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Overexpression of blood microRNAs 103, 30b and 29a in L-dopa treated Parkinson's patients.

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Supplemental data

Table e-1. Clinical Details of Treated IPD Patients vs Controls and of Drug-Naïve IPD Patients vs Controls, electronic file name: Table e-1.pdf

Table e-2. TaqMan® MicroRNA assays and relative amplification efficiencies for the studied samples, electronic file name: Table e-2.pdf

Figure e-1. Parallel line plots representing miRNA expression data; electronic file name: Figure e-1.jpg

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AUTHOR CONTRIBUTIONS

Ms. Serafin designed and performed the experimental work and wrote the manuscript. Dr. Foco designed and performed the statistical analyses and wrote the manuscript. Dr. Zanigni designed and contributed to the sample collection and clinical characterization of the patients. Mr. Blankenburg designed and performed the

bioinformatics analyses and assisted in the preparation of the manuscript. Ms. Anne Picard contributed to the experimental work, Ms. Zanon contributed to the sample collection and experimental work. Dr. Giannini contributed to the sample collection. Dr. Pichler critically revised the manuscript and contributed to the design of the sample collection. Dr. Facheris designed and contributed to the sample collection and clinical characterization of the patients. Prof. Cortelli revised the paper. Prof. Pramstaller designed and contributed to the sample collection and clinical characterization of the patients. Dr. Hicks critically revised the manuscript and contributed to the design of the sample collection. Dr. Domingues designed, performed, and supervised the bioinformatics analyses and wrote the manuscript. Dr. Schwienbacher designed and supervised the study, contributed to the design of the sample collection, and wrote the manuscript.

DISCLOSURE

Ms. Serafin, Dr. Foco, Dr. Zanigni, Mr. Blankenburg, Ms. Picard, Ms. Zanon, Dr. Giannini, Dr. Pichler, and Dr. Facheris report no disclosures. Prof. Cortelli received honoraria for speaking engagements or consulting activities from Allergan Italia, Boehringer Ingelheim Italia, Chelsea Therapeutics, GlaxoSmithKline S.p.A , Lundbeck Italy, Merck Sharp & Dohme (Italia), Teva, UCB Pharma S.p.A, Chiesi Farmaceutici, AbbVie srl. Prof. Pramstaller received honoraria for serving on scientific boards and speaking from Novartis, Boehringer, GlaxoSmithKline, Lundbeck and UCB. Dr. Hicks, Dr. Domingues and Dr. Schwienbacher report no disclosures.

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GLOSSARY

miRNAs = microRNAs

PD = Parkinson's disease

L-dopa = Levodopa (3,4-diidrossi-l-fenilalanina)

RT-qPCR = reverse transcription and quantitative real time polymerase chain reaction

RT = reverse Transcription

PBMC = peripheral blood mononucleated cells

RQI = RNA Quality Indicator

MIQE = Minimum Information for Publication of Quantitative Real-Time PCR Experiments

Ct = threshold cycle

ABSTRACT

Objective: The aims of the present study were to profile the expression of several candidate microRNAs (miRNAs) in blood from Levodopa (L-dopa) treated and drug-naïve Parkinson's disease (PD) patients versus unaffected controls and to interpret the miRNA expression data in a biological context.

Methods: We analyzed RNAs from peripheral blood of 36 L-dopa-treated, 10 drug-naïve PD patients and unaffected controls matched 1:1 by sex and age. We evaluated expression by RT-qPCR and we analysed data using a two-tailed paired t-test. To detect miRNA targets, several miRNA resources were combined to generate an overall score for each candidate gene using weighted rank aggregation.

Results: Significant overexpression of miR-103a-3p ($p<0.0001$), miR-30b-5p ($p=0.002$), and miR-29a-3p ($p=0.005$) in treated PD patients was observed and promising candidate target genes for these revealed by an integrated *in silico* analysis.

Conclusions: We revealed three candidate biomarkers for PD. MiRNAs 30b-5p and 29a-3p replicated a documented deregulation in PD albeit opposite to published data, while for miR-103a-3p we demonstrated for the first time an overexpression in treated PD patients. Expression studies in patients and/or in isolated PBMCs before and

following L-dopa administration are necessary to define the involvement of L-dopa treatment in the observed overexpression. Our *in silico* analysis to prioritize targets of deregulated miRNAs identified candidate target genes, including genes related to neurodegeneration and PD. Despite the preliminary character of our study, the results provide a rationale for further clarifying the role of the identified miRNAs in the pathogenesis of PD and for validating their diagnostic potential.

INTRODUCTION

Parkinson's disease (PD) is a chronic neurodegenerative disorders, characterized clinically by motor and non-motor symptoms. Dopamine replacement therapy improves motor symptoms, but does not slow disease progression and is associated with adverse effects including dyskinesias (1, 2). Several genes involved in PD are known to date (3), however its physiopathology is not completely defined and currently no reliable and clinically validated biomarker for PD has been identified (3, 4).

MicroRNAs (miRNAs) are small endogenous non-coding RNAs affecting gene expression, involved in most physiological functions and several pathologies, including PD (5). MiRNAs are promising candidate diagnostic markers for the pathologies they are involved in. Biomarkers from easily accessible tissues could be used to detect PD during preclinical or pre-motor phases and to monitor its progression. Peripheral blood can be considered a source of biomarkers because it is readily obtainable and peripheral blood mononucleated cells (PBMCs) share more

than 80% of the transcriptome with other tissues, including brain (6). To date, only two studies have assessed the expression of blood miRNAs in PD (7, 8), leading to the identification of two non-overlapping and not yet replicated sets of potentially deregulated miRNAs.

In this study, we profiled the expression of several PD candidate miRNAs in blood in Levodopa (L-dopa)-treated and drug-naïve PD patients and unaffected controls. Moreover, to better understand the potential role of these miRNAs in PD, we performed an integrated *in silico* analysis of the putative target genes of the deregulated miRNAs.

MATERIALS AND METHODS

Standard protocol approvals, registrations, and patients consensus.

We enrolled 36 L-dopa-treated and 10 drug-naïve PD patients presenting at the Movement Disorders outpatient clinic of the General Regional Hospital of Bolzano (Italy). We performed PD diagnosis according to Gelb criteria (9), and excluded all subjects with cognitive impairment, unable to sign the informed consent or affected by atypical Parkinsonisms. We enrolled among spouses or unrelated caregivers 36+10 disease-free controls matched 1:1 by sex and age (range ± 3 years), to remove the putative confounding effect exerted by these factors at design level. Demographic and clinical patients' features are summarized in Table e-1. The local ethics committee approved the study, and all participants provided written informed consent.

RNA isolation and quality control

We processed peripheral blood within six hours of blood collection. We extracted total RNA, including small RNAs, from PBMCs after red blood cells lysis in whole blood (36 L-dopa-treated patients and 36 matched controls) or in buffy coats obtained from 10 drug-naïve PD patients and 10 matched controls and frozen in 10% dimethyl sulfoxide (Cat.no. D2650; Sigma-Aldrich, Milano, Italy) using TRIzol® reagent (Cat.no. 15596-018; Life Technologies, Monza, Italy) according to the manufacturer's instructions. We assessed RNA quality and quantity with the Experion™ Automated Electrophoresis System (Bio-Rad Laboratories s.r.l., Milano, Italy) using the StdSens Analysis Kit (Cat.no. 7007103; Bio-Rad Laboratories s.r.l., Milano, Italy). All samples showed an RNA Quality Indicator (RQI) $\geq$ 7.5.

Reverse Transcription and quantitative real time PCR (RT-qPCR)

We analysed the expression of miRNAs 29a-3p, 29b-3p, 30a-5p, 30b-5p, and the candidate normalizers miRNA 103a-3p, RNUs Z30, 24, 6B (Table e-2).

We performed reverse transcription (RT) on 7 ng of total RNA using the TaqMan miRNA Reverse Transcription Kit (Cat.no. 4366597; Life Technologies, Monza, Italy) and miRNA-specific stem-loop primers (Table e-2) in a 10 µl reaction, according to the manufacturer's instructions. The thermal cycling parameters were 30 min at 16°C, 30 min at 42°C, 5 min at 85°C. We performed all RT-qPCR reactions in triplicate on the 96CFX instrument (Bio-Rad Laboratories s.r.l., Milano, Italy) in 10 µl using 0.7 µl of RT product per reaction, Universal MasterMixII no UNG, and TaqMan® small RNA assays (Cat.no. 4440048 and, 4427975 Life Technologies,

Monza, Italy, Table e-2), according to the manufacturer's instructions. The thermal cycling parameters were 10 min at 95°C followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. We reported efficiencies and correlation coefficients (r^2) for assays (Table e-2).

RT-qPCR normalization and relative quantification of blood miRNAs

We used the NormFinder (10) and GeNorm (11) algorithms, and the comparative delta-Ct approach (12) to assess the variance in expression levels and to identify the most stable and reliable reference genes. We assessed relative expression levels using the CFX Manager v1.6 (Bio-Rad Laboratories s.r.l., Milano, Italy) and qBasePlus (Biogazelle) software using RNU24 and Z30 as normalizers.

Statistical analysis

We generated means and standard deviations to summarize data and we checked the normality of the distribution of the differences of the relative expression levels within each matched pair using a Skewness-Kurtosis test. Since there was no departure from normality, we assessed the differential expression between matched cases and controls using a two-tailed paired t-test.

For each miRNA, we used the mean and the standard deviation of the differences of the relative expression as the input to calculate the appropriate sample size needed for a second experiment on drug-naïve patients. We calculated the sample size in order to achieve 80% power with alpha = 0.05 using the command sampsi implemented in

Stata 12. The analyses were performed using Stata 12 (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP).

We followed Minimum Information for Publication of Quantitative Real Time PCR Experiments (MIQE) guidelines (13) for description of samples, RNA extraction, RT-qPCR and data analysis.

Prioritization of miRNA targets

We applied a computational approach for the identification and prioritization of candidate targets of miRNAs 29a-3p, 30b-5p and 103a-3p, and used multiple miRNA target resources for the identification of candidate targets. We prioritized the candidates likely to be involved in PD based on a "guilt by association" principle (14) by searching for links to PD-related genes and processes. Different scores were computed from the multiple types of evidence (Figure 1). Moreover, we compared the ranked candidates to sets of PD-related genes and to results obtained with Endeavour, an established prioritization method (15).

We collected experimentally validated miRNA targets from multiple sources, in particular, a manual literature search of reported target genes (labeled CBM in Figure 1) and the public databases TarBase (version 6.0) (16), miRTarBase (retrieved on 2013-02-06) (17), miRWalk (retrieved on 2013-02-05) (18), and miRecords (retrieved on 2013-02-05) (19). We collected predicted miRNA targets from DIANA-microT-CDS (retrieved on 2012-01-18) (20), miRmap (retrieved on 2013-02-04) (21), miRDB (version 4.0) (22), TargetScan (retrieved on 2013-02-04) (23) and miRanda (release 2010-08) (24).

Custom panels of PD-related processes and genes were compiled and applied for prioritization. GOPD is a panel of PD-related processes, AVPD is a panel of genes related to PD, and CPD is a list of candidate PD genes. AVPD and CPD are non-overlapping sets. We compared the candidate miRNA targets to additional sets of PD-related genes, in particular sets of Parkin interactors from interaction screens (ParkinTAP, ParkinInt), or Parkin dependent ubiquitylation targets (ParkinUBQ). Given the known role of mitochondria function in PD (25), we used also a set of mitochondrion related genes (Mito) to annotate the results. These sets are described at https://gemex.eurac.edu/downloads/2013miRNA/.

We collected different scores from the multiple types of evidence (Figure 1). Several scores summarize the validated annotations (V_PMID) and predictions (PP and PF scores) from miRNA target resources. The GOSlim score allows the identification of candidate genes annotated to PD-related processes. BPscore quantifies the functional relationship to PD-related genes, PPI and Cpx summarize the number of protein interactions and complexes with PD-related proteins. We used the InSet score to identify candidates in PD panel, and used the different scores to compute a combined score (CRscore) applied to rank the candidates (https://gemex.eurac.edu/downloads/2013miRNA/).

RESULTS

miRNAs expression in PD patients and their matched controls

The results of the stability assessment of the expression of Z30, RNU24, RNU6B and miR-103a-3p genes among the analyzed samples indicated Z30 and RNU24 as the

best reference genes. MiR-103a-3p, previously used as normalizer for RT-qPCR quantification of blood miRNAs of PD patients (8), consistently demonstrated the lowest stability in our experiments with no obvious experimental causes. Due to marked differences in its expression levels between PD cases and matched controls, we included it amongst the other targets rather than as a reference gene. Expression analysis demonstrated overexpression of miR-29a-3p, miR-30b-5p and miR-103a-3p in L-dopa treated PD patients compared to their matched controls (Table 1, Figure e-1), while no significant difference was detected for miRNAs 29b-3p and 30a-5p.

MiRNA expression in drug-naïve PD patients and their matched controls

Since all analyzed PD patients were undergoing L-dopa therapy, the observed difference in miRNA expression could be the consequence of treatment, rather than disease pathogenesis. Therefore we performed a second experiment on an independent set of drug-naïve PD patients and their matched controls. Sample size needed to achieve a power of 80%, with alpha = 0.05, was calculated for the three deregulated miRNAs, based on the effect sizes and the standard deviations observed in the first experiment. Calculations indicated that 31 matched case-control pairs for miR-29a-3p, 294 pairs for miR-29b-3p, 299 pairs for miR-30a-5p, 26 pairs for miR-30b-5p and six pairs for miR-103a-3p were needed to achieve the desired power. Based on this and the currently available number of drug-naïve patients, we initially performed an exploratory expression analysis for miR-103a-3p on ten pairs of drug-naïve PD patients and their matched controls. No significant difference could be detected (mean difference 0.07, 95% CI -0.51; 0.65 p-value 0.785). Notably, this

dataset does not provide power to test the expression of the other miRNAs; nevertheless, it could be argued that data used to calculate the sample size come from a different population, the group of treated patients. The magnitude of the effects in the treated patients versus controls might not be totally comparable with the magnitude of the effects in the drug-naïve patients. Therefore, we also evaluated the expression of the remaining miRNAs. No significant differential expression was detected (miR-29a-3p: p=0.611, miR-29b-3p: p=0.1823, miR-30a-5p: p=0.6994, miR-30b-5p: p=0.3156). Due to the very likely lack of power, it cannot be excluded that a small, but statistically and clinically significant difference in expression, would be found in a larger sample size.

Prioritization of miRNA targets

Candidate targets were prioritized based on annotation and predictions from different miRNA target databases combined with different types of evidence relating candidates to PD genes and processes. For each miRNA, the results of the 20 top ranking candidates are summarized in Tables 2, 3, 4. Top ranking Endeavour prioritization candidates are also marked in the Tables, providing additional evidence for relation with PD molecular mechanisms. All prioritization and Endeavour results are available for download (https://gemex.eurac.edu/downloads/2013miRNA).

Top candidates in Tables 2, 3 and 4 tend to be annotated or predicted as targets according to multiple miRNA resources as expected. For example, *RUNX2* and *DICER* are annotated as targets of miRNAs 30b-5p and 103a-3p, respectively, according to several resources (V_PMID, PF, PP). The top candidates also tend to be

related to PD genes and processes according to different types of evidence. The miR-29a-3p candidate target *AKT1* illustrates this type of result, where multiple sources of evidence (GOSlim, BPscore, PPI, Cpx) indicate a link to PD genes and processes. A few candidates (*AKT1, MEX3B, BCL2*) rank among the top target candidates for more than one miRNA. Several top CRscore candidates also rank among the top Endeavour results, which is expected given that Endeavour relies on a similar strategy in relating candidates to AVPD genes. Nevertheless many top CRscore candidates are not among the top Endeavour results, which is not surprising given that CRscore prioritization takes into account the combined ranking from multiple miRNA target resources while Endeavour, being a generic prioritization method, does not consider the ranking from the miRNA target resources.

DISCUSSION

We found a significant overexpression of miRNAs 29a-3p, 30b-5p and 103a-3p in L-dopa treated PD patients. We then performed an exploratory expression analysis on ten pairs of drug-naïve PD patients and their matched controls to assess whether L-dopa therapy could affect miRNAs expression. No significant difference was detected, however, the results suggest a role of L-dopa treatment in miR-103a-3p expression only, due to the lack of power to detect significant changes for the remaining miRNAs. Additional expression analyses in patients and/or in isolated PBMCs before and following L-dopa administration are requested to define the involvement of the treatment in the observed deregulation. Other commercially available non-PD medications could also modify miR-103a-3p expression levels.

However there is no experimental evidence for this; moreover we can assume that their intake is randomly distributed among our matched pairs and consequently it should have not influenced our results. Different disease duration and stage could also have an impact, but their effect should be assessed in epidemiological studies performed on an independent sample of PD patients only.

Here we demonstrate for the first time a deregulation of miR-103a-3p blood levels in L-dopa treated PD patients. An altered expression of miRNAs 30b-5p (8) and 29a-3p (7) in peripheral blood of PD patients has been described, but not yet independently replicated. In contrast to these studies we found an increased expression for these miRNAs in PD. The two studies analysed partially overlapping miRNA sets, but no miRNA was found to be significantly deregulated in both studies. The discordant results could be explained by the different study design, the use of PD sample sets with different size and clinical features, by differences in the pharmacological treatment and distinct experimental conditions, including expression analysis approaches, and selection of reference genes (11).

For our RT-qPCR analyses we used a matched case-control experimental design on 36 PD cases versus their age- and sex- matched controls, whereas the previous studies followed an unmatched study design on relatively small sample sets (7, 8). Margis and collaborators observed a significant downregulation of miR-29a-3p in drug-naïve PD patients versus normal controls by RT-qPCR and only a trend to lower expression in treated patients (7). Here we studied L-dopa treated patients. Moreover, since no data are available regarding the reference genes used in their RT-qPCR study, the selection of the normalizer could also explain the different results.

Martins and collaborators found a downregulation of miR-30b-5p amongst 17 miRNAs in PD by microarray assays (8). To validate these results, they performed RT-qPCR on a subset of five miRNAs, but not including miR-30b-5p. MiR-103a-3p, used as normalizer, was instead analysed by us as a target gene due to the observed instability. It is interesting to observe that miR-103a-3p targets *CDK5R1*, a gene reported to be involved in neurodegenerative disorders, including PD (26). Moreover, miRNAs 103a and 29 have documented relationships to insulin resistance, a process possibly linked also to neurodegeneration in PD (27-29).

Recent data support an involvement of the deregulated miRNAs in PD pathogenesis (25-33), but their precise role remains to be defined. To better understand the role of the observed deregulated miRNAs in PD and the underlying biology in relation to the newly determined direction of expression we observed, we performed an integrated *in silico* analysis identifying candidate target genes for miRNAs 103a-3p, 30b-5p, and 29a-3p (Tables 2, 3, 4). One of the identified candidate targets of miR-29a-3p is *PARK7 (DJ-1)*, which protects cells against oxidative stress and cell death (34). Defects in this gene cause autosomal recessive early-onset PD, and are suggested to participate also in the onset and pathogenesis of sporadic PD (35). Moreover, the phosphorylation level of AKT is reduced in *PARK7* knockout mice, leading to neuronal cell death (35). The observed overexpression of miR-29a-3p in blood samples of PD patients might cause a downregulation of *PARK7* and therefore its reduced activity. PTEN, which is inhibited by PARK7 (35), and other members of the AKT/PTEN pathway, which is involved in the pathogenesis of PD and related to neuropsychiatric symptoms, like *IGF1*, *AKT1*, and *BCL2* were identified among the

first 20 ranked candidate targets for miRNAs 103a-3p, 29a-3p and 30b-5p (36). MiR-29a-3p is predicted to target also *GPR37*, coding for a Parkin substrate (37). Intracellular accumulation of GPR37 is neurotoxic and related to PD, whereas plasma membrane association is neuroprotective. The inactivation of GPR37 produces motor and non-motor phenotypes relevant for PD (37). Thus a hypothetical downregulation of *GPR37* due to the overexpression of miR-29a-3p could be relevant in PD. *CDC42*, a candidate gene for PD involved in neuronal death (38), was identified as potential target for miRNAs 29a-3p and 103a-3p. Interestingly, ROCK/CDC42-mediated microglial motility and gliapse formation leads to phagocytosis of degenerating dopaminergic neurons *in vivo*. The significance of microglial activation in PD is still controversial, and it is not clear whether this process exerts a protective or deleterious role (39). Further studies will be needed to clarify the role of microglial phagocytosis in the pathogenesis of PD and its potential deregulation by the repression of *CDC42* due to an overexpression of miRNAs 29a-3p and 103a-3p. *BCL2*, a target with a high-ranking score for all three deregulated miRNAs, was identified as a crucial player in dopaminergic neuron development and PD. Indeed, anti-apoptotic members of the BCL2 protein family, including BCL2, which is coded by the *BCL2* gene, are down-regulated in PD, whereas proapoptotic proteins of the same family are up-regulated resulting in extensive dopaminergic cell death (40). The observed overexpression of miRNAs 103a-3p, 30b-5p and 29a-3p in PD could therefore contribute to death of nigral dopaminergic neurons via *BCL2* suppression.

To conclude, in this study we performed for the first time a RT-qPCR expression analysis of selected miRNAs in PBMCs of a relatively large cohort of PD patients and

matched controls to replicate and validate previous findings. We identified miRNAs 103a-3p, 30b-5p, and 29a-3p as significantly overexpressed in PD. Further studies on larger sample sizes should be performed to assess the association of expression levels for miRNAs 29b-3p and 30a-5p with L-dopa treated PD patients, for which the sample used here is underpowered. Our results do not allow the conclusion that the identified candidate miRNAs are ready to be used as diagnostic markers, but contribute to the identification of a PD specific miRNA signature. Our target prioritization approach identified promising candidate target genes for all three miRNAs. Finally, our preliminary data provide a rationale for further clarifying the role of the identified miRNAs in the pathogenesis of PD and for validating their diagnostic potential.

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Figure legends

Figure 1 Prioritization of miRNA target candidates

Prioritization of miRNA target candidates based on annotation from multiple miRNA target resources and different types of evidence relating them to PD genes and processes. Candidate genes are represented by different geometric shapes, they are scored according to the different types of evidence (center), which are used to compute a combined rank (right).

Table 1 Results of miRNA expression analysis in L-dopa treated PD patients and their matched controls

microRNA	Mean difference within each pair (95% CI)	P value paired t-test
miR-29a-3p	0.69 (0.23; 1.15)	0.005
miR-29b-3p	0.26 (-0.28; 0.79)	0.339
miR-30a-5p	0.12 (-0.14; 0.37)	0.364
miR-30b-5p	1.76 (0.68; 2.84)	0.002
miR-103a-3p	1.80 (1.27; 2.33)	<0.0001

Results are referred to expression data elaborated using as reference genes Z30 and RNU24. CI = confidence interval

Table 2 miR-29a-3p target prioritization*

Rank	Gene ID	HGNC Symbol	Rank miR-30b-5p	Rank miR-103a-3p	V_PMID	#PF	#PP	InSet_AVPD	GOSlim	BPscore	PPI	Cpx	Mito	ParkinTAP	Endeavour100
1	1281	*COL3A1*			3	3	3								
2	3479	*IGF1*	232		1	3	3		●	●					
3	26959	*HBP1*			1	3	3								
4	2200	*FBN1*		399	2	2	3					●			
5	7837	*PXDN*	240		1	3	3		●	●					
6	207	*AKT1*	6		1				●	●	●	●			17
7	54454	*ATAD2B*	1592			3	3								
8	1287	*COL4A5*				3	3			●					
9	2006	*ELN*			1	2	3			●			●		
10	11315	*PARK7*					1	●	●		●	●	●		1
11	91404	*SESTD1*				3	2			●					
12	2861	*GPR37*				3	2			●	●	●			9
13	596	*BCL2*	32	17	2				●	●	●		●		
14	6018	*RLF*				3	3				●	●			
15	51050	*PI15*				3	3								
16	998	*CDC42*		159	2	1	2		●		●	●			
17	55048	*VPS37C*				3	3			●		●			
18	84206	*MEX3B*	7			2	3			●	●				
19	2597	*GAPDH*	28		1				●		●	●		●	8
20	5728	*PTEN*	851	190	2	2	3			●	●				14

*Rank: candidate rank according to CRscore. Rank miR-30b-5p, Rank miR-103a-3p = Rank in miR-30b-5p and miR-103a-3p target lists respectively. V_PMID = Number of distinct PubMed identifiers reporting candidate as miRNA target in validated target resources. #PF, #PP = PF scores above cutoff according to target prediction databases with full and incomplete identifiers respectively. InSet_AVPD = Candidates included in panel set AVPD are marked with ●. GOSlim = Candidate genes are marked if their annotation matches GO terms in GOPD panel or any of their children. BPscore = Candidate gene is marked if functionally related (BPScore >= 0.8) to genes in AVPD or CPD sets. PPI, Cpx = Candidate gene is marked if its encoded protein is reported to interact with proteins from AVPD or CPD sets or form a complex with AVPD or CPD proteins. Mitochondrion, ParkinTAP = Candidate gene is marked if included in Mito set with Mitochondrion related proteins and in set ParkinTAP obtained from a Parkin interaction screen, respectively. Endeavour100 = Candidate rank within Endeavour top 100. *COL3A1*= collagen, type III, alpha 1; *IGF1*= insulin-like growth factor 1; *HBP1*= HMG-box transcription factor 1; *FBN1* = fibrillin 1; *PXDN*= peroxidasin homolog (Drosophila); *AKT1* = v-akt murine thymoma viral oncogene homolog 1; *ATAD2B* = ATPase family, AAA domain containing 2B; *COL4A5* = collagen, type IV, alpha 5; *ELN*= elastin; *PARK7*= parkinson protein 7; *SESTD1* = SEC14 and spectrin domains 1; *GPR37* = G protein-coupled receptor 37; *BCL2* = B-cell CLL/lymphoma 2; *RLF* = rearranged L-myc fusion; *PI15* = peptidase inhibitor 15; *CDC42* = cell division cycle 42; *VPS37C* = vacuolar protein sorting 37 homolog C (S. cerevisiae); *MEX3B* = mex-3 RNA binding family member B; *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase; *PTEN*= phosphatase and tensin homolog

Table 3 miR-30b-5p target prioritization*

Rank	GeneID	HGNC Symbol	Rank miR-29a-3p	Rank miR-103a-3p	V_PMID	#PF	#PP	InSet_AVPD	GOSlim	BPscore	PPI	Cpx	Mito	ParkinUBQ	ParkinInt	Endeavour100
1	6622	*SNCA*			1			●	●	●	●		●			1
2	860	*RUNX2*	1560		2	3	3									
3	4734	*NEDD4*			1	2	2			●	●	●				
4	27327	*TNRC6A*		1849		3	3									
5	255520	*ELMOD2*			1	2	3			●				●		
6	207	*AKT1*	6		1				●	●	●	●				24
7	84206	*MEX3B*	18			3	3			●	●					
8	27252	*KLHL20*				3	3					●				
9	166968	*MIER3*			1	3	2									
10	8726	*EED*	1789		1	3	3									
11	4907	*NT5E*			1	3	3									
12	84002	*B3GNT5*	1380		1	3	3									
13	57551	*TAOK1*		820	1	3	2			●						
14	9140	*ATG12*			1	3	2		●							
15	51809	*GALNT7*		425	2	2	3									
16	120892	*LRRK2*		14		1	1	●	●		●		●			3
17	57763	*ANKRA2*				3	3									
18	5305	*PIP4K2A*			1	2	2			●						
19	8878	*SQSTM1*			1				●	●	●	●		●	●	12
20	92140	*MTDH*				3	2		●							

*See column description in table 2; Rank miR-29a-3p, Rank miR-103a-3p = Rank in miR-29a-3p and miR-103a-3p target lists respectively. ParkinUBQ = Candidates marked with ● are included in set ParkinUBQ consisting of Parkin dependent ubiquitylation targets. ParkinInt = Candidates marked with ● are included in set ParkinInt obtained from a Parkin interaction screen. *SNCA* = synuclein, alpha (non A4 component of amyloid precursor); *RUNX2* = runt-related transcription factor 2; *NEDD4* = neural precursor cell expressed, developmentally down-regulated 4, E3 ubiquitin protein ligase; *TNRC6A* = trinucleotide repeat containing 6A; *ELMOD2* = ELMO/CED-12 domain containing 2; *AKT1* = v-akt murine thymoma viral oncogene homolog 1; *MEX3B* = mex-3 RNA binding family member B; *KLHL20* = kelch-like family member 20; *MIER3* = mesoderm induction early response 1, family member 3; *EED* = embryonic ectoderm development; *NT5E* = 5'-nucleotidase, ecto (CD73); *B3GNT5* = UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 5; *TAOK1* = TAO kinase 1; *ATG12* = autophagy related 12; *GALNT7* = UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7 (GalNAc-T7); *LRRK2* = leucine-rich repeat kinase 2; *ANKRA2*= ankyrin repeat, family A (RFXANK-like) 2; *PIP4K2A* = phosphatidylinositol-5-phosphate 4-kinase, type II, alpha; *SQSTM1* = sequestosome 1; *MTDH* = metadherin

Table 4: miR-103a-3p target prioritization*

Rank	GeneID	HGNC Symbol	Rank miR-29a-3p	Rank miR-30b-5p	V_PMID	#PF	#PP	InSet_AVPD	GOSlim	BPscore	PPI	Cpx	Mito	ParkinTAP	Endeavour100
1	23405	*DICER1*	150	761	2	3	3								
2	4763	*NF1*		854	1	3	3		●						26
3	9683	*N4BP1*	692		1	3	3			●					
4	2247	*FGF2*		451	1	3	2			●					
5	63982	*ANO3*				3	3								
6	1983	*EIF5*				3	3			●	●	●			1
7	8313	*AXIN2*			1	3	3			●					
8	3267	*AGFG1*			1	3	2					●			
9	7533	*YWHAH*			1	2	2		●		●	●			72
10	11244	*ZHX1*			1	3	2								
11	93	*ACVR2B*			1	2	2			●					
12	8573	*CASK*				3	1			●	●				13
13	9441	*MED26*	1050			3	3								
14	120892	*LRRK2*		16			1	●	●		●		●		3
15	25777	*SUN2*			1	2	3					●			
16	23710	*GABARAPL1*			1				●		●	●			
17	596	*BCL2*	13	32	1				●	●	●		●		
18	81565	*NDEL1*		283	1	3	3			●					
19	1021	*CDK6*	26		2	2	1			●	●				60
20	3181	*HNRNPA2B1*		191	1	2	2					●		●	

*See column description in Table 2. *DICER1* = dicer 1, ribonuclease type III; *NF1* = neurofibromin 1; *N4BP1* = NEDD4 binding protein 1; *FGF2* = fibroblast growth factor 2 (basic); *ANO3* = anoctamin 3; *EIF5* = eukaryotic translation initiation factor 5; *AXIN2* = axin 2; *AGFG1*= ArfGAP with FG repeats 1; *YWHAH* = tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide; *ZHX1* = zinc fingers and homeoboxes 1; *ACVR2B* = activin A receptor, type IIB; *CASK* = calcium/calmodulin-dependent serine protein kinase (MAGUK family); *MED26* = mediator complex subunit 26; *LRRK2*: leucine-rich repeat kinase 2; *SUN2* = Sad1 and UNC84 domain containing 2; *GABARAPL1* = GABA(A) receptor-associated protein like 1; *BCL2* = B-cell CLL/lymphoma 2; *NDEL1*= nudE neurodevelopment protein 1-like 1; *CDK6* = cyclin-dependent kinase 6, *HNRNPA2B1* = heterogeneous nuclear ribonucleoprotein A2/B1.