG1, in which a temperature-sensitive point mutation prevents G1-S progression in the hamster cell line, tsBN462 (Sekiguchi et al., 1988, 1991). TFIID is involved in transcription initiation by RNA polymerase II (Grünberg and Hahn, 2013; Kandiah et al., 2014). It is a multi-subunit complex composed of TBP and at least 13 TAFs that collectively bind the TATA element upstream of the transcription start site in many gene promoters (Papai et al., 2011; Thomas and Chiang, 2006). The major isoform of TAF1 is a large, 250 kDa protein containing an N-terminal domain that binds TBP in competition with other TFIID components (Anandapadamanaban et al., 2013). TAF1 seems to be a negative regulator of TBP, inhibiting its DNA-binding activity and removing TFIID from the TATA element, which might be crucial in determining the balance between transcription initiation and termination (Thomas and Chiang, 2006). Structural analyses further suggest that TAF1 might have intrinsic DNA-binding activity itself. The conserved central core of TAF1 contains a winged helix (WH) domain that directly binds DNA and might mediate the effects of TAF1 on expression of various cell cycle genes (Wang et al., 2014). Thus, it is possible that TAF1 might act as a gene-specific transcription factor independent of its major role in modulating TBP within TFIID.

The SVA insertion within intron 32 of TAF1 is an intriguing candidate as the XDP genetic lesion, given the growing recognition of how mobile DNA elements might cause human disease. SVAs represent one of the many types of retrotransposon insertions, including LINEs (L1s), Alus, SINEs and human endogenous retrovirus (HERV) sequences, that comprise over 40% of the human genome (Hancks and Kazazian, 2012). Although evolutionarily millions of years old, these elements retain some potential to insert at new sites and cause disease. There are 2676 SVA elements comprising 0.13% of the human genome, with a new insertion in one out of every 916 births (Savage et al., 2013; Cordaux and Batzer, 2009; Hancks and Kazazian, 2010). SVA retrotransposition depends on endonuclease and reverse transcriptase activities derived from L1 elements, a process known as target site-primed reverse transcription. SVA inserts vary from 700 bp to 4 kb, with structures typically consisting of: (1) a 5′ to 3′ hexameric CCCTC repeat; (2) two anti-sense Alus; (3) a variable number of tandem GC-rich repeats; (4) shared sequence identity with the env gene and the right long terminal repeat of HERV-K10; and (4) a polyA signal sequence. The repetitive nature of these sequences throughout the genome renders them difficult to distinguish individually. There are over 12 human diseases, including cancer, known to be caused by SVA insertions, most of which are inserted in the sense orientation.

Fig. 4. Generation and characterization of neural stem cells. (A) Brightfield micrograph depicting characteristic morphology of a representative iPSC colony on Geltrex prior to neural conversion. (B) Epifluorescent image of neural stem cells (NSCs) generated by conversion of iPSCs in PSC Neural Induction Medium, labeled with calcein-AM (Life Technologies). NSCs on Geltrex formed discrete clusters of cells with occasional fine processes. Images in A and B captured at final magnification of 20×. (C-E) NSCs were also evaluated by immunofluorescence for expression of (C) nestin; (D) Sox1; and (E) musashi. Staining for each target was detected by a secondary antibody coupled to Alexa Fluor 594 (red), along with counterstaining to visualize cytoplasm (Wheat germ agglutinin-Alexa Fluor 488; green) and nuclei (Topro-3-iodide; blue). Images represent overlays of all three channels captured by laser confocal microscopy at final magnification of 100×. Immunoreactivities for nestin and musashi seem predominantly cytoplasmic, whereas Sox1 labeling was observed primarily within the nucleus. Scale bars: 10 µm. (F) Heatmap of ΔCt values depicting comparative expression of 22 neural marker genes in XDP and control NSCs vs corresponding iPSCs from which they were differentiated. Red, high expression; blue, low expression.
amplified by primer set TA14-391, was detected in NSCs at levels
Documented effects of such insertions include: (1) deletions of expression analysis of
geometric mean of housekeeping genes (pink); prolines (P; blue); and a threonine at position 1643 (T1643; green) that bromodomain motif (gray); acidic glutamate (E) and aspartate (D) residues alanine (A) and lysine (K) residues relative to: C-terminal segment of second
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telangiectasia-mutated (ATM) and ATM-RAD3-related (ATR) pathways (Katzenberger et al., 2006; Marengo and Wassarman, 2008). However, the signals governing neural-specific expression of N-TAF1 are not known. The decreased expression of N-TAF1 detected here seems consistent with the decrease reported in XDP caudate (Makino et al., 2007), indicating that this phenotype in XDP brain tissue is reproduced in the iPSC-derived NSCs. The neural cells differentiated here generally resembled early stage progenitors capable of generating both neuronal and glial cell types, which might suggest that aberrant N-TAF1 expression occurs at early stages that precede the development of neurodegeneration in XDP individuals. In addition to a selective loss of striatal medium spiny neurons (Goto et al., 2005), XDP brains also exhibit decreased numbers of proliferating cell nuclear antigen (PCNA)-positive neural progenitors within the subventricular zone, compared with control tissue (Goto et al., 2013). Thus, the recent neuropathology data and the present findings both seem to suggest that XDP-related functional defects might develop not only in mature neurons but also in neural precursor cells.

A curious feature of N-TAF1 is that it differs from the major isoform of TAF1 by only two amino acids. That these residues fall within an intrinsically disordered region of the protein prevents any structural modeling that might reveal a functional significance for this insertion. The surrounding cluster of prolines could indicate a polyproline helix, which can be a recognition motif involved in diverse cellular functions, including transcription (Adzhubei et al., 2013). If this segment forms a local helical structure, then the insertion of alanine and lysine would alter the configuration by changing the spacing of the prolines. Such a rearrangement might affect interactions with other proteins at this site. Nevertheless, such possibilities remain speculative at this point and will require empirical testing.

If the pathogenic defect in XDP is in fact reduced expression of several TAF1 isoforms, it might seem surprising that the medical phenotype is exclusively neurologic, given the role of different TAF1 isoforms in fundamental aspects of transcription in all cells. Yet such a scenario would not be unique, as sequence variations in TBP, the canonical binding partner for TAF1 and arguably the most crucial component of the TFIID complex, are also associated with neurodegeneration. Expanded trinucleotide repeat sequences in TBP have been shown to cause spinocerebellar ataxia-17 (SCA17) and to phenocopy Huntington’s disease (Nakamura et al., 2001; Fujigasaki et al., 2003; Fujigasaki et al., 2003; Hsu et al., 2014). This observation reinforces the hypothesis that at least some perturbations within TFIID have consequences specific to the central nervous system (CNS). Moreover, XDP might not be the only link between TAF1 and neurologic disease. Recent sequencing efforts have linked different coding mutations in TAF1 to X-linked intellectual disability (Hu et al., 2016). Thus, it is possible that XDP could be part of a spectrum of TAF1-related disorders in which different mutations that affect the protein to different extents induce CNS disorders of varying degrees of severity.

It should be noted that the lack of MTS expression in the cells profiled here does not in itself exclude the potential importance of these exons and/or DSC3 to XDP. It is still possible that these transcripts might be expressed at later neurodevelopmental stages and/or in specific neuronal subtypes that are involved in the disease process. Further studies will therefore be required to probe the possible contributions of these transcripts as well as the specific cellular pathways that might be affected by them and/or by decreased N-TAF1 expression. In that regard, the development of XDP patient-specific iPSC lines that can be differentiated toward

Fig. 5. TAF1 and MTS transcript expression levels in NSCs. Quantitative expression analysis of TAF1 transcript fragments in XDP (n=7) vs control (n=6) NSC clones based on comparative Ct method with targets normalized to the geometric mean of housekeeping genes HPRT1 and TFRIC. (A) N-TAF1, amplified by primer set TA14-391, was detected in NSCs at levels approximately threefold lower in XDP vs control cells. Data represent mean fold changes ± standard errors, analyzed by Student’s t-test. *P<0.05; N, not detected. (B) Amino acids 1556–1874 of N-TAF1 highlighted to indicate unique alieline (A) and lysine (K) residues relative to: C-terminal segment of second bromodomain motif (gray); acidic glutamate (E) and aspartate (D) residues (pink); prolines (P; blue); and a threonine at position 1643 (T1643; green) that is a potential phosphorylation site.

Documented effects of such insertions include: (1) deletions of endogenous gene sequences, as in NF1 (Vogt et al., 2014) and the promoter of FUS (Savage et al., 2013); (2) insertions associated with cancer (Szpakowski et al., 2009); (3) altered splicing, including exon skipping (SPTA1, BTK); (4) exonization of SVA into transcripts, which then become out-of-frame and dysfunctional (PKTN, ARH, LDLRAP1, PNPLA2, PMS2; Kwon et al., 2013; Hancks et al., 2009); and (5) transcription of SVA sequences driven by internal retrotransposon elements (Hancks and Kazazian, 2010).
specific neuronal cell fates in culture might be powerful tools that enable such investigations.

**MATERIALS AND METHODS**

**XDP and control subjects**

Subjects recruited for this study included: (1) individuals with a confirmed diagnosis of XDP based on prior genetic testing; (2) individuals suspected of XDP based on clinical history and Filipino ancestry; and (3) unaffected family members. The study was approved by the institutional review board at Massachusetts General Hospital, and all participants gave written informed consent. Clinical evaluation included comprehensive neurological exams with recorded scores for standard scales: Burke–Fahn–Marsden, Tsui–Torticollis, Toronto Western Spasmodic Torticollis Rating and Voice Disability Index (Albanese et al., 2013b).

**XDP and control fibroblasts**

For fibroblast derivation, skin biopsies were performed by standard procedures (Wray et al., 2012). Tissue explants were transferred to culture dishes containing fibroblast growth medium [Dulbecco’s Modified Eagle Medium (DMEM) with 20% fetal bovine serum (FBS) and 1% penicillin/streptomycin] and placed under sterile coverslips to promote cell attachment. Cultures were maintained in a humidified incubator at 37°C and 5% CO2 with medium exchanges every 3-4 days as fibroblasts migrated from the explant. Cultures were expanded by trypsinization, collecting cells by centrifugation, and resuspending in growth medium. All tissue culture media and supplements were obtained from Life Technologies (Grand Island, NY, USA) except where otherwise noted. All cell lines were confirmed to be free of contamination, including mycoplasma, prior to experimental use.

**Genotyping**

Genomic DNA (gDNA) was isolated from cells using DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA), as recommended. Genotyping was performed by PCR amplification of regions bearing six previously reported haplotype markers: five disease-specific single nucleotide changes (DSC1, -2,-3,-10,-12; Nolte et al., 2003) and the SVA in intron 32 of TAF1 (Makino et al., 2007). Primers used for PCR amplification of DSCs (Table S1) included previously published sequences (Nolte et al., 2003), as well as novel primers generated using NCBI Primer-BLAST software. PCR amplicons were resolved by gel electrophoresis, purified using QIAquick Gel Extraction kit (Qiagen), and sequenced to determine the presence or absence of XDP-specific nucleotide substitutions. To detect the SVA, long-range PCR was performed using previously described primers (Kawarai et al., 2013 and Takara’s PrimeSTAR GXL DNA polymerase (Clontech, Mountain View, CA, USA) with modified amplification conditions (Table S1). PCR amplicons were resolved by gel electrophoresis to identify either a 3229 bp product (XDP; containing the ~2.6 kb SVA) or a 599 bp product (control; no SVA).

**iPSC reprogramming and confirmation of pluripotency**

To generate iPSCs, XDP and control fibroblasts were seeded into six-well plates at an approximate density of 1×105 cells/well in fibroblast growth medium. Reprogramming was performed using the Cytotune-iPS Reprogramming kit (Life Technologies) consisting of non-integrating Sendai viruses encoding the classical Yamanaka factors, Oct3/4, Sox2, Klf4 and c-Myc (Takahashi and Yamanaka, 2006). When fibroblasts reached ~80% confluence, they were transduced with four viruses at approximate multiplicity of infection (MOI) of 3. Cells were maintained for one week with medium exchanges every other day before collecting and re-seeding into gelatin-coated dishes containing feeder layers of irradiated C1 mouse embryonic fibroblasts (MEFs; GlobalStem, Gaithersburg, MD, USA). Transduced cells on feeder layers were then switched to human embryonic stem cell (hESC) medium consisting of DMEM/F12 supplemented with 7 μl/1 2-mercaptoethanol (2-ME; Sigma, St Louis, MO, USA), 20% Knockout Serum Replacement (KOSR), 2× L-Glutamine, 1× Minimum Eagle’s Medium-Non-Essential Amino Acids (MEM-NEAA) and 10 ng/ml basic fibroblast growth factor (bFGF). Colonies were initially picked manually for transfer to fresh feeder layers. Subsequent expansion was achieved by digestion in collagenase IV, followed by scraping and centrifugation to collect cells. Pellets were triturated gently in fresh hESC medium to generate medium-small clumps that were seeded onto fresh feeder layers.

To confirm reprogramming and assess pluripotency, RNA was isolated from colonies using RNeasy reagents and spin columns (Qiagen) with on-column DNase digestion and then reverse transcribed using High Capacity cDNA Reverse Transcription kit (Life Technologies), all as recommended. Expression of endogenous Dmnt3B, hTERT, Nanog, Rex1, Oct4 and Sox2 genes, as well as housekeeping gene ACTB, was quantified by qRT-PCR in each iPSC clone as well as the corresponding parent fibroblast line. To generate differentiated EBs bearing cells of all three germ layers, colonies were collected from Matrigel-coated plates and seeded onto ultra-low attachment plates (Coming, Tewksbury, MA, USA) in hESC medium without bFGF and maintained for 1 week. Suspension culture EBs were then replated on 0.1% gelatin-coated plates in DMEM/10% FBS/1% penicillin/streptomycin and maintained for an additional week of adherent culture at which point RNA was isolated and reverse transcribed. The resulting cDNA samples, along with cDNA prepared from the undifferentiated, parent iPSC clones, were used for quantitative analysis of pluripotency using the TaqMan hPSC Scorecard Assay as recommended (Thermo Fisher Scientific). The assay panel consisted of 94 marker genes to evaluate trilineage potential. Quantitative analysis of gene expression data was performed using hPSC Scorecard analysis software (Thermo Fisher Scientific). All gene expression data were visualized as heatmaps generated using R software (www.R-project.org).

To further confirm pluripotency in vivo, ~1×106 cells of selected clones were resuspended in a mixture of phosphate-buffered saline (PBS) and Matrigel, then implanted transcutaneously at multiple sites in Fox Chase SCID mice (Charles River, Wilmington, MA, USA). Mice were euthanized after 6-8 weeks by CO2 inhalation. Tumors were collected, embedded in paraffin, sectioned, and stained with H&E to identify cellular morphologies characteristic of all three germ layers. Cell lines were confirmed to be karyotypically normal by Cell Line Genetics, Inc. (Madison, WI, USA). All animal experiments were performed according to procedures approved by the Institutional Animal Care and Use Committee (IACUC) of Harvard University.

For routine propagation of XDP and control iPSC lines, cells were adapted for growth under feeder-free conditions on Geltrex-coated tissue culture plates in mTESR-1 medium (Stemcell Technologies, Vancouver, BC, Canada). Passageing of colonies on Geltrex was performed by initial digestion in Accutase, followed by scraping, as described above. Cell lines were routinely tested for contamination, including mycoplasma, and confirmed to be negative.

**Neural differentiation**

XDP and control iPSC colonies on Geltrex were dissociated in Accutase to single cells that were then seeded into fresh coated plates at an approximate density of 2-3×103 cells/well in mTESR-1 medium containing 10 μM ROCK inhibitor, Y-27632. Medium was changed the next day to remove ROCK inhibitor. When cells reached ~25% confluence, they were placed in PSC Neural Induction Medium for seven days with medium exchanges every other day. On day eight, cells were detached using Accutase, collected by centrifugation, and seeded onto fresh coated plates in neural expansion medium consisting of Neurobasal Medium: DMEM/F12 (1:1) supplemented with 2% PSC Neural Induction Supplement and 5 μM Y-27632. After 24 h, medium was exchanged again to remove ROCK inhibitor. The resulting NSCs were maintained on Geltrex-coated plates in neural expansion medium for up to 4 or 5 passages in culture. NSCs were confirmed to be immune-positive for neural markers, nestin, musashi and Sox1, but negative for pluripotent marker, Oct3/4, by antibody staining. For a quantitative assessment of neural conversion, RNA from differentiated NSCs and the parent iPSC clones was reverse transcribed and subjected to qPCR using a panel of primers interrogating 22 marker genes for neural stem cells (Qiagen).

The human neuroblastoma cell line, SH-SY5Y (ATCC; Manassas, VA USA), was also used as a control to confirm neural specificity of N-TAF1. SH-SY5Y cells were cultured in DMEM containing 10% FBS and 1× specifically.
After induction of differentiation, XDP cells were cultured for 6 h in growth medium containing 50 μM of a specific NMD inhibitor, ethyl-2-[[6,7-dimethyl-3-oxo-1,2,3,4-tetrahydro-2quinoxalinyl]acetyl]amino]-4,5-dimethyl-3-thiophenecarboxylate (NMDI14; Martin et al., 2014), prior to RNA isolation. PCR products were evaluated based on melting temperature profiles, apparent size in gel electrophoresis, and Sanger sequencing.

Additional qRT-PCR was performed to amplify three genes also contained with the XDP chromosome region (CXXR3, ARC, OGT) using commercially available Tagman primers (Life Technologies).

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**Competing interests**

The authors declare no competing or financial interests.

**Author contributions**

D.C.B., X.O.B., N.S., N.I. and W.T.H. conceived of the study, designed and supervised experiments, interpreted data and wrote the manuscript. N.S., T.M.-B. and M.D. recruited subjects and performed all clinical evaluations. N.I., W.T.H., C.A.V., J.D., C.L., D.S., K.S. and N.W.-I. performed all experiments.

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**Supplementary information**

Supplementary information available online at http://dmm.biologists.org/lookup/suppl/doi:10.1242/dmm.022590/-/DC1

**References**

Adzhubei, A. A., Sternberg, M. J. E. and Makarov, A. A. (2013). Polyproline-II helix in proteins: structure and function. J. Mol. Biol. 425, 2100-2132.

Aguilar, J. A., Vesagas, T. S., Jamora, R. D., Teleg, R. A., Ledesma, L., Rosales, R. L., Fernandez, H. H. and Lee, V. L. (2011). The promise of deep brain stimulation in X-linked dystonia parkinsonism. Int. J. Neurosci. 121, 57-63.

Albanese, A., Bhatia, K., Bressman, S. B., DeLong, M. R., Fahn, S., Fung, V. S. C., Hallett, M., Jankovic, J., Jinnah, H. A., Klein, C. et al. (2013a). Phenomenology and classification of dystonia: a consensus update. Mov. Disord. 28, 863-873.

Albanese, A., Sorbo, F. D., Comella, C., Jinnah, H. A., Mink, J. W., Post, B., Vidalhiet, M., Volkman, J., Warner, T. T., Leentjens, A. F. et al. (2013b). Dystonia rating scales: critique and recommendations. Mov. Disord. 28, 873-884.

Anandapadamanaban, M., Andresen, C., Helander, S., Ohyama, Y., Siponen, M. I., Lundström, P., Kokubo, T., Ikura, M., Moche, M. and Sunnerhagen, M. (2013). High-resolution structure of TBP with TAF1 reveals anchoring patterns in transcriptional regulation. Nat. Struct. Mol. Biol. 20, 1008-1014.

Bock, C., Kiskinis, E., Verstappen, G., Gu, H., Boultling, G., Smith, Z. D., Ziller, M., Croft, G. F., Amoroso, M. W., Oakley, D. H. et al. (2011). Reference maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. Cell 144, 439-452.

Camargos, S., Lees, A. J., Singleton, A. and Cardoso, F. (2012). DYT16: the original cases. J. Neural. Neurosurg. Psychiatry 83, 1012-1014.

Cordaux, R. and Batzer, M. A. (2009). The impact of retrotransposons on human genome evolution. Nat. Rev. Genet. 10, 691-703.

Deng, H., Le, W.-D. and Jankovic, J. (2009). Impact of retrotransposons on human genome evolution. Nat. Rev. Genet. 10, 691-703.

Domingo, A., Lee, L. V., Bruiggemann, N., Freimann, K., Kaiser, F. J., Jamora, R. D. G., Rosales, R. L., Klein, C. and Westenberger, A. (2014). Woman with X-linked recessive dystonia-parkinsonism: clue to the epidemiology of parkinsonism in Filipino women? JAMA Neurol. 71, 1177-1180.

Domingo, A., Westenberger, A., Lee, L. V., Braenne, I., Liu, T., Vater, I., Rosales, R., Jamora, R. D., Pasco, P. M., Cuticchio-Dela Paz, E. M. et al. (2015). New insights into the genetics of X-linked dystonia-parkinsonism (XDP, DYT3). Eur. J. Hum. Genet. 23, 1334-1340.

Evidente, V. G. H., Advincula, J., Esteban, R., Pasco, P., Alfon, J. A., Natividad, F. F., Cuanang, J., Luis, A. S., Gwinn-Hardy, K., Hardy, J. et al. (2002a). Phenomenology of “Lubag” or X-linked dystonia-parkinsonism. Mov. Disord. 17, 1271-1277.
Evidente, V. G., Gwinn-Hardy, K., Hardy, J., Hernandez, D. and Singleton, A. (2002b). X-linked dystonia ("Lubag") presenting predominantly with a more benign phenotype? Mov. Disord. 17, 200-202.

Evidente, V. G. H., Nolte, D., Niemann, S., Advincula, J., Mayo, M. C., Natividad, F. F., Ruppert, S., Wang, E. H. and Tjian, R. (2004). Phenotypic and molecular analyses of X-linked dystonia-parkinsonism ("Lubag") in women. Arch. Neurol. 61, 1956-1959.

Evidente, V. G. H., Lyons, M. K., Wheeler, M., Hillman, R., Helepleoli, L., Beynen, F., Nolte, D., Müller, U. and Starr, P. A. (2007). First case of X-linked dystonia-parkinsonism ("Lubag") to demonstrate a response to bilateral pallidal stimulation. Mov. Disord. 22, 1790-1793.

Fujigasaki, H., Martin, J.-J., De Deyn, P. P., Camuzat, A., Defond, D., Stevanin, G., Dermat, B., Van Broeckhoven, C., Dürr, A. and Brice, A. (2001). CAG repeat expansion in the TATA-box binding protein gene causes autosomal dominant cerebellar ataxia. Brain 124, 1939-1947.

Geyer, H. L. and Bressman, S. B. (2011). Rapid-onset dystonia-parkinsonism. Harv. Clin. Neurool. 100, 547-552.

Goto, S., Lee, L. V., Munoz, E. L., Tooyama, I., Tamiya, G., Makino, S., Ando, S., Dantes, M. B., Yamada, K., Matsumoto, S. et al. (2005). Functional anatomy of the basal ganglia in X-linked recessive dystonia-parkinsonism. Ann. Neurol. 58, 7-11.

Goto, S., Kawai, T., Morigaki, R., Okita, S., Koizumi, H., Nagahiro, S., Munoz, E. L., Lee, L. V. and Kaji, R. (2013). Deficits in the striatal neuropeptide Y system in X-linked dystonia-parkinsonism. Brain 136, 1555-1567.

Graeber, M. B. and Müller, U. (1992). The X-linked dystonia-parkinsonism syndrome (XDP): clinical and molecular genetic analysis. Brain Pathol. 2, 193-205.

Grünewald, S. and Hahn, S. (2013). Structural insights into transcription initiation by RNA polymerase II. Trends Biochem. 83, 603-611.

Haberhausen, G., Schmitt, I., Köhler, A., Peters, U., Rider, S., Chelly, J., Tervilliger, J. D., Monaco, A. P. and Müller, U. (1995). Assignment of the dystonia-parkinsonism syndrome locus, DYT3, to chromosome region within a 1.5Mb YAC contig of Xq13.1. Am. J. Hum. Genet. 57, 644-650.

Hancks, D. C. and Kazazian, H. H. (2010). SVA retrotransposons: evolution and genetic instability. Semin. Cancer Biol. 20, 234-245.

Hancks, D. C. and Kazazian, H. H. (2012). Active human retrotransposons: variation and disease. Curr. Opin. Genet. Dev. 22, 191-203.

Hancks, D. C., Ewing, A. D., Chen, J. E., Tokunaga, K. and Kazazian, H. H. (2009). Exon-trapping mediated by the human retrotransposon SVA. Genome Res. 19, 1983-1991.

Hanna, J. H., Saha, K. and Jaenisch, R. (2010). Pluripotency and cellular reprogramming: facts, hypotheses, unresolved issues. Cell 143, 508-525.

Herzfeld, T., Nolte, D. and Müller, U. (2007). Structural and functional analysis of the human TAF1/DYT3 multiple transcript system. Mamm. Genome 18, 787-795.

Herzfeld, T., Nolte, D., Grznarova, M., Hofmann, A., Schultz, J. L. and Müller, U. (2013). X-Linked dystonia-parkinsonism syndrome (XDP, lubag): disease-specific sequence change DSC3 in TAF1/DYT3 affects genes in vesicular transport and dopamine metabolism. Hum. Mol. Genet. 22, 941-951.

Hornbeck, P. V., Kornherr, J. M., Tkachev, S., Zhang, B., Skrzypek, E., Murray, B., Latham, V. and Sullivan, M. (2012). phosphoSitePlus: a comprehensive resource for investigating the structure and function of experimentally determined post-translational modifications in man and mouse. Nucleic Acids Res. 40, D261-D270.

Hsu, T.-C., Wang, C.-K., Yang, C.-Y., Lee, L.-C., Hsieh-Li, H.-M., Ro, L.-S., Chen, C.-M., Lee-Hsiao, G. and Su, M.-T. (2014). Deactivation of TBP contributes to SCA17 pathogenesis. Hum. Mol. Genet. 23, 6878-6893.

Hu, H., Haas, S. A., Chelly, J., Van Esch, H., Raynaud, M., de Brouwer, A. P., Weiner, S., Frenoy, G., Frints, S. G. M., Laumonnier, F. et al. (2016). X-sequence sequencing of 405 unresolved families identifies seven novel intellectual disability genes. Mol. Psychiatry 21, 133-148.

Jacobson, R. H., Ladurner, A. G., King, D. S. and Tijan, R. (2000). Structure and function of a human TAF1/FI250 double bromodomain module. Science 288, 1422-1425.

Jambaldorj, J., Makino, S., Munkhbat, B. and Tamiya, G. (2012). Sustained response of dystonia and parkinsonism following globus pallidus internus deep brain stimulation in X-linked dystonia-parkinsonism (Lubag). Stereotact. Funct. Neurosurg. 88, 329-333.

Papai, G., Weil, P. A. and Schultz, P. (2011). New insights into the function of transcription factor TFIIID from recent structural studies. Curr. Opin. Genet. Dev. 21, 219-224.

Ruppert, S., Wang, E. H. and Tijan, R. (1993). Cloning and expression of human TAFII250: a TBP-associated factor implicated in cell-cycle regulation. Nature 362, 175-179.

Sako, W., Morigaki, R., Kaji, R., Tooyama, I., Okita, S., Kitazato, K., Nagahiro, S., Graybiel, A. M. and Goto, S. (2011). Structural and functional analysis of a human TAFII250 double bromodomain module. Curr. Opin. Genet. Dev. 21, 100-107.

Savage, A. L., Bubb, V. J., Breen, G. and Quinn, J. P. (2009). Complicated recessive dystonia Parkinsonism syndromes. Mov. Disord. 24, 490-499.

Seguchi, T., Miyata, T. and Nishimoto, T. (1988). Molecular cloning of the cDNA of human X chromosomeosomal gene (CCG1) which complements the temperature-sensitive G1 mutations, tsBN242 and ts13, of the BHK cell line. EMBO J. 7, 1683-1687.

Seguchi, T., Nohiro, Y., Nakamura, Y., Hisamoto, N. and Nishimoto, T. (1991). The human CCG1 gene, essential for progression of the G1 phase, encodes a 210-kilodalton nuclear DNA-binding protein. Mol. Cell. Biol. 11, 337-3352.

Sekiguchi, G., Fujigasaki, H., Lebre, A.-S., Camuzat, A., Jeannequin, C., Dode, T., Takahashi, J., San, C., Bellance, R., Brice, A. et al. (2003). Huntington’s disease Models & Mechanisms (2016) 9, 451-462 doi:10.1242/dmm.022590
disease-like phenotype due to trinucleotide repeat expansions in the TBP and JPH3 genes. *Brain* 126, 1599-1603.

Szpakowski, S., Sun, X., Lage, J. M., Dyer, A., Rubinstein, J., Kowalski, D., Sasaki, C., Costa, J. and Lizardi, P. M. (2009). Loss of epigenetic silencing in tumors preferentially affects primate-specific retroelements. *Gene* 448, 151-167.

Takahashi, K. and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663-676.

Thomas, M. C. and Chiang, C.-M. (2006). The general transcription machinery and general cofactors. *Crit. Rev. Biochem. Mol. Biol.* 41, 105-178.

Tsankov, A. M., Akopian, V., Pop, R., Chetty, S., Gifford, C. A., Daheron, L., Tsankova, N. M. and Meissner, A. (2015). A qPCR ScoreCard quantifies the differentiation potential of human pluripotent stem cells. *Nat. Biotechnol.* 33, 1182-1192.

Wang, H., Curran, E. C., Hinds, T. R., Wang, E. H. and Zheng, N. (2014). Crystal structure of a TAF1-TAF7 complex in human transcription factor IID reveals a promoter binding module. *Cell Res.* 24, 1433-1444.

Waters, C. H., Faust, P. L., Powers, J., Vinters, H., Moskowitz, C., Nygaard, T., Hunt, A. L. and Fahn, S. (1993). Neuropathology of lubag (X-linked dystonia parkinsonism). *Mov. Disord.* 8, 387-390.

Waters, C. H., Faust, P. L., Powers, J., Vinters, H., Moskowitz, C., Nygaard, T., Hunt, A. L. and Fahn, S. (1993). Neuropathology of lubag (X-linked dystonia parkinsonism). *Mov. Disord.* 8, 387-390.

Waters, C. H., Faust, P. L., Powers, J., Vinters, H., Moskowitz, C., Nygaard, T., Hunt, A. L. and Fahn, S. (1993). Neuropathology of lubag (X-linked dystonia parkinsonism). *Mov. Disord.* 8, 387-390.

Wilhelmsen, K. C., Weeks, D. E., Nygaard, T. G., Moskowitz, C. B., Rosales, R. L., dela Paz, D. C., Sobrevega, E. E., Fahn, S. and Gilliam, T. C. (1991). Genetic mapping of "Lubag" (X-linked dystonia-parkinsonism) in a Filipino kindred to the pericentromeric region of the X chromosome. *Ann. Neurol.* 29, 124-131.

Wray, S., Self, M., NINDS Parkinson’s Disease iPS Consortium, NINDS ALS iPS Consortium, Lewis, P. A., Taanman, J.-W., Ryan, N. S., Mahoney, C. J., Liang, Y. et al. (2012). Creation of an open-access, mutation-defined fibroblast resource for neurological disease research. *PLoS ONE* 7, e43099.

Wray, S., Self, M., NINDS Parkinson’s Disease iPS Consortium, NINDS ALS iPS Consortium, Lewis, P. A., Taanman, J.-W., Ryan, N. S., Mahoney, C. J., Liang, Y. et al. (2012). Creation of an open-access, mutation-defined fibroblast resource for neurological disease research. *PLoS ONE* 7, e43099.

Wray, S., Self, M., NINDS Parkinson’s Disease iPS Consortium, NINDS ALS iPS Consortium, Lewis, P. A., Taanman, J.-W., Ryan, N. S., Mahoney, C. J., Liang, Y. et al. (2012). Creation of an open-access, mutation-defined fibroblast resource for neurological disease research. *PLoS ONE* 7, e43099.

Wray, S., Self, M., NINDS Parkinson’s Disease iPS Consortium, NINDS ALS iPS Consortium, Lewis, P. A., Taanman, J.-W., Ryan, N. S., Mahoney, C. J., Liang, Y. et al. (2012). Creation of an open-access, mutation-defined fibroblast resource for neurological disease research. *PLoS ONE* 7, e43099.

Zech, M., Castrop, F., Schormair, B., Jochim, A., Wieland, T., Gross, N., Lichtner, P., Peters, A., Gieger, C., Meltinger, T. et al. (2014). DYT16 revisited: exome sequencing identifies PRKRA mutations in a European dystonia family. *Mov. Disord.* 29, 1504-1510.