Assessment of risk factor variants of *LRRK2, MAPT, SNCA* and *TCEANC2* genes in Hungarian sporadic Parkinson's disease patients

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Abbreviations

PD: Parkinson's disease

LRRK2: Leucine-rich repeat kinase 2 gene

LRRK2: Leucine-rich repeat kinase 2 protein, dardarin

SNCA: synuclein alpha gene

SNCA: alpha-synuclein protein

TCEANC2: transcription elongation factor A N-Terminal and central domain containing 2 gene

TCEANC2: transcription elongation factor A N-Terminal and central domain containing 2 protein

MAPT: microtubule associated protein tau gene

MAPT: microtubule associated protein tau protein

SNP: single nucleotide polymorphism

GWA: genome wide association

AD: Alzheimer's disease

PSP: progressive supranuclear palsy

EOPD: early-onset Parkinson's disease

LOPD: late-onset Parkinson's disease

PCR: polymerase chain reaction

RFPL: restriction fragment length polymorphism

OR: odds ratio

CI: confidence interval

LD: linkage disequilibrium

Cdk5: Cyclin-dependent kinase 5

iPSC: induced pluripotent stem cell

Abstract

Introduction: Parkinson's disease is the second most common neurodegenerative disease. Lifestyle, environmental effects and several genetic factors have been proposed to contribute to its development. Though the majority of PD cases do not have a family history of disease, genetic alterations are proposed to be present in 60 per cent of the more common sporadic cases.

Objective: The aim of this study is to evaluate the frequency of PD related specific risk variants of *LRRK2*, *MAPT*, *SNCA* and *PARK10* genes in the Hungarian population. Out of the ten investigated polymorphisms three are proposed to have protective effect and seven are putative risk factors.

Methods: For genotyping, TaqMan allelic discrimination and restriction fragment length polymorphism method was used. *LRRK2* mutations were investigated among 124 sporadic PD patients and 128 healthy controls. *MAPT* and *SNCA* variant frequencies were evaluated in a group of 123 patients and 122 controls, while *PARK10* variant was studied in groups of 121 patients and 113 controls.

Results: No significant difference could be detected in the frequencies of the investigated *MAPT* and *PARK10* variants between the studied Hungarian PD cases and controls. The minor allele of the risk factor S1647T *LRRK2* variant was found to be more frequent among healthy male individuals compared to patients. In the frequency of one of the investigated SNCA variant a significant intergroup difference was detected. The minor allele (A) of rs356186 is proposed to be protective against developing the disease. In accord with data obtained in other populations, the AA genotype was significantly more frequent among Hungarian healthy controls compared to patients. Similarly, a significant difference in genotype distribution was also found in comparison

of patients with late onset disease to healthy controls, which was due to the higher frequency of AG genotype among patients.

Conclusion: The frequencies of different gene variants show great differences in populations. Assessment of the frequency of variants of PD related genes variants is important in order to uncover the pathomechanisms underlying the disease, and to identify potential therapeutic targets. This is the first comprehensive study focusing on these genetic variants in the Hungarian population. Our results extend the knowledge on the world wide occurrence of these polymorphisms by demonstrating the occurrence of specific alleles and absence of others in Hungarian PD patients.

Keywords: Parkinson's disease, genetic risk factors, *LRRK2, MAPT, SNCA, PARK10*

Highlights

- G2385R and R1628P *LRRK2* variants are absent in the Hungarian population.
- The minor allele of the risk factor S1647T *LRRK2* variant is more frequent among healthy male individuals compared to patients.
- The protective rs356186 *SNCA* variant is significantly more frequent in homozygous form among controls than in PD patients.
- The rs356186 *SNCA* variant is significantly more frequent in heterozygous form among LOPD patients compared to controls.
- No signifcant dirrefence was detected in the frequency of rs1491923, R1398H, N551K, rs2583988, 1052553 and rs10788972 variants.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting millions of people worldwide [1]. It is a multifactorial disease: several environmental, lifestyle and genetic factors have been suspected to contribute to its development. 5-10 % of PD cases are familial of which 30 percent is monogenic [2]. Regarding sporadic PD, only 3-5 percent of the cases are caused by single gene mutations. However, growing body of evidence suggest the involvement of genetic factors in 60 % of the more common idiopathic PD cases as well [3].

So far over 40 human genomic loci have been proven or proposed to be related to PD [3]. Several of these are also referred as 'PARK' and a number reflecting the order of their discovery to indicate the association with the disease. In this study, we investigated the presence of ten variants of four PARK genes: Leucine-rich repeat kinase 2 (*LRRK2*; PARK8), synuclein alpha (*SNCA*; PARK1 and 4), transcription elongation factor A N-Terminal and central domain containing 2 (*TCEANC2*; PARK10) and microtubule associated protein tau (*MAPT)* in Hungarian PD patients.

The involvement of *LRRK2* in PD was first described in a large Japanese family in 2002 [4]. Since then, several *LRRK2* mutations have been identified, and alterations of this gene have been shown to be among the major causes of both familial and sporadic PD cases. Intensive research is ongoing to identify variants of the gene that act as risk factors in the disease. Two single nucleotide polymorphisms (SNPs) have been found to increase the risk of PD in Asian populations. One of them is a Gly to Arg substitution (G2385R), the other one an Arg to Pro change (R1628P). While both of these have been proven to be risk factors among Han Chinese, to date none of these variants has been found among Caucasians [5][6].

In addition to G2385R and R1628P, a change of the 1647th amino acid Ser to Thr (S1647T) is also proposed to be a susceptibility factor for PD [7]. Its effect of increasing PD risk was reported in Asian populations [7], however, such association has not been found in the Caucasian populations investigated so far [8].

Some of the *LRRK2* polymorphisms on the other hand have been proposed to be protective against PD. Such variants are the Arg to His and Asn to Lys changes at the 1398th and 551th positions of the protein (R1398H and N551K). The occurrence of either of these in combination with the G2385R and R1628P allele is reported to diminish the otherwise elevated risk of the disease [7].

Recently a Genome Wide Association (GWA) Study revealed that a common variability near the *LRRK2* gene affects the risk of PD. The minor allele resulting from an A to G change (indicated in forward orientation, rs1491923) was found to be more common among both Caucasian and Asian PD patients then their healthy controls [9].

Similarly to *LRRK2*, several variants of the *SNCA* gene have been proposed to be risk-, or protective factors regarding PD. In fact, mutations of the *SNCA* gene were the first genetic variants identified as causes of autosomal dominantly inherited familial PD. Two intronic variants of the gene: rs2583988 and rs356186 are proposed risk-, and protective factors against PD, respectively. The role of these variants among sporadic PD patients of Caucasian origin is controversial. Association and also the lack of it between these variants and the disease have been reported in several studies involving Caucasian subjects [10][11][12][13][14].

The *MAPT* gene is located on the long arm of chromosome 17, at a site of an approximately 900 kb common inversion [15] that results in two distinct haplotypes: the non-inverted H1 and the

inverted H2. The H1 haplotype has been associated with numerous diseases which are often referred as taupathies: Alzheimer's disease (AD), sporadic fronto-temporal dementia, progressive supranuclear palsy (PSP) and PD. A common pathological hallmark of these is the accumulation of MAPT neurofibrillary tangles in nerve cells [15]. The association of H1 with PD is, however, still an intriguing question. Several studies involving subjects of different nationalities reported no, or marginal association between the occurrence of the H1 haplotype and PD (reviewed in [16]). SNPs suitable of marking the inversion have been identified: a G to A change (rs1052553) is an indicator of the H1 haplotype [15].

The long arm of chromosome 1 containing the PARK10 region with the locus of *TCEANC2* gene has also gathered interest concerning its role in PD. The link between PD and this region was identified first approximately 15 years ago [17], and since then, a linkage disequilibrium (LD) for a block of 100 kb was identified in the region [18]. The SNP rs10789972, located in the *TCEANC2* gene, was found to show association with sporadic PD in American population [18] but there was no association detected among subjects of Han Chinese origin [19][20].

Allelic variants of PD-related genes are found in widely different frequencies among different populations, making it difficult to clarify the genuine effect of specific variants on the development of PD in distinct populations. It is important therefore to evaluate the occurrence of specific genetic alterations in homogenous study groups of different nationalities. Information on the occurring mutations in a population can be beneficial for understanding more of the pathological mechanisms underlying the disease. Moreover, the identification of gene variants characteristic for a population might be useful also in applying the most fitting therapeutic methods and developing new therapeutic approaches.

The aim of our study was to assess the frequency of *LRRK2*, *SNCA*, *MAPT* and *TCEANC2* mutations in sporadic PD patients in Hungary. All combined, we assessed the occurrence of ten mutations which vary in their effects as some are risk factors and others protective. We selected SNPs that are either the most intensively studied (as they have been proven to play a role in the disease in certain populations) or have been recently identified as potential risk factors. To our knowledge, this is the first throughout study focusing on the prevalence of these PARK gene variants in Hungary.

2. Material and methods

2.1. Subjects

2.1.1. *LRRK2* variants

124 sporadic PD patients were involved in the study (mean age: 66.5 ± 9.5 years, male-female ratio 61:63) (Table 1). Depending on the first appearance of symptoms, two groups were formed: early-onset (EOPD; disease onset ≤ 60 years) and late-onset (LOPD; disease onset > 60 years) PD patients. The EOPD group comprised 68, the LOPD 56 individuals. The age at disease onset was 51.1 ± 7.4 and 68.7 ± 5 years, respectively. The control group consisted of 128 healthy volunteers (mean age of 64.5 ± 9.6 years, male-female ratio 61:67).

2.1.2. *SNCA* and *MAPT* variants

The frequencies of the rs2583988 and rs356186 SNPs of *SNCA* and rs1052553 variant of *MAPT* were assessed in the groups of 123 sporadic PD patients (mean age: 66,5 ± 9,5 years, male-female ratio 60:63) and 122 healthy controls (mean age: 64,3 ± 8,8 years, male-female ratio

56:66) (Table 1). Based on the appearance of the first symptoms, the patient's group was divided into two subgroups. The EOPD (disease onset ≤ 60 years) group comprised 67, the LOPD (disease onset > 60 years) group 56 patients. The age at disease onset was 51,1 ± 7,5 and 68,7 ± 5 years, respectively

2.1.3. *TCEANC2* polymorphism

The frequency of the rs10789972 SNP of the *TCEANC2* gene in the PARK10 locus was evaluated among 121 sporadic PD patients (mean age: 66,5 ± 9,6 years, male-female ratio 59:62) and 113 healthy controls (mean age: 64,9 ± 8,1 years, male-female ratio 50:60) (Table 1). Among PD patients, 66 individuals reported the first disease symptoms at or under the age of 60 years (EOPD, disease onset 51 ± 7,5 years). In the case if the other 55 patients the first symptoms appeared after the age of 60 years (LOPD, disease onset 68,7 ± 5,1 years).

In all study groups, the diagnosis of PD was set up based on medical history and physical examination by movement disorder specialists. All control individuals lacked history of neurological and psychiatric disorders.

Informed consent was obtained from all study participants. The study is in full accordance with the Helsinki Declaration and was approved by the Medical Research Council Scientific and Research Ethics Committee.

2.2. DNA isolation

The standard desalting method [21] was used for genomic DNA isolation from peripheral blood. The extracted DNA was stored at -20 ^{o}C.

2.3. Restriction fragment length polymorphism

For the genotyping of R1628P and G2385R variants polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis was implemented. The sequences of the primers used for generating PCR products and annealing temperatures can be provided on request (please contact the Corresponding Author). For the investigation of G2385R and R1628P SNPs 170 bp and 419 bp PCR products were generated, respectively. After amplification, the PCR products were digested with restriction enzymes at 37 ˚C overnight. *Acc*I restriction enzyme was used for the detection of G2385R, and *Bst*UI for R1628P. DNA fragments were then detected on 2% agarose gel electrophoresis, visualizing the bands with ECO Safe alternative gel stain. Wild-type G2385R samples remained undigested, resulting in one, 170 bp DNA fragment. In the case of heterozygous samples three fragments (170,123 and 47 bp), while in the case of homozygous mutants, two (123 and 47 bp) fragments could be detected. Opposite to this, in the case of the R1628P SNP digestion of homozygous wild-type samples resulted in the generation of two (263 and 156 bp) DNA fragments. The partial digestion of heterozygous samples yielded three bands (419, 263 and 156 bp), while PCR products of homozygous mutant samples remained undigested resulting in one detectable band (419 bp).

2.4. TaqMan allelic discrimination method

The analysis of R1398H, N551K, S1647 and rs1491923 *LRRK2* variants and all the investigated *MAPT*, *SNCA* and *TCEANC2* variants was performed with the use of TaqMan allelic discrimination assays obtained from Thermo Fisher Scientific. PCR reactions were run on Bio-Rad real-time thermal cycler CFX96. The reaction conditions can be obtained on request (please contact the Corresponding Author).

2.5. Statistical analysis

For statistical analysis GraphPad Prism 6.01 statistics software was used. For the analysis of genotype and allele frequencies Chi-square (χ^2) test or Fisher's test was used. Odds ratio (OR) with a 95% confidence interval (95% CI) was implemented for the analysis of the association between PD and genotype frequencies. P less than 0,05 was considered statistically significant.

3. Results

3.1. Putative risk factor *LRRK2* mutations (G2385R, R1628P, S1647T and rs1491923)

The G2385R and R1628P variants were found to increase the risk of developing in PD, however, seem to be absent or extremely rare in Caucasian populations. In accord with this, we did not found any of these SNPs to be present in either of our study groups (Suppl. Table 2.).

The S1647T substitution results from a T to A change in exon 34. The minor allele (A) of the variant was found to be a risk factor of PD in several Asian populations. However, such relation has not been identified in Caucasian populations. The genotype and allele distribution of this variant was similar in both study groups (Suppl. Table 3.). The difference was not significant when comparing EOPD and LOPD patient subgroups to controls. When analyzing the genders separately, the comparison of female patients to healthy controls showed no significant difference regarding both genotype and allele frequencies. However, when analyzing the genotype distribution of male patients in comparison with the corresponding control group, a trend towards higher AA frequency could be observed in healthy controls. Comparing allele frequencies of the same groups revealed the minor (A) allele to be significantly more frequent among healthy male individuals ($\chi2=6,06$; $p=0,014$) (Suppl. Table 3.).

The SNP rs1491923 is an A to G change (indicated in reverse orientation), affecting a site 0.17Mb upstream of *LRRK2* gene [9]. Its role as a predisposing factor PD was proposed recently based on the results of a GWAS study [9]. We found both genotype and allelic distribution of this variant to be similar in our patient and control group (Suppl. Table 4.). Comparison of subgroups generated by separating our two main study groups (PD and control) either by gender or by the age of disease onset did not reveal significant difference either in genotype or in allele frequencies (Suppl. Table 4.).

3.2. Protective *LRRK2* variants (R1398H and N551K)

The R1398H and N551K *LRRK2* variants were found to diminish the increased risk of the disease in G2385R and R1628P carriers [7]. We did not find any significant difference between either the genotype or allele frequencies of the R1398H or N551K variants between the control and PD group (Suppl. Table 5.). Allele and genotype frequencies were also similar after stratification by gender or by age at disease onset. We found these variants to be in LD, as except for one case in our group of healthy controls, the R1398H and N551K substitution always occurred simultaneously (Suppl. Table 5.).

3.3. *SNCA* and *MAPT* gene variants

The rs356186 SNP is an intronic G to A change in the *SNCA* gene, of which the minor A allele is proposed to be protective in PD. Comparing the genotype distribution of our control and patients' group there was a significant difference (χ^2 =7,65; p=0,022) (Suppl. Table 6.). This intergroup difference was due to the higher relative frequency of the AA genotype among healthy participants in comparison to patients (AA vs. GG+AG, Fisher's test: p=0,019, OR: 0,12, CI (95%): 0,014-0,95). A significant difference in genotype distribution was also found when

comparing the LOPD group to healthy controls (χ^2 =6,14; p=0,046) (Suppl. Table 6.). This difference is a consequence of higher frequency of AG genotype among LOPD patients (AG vs. GG+AA; χ^2 =5,07; p=0,024). No significant difference in genotype or allele distribution could be detected in other study setups.

No significant difference was found in genotype or allele frequency of rs2583988 SNP of *SNCA* and the studied *MAPT* variant (rs1052553) in either comparison (Suppl. Table 7. and 8.).

3.4. *TCEANC2* gene variant

Both allele and genotype distribution of the rs10789972 SNP was similar in the PD and control group, revealing no significant difference (Supp. Table 9.). Similarly, no significant difference was found when comparing the EOPD, LOPD, male or female patients to the corresponding control groups.

4. Discussion

The aim of our study was to assess the frequency of six *LRRK2*, two *SNCA*, a haplotype marking *MAPT* and *PARK10* variants in Hungarian sporadic PD patients. To our knowledge this is the first comprehensive study focusing on these gene variants in Hungary.

The *LRRK2* gene is localized on the long arm of chromosome 12. LRRK2 – also known as dardarin – is a large protein, built up of more, than 2500 amino acids. It is a representative of the ROCO superfamily and consists several domains, of which two (a kinase and GTPase) are enzymatic. Though the exact physiological function of the protein needs further elucidation, LRRK2 is suggested to serve as a scaffolding protein, to be involved in the process of neurite

outgrowth, maintenance of the cytoskeleton, vesicle transport and degradation of autophagic protein (reviewed in [22]).

Among the investigated *LRRK2* variants four are putatively, or among some populations proven risk factor variants, and two SNPs have been found to have protective effects among certain circumstances.

Even today, data regarding the *LRRK2* mutations that might act as risk factors in PD is inconclusive. Out of the more, than 100 SNPs in *LRRK2* gene G2385R and R1628P are the only validated coding susceptibility alleles for PD [23]. The Gly to Arg substitution at the 2385th amino acid position (G2385R) causes a two-fold increase in PD risk, while the Arg to Pro amino acid change at position 1628 (R1628P) causes an even bigger increase in the possibility of developing the disease [24]. Our results showing that both of these variants are absent in our study groups are in accord with literature data. The R1628P and G2385R substitutions have been found only in the Asian, but not in Caucasian populations [25][23][26].

The G3285R substitution is located towards the C terminus of the protein in the WD40 domain. As this domain takes part in protein-protein interactions, one might suppose that the amino acid change leads to alterations in the interactions with substrates and other regulatory proteins [5]. Functional studies revealed that under oxidative stress cells with the G2385R substitution showed a higher rate of apoptosis compared to the wild type [27]. The mutation might also increase the kinase activity of the protein, however, the data regarding this issue are inconclusive [7][28]. The R1628P mutation affects the COR domain of the protein and there is data suggesting it to cause a diminishment in GTPase activity [28]. Besides changes in the GTPase, the R1628P substitution was also found to increase the kinase activity of dardarin [7]. This is probably because of the

increased binding affinity of LRRK2 with Cyclin-dependent kinase 5 (Cdk5) due to the amino acid substitution, which leads to the phosphorylation of LRRK2 at the S1627 site, resulting in increased kinase activity of the protein [29]. Similarly, to G2385R, R1628P mutant cells were found to be more prone to apoptosis under oxidative stress when compared to wild type [30].

S1647T is another variant, which the effect of increasing the risk towards PD was first spotted in a Han Chinese population [7]. This Ser to Thr substitution is located in the COR protein domain, which together with the adjacent Roc domain forms the tandem Roc-COR domain, accounting for the GTPase function of LRRK2. Existing data suggest that GTP binding is essential for the activation of kinase function of this protein, therefore mutations affecting the GTPase domain might have effects on kinase function as well [28]. Other reports indicate that the dimeric form is essential for kinase activity [28]. Considering that the COR domain is a core element in protein dimerization [31], mutations affecting this domain could have effects on kinase activity either by changes in autophosphorylation or protein conformation. However, further studies are necessary for the elucidation of the effects of the S1647T mutation, as so far no changes have been found in kinase activity in relation with this variant [7].

Our findings, that there is no significant association between the ST1647T *LRRK2* variant and PD in our cohort, is in accord with literature data available regarding Caucasian populations, as no significant association was found in Finnish and Greek study groups either [8]. Our result of higher frequency of the minor allele among male controls compared patients is in contrast with literature data. However, this conflicting result might be due to the relatively small sample size.

Rs1491923 is an A to G (forward orientation) change 0.17 Mb upstream the gene. The possible significance on developing PD of this common intronic variant was proposed by a GWA study. It

was found that the minor allele of this SNP was more common among American, German and British PD patients [9]. Findings obtained by the use of an induced pluripotent stem cell (iPSC) model of idiopathic PD suggest that this variant might have detrimental effect on mitochondrial protein clearance and autophagy [32]. Though our results do not add to these findings, the possible risk effect of this variant on the disease cannot be excluded. In order to clarify such associations further genotype analysis of independent sample groups of different populations is clearly warranted.

Besides risk factor mutations there are variants of the *LRRK2* gene which seem to have a protective effect against the development of PD. Such variants are the R1398H and N551K substitutions, located in the ROC domain and armadillo repeat region of the protein, respectively. A study involving Asian patients and controls found these variants to be in LD and were significantly more frequent among PD patients [7]. The same study revealed a prominent reduction in the otherwise increased disease risk due to the presence G2385R and R1628P polymorphisms in individuals who simultaneously were carriers of either the R1398H or N551K SNPs [7]. Moreover, appearance of either of the protective variants could largely negate the risk of a R1628P carrier, resulting in an OR 1.5-1.6 instead of 1.9 [7]. This could partly be explained by the diminished kinase activity of R1398H mutant dardarin, which might be able to compensate the elevated enzyme function, a result of R1628P and/or G2385R substitutions [7]. In Caucasian population no significant difference was found in the frequencies of these variants between PD patients and healthy individuals [8]. Our observations corroborate with data published on Greek and Finnish populations [8] in finding no significant difference among PD patients and controls. Our data are also consistent with the findings of others in regard the LD these gene variants show [7].

The *SNCA* gene is located on the long arm of chromosome 4 and consisting of 10 exons it spans over 114 kb. The product of the gene is the 140 amino acid alpha-synuclein (SNCA), a major component of the PD-related Lewy bodies. Accumulation of the protein is proposed to contribute to the selective loss of dopaminerg neurons seen in PD due to the increased sensitivity of the cells to dopamine toxicity [33]. Mutations of the *SNCA* gene were the first genetic alterations identified to cause autosomal dominant PD. Since then several SNPs within the gene have been proposed to contribute to, or, in some cases, decrease the risk of developing the disease. The SNP rs2583988 is an intronic C/T base change. The minor allele of the variant was found to occur at a significantly higher frequency among PD patients compared to controls in studies involving individulals of Caucasian origin [10][11][12]. However, there are also data representing for the lack of such association between PD and the polymorphism [13]. In accord with our findings, association between the minor allele frequency of rs2583988 and PD was not found among German [13] or Irish [14] patients.

Rs356186 is an A/G change (indicated in forward orientation) which is also located in the intronic region of the *SNCA* gene. The presence of the minor allele is proposed to be a protective factor against developing PD. This assumption is based on the detection of the minor allele significantly more frequent among healthy controls compared to PD patients in Irish [14], Italian [10] and populations of Northern Central and Southeastern European origin [11]. However, no significant difference was detected between controls and patients in a study involving German participants (except when comparing the frequency between female PD patients and the corresponding control individuals) [13]. Recently a meta-analysis was conducted with the aim to find the most relevant *SNCA* SNPs in PD [34]. Zang *et al.* analysed the significance level of the different variants from various studies, and based on that, defined the polymorphisms rs2583988

and rs356186 as recommended and most recommended *SNCA* SNPs, respectively [34]. The same study also concluded that heterozygotes of the protective *SNCA* variant (rs356186) greatly contribute to the effect of this SNP since in the overall analyzed populations the dominant model of the variants showed significant difference [34]. These findings are in accord with our data, as we found that the significant difference in genotype distribution between LOPD group and healthy controls was a consequence of higher frequency of AG genotype among LOPD patients. We also detected a significantly higher relative frequency of the AA genotype among healthy participants in comparison to patients.

MAPT gene on the long arm of chromosome 17 is located at a site of a common inversion that results in two haplotypes. The more common haplotype referred as H1, the rarer as H2 [35]. Several genes are localized in the approximately 900 kb affected region of chromosome that results in H1 and H2 haplotype formation [36]. One of the most studied one of these genes is *MAPT* due to its linkage with several disorders including neurodegeneration [37]. The H1 haplotype was found to show higher transcriptional activity being stronger at initiating transcription thus resulting in increased expression of the *MAPT* gene [38]. In accord with this, the H1 haplotype has been associated with neurological diseases such as sporadic frontotemporal dementia, PSP, AD and PD – which all share a pathological hallmarks of accumulated MAPT neurofibrillary tangles in neurons [39]. However, the role of H1 haplotype in PD risk is controversial. Several studies of various populations reported no, or only marginal significance of the variant in PD (reviewed in [37]). Our results are in accord with those which found no significant association between the H1 haplotype and PD involving British [40], Swedish [41] and Taiwanese [42] populations (reviewed: [16]).

The *TCEANC2* is one of the genes located in the *PARK10* region on chromosome 1. The gene spans approximately 58 kilobases and contains 6 exons. The exact function of TCEANC2 is still unknown. Data suggest its involvement in RNA processing [18]. The relationship of the *PARK10* locus and PD was first described in a large Icelandic family [17]. Since then, a LD block of 100 kilobase in this region was found to be associated with the disease [18]. The SNP rs10789972, located in the *TCEANC2* gene, was found to show the strongest association with sporadic PD in American population [18]. However, associaton between the variant and PD has not been found in Han-Chinese population [19][20]. Our findings do not indicate association of the variant and PD, however, further studies focusing on elucidating this question are strongly warranted.

5. Conclusions

A growing body of evidence suggests contribution of genetic factors in the development of PD. Besides the well established pathogenic mutations, several gene variants have been proposed to be risk factors, or, on the contrary, to play a protective role in the disease. Such assumptions are mainly based on genome wide association studies. The heterogeneity of the study groups included in these studies might cover frequency differences that might exist among different populations in respect of specific gene variants. Therefore it is important that findings of GWA studies are tested in specific populations. Our results are from the first comprehensive study focusing on the *LRRK2*, *SNCA*, *MAPT* and *PARK10* risk and protective variants in the Hungarian population. We believe that these results represent a valuable contribution to the evaluation of the world wide significance of these genetic variants.

6. Conflict of interest

The authors declare no conflict of interest.

7. Acknowledgements and funding

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Table 1.

Gene		Total number of participiants	Age (mean±SD; years)	Male/female ratio	Disease onset (EOPD/LOPD ratio)
LRRK2	PD	124	66,5 ± 9,5	61/63	68/56
	Control	128	64,5 ± 9,6	61/67	
SNCA and *MAPT*	PD	123	66,5 ± 9,5	60/63	67/56
	Control	122	64,3 ± 8,8	56/66	
TCEANC2	PD	121	66,5 ± 9,6	59/62	66/55
	Control	113	64,9 ± 8,1	50/60	

Demographic data of the study groups. Abbreviations: PD: Parkinson's Disease; EOPD: early-onset Parkinson's Disease; LOPD: late-onset Parkinson's Disease; SD: standard deviation.