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BRIEF COMMUNICATION

Association of *HIF1A* and Parkinson's disease in a Han Chinese population demonstrated by molecular inversion probe analysis

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Abstract

Parkinson's disease (PD) is a common neurodegenerative disorder with multiple factors contributing to disease pathogenesis. Previous studies implicated the involvement of the transcription factor hypoxia inducible factor 1 alpha (HIF1A) in PD through its transcriptional regulation of PD-associated genes. This study uses molecular inversion probes (MIPs) followed by high-throughput sequencing for the genetic analysis of *HIF1A* in a large cohort including 1692 ethnic Han Chinese PD patients and 1419 neurologically normal control subjects matched for age, gender, and ethnicity. Common *HIF1A* variant rs11549465 was found to be associated with increased late-onset PD (LOPD) risk (OR (95%CI) = 1.531(1.068–2.194), $P = 0.03828$ for trend test, $P = 0.03948$ for analyses using the allelic model and $P = 0.04196$ for logistic regression analyses (sex + age as covariates)). Though the gene-based variants burden test is negative, seven rare non-synonymous, predicted-pathogenic point variants were identified. In conclusion, our study further indicates that HIF1A plays a role in PD pathogenesis.

Keywords *HIF1A* · Molecular inversion probes · rs11549465 · Parkinson's disease

Introduction

Parkinson's disease (PD) is a common neurodegenerative disease, affecting more than 1% of the population over 60 years of age [1]. As a genetically complex disease, PD is associated with multiple genetic risk factors [2]. Monogenic PD with mutations in the known risk factors *SNCA*, *LRRK2*, and *PINK1* accounts for only 5–10% of PD patients, suggesting that numerous other susceptibility loci are yet to be found. Current PD research focuses on the mechanistic analysis of discovered PD genes and variants as well as the identification of potential new PD candidate genes.

Hypoxia inducible factor 1 alpha (HIF1A), encoded by the *HIF1A* gene, is a transcriptional activator of various genes related to adaptive cellular responses to hypoxia [3]. A large body of research has demonstrated that HIF1A plays an important role in the pathogenesis of multiple diseases including leukemia, immune response dysregulation, infectious disease, trauma, neurodegeneration, and cancer [4, 5]. HIF1A alters the expression of target genes involved in critical molecular

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and cellular processes, such as angiogenesis, iron metabolism, cell proliferation, and energy metabolism, which can contribute to disease pathogenesis when aberrant.

The transcription factor HIF1A can be further attributed in PD pathogenesis through its modulation of downstream gene expression. For example, HIF1A can upregulate the expression of *ATP13A2* by binding to its promotor region [6]. The presence of HIF1A rescues *DJ-1*-deficient neurons following 1-methyl-4-phenylpyridinium (MPP+) treatment [7]. Furthermore, dysfunction in the HIF1A pathway is involved in phosphatase and tensin homolog (PTEN)-induced putative kinase 1(PINK1)-associated PD pathogenesis [8]. Additionally, HIF1A increases the transcription of the tyrosine hydroxylase (TH) gene, which encodes an important dopamine rate-limiting enzyme in the biosynthesis of dopamine, by binding to cis-acting regulatory elements during hypoxia [9].

In order to assess the importance of HIF1A in PD and to determine whether the *HIF1A* gene is associated with PD risk, we performed a genetic analysis of *HIF1A* in a large Chinese PD cohort.

Materials and methods

The study was conducted in accordance with the declaration of Helsinki and was approved by the Ethics Committee of Xiangya Hospital. Details of subjects, genomic DNA isolation, molecular inversion probe (MIP) design, capture, and sequencing; quality control; raw data processing; and variant validation, bioinformatics, and statistical analysis are included in Supplementary Materials and Methods.

Results

A MIP-based targeted sequencing assay of *HIF1A* in Han Chinese (1692 sporadic PD patients and 1419 healthy controls) identified a total of 45 single nucleotide variations (SNVs) within exons (Supplementary Table 1), among which 2 SNVs (rs11549465 and rs11549467) were common variants with MAF > 0.01. As for rare SNVs, there were 28 rare non-synonymous SNVs, all of which were heterozygous. The frequency of rare non-synonymous *HIF1A* variants in patients was 4.0% (68/1692), and 3.5% (49/1419) in healthy controls. The location of common variants and 7 rare non-synonymous SNVs predicted to be damaging by in silico prediction tools is presented in Fig. 1.

Rs11549465 in *HIF1A* was associated with increased LOPD risk

Two SNPs (rs11549465 and rs11549467) with MAF > 0.1 were found in *HIF1A*. The SNPs were in accordance with

Table 1 The association of common variants with PD and LOPD

SNPs	MAF*	Group	Genotype *n* %			P_{HWE}	MAF	Allelic model				Trend test		Logistic regression (sex + age covariate)			
			TT	TC	CC			OR	L95 OR	U95 OR	*P* value adjusted by FDR	*P* value adjusted by BONF	*P* value adjusted by FDR	*P* value adjusted by BONF	*P* value adjusted by BONF	*P* value adjusted by FDR	*P* value adjusted by BONF	*P* value adjusted by FDR
rs11549465	8.7652%	Patients total	1545	145	2	0.46	0.044	1.199	0.9291	1.547	0.1588	0.3252	0.1626	0.322	0.3176	0.161		
		Controls total	1315	103	1	0.48	0.037											
		LOPD	648	70	1	0.53	0.05	**1.531**	**1.068**	**2.194**	**0.03828**	**0.03948**	**0.03948**	**0.04196**	**0.03828**	**0.04196**		
		Controls ≥ 50 y	758	52	1	0.91	0.033											
			AA	AG	GG													
rs11549467	0.6074%	Patients total	1547	145	0	0.07	0.043	1.239	0.9545	1.607	0.1588	0.2139	0.1626	0.2046	0.1995	0.161		
		Controls total	1320	99	0	0.17	0.035											
		LOPD	660	59	0	0.25	0.041	1.292	0.8836	1.888	0.177	0.3706	0.1853	0.44	0.354	0.22		
		Controls ≥ 50 y	759	52	0	0.35	0.032											

PD, Parkinson's disease; LOPD, late-onset PD; SNPs, single nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; OR, odds ratio; L95 OR and U95 OR, lowest and highest OR; BONF, Bonferroni; FDR, false discovery rate. Bold data represented the statistical significant results. *, indicates MAF at Exome Variant Server

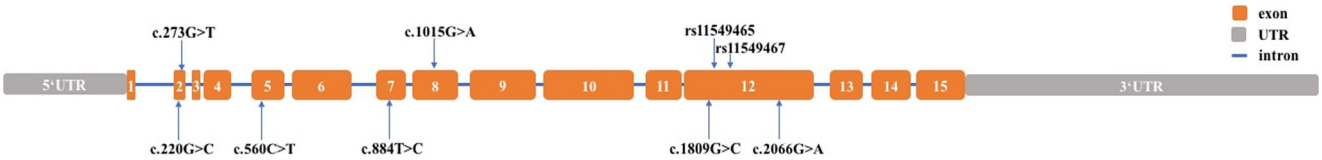

Fig. 1 Schematic representations of HIF1A with orange boxes indicating exons 1–15. Common variants and 7 rare non-synonymous SNVs predicted to be damaging by in silico prediction tools were indicated by arrows

the Hardy-Weinberg equilibrium in PD patients and health controls. We found that the SNP rs11549465 was associated with increased LOPD risk ($P = 0.03828$ for the Trend test, $P = 0.03948$ for the allelic model, $P = 0.04196$ for logistic regression (sex + age covariate), odds ratio (OR) (95% CI) = 1.531 (1.068–2.194)). There was no association between rs11549467 and PD (Table 1).

Rs11549465 was a non-synonymous SNV located in exon 12. The MAFs for rs11549465 were 0.04285 in PD patients and 0.05007 in LOPD, which are similar to values in the gnomAD database (MAF = 0.04330 in the East Asian population). By eQTL analysis with the Braineac database, we found the eQTLs to be related to rs11549465 for *SYT16* ($P = 2.60E-02$ in the whole brain, $P = 1.50E-03$ in cerebellum), *HIF1A* ($P = 1.90E-03$ or $2.10E-03$ in cerebellum), *PRKCH* ($P = 3.50E-02$ in hippocampus, $P = 2.20E-03$ in medulla), *MNAT1* ($P = 2.70E-03$ in medulla), *MAD2L1* (P = 2.70E−03 in medulla), *FLJ43390* ($P = 4.60E-03$, $6.70E-03$, or $8.90E-03$ in cerebellum), *FLJ22447* ($P = 7.30E-03$ in medulla), and *KCNH5* ($P = 1.00E-02$ in cerebellum) (Table 2). Moreover in the GTEx database, *HIF1A* eQTLs related to rs11549465 and were significantly different in the frontal cortex ($P = 0.02$) (Table 3).

Rare predicted pathogenic SNVs

Seven out of 28 rare non-synonymous SNVs were predicted to carry disease risk by in silico prediction tools (Table 4). Among these, the SNVs c.T884C and c.G1015A have not been reported previously. Notably, c.G1809C was only detected in PD patients. Three cases with c.G1737C were classified as early-onset PD (EOPD) patients. They presented with resting tremor, bradykinesia, and rigidity in motor symptoms. In terms of non-motor symptoms, none of the three PD patients had cognitive impairment, rapid eye movement sleep behavior disorder (RBD), hyposmia, or constipation. All responded well to the dopamine promoter drug levodopa. Detailed clinical findings are summarized in Supplementary Table 2.

A gene-based variants burden test was accomplished to analyze the multiple variants identified in this study. The results do not support a positive association between *HIF1A* and PD in this Han Chinese population ($P = 0.804$, OR (95%CI) = 0.838 (0.314–2.238)).

Discussion

Even though PD is the most prevalent movement disorder, the mechanistic basis for the pathogenesis of PD remains elusive. However, genetic factors do play a central role in PD. Causative genes such as *SNCA*, *LRRK2*, *DJ-1*, and risk genes such as *GBA* have been demonstrated to contribute to PD development [2]. HIF1A has been found to be involved in the oxidative stress associated with PD pathogenesis and has been shown to interact with multiple PD causative genes such as *DJ-1*, *PINK1*, and *ATP13A2*. Therefore, we conducted a genetic analysis of *HIF1A* in a Han Chinese PD cohort with a sample size of more than 1000 PD patients and controls.

By analysis of common variants, we found that *HIF1A* rs11549465 was associated with increased risk for PD in the LOPD cohort. Some eQTLs of rs11549465 were identified in brain transcripts. Moreover, in our analysis, we found a predicted pathogenic non-synonymous rare variant, c.G1809C, in three PD patients but not in the controls. This has not been reported previously in an East Asian population.

With regard to a possible molecular mechanism, HIF1A can bind to hypoxia-response elements (HREs) in the promoter region of genes. As a transcription factor, it could regulate the expression of PD predicted pathogenic genes such as *DJ-1*, *PINK1*, and *ATP13A2* [6–8]. Moreover, gene dysfunction could generate excessive reactive oxygen species (ROS), contributing to PD pathogenesis [10]. We speculated that variants of *HIF1A* may be involved in PD pathogenesis either by affecting the expression of genes or downstream PD pathogenic genes, modulating the ROS pathway.

However, there are limitations to this study. Although our analyses included a large sample of PD patients and is the first population-based study of the role of *HIF1A* in PD, future population-based validation analyses and functional assessments are needed to elucidate the pathogenic, mechanistic-relationship of *HIF1A* to PD. Additionally, this analysis was a cross-sectional case-control study. Future prospective multicenter trials are necessary to fully elucidate the role for *HIF1A* in PD.

In conclusion, this genetic analysis of *HIF1A* in a large Chinese cohort suggests its possible involvement in PD pathogenesis.

Table 2 eQTLs related to rs11549465 in Braineac

ID	Gene	Chr	Start	End	AveAll	CRBL	FCTX	HIPP	MEDU	OCTX	PUTM	SNIG	TCTX	THAL	WHMT
3539239	*SYT16*	chr14	62278712	62568427	**2.60E−02**	**1.50E−03**	1.50E−01	2.10E−01	1.30E−01	6.60E−01	3.70E−01	7.60E−01	8.80E−01	1.70E−01	9.10E−01
3539075	*HIF1A*	chr14	62155742	62215469	8.10E−01	**1.90E−03**	2.30E−01	7.60E−01	4.00E−01	1.50E−01	2.90E−01	2.70E−01	8.20E−01	3.90E−01	5.40E−01
3539080	*HIF1A*	chr14	62155742	62215469	8.10E−01	**2.10E−03**	3.70E−01	3.50E−01	3.20E−01	3.40E−01	9.90E−01	9.00E−01	2.50E−01	6.90E−01	7.50E−01
3538956	*PRKCH*	chr14	61747205	62017687	1.20E−01	7.60E−01	5.40E−01	**3.50E−02**	**2.20E−03**	5.90E−01	7.20E−01	8.00E−01	1.10E−01	4.10E−01	7.70E−01
3538763	*MNAT1*, *MAD2L1*	chr14	61201460	61435480	6.60E−01	5.70E−01	4.70E−01	3.50E−01	**2.70E−03**	2.60E−01	9.90E−01	4.30E−01	4.40E−01	5.30E−01	6.50E−01
3539317	*FLJ43390*	chr14	62581091	62600898	5.00E−01	**4.60E−03**	5.10E−01	6.00E−01	7.70E−01	8.60E−01	8.80E−01	5.40E−01	5.20E−01	3.60E−01	6.30E−01
3539309	*FLJ43390*	chr14	62581091	62600898	5.20E−01	**6.70E−03**	4.50E−01	8.90E−01	9.30E−01	9.80E−01	7.40E−01	7.50E−01	9.40E−01	5.70E−01	2.70E−01
3539051	*FLJ22447*	chr14	62037188	62125404	5.20E−01	9.40E−01	7.30E−01	9.60E−01	**7.30E−03**	7.50E−01	8.10E−01	4.90E−01	7.80E−02	8.30E−01	1.70E−01
3539319	*FLJ43390*	chr14	62581091	62600898	8.50E−01	**8.90E−03**	2.80E−01	7.20E−01	4.80E−01	8.40E−01	8.90E−01	8.70E−01	2.80E−01	5.80E−01	6.60E−01
3567904	*KCNH5*	chr14	63089146	63568752	1.40E−01	**1.00E−02**	4.50E−01	3.40E−01	3.90E−01	1.00E+00	7.90E−01	1.80E−01	5.90E−01	5.50E−01	4.30E−01

The bold words represented the statistical significant results

AveAll, average across all 10 regions; CRBL, cerebellum; FCTX, frontal cortex; HIPP, hippocampus; MEDU, medulla; OCTX, occipital cortex; PUTM, putamen; SNIG, substantia nigra; TCTX, temporal cortex; THAL, thalamus; WHMT, white matter

Table 3 eQTLs related to rs11549465 in GTEx

Gene	*P* value	Effect size	*T* statistic	Standard error	Tissue
HIF1A	0.52	− 0.065	− 0.65	0.1	Caudate (basal ganglia)
HIF1A	0.29	− 0.14	− 1.1	0.13	Amygdala
HIF1A	0.051	− 0.22	− 2	0.11	Anterior cingulate cortex (BA24)
HIF1A	0.73	0.04	0.35	0.11	Cerebellar hemisphere
HIF1A	0.7	− 0.035	− 0.39	0.089	Cerebellum
HIF1A	0.54	− 0.056	− 0.61	0.091	Cortex
HIF1A	0.02	− 0.23	− 2.4	0.098	Frontal cortex (BA9)
HIF1A	0.6	0.066	0.52	0.13	Hippocampus
HIF1A	0.6	− 0.073	− 0.52	0.14	Hypothalamus
HIF1A	0.52	− 0.08	− 0.65	0.12	Nucleus accumbens (basal ganglia)
HIF1A	0.2	− 0.13	− 1.3	0.1	Putamen (basal ganglia)
HIF1A	0.89	0.019	0.14	0.13	Spinal cord (cervical c-1)
HIF1A	0.77	0.034	0.3	0.11	Substantia nigra

Table 4 Rare pathogenic variants in PD patients and controls

Variants	Heterozygous/homozygous	SIFT	Polyphen2	MutationTaster	1000G	ExAC	gnomAD	dbSNP ID	PD	Controls	Gene burden test
NM_001243084.1:c.220G > C	Heterozygous	Damaging	Probably damaging	Disease causing	0.000998403	0.00182	1.969E−3	rs61755705	0	1	*P* value = 0.804 OR (95%CI) = 0.838 (0.314–2.238)
NM_001243084.1:c.273G > T	Heterozygous	Damaging	Probably damaging	Disease causing	-	8.24E−06	3.312E−5	rs765076295	1	1	
NM_001243084.1:c.560C > T	Heterozygous	Damaging	Probably damaging	Disease causing	-	3.30E−05	4.139E−6	rs770277757	4	3	
NM_001243084.1:c.884 T > C	Heterozygous	Damaging	Probably damaging	Disease causing	-	-	-	-	0	1	
NM_001243084.1:c.1015G > A	Heterozygous	Damaging	Probably damaging	Disease causing	-	-	-	-	0	1	
NM_001243084.1:c.1809G > C	Heterozygous	Damaging	Possibly damaging	Disease causing	-	-	4.061E−6	rs574603732	3	0	
NM_001243084.1:c.2066G > A	Heterozygous	Damaging	Probably damaging	Disease causing	-	-	3.229E−5	Novel	0	1	

1000G, 1000 genomes; ExAC, exome aggregation consortium; gnomAD, Genome Aggregation Database; CI = confidence interval

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Compliance with ethical standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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