Review Article

Alternative Splicing Generates Different Parkin Protein Isoforms: Evidences in Human, Rat, and Mouse Brain

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Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene mutations are the most frequent causes of autosomal recessive early onset Parkinson’s disease and juvenile Parkinson disease. Parkin deficiency has also been linked to other human pathologies, for example, sporadic Parkinson disease, Alzheimer disease, autism, and cancer. PARK2 primary transcript undergoes an extensive alternative splicing, which enhances transcriptomic diversification. To date several PARK2 splice variants have been identified; however, the expression and distribution of parkin isoforms have not been deeply investigated yet. Here, the currently known PARK2 gene transcripts and relative predicted encoded proteins in human, rat, and mouse are reviewed. By analyzing the literature, we highlight the existing data showing the presence of multiple parkin isoforms in the brain. Their expression emerges from conflicting results regarding the electrophoretic mobility of the protein, but it is also assumed from discrepant observations on the cellular and tissue distribution of parkin. Although the characterization of each predicted isoforms is complex, since they often diverge only for few amino acids, analysis of their expression patterns in the brain might account for the different pathogenetic effects linked to PARK2 gene mutations.

1. Introduction

Homzygous or compound heterozygous mutations of Parkinson protein 2, E3 ubiquitin protein ligase (PARK2) gene are cause (50% of cases) of autosomal recessive forms of PD, usually without atypical clinical features. PARK2 mutations also explain ~15% of the sporadic cases with onset before 45 [1, 2] and act as susceptibility alleles for late-onset forms of Parkinson disease (2% of cases) [3]. Along with Parkinsonism forms, PARK2 gene has been linked to other human pathologies, such as Alzheimer disease [4], autism [5], multiple sclerosis [6], cancer [7, 8], leprosy [9], type 2 diabetes mellitus [10], and myositis [11].

PARK2 gene is located in the long arm of chromosome 6 (6q25.2-q27) and spans more than 1.38 Mb [12, 13]. From the cloning of the first human cDNA [12, 13], PARK2 genomic organization was thought to include only 12 exons encoding one transcript. Many evidences now demonstrate the existence of additional exonic sequences, which can be alternatively included or skipped in mature mRNAs. To date, dozens of PARK2 splice transcripts have been described [14] and have been demonstrated to be differentially expressed in tissue and cells [15–21]. These multiple PARK2 splice variants potentially encode for a wide range of distinct protein isoforms with different structures and molecular architectures. However, the characterization and the distribution of these isoforms have not been deeply detailed yet. While studying PARK2 splice variants mRNAs is relatively simple, differentiating protein isoforms is more complex, since they often diverge only for few amino acids. The complexity of this task could
explain the small number of scientific papers on this topic. However, solving this riddle is fundamental to comprehend the precise role of PARK2 in human diseases. The tissue and cell specific expression pattern of PARK2 isoforms, in fact, might account for the different pathogenetic effects linked to this gene.

In this review, we briefly describe the structure of PARK2 gene, its currently known transcript products, and the predicted encoded protein isoforms expressed in human, rat and mouse; the latter are two commonly used animal models for studying human diseases. Then, we illustrate the expression of these isoforms by recapitulating the major literature evidences already available, which have previously unknowingly demonstrated their existence. We focus on the expression and cellular distribution of parkin isoforms in the brain. Finally, we collect in a panel the different parkin antibodies, commercially available, which could be useful for the characterization of the isoforms expression and distribution.

2. PARK2 Alternative Splice Transcripts Produce Isoforms with Different Structures and Functions

To date, 26 human different cDNAs, corresponding to 21 unique PARK2 alternative splice variants, have been described and are summarized in Figure 1 and Table 1. These mature transcripts are derived from the combination of 17 different exonic regions. Similarly, 20 PARK2 transcripts (20 exons) have been characterized in rat (Figure 2 and Table 2) and 9 (15 exons) in mouse (Figure 3 and Table 3). All of them have been carefully described in our previous paper [14].

For each of these variants, the encoded protein isoform, the corresponding molecular weight, and isoelectric point have been predicted and reported in Tables 1, 2, and 3. H8/H17, H9/H13, and H7/H18 isoforms show the same molecular weight and isoelectric point (Table 1), since they have the same amino acid composition; similarly, R2/R7/R14, R17/R18, and R3/R16 show the same primary structure, as shown in Table 2. Although equal, these proteins are encoded by different splice variants which probably produce the same protein with different efficiency.

In addition to primary structures, molecular architectures and domains composition have also been evaluated (Figures 1, 2, and 3 panels (b) and (c)). As previously described, the original (canonical) PARK2 protein (Accession number BAA25751.1) [12] comprises an N-terminal ubiquitin-like (UBQ) domain and two C-terminal in-between ring fingers (IBR) domains. The UBQ domain targets specific protein substrates for proteasome degradation, whereas IBR domains occur between pairs of ring fingers and play a role in protein quality control. PARK2 encoded isoforms structurally diverge from the canonical one for the presence or absence of the UBQ domain and for one of or both IBR domains. Moreover, when the UBQ domain is present, it often differs in length from that of the canonical sequence. Interestingly, some isoforms miss all of these domains.

The different molecular architectures and domain composition of isoforms might roughly alter also their functions. Parkin protein acts as an E3 ubiquitin ligase and is responsible of substrates recognition for proteasome-mediated degradation. PARK2 tags various types of proteins, including cytosolic (Synphilin-1, Pael-R, CDCrel-1 and 2a, α-synuclein, p22, and Synaptotagmin XI) [25–29], nuclear (Cyclin E) [15], and mitochondrial ones (MFN1 and MFN2, VDAC, TOM70, TOM40 and TOM20, BAK, MIRO1 and MIRO2, and FISI) [30–34]. The number of targets is so high that parkin protein results involved in numerous molecular pathways (proteasome-degradation, mitochondrial homeostasis, mitophagy, mitochondrial DNA stability, and regulation of cellular cycle). To date it is unknown if all these functions are mediated by a single protein or by different isoforms. However, considering that parkin mRNAs have a different expression and distribution in tissues and cells [14], which should be also mirrored at the protein level, it is reasonable to hypotize that these distinct isoforms might perform specific functions and could be differentially expressed in each cellular phenotype. Each PARK2 splice variants may acts in different manner to suit cell specific needs. This hypothesis is supported by previous evidences showing different and even opposite functions of other splice variants, such as BCL2L12 pattern expression related to cellular phenotype [35]. Finally, based on the extensive alternative splicing process of PARK2 gene, we cannot rule out that additional splice variants with different functions (beyond those listed) may exist.

3. Evidences of Multiple Parkin Isoforms in Brain

A remarkable number of papers have demonstrated the existence, in human and other species, of different mRNA parkin variants [15–21]. However, few of them have investigated parkin isoforms existence, and some have done it without the awareness of PARK2 complex splicing [23, 36, 37]. In fact, although many mRNA parkin splice variants have been cloned, the corresponding proteins have been only deduced through the analysis of the longest open reading frame and uploaded on protein databases as predicted sequences. To date many questions are still unanswered: Are all mRNA parkin splice variants translated? Does a different expression pattern of parkin proteins, in tissue and cells, exist? Does each protein isoform have a specific function? In the following paragraphs we try to answer these questions by summarizing the knowledge accumulated over the last three decades on parkin expression and distribution in human, rat, and mouse brain. Existing data are reinterpreted by considering the complexity level of PARK2 gene splicing described above.

Many conflicting data emerges in the literature regarding the number and relative electrophoretic mobility of parkin proteins. While the majority of papers reported only a band of ∼52 kDa corresponding to the canonical parkin isoform, also known as full length parkin, additional bands (from ∼22 kDa to ∼100 kDa) both in rodent [23, 28, 36–37] and human brain regions were also detected [22–25, 39, 42–45].
Figure 1: Chromosomal localization, exonic structure of alternative splice variants, and corresponding predicted protein isoforms of human PARK2. (a) Cytogenetic location of human PARK2 gene (6q26). (b) Exon organization map of the 21 human PARK2 splice variants currently known. Exons are represented as red bars. The size of introns (black line) is proportional to their length. The codes on left refer to gene identifiers reported in Table 1. (c) Predicted molecular architecture of PARK2 isoforms. Red boxes represent UBQ domain and blue boxes represent IBR domains.

Figure 2: Chromosomal localization, exonic structure of alternative splice variants, and corresponding predicted protein isoforms of rat PARK2. (a) Cytogenetic location of rat PARK2 gene (1q11). (b) Exon organization map of the 20 rat PARK2 splice variants currently known. Exons are represented as red bars. The size of introns (black line) is proportional to their length. The codes on left refer to gene identifiers reported in Table 2. (c) Predicted molecular architecture of PARK2 isoforms. Red boxes represent UBQ domain and blue boxes represent IBR domains.
Figure 3: Chromosomal localization, exonic structure of alternative splice variants, and corresponding predicted protein isoforms of mouse PARK2. (a) Cytogenetic location of mouse PARK2 gene (A3.2-A3.3). (b) Exon organization map of the 9 mouse PARK2 splice variants currently known. Exons are represented as red bars. The size of introns (black line) is proportional to their length. The codes on left refer to gene identifiers reported in Table 3. (c) Predicted molecular architecture of PARK2 isoforms. Red boxes represent UBQ domain and blue boxes represent IBR domains.

Table 1: Homo sapiens parkin isoforms.

| New code identifier | GI          | Protein accession number | aa sequence | Predicted MW | pI  |
|---------------------|-------------|--------------------------|-------------|--------------|-----|
| H20                 | 4696099976  | AGH62057I                | 530 aa      | 58,127       | 6,41|
|                     | 3063387     | BAA25751I BAF43729I      | 465 aa      | 51,65        | 6,71|
| H1                  | 158258616   | BAF85279I BAF84553I      | 437 aa      | 48,713       | 7,12|
|                     | 169790968   | NP004553.2               | 415 aa      | 46,412       | 6,91|
|                     | 125630744   | ABN46990.1               | 387 aa      | 43,485       | 7,43|
| H5                  | 284468410   | ADB90270I                | 386 aa      | 42,52        | 6,65|
|                     | 169790970   | NP054642.2               | 386 aa      | 42,52        | 6,65|
|                     | 284468412   | ADB90271I                | 358 aa      | 39,592       | 7,08|
|                     | 284516985   | ADB91979I                | 316 aa      | 35,63        | 6,45|
|                     | 31491069    | AAB22014.1               | 274 aa      | 30,655       | 6,3 |
|                     | 284468407   | *                        | 203 aa      | 22,192       | 5,68|
| H21                 | 284516991   | *                        | 172 aa      | 19,201       | 6,09|
|                     | 284516982   | *                        | 143 aa      | 15,521       | 5,54|
|                     | 284468408   | *                        | 139 aa      | 15,407       | 6,10|
| H3                  | 284516983   | ADB90269I                | 139 aa      | 15,393       | 6,41|
|                     | 284516984   | ADB91978I                | 95 aa       | 10,531       | 8,74|
| H7                  | 194378189   | BAG57845.1               | 61 aa       | 6,832        | 10,09|
|                     | 469609974   | AGH62056.1               | 51 aa       | 5,348        | 7,79|

H1 represents the canonical sequence cloned by Kitada et al., 1998 [12].
* The protein accession number is not present in database.
Parkin was observed both in rat central and peripheral nervous system. Two major bands of ~50 and ~44 kDa were recognized in cell extracts from rat Substantia Nigra (SN) and cerebellum by western blot analysis. In adrenal glands there were visualized several immunoreactive bands of 50, 69–66, and 89 kDa [36]. Additional bands were also observed in primary cultures of cortical type I astrocytes [37].

Similar result was observed in mouse brain homogenate: a major band of 50 kDa and fainter bands of ~40 and 85/118 kDa were identified on immunoblot. In all these papers, lower and higher molecular weight bands were described as posttranslational modification or proteolytic cleavage of 52 kDa canonical protein or heterodimers resulting from the interaction of parkin with other proteins.

Table 2: *Rattus norvegicus* parkin isoforms.

| New code identifier | GI          | Protein accession number | aa sequence | Predicted MW | pI  |
|---------------------|-------------|--------------------------|-------------|--------------|-----|
| R13                 | 284810438   | ADB96019.1               | 494 aa      | 54,829       | 6.46|
| R4                  | 20385787    | AAM21452.1               | 489 aa      | 54,417       | 6.46|
| R1                  | 7229096     | BAA92431.1               | 465 aa      | 51,678       | 6.59|
| R4                  | 11464986    | AAG37013.1               | 465 aa      | 51,678       | 6.59|
| R5                  | 20385789    | AAM21453.1               | 446 aa      | 49,367       | 6.59|
| R8                  | 284066979   | AAM21456.1 ADB77772.1    | 437 aa      | 48,734       | 6.74|
| R5                  | 520845533   | AGP25367.1               | 421 aa      | 46,854       | 6.59|
| R10                 | 284066981   | ADB77773.1               | 394 aa      | 43,297       | 6.06|
| R9                  | 520845539   | AGP25371.1               | 344 aa      | 38,558       | 6.13|
| R2                  | 18478865    | AAL73348.1               | 274 aa      | 30,641       | 6.2 |
| R7                  | 20385793    | AAM21455.1 ADB96018.1    | 274 aa      | 30,641       | 6.2 |
| R4                  | 520845525   | AGP25364.1 AGP25365.1    | 274 aa      | 30,641       | 6.2 |
| R12                 | 284468405   | ADB90268.1               | 256 aa      | 28,006       | 6.44|
| R6                  | 20385791    | AAM21454.1               | 203 aa      | 22,288       | 5.42|
| R11                 | 284468403   | ADB90267.1               | 193 aa      | 21,253       | 8.54|
| R9                  | 20385803    | AAM21460.1               | 177 aa      | 19,84        | 5.97|
| R17                 | 520845535   | AGP25369.1               | 139 aa      | 15,404       | 6.29|
| R18                 | 520845537   | AGP25370.1               | 139 aa      | 15,404       | 6.29|
| R3                  | 18478869    | AAL73349.1               | 111 aa      | 12,329       | 6.92|
| R16                 | 520845533   | AGP25368.1               | 111 aa      | 12,329       | 6.92|
| R20                 | 520845541   | AGP25372.1               | 86 aa       | 9,929        | 7.5 |

Table 3: *Mus musculus* parkin isoforms.

| New code identifier | GI          | Protein accession number | aa sequence | Predicted MW | pI  |
|---------------------|-------------|--------------------------|-------------|--------------|-----|
| M1                  | 10179808    | AAG13890.1               | 464 aa      | 51,617       | 6.9 |
| M2                  | 74685929    | BAA82404.1               | 274 aa      | 30,631       | 6.54|
| M3                  | 86577675    | AAI13205.1               | 274 aa      | 30,631       | 6.54|
| M5                  | 220961631   | *                        | 262 aa      | 28,7         | 7.57|
| M6                  | 10179810    | AAG13891.1               | 255 aa      | 28,154       | 8.49|
| M7                  | 10179812    | AAG13892.1               | 214 aa      | 23,388       | 6.51|
| M8                  | 220961637   | ACL93281.1               | 106 aa      | 11,482       | 9.3 |
| M9                  | 74227131    | *                        | 75 aa       | 8,053        | 8.85|
| M10                 | 220961633   | ACL93281.1               | 65 aa       | 7,181        | 5.62|
| M11                 | 284829878   | ADB99567.1               | 63 aa       | 6,967        | 6.53|

*The protein accession number is not present in database.
However, we speculate that they might correspond to multiple parkin isoforms with different molecular weight. In knocked-out mice for parkin exon 2, several unexpected bands were also observed on immunoblot. This was interpreted as antibody cross-reactivity with nonauthentic parkin protein [46]. However, as shown in Figure 3, these bands might represent isoforms encoded by splice variants not containing the deleted exon (i.e., M5 and M4).

Parkin expression was also demonstrated in human brains of normal and sporadic Parkinson disease (PD) subjects, but it was absent in any regions of AR-JP brain [22, 23]. A major band of 52 kDa and a second fainter band of ~41 kDa were observed on immunoblot from human frontal cortex of PD patients and control subjects [22]. Parkin expression was also observed in Lewy bodies (LBs), characteristic neuronal inclusions in PD brain. However, in this regard we highlight widely varying results. Initially, the parkin protein expression was reported in neurons of the SN, locus coeruleus, putamen, and frontal lobe cortex of sporadic PD and control individuals but no parkin-immunoreactivity (IR) was found in SN LBs of PD patients [22, 23]. Later on, parkin-IR was described in nigral LBs of four related human disorders, sporadic PD, α-synuclein-linked PD, LB positive parkin-linked PD, and dementia with LBs (DBL) [24]. These discrepant results might be due to the antibodies used. In fact, as shown in Table 4, aligning the epitope sequence recognized by the antibody to each isoform sequence, we discovered that every antibody identifies a pool of different isoforms.

In accord with this hypothesis, we also explain discordant results observed by Schlossmacher et al. (2002) regarding the cellular distribution of the protein. In fact, they described strongly labeled cores of classical intracellular LBs in pigmented neurons of the SN in PD and DBL patients by using HP2A antibody, whereas HPIA and HP7A antibodies intensively labeled cytoplasmic parkin, in a granular pattern, of cell bodies and proximal neurites of dopaminergic neurons in both diseased and normal brains [24]. These results might represent a different cellular expression profile of parkin isoforms in healthy and diseased human brains. This hypothesis is supported by another study demonstrating a different expression profile of parkin mRNA splice variants in frontal cortex of patients with common dementia with LB, pure form of dementia with LB, and Alzheimer disease suggesting the direct involvement of isoform-expression deregulation in the development of such neurodegenerative disorders [17]. To date there exists only one paper that has dealt with parkin amino acid sequencing [47]. Trying to ensure that the signal observed on human serum by western blot analysis belongs to parkin protein, they cut off the area on the blot between 50 and 55 kDa in two separate pieces and performed a MALDI-TOF analysis on each. Peptides peaks analysis revealed the presence of six other proteins with similar sequence to canonical one. However, authors did not even speculate that they could represent additional parkin isoforms. Further evidence of the existence of multiple isoforms come from the conflicting data on their tissue and cellular distribution. Parkin protein is particularly abundant in the mammalian brain and retina [22, 23, 36, 48, 49]. In human, parkin immunoreactivity (IR) has been observed in SN, locus coeruleus, putamen, and frontal lobe cortex [22, 23]. Similarly, it has been strongly measured in rat hippocampus, amygdaloid nucleus, endopiriform nucleus, cerebral cortex, colliculus, and SN (pars compacta and pars reticulata) [37, 50].

Analog parkin distribution was reported in mouse. Most immunoreactive cells were found in the hindbrain. In the cerebellum only the cells within the cerebellar nuclei were positive, while the structures located in the mesencephalon presented moderate to strong immunoreactivity. In the ventral part of the mesencephalon the red nucleus showed large strongly stained cells. In the SN moderate parkin immunoreactivity was confined to the pars reticulata. In the dorsal mesencephalon, immunopositive cells were found in the intermediate and deep gray layer of the superior colliculus and in all parts of the inferior colliculus [12, 36, 41, 51]. Although in most brain regions good correlations between parkin-IR and mRNA were observed, incongruent data emerged from some paper in rat SNc (substantia nigra pars compacta), hippocampus, and cerebellar Purkinje cells distribution, where mRNA was detected but no parkin-IR was revealed [23, 36].

Furthermore, in an early study, parkin was described in cytoplasm, in granular structure, and in neuronal processes but was absent in the nucleus [22]. Subsequently other studies reported also its nuclear localization [23, 37, 48, 52–54]. Finally, some papers have also observed a small mitochondrial pool of the protein [55, 56]. All these evidences have suggested that protein could localize to specific subcellular structure under some circumstances. However, it is also reasonably hypothesized that a specific pattern of subcellular distribution of parkin isoforms is related to each cellular phenotype, since in all these papers, protein immunolocalization was performed by using antibodies recognizing different epitopes. Some discrepancies are also observed in the expression of parkin in the SNc of patients affected by other forms of parkinsonism [23]. Brain isoforms might have different species-specific biochemical characteristics, when comparing murine versus human parkin. In fact, it has been shown that mouse protein is easily extracted from brain by high salt buffer, instead human parkin is only extracted with harsher buffers, especially in elderly. This suggested that human parkin becomes modified or interacts with other molecules with age, and this alters its biochemical properties [42]. However, we cannot rule out that this may correlate to a specific expression pattern of isoforms with different biochemical properties in the brains of rodents and humans relative to age. All of these observations were also supported by contradictory results emerging from clinical studies. Initially, recessive mutations in the parkin gene were related to sporadic early onset parkinsonism [2]; however, the mode of transmission was subsequently rejected by other genetic studies with not only homozygous or compound heterozygous mutations, but also single heterozygous mutations, affecting only one allele of the gene [2, 57–61]. It has been suggested that haploinsufficiency is a risk factor for disease, but certain mutations are dominant, conferring dominant-negative or
Table 4: Parkin isoforms recognized by antibodies used in some studies.

| Name                      | Target | Recognized Parkin isoforms |
|---------------------------|--------|----------------------------|
| M73 (Shimura et al., 1999) [22] | 124–137 | H1, H4, H5, H8, H9, H10, H13, H14, H17, H20, H21 |
| M74 (Shimura et al., 1999) [22] | 293–306 | H1, H2, H3, H4, H5, H6, H8, H10, H11, H14, H17, H20, H21 |
| ParkA (Huynh et al., 2000) [23] | 96–109 | H1, H2, H3, H4, H5, H6, H8, H9, H10, H11, H13, H14, H17, H20, H21 |
| ParkB (Huynh et al., 2000) [23] | 440–415 | H1, H2, H5, H6, H7, H8, H10, H11, H12, H14, H17, H18, H20, H21 |
| HP6A (Schlossmacher et al., 2002) [24] | 6–15 | H1, H4, H5, H6, H9, H10, H13, H14, H16, H20 |
| HP7A (Schlossmacher et al., 2002) [24] | 51–62 | H1, H4, H5, H6, H9, H10, H13, H14, H15, H20 |
| HP1A (Schlossmacher et al., 2002) [24] | 84–98 | H1, H2, H3, H4, H5, H6, H8, H9, H10, H11, H13, H14, H17, H20, H21 |
| HP2A (Schlossmacher et al., 2002) [24] | 342–353 | H1, H2, H3, H4, H5, H6, H7, H8, H11, H12, H17, H18, H20, H21 |
| HP5A (Schlossmacher et al., 2002) [24] | 453–465 | H1, H2, H5, H6, H7, H8, H10, H11, H12, H14, H17, H18, H20, H21 |

Figure 4: Differential detection of parkin isoforms in rat brain using five anti-parkin antibodies. (a) Representative immunoblot of parkin isoforms in rat brain visualized by using five different antibodies. Ab1, Ab2, Ab3, Ab4, and Ab5 correspond to groups #3, #4, #5, #8, and #9 of Table 5. Immunoblot for β-tubulin was used as loading control. (b) Canonical parkin sequence domains recognized by the five antibodies.

Toxic gain of functions of parkin protein [61]. However, in light of the evidence outlined above, it is possible that some single heterozygous mutation might affect gene expression by inducing loss of function of some isoforms and gain of function of other.

4. The Diversified Panel of Antibodies

Commercially Available against PARK2

To date more than 160 PARK2 antibodies are commercially available. They are obtained from different species (generally rabbit or mouse) and commercialized by various companies. Table 5 lists 32 commercially available PARK2 antibodies whose immunogens used are specified by providers in datasheet. Some of them recognize a common epitope, therefore, have been included in the same group. Tables 6, 7, and 8 report, respectively, human, rat, and mouse parkin isoforms recognized by these antibodies. When the amino acid sequence recognized by the antibody perfectly match with the sequence of the protein, it is very likely to get a signal by western blot or immunohistochemistry analysis (this is indicated in the table by “Yes”). Instead, if the antibody recognizes at least 8 consecutive amino acids on the protein, it is likely to visualize a signal both by western blot or immunohistochemistry analysis (this is indicated in the table by “May be”). Finally, if the antibody recognizes less than 8 consecutive amino acids, it could rule out the possibility to visualize a signal on immunoblot or immunohistochemistry analysis (this is indicated in the table by “No”). The use of these 32 antibodies may allow the identification of at least 15 different PARK2 epitopes (Table 5). Although no epitope is isoform specific, the combinatorial use of antibodies targeting different protein regions may provide a precious aid to decode the exact spectrum of PARK2 isoforms expressed in tissues and cells. An example of combinatorial use of antibodies has been reported in Figure 4. On rat brain homogenate, these five antibodies raised against different parkin epitopes, revealed the canonical ~50 kDa band, but additional putative bands of higher and lower molecular weight were visualized. This experimental data reinforce the existence of more than one parkin isoform and confirm that the investigation of
| Antibody group # | Trade name | Generic name | Companies | Target domain |
|----------------|------------|--------------|-----------|---------------|
| #1             | H00005071-B01P | Abnova       | Abnova    | 1 aa–387 aa   |
|                | H00005071-D01P | Abnova       | Abnova    |               |
|                | H00005071-D01 | Abnova       |           |               |
| #2             | OASA06385   | Aviva System biology |           |               |
|                | AHP495      | AbD Serotec  |           |               |
|                | MD-19-0144  | Raybiotech, Inc. |           |               |
|                | DS-PB-01562 | Raybiotech, Inc. |           |               |
|                | PAB14022    | Abnova       |           |               |
| #3             | MCA3315Z    | AbD Serotec  | Abnova    | 288 aa–388 aa |
|                | H00005071-M01 | Abnova       |           |               |
| #4             | PAB1105     | Abnova       | Fitzgerald| 62 aa–80 aa   |
|                | 7R-PR059    |              |           |               |
| #5             | PAB0714     | Abnova       | Millipore Chemicon | 305 aa–323 aa |
|                | AB512       |              | Novus biologicals |               |
|                | R-113-100   |              |           |               |
| #6             | P5748       | Sigma        |           |               |
|                | GTX25667    | GeneTex International Corporation |           |               |
|                | Parkin antibody CR20121213, GTX25667 |           |           |               |
|                | ABIN122870  | Antibodies on-line |           |               |
|                | PAI-751     | Thermo Fisher Scientific, Inc. |           |               |
| #7             | R-114-100   | Novus biologicals |           | 295 aa–311 aa |
|                | Anti-Parkin, aa295-311 h Parkin, C-terminal | Millipore Chemicon |           |               |
| #8             | MAB5512     | Millipore Chemicon |           |               |
|                | Anti-Parkin antibody, clone PRK8/05882 Parkin (PRK8): sc-32282 | Millipore Upstate |           | 399 aa–465 aa |
|                |             | Santa Cruz   |           |               |
| #9             | Parkin (H-300): sc-30130 Parkin (D-1): sc-133167 Parkin (H-8): sc-136989 | Santa Cruz |           | 61 aa–360 aa |
| #10            | EB07439     | Everest Biotech |           |               |
|                | GTX89242 PARK2 antibody, internal CR20121213, GTX89242 NB100-53798 | GeneTex International Corporation |           | 394 aa–409 aa |
|                |             | Novus biologicals |           |               |
| #11            | GTX113239 Parkin antibody [NIC1] CR20121213, GTX113239 | GeneTex International Corporation |           | 28 aa–258 aa |
| #12            | 10R-3061    | Fitzgerald   |           | 390 aa–406 aa |
| #13            | A01250-40   | GenScript    |           | 300 aa–350 aa |
| #14            | NB600-1540  | Novus biologicals |           | 399 aa–412 aa |
| #15            | ARP43038_P050 | Aviva System biology |           | 311 aa–360 aa |

Antibodies against canonical PARK2 isoform (NP_004533.2) were grouped if they recognize the same epitope. To each group was assigned a new identification code (#).
| New code identifier | Ab #1 | Ab #2 | Ab #3 | Ab #4 | Ab #5 | Ab #6 | Ab #7 | Ab #8 | Ab #9 | Ab #10 | Ab #11 | Ab #12 | Ab #13 | Ab #14 | Ab #15 |
|---------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|--------|
| H20                 | May be (360 aa) | Yes | May be (64 aa) | Yes | Yes | Yes | Yes | Yes | May be (299 aa) | May be (17 aa) | May be (230 aa) | Yes | Yes | Yes | May be (47 aa) |
| H1                  | May be (360 aa) | Yes | May be (64 aa) | Yes | Yes | Yes | Yes | Yes | May be (17 aa) | Yes | Yes | Yes | Yes | May be (47 aa) |
| H5                  | May be (333 aa) | Yes | May be (64 aa) | Yes | Yes | Yes | Yes | Yes | May be (271 aa) | May be (17 aa) | May be (202 aa) | Yes | Yes | Yes | May be (47 aa) |
| H10                 | May be (311 aa) | Yes | May be (22 aa) | Yes | No | May be (14 aa) | Yes | Yes | May be (250 aa) | May be (17 aa) | May be (230 aa) | Yes | No | Yes | No |
| H14                 | May be (283 aa) | Yes | May be (22 aa) | Yes | No | May be (14 aa) | Yes | Yes | May be (222 aa) | May be (17 aa) | May be (202 aa) | Yes | May be (12 aa) | May be (15 aa) | No |
| H4                  | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No | May be (299 aa) | No | May be (230 aa) | No | Yes | No | May be (47 aa) |
| H8                  | May be (274 aa) | Yes | May be (64 aa) | No | Yes | Yes | Yes | Yes | May be (281 aa) | May be (17 aa) | May be (178 aa) | Yes | Yes | May be (15 aa) | May be (47 aa) |
| H17                 | May be (274 aa) | Yes | May be (64 aa) | No | Yes | Yes | Yes | Yes | May be (280 aa) | May be (17 aa) | May be (178 aa) | Yes | Yes | May be (15 aa) | May be (47 aa) |
| H21                 | May be (254 aa) | Yes | May be (64 aa) | No | Yes | Yes | Yes | Yes | May be (252 aa) | May be (17 aa) | May be (150 aa) | Yes | Yes | May be (15 aa) | No |
| H6                  | May be (148 aa) | No | May be (64 aa) | No | Yes | Yes | Yes | Yes | May be (252 aa) | No | May be (52 aa) | Yes | Yes | Yes | Yes |
| H11                 | May be (162 aa) | No | May be (64 aa) | No | Yes | Yes | Yes | Yes | May be (66 aa) | Yes | Yes | Yes | Yes | Yes | Yes |
| H2                  | May be (161 aa) | No | May be (64 aa) | No | Yes | Yes | Yes | Yes | May be (67 aa) | Yes | Yes | Yes | Yes | Yes | Yes |
| H3                  | May be (161 aa) | No | May be (64 aa) | No | Yes | Yes | Yes | No | May be (67 aa) | No | Yes | No | Yes | No |
| H12                 | May be (42 aa) | No | May be (42 aa) | No | May be (12 aa) | No | No | Yes | Yes | No | May be (39 aa) | Yes | Yes | Yes | Yes |
| H9                  | May be (137 aa) | Yes | No | Yes | No | No | No | No | Yes | No | May be (110 aa) | No | No | No | No |
| H13                 | May be (137 aa) | Yes | No | Yes | No | No | No | No | Yes | No | May be (110 aa) | No | No | No | No |
| H7                  | May be (27 aa) | No | May be (27 aa) | No | No | No | No | Yes | May be (30 aa) | Yes | No | Yes | May be (24 aa) | Yes | Yes |
| H18                 | May be (27 aa) | No | May be (27 aa) | No | No | No | No | Yes | May be (30 aa) | Yes | No | Yes | May be (24 aa) | Yes | Yes |
| H15                 | May be (65 aa) | No | No | No | No | No | No | No | No | May be (38 aa) | No | No | No | No |
| H19                 | No | No | No | No | No | No | No | No | No | No | No | No | No | No | No |
| H16                 | No | No | No | No | No | No | No | No | No | No | No | No | No | No | No |

Yes = perfect match between predicted protein sequence and antibody epitope.
May be = partial match between predicted protein sequence and antibody epitope; in parenthesis number of amino acid matching/total number of amino acid recognized by antibody epitope.
No = matching between predicted protein sequence and antibody epitope is less than 8 consecutive amino acids.
| New code identifier | Ab #1 | Ab #2 | Ab #3 | Ab #4 | Ab #5 | Ab #6 | Ab #7 | Ab #8 | Ab #9 | Ab #10 | Ab #11 | Ab #12 | Ab #13 | Ab #14 | Ab #15 |
|---------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|--------|
| R13                | May be | May be | May be | May be | May be | Yes | Yes | Yes | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be |
| R14                | May be | May be | May be | May be | May be | Yes | Yes | Yes | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be |
| R15                | May be | May be | May be | May be | May be | Yes | Yes | Yes | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be |
| R16                | May be | May be | May be | May be | May be | Yes | Yes | Yes | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be |
| R17                | May be | May be | May be | May be | May be | Yes | Yes | Yes | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be |
| R18                | May be | May be | May be | May be | May be | Yes | Yes | Yes | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be |
| R19                | May be | May be | May be | May be | May be | Yes | Yes | Yes | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be |
| R20                | May be | May be | May be | May be | May be | Yes | Yes | Yes | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be | May be |

Yes = perfect match between predicted protein sequence and antibody epitope.
May be = partial match between predicted protein sequence and antibody epitope; in parenthesis number of amino acid matching/total number of amino acid recognized by antibody epitope.
No = matching between predicted protein sequence and antibody epitope is less than 8 consecutive amino acids.
| New code identifier | Ab #1 | Ab #2 | Ab #3 | Ab #4 | Ab #5 | Ab #6 | Ab #7 | Ab #8 | Ab #9 | Ab #10 | Ab #11 | Ab #12 | Ab #13 | Ab #14 | Ab #15 |
|---------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|--------|
| M1                  | May be (294 aa) | No | May be (61 aa) | May be (13 aa) | May be (18 aa) | Yes | Yes | May be (70 aa) | May be (244 aa) | No | May be (176 aa) | May be (15 aa) | May be (48 aa) | May be (14 aa) | Yes |
| M5                  | May be (147 aa) | No | No | May be (62 aa) | No | May be (18 aa) | Yes | Yes | May be (70 aa) | May be (153 aa) | No | May be (55 aa) | May be (15 aa) | May be (48 aa) | May be (14 aa) | Yes |
| M2                  | May be (191 aa) | No | No | No | May be (13 aa) | No | No | No | May be (134 aa) | No | May be (164 aa) | No | No | No | No |
| M3                  | May be (192 aa) | No | No | No | May be (13 aa) | No | No | No | May be (135 aa) | No | May be (165 aa) | No | No | No | No |
| M8                  | May be (161 aa) | No | No | No | May be (13 aa) | No | No | No | May be (106 aa) | No | May be (136 aa) | No | No | No | No |
| M7                  | May be (53 aa) | No | No | No | No | No | No | No | No | No | May be (27 aa) | No | No | No | No |
| M4                  | No | No | No | No | No | No | No | No | No | No | No | No | No | No | No |
| M6                  | May be (53 aa) | No | No | No | No | No | No | No | No | No | May be (27 aa) | No | No | No | No |
| M9                  | May be (53 aa) | No | No | No | No | No | No | No | No | No | May be (27 aa) | No | No | No | No |

Yes = perfect match between predicted protein sequence and antibody epitope.
Maybe = partial match between predicted protein sequence and antibody epitope; in parenthesis number of amino acid matching/total number of amino acid recognized by antibody epitope.
No = matching between predicted protein sequence and antibody epitope is less than 8 consecutive amino acids.
parkin expression profile should not be restricted to the use of a single antibody. The latter approach, in fact, could not reveal the entire spectrum of parkin variants.

5. Conclusion

Alternative splicing is a complex molecular mechanism that increases the functional diversity without the need for gene duplication. Alternative splicing performs a crucial regulatory role by altering the localization, function, and expression level of gene products, often in response to the activities of key signaling pathways [62]. PARK2 gene, as the vast majority of multiexon genes in humans, undergoes alternative splicing [14, 63, 64]. The importance of alternative splicing in the regulation of diverse biological processes is highlighted by the growing list of human diseases associated with known or suspected splicing defects, including PD [65].

Mutations that affect PARK2 splicing could modify the levels of correctly spliced transcripts, alter their localization, and lead to a loss of function of some of them and/or gain of function of others in time- and cell-specific manner. Even if few, some evidences supporting this hypothesis have been already described. Preliminary studies reported PARK2 isoforms with defective degradation activity of cyclin E and control of cellular cycle [15] or characterized by altered solubility and intracellular localization [66]. No evidence of gain of function has been reported, but it is plausible, because a functional screen of the PARK2 splice variants has not been done yet. The huge number of molecular targets attributed to full-size parkin protein could be shared by the others parkin isoforms which could have additional biological activities that until now are uncisidered. In light of this consideration, alteration of the natural splicing of PARK2 and deregulation in the expression of parkin isoforms might lead to the selective degeneration of dopaminergic neurons in SN of ARJP. However this is a hypothesis, since the functional screen of the PARK2 splice variants is not available and this field is still unexplored.

All these could, at least in part, justifying the conflicting and heterogeneous data of studies revised in this work, which preceded the knowledge of PARK2 alternative splicing and expression of multiple isoforms for this gene. Understanding PARK2 alternative splicing could open up new scenarios for the resolution of some Parkinsonian syndrome.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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