

# Increasing the Coding Potential of Genomes Through Alternative Splicing: The Case of *PARK2* Gene

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**Abstract:** The completion of the Human Genome Project aroused renewed interest in alternative splicing, an efficient and widespread mechanism that generates multiple protein isoforms from individual genes. Although our knowledge about alternative splicing is growing exponentially, its real impact on cellular life is still to be clarified. Connecting all splicing features (genes, splice transcripts, isoforms, and relative functions) may be useful to resolve this tangle. Herein, we will start from the case of a single gene, Parkinson protein 2, E3 ubiquitin protein ligase (*PARK2*), one of the largest in our genome. This gene is implicated in the pathogenesis of autosomal recessive juvenile Parkinsonism and it has been recently linked to cancer, leprosy, autism, type 2 diabetes mellitus and Alzheimer's disease. *PARK2* primary transcript undergoes an extensive alternative splicing, which enhances transcriptomic diversification and protein diversity in tissues and cells. This review will provide an update of all human *PARK2* alternative splice transcripts and isoforms presently known, and correlate them to those in rat and mouse, two common animal models for studying human disease genes. Alternative splicing relies upon a complex process that could be easily altered by both *cis* and *trans-acting* mutations. Although the contribution of *PARK2* splicing in human disease remains to be fully explored, some evidences show disruption of this versatile form of genetic regulation may have pathological consequences.

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## INTRODUCTION

With the completion of the Human Genome Project, it came as a surprise to discover that human genome contains only a fraction of genes than originally predicted. It was clear that the small number of human genes could not account for the complexity of the proteome. Therefore, the biological paradigm "one gene to one protein" was groundless. Among several proposed mechanisms, alternative splicing is considered the major driving force for transcriptome and proteome diversity. Because of its ability in increasing the coding potential of a genome, alternative splicing represents a cheap and powerful tool that allows cells to expand their proteome, producing multiple protein products from a single gene. Although our knowledge about alternative splicing is growing exponentially, its real impact on cellular life is still under debate. Connecting each gene to its splice transcripts, corresponding isoforms and relative functions may be useful to resolve this tangle and decipher how splicing acts in physiological and pathological conditions. Indeed, this is not an easy task. In this review, we will represent the

splicing features of a single gene, Parkinson protein 2, E3 ubiquitin protein ligase gene (*PARK2*), one of the largest genes in human genome [1].

Mutations in *PARK2* gene are responsible for the development of a form of autosomal recessive juvenile Parkinsonism (AR-JP) characterized by all the classical symptoms of Parkinson disease (PD), such as tremor, rigidity and bradykinesia [2]. In addition to AR-JP, *PARK2* has been recently linked to cancer [3, 4], leprosy [5], autism [6], type 2 diabetes mellitus [7] and Alzheimer's disease [8].

*PARK2* gene spans more than 1.38 Mb of genomic DNA in the long arm of chromosome 6 (6q25.2-q27) [1, 9]. To date, homologous *PARK2* genes have been characterized in twelve different organisms, including rat [10], mouse [11, 12], fruit fly [13], zebrafish [14] and worm [15].

The first isolated human *PARK2* transcript was of 2,960 bases with an open reading frame (1,395 bases) encoding a protein of 465 amino acids [1]. Based on this transcript, the genomic organization and exon/intron boundary sequences of *PARK2* consisted of 12 exons [1]. In the last fifteen years, these 12 exons have been the focus of hundreds of different screenings. The Parkinson Disease Mutation Database (<http://www.molgen.vib-ua.be/PDmutDB>) [16] currently lists 214 *PARK2* mutations: exon rearrangements (deletions, duplications) or, more often, point mutations.

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While many studies concentrated on the genetic variations present in the 12 originally established *PARK2* exons and in their exon/intron boundaries, there is now enough evidence that additional exonic sequences exist in human and other species, and that they can be alternatively spliced to produce different variants [11, 17-22]. These transcripts show different patterns of expression and encode proteins with different functions [11, 17-22].

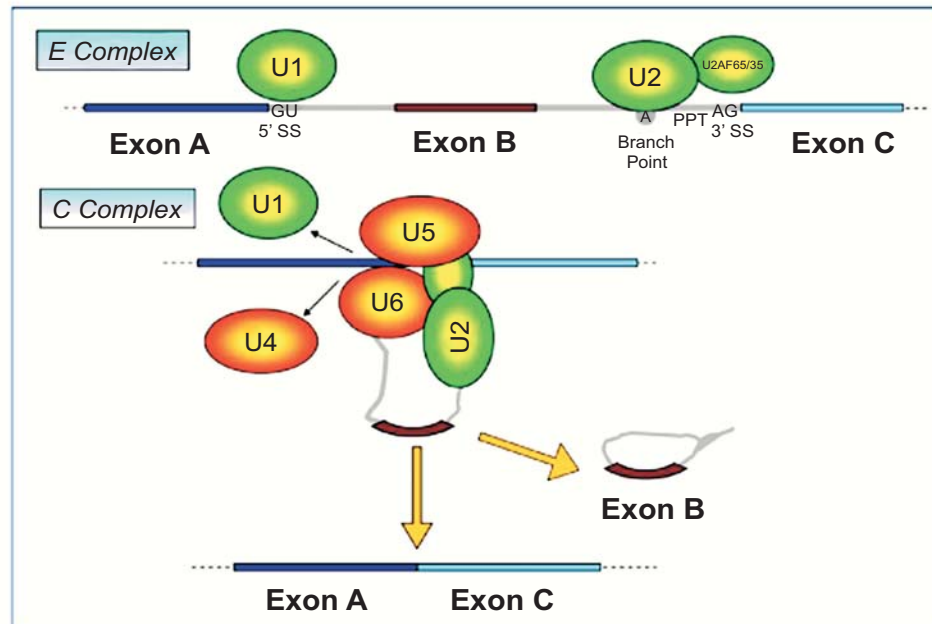
This review will provide an update of all human *PARK2* alternative splice variants presently known and correlate them to those in rat and mouse, two common animal models for studying human disease genes. Before describing *PARK2* splice variants, the next paragraph will briefly introduce the process of alternative splicing.

### ALTERNATIVE SPLICING, BASIC CONCEPTS

A crucial regulatory stage in the pathway of gene expression is splicing of precursor mRNA (pre-mRNA). In this process, introns are removed and exons are joined to form a mature mRNA, which is then polyadenylated, exported to cytoplasm, and translated into protein. Four conserved regions are important for the splicing process: the 5' (GU) and the 3' (AG) splice sites, the branch point placed upstream of the 3' splice site and the polypyrimidine tract (PPT) placed between the 3' splice site and the branch point (Fig. 1). Splicing regions are recognized by a large macromolecular complex, the spliceosome, which models the pre-mRNA while it is synthesized by RNA polymerase II in the nucleus (Fig. 1). The splicing machinery is composed of five small nuclear RNAs (U1, U2, U4, U5 and U6) that tie with proteins to form small nuclear ribonucleoproteins (snRNPs) [23]. During the first step of spli-

ceosome assembly (E complex), U1 base-pairs with the 5'-splice site, whereas U2 base-pairs with the branch-point. Then, the tri-snRNP complex U4, U5 and U6 associates with the forming spliceosome (now called B complex) and U4 is ejected. This allows U6 to replace U1 at the 5' splice site (C complex) and leads to a U6-U2 interaction that gets close together to the 5'-splice site and the branch point, allowing for a transesterification step. At the end, U5 brings near the two exons and allows for the second step of splicing, joining the two exons [24].

Splicing of exons does not always proceed in the same manner and different combinations of exons can be joined by a process known as alternative splicing. Alternative splicing of coding exons may generate protein isoforms with different biological properties, protein-protein interactions, subcellular localization, signaling pathway or catalytic ability [25]. Alternative splicing in non-coding sequences, instead, can affect the efficiency of mRNA translation, stability or localization [26]. By modifying the reading frame or adding premature stop codons, some splicing events lead to truncated proteins or "Nonsense-Mediated mRNA Degradation" (NMD mechanism) [27]. In this manner, splicing works as an on-off switch in gene expression. Alternative splicing is regulated in time and space, allowing a particular mRNA to be expressed in a specific cell and physiological condition [28]. This process, therefore, represents an extremely economical mean of increasing protein diversity, which can finely tune genomic information to meet the unique needs of each cell. The process of alternative splicing increases the number of the mRNA expressed and explains the divergence between the estimated 24,000 human protein-coding genes and the 100,000 different proteins that are supposed to be generated [29].



**Fig. (1). The alternative splicing mechanism.** The spliceosome machinery (U1, U2, U4, U5 and U6) assembles on the nascent pre-mRNA. The conserved sequences that enable recognition of the mRNA by the spliceosome are: the 5' splice site (GU), the 3' splice site (AG), the branch point and the polypyrimidine tract (PPT). In E complex, U1 forms a base-pairing interaction with the 5'-splice site, whereas U2 base-pairs with the branch-point. Then, a tri-snRNP complex containing U4, U5 and U6 associates with the forming spliceosome, removing U1 and U4 (C complex). These steps allow the two transesterification reactions and join the exons.

### ALTERNATIVE SPLICING OF PARK2

In the following paragraphs we will first describe all *PARK2* alternative splice variants presently known in human, followed by those in rat and mouse, two common animal models for studying human disease genes.

#### Human PARK2 Alternative Splice Variants

*PARK2* is one of the largest genes in the human genome and spans more than 1.38 Mb of genomic DNA in the long arm of chromosome 6 (6q25.2-q27).

To date, GenBank (Unigene cluster Hs.132954) currently lists 26 human *PARK2* transcripts corresponding to 21 different alternative splice variants. Each of these alternative splice variants is shown in (Table 1). The alignment of genomic and transcript sequences (Fig. 2) indicates these alternative splice variants are composed of 17 exons, whose exact length and specific coordinates on *PARK2* gene are indicated in (Table 2). With the exception of few exons (9, 11, and 14), each exclusively expressed in a single splice variant, the others are not associated to specific transcripts (Fig. 2). The joining of different exons does not seem to follow any specific order. Indeed, no exonic cluster (i.e. exonic sequences lying close in gene and spliced always, in or out, together) is evident.

The cDNA clone (2,960 bp) submitted by Kitada *et al.* (Accession number AB009973.1) [1] represents the longest transcript sequence present in GenBank, although the same repository contains three records of *PARK2* Reference Sequences with a length between 3 and 4 Kbp. These RefSeq sequences have been generated by NCBI staff by assembling transcript and genomic sequences, and their existence remains uncertain (for further details see Table 1). The shortest human *PARK2* transcript variant is 454 bp. This means that *PARK2* undergoes a complicated pattern of splicing assembling that greatly reduces primary transcript length up to 3000 times.

#### Rat Park2 Alternative Splice Variants

Rat *Park2* gene is located in the long arm of chromosome 1 and spans more than 1.18 Mb. The cloned rat *Park2* transcripts range between 1670 and 534 bp, and thus the splicing assembling strongly reduces the length of the primary transcript more than 2200 times.

GenBank (Unigene cluster Rn.207194) currently lists 27 rat *Park2* transcripts (Table 3) that correspond to 20 unique alternative splice variants (Fig. 3). These alternative splice variants are composed of 20 exons, which are reported in (Table 2). As in human, some exons are uniquely present in a single splice variant (exons 3, 4, 5, 8, 9, 12, 18, and 19). Based on the exonic composition of transcript variants, no exonic cluster or preferential rearrangement is evident.

#### Mouse Park2 Alternative Splice Variants

*Park2* splicing has been less investigated in mouse. To date, only 12 transcripts have been cloned and are collected in Unigene cluster Mm.311110 (Table 4). These transcripts arise from splicing of 15 different exons (Table 2) and correspond to 9 unique alternative splice variants (Fig. 4). Among these, a splice variant (M3) is generated by the use of an alternative 5' donor site inside exon 7 (Fig. 4). Exons 10 and 12 are exclusively expressed in a single splice variant.

Table 1. *Homo sapiens* PARK2 splice variants.

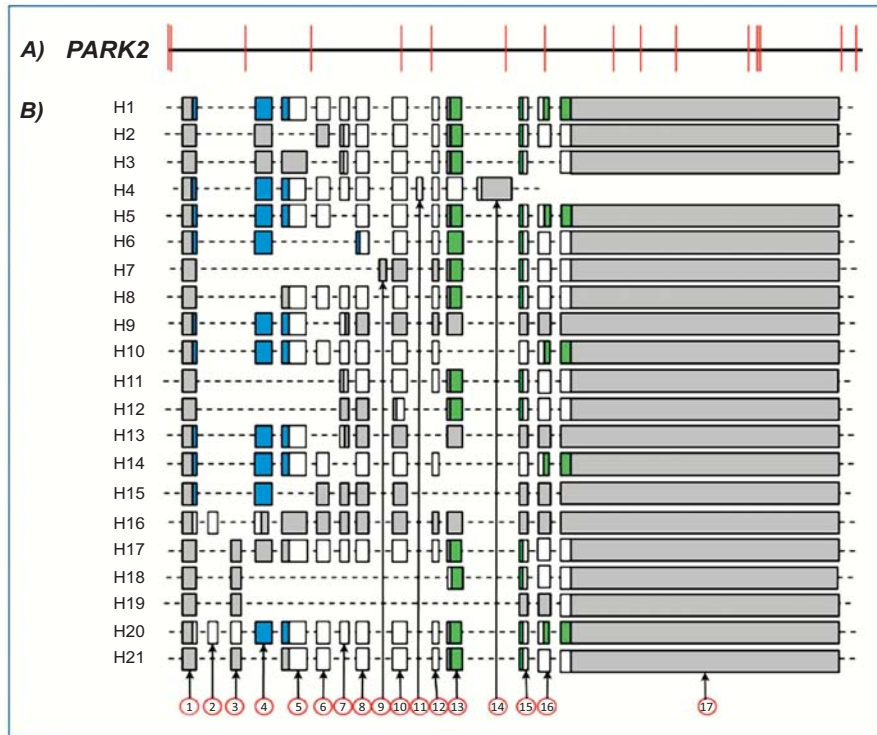
GI PARK2 mRNAs	Code Identifier	Corresponding Homologs
3063387; 121308969; 125630744; 158258616; 169790968*	H1	R1 - M1
20385797	H2	R2 - M7
20385801	H3	R6
34191069	H4	
169790970*; 284468410	H5	R8
169790972*	H6	
194378189	H7	
284468407	H8	R14 - M5
284468408	H9	
284468412	H10	
284516981	H11	
284516982	H12	
284516983	H13	
284516985	H14	
284516987	H15	
284516989	H16	
284516991	H17	
284516993	H18	
469609974	H19	
469609976	H20	
520845529	H21	

Gene identifiers corresponding to human (H) *PARK2* splice variants currently known are reported. For convenience, a new code identifier based on submission date has been assigned to each variant. In some cases, different submissions (with different Gene Identifiers) exist for the same splice variant. Three records (marked with an asterisk) are Reviewed Reference Sequences (RefSeq) that have been curated by NCBI staff, by assembling transcript and genomic sequences derived from DB023187.1, AK292590.1 and AL32982.12. These RefSeq records include a subset of the publications that are available for *PARK2* gene. However, we have no certainty of these full lengths, because they are not supported by direct cloning and submitted sequences. Homologous transcripts in rat (R) and mouse (M) are reported in the table; for their Gene Identifiers the reader is referred to Tables 3 and 4.

Mouse *Park2* gene is located in chromosome 17 where it spans 1.22 Mb. Cloned transcripts range from 3226 to 793 bp and, therefore, alternative splicing process reduces primary transcript length by about 1500 times.

### SPECIES-SPECIFIC ALTERNATIVE SPLICING OF PARK2 IN HUMAN, RAT AND MOUSE

Alternative splicing is thought to be the major source of phenotypic diversity in higher eukaryotes, especially in mammals. It contributes to enhance transcriptomic diversification, and thus plays an important role in speciation and in the dynamic evolution of genome structure [30].



**Fig. (2).** Human *PARK2* gene and exon organization of alternative splice variants. **A:** Exonic and intronic organization of human *PARK2* gene. Exons are represented as red bars. The size of introns (black line) is proportional to their length. **B:** Exon organization map of the 21 human *PARK2* splice variants currently known. Exons are represented by shaded boxes (gray for non coding sequence, white for coding sequence, blue for UBQ domain and green for IBR domains) with a size proportional to their length. The first (1) and last (17) exons are represented entirely, although their sequence is partial in some variants (H1-H5, H7-H21).

**Table 2.** Names, gene coordinates and length of human, rat and mouse *PARK2* exons.

<i>Homo Sapiens</i>			<i>Rattus Norvegicus</i>			<i>Mus Musculus</i>		
Exon	Length	Coordinates on <i>PARK2</i> Gene	Exon	Length	Coordinates on <i>Park2</i> Gene	Exon	Length	Coordinates on <i>Park2</i> Gene
1	141	5.001-5.141	1	77	178.383-178.459	1	106	183.449-183.554
2	97	61.313-61.409						
3	98	161.506-161.603						
4	164	289.330-289.493	2	164	403.508-403.671	2	164	410.194-410.357
			3	72	427.602-427.673			
			4	154	438.685-438.838			
			5	156	503.452-503.607			
5	241	470.038-470.278	6	241	564.217-564.457	3	241	580.527-580.767
6	122	531.551-531.672	7	122	627.620-627.741	4	122	646.655-646.776
			8	237	659.191-659.427			
			9	90	673.136-673.225			
7	84	678.629-678.712	10	84	754.421-754.504	5	84	777.615-777.698
8	116	759.386-759.501	11	116	819.899-820.014	6	116	835.744-835.859
						7	587	899.713-900.299
			12	35	950.478-950.512			

(Table 2) contd....

<i>Homo Sapiens</i>			<i>Rattus Norvegicus</i>			<i>Mus Musculus</i>		
Exon	Length	Coordinates on <i>PARK2</i> Gene	Exon	Length	Coordinates on <i>Park2</i> Gene	Exon	Length	Coordinates on <i>Park2</i> Gene
9	70	895.786-895.855						
10	137	946.895-947.031	13	137	953.668-953.804	8	137	978.375-978.511
11	57	1016.672-1016.728						
12	62	1163.387-1163.448	14	62	1159.331-1159.392	9	62	1181.667-1181.728
						10	154	1188.275-1188.428
13	150	1183.800-1183.949	15	150	1174.875-1175.024	11	150	1197.792-1197.941
14	326	1187.366-1187.691						
						12	895	1209.731-1210.625
15	84	1345.926-1346.009	16	84	1323.441-1323.524	13	84	1347.110-1347.193
16	118	1372.598-1372.715	17	118	1356.481-1356.598	14	118	1393.691-1393.808
			18	8	1362.100-1362.107			
			19	172	1362.156-1362.327			
17	2654	1382.592-1385.245	20	209	1367.384-1367.592	15	1821	1404.606-1406.426

Names, coordinates on human (NG\_008289.1), rat (NC\_005100.3, selected region from base 49505976 to 51051947) and mouse (NC\_000083.6, selected region from base 10656936 to 12246807) genes and length (bp) of *PARK2* exons are reported. For convenience, in this work, exons have been renamed consecutively and in ascending order. Homologous exons among different species are reported on the same row.

Inter-species comparison of *PARK2* genes is useful to identify the role of alternative cassette exons during evolution. In addition, investigating the analogies and the divergences between species may be fundamental in creating a valid animal model for Parkinson’s disease. To this regard, knocking out *Park2* function in mice has been achieved by deletion of exon 2, 3, and 8 in *Park2* gene, but no loss of nigrostriatal dopaminergic neurons has been reported [31-34].

The genomic structures of human *PARK2* gene and its homologs in rat and mouse are shown in (Fig. 5). Most of the exons have been conserved during evolution (e.g rat exon 2, human exon 4 and mouse exon 2; rat exon 14, human exon 12 and mouse exon 9), and their sequences have a high degree of homology (up to 95%). Differently, other exons are species-specific (e.g. human exons 2, 3, 9, 11 and 14).

Species-specific cassette exons may originate by two different mechanisms: i) exonization of common ancestral intronic sequences, and ii) exon shuffling, in which a new exon is inserted into an existing gene [30]. Rat and mouse specific *Park2* exons may have originated by the first mechanism (exonization). Gene-comparison, in fact, reveals that some rat-specific *Park2* exons (e.g., rat exon 3, 5, 8, 9 and 19) have a high degree of identity level with intronic sequences of mouse and *vice versa* (e.g. mouse exon 7, 10 and 12). During evolution, therefore, these sequences may have gained splice sites (AG/GT) and been expressed in one species but not in the other one. Conversely, human-specific *PARK2* exons might have originated by exon shuffling, since their sequences are unique in human *PARK2* gene and do not match to any of the corresponding *Park2* intronic regions of rat and mouse (Fig. 5).

Analyzing the exonic structures of *PARK2* alternative splice variants of the three species (Figs. 2, 3 and 4), we can separate exons with a low (e.g. human exons 2, 3, 9, 11 and 14) or high inclusion level (e.g. human exons 1, 4-8, 10, 12, 13, 15-17) in splice variants. Exons with a high inclusion level in splice variants coincide with conserved exons (with the exception of the first and the last exons, whose evolutionary conservation is relatively modest), while those having a low inclusion level coincide with species-specific exons (Fig. 5).

**ALTERNATIVE SPLICING OF *PARK2* PRODUCES DIVERSITY**

The combinatorial arrangement of *PARK2* exons generates a large number of alternatively spliced mRNAs that may diverge for untranslated regions, half-life, regulation or degradation modality (e.g. the NMD mechanism seen above). Moreover, their different coding regions may lead the expression of distinct *PARK2* protein isoforms, which are different in amino acid sequence, post-translational modifications and functional domain composition (Fig. 6).

The original (canonical) *PARK2* protein (Accession number BAA25751.1) [1] comprises an N-terminal ubiquitin-like (UBQ) domain and two C-terminal in-between ring fingers (IBR) domains, encoded by specific *PARK2* exons (Figs. 2, 3 and 4). The UBQ domain targets specific protein substrates for degradation by the proteasome, whereas IBR domains occur between pairs of ring fingers and play a role in protein quality control. *PARK2* isoforms, encoded by the alternative splice transcripts currently known, structurally diverge from the canonic one for the presence or absence of

the UBQ domain and for one or both IBR domains (Fig. 6). Moreover, when UBQ domain is present, it often differs in length from the canonic one. Interestingly, some isoforms miss some of these domains (Fig. 6).

**Table 3.** *Rattus norvegicus Park2* splice variants.

GI <i>Park2</i> mRNAs	Code Identifier	Corresponding Homologs
7001383; 7229096; 7717034; 11464986*; 11527823	R1	H1 - M1
18478865	R2	H2 - M7
18478869	R3	
20385787	R4	
20385789	R5	
20385791	R6	H3
20385793; 284810436	R7	
20385795; 284066979	R8	H5
20385803	R9	
284066981	R10	
284468403	R11	
284468405	R12	
284810438	R13	
520845525; 520845527	R14	H8 - M5
520845531	R15	
520845533	R16	
520845535	R17	
520845537	R18	
520845539	R19	
520845541	R20	

Gene identifiers corresponding to rat (R) *Park2* splice variants currently known are reported. For convenience, a new code identifier based on submission date has been assigned to each variant. In some cases, different submissions (with different Gene Identifiers) exist for the same splice variant. One record (marked with an asterisk) is a Provisional Reference Sequence (RefSeq) identical to 7229096, which has not yet been subjected to NCBI final review. Homologous transcripts in human (H) and mouse (M) are reported in the table; for their Gene Identifiers the reader is referred to Tables 1 and 4.

Alternative splicing also affects intrinsically disordered protein regions (e.g. regions lacking of stable tertiary structure), thus playing a critical role in remodeling protein-protein interactions [35]. Alternative splicing events on intrinsically disordered protein regions could regulate interactions of *PARK2* isoforms with specific cellular targets. In addition, *PARK2* isoforms generated by different alternative splice transcripts could interact with each other mutually regulating their functions, as it has been reported for RBCK1, a protein with IBR and E3 ubiquitin ligase domains, whose migration in the nucleus is inhibited by interaction with RBCK2, an isoform lacking IBR domain [36].

In addition to molecular architectures, alternative splicing may also influence stability, localization and catalytic efficiency of *PARK2* isoforms. Although scientific evidences concerning this are still few, preliminary studies reported the identification of a *PARK2* isoform, missing exons 5-8, with a defective degradation activity of Cyclin E and control of cellular cycle [19]. Another study detected a splice variant of pdr-1 (a *Caenorhabditis elegans* homologous of *PARK2*) with an in frame deletion, characterized by altered solubility and intracellular localization [15].

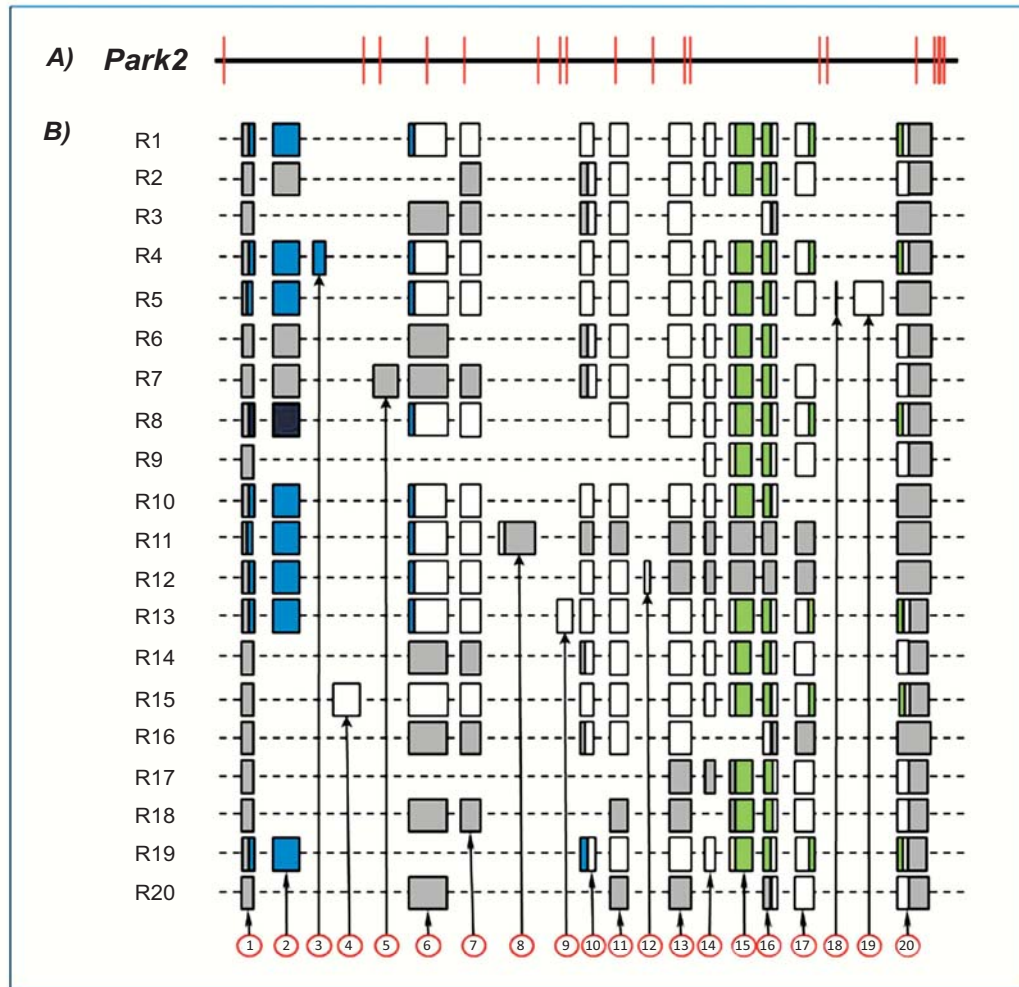
Besides the well-known involvement in proteasome-dependent degradation of target proteins [37, 38], *PARK2* has been implicated in apoptosis regulation [39], mitochondrial homeostasis, mitophagy [40, 41] and mitochondrial DNA stability [42]. In addition, UBQ proteins such as *PARK2*, are implicated in endocytosis, cellular trafficking, signal transduction, transcriptional regulation and DNA repair, in ubiquitin degradation independent manner [43]. It may not be excluded that *PARK2* alternative splice isoforms (included those missing functional domains) act in a different cellular context, operating in a still, not yet characterized manner.

#### DIFFERENTIAL EXPRESSION OF *PARK2* IN TISSUES AND CELLS

Alternative splicing events are finely regulated in time and space, and thus contribute to cell specialization and tissue definition. Although we are not yet able to define the tissue and cell specific spectrum of expression of *PARK2* and its homologs, the few evidences reported to date undoubtedly demonstrate that a regional and cellular differential expression of transcripts and isoforms exists. Distinct transcript-expression profiles occur in human brain regions [44] and leukocytes [17], in rat brain and isolated nerve cells [18] and in a wide variety of mouse tissues including brain, heart, lung, liver, skeletal muscle, kidney, and testis [11]. (Fig. 7), for example, shows the different expression patterns of *Park2* splice variants in rat cerebellar granule cells, cortical neurons and type I astrocytes [18].

Differential expression of *PARK2* transcripts is also mirrored at the protein level. In mice, *Park2* protein isoforms show a differential distribution in different nervous system areas (cerebral cortex/diencephalons, hippocampus, cerebellum, brainstem, striatum, spinal cord), peripheral tissues (heart, liver, spleen, pancreas, kidney), and developmental stages [45, 46]. Distinct expression patterns occur also in rat brain regions (*substantia nigra* and cerebellum) [46-48] and isolated nervous cells [49]. To this regard, a representative example is shown in (Fig. 8), where *Park2* isoforms are clearly differentially expressed in rat cortical neurons and type I astrocytes. Both western blot and immunofluorescence assays demonstrate not only a quantitative difference in *Park2* expression levels between these cell types, but also a different expression pattern of splice isoforms. The expression of *PARK2* has been recently assessed in human blood [50]. This study revealed the presence of different splice variants and protein isoforms.

When the extensive *PARK2* alternative splicing was still unknown, correlation between mRNA and protein expression patterns was not an easy task. The presence of unexpected immunoreactive bands on western blot, for example,



**Fig. (3).** Rat *Park2* gene and exonic structure of alternative splice variants. **A:** Exons and introns organization of rat *Park2* gene. **B:** Exon organization map of the 20 rat *Park2* splice variants currently known. For details, see Fig. 2. Exon 1 and exon 20 sequences are partial in some variants (R1-R13).

was very often explained as the result of partially translated forms of *PARK2* full-length, post-translational modifications [46, 47] or cross reactivity with other proteins [51, 52]. Interestingly, the datasheet of many commercially available *PARK2* antibodies (see below for further details) shows the presence of multiple immunoreactive bands without providing sufficient explanations. Unlike the past, splicing of *PARK2* can now be investigated at the protein level in more details by the use of different antibodies. To date more than 160 *PARK2* antibodies are commercially available. They are generally raised from rabbit or mouse and commercialized by various companies. (Table 5) list 35 commercially available *PARK2* antibodies whose immunogens used are known. Some of them have been raised against the same immunogen, and thus recognize common epitopes. These 35 antibodies may allow recognizing 15 different *PARK2* epitopes. Although no epitope is probably isoform specific, the combinatorial use of antibodies targeting different protein regions, together with the use of different techniques such as two dimensional gel assays, may provide a precious aid to decode the exact spectrum of *PARK2* isoforms expressed in tissues and cells.

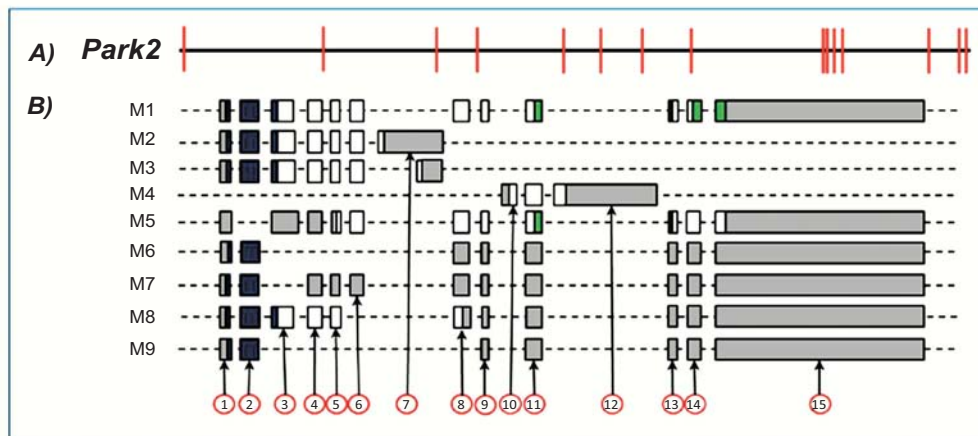
### ALTERNATIVE SPLICING OF *PARK2* AND PATHOLOGY

Alternative splicing process is a key element in *PARK2* gene expression and could be easily disrupted through multiple errors. An aberrant alternative splicing may arise from changes of regulatory sequences required for correct pre-mRNA processing, such as splice sites, branch point, polypyrimidine tract, exonic splicing enhancers/silencers and intronic enhancers/silencers, which are called *cis-acting* mutations. To this regard, a number of *PARK2 cis-acting* mutations identified in patients with Parkinson’s disease have been collected in Parkinson Disease Mutations database. To date, point mutations localized in splice acceptor or donor sites of *PARK2* introns 1, 6, 7, 10, 12, 13 and 16 have been investigated [53-59], while *cis-acting* mutations in splice sites of exons 2-5, 8, 9, 11, 14, 15, 17 and in the other splicing regulatory regions have not yet been explored and need to be assessed. Moreover, deregulation of alternative splicing may be the result of changes in the components of spliceosome machinery (*trans-acting* mutations). Further studies are required to discover their possible pathogenic influence on *PARK2* alternative

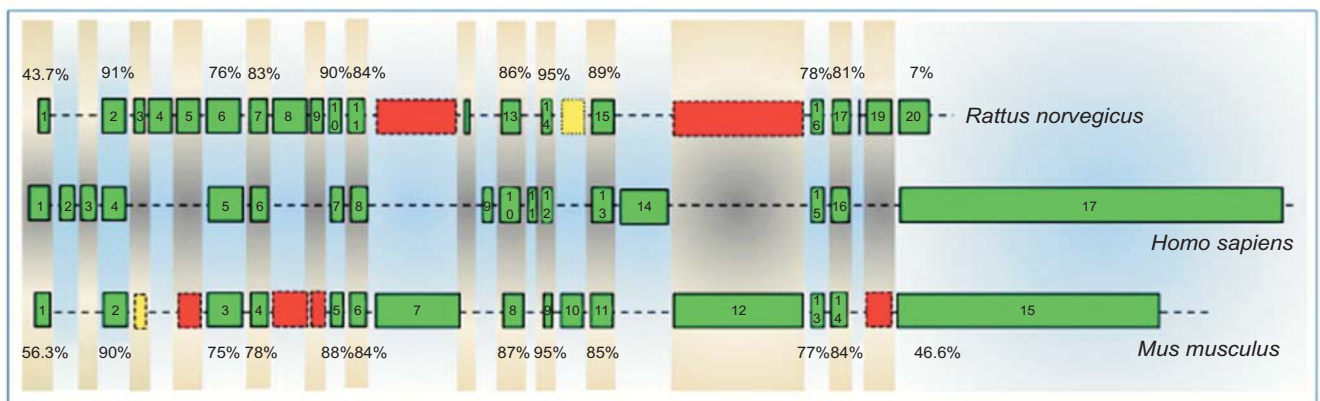
**Table 4. *Mus musculus Park2* splice variants.**

GI <i>Park2</i> mRNAs	Code Identifier	Corresponding Homologs
5456929; 86577675; 10179808; 118131140*	<b>M1</b>	<b>H1 - R1</b>
10179810	<b>M2</b>	
10179812	<b>M3</b>	
74227131	<b>M4</b>	
220961631	<b>M5</b>	<b>H8 - R14</b>
220961633	<b>M6</b>	
220961635	<b>M7</b>	<b>H2 - R2</b>
220961637	<b>M8</b>	
284829878	<b>M9</b>	

Gene identifiers corresponding to mouse (M) *Park2* splice variants currently known are reported. For convenience, a new code identifier based on submission date has been assigned to each variant. In some cases, different submissions (with different Gene Identifiers) exist for the same splice variant. One records (marked with an asterisk) is a Provisional Reference Sequence (RefSeq), derived from AC105305.8, AC091254.77, AC091484.8, AC091777.26, AC093450.20, AC122259.2, CT009575.8 and AC163687.5, that has not yet been subjected to NCBI final review. Although this record has been generated by genomic sequence alignments, it perfectly matches to 10179808. Homologous transcripts in human (H) and rat (R) are reported in the table; for their Gene Identifiers the reader is referred to Tables 1 and 3.

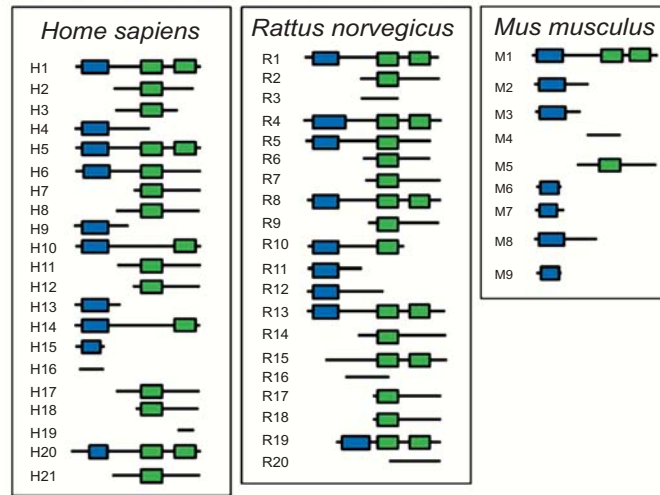


**Fig. (4). Mouse *Park2* gene and exonic structure of splice variants.** **A:** Exons and introns organization of mouse *Park2* gene. **B:** Exon organization map of the 9 mouse *Park2* splice variants currently known. For details, see Fig. 2. Exon 1 and exon 15 sequences are partial in some variants (M5-M9).

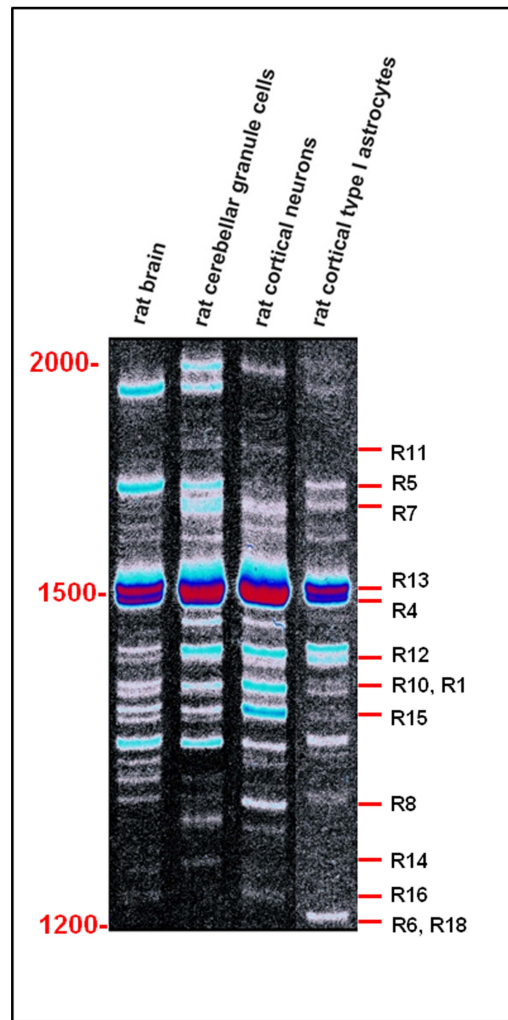


**Fig. (5). Exonic structures of human, rat and mouse *PARK2* genes.** Homologous sequences are shown in the same column. Green boxes represent the known exons of *PARK2* genes. Yellow boxes represent homologous sequences that could be potentially expressed, since they are provided with splice sites (AG/GT). Red boxes represent homologous sequences without splice sites and, therefore, not expressible. Numbers in the top of the figure indicate percentages of homology between human and rat exons, while those at the bottom denote percentages of homology between human and mouse exons.

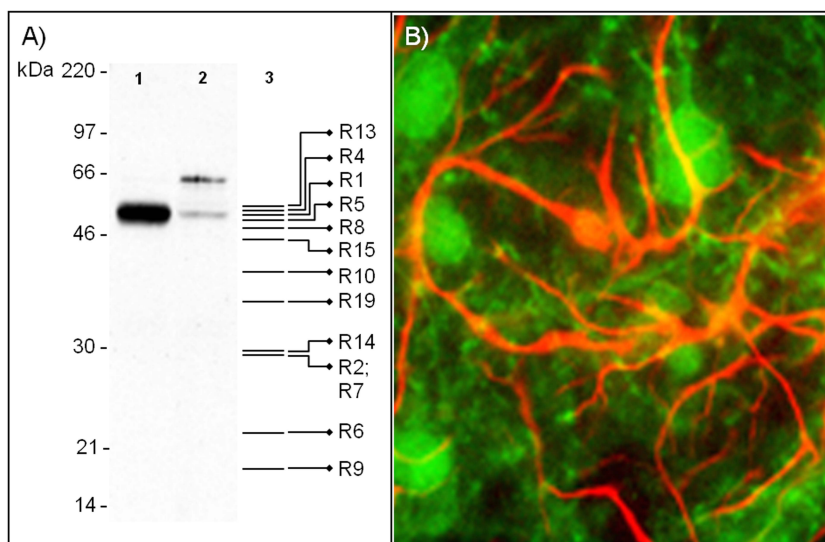




**Fig. (6). Predicted molecular architecture of *PARK2* isoforms.** *PARK2* isoforms contain one or more of the following domains: an N-terminal Ubiquitin-like domain (UBQ, in blue) and one or two In Between Ring finger domains (IBR, in green). The code identifier of each isoform, reported on their left, corresponds to that of the encoding splice variants listed in (Tables 1, 3 and 4).



**Fig. (7). Differential expression of *Park2* transcript variants in rat brain and isolated cells.** Single-stranded cDNAs from adult rat brain, rat cortical neurons, rat cerebellar granule cells and rat cortical type I astrocytes mRNAs was PCR amplified with primers flanking the start and the stop codon of *Park2*. The resulting splicing patterns clearly show a regional and cellular differential expression among different rat neuronal cells, producing spatial and functional diversification. Marker length (bp) is shown on the left. Known rat splice variants with a length between 1200 and 2000 bp are shown on the right (for codes, see Table 3). All the other products have not yet been cloned and remain unknown.



**Fig. (8). Differential expression of *Park2* isoforms in rat cortical neurons and type I astrocytes.** **A:** Western Blot analysis of *Park2* isoforms in rat neurons (lane 1) and type I astrocytes (lane 2). The assay was performed using a rabbit anti-*Park2* polyclonal antibody (AB5112, Millipore) as previously described [49]. Expected molecular weights of *Park2* isoforms potentially recognized by this antibody are drawn on lane 3. The very intense immunoreactive band of about 51 kDa in cortical neurons overlaps with the expected molecular weight of several isoforms (R1, R2, R4-R10, R13-R15, and R19). Instead, type I astrocytes express a faint band of 51 kDa which may correspond to R1 isoform, and a 65 kDa band that corresponds to a still uncharacterized variant. It should be noted that the antibody used in this western blot analysis recognizes only one epitope, which is not present in all isoforms. Other isoforms, therefore, may be expressed in these cell types and not be immunoreactive to the antibody used. **B:** *Park2* and GFAP immunoreactivity in mixed cortical cultures. Primary cultures of cortical neurons and type I astrocytes were double labeled with antibodies against *Park2* protein (green signal) and GFAP (red signal), as previously described [49], and observed by fluorescence microscopy.

**Table 5. List of commercially available antibodies against human, rat and mouse *PARK2*.**

Product Number	Provider	Immunogen (aa)	Recognized Isoforms
H00005071-B01P	Abnova	1-387	H4
H00005071-D01P	Abnova		
H00005071-D01	Abnova		
OASA06385	Aviva Systems biology	83-97	H1, H4, H5, H8, H9, H10, H13, H14, H17, H20, H21
AHP495	AbD Serotec		
MD-19-0144	Raybiotech		
DS-PB-01562	Raybiotech		
PAB14022	Abnova		
MCA3315Z	AbD Serotec	288-388	H4
H00005071-M01	Abnova		
PAB1105	Abnova	62-80	H1, H4, H5, H9, H10, H13, H14, H20
70R-PR059	Fitzgerald		
PAB0714	Abnova	305-323	H1-H6, H8, H11, H17, H20, H21, R1, R2, R4-R10, R13-R15, R19
AB5112	Millipore		
R-113-100	Novus biologicals		

(Table 5) contd....

Product Number	Provider	Immunogen (aa)	Recognized Isoforms
P5748	Sigma	298-313	H1-H6, H8, H11, H17, H20, H21, R1, R2, R4-R10, R13-R15, R19, M1, M5
GTX25667	GeneTex		
ABIN122870	Antibodies online		
PA1-751	Thermo Scientific		
R-114-100	Novus biologicals	295-311	H1-H6, H8, H10, H11, H14, H17, H20, H21, R1, R2, R4-R10, R13-R15, R19, M1, M5
AB5978	Millipore		
MAB5512	Millipore	399-465	H1, H2, H5-H8, H10-H12, H14, H17-H21
05-882	Millipore		
sc-32282	Santa Cruz		
sc-30130	Santa Cruz	61-360	H1-H3, H6, H9, H11-H13
sc-133167	Santa Cruz		
sc-136989	Santa Cruz		
EB07439	Everest Biotech	394-409	H2, H6, H7, H11, H12, H18
GTX89242	Gene Tex		
NB100-53798	Novus biologicals		
GTX113239	GeneTex	28-258	H1
10R-3061	Fitzgerald	390-406	H1, H2, H5-H8, H10-H12, H14, H17, H18, H20, H21
A01250-40	GenScript	300-350	H1-H6, H8, H11, H17, H20, H21
NB600-1540	Novus biologicals	399-412	H1, H2, H5-H7, H10-H12, H18, H20
ARP43038_P050	Aviva Systems biology	311-360	H2, H3, H6, H7, H11, H12, H18, M1, M5

Some *PARK2* antibodies have been raised against the same immunogen. Amino acids positions refer to *PARK2* isoform NP\_004553.2. Human (H), rat (R), and mouse (M) recognized isoforms are indicated in the right column.

splicing, and to elucidate the exact splice pattern/phenotype correlations. Disruption of *PARK2* alternative splicing by both *cis*- and *trans-acting* mutations, in fact, could result in a functionally harmful *PARK2* expression pattern, creating aberrant events with pathological consequences that may provide an explanation for the broad spectrum of phenotypic abnormalities observed in patients with *PARK2* mutations [60-62].

Both *cis*- and *trans-acting* mutations have been previously associated to human diseases. For example, *cis-acting* mutations have been found in genes involved in Alzheimer’s Disease (*PSEN1*, *MAPT*, *GRN*) [63], while *trans-acting* mutations have been detected in Retinitis pigmentosa (U4/U5/U6 protein complex) [64], in Spinal Muscle Atrophy [65], and in Dystrophia Myotonica [66].

Emerging evidences support the importance of *PARK2* splice variants expression changes in disease development. When compared to control patients, distinct *PARK2* splice patterns have been identified in frontal cortex of Parkinson’s disease, pure dementia with Lewy bodies, common Lewy

body disease and Alzheimer’s disease patients [20-22]. The disease-specific expression profiles of *PARK2* isoforms suggest a role for splicing deregulation in the development of neurodegenerative disorders. In addition, aberrant *PARK2* transcripts have been reported in some human cancers (ovarian and colorectal cancer, chronic myeloid leukemia and other cancer-derived cell lines) [67]. Based on these evidences, the possibility that *PARK2* aberrant alternative splicing may be involved in PD and other human diseases represents an interesting hypothesis that needs to be further investigated.

**CONCLUSION**

*PARK2* gene provides a fascinating example of the use of alternative splicing to create different variants within single cell types. Disruption of this versatile form of genetic regulation may alter the fine-tuning of the encoded proteins to suit specific cellular needs. Investigating the full spectrum of *PARK2* alternative spliced mRNAs, studying the complete pattern of expression of alternative splicing transcripts and isoforms in tissue and cell-types, and determining each of their functions will require additional studies. Furthermore,

investigating *cis*- and *trans*-acting mutations may provide novel insights into the pathogenesis of human diseases associated to *PARK2* and their cure.

### CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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