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Predictors of Parkin Mutations in Early Onset Parkinson disease: the CORE-PD Study

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Abstract

Background—Mutations in the parkin gene are the most common genetic cause of early-onset Parkinson's disease (EOPD). Results from a multi-center study of cases with PD systematically sampled by age at onset (AAO) have not been reported.

Objective—To determine risk factors associated with carrying mutations in the parkin gene.

Design—Cross-sectional observational study

Setting—13 movement disorders centers

Participants—956 EOPD cases defined as AAO <51.

Main Outcome Measures—Presence of heterozygous, homozygous or compound heterozygous parkin mutations.

Results—14.7% of cases reported a family history of PD in a first-degree relative using a previously validated interview. Sixty-four cases (6.7%) had parkin mutations (3.9% heterozygous, 0.6% homozygotes, 2.2% compound heterozgyotes). Copy Number Variation (CNV) was present in 52.3% (31.6% of heterozygotes, 83.3% of homozygotes, 81.0% of compound heterozygotes). Deletions in exons 3–4 and 255delA, were common in Hispanics, and specifically, in the Puerto Rican population. Earlier AAO, Hispanic ethnicity (OR compared to White non-Hispanic 2.7 95% CI 1.3–5.7, p<0.009) and family history of PD in a first-degree relative (OR 2.8 95%CI 1.5–5.3, p<0.002) were associated with carrying any mutation in the parkin gene (heterozygous, homozygous, compound heterozygous). Hispanic ethnicity was associated with carrying a heterozygous mutation (OR compared to non-Hispanic Caucasian 2.8 95%CI 1.1–7.2, p<0.03) after adjustment for covariates.

Conclusion—AAO, Hispanic ethnicity and family history of PD are associated with carrying any parkin mutation (heterozygous, homozygous, compound heterozygous) and heterozygous mutations alone. The increased odds of carrying a parkin mutation in Hispanics warrants further study.

Introduction

Mutations in the parkin gene (PARK2;OMIM #600116)[1, 2] are the most common genetic risk factors for early-onset Parkinson's disease (EOPD).[3–13] EOPD has been defined variably as age at onset (AAO) ≤45 years or ≤55 years. Cases with parkin mutations with AAO > 70 years have also been described. [7, 14–16] In PD cases with AAO ≤ 45 years from families with an autosomal recessive mode of inheritance, the frequency of parkin mutations may be as high as 49%[3], while in cases without a family history of PD, the reported range is 15–18%.[4, 6, 17] AAO is inversely correlated with the frequency of parkin mutations in both familial[3] and sporadic[6] cases. The role of heterozygous parkin mutations as causative or susceptibility factors remains controversial.[18–20] Studies in both familial and sporadic cases have consistently found that heterozygotes have older AAO and are more likely to be represented in sporadic samples than homozygotes or compound heterozgygotes. [3–7, 14–16]

In 2004, we initiated the Consortium on Risk For Early-Onset PD study (CORE–PD), a multi-site study to systematically determine the range of phenotypic manifestations in EOPD cases who carry parkin mutations and their family members. Here, we present the baseline characteristics of 956 cases recruited at 13 sites in CORE-PD, and the features associated with carrying heterozygous, homozygous and compound heterozygous parkin mutations.

Methods

CORE-PD was built upon the infrastructure created for the Genetic Epidemiology of PD study (GEPD), using many of the same instruments.[21–23] PD cases recruited in GEPD between 1998 and 2003 were ascertained based on AAO of motor signs <51(EOPD) or >51 late-onset PD (LOPD), regardless of the presence or absence of a family history of PD. Cases with AAO <51 (EOPD) were oversampled and included 247 PD cases.[21]

All cases were recruited from the Center for Parkinson's Disease and Other Movement Disorders at Columbia University (CPD) and underwent an identical evaluation that included a medical history, Unified Parkinson's Disease Rating Scale (UPDRS)[24] and videotape of PD and essential tremor (ET). Only the PD cases with AAO <51 on whom DNA was available (n=247) were included in CORE-PD Additional cases (n= 709) were recruited from 2004 through 2009 as part of CORE-PD. Institutional review boards at all participating sites approved the protocols and consent procedures. PD cases were recruited from each of 13 sites based on the AAO requirement of <51 and performance on the mini-mental state exam (MMSE)[25] >23 to ensure that a reliable history could be obtained from each subject. In addition to the MMSE, Part I of the CORE-PD assessment included collection of demographic information, UPDRS, a family history interview [22], and a blood sample for DNA sent to the NINDS Human Genetics Resource Center DNA and Cell Line Repository (http://ccr.coriell.org). An aliquot of DNA was subsequently sent to Columbia University for analysis. All examiners were unaware of the genetic status of the participants at the time of recruitment and thereafter. While the identity of each PD subject was known to each site, information sent to the coordinating site at Columbia and the Coriell repository was de-identified. In Part II of CORE-PD, cases who carried parkin mutations and a sample of those who did not carry parkin mutations were given a detailed neuropsychological, psychiatric and risk factor assessment We performed identical examinations on first-degree relatives of all cases in Part II. Families were expanded sequentially, by collection of the same information on first-degree relatives of each newly discovered family member who had PD or carried a parkin mutation. These data derived from the Part II evaluation will be presented separately.

Molecular Genetic Analysis

In this study we report data on all 956 cases with PD including 247 cases previously reported from GEPD.[26, 27] and 709 newly recruited cases from CORE-PD. In GEPD, the parkin gene was completely sequenced in the first 101 PD cases [26]. The next 246 cases were screened for point mutations using denaturing high performance liquid chromatography (DHPC). Amplicons were either directly sequenced (n=126) or analyzed using a parkin genotyping array (n=20) [28] in DNA samples with abnormal elution profiles.

Primers and DHPLC conditions used for analysis of the parkin gene have been described previously.[29] To identify CNV (exon deletions and duplications) within the parkin gene, semi-quantitative multiplex PCR was performed on all samples.[26]

In CORE-PD, we screened 709 samples for point mutations using DHPLC and the parkin genotyping array[28] and for CNV (exon deletions and duplications) using semi-quantitative multiplex PCR.[26] The genotyping array was used to analyze amplicons in DNA samples with abnormal elution profiles and has excellent sensitivity and specificity for detection of sequence variants when compared to the gold standard of sequencing.[28] The primers used for PCR amplification of parkin exons 1–12 and intron-exon boundaries and sequencing have been described previously.[30] Cycle sequencing was performed on the purified PCR product as per the manufacturer's instructions (BigDye, Applied Biosystems). Products were analyzed on an ABI3700 genetic analyzer. Chromatograms were viewed using Sequencher

(Genecodes) and sequence variants determined. All sequence variants identified were confirmed by analysis in a separate PCR followed by bi-directional sequencing.

We previously sequenced the parkin gene in 105 White non-Hispanic controls.[26] To determine whether novel variants identified in Black non-Hispanics or Hispanics in this study were mutations, we sequenced 139 Hispanic and 119 Black non-Hispanic non-demented controls from the Washington Heights Columbia Aging Project who had normal neurological exams.[31, 32] Based on the published literature and the sequence data from controls, variants with a frequency of ≤1% were classified as mutations. Additional criteria used to classify new variants as mutations included predicted effect on the encoded protein (null, truncation, missense, splice, synonymous), evolutionary conservation of the impacted amino acid residue or region, and location in conserved functional domains. We classified sequence variants with no known functional significance as polymorphisms if their frequency was ≥1% in ethnically matched controls in published studies[2, 4, 28, 33] or in the current study. We defined a variant as "variant of uncertain significance" if it had been previously reported as a mutation in at least one ethnic group, but had a similar frequency in cases and controls in another ethnic group and the variant was predicted to affect protein function using the analysis software SIFT (http://blocks.fhcrc.org/sift/). [34] Nine cases carrying seven variants of uncertain significance were identified. In this analysis, we consider these variants of uncertain significance as parkin non-carriers. We also performed all analyses excluding these variants.

Diagnosis of PD in Relatives

Information on the family history of PD in first-degree relatives was obtained by administering a reliable, validated interview to each subject.[22] An algorithm was created to generate a final diagnosis for PD in each first-degree relative based on the family history interview. For relatives diagnosed with PD, a level of certainty was assigned as definite, probable, possible, uncertain, and unlikely. A best estimate diagnosis of PD was assigned for each relative.[21] We previously demonstrated that a conservative diagnosis of PD (definite, probable or possible PD) had the best combination of sensitivity and specificity of PD.[21] In this study, if any first-degree relative met the conservative definition of PD, the family history of PD was considered positive.

Statistical methods

Demographic and clinical characteristics of PD cases who carried a parkin mutation (heterozygote, homozygote, compound heterozygote) and those who did not (non-carriers) were compared using chi square tests for categorical variables and Student's t-test for continuous variables. Logistic regression models were constructed to examine whether demographic features including AAO, ethnic group, and family history of PD, education and gender were associated with carrying a parkin mutation. AAO was categorized as <40 (n=299) or 40–50 (n=624). Ethnic group was categorized as White non-Hispanic, Black, non-Hispanic, Hispanic or "other". Multiple ethnic groups were included in the "other" category; 70% described themselves as Asian. Family history of PD was categorized using a 'conservative' definition of PD in any first-degree relative.[22] Additional models were constructed to examine the association of these characteristics with parkin heterozygosity compared to parkin non-carrier (reference) and homozygosity or compound heterozygosity (combined) compared to parkin non-carrier as the reference.

Results

Demographic and clinical characteristics of the cases are shown in Table 1. Sixty-four PD cases (6.7%) had parkin mutations (3.9% heterozygous, 0.6% homozygotes, 2.2%

compound heterozgyotes). The prevalence of mutations declined with AAO from 57% (8/14) in cases with AAO <20, 30% (13/43) in cases with AAO 20–29, 9% (23/254) in cases with AAO 30–39 and 3% (20/644) in those with AAO 40–50 (test for linear trend p<0.001). None of 12 Black non-Hispanics carried a parkin mutation, while 5.7% (48/838) White non-Hispanic, 15.6% (12/77) of Hispanics and 14.8 (4/27) of cases in "other" ethnic groups combined did (p<0.002). No Blacks, but nine Whites endorsed Hispanic ethnicity (Mexican) and were classified in the Hispanic category. One carried a heterozygous exon 6 deletion. Among those who reported a family history of PD in a first degree relative, 11.8% carried mutations compared to 5.7% of those who did not have a family history of PD (p=0.007). CNV was present in 52.3% of carriers (31.6% heterozygotes, 83.3% homozygotes, 81.0% compound heterozygotes). There was no reported consanguinity.

All mutation carriers were similar in age but compound heterozygotes and heterozgyotes were significantly younger than parkin non-carriers. Each mutation carrier group had significantly younger AAO than the non-carrier group Compound heterozygotes and homozygotes each had a significantly younger AAO than heterozygotes. The mean age of White non-Hispanics was 41.7(sd 6.7) years, Black non-HIspanics 36.6 (sd 6.9) years, Hispanics 39.7 (sd 8.1) years and "other" 40.4 (sd 8.3) years. White Non-Hispanics were significantly older than Black non-Hispanics (p <0.05).

Dystonia, as a presenting sign did not differ between carriers (0%) and non-carriers (1.7%) of parkin mutations (p=0.4) Similarly there was no difference in reported response to levodopa; 93.4% of carriers compared to 90.5% of non-carriers reported a response to anti-parkinson medications when tried in an adequate does.(p=0.60).

Logistic models examining the association of demographic risk factors with the presence of any parkin mutation or the presence of heterozygous mutations compared to non-carriers are shown in Tables 2 and 3. AAO was inversely related to the presence of any parkin mutation, after adjustment for ethnicity and family history of PD in a first-degree relative. Education and gender were not associated with mutation status in this model. Compared to White non-Hispanic, Hispanic ethnicity was associated with the presence of a parkin mutation in both the model examining the presence of any mutation (OR 2.7 95% CI 1.3–5.7, p<0.009) (Table 2) and the model examining heterozygotes compared to non-carriers OR 2.8 95%CI 1.1–7.2, p<0.03 (Table 3). When cases with mutations in both alleles (compound heterozygote and homozygotes) were compared to non-carriers, AAO (OR 18.6 95%C I5.5–63.8 p<0.001) and family history of PD 3.5 95% CI 1.4–9.2 p <0.01 were significant but Hispanic ethnicity was no longer significant (data not shown). When those with two mutations were compared to those with a single mutation (heterozygotes), AAO was inversely associated with carrying two mutations (OR 6.6 95%CI 1.6–27.1 p<0.008) but neither ethnicity nor family history was associated with carrying two mutations.

All analyses were repeated excluding 35 LRRK2 G2019S mutation carriers, 45 Glucocerebrosidase N370S carriers and 23 Glucocerebrosidase L444P carriers. . One heterozygous parkin carrier (deletion) also carried a G2019S mutation and 3 heterozygous parkin carriers had GBA mutations (2 L444P and 1 N370S). The inverse relationship between AAO and carrying any parkin mutation (n=57) or a single mutation and the relationship between family history of PD and carrying either 1 or 2 mutations remained. Adjusting for AAO and family history of PD, the association between Hispanic ethnicity and carrying any parkin mutation (OR 2.6 95%CI 1.1–5.9 p<0.03) or a heterozygous mutation (OR 3.7 95%CI 1.3–10.08.3p<0.01) persisted.

The specific parkin mutations detected in heterozygotes, homozygotes and compound heterozygotes are listed in Table 4. The seven variants of uncertain significance detected

among nine cases included Asp18Asn, Ala82Glu (n=2), Pro437Leu (n=2), Pro153Arg, ATG-23C>T and ATG-43T>C and Met192Leu. Findings were not significantly different when these variants were excluded from all analyses.

The nationalities of the 77 Hispanic cases included 21 from the Dominican Republic, 20 from Puerto Rico, 15 from Mexico, eight from Ecuador and fewer than five cases each from Cuba, Peru, Columbia, Chile and Ecuador. The 12 Hispanic carriers included seven Puerto Ricans, two Mexicans, one Cuban, one Dominican, and one Peruvian. Six cases (5 Puerto Rican and one Mexican carried deletions of exon 3–4. Both exon 3–4 homozygotes were of Puerto Rican descent. Family history of PD was reported by one homozygote, but was not available for the other. None of the other carriers of exon 3–4 deletions reported a family history of PD. The second most common mutation in Hispanics was 255delA, present in three Puerto Ricans in association with the exon 3–4 deletion and one Mexican heterozygote.

Discussion

This is the largest systematically collected sample of EOPD cases recruited solely based on AAO. We demonstrated that among cases with EOPD, carrying any parkin mutation or a heterozygous mutation is inversely related to AAO and that parkin mutations are more common in those with a family history of PD in a first degree relative. Parkin mutations, in particular deletion 3–4 and 255delA, are common in the Hispanic, and specifically, the Puerto Rican population.

The low frequency of parkin mutations in this sample (6.7%) may reflect the reduced penetrance of parkin mutations, particularly among heterozgygotes that represent 58% of mutation carriers, and the fact that 94% of the sample had an AAO >30 years. Using the kin cohort method, in a sample of cases that was 72% heterozygote, we previously reported a penetrance of 7% at age 65[27] for first-degree relatives estimated to be parkin heterozygotes. This was not significantly different from those estimated to be non-carrier relatives or control relatives. The frequency of parkin mutation carriers in the current study, 36.8% (21/57) of those with AAO<30 and 6.1% (35/572) of those with AAO 30–45, is similar to a large sporadic series[6] that reported 33.8% (23/68) with AAO <30 and 8% (14/175) of those with AAO 30–45. In addition, we and others [26, 33] have reported that variants previously considered to be mutations have now been identified in similar frequency in ethnically matched control groups. We now consider these normal variants, further reducing the frequency of reported mutations.

The role of heterozygotes has remained controversial; some authors believe that heterozygous point mutations are not pathogenic,[20] and others, that deletions rather than point mutations are more likely to have functional consequences.[35] Our finding that cases with a parkin mutation in the heterozygous state have younger AAO of PD than those who do not have mutations after adjustment for ethnicity and family history of PD supports the concept that parkin heterozygosity is a susceptibility factor for PD. Heterozygosity may lead to disease by means of haploinsufficiency, dominant negative effects, or gain of function.[19, 36] PET studies show reduced FDOPA uptake in nigrostriatal terminals in the caudate and posterior putamen of both symptomatic and asymptomatic heterozygotes compared to controls, a reduction similar to that found in sporadic PD.[37–39] Transcranial sonography demonstrated greater substantia nigra hyperechogenicity in both symptomatic homozygotes and heterozygotes compared to controls.[40] These functional and structural imaging studies suggest that parkin heterozygotes can compensate to maintain motor function in the face of mild dopaminergic deficits.[41]

Eight studies have now reported genetic variants that could lead to functionally relevant alterations of protein structure among controls with mutation frequencies in the parkin gene ranging from 0 to 3.9%.[20, 26, 33, 42–46] Most of these studies were limited by relatively small sample size and controls that were not ethnically matched to cases. In the largest study to date[44] in addition to missense and frameshift mutations, four different dosage mutations were seen in 356 controls from South Tyrol and Germany. One control with a missense mutation was examined three years after the first exam and had mild parkinsonism and evidence of increased echogenicity on transcranial sonography of the substantia nigra, consistent with mild parkinsonism. These findings suggest that parkin heterozgyosity may increase PD susceptibility rather than directly causing PD. A similar pathogenic role has been proposed for PINK1[19] and GBA.[47, 48] In addition to the possibility of interaction with functional variants in other genes, there is new evidence that environmental factors such as exposure to both maneb and paraquat during critical periods early in life may be associated with early onset PD.[49]

Although the numbers were small, it appears that the exon 3–4 deletion and the frameshift mutation 255delA are common among Hispanic PD cases, in particular Puerto Ricans, after adjustment for AAO and family history of PD. These two mutations and three others (deletions in exon 3, deletions in exon 4 and the Arg275Trp) were reported to account for 35% (133/379) unrelated mutation carriers reported from 1998 through 2003 and suggest hot spots for ‘small’ mutations in exons 2 and 7 and rearrangements most commonly in exons 2 through 4.[2] We previously reported a potential founder mutation in 3 families of Puerto Rican descent who carried an exon 3–4 deletion including 1 homozygote. [2, 4] Using 10 microsatellite markers, a haplotype was identified. All 3 Puerto Rican carriers of the exon 3–4 deletion shared at least one common allele at all markers except D6S1277 and D6S2436 that flanked the gene. [2] A PD case from Northern Germany who carried this deletion[50] did not share the common haplotype. [2] One of these three families reported previously, a compound heterozygote[2] is included in the current study.

The 255delA was the second most common mutation recognized in Hispanics in this study. In a series of 37 cases from Spain with AAO ≤ 40 or a recessive pattern of inheritance, seven PD cases (19%) carried a parkin mutation including four PD cases with homozygous 255delA mutations. Three of these four cases reported a family history of PD. [51] The 255delA was seen in 1/200 control chromosomes in that series. [51] We did not detect this mutation in 139 controls of Caribbean Hispanic descent. It is suggested[52] that the 255delA frameshift mutation may be an ancestral European mutation. Further exploration of Hispanic PD cases with respect to genetic modifiers of age at onset of PD and phenotypic variability is warranted.

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Table 1

Demographics of the cohort

	Heterozygous N=37 3.9%	**Homozygous N=6 0.6%**	**Comp Het N=21 2.2%**	**Non-carriers N=892 93.3%**	**Total N=956**
Age (sd)	49.5(7.8)	52.2 (14.5)	47.4 (12.7)	52.6 (8.6)	52.4 (8.7)**
Age at onset (AAO) (sd)	37.2 (9.2)	28.5 (2.7)	28.9 (10.2)	42.0 (6.3)	41.4 (6.9)**
AAO <20 years	2	0	6	7	14 *
20–29	7	4	2	30	43
30–39	11	2	10	231	254
40–50	17	0	3	624	644
Duration (sd) years	12.0 (9.0)	23.7 (13.7)	18.5 (11.7)	10.7 (7.5)	11.0 (7.8)**
UPDRS III (sd)	21.6 (15.6)	23.6 (11.0)	18.6 (13.8)	20.3 (11.8)	20.3 (12.0)
Levodopa (mg)	247.2(291.2)	335.0 (391)	576.3 (919)	477.8 (510.2)	469.7(516.0)
%Family History of PD(n=923)	25.0	60.0	20.0	13.9	14.7 **
Mini mental (sd)	28.8 (1.6)	26.8 (3.1)	28.4 (2.3)	28.8 (2.0)	28.8 (2.0)
Education (sd)	15.0(3.4)	11.7(5.5)	13.7 (2.6)	15.6 (3.0)	15.5 (3.0)**
% men (n)	54.1 (20)	83.3. (5)	52.4 (11)	62.4 (556)	62.0 (592)
% White non-Hisp(n)	73.0 (27)	66.7 (4)	81.0 (17)	88.8 (790)	87.8 (838)**#
% Black non-Hispanic	0	0	0	1.3 (12)	1.3 (12)
% Hispanic (n)	16.2 (6)	33.3 (2)	19.0 (4)	7.3 (65)	8.2 (77)
% Other	10.5 (4)	0	0	2.6 (23)	2.7 (27)

Overall significance

** $p < 0.001$ There were significant pairwise comparisons for age at onset for all categories except homozygotes compared to compound heterozygotes. There were significant pairwise comparisons for disease duration for all but homozygotes compared to compound heterozygotes and heterozygotes compared to non-carriers.

* Age at onset missing for 1 subject

Race/ethnicity missing in 2 subjects

Table 2

The Odds of carrying any mutation in the parkin gene (heterozygous, homozygous, compound heterozygous) compared to probands who are non-carriers of parkin mutations

	Odds Ratio: any parkin mutation compared to non-carriers	95% confidence interval	P value
Age onset 40–50 (n=624)	reference		
Age onset 40 (n=299)	5.0	2.8–8.8	0.001
White non-Hispanic (n=811)	reference		
Black non-Hispanic (n=12)*	0	0	0.99
Hispanic (n=73)	2.7	1.3–5.7	0.009
Other (n=27)	2.4	0.8–7.5	0.14
Family History of PD (n=923)**	2.8	1.5–5.3	0.002

* No Black non-Hispanics had parkin mutations

** 923 cases including 61 carriers of any parkin mutation had a family history interview.

Table 3

The Odds of carrying a single heterozygous mutation in the parkin gene compared to probands who are non-carriers of parkin mutations

	Odds Ratio: heterozygote Compared to non-carriers	95% confidence interval	Significance
Age onset 40–50 (621)	reference		
Age onset <40 (277)	2.7	1.3–5.3	0.005
White non-Hispanic (791)	reference		
Black non-Hispanic (12)*	0	0	0.99
Hispanic (68)	2.8	1.1–7.2	0.03
Other (27)	4.3	1.3–13.7	0.01
Family History of PD (n=898)**	2.5	1.1–5.5	0.05

* No Black non-Hispanics had parkin mutations

** 898 cases including 36 heterozygotes had a family history interview

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Table 4

Mutations by ethnic group and zygosity

Exon	Mutation	Type of Mutation	Location within functional protein domain	Zygosity	Ethnic Groups
1	c.81G>T	Point	NA	heterozygote	Hispanic (1)
2	Arg42Pro	Point/missense	Ubiquitin	heterozygote	White (2)
2	Exon 2 deletion	CNV	Ubiquitin	heterozygote	White (1)
2	Exon 2 duplication	CNV	Ubiquitin	heterozygote	White (1)
2	255delA	Small nucleotide deletion/insertion	Ubiquitin	heterozygote	Hispanic (1)
2	202-3delAG	Small nucleotide deletion/insertion	Ubiquitin	heterozygote	White (3)
2	Ala46Pro	Point/missense	Ubiquitin	heterozygote	White (1)
2	Gln34Arg	Point/missense	Ubiquitin	heterozygote	Other (1)
2	Asp53Glu	Point/missense	Ubiquitin	heterozygote	White (1)
3	Exon 3 40 bp del	Small nucleotide deletion/insertion	Ubiquitin	heterozygote	White (3)
3	Exon 3 deletion	CNV	Ubiquitin	heterozygote	White (1)
3	Arg128Lys	Point/missense	Ubiquitin	heterozygote	White (1)
3,4	Exon 3–4 deletion	CNV	Ubiquitin	heterozygote	White (2)
5,6	Exon 5–6 deletion	CNV	unknown	heterozygote	White (1)
6	Exon 6 deletion	CNV	unknown	heterozygote	White(1), Hispanic(1)
6	Thr240Met	Point/missense	RING1	heterozygote	Hispanic(1)
7	Arg275Trp	Point/missense	RING1	heterozygote	White (6), Hispanic(1), other (1)
7	Arg256Cys	Point/missense	RING1	heterozygote	White (1)
7	Leu272Leu	Point/missense	RING1	heterozygote	Other (1)
8	Iso298Leu	Point/missense	unknown	heterozygote	White (1)
8	Exon 8 deletion	CNV	unknown	heterozygote	Hispanic (1)
10	Exon10 deletion	CNV	unknown	heterozygote	White (1)
12	Pro437Ala	Point/missense	RING2	heterozygote	Other (1)
2	255delA	Small nucleotide deletion/insertion	Ubiquitin	homozygote	White (1)
3	Exon 3 40 bp deletion	Small nucleotide deletion/insertion	Ubiquitin	homozygote	White (2)
3,4	Exon 3–4 deletion	CNV	Ubiquitin	homozygote	Hispanic (2)

Exon	Mutation	Type of Mutation	Location within functional protein domain	Zygosity	Ethnic Groups
6	Exon 6 deletion	CNV	unknown	homozygote	White (1)
2 and 2–4	Arg 256Cys + Exon 2–4 deletion	Point/missense, CNV	RING1, Ubiquitin	Comp het	White (1)
2 and 2–4	255delA+exon 2–4 deletion	Small nucleotide deletion/insertion, CNV	Ubiquitin	Comp het	White (1)
2 and 3–4	255delA+exon 3–4 deletion	Small nucleotide deletion/insertion, CNV	Ubiquitin	Comp het	Hispanic (3)
2 and 7	255delA +Arg275Trp	Small nucleotide deletion/insertion, Point/ missense	Ubiquitin, RING1	Comp het	White (1)
2 and 7	Arg42Pro + Arg275Trp	Point/missense	Ubiquitin, RING1	Comp het	White (1)
2 and 3–4	202delAG + Exon 3–4 deletion	Small nucleotide deletion/insertion, Point/ missense	Ubiquitin	Comp het	White (1), Hispanic (1)
2 and 3	Arg42Pro + exon 3 deletion	Point/missense, CNV	Ubiquitin	Comp het	White (1)
3 and 4	Exon 3 40bp deletion, Exon 3 40bp deletion +exon 4 deletion	Small nucleotide deletion/insertion, CNV	Ubiquitin	Comp het	White (1)
3 and 5	Exon 3 deletion + Exon 5 deletion	CNV	Ubiquitin, unknown	Comp het	White (1)
3 and 7	Exon 3 40bp deletion +Arg275Trp	Small nucleotide deletion/insertion, Point/ missense	Ubiquitin, Ring 1	Comp het	White (1)
3 and 12	Exon 3 deletion +Exon 12 deletion	CNV	Ubiquitin, RING2	Comp het	White (1)
3 and 12	Exon 3 40bp deletion + Gly430Asp	Small nucleotide deletion/insertion, Point/ missense	Ubiquitin, RING2	Comp het	White (1)
3–4 and 12	Exon 3–4 deletion, Gly 430Asp	CNV, Point/missense	Ubiquitin, RING2	Comp het	White (1)
4 and 7	Exon 4 deletion + Arg 275Trp	CNV, Point/missense	Ubiquitin, RING1	Comp het	White (1)
4 and 10	Exon 4 deletion+ Arg366Gln	CNV, Point/missense	Ubiquitin,	Comp het	White (1)
7 and 12	Arg275Trp + Gly430Asp	Point/missense	RING1, RING2	Comp het	White (1)
7–8, 10	Exon 7–8 duplication, exon 10 deletion	CNV	Ring 1 Unknown	Comp het	White (1)
7 and 6	Arg275Trp +Cys212Tyr	Point/missense	Ring 1 unknown	Comp het	White (1)