INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease (Calabrese, 2007). With technological advancement, a growing list of genes have been confirmed to cause familial PD (Deng, Wang, & Jankovic, 2018; Lill, 2016; Puschmann, 2017). Next-generation sequencing technology has been applied worldwide to identify the causative genes for various neurological disorders (Bahassi & Stambrook, 2014). The glucocerebrosidase gene (GBA) has been a candidate gene for PD for a decade (Deng et al., 2018). It is involved in lysosomal sphingolipid degradation. The heterozygous GBA L444P mutation is a high-risk mutation for PD (O'Regan, deSouza, Balestrino, & Schapira, 2017). Moreover, polymerase gamma (POLG) is an enzyme responsible for the replication and repair of mitochondrial DNA. Mutations in POLG may cause variable clinical manifestations, including parkinsonism, epilepsy, cerebellar ataxia, neuropathy, and progressive external ophthalmoplegia. However, mutations of this gene are rare in patients with typical Parkinson's disease (PD). We report a man (current age: 59 years) without any underlying disease presenting with right-hand tremor at the age of 39 years, followed by slow movement, rigidity, and postural instability. He developed motor fluctuation and levodopa-induced dyskinesia 8 years later. At the age of 58 years, cognitive decline and visual hallucination ensued; he was institutionalized thereafter. We used multiplex ligation-dependent probe amplification, which demonstrated no large deletions or duplications of relevant PD genes. Next, targeted sequencing panel covering 51 genes causative for PD was applied for the proband; it revealed a heterozygous missense substitution R964C in POLG and a heterozygous missense substitution L444P in GBA. The patient's father, who had been diagnosed as having PD and type 2 diabetes mellitus at the age of 70 years, demonstrated identical mutations. This is the first report of familial PD combined with POLG R964C and GBA L444P mutations. Two pathogenic gene mutations potentially cause double hit in pathological neurodegeneration. This finding extends our understanding of the PD genotype–phenotype correlation.

KEYWORDS
GBA, missense substitution, next-generation sequencing, Parkinson's disease, POLG
the replication and repair of mitochondrial DNA (Chan & Copeland, 2009) and mutation in the POLG may cause various clinical manifestations, including parkinsonism (Miguel et al., 2014), epilepsy (Stricker et al., 2009; Stumpf, Saneto, & Copeland, 2013), cerebellar ataxia (Stricker et al., 2009; Stumpf et al., 2013), and progressive external ophthalmoplegia (Luoma et al., 2004; Miguel et al., 2014). R964C, a missense substitution POLG mutation, was considered to be related to manifestations of the central nervous system other than typical PD. Herein, we report the first case of a patient with young-onset PD (YOPD) carrying both POLG R964C and GBA L444P mutations.

### 2 | CASE PRESENTATION

A man (current age: 59 years), without any underlying disease, presented with a right-hand tremor at the age of 39 years, followed by loss of facial expression, slow movement, rigidity, and postural instability. He also had rapid eye movement sleep behavior disorder (RBD), but no hyposmia or orthostatic dizziness. At the age of 45 years, his neurological examination revealed free ocular movement, no ptosis, and normal deep tendon reflex. He had right-side predominant rigidity, bradykinesia, mild neck dystonia, and festinating gait. He generally responded well to levodopa. His Unified Parkinson Disease Rating Scale (UPDRS) III scores revealed more than 50% improvement under a levodopa equivalent dose of 790 mg. He then developed motor fluctuation and levodopa-induced dyskinesia after 7 years of symptom onset. At the age of 58 years, he demonstrated progressive cognitive decline, visual hallucination, and was required to live in a nursing home. His Mini Mental State Examination score was 10 and his Clinical Dementia Rating was 2. We obtained patient's serum lactate and pyruvate level and all revealed normal. Nerve conduction study showed right deep peroneal motor axonal neuropathy and right ulnar nerve neuropathy cross elbow, which suggested an entrapment neuropathy. Electroencephalogram revealed no epileptiform discharge. His father was diagnosed as having PD, along with type 2 diabetes mellitus, at the age of 70 years; his neurological examination revealed resting tremor, rigidity, and bradykinesia on the left side, all of which diminished after levodopa treatment. He had...
symptom of chronic insomnia, bilateral lower limbs pain, and anxiety but no RBD. Both patient and his family had no symptom of ataxia, proximal weakness, epilepsy, or ophthalmoparesis.

We could not obtain his mother’s DNA sample because the patient had not been in contact with his mother and sister for many years. His younger brother died in a traffic accident without any history of parkinsonian symptoms. The rest of his family members did not have any extrapyramidal symptom, epilepsy, myopathy, or ataxia. Figure 1 presents the family pedigree of the patient’s family.

Genomic DNA was extracted from peripheral venous blood lymphocytes of the patient and his father. Next, multiplex ligation-dependent probe amplification was used to detect large deletions or duplication in the DNA (Jeuken, Cornelissen, Boots-Sprenger, Gijsen, & Wesseling, 2006). We then used target exome sequencing with a TruSeq Custom Amplicon Low Input panel (Illumina) to determine the 51 PD-causative genes mutation sites in patients (Deng et al., 2018; Lill, 2016; Puschmann, 2017). Target regions of patients’ blood genomic DNA were amplified with specific primers, ligated of adaptors to the amplified PCR products, and finally generated the libraries. Paired-end 150-bp NGS were performed on an Illumina MiSeq system at the Genomic Medicine Core Laboratory, Chang Gung Memorial Hospital. The validation of NGS results was performed with automatic sequencer ABI 3730 (Thermo Fisher, USA).

Nonsynonymous single-nucleotide polymorphisms, insertions-deletions, stop–gain, and frameshift variants were picked up. Next, Sorting Intolerant from Tolerant, Mutation Taster (http://www.mutationtaster.org/), and Polymorphism Phenotyping (version 2) were performed to detect amino acid substitutions affecting protein function. In addition, to determine potential candidate genes, we assessed the frequency of the variants in the general population (Exome Aggregation Consortium, dbSNP, 1000 Genomes Project). We considered variants with a minor allele frequency of ≤0.1% (rare variants). The mutation was classified as a pathogenic mutation if previous literature reported it as causative.

We confirmed the presence of heterozygous missense substitutions in POLG [c.2890G > A (p.R964C)] as well as GBA [c.1187A > G (p.L444P)] in the patient and his father (Figure 2). POLG R964C signifies alteration in a highly conserved site (Figure 3). According to the American College of Medical Genetics guidelines, POLG R964C meets the pathogenic criteria as one strong pathogenic evidence, (PS3, well-established in vitro functional studies supportive of a damaging effect on the R964C mutation), and two moderate pathogenic evidence (PM1, located in a mutational hotspot and functional domain without benign variation, and PM2, absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or ExAC. Combining the two criteria, this
### TABLE 1  POLG mutation phenotype without progressive external ophthalmoplegia

| Genotype             | Onset age of parkinsonism | Family history | Gender | Resting tremor | Rigidity | Bradykinesia | Seizure | Neuropathy | Levodopa response | Reference          |
|----------------------|---------------------------|----------------|--------|----------------|----------|--------------|---------|------------|-------------------|--------------------|
| G737R R853W          | 26                        |               | +      | F              | -        | +            | -       | +          | Good              | Davidzon et al. (2006) |
| G737R R853W          | 20                        |               | +      | F              | -        | +            | -       | +          | Good              | Davidzon et al. (2006) |
| R722H                | 57                        |               | -      | F              | +        | +            | -       | -          | Good              | Luoma et al. (2007)   |
| Y831C Q1236H         | 70                        |               | -      | F              | +        | +            | -       | -          | Good              | Luoma et al. (2007)   |
| R722H Q1236H         | 66                        |               | -      | F              | +        | +            | -       | -          | Good              | Luoma et al. (2007)   |
| S1230F               | 65                        |               | -      | M              | +        | +            | -       | -          | Good              | Luoma et al. (2007)   |
| P587L W748S          | 49                        |               | -      | M              | -        | +            | +       | -          | Good              | Ylönen et al. (2013)  |
| Y831C R722H          | 56                        |               | -      | F              | +        | +            | -       | -          | Good              | Ylönen et al. (2013)  |
| W748S R993C E1143G   | 72                        |               | -      | F              | +        | +            | -       | -          | Good              | Ylönen et al. (2013)  |
| E856K                | 18                        | +             | M      | -              | +        | +            | -       | -          | Good              | Mehta et al. (2016)   |
| R964C, GBA L444P     | 39                        | +             | M      | +              | +        | +            | -       | -          | Good              | Our case            |
| R964C, GBA L444P     | 70                        | +             | M      | +              | +        | +            | -       | -          | Good              | Our case            |

### TABLE 2  POLG R964C mutation phenotype

| Phenotype                | Onset age | Clinical manifestation | Lactate acidosis | Epilepsy | Ataxia | Sensory neuropathy | PEO | Reference          |
|--------------------------|-----------|------------------------|------------------|----------|--------|-------------------|-----|--------------------|
| Homozygous R964C mutation| 6 and 15  | NRTI toxicity          | +++              |          |        |                   |     | Bailey et al. (2009), Yamanaka et al. (2007) |
| Homozygous R964C mutation| 34        | Nonsyndromic Ovarian dysfunction |        | -       | -      |                   |     | Chen et al. (2018) |
| Compound heterozygous R964C and A862T mutations | 17 | ANS | - | + | + | + | - | Wong et al. (2008) |
| Compound heterozygous R964C and A862T mutations | 17 | ANS | - | + | + | + | - | Wong et al. (2008) |
| Compound heterozygous R964C and A862T mutations | 17 | ANS | - | + | + | + | - | Wong et al. (2008) |
| Heterozygous R964C and GBA L444P mutations | 39 | Parkinson's disease | - | - | - | - | - | Our case |
| Heterozygous R964C and GBA L444P mutations | 70 | Parkinson's disease | - | - | - | - | - | Our case |

Note. PEO: Progressive external ophthalmoplegia, ANS: Ataxia Neuropathy Spectrum, NRTI: nucleotide reverse transcriptase inhibitor.
Homozygous R964C mutation can present as early ovarian fail‐
ure or nucleotide reverse transcriptase inhibitor toxicity when
anti‐human immunodeficiency virus‐1 medication is taken (Bailey,
2009). The recombinant R964C Pol γ activity had only 14% polymerase activity compared to Wide type. In the presence of nucleoside reverse transcriptase inhibitor, both heterozygously and homozygously harboring mutant R964C Pol γ lymphoblastoid cell lines contained significantly reduced mtDNA levels, compared with those wild type Pol γ (Yamanaka et al., 2007).

On the other hand, GBA mutation is known as loss of lysosomal hydrolase glucocerebrosidase (GCase) activity causing impairment of the autophagy lysosome pathway. Dysfunction of the mitophagy can be caused by impairment of autophagy lysosome pathway (Gegg & Schapira, 2016; Kim, Rodriguez‐Enriquez, & Lemasters, 2007). In animal model, heterozygous GBA L444P mutation mice exhibited reduction in GCase activity and impairment autophagic delivery of mitochondria to lysosomes and mitochondrial priming dysfunction (de la Mata et al., 2015; Li et al., 2019).

Moreover, accumulating evidence has indicated that harboring more than two mutational loci in two allele may cause a synergetic effect, leading to early neurodegeneration (Cady et al., 2015; Giri, Zhang, & Lü, 2016). Also, polygenic factors contribute to the impairment of mitochondrial replication and repair may result in PD (Gaare et al., 2018). We suspect that these two gene mutations could both influence repairing mitochondria and increase oxidative stress causing early neurodegeneration.

In our patient’s family, only one patient developed YOPD, whereas his father developed late-onset PD. No literature has reported PD in POLG R964C mutation. Furthermore, the same mutations could reveal variable presentations, suggesting that epigenetic or environmental factors, as well as other modifiers may influence the clinical manifestation.

4 | CONCLUSION

We reported a first familial PD of combined POLG R964C and GBA L444P mutations. This finding extends our understanding of the PD genotype–phenotype correlation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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