Woman With X-Linked Recessive Dystonia-Parkinsonism
Clue to the Epidemiology of Parkinsonism in Filipino Women?

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X-linked dystonia-parkinsonism (XDP, Lubag disease) is a hereditary movement disorder indigenous to the Philippines. The condition typically presents in men and as a focal or segmental dystonia that generalizes within 5 years after onset. Subsequently, parkinsonism sets in, overlaps with the dystonia, and then predominates.1 Notably, patients with late-onset disease may present with a purely parkinsonian syndrome.

Despite X-linked recessive inheritance, women manifesting the disease have been described. In our recent report of a woman with XDP,2 genetic analysis revealed an undiagnosed X-chromosome monosomy in a subset of cells, as seen in atypical Turner syndrome. In other affected women, nonrandom (skewed) X-chromosome inactivation (XCI; ie, >80% of cells with the wild-type [wt] chromosome inactivated) was previously offered to explain the occurrence of the phenotype3 but this has never been investigated. Here we describe, to our knowledge, the first woman with XDP with genetically proven skewed XCI and hypothesize that this epigenetic factor might contribute to late-onset parkinsonism in elderly Filipino women and possibly even to the observation of a slight female predominance in the prevalence of parkinsonism in the Philippines.

**Methods**

This study was approved by the ethics committee of the University of Lübeck. After obtaining written informed consent for genetic testing and for disclosure of results and videos, genomic DNA was extracted from blood using standard procedures. Blood RNA was collected in PAXGene tubes (PreAnalytIX, QiagenBD) and extracted according to the manufacturer’s protocol, and complementary DNA (cDNA) was reversely transcribed using a standard kit (Fermentas, Thermo Fisher Scientific). Sanger sequencing of relevant polymerase chain reaction–amplified DNA and/or cDNA segments was performed on an ABI3500XL Genetic Analyzer (Applied Biosystems). The degree of XCI skewing was estimated by an assay4 that uses a methylation-sensitive HpaII restriction enzyme that is unable to cut methylated DNA. The HpaII restriction site located in exon 1 of the androgen receptor gene on the X chromosome is in the proximity of a polymorphic CAG repeat that can be used to distinguish between the 2 alleles in a woman. This restriction site is methylated on the inactive X chromosome and unmethylated on the active X chromosome. The region containing the HpaII restriction site and the polymorphic

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**IMPORTANCE** Despite recessive inheritance, X-linked dystonia-parkinsonism (Lubag disease) has also been described in women presenting with a late-onset isolated parkinsonian syndrome. Interestingly, unlike in other populations, there is a slight female predominance in the prevalence of parkinsonism in the Philippines.

**OBSERVATIONS** In a Filipino woman with suspected Parkinson disease, we confirmed the presence of all changes specific for X-linked dystonia-parkinsonism in genomic DNA. Subsequently, we analyzed complementary DNA and evaluated the methylation status of the androgen receptor gene. Owing to extremely skewed (98%:2%) X-chromosome inactivation, the patient expressed almost solely the mutated allele in a disease-specific change, rendering her molecularly comparable with a hemizygously affected man.

**CONCLUSIONS AND RELEVANCE** Skewed X-chromosome inactivation is the likely cause of parkinsonism in this heterozygous mutation carrier. Because women carriers of the genetic changes specific for X-linked dystonia-parkinsonism are common in the Philippines, the epigenetic factor of nonrandom X-chromosome inactivation may contribute to the skewing of the sex prevalence of parkinsonism toward women in this country, warranting further investigation.
CAG repeat was amplified via polymerase chain reaction prior to and after the digestion of the genomic DNA with HpaII (New England Biolabs). After restriction, only the methylated (and thus uncut) allele in the androgen receptor locus mentioned, representing the inactive X chromosome, was amplified. The amplicons were then genotyped on an ABI3030 Genetic Analyzer (Applied Biosystems). Densitometric analysis of the alleles was performed at least twice for each sample using GeneMapper Software 5 (Applied Biosystems). The volume of the HpaII-digested shorter allele was divided by the ratio of the nondigested shorter/longer allele volumes to compensate for preferential amplification of the shorter allele.

**Report a Case**

The patient was a 73-year-old previously healthy Filipino woman who developed tremors in both hands at the age of 57 years. On consult, she was diagnosed as having idiopathic Parkinson disease (PD) and was given levodopa/carbidopa, offering no relief. At age 64 years, she started to have gait problems. Family history was negative for any movement disorder; however, both her parents were from Panay Island in the Philippines. On examination, she was noted to have mild right shoulder elevation and internal rotation of the arm but no overt dystonia. More prominently, she exhibited symmetric hand tremors at rest, masked facies, cogwheel rigidity on all extremities, and generalized bradykinesia. Her gait was markedly impaired by festination (Video). Cranial magnetic resonance imaging revealed typical features seen in XDP of symmetric linear hyperintense T2 signals involving the caudate rims bilaterally, with moderate caudate head atrophy (Figure). Our genetic analysis first centered on determining whether the patient carried the previously described XDP-specific genetic changes. There are currently 7 reported genetic alterations on Xq13.1 including 5 disease-specific single-nucleotide changes, one 48-bp deletion, and 1 SVA (SINE/VNTR/Alu) retrotransposon insertion; all are situated either in introns of the TAF1 gene or in the DYT3 multiple transcript system. The patient was heterozygous for all 7 changes. Next, we aimed to determine the pattern of expression of the wt vs the affected X chromosome. Among the 7 changes within the XDP haplotype, only disease-specific single-nucleotide change 3 can be detected in a mature transcript; therefore, we investigated its expression in blood-derived cDNA. Sequencing of cDNA revealed expression of only the mutated (C>T) allele (Figure in the Supplement), implying complete inactivation of the wt X chromosome and skewing of expression to the direction of the chromosome carrying the disease-specific changes. To confirm this, we evaluated the methylation status of the CAG microsatellite locus at the 5' end of the androgen receptor gene, which revealed extremely (98%:2%) skewed XCI.

**Discussion**

There are currently more than 500 men diagnosed as having XDP in the Philippines compared with only 14 cases of affected women in the literature to date. Most women with genetically confirmed XDP are heterozygous for the XDP haplotype (Table). Our patient likewise carried both wt and mutated alleles in her DNA but is molecularly comparable with a hemizygous XDP man because of extremely skewed XCI. Although XCI varies to some degree across tissues, patterns in tissues do not differ significantly (ie, XCI data are overall comparable between accessible [eg, blood] and inaccessible tissues [eg, brain] at various ages). Thus, it is likely that the data we obtained from blood-derived DNA/RNA reflects the XCI pattern in the brain.

Our review of all cases of XDP in women published to date revealed that women with XDP presenting with isolated or predominant parkinsonian symptoms (ie, tremor, rigidity, or gait problems) have an older age at onset (AAO; mean, 66 years; range, 42-75 years; cases 4-6 and 8 in the Table) compared with those presenting primarily with hyperkinetic symptoms (ie, focal or segmental dystonia and chorea) (mean, 44 years; range, 26-59 years; cases 1-3, 7, and 9-14). In this descriptive review, there were wide variations in presentation and AAO; however, parkinsonism was prominent in all women with an AAO beyond the age of 60 years, paralleling the typical phenomenology in affected men. Of note, the effect of advanced AAO on the presentation, regardless of sex, resembles descriptions in other forms of dystonia-parkinsonism. For example, in dopa-responsive dystonia, affected individuals manifesting at an older age have also been observed to exhibit parkin-
sonian symptoms more frequently than patients with a younger AAO and they may even be clinically indistinguishable from idiopathic PD. While there is only a small percentage of women who will have skewed XCI (8%-15%), and even more so the extremely skewed pattern (<1% of women), XCI skewing ratios in blood have been found to correlate with increasing age. Increasing XCI skewing is particularly observed at ages 50 to 60 years, which corresponds to our patient’s AAO (57 years). These observations have led us to hypothesize

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient No.</th>
<th>Age at Onset, y</th>
<th>Age at Last Examination, y</th>
<th>Clinical Features</th>
<th>Genetic Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waters et al, 1993</td>
<td>1</td>
<td>59</td>
<td>61</td>
<td>Dystonic posturing in left foota</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>Evidente et al, 2004 and Waters et al, 1993</td>
<td>2</td>
<td>51</td>
<td>66</td>
<td>Eyebrow elevation Shuffling gait, breakdown of rapid alternating movements, retropulsion Chorea,* initially limb then progressed to generalized chorea</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>Evidente et al, 2004</td>
<td>3</td>
<td>42</td>
<td>46</td>
<td>Cervical dystoniaa</td>
<td>Myoclonus</td>
</tr>
<tr>
<td>Evidente et al, 2004</td>
<td>4</td>
<td>75</td>
<td>76</td>
<td>Shuffling gait,* breakdown of rapid alternating movements</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>Evidente et al, 2004</td>
<td>5</td>
<td>42</td>
<td>47</td>
<td>Perioral tremora</td>
<td>Impairment of tandem gait</td>
</tr>
<tr>
<td>Evidente et al, 2004</td>
<td>6</td>
<td>75</td>
<td>76</td>
<td>Shuffling gait,* breakdown of rapid alternating movements, hypomimia, stooped posture, microphagia, absent arm swing, postural and action tremor</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>Evidente et al, 2004</td>
<td>7</td>
<td>26</td>
<td>26</td>
<td>Intermittent upper body and facial chorea</td>
<td>Heterozygous</td>
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<td>Evidente et al, 2004</td>
<td>8</td>
<td>72</td>
<td>74</td>
<td>Stooped posture, breakdown of rapid alternating movements, shuffling gait, retropulsion</td>
<td>Left arm chorea*</td>
</tr>
<tr>
<td>Evidente et al, 2004</td>
<td>9</td>
<td>35</td>
<td>36</td>
<td>Upper limb action and postural tremora Breakdown of rapid alternating movements, retropulsion</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>Lee et al, 2011</td>
<td>10</td>
<td>47</td>
<td>63</td>
<td>Blepharospasm,* oromandibular dystonia, leg dystonia, then generalized dystonia</td>
<td>Hypomimia, bradykinesia</td>
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<td>Lee et al, 2011</td>
<td>11</td>
<td>49</td>
<td>67</td>
<td>Torticollis,* leg dystonia, dysphagia, then generalized dystonia</td>
<td>Leg cramps</td>
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<tr>
<td>Lee et al, 2011</td>
<td>12</td>
<td>37</td>
<td>39</td>
<td>Flexion and upgoing of toes,* torticollis, facial dystonia, jaw opening, then generalized dystonia</td>
<td>Festinating gait, writing difficulty, Slurred speech</td>
</tr>
<tr>
<td>Lee et al, 2011 and Westenberger et al, 2013</td>
<td>14</td>
<td>50</td>
<td>57</td>
<td>Leg dystonia,* then generalized dystonia Generalized rigidity, bradykinesia in hand and finger movements</td>
<td>Slurred speech</td>
</tr>
<tr>
<td>Current case</td>
<td>15</td>
<td>57</td>
<td>73</td>
<td>Shoulder dystoniaa Hand tremors,* shuffling gait, festination, cogwheel rigidity, bradykinesia</td>
<td>Heterozygous, extremely skewed X-chromosome inactivation</td>
</tr>
</tbody>
</table>

*a Initial symptom.
that aging-related changes in XCI skewing are contributory to the AAO in our patient and also possibly in other elderly, affected women who are heterozygous for the XDP haplo- type. Supporting this idea, in Rett syndrome, another X-linked disorder, a correlation has been shown between the degree of XCI skewing and clinical phenotype in women carrying the same mutations. 12

Epidemiologic studies on PD report a higher prevalence in men than in women. 13 Intriguingly, in the Philippines, the ratio of parkinsonism 14 in men vs women is 1:1.07. This slight skewing of the ratio to the other side might be an important epidemiologic clue given that XDP is indigenous to this country and may actually represent an underdiagnosed cause of parkinsonism in elderly women. That our case was initially diagnosed as having idiopathic PD and was given a trial of levodopa/carbidopa but turned out to be a heterozygous manifesting carrier of the XDP-associated changes is illustrative of this point. Thus, it will be interesting to determine in future population-based studies the possible contribution of this epigenetic factor of nonrandom XCI to the sex prevalence of parkinsonism in the Philippines.

Conclusions

Here, we revealed the molecular underpinnings of a woman manifesting XDP due to skewed XCI and related late-onset disease to parkinsonism in women with XDP. The notion that XDP is an underdiagnosed cause of parkinsonism in Filipino women warrants further investigation.

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Drafting of the manuscript: Domingo, Westenberger.
Critical revision of the manuscript for important intellectual content: Lee, Brüggemann, Freimann, Kaiser, Jamora, Rosales, Klein.
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REFERENCES