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Single Nucleotide Polymorphisms of PARKIN Gene in Ten Indian Populations

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Abstract

Parkinson's disease (PD) is the second most common progressive neurodegenerative brain disorder after Alzheimer's disease. Due to the complex etiology of PD, there is possibility that single nucleotide polymorphisms (SNP) in PARKIN gene could be associated with the disease and lead to the pathogenesis by genoenvironmental interactions. Role of PARKIN polymorphisms as risk factors varies in different populations among various ethnic groups. Indian populations, known for their rich diversity, are not included in the genotyping of single nucleotide polymorphisms in the global survey for all the genes associated with PD. Further detailed study in this field will give a greater insight to analyze the haplotypic and Linakage Disequilibrium (LD) and decipher the pathogenesis of PD patterns in this region. A total of 1000 individuals belonging to ten ethnic populations of India were included in the present study. Five PARKIN gene polymorphisms (rs1801474, rs72480421, rs1801582, rs1801334 and rs35125035) were screened by PCR and sequencing. The present study shows that the rs72480421 (His200Gln) is monomorphic for all populations. Five major haplotypes accounted for almost all chromosomes (90-98%) in all populations studied. LD was more fragmented across PARKIN locus in all populations. The haplotype diversity and the fragmented LD across PARKIN gene in all populations of the present study suggest the existence of frequent recombination within the large introns of the PARKIN gene.

Key Words: allelic variation, linkage disequilibrium, *PARKIN*, SNP.

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Introduction

Parkinson's disease (PD) is the second most common progressive neurodegenerative brain disorder after Alzheimer's disease affecting at least 2% of the world population above 65 years and up to 3 to 5% of people 85 years of age and older (Alves et al. 2008). As these demographic age groups are growing rapidly due to general aging of the population and increasing life spans, neurodegenerative diseases will represent an ever-growing economic and social burden for society (Dowding et al. 2006; Winter et al. 2009). Due to the observation that only 15 to 20% of PD patients have a clear positive family history of PD, research shows that majority of PD patients have a complex etiology (Lang et al. 1998) including both genetic and environmental component (Hardy et al. 2003). In India, PD is much higher among the Parsis (328 per 100,000) living in India (Bharucha et al. 1987; Bharucha et al. 1988; Singhal et al. 2003).

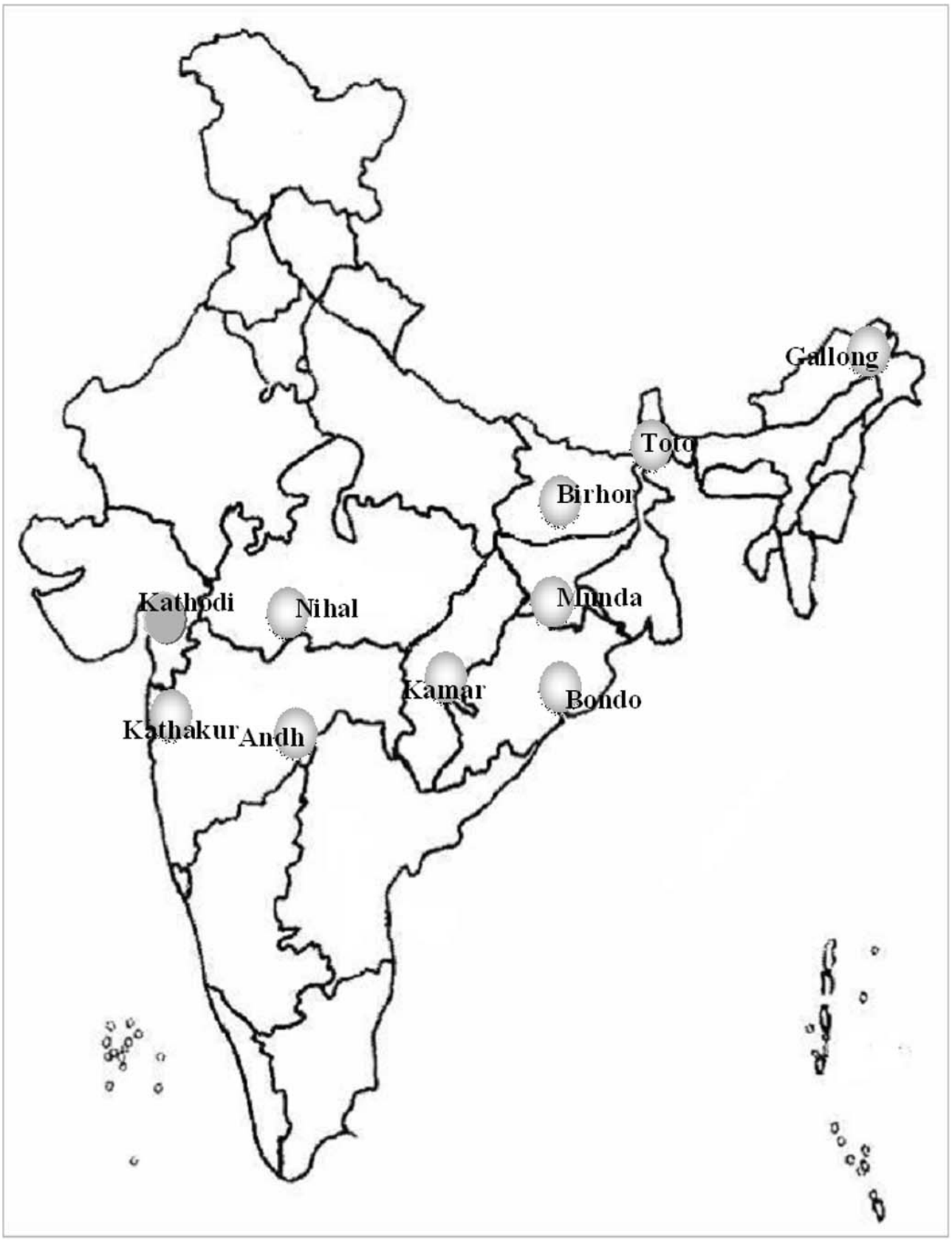
Among all the genes causal to PD, largest numbers of mutations have been detected in the PARKIN gene (PARK2 locus). In context to the case-control data from Eastern India, research study shows that pathogenic mutations for PD are absent in LRRK2 and DJ-1 (Sanyal et al. 2010), but the prevalence of PARKIN mutations is 7.24% among the PD patients (Biswas et al. 2006). However, the role of PARKIN polymorphisms as risk factors for PD still needs to be explored because of apparent inconsistency in association with the disease studied in different populations, which however, might result from overall genomic variation in the ethnic groups (http://alfred.med.yale.edu). Till date, PARKIN mutations have been reported at different frequencies throughout worldwide with respect to case-control association studies in various population groups (Slominskii et al. 2003; Groen et al. 2004; Li et al. 2005; Ross et al. 2007; Deng et al. 2008; Lesage et al. 2008). But, there is a lack of knowledge of the type and frequency of polymorphisms in PARKIN gene among the endogamous tribal populations.

Unlike many other populations of the world, India consists of ethnically, geographically and genetically diverse populations, comprising of more than a billion people of four major linguistic lineages, consisting of 4693 communities with several 1000 endogamous groups (IGVC 2005). Although PARKIN gene was analyzed in 55 ethnic groups of India (Biswas et al. 2007), the smaller sample size that were used in each ethnic groups and the number of single nucleotide polymorphisms (SNPs) screened has limited the study to draw a definite inference. With an ongoing project on Parkinson's disease in Eastern India on patient-control cohort (data unpublished), in the present study, we examined five PARKIN gene polymorphisms (these SNPs were found in the case-control study) in 10 Indian populations to study the Indian gene pool.

Materials And Methods

As part of a larger effort to study genomic variation in Indian population, a total of 1000 adult individuals from three linguistic groups belonging to 10 ethnic groups who inhabit geographically diverse regions of India were selected. The identification of populations as well as collection of samples has been carried out with the help of trained anthropologists and community health workers. Names of the populations, sample sizes, linguistic affiliations and place of inhabitation are presented in Table 1 and the geographical location of the sampled area is shown in Figure 1 (supplementary material online). All the subjects were apparently normal healthy volunteers and no diagnosis of Parkinson's disease or related disorders performed on them. Following written informed consent from the participants, venipuncture on each study subjects was performed and blood specimen was collected into EDTA-Vacutainer (Beckton-Dickinson, Franklin Lanes, NJ, USA). Genomic DNA was extracted from all the participants using standard procedures (Sambrook et al. 1989). The institutional review board of the Anthropological Survey of India, Kolkata, approved this study.

Fig. 1


Gallong
Toto
Birhor
Kathodi
Nihal
Munda
Kamar
Bondo
Kathakur
Andh

PARKIN genotyping

Five PARKIN gene-specific primers were used to amplify the exons and adjacent flanking regions. PCR was carried out to in a total volume of 10.0 µl containing 40 -100 ng genomic DNA, 0.4 µM of each primer, 0.2 mM of each dNTP, 0.5 -1.5 mM of MgCl2 (as appropriate) and 0.5 unit of Taq polymerase (Invitrogen, Carlsbad, California) in a GeneAmp-9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). Annealing temperatures were calculated based on Tm of the primer pairs. The primer pairs were used to amplify the PARKIN exons 4,5,10,11 and 12. PCR amplified DNA fragments were resolved on 2% agarose gel and visualized by ethidium bromide staining. The PCR products were directly sequenced in forward and reverse direction using the Big Dye Terminator Cycle Sequencing Ready Reaction kit and an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Nucleotide changes were detected by comparing sequence obtained in chromatogram with the normal PARKIN gene sequence (GenBank Accession No. AB009973) using pair-wise BLAST (Tatusova et al. 1999) and SeqScape software v2.5.

Statistical Analysis

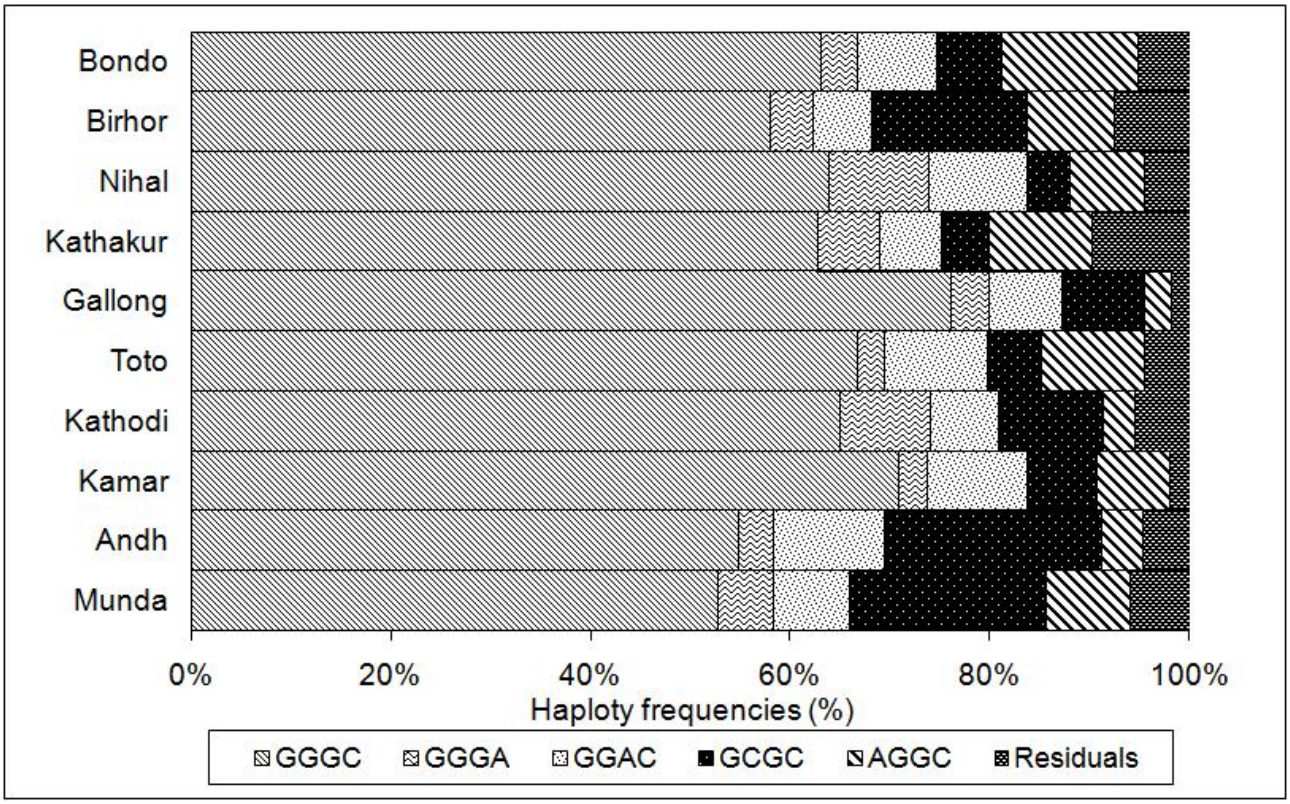
Allele frequencies at rs1801474 (Ser167Asn), rs72480421 (His200Gln), rs1801582 (Val380Leu), rs1801334 (Asp394Asn) and rs35125035 (3'UTR) were estimated by the gene counting method and their distribution was listed for Hardy-Weinberg Equilibrium by χ^2 test using the HWSIM programme (Cubells et al. 1997). Haplotype frequencies were established by Arlequin v2.0 (Schneider et al. 2000). The Haploview 3.12 (Barrett et al. 2005), with default settings was used to assess the Linkage disequilibrium (LD) (D' and r^2) between each pair of SNPs and also to define haploblocks.

Results

In the present study, we designated the wild and mutant alleles with their respective nucleotides for all the SNPs. For haplotype analysis, we used these designations in order from 5' to 3'. Table 2 shows the location, sequence and ancestral allele for the polymorphic sites and their NCBI reference IDs. Resulting population-specific individual allele frequencies and counts of genotypes are shown in Table 3.

The rs72480421 (His200Gln) was found to be monomorphic for all the samples and therefore was excluded from further analysis of this study. The four marker systems are polymorphic in all studied populations. The rs1801474 minor allele frequency is found to be greater than 3% in all study populations, with a minimum 3% (Gallong) and maximum of 17.5% in Kathakur populations. The rs1801582 site shows a minimum 6.6% (Nihal) and maximum 26% (Andh) minor allele frequencies. However, the rs1801334 and rs35125035 exhibited slightly less MAF than the above two SNPs. The MAF of rs1801334 is minimum 9% in Gallong and Kathodi and maximum 13.5% in Andh populations. The rs35125035 shows the minimum frequency of 4% in Gallong and maximum of 14% in Kathakur populations (Table 3). Hardy–Weinberg proportions for each of the four PARKIN sites are also presented in Table 3. Some population for rs1801474 (Andh, Toto and Kathakur) and rs1801582 (Kathodi, Kathakur, Nihal and Bondo) SNPs deviated significantly from Hardy-Weinberg equation. Four-site (rs1801474, rs1801582, rs1801334 and rs35125035) haplotype frequencies in the study populations are presented graphically in Figure 2.

Fig. 2

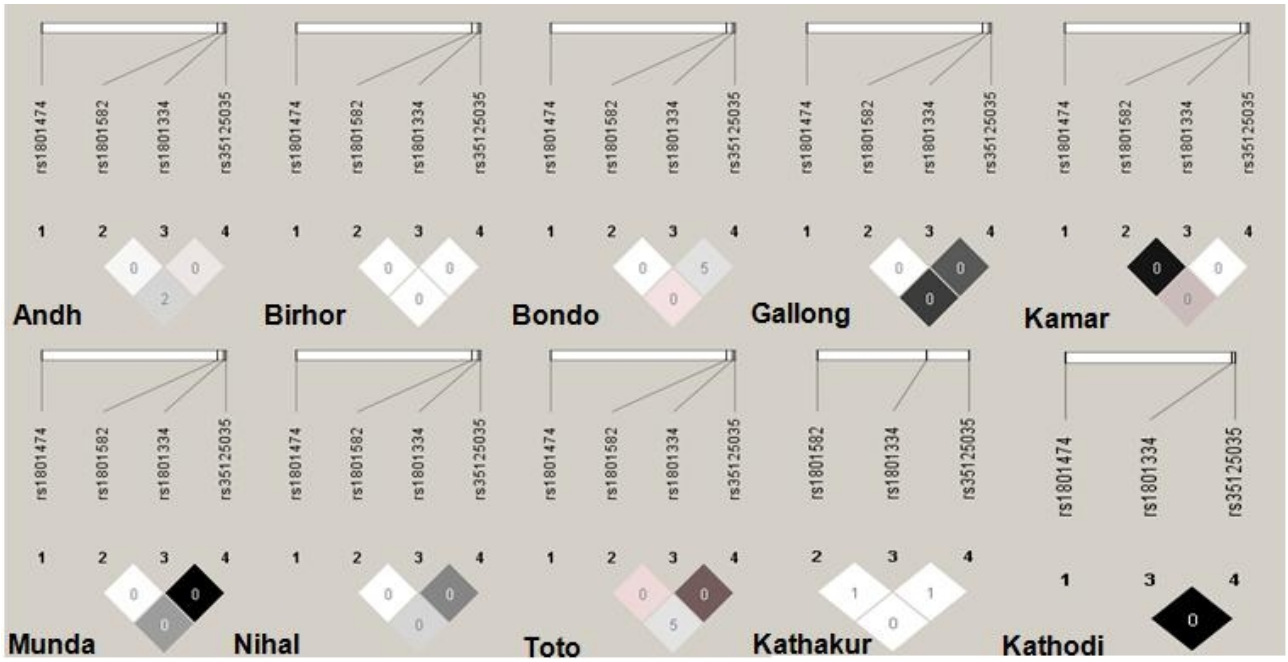


MAF of rs1801334 is the highest in Indian populations (0.109) than those of the other populations who participated in Hapmap project yielding a very high heterozygosity. However, with respect to rs1801474, MAF was found to be similar between YRI (0.102) and Indian populations (0.103). MAF of the variant rs1801582 is low in Indian populations (0.135) as compared to Luhya (0.218) and Maasai (0.196) of Kenya, Mexicans (0.224), Gujarati Indians (0.292), but higher than the Japanese (0.058), Hans Chinese (0.08) populations.

Out of sixteen possible haplotypes, five major haplotypes having frequencies greater than 5% accounted for almost all chromosomes (90–98%) in all populations studied. However haplotype frequencies showed striking variation among populations. In all populations, GGGGC is the major haplotype with the frequency ranging from 52.8% (Munda) to 76.2 (Gallong), GCGC was the second major haplotype in Birhor (15.6%), Munda (19.7%) and Andh (21.8%). The other haplotype with more than 5% frequency in at least one population are GGAC, GGGA and AGGC. The extremely uncommon haplotypes with the frequency less than 5.0% in all populations was considered as residuals. We evaluated pairwise LD for all four SNPs in all ten populations (Figure 3).

When measured by D', strong LD was observed between rs1801582 and rs1801334 in Kamar (D'=0.934), between rs1801582 and rs35125035 in Gallong (D'=0.81), between rs1801334 and rs35125035 in Munda (D'=1.0), Nihal (D'=0.577), Gallong (D'=0.712) Toto (D'=1.0). However, the r^2, which is the representative measure for differences in allele frequencies, LD, was more fragmented across the PARKIN locus in all populations (Figure 3). LD between rs1801474 and any other loci was not significant in any population.

Fig. 3



Discussion

As a part of overall larger goal to examine genomic variation in the Indian population, we selected five SNPs, which included four exonic non-synonymous SNPs. The present study shows that the rs72480421 (His200Gln) is monomorphic for all populations. The other polymorphic loci have shown lot of variation in their allele frequency spectrum. Some populations significantly deviated from HWE for rs1801474 and rs1801582. Haplotype distribution has shown remarkable variations among the populations, only five major haplotypes accounting for 90–98% of chromosomes in all populations. LD was more fragmented across the gene in all populations.

PARKIN gene is one of the largest genes in the human genome (2 million base pairs) and is located on chromosome six with 12 exons encoding 465 amino acid residues. PARKIN gene mutations are the most frequent cause of autosomal recessive Parkinson's disease in the populations from all over the world. Till date a wide variety of mutations in the PARKIN gene have been detected, including exon deletions and duplications, as well as point mutation (Klein et al. 2005; Madegowda et al. 2005; Biswas et al. 2006; Abbas et al. 1999; Chaudhary et al. 2006; Mena et al. 2008). A most recent study has identified a deletion hot spot between exons 2 and 5. Further analysis of the deletion breakpoints of the PARKIN mutations in 22 families with autosomal-recessive juvenile Parkinson's has identified 18 deletion breakpoints at the DNA nucleotide sequence level. Furthermore, a possible association between these deletion events and meiotic recombination was documented (Asakawa et al. 2009). In general, strong association between pairs of markers suggests lack of recombination, whereas weak association may be evidence for a history of active recombination between them, i.e. possible hotspots (Jeffreys et al. 2005). Analysis of Ser167Asn, Arg366Trp, Val380Leu, and Asp394Asn polymorphisms in 194 patients with Parkinson's patients and 125 control subjects of European origin did not find the LD between the last three SNPs (Lucking et al. 2003).

Although the present study includes only rs1801474 (Exon 4) and rs72480421 (Exon 5) are in the so-called deletion hot spot, remaining 3 SNPs are also separated by large introns. The distance between rs1801582 (exon 10) and rs1801334 (exon 11) is 26.6 kb, likewise between rs1801334 and rs35125035 (exon 12) is 10.1 kb. Contemporary ethnic populations of India are highly variable both culturally and

biologically. The origins of the genetically and culturally diverse populations of India have been subject to numerous genetic studies based on blood group, serum protein and red-cell enzyme polymorphisms (Cavalli-Sforza et al. 1994; Papiha 1996). Unfortunately, Indian populations, known for their rich diversity, are not included in the genotyping of single nucleotide polymorphisms in the global survey for all the genes associated with PD. Further detailed study in this field will give a greater insight to analyze the haplotypic and LD and decipher the pathogenesis of PD patterns in this region.

This study is an eye opener to associate the correlation of genetic variation with epidemiological data with respect to Indian population. The haplotype diversity and the fragmented LD across PARKIN gene in all populations of the present study are suggesting the existence of frequent recombination within the large introns of the PARKIN gene[1].

Table 1: Name of the population, their linguistic affiliation, geographic location and number of samples analyzed.

Sl. No.	Population name	Linguistic group	Geographic region	Total chromosomes
1	Munda	Austro-Asiatic	Jharkhand	200
2	Andh	Dravidian	Maharashtra	200
3	Kamar	Austro-Asiatic	Chattisgarh	200
4	Kathodi	Austro-Asiatic	Gujarat	200
5	Toto	Austro-Asiatic	West Bengal	200
6	Gallong	Tibeto Burman	Arunachal Pradesh	200
7	Kathakur	Austro-Asiatic	Maharashtra	200
8	Nihal	Austro-Asiatic	Madhya Pradesh	200
9	Birhor	Austro-Asiatic	Bihar	200
10	Bondo	Austro-Asiatic	Orissa	200

Table 2. SNP location, relative position on gene, amino acid change, ancestral allele and reference ID for all SNPs

SNP location	Relative mRNA position on gene	Amino acid change	Ancestral allele	dbSNP reference ID
Exon 4	634	Ser167Asn	G	rs1801474
Exon 5	734	His200Gln	C	rs72480421
Exon 10	1272	Val380Leu	G	rs1801582
Exon 11	1314	Asp394Asn	G	rs1801334
UTR 3'	NA	NA	C	rs35125035

NA-Not applicable

Table 3. Genotype, allele frequencies and Hardy-Weinberg Chi-Square in PARKIN gene among 10 Indian populations

Populations	Genotype	MAF	HW χ^2	*P* value

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rs1801474						
	GG	GA	AA	rs1801474 (A)	HW χ^2	*P* value
Munda	79	19	2	0.115	0.443	0.506
Andh	92	5	3	0.055	26.936	0.000*
Kamar	83	17	0	0.085	0.863	0.353
Kathodi	87	13	0	0.065	0.483	0.487
Toto	80	15	5	0.125	9.878	0.002*
Gallong	94	6	0	0.030	0.096	0.757
Kathakur	74	17	9	0.175	16.913	0.000*
Nihal	81	17	2	0.105	0.912	0.340
Birhor	77	20	3	0.130	1.342	0.247
Bondo	72	27	1	0.145	2.468	0.116
rs1801582						
	GG	GC	CC	rs1801582 (C)	HW χ^2	*P* value
Munda	60	31	9	0.245	2.626	0.105
Andh	58	32	10	0.260	2.836	0.092
Kamar	87	11	2	0.075	4.293	0.038
Kathodi	79	13	8	0.145	22.629	0.000*
Toto	86	12	2	0.080	3.414	0.065
Gallong	81	19	0	0.095	1.102	0.294
Kathakur	85	12	3	0.090	7.150	0.007*
Nihal	89	9	2	0.065	6.737	0.009*
Birhor	64	31	5	0.205	0.239	0.625
Bondo	86	10	4	0.090	15.171	0.000*
rs1801334						
	GG	GA	AA	rs1801334 (A)	HW χ^2	*P* value
Munda	81	17	2	0.105	0.912	0.340
Andh	75	23	2	0.135	0.023	0.879
Kamar	80	19	1	0.105	0.012	0.913
Kathodi	82	18	0	0.090	0.978	0.323
Toto	79	18	3	0.120	2.182	0.140
Gallong	83	16	1	0.090	0.054	0.817
Kathakur	80	20	0	0.100	1.235	0.267
Nihal	76	22	2	0.130	0.075	0.784
Birhor	82	16	2	0.100	1.235	0.267
Bondo	79	19	2	0.115	0.443	0.506
rs35125035						
	CC	CA	AA	rs35125035 (A)	HW χ^2	*P* value
Munda	88	11	1	0.065	0.903	0.342
Andh	87	13	0	0.065	0.483	0.487
Kamar	92	7	1	0.045	3.444	0.063
Kathodi	79	19	2	0.115	0.443	0.506
Toto	90	9	1	0.055	1.801	0.180
Gallong	92	8	0	0.040	0.174	0.677
Kathakur	75	22	3	0.140	0.746	0.388
Nihal	80	17	3	0.115	2.717	0.099
Birhor	85	13	2	0.085	2.698	0.100
Bondo	86	14	0	0.070	0.567	0.452

**P< 0.01 was considered statistical significant. d.f.=1 for all tests.*

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