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Analysis of common and rare *VPS13C* variants in late onset Parkinson disease

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Supplemental Data: Table e-1 – e-6

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Abstract

Objective: *VPS13C* is within a risk locus for Parkinson Disease (PD), and bi-allelic *VPS13C* mutations are a rare cause of early onset, rapidly progressive PD. In the current study we aimed to study the role of coding *VPS13C* variants in a large cohort of late-onset PD (LOPD) patients.

Methods: *VPS13C* and its untranslated regions were sequenced using targeted next-generation sequencing in 1,567 PD patients and 1,667 controls from 3 cohorts. Association tests of rare homozygous and compound heterozygous mutations and burden tests for rare heterozygous mutations were performed. Common variants were analyzed using logistic regression adjusted for age and sex in each of the cohorts, followed by a meta-analysis.

Results: No bi-allelic carriers of rare *VPS13C* variants were found among patients and two carriers of compound heterozygous variants were found in two controls. There was no statistically significant burden of rare (MAF<1%) or very rare (MAF<0.1%) coding *VPS13C* variants in PD. A *VPS13C* haplotype including the p.R153H-p.I398I-p.I1132V-p.Q2376Q variants was nominally associated with a reduced risk for PD (meta-analysis of the tagging SNP p.I1132V (OR=0.48, 95%CI=0.28-0.82, p=0.0052). This haplotype was not in linkage disequilibrium (LD) with the known genome-wide association study (GWAS) top hit.

Conclusions: Our results do not support a role for rare heterozygous or bi-allelic *VPS13C* mutations in LOPD. Additional genetic replication and functional studies are needed to examine the role of the haplotype identified here associated with reduced risk for PD.

Keywords: Parkinson's disease; genetic variants; VPS13C; association study

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Introduction

The vacuolar protein sorting 13C (*VPS13C*) gene is located within a risk locus for Parkinson Disease (PD), reported in large genome-wide association studies (GWAS) of European population [1, 2]. The SNP reported in the GWAS (rs2414739, chr15.hg19:g.61994134G>A) was also studied in several Asian populations and in Iranians, with conflicting results [3-7], possibly due to ethnicity-related differences.

Subsequently, homozygous and compound-heterozygous *VPS13C* mutations were identified as a rare cause of early onset PD (EOPD) characterized by rapid progression and early cognitive dysfunction. It was demonstrated that *VPS13C* is partially localized at the mitochondrial membrane, and its silencing led to mitochondrial dysfunction and increased PINK1/Parkin-dependent mitophagy [8]. A follow-up study in 80 EOPD patients identified an additional patient with compound-heterozygous mutations with similar clinical features to the previously reported patients [9]. In another recent study, a homozygous deletion in *VPS13C* was reported to be the probable cause of an early onset parkinsonism in one patient [10]. Thus far, full sequencing studies of *VPS13C* have not been reported in late onset PD (LOPD).

To further study the potential role of *VPS13C* variants in PD, we sequenced its coding and regulatory regions using targeted next-generation sequencing in three cohorts of PD (predominantly LOPD) and in controls. We examined the association of common, rare and bi-allelic *VPS13C* variants on the risk for PD. We further tested whether any coding variant or variants in the untranslated regions of *VPS13C* are in linkage disequilibrium (LD) with the top *VPS13C*-associated GWAS hit, to determine if any of these variants can explain the GWAS association of this locus.

Methods

Study population

Three cohorts, with a total of 1,567 unrelated PD patients and 1,667 controls, were included in this study, detailed in Table 1. First cohort was composed of French and French-Canadian participants recruited in Quebec (Canada) and in France. The second cohort was recruited in New York - Columbia University,

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and was previously described[11]. The majority of participants from New York are of European descent and 38% are Ashkenazi Jewish (AJ). The third cohort was recruited in Israel (Sheba Medical Center) and all participants included in this study from the Israeli cohort are AJ. All samples were consecutively recruited. As detailed below, due to the differences in age and sex (Table 1), statistical analysis was adjusted and included age and sex as co-variates. To account for different ethnicities in the New York cohort, an ethnicity covariate was also introduced in this cohort (GWAS data was not available for this cohort, therefore the reported ethnicity was used and not principal components). All three cohorts were sequenced in the same lab (McGill University), following the same protocol. All PD patients were diagnosed by movement disorder specialists according to the UK brain bank criteria [12], without excluding patients with family history of PD.

Compliance with Ethical Standards

The institutional review board (McGill University Health Center Research Ethics Board - MUHC REB) approved the study protocols (reference number IRB00010120). Informed consent was obtained from all individual participants before entering the study.

DNA extraction and *VPS13C* sequencing

DNA was extracted using a standard salting out protocol. The coding sequence and regulatory regions of *VPS13C* were targeted using molecular inversion probes (MIPs), that were designed as previously described [13]. MIPs were selected based on their predicted coverage, quality and overlap. All MIPs used to sequence *VPS13C* in the present study are included in Table S-1. Targeted DNA capture and amplification was done as previously described [14], and the full protocol is available upon request. The library was sequenced using Illumina HiSeq 2500 platform at the McGill University and Genome Quebec Innovation Centre. Reads were mapped to the human reference genome (hg19) with Burrows-Wheeler Aligner [15]. Genome Analysis Toolkit (GATK, v3.8) was used for post-alignment quality control and variant calling [16] , and ANNOVAR was used for annotation [17]. Data on the frequency of each *VPS13C*

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variant were extracted from the public database Genome Aggregation Database (GnomAD) [18]. Validation of the tagging variant p.I1132V was performed using Sanger sequencing, with the following primers: forward 5' – CCGGGAAGGTAATGACAAAA – 3', reverse 5' – CCCCTGATTGAAAAGTCACA– 3'

Quality control

During quality control (QC) filtration using PLINK software v1.9 [19], SNPs with genotyping rate lower than 90% were excluded. Genotyping rate cut-off for individuals was 90%, and individuals with a lower genotyping rate were excluded. SNPs that deviated from Hardy-Weinberg equilibrium set at p=0.001 threshold were filtered out. Threshold for missingness difference between cases and controls was set at p=0.05 and the filtration script adjusted it with Bonferroni correction. After these QC steps, cohort composition was as described in Table 1. To be included in the analysis, minimum quality score (GQ) was set to 30. Rare variants (minor allele frequency, MAF<0.01 or 0.001) had to have a minimal coverage of >50x to be included, and common variants had to have a minimal coverage of >15x to be included in the analysis.

Statistical Analysis

The association between common *VPS13C* variants and PD was examined by using logistic regression models using PLINK v1.9, with the status (patient or control) as a dependent variable, age and sex as covariates in all cohorts, and AJ ancestry as an additional covariate in the New York cohort. To analyze rare variants (MAF < 0.01) and very rare variants (MAF < 0.001), an optimised sequence Kernel association test (SKAT-O, R package) was performed [20]. The effects of SNP genotypes on the age at onset (AAO) was tested using analysis of variance (ANOVA; in R software). Meta-analysis of common variants in the three cohorts was performed using Metafor Package in R software [21]. Linkage disequilibrium in our data was examined by PLINK v1.9 and LD between discovered SNPs and the GWAS top hit rs2414739 was tested using LDlink application, selecting all non-Finish Europeans [22].

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Data availability statement

Anonymized data is available upon request by any qualified investigator.

Results

Rare *VPS13C* mutations and homozygous or compound heterozygous *VPS13C* mutations are not associated with late onset PD.

The average coverage of *VPS13C* with the MIPs was 94% of nucleotides covered at > 10x, and 90% covered at > 50x. There were no differences in coverage between the cohorts and between patients and controls. Table S-2 details the rare variants that are either nonsynonymous or potentially affect a splicing site (n=60) and Table S-3 details the common variants in coding regions (n=18) identified in the three cohorts.

In order to examine whether rare homozygous or compound heterozygous *VPS13C* mutations may cause LOPD, and since patients with *VPS13C* bi-allelic mutations are very rare, we included in this analysis only rare mutations with allele frequency < 0.001. Only two carriers of compound heterozygous mutations were identified, and both were controls, suggesting that bi-allelic mutations are not common in LOPD (Table S-6).

To further study a potential role for rare (allele frequency < 0.01) or very rare (allele frequency < 0.001) *VPS13C* nonsynonymous or splice variants in LOPD, a SKAT-O was performed. There was no association between rare variants (French/French Canadian cohort p=0.44, New York cohort p=0.34, Israel cohort p=0.91) or very rare variants (French/French Canadian cohort p=0.17, New York cohort p=0.85, Israel cohort p=0.89) and PD.

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A *VPS13C* haplotype including the p.R153H-p.I398I-p.I1132V-p.Q2376Q coding variants is nominally associated with reduced risk for PD.

To test whether common coding variants in *VPS13C* are associated with LOPD, logistic regression models adjusted for age and sex were performed, and additional adjustment for ethnicity was included in the New York cohort (see methods). A nominal association was observed in four variants (p.R153H [rs12595158, chr15.hg19:g.62316035C>T], p.I398I [rs9635356, chr15.hg19:g.62299603T>G], p.I1132V [rs3784635, chr15.hg19: g.62254989T>C] and p.Q2376Q [rs17238189, chr15.hg19: g.62212781T>C]) with reduced risk for PD in the New York cohort (Table 2). These remained nominally significant with and without including adjustment for ethnicity, and only one of them, p. I1132V (OR 0.28, 95% CI 0.12-0.64, p=0.0025), remained statistically significant after correction for multiple comparisons. In the two other cohorts, these variants showed the same directionality as in the New York cohort, but did not reach statistical significance (Table 2). These four variants, p.R153H, p.I398I, p.I1132V and p.Q2376Q, are in strong or even complete LD (Table S-4). The most significant tagging SNP of this haplotype, p. I1132V, was validated using Sanger sequencing in all three cohorts. Meta-analysis of all the common coding variants showed an association of these four linked SNPs with reduced risk for PD (Figure 1), with similar directionality across all cohorts. To examine whether this haplotype may affect AAO of PD, ANOVA with the status of the p. I1132V variant was performed. This variant was not associated with AAO in all three cohorts (French/French Canadian cohort p=0.65, New York cohort p=0.34, Israel cohort p=0.99).

None of these variants were in LD with the known GWAS top hit, rs2414739, and therefore these associations do not explain the GWAS hit in the *VPS13C* locus. Of all the other common coding variants, only one variant, p.E2008D (rs78071599, chr15.hg19: g.62223303C>G), was in some LD with the GWAS top hit (D'=0.808, r^2=0.006, Table S-5), but this variant was not associated with LOPD, and therefore it also cannot explain the GWAS hit in our populations. Interestingly, an intronic variant, rs78530361 (chr15.hg19: g.62214265C>T), was in strong LD with the top GWAS SNP (D'=1, r^2=0.003). Of note, r^2 is low since this intronic SNP has a much lower allele frequency than the top GWAS hit, but

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every time the rs78530361 SNP is found, it is on an allele which harbors the top GWAS hit rs2414739. However, in our cohorts, this variant was not associated with PD (p=0.66), likely due to its very low r^2 with the GWAS hit.

Discussion

Our study, which included full sequencing of *VPS13C* in three cohorts, identified 60 rare *VPS13C* variants (MAF<0.01) that are nonsynonymous or affect splicing, and 18 common variants (MAF>0.01) in coding regions of the gene and splice sites. Our results suggest that rare homozygous and compound heterozygous variants are rare in LOPD and probably have importance mainly in early onset PD, as previously described. We have identified a potentially protective haplotype, which includes four variants, two of which are substitutions of amino acids, p.R153H and p.I1132V. Interestingly, this haplotype is not in LD with the top GWAS SNP, rs2414739, suggesting that this may be a secondary association in the *VPS13C* locus, which was not identified in previous studies. However, this should be considered as preliminary results and needs to be examined in additional cohorts in order to conclude whether this haplotype is indeed associated with reduced risk for PD. Furthermore, the full sequencing analysis did not identify any coding variant that is in LD with the original GWAS hit that can explain the GWAS association in this locus. This may suggest that the variant that has the main effect on the risk for PD in the *VPS13C* is outside of the coding and untranslated regions of the gene, likely being a regulatory element.

Previous studies on the top GWAS hit in the *VPS13C* locus demonstrated conflicting results. While significant associations of the top GWAS hit in this locus (rs2414739) with PD were found in Iranian [5] and East Asian [3] populations, negative results were reported in Taiwanese [4] and Han Chinese [6, 7] populations. Therefore, it is possible that the association of *VPS13C* with PD is population dependent. Of note, that the association in the current study was mainly driven by populations enriched in Ashkenazi Jews, while in the French/French Canadian cohort the differences between patients and controls were

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much smaller and not statistically significant (Table 2). This may suggest that this haplotype association is population specific, and additional studies in different populations are required to answer this question.

In our three cohorts, we did not find any very rare homozygous mutations with MAF<0.001 and found only two carriers of compound heterozygous mutations, both of which were controls (Table S-6). We were unable to determine if the mutations were in cis or trans. Previously reported cases of PD in carriers of compound heterozygous or homozygous mutations all shared a specific clinical presentation of PD: early onset, rapid progression and early cognitive dysfunction [8, 9]. In one study, the patient's AAO was 39, and disease progression was moderately severe with psychiatric symptoms and impaired cognition [9]. In another study, the three patients showed severe phenotypes: AAO of 25, 33, and 46 years, severe and early cognitive dysfunction, and became bedridden at 31, 43, and 58 years, respectively. Considering the young age of one of the compound heterozygous *VPS13C* variant carriers in our cohort (30 years), it is still possible, although unlikely that this individual will develop PD in the future. The negative results of the SKAT-O analyses demonstrate that rare heterozygous variants in *VPS13C* do not have an important role in PD in our cohorts.

Our study has several limitations. The differences between PD patients and controls in sex and mainly age are significant in some of our cohorts. To address this limitation, we included age and sex as covariates in the regression models. Therefore, if the association of the protective haplotype was related to age and not to disease status, we would likely not observe an association in the adjusted model. There was no significant difference in the percentage of Ashkenazi Jews between patients and controls in the New York cohort, yet we still performed the regression model with and without ethnicity as a covariate, and in both analyses the results remained significant. Nevertheless, the fact that our populations are enriched with relatively homogeneous populations such as Ashkenazi Jews and French Canadians requires additional studies in other populations.

In conclusion, our results suggest that *VPS13C* variants have a limited role in late-onset PD. The potentially PD protective haplotype located within *VPS13C*, which requires additional replications, may suggest that *VPS13C* could be a future target for PD therapeutic development. If naturally occurring

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genetic variants may reduce PD risk, it is conceivable that drugs that can mimic their effects could be developed. Additional genetic and functional studies will be required to determine if *VPS13C* may be a viable target for PD drug development.

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Figure legends

Figure 1 Legend: Forest plots – meta-analyses of four *VPS13C* coding variants associated with reduced risk for Parkinson Disease. The forest plots depict the effects of the four *VPS13C* coding variants that create the haplotype associated with reduced PD risk in the three cohorts studied, and their meta-analyzed effect on risk for PD. The results in the random effect model are nearly identical. CI, confidence interval; FE, fixed effect

Table 1: Study population details

Sequenced		Cohort	Min Depth of Coverage	Analyzed						Genotyping call rate %
				Cases			Controls			
N (cases)	N (controls)			N	Mean Age (SD) y	%Male	N	Mean age (SD) y	%Male	
543	866	French/French-Canadian	15x	534	59.7(11.4)	63.7	858	42.0(13.6)	51.6	99.6
			50x	498	59.8(11.2)	63.5	785	42.3(13.5)	52.0	99.2
533	270	New York	15x	520	59.3(11.8)	64.0	262	65.0(9.8)	34.0	99.9
			50x	502	59.3(11.8)	64.1	245	65.4(9.6)	32.7	99.5
491	531	Israel	15x	482	60.6(11.7)	61.8	488	33.9(7.2)	57.8	99.3
			50x	478	60.7(11.7)	61.7	487	33.9(7.2)	57.7	99.3

N, number; Min, minimum; y, year

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Table 2: Four variants forming the protective haplotype found in *VPS13C* and the results of the logistic regression in three cohorts

Variant	rs number	nt-substitution	Cohort	N (cases)	N (controls)	Affected MAF	Unaffected MAF	GnomAD MAF†	OR (95%CI)	P
p.R153H	rs12595158	c.G458A	F/FC	15	27	0.0143	0.0157	0.0734	0.56 (0.26-1.20)	0.1358
			NY	14	16	0.0135	0.0324		0.38 (0.17-0.84)	0.0172
			Israel	16	22	0.0166	0.0225	0.0236	0.70 (0.19-2.6)	0.5940
p.I398I	rs9635356	c.A1194C	F/FC	15	28	0.0143	0.0163	0.0737	0.56 (0.26-1.20)	0.1330
			NY	16	16	0.0154	0.0324		0.42 (0.20-0.91)	0.0274
			Israel	15	19	0.0156	0.0195	0.0214	0.75 (0.19-2.89)	0.6752
p.I1132V	rs3784635	c.A3394G	F/FC	15	26	0.0143	0.0152	0.0726	0.69 (0.32-1.48)	0.3404
			NY	11	18	0.0106	0.0363		0.28 (0.12-0.64)	0.0025
			Israel	14	19	0.0145	0.0195	0.0233	0.65 (0.16-2.65)	0.5444
p.Q2376Q	rs17238189	c.A7128G	F/FC	10	24	0.0095	0.0140	0.0698	0.51 (0.21-1.23)	0.1344
			NY	16	17	0.0154	0.0344		0.42 (0.20-0.90)	0.0245
			Israel	16	20	0.0166	0.0215	0.0212	0.47 (0.07-3.10)	0.4350

nt: nucleotide; N, number; MAF, minor allele frequency; GnomAD, Genome Aggregation Database; OR, odds ratio; CI, confidence interval; P, p value; F/FC, French/French-Canadian; NY, New York.
†GnomAD_ASJ frequencies were used for the Israeli cohort; all samples are Ashkenazi Jewish.

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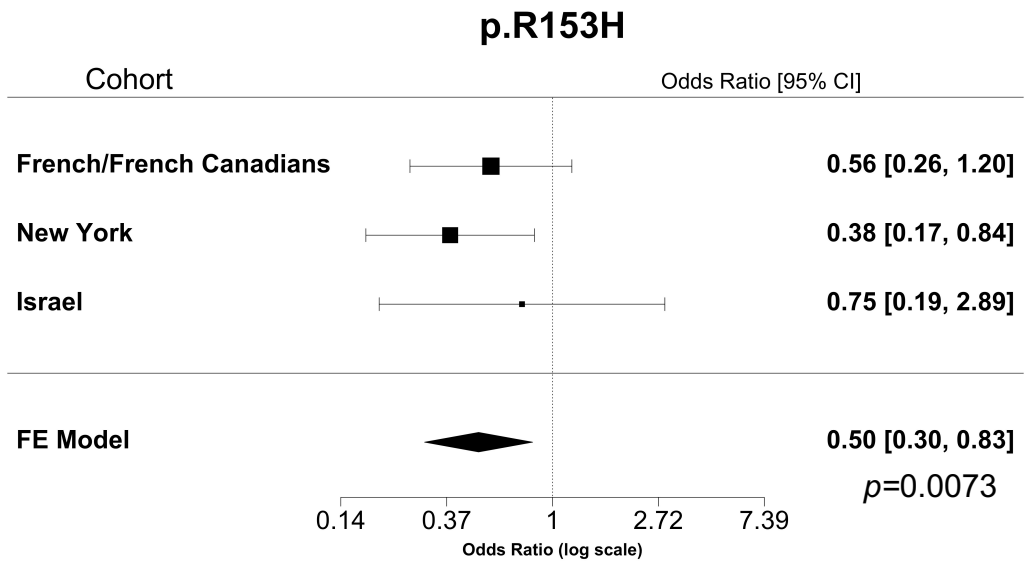
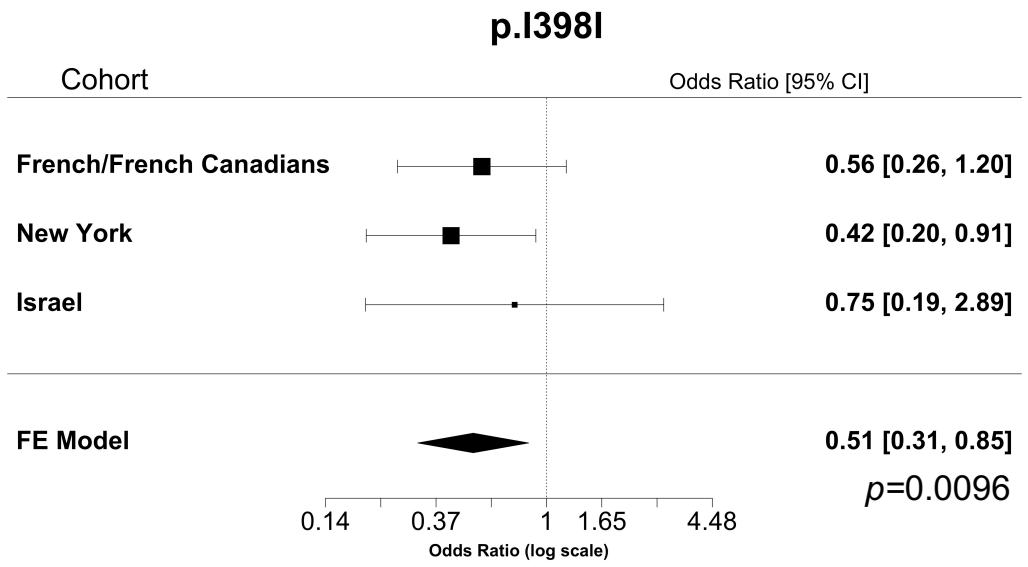
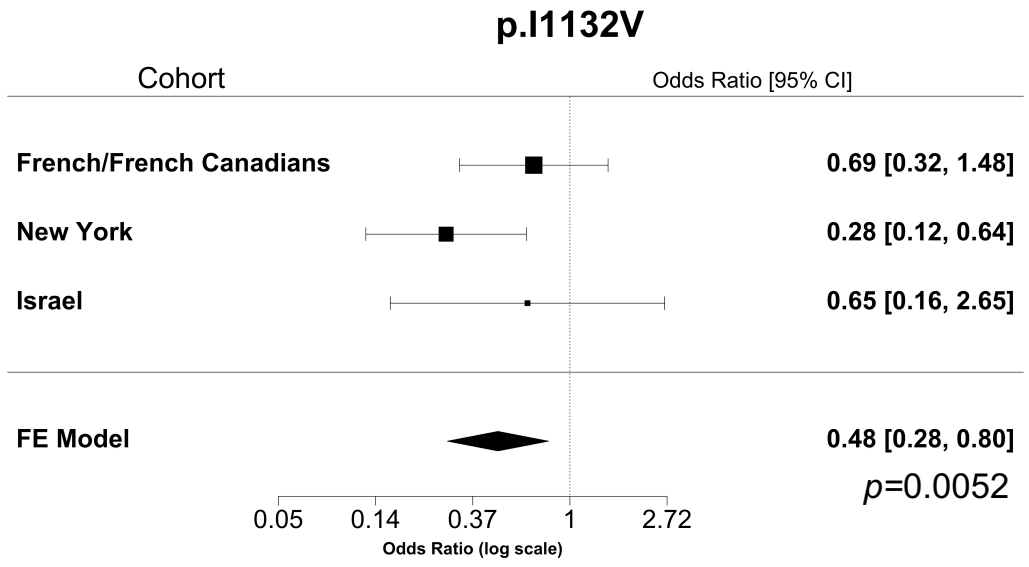
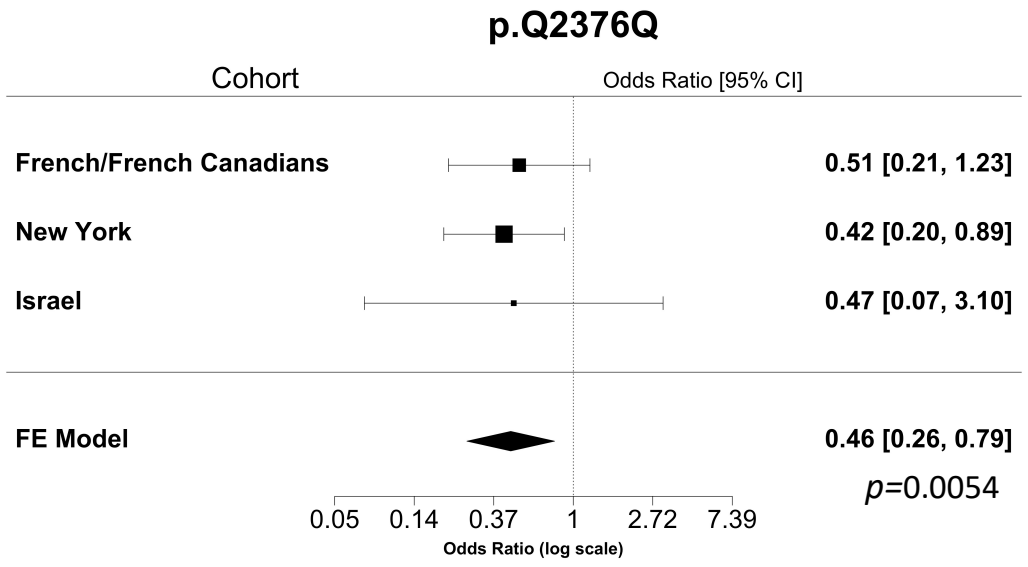

p.R153H
Cohort
Odds Ratio [95% CI]
French/French Canadians
0.56 [0.26, 1.20]
New York
0.38 [0.17, 0.84]
Israel
0.75 [0.19, 2.89]
FE Model
0.50 [0.30, 0.83]
p=0.0073
0.14
0.37
1
2.72
7.39
Odds Ratio (log scale)


p.I398I
Cohort
Odds Ratio [95% CI]
French/French Canadians
0.56 [0.26, 1.20]
New York
0.42 [0.20, 0.91]
Israel
0.75 [0.19, 2.89]
FE Model
0.51 [0.31, 0.85]
p=0.0096
0.14
0.37
1
1.65
4.48
Odds Ratio (log scale)


p.I1132V
Cohort
Odds Ratio [95% CI]
French/French Canadians
0.69 [0.32, 1.48]
New York
0.28 [0.12, 0.64]
Israel
0.65 [0.16, 2.65]
FE Model
0.48 [0.28, 0.80]
p=0.0052
0.05
0.14
0.37
1
2.72
Odds Ratio (log scale)


p.Q2376Q
Cohort
Odds Ratio [95% CI]
French/French Canadians
0.51 [0.21, 1.23]
New York
0.42 [0.20, 0.89]
Israel
0.47 [0.07, 3.10]
FE Model
0.46 [0.26, 0.79]
p=0.0054
0.05
0.14
0.37
1
2.72
7.39
Odds Ratio (log scale)