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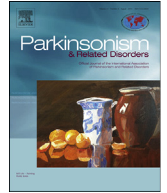

Short communication

Early-onset parkinsonism caused by alpha-synuclein gene triplication: Clinical and genetic findings in a novel family

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ABSTRACT

Introduction: Triplications of *SNCA*, the gene encoding for α-synuclein, cause a very rare Mendelian form of early-onset parkinsonism combined with cognitive and autonomic dysfunctions. Only six families with *SNCA* triplications have been described so far, limiting our knowledge of the associated phenotype. In this study, we report clinical and genetic findings in a new Italian family with *SNCA* triplication.

Methods: The patients' phenotype was assessed by neurological examination, neuropsychological tests, and brain imaging (MRI and SPECT-DaTSCAN). For the genetic investigation, we used three independent techniques: genome-wide SNP microarrays, fluorescence in situ hybridization (FISH), and multiplex ligation-dependent probe amplification (MLPA).

Results: Genetic studies documented the presence of four copies of the *SNCA* gene in the affected family members. FISH experiments and the segregation in the family were consistent with a heterozygous triplication of the *SNCA* locus. The patients carrying the SNCA triplication developed early-onset parkinsonism combined with depression, behavior disturbances, sleep disorders, and cognitive decline; marked autonomic dysfunctions were not observed. Brain imaging revealed fronto-parietal atrophy and a severe striatal dopaminergic deficit.

Conclusion: The identification of this novel family contributes to the genetic and clinical characterization of this rare form. Our data reinforce the view that *SNCA* triplications cause early-onset parkinsonism, with prominent non-motor features.

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1. Introduction

Genetic studies of the familial forms of Parkinson's disease (PD) have led to the identification of disease-causing mutations in six genes: *SNCA*, *LRRK2*, *VPS35*, *Parkin*, *PINK1*, and *DJ-1* [1]. The first to be discovered was the gene encoding for α-synuclein, *SNCA*.

The pathogenic role of *SNCA* mutations was first identified through the study of large families with autosomal dominant PD from Italy and Greece [2]. Subsequent studies identified additional pathogenic mutations, including missense mutations and multiplications (duplications and triplications) [3,4]. While *SNCA* duplications are relatively common in familial PD cases (1.5% in autosomal dominant PD), missense mutations and triplications are extremely rare [4].

To our knowledge, *SNCA* triplications have been reported in only four extended families [3–6] and two single patients [7,8]. The patients reported so far developed an aggressive form of early-onset parkinsonism, combined with additional non-motor signs, such as cognitive and autonomic dysfunctions. These clinical presentations support the idea that *SNCA* triplications cause a more severe phenotype than *SNCA* duplications, consistently with the underlying genetic defects (i.e., triplications result in one more copy of the *SNCA* gene than duplications). Nevertheless, since the number of reported families is limited, more data are required to derive any solid conclusions.

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Here, we report the identification of a triplication at the *SNCA* locus in a new family from the Abruzzi region, Italy. We provide clinical descriptions, instrumental evaluations, and videos of the affected family members, and we use three independent techniques to support our genetic findings.

2. Methods

We performed clinical and genetic studies on a family from the Italian region of Abruzzi. The four-generation pedigree of the family is provided in Fig. 1A. This study was approved by the appropriate institutional review boards and all participants provided written informed consent.

Genomic DNA was isolated from peripheral blood with standard protocols. We performed gene dosage of *SNCA*, *parkin*, *PINK1*, *DJ-1*, *GCH1*, and *ATP13A2* using multiplex ligation-dependent probe amplification (MLPA) kits P051 and P052 (MRC Holland, Amsterdam, The Netherlands) in all the participating family members. MLPA experiments were conducted following the manufacturer's protocols.

In order to confirm the aberrant number of copies at the *SNCA* locus, we employed two additional techniques. First, we performed a copy-number variant analysis of the *SNCA* locus in the index case IV-6 using Illumina HumanOmniExpress BeadChip array (Illumina, San Diego, California; 730,525 SNPs at a median distance of 2.1 kb). The data were analyzed using Nexus Copy Number, Discovery Edition, Version 7 (BioDiscovery, El Segundo, California). Second, Fluorescence in situ hybridization (FISH) was carried out on blood mononuclear cells of subject IV-6. Bacterial artificial chromosome (BAC) DNA clones (RP11-502A23, RP11-115D19, RP11-188M16, and RP11-252C9) were labeled using BioPrime DNA Labeling System (Life Technologies, Carlsbad,California) with either digoxigenin-11-dUTP (Roche, Basel, Switzerland), biotin-16-dUTP (Roche, Basel, Switzerland), or cyanine 3-dCTP (Perkin Elmer, Waltham, Massachusetts). For the first two labelling methods, probes were detected using anti-digoxigenin FITC (Roche, Basel, Switzerland) or Streptavidin Alexa 594 (Life Technologies, Carlsbad,California). The FISH experiments were performed according to standard protocols. Genomic locations of the probes were retrieved from NCBI Clone DB, or using eFISH (http://projects.tcag.ca/cgi-bin/efish/index.cgi).

3. Results

3.1. Case reports

The index patient (IV-6) developed the first symptoms of the disease in 2003, at the age of 28 years (Table 1). At that time, he presented facial hypomimia, rigidity, and bradykinesia more prominent on the left side; depression and REM sleep behavior disorder were also present. His Hoehn/Yahr stage was 2, and his UPDRS III score was 7. Brain MRI revealed bilateral fronto-parietal atrophy, while SPECT-DaTSCAN (Suppl. Fig. A) showed reduced tracer binding in the caudate nuclei and in the putamen (more prominent to the right). The binding reduction was more severe than the reduction usually observed in early stage PD, especially in the caudate nuclei. After a year, despite the treatment with dopamine agonist, the UPDRS III score increased to 15 and L-Dopa was introduced in the therapy (400 mg/day). In the following years, the patient developed hypersexuality, delirium of jealousy, and aggressive behavior. The therapy was modified removing the dopamine agonist and adding clozapine (50 mg/day). In 2012 (Video1), the Hoehn/Yahr stage was 3.5, the UPDRS III score was 40, and cognitive testing showed a deficit in the executive functions, cognitive flexibility, and planning (mini-mental state examination 23/30, frontal assessment battery 5/18, dementia rating scale-2 112). At our last examination in 2014, after disease duration of 11 years, Hoehn/Yahr stage was 4, UPDRS III score was 63, and therapy consisted of L-Dopa in combination with atypical antipsychotics.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2015.06.005.

The patient's sister (IV-5) developed the symptoms of the disease later than her brother, at 42 years old (Table 1). At first examination, she had facial hypomimia, rigidity, and bradykinesia more prominent on the right side (Video 2). She also reported REM sleep behavior disorder since adolescence, depressive episodes during the last five years, and three spontaneous abortions in the second trimester of pregnancy from 2010 to 2012. Her Hoehn/Yahr stage was 2 and UPDRS III score was 9. She refused to receive therapy for her motor symptoms. Neuropsychological tests were within normal limits. MRI was normal, while SPECT-DaTSCAN showed bilateral reduced binding in the putamen (left side > right side) and no significant alterations in the caudate nuclei (Suppl. Fig. B).

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2015.06.005.

Information about the family history was reported by subject IV-5 and also documented, in part, by hospital records. The pattern of inheritance was compatible with a Mendelian autosomal dominant trait (Fig. 1A). The mother (III-4) of IV-5 and IV-6 developed PD at the age of 30. Her clinical signs included rigidity, bradykinesia and tremor of the left hand. From the onset of the disease, she also suffered from depression and anxiety. Her treatment consisted in biperiden, L-Dopa, and benzodiazepines. She died at the age of 43 years old, after disease duration of 13 years; her last hospital records reported cognitive decline, psychomotor agitation, and severe behavior disturbances.

Only few information were available for the previous generations. The maternal grandmother of the index case (II-4) was reported with early-onset PD, and died at 45 years old by unknown causes. The grandmother's sister (II-2) was reported with symptoms of PD at the age of 55 and died at the age of 72. The maternal grand grandmother (I-2) had symptoms compatible with PD and died at the age 40 by unknown causes.

3.2. Genetic results

The MLPA experiments identified an aberrant number of copies of *SNCA* in the two affected siblings (IV-5 and IV-6). The signal intensity for all the *SNCA* probes included in the MLPA kits (exons 2, 3, 4, 5, 6, and 7) was double than expected in a diploid genome, supporting the existence of four copies of the gene (Fig. 1B and C). This abnormal copy number was not detected in any other family members (Fig. 1A). Our copy-number analysis using SNP arrays data confirmed the copy-number gain in the chromosomal region 4q22.1. The analysis showed both an increased Log R ratio and a deviation from the expected values of the B-allele frequency (Fig. 1D), two abnormalities that support increased gene dosage and deviation from diploidy. The centromeric border of the triplication was predicted between rs13148714 and rs12649385, while the telomeric side was between rs1903577 and rs7688033: a region with maximum size of 1.30 Mb. The analysis of this genomic region using the UCSC Genome Browser revealed the presence of five protein-coding genes: *TIGD2*, *GPRIN3*, *SNCA*, *MMRN1*, and *CCSER1*.

To further investigate this genomic rearrangement, we performed FISH experiments in blood cells of subject IV-6 using four different probes that annealed within, or just outside the genomic region indicated by our copy-number analysis (Fig. 1D). Representative results are provided in Fig. 1E and F. The probes annealing outside the region yielded two signals, while the probes annealing

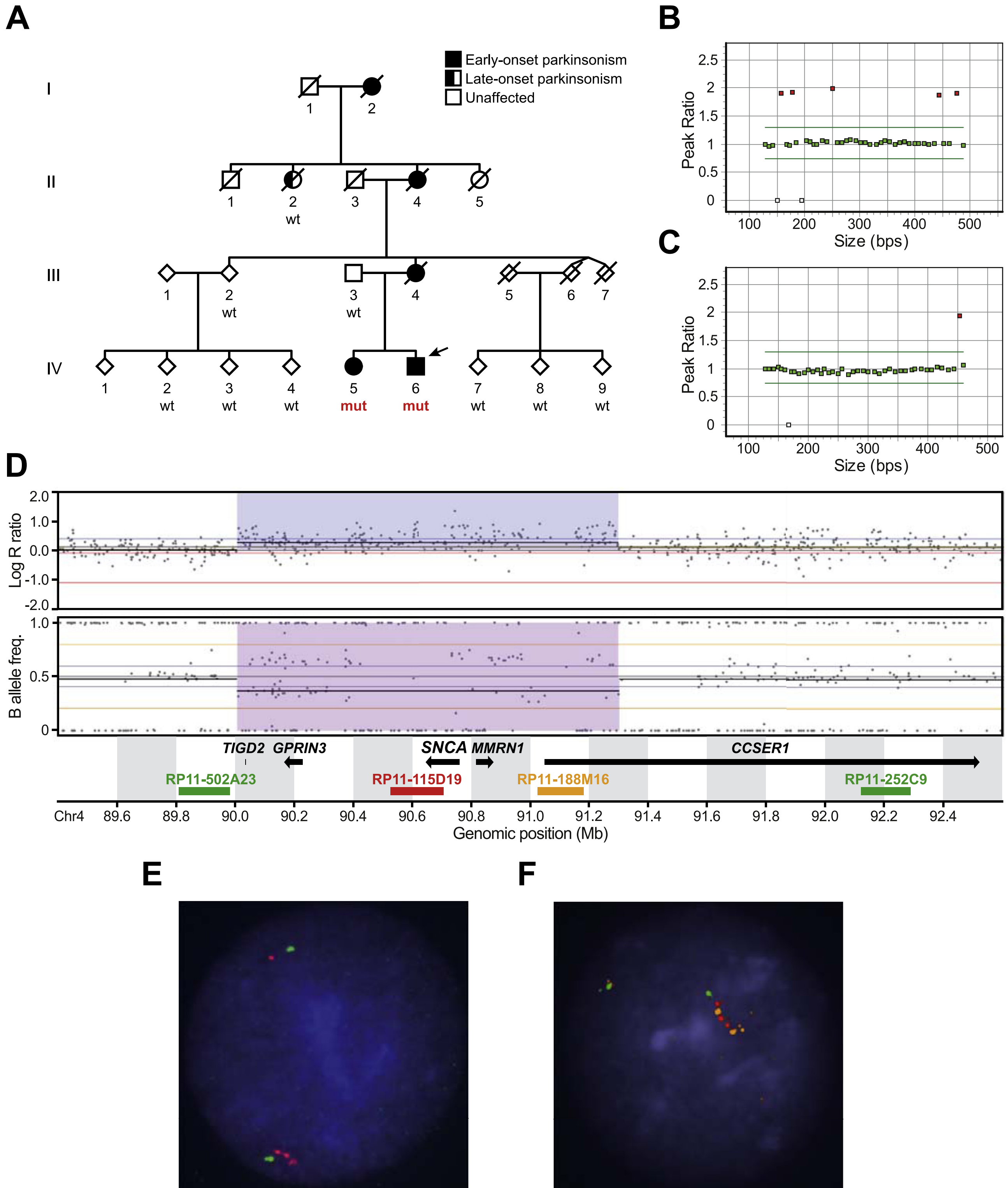

Fig. 1. Genetic findings in a family with SNCA triplication. A—Pedigree of the Italian family with *SNCA* triplication. To protect subjects' privacy, the gender of some subjects has been disguised. mut, *SNCA* triplication; wt, wild type (normal number of *SNCA* copies). B,C—MLPA experiments in subject IV-6 support the presence of four copies of *SNCA*. Gene dosage is expressed as Peak Ratio (vertical axes): the ratio between measured signal and a control sample. A Peak Ratio of one corresponds to a gene dosage of two copies, while a Peak Ratio of two corresponds to a gene dosage of four copies. Squares represent the results of the MLPA probes; *SNCA* probes are depicted in red; probes of additional genes are depicted in green; white squares represent the probes detecting point mutations. D—Copy-number variant analysis of chromosome 4 using SNP arrays data from subject IV-6. Log R ratio and B-allele frequency (vertical axes) are represented as a function of the genomic position (horizontal axis). Blue and purple boxes indicate the triplicated region. Black arrows indicate the positions of the five genes involved in the triplication; green, red, and orange rectangles display the position of the FISH probes. E,F—Representative results of the FISH experiments supporting the increased dosage of the *SNCA* gene. Control probes are labeled in green, and display two signals, while the probes within the triplicated region are labelled in orange or red, and display four signals. Also, the clustering of the signals suggests that three copies of *SNCA* are on the same chromosome, supporting a heterozygous triplication. Green (panel D) RP11-252C9; green (panel E) RP11-502A23; orange RP11-188M16; red RP11-115D19.

Table 1
Clinical and genetic features of patients with *SNCA* triplications.

Reference	This study			Waters et al., 1994; Muenter et al., 1998; Singleton et al., 2003; Ross et al., 2008; Gwinn et al., 2011	Farrer et al., 2004; Fuchs et al., 2007; Ross et al., 2008	Ibanez et al., 2009	Sekine et al., 2010	Keyser et al., 2010	Byers et al., 2011
Subject ID	IV-5	IV-6	III-4	14 affected Relatives	4 affected Relatives	3 affected Relatives	3 affected Relatives	1 familial PD patient	1 PD patient
Gender	F	M	F	6 M, 8 F	2 M, 2 F	2 M, 1 F	3 M	M	M
Genotyped individuals	Yes	Yes	No	4	1	1	1	1	1
Size of the triplicated region (Mb)	1.29 to 1.30			1.69 to 1.83	0.82 to 0.84	2.61 to 2.64	>0.40	N/A	N/A
Genes in triplication	TIGD2, GPRIN3, SNCA, MMRN1, CCSER1			*HERC6*, *HERC5*, *PIGY*, *PYURF*, *HERC3*, *NAP1L5*, *FAM13A*, *TIGD2*, *GPRIN3*, *SNCA*, *MMRN1*, *CCSER1*	*SNCA*, *MMRN1*, *CCSER1*	*GPRIN3*, *SNCA*, *MMRN1*, *CCSER1*	*SNCA*, *MMRN1*, *CCSER1*	*SNCA*	*SNCA*
Onset age (y.rs)	42	28	30	24–48 (range)	31	36–61 (range)	28–49 (range)	46	38
Disease duration (y.rs)	2	11	13	~8 (average)	21	5–10 (range)	3–8 (range)	9	2
Rigidity	+	+	+	+	+	3/3	3/3	+	+
Bradykinesia	+	+	+	+	+	3/3	3/3	+	+
Rest tremor	+	–	+	+	+	2/3	3/3	+	+
Response to L-Dopa	N/A	+	+	+ (initially)	+	3/3	3/3	+	+
Motor fluctuations	–	–	N/A	+	+	N/A	N/A	N/A	+
REM sleep behavior disorder	+	+	N/A	+	+	N/A	0/3	N/A	+
Orthostatic hypotension	–	–	–	+	+	N/A	3/3	+	–
Urinary incontinence	–	–	N/A	+	+	3/3	0/3	N/A	–
Depression	+	+	+	+	+	N/A	2/3	N/A	+
Cognitive decline	–	+	+	+	+	2/2	2/3	+	+
Psychotic disturbances	–	+	+	+	+	N/A	2/3	+	–

F, female; M, male; +, present; -, absent; N/A, not available.
Byers B et al., PLoS One 2011; 6:e26159. Farrer M et al., Ann Neurol 2004; 55:174–9. Fuchs J et al., Neurology 2007; 68:916–22. Gwinn K et al., Mov Disord 2011; 26:2134–6. Ibanez P et al., Arch Neurol 2009; 66:102–8. Keyser RJ et al., Neurogenetics 2010; 11:305–12. Muenter et al., Ann Neurol 1998; 43:768–81. Ross OA et al., Ann Neurol 2008; 63:743–50. Sekine T et al., Mov Disord 2010; 25:2871–5. Singleton AB et al., Science 2003; 302:841. Waters CH et al., Ann Neurol 1994; 35:59–64.

inside the genomic region yielded four signals. Also, the location of the four signals in the nuclei was consistent with having three copies of *SNCA* on one chromosome and one copy on the other chromosome.

4. Discussion

Here, we report on the identification of a novel family with early-onset parkinsonism and triplication of the *SNCA* gene. The existence of four copies of the *SNCA* gene was consistently detected using three different genetic techniques, and the mutation segregated with the disease in the two patients with early-onset PD available for testing, while unaffected subjects did not carry this mutation. The pattern of familial transmission of the disease, the presence of a normal number of copies of the *SNCA* gene in the unaffected father (III-3), and the results of the FISH experiments support the contention that the aberrant number of copies is the result of a heterozygous triplication, rather than a homozygous duplication. Of note, subject II-2 did not carry the mutation. The clinical data available for this patient are very limited, but she developed the disease later than the rest of the affected family members and had prolonged disease duration. For these reasons, we consider her as a phenocopy.

As *SNCA* triplications are very rare, the clinical description of such families is valuable. The affected family members developed an early-onset form of parkinsonism combined with sleep disorders, cognitive decline, and behavioral disturbances. Brain imaging revealed fronto-parietal atrophy in one of the affected siblings, and a severe dopaminergic deficit at the level of the caudate nuclei and putamen. Unlike the cases previously reported, these new patients with *SNCA* triplication did not suffer from severe, symptomatic autonomic dysfunctions such as urinary incontinence, or postural hypotension, and did not experience visual, or auditory hallucinations. Nevertheless, the presentation, progression, and onset age of the disease in this novel family support the notion that *SNCA* triplication causes early-onset parkinsonism with additional prominent non-motor signs. This phenotype is more severe than the phenotype of patients with *SNCA* duplication, which may resemble idiopathic PD [9]. Recently, the combination of data from multiple families [3,4,10] was used to propose an explanation for the phenotypic differences between *SNCA* duplications and triplications. These studies suggested that, rather than affecting the severity of the disease, the different gene dosage can modify the penetrance and variability of the phenotype, with triplications causing fully-penetrant forms with earlier onset and prominent non-motor features. Our new family adds new data and reinforces this notion.

Few studies have shed light on the effects of these mutations. Triplications of the *SNCA* gene are known to increase α-synuclein protein levels in the blood and in postmortem brain tissue [11]. Also, increased expression and accumulation of α-synuclein have been observed in midbrain dopaminergic neurons differentiated from induced pluripotent stem (iPS) cells with *SNCA* triplications [8,12]. These studies demonstrate that the gene dosage of *SNCA* can modify the expression levels of the corresponding protein.

Not all the *SNCA* triplications described so far are of the same size or involve the same genes, but the comparison of the different triplications reported is made difficult by incompleteness of the data. If we consider the three families with available detailed information, the triplication in two families [3,4] extends to a bigger genomic region than in our family, while the genomic region in the third family [5] is smaller than in the novel family. Also, like in the previous families, *SNCA* is not the only gene altered by the mutation, and the other genes involved might act as modifiers, thereby possibly explaining part of the variability of the phenotype.

The identification of a novel family with *SNCA* triplication is an important step towards a better characterization of the phenotype associated with this very rare Mendelian form of parkinsonism. Despite the rarity of triplications at the *SNCA* locus, duplications are relatively common (1.5% in autosomal dominant PD [4]), and diagnostic laboratories should consider testing the *SNCA* copy number in patients with autosomal dominant PD.

Role of the funding source

The founding source had no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Author contributions

1: conception and design of the study (A), acquisition of data (B), analysis and interpretation of data (C);
2: drafting the article (A), revising it critically for important intellectual content (B);
3: final approval of the version to be submitted;
Simone Olgiati: 1BC, 2AB, 3.
Astrid Thomas: 1BC, 2AB, 3.
Marialuisa Quadri: 1C, 2B, 3.
Guido J. Breedveld: 1C, 2B, 3.
Josja Graafland: 1B, 2B, 3.
Hubertus Eussen: 1C, 2B, 3.
Hannie Douben: 1B, 2B, 3.
Annelies de Klein: 1C, 2B, 3.
Marco Onofrj: 1AC, 2AB, 3.
Vincenzo Bonifati: 1AC, 2AB, 3.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2015.06.005.

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