the antigens recognized by the antisera present in these ovarian teratoma patients, it is conceivable that a functionally related group of antigens are recognized. Such a finding would extend the already great interest the current work holds for both the clinician and the basic neuroscientist.

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Parkin Gene Variations and Parkinsonism: Association Does Not Imply Causation

In 1998, two Japanese families with "autosomal recessive juvenile parkinsonism" were found to have homozygous deletions in a gene that is now known as *Parkin.*[1] This represented the second monogenic form of parkinsonism in which the causative gene had been found *(PARK2)* and the first of the autosomal recessive forms. Typically, *PARK2* has an early age at onset, as well as other clinical and pathological features that tend to differentiate it from sporadic Parkinson's disease,[2] although the phenotype can be indistinguishable between the two disorders. Like the *DMD* gene in

Duchenne's muscular dystrophy, the *Parkin* gene is large and unstable[3] and mutations of *PARK2* are numerous. In fact, more than 100 different *Parkin* gene variations have been observed in persons with parkinsonism that have been proposed to be disease-causing, even when only a single copy of the variant is present (heterozygotes). These observations pose a fundamental dilemma for clinicians and researchers: How does one dissect out normal allelic variants from pathological allelic variants in the *Parkin* gene? A second issue relates to the problem of proving the increasingly popular notion that only one allelic variant is sufficient to cause Parkinson's disease in those patients and/or families in whom a *Parkin* mutation is found on only one allele.

In this issue of *Annals,* Kay and colleagues[4] take a fresh approach to these questions by performing intensive genetic screening for variants in the *Parkin* gene not only in cases with parkinsonism, but also in an equal number of control subjects at the same time. In a two-staged study, they first began by sequencing all 12 exons and exon-intron boundaries in the *Parkin* gene of 302 sequentially enrolled patients in a movement disorders clinic and 301 control subjects. Of the 34 heterozygous sequence variants that were identified, many would not be predicted to result in functional changes in the protein, and only 1 of the variants (p.P437L) was more common in patients compared with control subjects. When the much larger replication phase was conducted (with 1,260 cases and 1,657 control subjects), even the p.P437L variant was found to be no more common in patients than control subjects.

These observations should sound a note of caution when interpreting the significance a heterozygous variant in the Parkin gene. In a setting where both patients and control subjects were similarly studied, no evidence of a causative association between any of the *Parkin* variants and parkinsonism was found. The take-home message is clear: Not all changes in the *Parkin* gene can be assumed to be disease-causing. Because many (even most) studies do not include control subjects, but instead focus on persons with parkinsonism and their first-degree relatives (who will, on average, share 50% of genes), erroneous assumptions regarding causation may result.

One important issue regarding the *Parkin* gene was not fully addressed by the current study. Whereas half of the reported variants in *Parkin* are point mutations, which are the focus of Kay and colleagues'[4] article, the remaining *Parkin* sequence changes are constituted of large rearrangements, such as deletions or multiplications of one or more exons. Importantly, quantitative exon-dosage analysis, rather than the sequencing approach chiefly used here (except for the retesting of individuals with variations found in the first round of testing), must be used to identify most large rearrangements. In the large, unstable *Parkin* gene, such analyses can be particularly important. West and colleagues[5] have provided a good example for the need for a thorough sequencing and exon-dosage analysis. These investigators reevaluated 20 heterozygous *Parkin* mutation carriers with both techniques and detected 8 previously undetected mutations (4 point mutations and 4 larger rearrangements) illustrating the importance of a careful comprehensive analysis, including both sequencing and exon-dosing techniques.

Notably, in a population of exclusively young-onset ($<$50 years) patients, the mutation frequency and the phenotypic outcome might be different. Clark and colleagues[6] used a similar comprehensive approach in their investigation of *Parkin* mutations in control subjects, as well as cases with early-onset parkinsonism. Using both complete sequencing and gene-dosing analyses, they found a total of 5 different point mutations and 3 larger deletions in 101 patients, but none in control subjects except for 1 synonymous substitution (p.L261L). One patient carried a homozygous deletion, and another patient was compound heterozygous for a point mutation and the p.L261L sequence change. In the remaining eight *Parkin*-positive patients, only a single allelic variant (five point mutations and three exon deletions) was observed. The authors speculated that these variants may increase susceptibility for parkinsonism. In fact, studies in familial parkinsonism suggest that heterozygous *Parkin* mutations may indeed affect clinical features of parkinsonism, such as age at onset.[2,5,7–10]

Although the current work offers important insights into the role of *Parkin* changes in parkinsonism, many questions remain unanswered. Chief among these is how to interpret a heterozygous change in the *Parkin* gene in a patient with parkinsonism. Kay and colleagues' work rightly argues against the unquestioning assumption that any *Parkin* sequence variant affects disease risk, but they found few previously reported mutations in either cases or control subjects. More work is needed to determine whether individuals heterozygous for mutations known to cause disease in homozygotes are at greater risk for parkinsonism. Alternatively, those with heterozygous mutations may be more likely to develop parkinsonism in the presence of a second, non-*Parkin* genetic abnormality[11,12] or an environmental insult. As few studies have investigated the latter questions, these may provide a fruitful direction for future study.

It is also important to point out that carefully done functional studies in cell-culture systems provide important insights in the pathogenicity of *Parkin* sequence changes.[13–16] Such studies should also be taken into account when determining the clinical and functional consequences of a given variation.

In summary, no study has yet provided full insight

into the relation between *Parkin* heterozygotes and parkinsonism. Critical to our understanding is careful investigation not only in individuals with parkinsonism, but also in adequately sized control populations. Kay and colleagues' article in this issue provides a provocative reminder of this point. As genetic testing moves from the research laboratory to the clinic, accurate interpretation of novel *Parkin* variants is imperative.

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