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Association between *AKT1* gene and Parkinson's disease: A protective haplotype

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Abstract

Variation in *AKT1* has been associated with schizophrenia, bipolar disease and type II diabetes. The aim of the present study was to investigate the potential role of variability within *AKT1* as a risk factor for Parkinson's disease (PD). We performed a case-control association analysis of *AKT1* in a Greek cohort of PD using four tagging SNPs and five constructed haplotypes. To assess the structure of this locus in different populations we have performed linkage disequilibrium (LD) analysis using these variants [dunning]. In multilocus analysis, the frequency of a four-SNP1/2/3/4 haplotype was significantly higher in controls in comparison with PD patients ($\chi^2 = 19.76$, $p = 0.00009$, OR= 0.11 C.I. = 0.03–0.35). The association remained significant even after Bonferroni correction for the number of haplotypes ($p = 0.0002$). So, this certain haplotype was significantly associated with reduced risk of the disease. The data presented here suggest the involvement of *AKT1* in protection of PD through many possible mechanisms involving different signaling pathways that could be potential therapeutic targets in the future.

Keywords

AKT1 ; Parkinson's disease; Association; Haplotypes

The murine thymoma viral oncogene homologue *AKT1*, also termed protein kinase B, is a serine/threonine protein kinase homologue to protein kinase A and C. *AKT1* is a downstream target of the insulin-signaling pathway with both anti-apoptotic and peripheral metabolic effects [16]; in addition, *AKT1* has been implicated as a mediator of the phosphoinositide signal transduction system and its activation generates phosphorylation of many cellular proteins that are involved in processes of metabolism, apoptosis and proliferation of neuronal cells [13,16]. Variation in *AKT1* has been associated with schizophrenia [11,19], bipolar disease [22] and type II diabetes [18].

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The aim of the present study was to investigate the potential role of variability within *AKT1* as a risk factor for Parkinson's disease (PD). We genotyped four SNPs (SNP1–4) in *AKT1* and studied the association of this variability at the single locus and haplotype level in a series of patients from Central Greece with PD. Here we provide initial data that suggest an association between *AKT1* haplotypes and risk for disease.

Two hundred and eighty one PD patients (121 women, 160men) and 220 healthy controls (96 women, 124men) age-, gender- and ethnicity-matched were included in the Greek cohort. All patients were residents of Thessaly (Central Greece) and were identified prospectively during a 3-year period (2001–2004) in the outpatient clinic for movement disorders in Larissa University Hospital and were followed up for at least 1 year and up to 3 years. PD patients were on average 69.8 ± 8.7 (range: 44–95) years old at time of initial examination, while their mean age-at-onset of disease was 63.3 ± 9.6 (range: 30–88). Healthy controls had a mean age of 68.3 ± 12.8 (range: 32–93). The diagnosis of PD was based on established criteria [9]. All patients were sporadic based on pedigree analysis. Patients with onset at or before 30 years were also excluded. Age-at-onset was defined as the age at which the patient noticed the first symptom indicative of PD. Controls were normal subjects living in the same geographical area as the patients who visited our outpatient clinic and finally were found free of any neurological disease (PD included). After approval from Hospital Scientific Committee and informed consent, blood samples were drawn for DNA extraction from patients and controls. Certified neurologists who were blind to genotyping results performed all clinical assessments (such as PD diagnosis, age at onset, etc.).

SNPs used in the present study were selected from 39 SNPs across the *AKT1* locus from a data dump of Caucasian data available through the international HapMap project web page (http://www.hapmap.org). A total of four tagging SNPs (tSNP) were identified using the genetic programme Tagger (http://www.broad.mit.edu/mpg/tagger/) covering almost 8 kb across *AKT1* (Table 1). The selected SNPs captured 85% of the genetic variation across the gene. One apparent block of linkage disequilibrium (LD) was identified containing the first three tagging tSNPs (Table 2).

Taqman ® Assays-by-Design SM SNP Genotyping (Applied Biosystems) based assays were employed for allelic discrimination of the four SNPs. Thermal cycling and end-point PCR analysis was performed on an ABI PRISM® 7900 Sequence Detection System and analysed with SDS software (Applied Biosystems).

Data was stored and manipulated for genetic analysis using the database GERON genotyping.

GERON genotyping was used to perform x^2 tests of association. Haplotype construction was performed using the Shesis program.

Pair-wise D', r^2, and Hardy-Weinberg equilibrium measurements were made using the program Arlequin 3.01.

Based on x-test, we applied a Bonferroni correction of x^2 and this was considered significant.

The genotype frequencies of all SNPs were at or near Hardy–Weinberg equilibrium. The selected SNPs captured a minimum of 85% of the genetic variation across the gene which is a common allelic variation that can be captured with this methodology. All studied SNPs showed a minor allele frequency of more than 5% for their minor alleles, which improves their comparability to common disease-susceptibility polymorphisms [4] and the power to detect LD [21]. Pair-wise LD is shown in Table 2. LD revealed a strong LD between SNPs 1, 2, 3 (p-values < 0.01 in all comparisons). We found no association between each SNP and PD (Table 3). The global tests of association taking into account all alleles were not significant. In multilocus analysis, the frequency of a four-SNP1/2/3/4 T T G G haplotype was significantly higher in controls (5.2%) in comparison with PD patients (0.6%) (χ^2 = 19.76, p = 0.00009, OR= 0.11 C.I. = 0.03–0.35). The association remained significant even after Bonferroni correction for the number of haplotypes (p = 0.0002). The four-SNP haplotypes and their frequencies (if the frequency >2%) are shown in Table 4.

Recent studies showed that the neuroprotection of β-synuclein against neurotoxins such as rotenone requires the activation of the Akt signaling pathway, a molecule centrally involved in neuronal survival and plasticity [7]. More specifically β-synuclein overexpression is associated with increased akt phosphorylation and this Kinase co-precipitates only with b-synuclein in the brains of tg mice. In the present study, we found that in multilocus analysis of *AKT1*, four SNP haplotype T-T-G-G was significantly associated with reduced risk of the disease.

Another study that shows very clearly the involvement of Akt in PD is by Seo J.H. et al. [20]. In this study, there is evidence that a-synuclein could have a protective effect against neurotoxicity mediated by activation of the pro-survival PI13K/Akt pathway followed by Bcl-2 family anti-apoptotic expression.

Akt phosphorylates and regulates a variety of proteins that have been implicated in cell survival, including the proapoptotic proteins Bad and caspase-9, protein kinases such as GSK3 and transcription factors [6,12]. All these Akt targets mediade cell survival. The central role of Akt that makes it a convergence point for diverse survival signals maybe could explain a possible role in the pathogenesis of PD.

Additional support for involvement of Akt in PD came from several studies that show that this is a candidate gene for schizophrenia [8,11]. From these studies it was concluded that modulation of the AKT/GSK3b signalling pathway might play a role in dopamine related disorders [5]. Numakawa et al. reported also a significantly suppressed phosphorylation of AKT in primary cortical neuronal cultures after silencing of another gene, named, dysbindin [17]. A dopamine-induced dysbalance of basal ganglia neurocircuitries may be an important pathophysiological component in PD and schizophrenia [14]. There is a link between these two different entities and a lot of genes have been studied in both diseases with controversial results. Polymorphisms in PHOX2M, a gene for a transcription factor that plays an important role in the development of catecholaminergic neurons and regulates the expression of both tyrosine hydroxylase and dopamine β-hydroxylase genes have been also associated with both PD and schizophrenia [10]. Other genes, like Nurr1 [2], adenosine A2 receptor and calcitoni/alpha-CGRP have presented conflicting associations [1].

A major finding that supports the protective role of certain haplotypes in this gene against PD is that a specific haplotype in *AKT1* is relatively resistant to apoptosis through p53 pathway [6] P53 signaling mediates apoptosis in dopaminergic cells [15] and has been implicated in the death of neurons observed in experimental models of PD [3] supporting the importance of the previous finding. The set of SNPs that were studied [6] were different from the SNPs that we used in the present study but they support our basic finding. Maybe these SNPs that we have studied are in LD with a functional SNP that has not been detected yet. The need for screening more SNPs and performing functional studies is obvious.

The data presented here suggest the involvement of *AKT1* in protection of PD through many possible mechanisms involving different signaling pathways that could be potential therapeutic targets in the future.

References

[1]. Buervenich S, Xiang F, Sydow O, Jonsson EG, Sedvall GC, Anvret M, Olson L, Identification of four novel polymorphisms in the calcitonin/alpha-CGRP (CALCA) gene and an investigation of their possible associations with Parkinson disease, schizophrenia, and manic depression, Hum. Murat 17 (2001) 435–436.

[2]. Carmine A, Buervenich S, Galter D, Jonsson EG, Sedvall GC, Farde L, Gustavsson JP, Bergman H, Chowdari KV, Nimgaonkar VL, Anvret M, Sydow O, Olson L, NURR1 promoter polymorphisms: Parkinson's disease, schizophrenia, and personality traits, Am. J. Med. Genet. B Neuropsychiatr. Genet 120 (2003) 51–57.

[3]. Duan W, Zhu X, Ladenheim B, Yu QS, Guo Z, Oyler J, Cutler RG, Cadet JL, Greig NH, Mattson MP, p53 inhibitors preserve dopamine neurons and motor function in experimental parkinsonism, Ann. Neurol 52 (2002) 597–606. [PubMed: 12402257]

[4]. Dunning AM, Durocher F, Healey CS, Teare MD, McBride SE, Carlomagno F, Xu CF, Dawson E, Rhodes S, Ueda S, Lai E, Luben RN, Van Rensburg E,J, Mannennaa A, Kataja V, Rennart G, Dunham I, Purvis I, Easton D, Ponder BA, The extent of linkage disequilibrium in four populations with distinct demographic histories. Am. J. Hum. Genet 67 (2000) 1544–1554. [PubMed: 11078480]

[5]. Emamian ES, Hall D, Birnbaum MJ, Karayiorgou M, Gogos JA, Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia, Nat. Genet 36 (2004) 131–137. [PubMed: 14745448]

[6]. Harris SL, Gil G, Robins H, Hu W, Hirshfield K, Bond E, Bond G, Levine AJ, Detection of functional single-nucleotide polymorphisms that affect apoptosis, Proc. Natl. Acad. Sci. U.S.A 102 (2005) 16297–16302. [PubMed: 16260726]

[7]. Hashimoto M, Bar-On P, Ho G, Takenouchi T, Rockenstein E, Crews L, Masliah E, Beta-synuclein regulates Akt activity in neuronal cells.A possible mechanism for neuroprotection in Parkinson's disease, J. Biol. Chem 279 (2004) 23622–23629. [PubMed: 15026413]

[8]. J Hong C, Liu HC, Liu TY, Liao DL, Tsai SJ, Association studies of the adenosine A2a receptor (1976T > C) genetic polymorphism in Parkinson's disease and schizophrenia, J. Neural. Transm 112 (2005) 1503–1510. [PubMed: 15719154]

[9]. Hughes AJ, Daniel SE, Kilford L, Lees AJ, Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases, J. Neural. Neurosurg. Psychiatry 55 (1992) 181–184.

[10]. Ide M, Yamada K, Toyota T, Iwayama Y, Ishitsuka Y, Minabe Y, Nakamura K, Hattori N, Asada T, Mizuno Y, Mori N, Yoshikawa T, Genetic association analyses of PHOX2B and ASCL1 in neuropsychiatric disorders: evidence for association of ASCL1 with Parkinson's disease, Hum. Genet 117 (2005) 520–527. [PubMed: 16021468]

[11]. Ikeda M, Iwata N, Suzuki T, Kitajima T, Yamanouchi Y, Kinoshita Y, Inada T, Ozaki N, Association of AKT1 with schizophrenia confirmed in a Japanese population, Biol. Psychiatry 56 (2004) 698–700. [PubMed: 15522255]

[12]. Kim AH, Yano H, Cho H, Meyer D, Monks B, Margolis B, Birnbaum MJ, Chao MV, Akt1 regulates a JNK scaffold during excitotoxic apoptosis, Neuron 35 (2002) 697–709. [PubMed: 12194869]

[13]. Lin CH, Yeh SH, Lu KT, Leu TH, Chang WC, Gean PW, A role for the PI-3 kinase signaling pathway in fear conditioning and synaptic plasticity in the amygdala, Neuron 31 (2001) 841–851. [PubMed: 11567621]

[14]. Mehler-Wex C, Riederer P, Gerlach M, Dopaminergic dysbalance in distinct basal ganglia neurocircuits: implications for the pathophysiology of Parkinson's disease, schizophrenia and attention deficit hyperactivity disorder, Neurotox. Res 10 (2006) 167–179. [PubMed: 17197367]

[15]. Nair VD, Activation of p53 signaling initiates apoptotic death in a cellular model of Parkinson's disease, Apoptosis 11 (2006) 955–966. [PubMed: 16544096]

[16]. Nicholson KM, Anderson NG, The protein kinase B/Akt signalling pathway in human malignancy, Cell Signal. 14 (2002) 381–395. [PubMed: 11882383]

[17]. Numakawa T, Yagasaki Y, Ishimoto T, Okada T, Suzuki T, Iwata N, Ozaki N, Taguchi T, Tatsumi M, Kamijima K, Straub RE, Weinberger DR, Kunugi H, Hashimoto R, Evidence of novel neuronal functions of dysbindin, a susceptibility gene for schizophrenia. Hum. Mol. Genet 13 (2004) 2699–2708. [PubMed: 15345706]

[18]. Otieno CJ, Bastiaansen J, Ramos AM, Rothschild MF, Mapping and association studies of diabetes related genes in the pig, Anim. Genet 36 (2005) 36–42. [PubMed: 15670129]

[19]. Schwab SG, Hoefgen B, Hanses C, Hassenbach MB, Albus M, Lerer B, Trixier M, Maier W, Wildenauer DB, Further evidence for association of variants in the AKT1 gene with schizophrenia in a sample of European sib-pair families, Biol. Psychiatry 58 (2005) 446–450. [PubMed: 16026766]

[20]. Seo JH, Rah JC, Choi SH, Shin JK, Min K, Kim HS, Park CH, Kim S, Kim EM, Lee SH, Lee S, Suh SW, Suh YH, Alpha-synuclein regulates neuronal survival via Bcl-2 family expression and Pl3/Akt kinase pathway, FASEB J. 16 (20021826-) 8.

[21]. Terwilliger JD, Zollner S, Laan M, Paabo S, Mapping genes through the use of linkage disequilibrium generated by genetic drift: 'drift mapping' in small populations with no demographic expansion, Hum. Hered 48 (1998) 138–154. [PubMed: 9618061]

[22]. Toyota T, Yamada K, Detera-Wadleigh SD, Yoshikawa T, Analysis of a cluster of polymorphisms in AKT1 gene in bipolar pedigrees: a family-based association study, Neurosci. Lett 339 (2003) 5–8. [PubMed: 12618287]

Table 1

SNPs genotyped in the current study

SNP	rs number	Chromosome position	Minor allele (frequency)	Tagging/coding
1	rs2494743	85306793	C (0.092)	Tagging/intronic
2	rs2498788	85308082	T (0.083)	Tagging/intronic
3	rs2494746	85312792	C (0.083)	Tagging/intronic
4	rs1130214	85314807	T (0.275)	Tagging/intronic

Minor allele frequency and chromosome position are based on the NCBI reference sequence in dbSNP build 124.

Table 2

Pair-wise linkage disequilibrium measurements using *D*' and r^2

D' / r^2	SNP1	SNP2	SNP3	SNP4
SNP1		0.901	0.939	0.895
SNP2	0.152*		1.00	0.993
SNP3	0.832**	0.130**		1.00
SNP4	0.025*	0.003**	0.025**	

* *p*-value < 0.01.

** *p*-value < 0.001.

Table 3

Allele frequencies and *p*-values of single-locus association in the study

		Controls	Cases	x^2	*p*-value	Odds ratio (C.I: 95%)
rs2494743						
Genotype	T/T	164	206			
	T/C	46	63			
	C/C	5	12	1.54	0.46	
Allele	T	374	475			
	C	56	87	1.19	0.27	1.22 (0.85–1.75)
rs2498788						
Genotype	C/C	168	231			
	C/T	47	46			
	T/T	3	0	6.01	0.049	
Allele	C	383	508			
	T	53	46	4.02	0.044	1.52 (1.0–2.32)
rs2494746						
Genotype	G/G	156	203			
	G/C	51	65			
	C/C	4	11	1.70	0.42	
Allele	G	471	363			
	C	87	59	0.49	0.48	1.13 (0.79–1.62)
Rs1130214						
Genotype	G/G	120	154			
	G/T	77	100			
	T/T	17	22	0.003	0.99	
Allele	G	317	408			
	T	111	144	0.002	0.95	0.99 (0.74–1.32)

Odds ratios and their 95% confidence intervals are presented for the minor allele versus the major allele for all ht SNPs.

Table 4

Haplotype frequencies in PD patients and controls in studied populations

Haplotype ID	rs2494743	rs2498788	rs2494746	rs1130214	Case (%)	Control (%)	Chi-square	*p*-value	Odd ratio (95%C.I.)
A		C	G	G	59	55.8	1.0	0.31	1.14 (0.88–1.48)
B	T	C	G	T	23.8	21.5	0.65	0.41	1.13 (0.83–1.54)
C	C	C	C	G	7.4	7.9	0.085	0.77	0.93 (0.57–1.50)
D	C	T	C	G	6.1	3.4	3.55	0.059	1.83 (0.96–3.49)
F	T	T	G	G	0.6	5.2	19.76	0.00009	0.11 (0.03–0.35)
					Global x^2 (df = 4) 25.94		Fisher's *p*-value 0.0000069		