Short Communication

Pathogenic Lrrk2 substitutions and Amyotrophic lateral sclerosis

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Summary Pathogenic Lrrk2 Y1699C substitution observed in a large German-Canadian kindred presents a neurodegenerative disorder that is reminiscent of amyotrophic lateral sclerosis and Parkinsonism-Dementia Complex. We screened 54 patients with ALS for seven known Lrrk2 pathogenic substitutions in the Roc, COR and kinase domains. No mutations were observed suggesting that this locus does not have a major influence on the ALS phenotype. However we can not rule out other genetic variation at the LRRK2 locus may play a role in parkinsonian disorders with amyotrophic lateral sclerosis and may be considered candidates for genetic screening.

Keywords: Amyotrophic lateral sclerosis, Lrrk2, pathogenic substitutions

Introduction

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset paralytic disease characterized by a loss of motor neurons in the cerebral cortex, brainstem, and spinal cord (Rowland and Shneider, 2001). It is invariably fatal, with an average mortality of 3–5 y after onset. As with many other prevalent neurodegenerative disorders, ALS is essentially sporadic and in less than 10% familial (Rowland and Shneider, 2001). At this point, neither the cause nor the mechanism by which motor neurons degenerate in ALS is known.

Over the last decade a number of genetic loci have been identified in familial forms of ALS (Kunst, 2004), and 5–10% of ALS cases have a documented family history, suggesting there is a strong genetic component to disease in these cases. There are at least six loci for autosomal dominant ALS. Mutation of the gene copper-zinc superoxide dismutase (SOD1 [MIM 147450]) is reported. Over one hundred SOD1 mutations have been described and are thought to account for ~20% of familial ALS (Kunst, 2004). However, the influence of other genetic loci in ALS is yet to be resolved. A recent study identified two pedigrees with individual family members presenting with ALS or Parkinson’s disease (PD), these findings may be co-incident or indicate a genetic overlap between these two neurodegenerative disorders (Stewart, 2005).

Mutations in the gene coding for the leucine-rich repeat kinase 2 (LRRK2) are linked to an atypical forms of parkinsonism (Zimprich et al., 2004). A LRRK2 5096A>G mutation that results in a tyrosine (Y) to cysteine (C) pathogenic substitution, at position 1699, was originally identified in a large German-Canadian kindred, Family A. Affected family members present with a parkinsonian syndrome, with some members having dementia, possibly co-existent with their parkinsonism (Wszolek et al., 1997). Two individuals had parkinsonism associated with a distal limb muscle weakness, atrophy and fasciculation. Neuro-pathological examination of the latter showed evidence of
nigral neuronal loss with gliosis and spinal cord anterior horn neurodegeneration with ubiquitin-immunoreactive axonal spheroids, reminiscent of ALS.

Since the initial reports of LRRK2 mutations in familial PD, we learned that they are primarily located in the functional Roc, COR and MAPKKK domains of the gene and that seven pathogenic variants can be found in these regions (Mata et al., 2005). Strikingly it is observed that common LRRK2 mutations may also be found in 1–2% of presumed sporadic cases of PD (Gilks et al., 2005; Kachergus et al., 2005). The association of LRRK2 mutations with an ALS-like phenotype together with their possible occurrence in sporadic disease prompted us to determine if LRRK2 variants are common in ALS.

Subjects and methods

For this study, DNA samples from 55 subjects were used. These were 28 men and 27 women, with mean age at symptom-onset of 54 y (range 18–79 y). One patient was diagnosed with multifocal motor neuropathy on follow-up evaluation, and blood was drawn from one normal control; the remaining patients all had ALS. One ALS patient also had dementia and no patients in this study reported a family history of parkinsonism. Of those with ALS, weakness began in the legs (48%), arms (22%), bulbar region (26%), or respiratory muscles (4%). The average ALS Functional Rating Scale score was 30.6 at the most recent follow-up visit, and average forced vital capacity was 59% of predicted. Twenty-two patients eventually required gastrostomy, 41 used non-invasive ventilation, and 27% died. Of those with ALS, 11 had a positive family history, and 6 were SOD gene positive. The average disease duration at time of follow-up was 3.6 y.

The Roc, COR and MAPKKK domains contain seven known LRRK2 mutations that result in pathogenic substitutions 4321C>T, 4321C>G, 4322G>A (R1441G/C/H), 5096A>G (Y1699C), 6035T>C (I2020T), 6055G>A (G2019S) and 6059T>C (R1441G). PCR amplification of three exons 31, 35 and 41 encoding these domains was performed on each subject using cycling conditions 57–52 touchdown. The following primer exons 31, 35 and 41 encoding these domains was performed on each subject with BigDye chemistry on an ABI 3100 (Applied Biosystems).

Direct sequencing of the three exons was performed with these primers at a common LRRK2 regions (Mata et al., 2005). Strikingly it is observed that seven pathogenic variants can be found in these functional Roc, COR and MAPKKK domains of the gene (Ross and Farrer, 2005); clinically patients have presented with parkinsonism, dementia, supranuclear gaze palsy, and pathologically tauopathy or synucleinopathy with nigral neuronal cells loss (Wszolek et al., 1997, 2004). The clinical and pathological variation that has been observed for Lrrk2 mutations at positions R1441, Y1699 and G2019 suggests common disease mechanisms may exist in a number of neurodegenerative disorders.

Family A harboring a pathogenic Y1699C substitution presented with parkinsonism, ALS and dementia (Wszolek et al., 1997; Zimprich et al., 2004). This complex phenotype is reminiscent of the Western Pacific parkinsonism-ALS-dementia complex found on the island of Guam (Plato et al., 2002). Pathologically, this multisystemic neurodegenerative disorder falls into the group of tauopathies, characterized by neurofibrillary tangles (NFT) (Chen, 1981; Wada et al., 1999). However, there are no NFT in the spinal cord of ALS patients outside of Guam (Chen, 1981; Wada et al., 1999), but instead ubiquitin-immunoreactive inclusions (Matsumoto et al., 1990a, b). Similar ubiquitin-immunoreactive inclusions to those observed in ALS were observed in the spinal cord and brain of ALS patients (Matsumoto et al., 1990a, b).

There was an absence of pathogenic substitutions in these three exons of Lrrk2 in the ALS patients of this study indicating no role for variation at this locus in ALS. However, the mixed presentation of Family A suggests variation in this locus may influence complex neurodegenerative disorders such as parkinsonism-ALS-dementia. It is now (Fig. 1), although no evidence of splicing at this position in LRRK2 has been observed. There was no evidence of any exonic variants in the three exons, 31, 35 and 41, or any SNPs in the intronic regions around exons 31 and 41.

Results and discussion

No evidence of any known Lrrk2 pathogenic substitutions R1441G/C/H, Y1699C, I2020T, G2019S and I2020T, was observed in our sample of ALS patients. One rare heterozygous single nucleotide polymorphism (SNP) was observed in the flanking intronic sequence of exon 35 (nucleotide +92) in one sporadic ALS patient. Two common SNPs were also observed in the intronic region surrounding exon 35

![Fig. 1. The sequence of exon 35 is highlighted in bold and the Y1699C A>G variant is shown in brackets. The arrows indicate the sequence of the primers that were used in PCR amplification of the region. The exon 35 variations in sequence that were observed are shaded in grey. SNP genotypes are shown in brackets.](image-url)
crucial that the role of LRRK2 is clarified in atypical clinical presentation as genetic testing for these variants becomes closer to being established. Therapies targeted at specific genetic variants and tailored to patient genetic profiles will determine treatment.

Our group recently published a comprehensive screening in one hundred families with an autosomal dominant mode of inheritance, identifying 26 exonic DNA variations in the LRRK2 gene (Mata et al., 2005). We are presently examining the frequency of these non-pathogenic polymorphisms within the coding region of the gene in an attempt to resolve the influence of common LRRK2 variation in susceptibility to ALS, neurodegeneration and related disorders.

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