Parkin western blotting is useful for identification of patients with Parkin-related Parkinson's disease

Parkinson's disease (PD) is a progressive neurological movement disorder characterised pathologically by degeneration of dopaminergic neurons in the substantia nigra pars compacta and the presence of Lewy bodies. Cardinal clinical features of PD are bradykinesia, rigidity, resting tremor, postural instability and responsiveness to levodopa. Identification of patients with causative variations in the PD-related

genes (eg, *SNCA*, *LRRK2*, *Parkin*, *PINK1*, *DJ-1* and *ATP13A2*) has been challenging given that only some patients have distinctive clinical phenotypes or a suggestive family history. Although clinical clues to a specific monogenic form of PD may be observed in some cases, for instance, behavioural problems and postural hypotension in patients with missense mutations in *SNCA*, early-onset dystonia in patients with mutations in *Parkin* and vertical gaze palsy, spasticity and facial-faucal mini-myoclonus in patients with mutations in *ATP13A2*,^{1,2} these manifestations are not universally present. Moreover, patients with mutations in *LRRK2* or multiplications in *SNCA* can be clinically indistinguishable from idiopathic PD. Additionally, variable disease expressivity³ and overlapping clinical presentations in the monogenic and idiopathic cases also complicate the diagnostic process to identify each monogenic form of PD. Identification of mutations in *Parkin* (MIM# 602544), which are the commonest genetic cause of autosomal recessive PD (MIM# 600116), requires a combination of direct sequencing and MLPA genetic analysis, given that missense mutations, microdeletions/duplications and large-scale rearrangements and gene dosage effects can all cause Parkin-related PD.⁴

In this study, we determined Parkin expression in PD patient-derived skin

fibroblasts, instead of Parkin-deficient blood leucocytes, to investigate its usability in the diagnosis of Parkin-related PD. Western blotting of Parkin was performed in fibroblasts from individuals with known mutations in PD-related genes (ie, *Parkin*, *PINK1*, *ATP13A2*, and *LRRK2*), idiopathic PD and healthy controls (figure 1A–C; detailed description of the samples and method has previously been published^{2,3,5}).

Full-length wild-type Parkin protein (50 kDa) was present in all controls. Consistent with previous findings,^{6,7} the absence of Parkin was observed in the homozygous and compound heterozygous *Parkin* mutation carriers. The expression of Parkin was significantly decreased in the single heterozygous *Parkin* mutation carriers to $58 \pm 4.5\%$ compared to controls. Parkin was present in *PINK1*, *ATP13A2*, *LRRK2* mutation carriers and in idiopathic PD patients at comparable levels to the healthy controls (figure 1D). These results indicate that Parkin western blotting can be used to efficiently identify Parkin-related PD from other forms of monogenic PD that may similarly present with an autosomal recessive family history, younger age of onset or overlapping phenotype. The possibility of patients harbouring missense mutations in *Parkin* cannot be excluded entirely by this method since Parkin levels

Figure 1 Determination of Parkin protein in human skin fibroblasts. Western blotting using whole-cell lysates from healthy controls, individuals with mutations in Parkinson's disease (PD)-related genes and idiopathic PD. (A) Full-length wild-type Parkin protein (~50 kDa) was absent in homozygous and compound heterozygous *Parkin* mutation carriers. A reduced Parkin expression (~50%) was observed in the single heterozygote, compared to the healthy controls. The expression of Parkin was comparable between the healthy controls and (B) *PINK1*, *ATP13A2*, (C) *LRRK2* mutation carriers and idiopathic PD; β -actin (42 kDa) was used as loading control. (D) Densitometric analysis of the immunoblots. The intensity of each band was normalised to β -actin and presented as mean $\pm$ SD*; $p < 0.001$ in two-tailed Student's *t* test compared with the healthy controls.

may not always be altered in this setting.⁸ Additionally, it can be used to confirm the functional deficit on next-generation sequencing protocols when a *Parkin* mutation has been identified.

In summary, western blotting, to identify *Parkin* protein expression in skin fibroblast cultures, may be used as a supportive tool to identify patients suspected of having mutations in the *Parkin* gene and justify further mutational analysis and gene dosage studies for the identification of mutations in *Parkin* versus other genes that cause monogenic young onset Parkinsonism. Further validation to fully assess the usability of *Parkin* western blotting in differential diagnosis for *Parkin*-related PD using a larger cohort of healthy controls, idiopathic and monogenic PD patients including with mutations in other PD-related genes (eg, *SNCA* and *DJ-1*) is warranted.

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