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Assessment of Parkinson's disease risk loci in Greece

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Abstract

Genome wide association studies (GWAS) have been shown to be a powerful approach to identify risk loci for neurodegenerative diseases. Recent GWAS in Parkinson's disease (PD) have been successful in identifying numerous risk variants pointing to novel pathways potentially implicated in the pathogenesis of PD. Contributing to these GWAS efforts, we performed genotyping of previously identified risk alleles in PD patients and controls from Greece. We showed that previously published risk profiles for Northern European and American populations are also applicable to the Greek population. In addition, while we were largely underpowered to detect individual associations we replicated 5 of 32 previously published risk variants with nominal p-values <0.05. Genome-wide complex trait analysis (GCTA) revealed that known risk loci explain disease risk in 1.27% of Greek PD patients. Collectively, these results indicate that there is likely a substantial genetic component to PD in Greece similarly to other worldwide populations that remains to be discovered.

Keywords

Parkinson's disease; GWAS; GCTA; genetics; Greece; risk profiles

1. Introduction

The dissection of the genetic basis of Parkinson's disease (PD) started with the identification of *a-synuclein (SNCA)* mutations in 1997 (Polymeropoulos, et al., 1997). Fifteen years later, the cause of most PD cases still remains unknown as Mendelian mutations collectively account for less than 5% of the disease (Pankratz, et al., 2012). More recently, driven by the common disease-common variant (CDCV) hypothesis (Reich and Lander, 2001), several PD genome wide association studies (GWAS) (Edwards, et al., 2010, Hernandez, et al., 2012, Pihlstrom, et al., 2013, Saad, et al., 2011, Satake, et al., 2009, Simon-Sanchez, et al., 2009, Simon-Sanchez, et al., 2011) and large scale meta-analyses (International Parkinson's Disease Genomics Consortium and Wellcome Trust, Case Control Consortium 2, 2011, Do, et al., 2011, Lill, et al., 2012, Nalls, et al., 2011, Pankratz, et al., 2012) have shown that variants within 26 loci increase the risk for PD. Despite these advances, there is evidence that a large number of causative loci still remain to be discovered (Keller, et al., 2012).

It has been previously argued that studies in isolated populations with limited genetic heterogeneity are valuable for studying the genetic basis of disease (Hernandez, et al., 2012) with an illustrative example being the Finnish population (Kere, 2001, Peltonen, et al., 1999) in which ALS GWA studies (Laaksovirta, et al., 2010) paved the road to the discovery of *C9orf72* repeat expansions as a major cause of ALS/FTD (DeJesus-Hernandez, et al., 2011, Renton, et al., 2011, Traynor, 2012). However, a recent PD GWAS completed in the Finnish population following a similar rationale failed to identify such high risk variants (Hernandez, et al., 2012).

Similarly to the Finnish population, there is evidence that the Greek population is an isolated population (Mok, et al., 2012) with subtle genetic intricacies when compared to other European populations (International HapMap Consortium, 2003, Stathias, et al., 2012). This, in combination with the location of Greece in the crossroad between Europe, Africa and the Middle East serving as a "genetic pool" for transiting populations (Di Giacomo, et al., 2004, Hughey, et al., 2013, King, et al., 2011, Semino, et al., 2004, Stathias, et al., 2012) renders genetic studies in the Greek population both promising and informative for other European populations. Motivated by these observations, we undertook a PD case-control analysis targeting variants previously implicated in risk for PD by GWA studies.

2. Materials and methods

All samples were collected in accordance to institutional ethical procedures after providing written informed consent. Individuals originated from 4 geographic locations in Greece (Athens, Crete, Syros, Thessaly for details see Table 1 and Table S1). The total number of samples was 1154 cases and 997 controls. Parkinson's disease patients were diagnosed according to the Queen Square brain bank criteria (Gibb and Lees, 1988, Gibb and Lees, 1989). Controls were healthy individuals with no signs or symptoms of parkinsonism whose close relatives were also free from parkinsonism based on self-report or available clinical data if possible.

All samples were genotyped as part of a larger study using the NeuroX Array (Illumina) which is an exome plus custom content genotyping array. The NeuroX contains 267,607 probes densely covering previously published PD GWAS associated loci, rare variants identified through exome sequencing studies of neurodegenerative diseases, ancestry informative markers, markers for determination of identity by descent, X chromosome SNPs for gender determination, candidate loci for neurodegenerative disease GWAS, as well as standard Illumina exome array content. After initial genotyping, genotypes were clustered using Illumina GenomeStudio on default parameters. For SNPs previously associated with PD, genotype clusters were manually inspected (see Figure S1).

Sample quality control (QC) was slightly more rigorous than standard GWAS due to the use of an exome-based array with abundant rare variants and experimental content. All sample QC was based on SNPs with Illumina GenTrain scores > 0.7, indicative of generally higher quality genotyping. Initially, samples with less than 95% successful calls on a genome-wide scale and gender estimated from X chromosome heterogeneity not matching clinical reports of gender were excluded. X heterogeneity calculations were based on common SNPs from the International HapMap Project that had genotypes with missingness < 5% and Hardy-Weinberg equilibrium (HWE) p-values > 1E-5. For further data cleaning, a subset of the genotype data was used, including only SNPs present in HapMap3 populations with genotype missingness < 5%, HWE p-values > 1E-5 and a pairwise r2 < 0.5 across sliding windows of 50 SNPs. Using this reduced dataset we estimated genome-wide rates of heterozygosity, excluding any samples with observed heterozygosity divergence more than 3 standard deviations from the expected population mean. Following this exclusion, samples were clustered using principal components analysis to evaluate European ancestry as compared to HapMap3 populations at overlapping SNPs (International HapMap Consortium, 2003, Patterson, et al., 2006, Price, et al., 2006, Yang, et al., 2011). At this stage, samples were excluded if they were outside of 6 standard deviations from the means of eigenvectors 1 or 2 based on the combined CEU (CEPH) and TSI (Tuscan) reference samples (see Figure S2). Confirmed European ancestry samples were extracted and identity by descent was quantified, allowing us to exclude any samples sharing proportionately more than 12.5% of alleles indicating cryptic relatedness at the level of cousins. Within related pairs, individuals were retained to maximize a 1:1 ratio of cases to controls and preserve study power. At this time, 10 eigenvectors were estimated to account for population substructure and to be used as covariates in all analyses.

Once sample quality control was completed, genotype data on all attempted SNPs was extracted for samples meeting inclusion criteria. At this point, we excluded all SNPs with MAF < 0.01, HWE p-values < 1E-5, differential missingness between cases and controls at pvalues < 1E-5, differential missingness by haplotypes at p-values < 1E-5 and GenTrain scores < 0.7. SNPs at MAF < 0.01 were not retained due to concerns about study power. For analyses in this manuscript, we utilized a working sample size of 960 cases and 876 controls genotyped at 48,805 SNPs.

The purpose of this project was to investigate whether known PD-associated SNPs contribute to PD risk in the Greek population both through mining data generated on the single SNP level, but also by using genetic risk profiling to aggregate risk across all known loci. We also attempted to estimate PD heritability in this population based on all available SNP data and also only focusing in on known GWAS loci.

For all SNPs and samples passing quality control as described above, logistic regression analyses were used to estimate risk associated with each SNP while adjusting for eigenvectors 1 – 10 as covariates. All loci summarized in Keller, et al., 2012 were also extracted to evaluate risk associated with previously discovered GWAS loci in our Greek cohort (Table 2). Loci that reached genome-wide significance in previously published PD GWASs were matched based on position to their corresponding NeuroX probes. Prior to matching, human build (HB) 36 positions of published loci were converted to HB37 through dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) when an rsID was available, or else through UCSC genome lift (http://genome.ucsc.edu/cgi-bin/hgLiftOver). Suitable proxies were located through SNAP (http://www.broadinstitute.org/mpg/snap/) or 1000 genomes (http://www.1000genomes.org/) for published SNPs that were absent from the NeuroX, did not pass quality control or had non-satisfactory cluster plots. Proxies selected fulfilled all of the following criteria: r2>0.5 and distance <500kb from SNP of interest as derived from calculations in the European ancestry populations with which imputations were conducted in the initial discovery GWAS, or the 1000 Genomes Project's phase 1 alpha freeze if no imputation was used in the original report or the imputation reference was unavailable. If more than one suitable proxy were located for a candidate SNP, proxies with the largest r2 and smallest distance were preferred. Previously published SNPs or their proxies with a MAF< 0.01 were included in the study if the MAF was similar to the one catalogued in 1000 genomes and if the cluster plot was satisfactory. After this step, SNPs remaining without suitable proxies were excluded from the study. Power calculations were undertaken with the online tool CaTS (http://www.sph.umich.edu/csg/abecasis/CaTS/index.html) (Skol, et al., 2006) for 3 levels of significance (0.05, 0.002, 5E-8) assuming a disease prevalence of 0.002 under an additive disease model; the power to detect association was calculated separately for each of the 32 variants included in our replication study using the smallest ORs and MAFs reported in previous PD GWAS meta-analyses or in the meta-analyses results cataloged in PD gene (http://www.pdgene.org/) (Lill, et al., 2012). QQ plot and genomic inflation factor were also calculated for all SNPs passing QC (figure S3).

Risk profiles were calculated incorporating 30 of the 32 published SNPs (or their proxies) included in our study (Table 2) as previously described, one monomorphic and a second near monomorphic SNP from the Greek dataset without sufficient proxies were excluded (rs2102808, rs34637584). For the SNPs from published GWAS, aggregate risk allele frequencies were calculated, weighted by the published odds ratio in a method described in detail elsewhere (International Parkinson's Disease Genomics Consortium and Wellcome Trust, Case Control Consortium 2, 2011, Hernandez, et al., 2012, Nalls, et al., 2011, Ripatti, et al., 2010). In brief, risk allele dosages were counted and a composite score across all loci was generated. Per SNP risk alleles are scaled by their published odds ratios, or using available data for proxy SNPs, giving larger weights to alleles with higher risk estimates. Overall trend estimates were used to evaluate the significance of the risk score's association with PD status across the Greek cohort using logistic regression. At this stage, receiver operator curves were generated to assess the clinical predictability of PD associated with the cumulative risk score indicated by the area under the curve (AUC) (figure S4). In addition, the dataset was divided into quintiles based on the genetic risk score. More logistic regression analyses were conducted comparing the lowest risk quintile to the 2nd through 5th highest risk quintiles, always using the lowest quintile as a reference group in the model

(Table 3). All risk profiling analyses were adjusted for eigenvectors to account for population substructure.

To ascertain narrow sense heritability estimates from this outbred sample series, the restricted maximum likelihood method within the Genome-wide complex trait analysis (GCTA) package was utilized (Lee, et al., 2011, Lee, et al., 2012, Yang, et al., 2010, Yang, et al., 2012, Yang, et al., 2011). We calculated the variance in PD risk explained by all genotyped SNPs passing quality control as well as second modeling scenario based on a subset of all SNPs passing quality control limited to those within 1 MB of previously identified GWAS loci assuming a PD prevalence in the general population of 0.002(Keller, et al., 2012). These analyses were also adjusted for principal components 1–10 to account for population substructure. This allows us to estimate heritability within the Greek population attributable to genome/exome-wide assayed variation, as well as that attributable to GWAS loci.

Finally, in order to assess the contribution to PD risk of loci previously identified through candidate gene studies in the Greek population (table 4), association results were extracted for suitable NeuroX SNPs or proxies selected as described above from the previously generated logistic regression dataset.

3. Results

Based on prior knowledge, a number of recent GWAS identified loci show marginal associations at p-values < 0.05 (Table 2). This could technically be viewed as a form of replication if prior knowledge of these robust associations is considered, even though this study itself is immensely underpowered compared to the initial discovery and replication cohorts within the original reports. While the astounding strength of the *STK39* association is impressive, lower significance associations are seen at *SNCA* (p-value 0.019), *RIT2*/*SYT4* (p value 0.002), *GAK* (p-value 0.025), and *CCDC62*/*HIP1R* (p-value 0.048), all agreeable with the directionality of allelic effect as seen in previous studies (International Parkinson's Disease Genomics Consortium and Wellcome Trust, Case Control Consortium 2, 2011, Do, et al., 2011, Lill, et al., 2012, Nalls, et al., 2011).

Our risk profiling analysis yielded results quite similar to those published in (International Parkinson's Disease Genomics Consortium and Wellcome Trust, Case Control Consortium 2, 2011, Hernandez, et al., 2012, Nalls, et al., 2011) (Table 3). We show a highly significant trend for risk profile scores calculated to assess the cumulative risk attributable to all known GWAS loci associated with PD (p-value < 1E-12) with an odds ratio of 2.44 associated with membership in the highest quintile of PD risk compared to those in the lowest quintile of PD risk. Like previous studies of PD GWAS, the predictability of risk profiles based on GWAS data does not rise to clinical utility we would have hoped for, with an area under the curve (AUC) from receiver operator curve analyses being only 0.5934.

Heritability analyses show roughly 1.27% of the variance in PD risk is attributable to the regions surrounding known GWAS loci. On the other hand, heritability estimates from all assayed SNPs passing quality control suggest that there is a total variance explained by the SNPs assayed on the NeuroX array to be around 17.55%. This suggests that future studies in larger samples sizes with dense sequencing data (among other sources of genetic data) may explain this remaining 16.28% genetic variation in risk similar to what was seen in Keller, et al., 2012.

We failed to replicate the results of previous candidate gene studies in the Greek population at a nominal significance level of <0.05 though the association was of similar directionality

and effect size for all 3 previously identified significant SNPs (Fung, et al., 2006, Michelakakis, et al., 2012) (table 4).

4. Discussion

Even though the existence of common variants of large effect size is unlikely in the Greek population based on this relatively underpowered analysis, we have replicated the association of 5 previously reported variants of lower effect size within the *SNCA*, *STK39*, *RIT2*/*SYT4*, *GAK*, and *CCDC62*/*HIP1R* loci (International Parkinson's Disease Genomics Consortium and Wellcome Trust, Case Control Consortium 2, 2011, Do, et al., 2011, Lill, et al., 2012, Nalls, et al., 2011, Pankratz, et al., 2012) with nominal p-values <0.05. There are two possible explanations for the failure to replicate the association for the remaining individual risk variants. First, our study had limited power to detect associations with variants of small MAF and effect size (table 2). Second, as it is likely that the variants identified in previous GWA studies are just proxies for the putative functional variants, population-specific differences in linkage disequilibrium patterns and allele frequencies could be responsible for the lack of replication (Singleton, et al., 2013).

We were able to replicate the previously reported risk profiles in the current dataset, and the observed effects are relatively consistent with previous work (International Parkinson's Disease Genomics Consortium and Wellcome Trust, Case Control Consortium 2, 2011, Hernandez, et al., 2012, Nalls, et al., 2011) indicating that there probably is a contribution of previously reported variants to PD risk in the Greek population.

In conclusion, the results from GCTA and the interpretation of our findings in the context of previous GWASs, coupled with positivity of family history for PD in 17.2% of our cases show that there probably is a substantial, unknown genetic component for PD in the Greek population which should be addressed in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Descriptive statistics of the Greek cohort. PD=Parkinson's disease, QC= Quality control.

Samples	Number of subjects passing QC	Male(%)	Female(%)	Mean age+/−sd	Positive PO family history(%)*	Negative PO family history(%)*
All samples	1836	1036 (56.4%)	800 (43.6%)	63.18519 +/−11.4321	115 (17.2%)	555 (82.8%)
Cases	960	553 (57.6%)	407 (42.4%)	64.04325 +/−10.91366 (age at onset)	115 (17.2%)	555 (82.8%)
Controls	876	483 (55.1%)	393 (44.9%)	62.14231 +/−11.95655 (age at study enrolment)	NA	NA

*Note: family history statistics calculation was based on 670 PO cases and 0 controls with available data.

Table 2

A summary of known PD risk loci in the Greek population.

Risk estimates are based on the dosage of Allele 1 (minor allele). MAF refers to the minor allele frequency, comparisons with 1000 Genomes Project data were based on European samples available from [http://1000genomes.org]. For directionality comparisons, previously published minor alleles with corresponding ORs per minor allele dose and p-values are listed. Power to detect association at 3 significance levels for each variant individually is also listed.

candidate gene	Corresponding SNP	NeuroX SNP	Reference	CHR	BP (HB37)	Allele 1 (minor allele)	Allele2	Genotype quality (GenTrain score)	MAF	MAF in Project European 1000 Genomes	ORper allele 1 (minor allele} dose (95% CI)	SE	P-value	Published minor allele	Published OR per minor allele dose (95% CI)	Published p-value	POWER a=0.05/0.002/5E-8
GBA	i4004 16	exm lO62 17	(Do, et al., 2011)	1	155205634	C	T	0.6468	0.005719	NA	1.491 (0.6089–3.65)	0.4568	0.382 1	C	4.048(3.08–5.32)	5. 17E-21	99%/89% /7%
GBA	N370S(proxy)	NeuroX_rs71628662	(Lill, et al., 2012)	1	155359992	C	T	0.9056	0.002996	0.03	1.598 (0.4622–5.525)	0.6329	0.4589	C	3.37 (2.67–4.25)	1.44E-14	100%/97% /26%
SYfI1/RAB25	chr l: 154 105678 (proxy)	exm-rs34372695_ver3	(Lilt et al., 2012, Nalls, et al., 2011)	1	156030037	T	C	0.7682	0.005719	0.03	1.198 (0.5 171–2.774)	0.4285	0.6739	T	1.67 (1.40–1.98)	2.35E- 12	69%/26% /0%
RAB7LljPARK16	rs708723	NeuroXJs708723	(Plagnol et al., 2011)	1	205739266	C	T	0.9013	0.40 11	0.47	0.9454 (0.8261–1.082)	0.0688	0.4144	C	0.89(0.85–0.92)	8.82 E-15	99%/86% /11%
RAB7LljPARK16	rs947211	exm-rs947211	(Lill, etal.,2012)	1	205752665	A	G	0.9262	0.2587	0.25	0.88 15 (0.7596–1.023)	0.07593	0.09669	A	0.87(0.83–0.92)	8.00E- l0	44%/10% /0"10
SLC41Al	rs823 156	exm-rs823156	(Do, et al., 20 11)	1	205764640	G	A	0.8 105	0.2072	0.18	0.8822(0.7518 –1.035)	0.08 163	0.1247	G	0.827 (0.77 –0.89)	1.27E-07	47%/12% /0%
ACMSD	rs6710823 (proxy)	NeuroX_rs6430538	(Nalls, et al., 20 11)	2	135539967	C	T	0.8185	0.27 15	0.49	1.035 (0.8917 –1.201)	0.07604	0.6507	A	1.4 (1.20–1.63)	1.35 E-09	98%/82% /8%
STK39	rs2 102808	exm-rs2 102808_ver4	(Nalls, et al., 20 11)	2	16911 7025	T	G	0.712	0	0.12	NA	NA	NA	T	1.28 (1.1 9 –1.38)	3.3 1E-11	75%/33%/ 0%
STK39	rs2390669 (proxy)	NeuroX_rs 1955337	(Lill, etal.,2012)	2	1691 29145	T	G	0.9128	0.1394	0.12	1.47 (1.212 –1.783)	0.09843	8.98E-05	C	1.14(1.06 –1.22)	1.37E-09	28%/4%/ 0%
NMD3	rs340 16896	NeuroX_rs340 16896	(Plagnol et al., 2011)	3	160992864	T	C	0.862	0.3562	0.3 1	1.039(0.906 –1.192)	0.06997	0.583 1	T	1.08 (1.02- 1.14)	1.31E-06	19%/2%/ 0%
MCCCljLAMP3	rsl0513789	exm-rsl0513789_ver4	(Do, et al., 2011)	3	182760073	G	T	0.9032	0.2075	0.22	0.9603(0.8166 –1.129)	0.08271	0.6244	G	0.803(0.75 –0.86)	2.67E- l0	58%/18%/ 0%
MCCCljLAMP3	rsl171 1441	exm-rs l171 144 1	(Lill, et al., 2012, Nalls, et al., 20 11)	3	182821275	A	G	0.82 19	0.146	0.17	1.007 (0.8358- 1.2 14)	0.0952	0.9399	A	0.84(0.80 –0.89)	9.20E- l0	32%/6%/ 0%
GAK	rs6599389	exm-rs6599389	(Do, et al., 2011)	4	939 113	A	G	0.794 1	0.1198	0.06	1.258 (1.03- 1.537)	0.1022	0.02478	A	1.311 (1.19 –1.44)	3.87E-08	63%/2 1%/ 0%
DGKQ	rs11248060	exm-rs 11248060	(Lill, etal.,2012)	4	964359	T	C	0.9079	0.1168	0.13	1.221 (0.9917 –1.503)	0.1061	0.05989	T	1.22 (1.1 3 –1.32)	3.04 E-12	50%/13%/ 0%
BSTl	rsl1724635	NeuroX_rs l1724635	(Lill, et al., 2012, Nalls, et al., 2011)	4	15737101	C	A	0.898	0.4314	0.44	0.9566(0.8382 –1.092)	0.06739	0.5099	C	0.86(0.82 –0.91)	1.87E- l0	90%/12%/ 0%
STBDl	rs6812 193	exm-rs6812193	(Plagnol et al., 2011)	4	77198986	T	C	0.9278	0.3597	0.4	1.007 (0.8793 –1.1 53)	0.069 15	0.9205	T	0.89(0.85 –0.93)	1.17E-17	79%/5%/ 0%
SNCA	rs356220	exm-rs356220	(Do, et al., 2011)	4	90641340	T	C	0.8228	0.3905	0.49	1.172 (1.026- 1.339)	0.0679 1	0.0 1923	T	1.285 (1.22 –1.36)	2.29E- 19	96%/74%/ 4%
SNCA	rs6532 194	NeuroX_rs6532 194	(Lill, etal.,2012)	4	90780902	T	C	0.9136	0.1155	0.1	1.122 (0.9125 –1.381)	0.1056	0.2743	T	1.23 (1.14- 1.32)	4.9 1E-11	87%/5 1%/ 1%
HIA	rs2395 163	exm-rs2395163	(Pankratz, etal.,2012)	6	32387809	C	T	0.7276	0.1408	0.2 1	0.9702(0.774 –1.216)	0.1153	0.7928	C	0.8 1(0.78–0.84)	3.00E- 11	66%/23%/ 0%

candidate gene	Corresponding SNP	NeuroX SNP	Reference	CHR	BP (HB37)	Allele 1 (minor allele)	Allele2	Genotype quality (GenTrain score)	MAF	MAF in Project European 1000 Genomes	ORper allele 1 (minor allele) dose (95% CI)	SE	P-value	Published minor allele	Published OR per minor allele dose (95% CI)	Published p-value	POWER a=0.05/0.002/5E-8
GPNMB	rs156429 (proxy)	NeuroXJs 199347	(Plagnol et al., 2011)	7	23293746	C	T	0.8652	0.43 19	0.37	0.9868(0.8651 –1.126)	0.06712	0.8429	G	0.89(0.86 –0.93)	3.05 E-13	4 1%/9%/ 0%
FGF20	rs591323	NeuroXJs591323	(Plagnol et al., 2011)	8	16697091	A	G	0.8602	0.2508	0.29	1.046 (0.8982 –1.2 19)	0.07793	0.5603	A	0.89(0.86 –0.93)	1.92E-11	35%/7%/ 0%
ITGAB	rs707736 1	exm-rs707736 1	(Lill, etal.,2012)	10	15561543	C	T	0.907	0.1443	0.1	1.097 (0.9128 –1.318)	0.09368	0.3239	C	0.86(0.79 –0.93)	1.51E-08	25%/4%/ 0%
IRRK2	rs1491942	exm-rs 1491942	(Lill, et al., 2012, Nalls, et al., 2011)	12	40620808	C	G	0.7824	0.2467	0.18	1.11 7 (0.9619 –1.297)	0.07629	0.1468	C	1.2 (1.11 –1.29)	6.44E- 15	51%/14%/ 0%
IRRK2	rs34637584	exm994671	(Do, et al., 2011)	12	40734202	A	G	0.7749	0.000545	0	1.45 E +09 (O-Inf)	17240	0.999	A	9.6 15 (6.43- 14.37)	1.82E-28	100%/1 00%/ 35%
CCDC62jHIP1R	rs12817488 (proxy)	NeuroX_dbSNP *Js 110*60 180_replciate_l	(Nalls, et al., 2011)	12	123303586	G	A	0.9306	0.4662	0.45	0.9075(0.7963 –1.034)	0.06668	0.1456	A	1.17 (1.09 –1.24)	4.43E-09	61%/20%/ 0%
CCDC62jHIP1R	rsl0847864	NeuroX_rsl0847864	(Lill, etal.,2012)	12	123326598	T	G	0.879 1	0.3453	0.34	1.147 (1.00 1 –1.314)	0.06939	0.04871	T	1.12 (1.07 –1.18)	4.37 E-17	55%/16%/ 0%
STX1B	rs4889603	NeuroX_rs4889603	(Plagnol et al., 2011)	16	30982225	A	G	0.7141	0.4438	0.41	0.8985(0.7878 –1.025)	0.06708	0.1105	G	1.14 (1.09 –1.1 9)	6.98E-13	40%/8%/ 0%
SREBFljRA1I	rs11868035	exm-rs 11868035	(Do, et al., 2011)	17	17715101	A	G	0.7445	0.3769	0.34	0.9303(0.8114- 1.067)	0.06977	0.3004	A	0.85 1(0.80 –0.90)	5.6 1E-08	63%/2 1%/ 0%
MAPT	rs2942 168	exm-rs2942168	(Nalls, et al., 2011)	17	43714850	T	C	0.9049	0.2067	0.23	0.8524(0.6366 –1.141)	0.1489	0.2837	T	0.78(0.74 –0.81)	1.62E-18	82%/42%/ 1%
MAPT	rs12 185268	exm1330895	(Do, et al., 2011)	17	43923683	G	A	0.7038	0.2033	0.23	0.899 1(0.6716 –1.204)	0.1488	0.4749	G	0.769 (0.72 –0.82)	2.72 E-14	92%/60%/ 2%
RIT2jSYT4	rs4130047	exm-rs4130047	(Do, et al., 2011)	18	40678235	C	T	0.853	0.32 19	0.33	1.239 (1.08 –1.422)	0.070 14	0.00222	C	1.16 1 (1.10- 1.23)	2.44E-07	57%/17%/ 0%
USP25	rs2823357	exm-rs2823357	(Do, et al., 2011)	21	16914905	A	G	0.9 151	0.3736	0.39	1.021 (0.8938 –1.1 67)	0.06799	0.758	A	1.149 (1.09 –1.21)	6.32E-07	54%/15%/ 0%

BP=base pair, OR=Odds ratio, SE=standard error, CI=confidence interval, HB=Human Build.

Table 3

Genetic risk profiles in the Greek cohort.

Profile based on **Table 2 SNPs**	**1st quintile**	**2nd quintile**	**3rd quintile**	**4th quintile**	**5th quintile**
Odds ratio	1	1.06	1.25	1.59	2.44
Lower limit ofthe 95% CI		0.79	0.94	1.18	1.81
Higher limit ofthe 95% CI		1.42	1.68	2.12	3.3
Trend p-vaIue	7.82E-13				
AUC	0.5934				

AUC= predictive area under the curve, CI=confidence interval.

Table 4

Study of variants identified through previous candidate gene studies in the Greek population.

Gene	SNP	NeuroX SNP	CHR	BP (HB37)	A1 (minor allele)	OR (95% Cl) per copy of A1	SE	p-value	Published minor allele	Published OR (95% Cl) per copy of minor allele	Published p-value	Sample size (cases/controls)	Reference
SCARB2	rs6824953	NeuroX_rs35873788 (proxy)	4	77096606	T	1.061 (0.9138–1.233)	0.07636	0.436	G	1.26 (1.01–1.57)	0.042	347/329	(Michelakakis, et al., 2012)
SCARB2	rs6825004	Neu roxd b5NP_rs6825004_replciate_1	4	77110365	G	0.8764 (0.763–1.007)	0.0707	0.06197	G	0.71 (0.56–0.90)	0.006	347/329	(Michelakakis, et al., 2012)
SCARB2	rs6825004	Neu rOX_db5NP_rs6825004_replciate_2	4	77110365	G	0.8764 (0.763–1.007)	0.0707	0.06197	G	0.71 (0.56–0.90)	0.006	347/329	(Michelakakis, et al., 2012)
SCARB2	rs6825004	Neu roxd b5NP_rs6825004_replciate_3	4	77110365	G	0.8764 (0.763–1.007)	0.0707	0.06197	G	0.71 (0.56–0.90)	0.006	347/329	(Michelakakis, et al., 2012)
SCARB2	rs4241591	proxy tors6824953	4	77121346	NA	NA	NA	NA	A	0.99 (0.79–1.23)	0.93	347/329	(Michelakakis, et al., 2012)
SCARB2	rs9991821	proxy tors6825004	4	77130285	NA	NA	NA	NA	A	0.94 (0.72–1.24)	0.69	347/329	(Michelakakis, et al., 2012)
SCARB2	rsl7234715	NeuroX_rs11097314 (proxy)	4	77187556	A	1.071 (0.938–1.222)	0.06752	0.3118	C	0.95 (0.74–1.22)	0.69	347/329	(Michelakakis, et al., 2012)
LRRK2	rs10878258	NeuroX_rs6581622 (proxy)	12	40634158	C	0.9141 (0.7935–1.053)	0.07222	0.2136	NA	NA	0.05	217/221	(Paisan-Ruiz, et al., 2006)
AKTI	rs2494743	NA	14	105251720	NA	NA	NA	NA	C	1.22 (0.85–1.75)	0.27	281/220	(Xiromerisiou, et al., 2008)
AKTI	rs2498788	NA	14	105253009	NA	NA	NA	NA	T	1.52 (1.0–2.32)	0.044	281/220	(Xiromerisiou, et al., 2008)
AKTI	rs2494746	NA	14	105257719	NA	NA	NA	NA	C	1.13 (0.79–1.62)	0.48	281/220	(Xiromerisiou, et al., 2008)
AKTI	rsl130214	NA	14	105259734	NA	NA	NA	NA	T	0.99 (0.74–1.32)	0.95	281/220	(Xiromerisiou, et al., 2008)
MAPT	rs1467967	NA	17	43986179	NA	NA	NA	NA	C	0.89 (0.67–1.18)	0.43	100/94	(Fung, et al., 2006)
MAPT	rs242557	exm-rs242557	17	44019712	A	1.044 (0.906–1.204)	0.0725	0.5493	A	1.28 (0.96–1.71)	0.09	100/94	(Fung, et al., 2006)
MAPT	rs3785883	NeuroX_dbSNP- rs116686818	17	44054433	A	1.069 (0.9062–1.261)	0.08429	0.4284	A	1.42 (0.99–2.03)	0.05	100/94	(Fung, et al., 2006)
MAPT	rs2471738	NA	17	44076063	NA	NA	NA	NA	A	0.97 (0.67–1.41)	0.88	100/94	(Fung, et al., 2006)
MAPT	del-ln9	NA	17	44086651	NA	NA	NA	NA	H2	0.81 (0.59–1.11)	0.19	100/94	(Fung, et al., 2006)
MAPT	rs7521	NeuroX- rs7521	17	44105395	G	1.001 (0.8662–1.157)	0.07395	0.9859	G	0.82 (0.62–1.05)	0.15	100/94	(Fung, et al., 2006)

CHR=Chromosome, BP=Base pair, HB=Human Build, OR=Odds ratio, SE=Standard error, CI=Confidence intervals.