Version 1

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SNP Genotyping and ApoE Genotyping V.1

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ABSTRACT

This protocol details the steps for DNA extraction from a human blood sample, quality control, and SNP and APOE genotyping.

The protocol has been adapted from the **PRoBaND SNP Genotyping and ApoE Genotyping Protocol.**

The overall protocol for PRoBaND /Tracking Parkinson's is published:

Malek, N., Swallow, D. M. A., Grosset, K. A., Lawton, M. A., Marrinan, S. L., Lehn, A. C., Bresner, C., Bajaj, N., Barker, R. A., Ben-Shlomo, Y., Burn, D. J., Foltynie, T., Hardy, J., Morris, H. R., Williams, N. M., Wood, N., & Grosset, D. G. (2015). Tracking Parkinson's: Study Design and Baseline Patient Data. *Journal of Parkinson's Disease*, *5*(4), 947–959. https://doi.org/10.3233/JPD-150662

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EXTERNAL LINK

https://doi.org/10.1136/jnnp-2021-328365

PROTOCOL CITATION

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

https://content.iospress.com/articles/journal-of-parkinsons-disease/jpd150662

KEYWORDS

Parkinson's disease, Blood, DNA, Genotyping, Apolipoprotein E (APOE), Single Nucleotide Polymorphisms (SNPs), ASAPCRN

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GUIDELINES

The 1000 genomes reference panel:

1000 Genomes Project Consortium, Auton, A., Brooks, L. D., Durbin, R. M., Garrison, E. P., Kang, H. M., Korbel, J. O., Marchini, J. L., McCarthy, S., McVean, G. A., & Abecasis, G. R. (2015). A global reference for human genetic variation. *Nature*, *526*(7571), 68–74.https://www.nature.com/articles/nature15393

MATERIALS TEXT

BD Vacutainer EDTA Blood Collection Tubes:
https://www.camlab.co.uk/bd-vacutainer-edta-blood-collection-tubes

Illumina HumanCore Exome Array
https://www.illumina.com/products/by-type/microarray-kits/infinium-exome.html

SAFETY WARNINGS

The process of venepuncture has the potential to expose research and clinical personnel and other health care professionals and participants to blood from other people, thereby putting them at risk from blood borne pathogens.

In order to minimise risk to the participants, research and clinical personnel and other health care professionals it is imperative to ensure that personnel performing a venepuncture have received appropriate training and education to ensure they are competent. The procedure, the use of PPE and disposal of sharps etc., must be in accordance institution policies, approved risk assessments and procedures.

BEFORE STARTING

Check the planned procedure is covered by existing Research Ethics Committee. Check that you have the correct PPE, supplies and consumables.

Sample Collection and Genotyping

1 At study entry (baseline), collect a **4 mL** blood sample in an ethylenediaminetetraacetic acid (EDTA) vacutainer for DNA extraction.

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use and disposal of sharps etc., must be in accordance institution policies, approved risk assessments and procedures.

2 Perform DNA extraction.

DNA extraction was carried out at K biosciences, UK

3 Genotype DNA samples using the Illumina HumanCore Exome array with custom content following manufacturers instructions : https://www.illumina.com/products/by-type/microarray-kits/infinium-exome.html

This covered approximately 250,000 common variants, 250,000 rare variants, and over 27,000 custom variants that have been implicated in neurological and psychiatric disorders.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., de Bakker, P. I. W., Daly, M. J., & Sham, P. C. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. American Journal of Human Genetics, 81(3), 559–575. https://doi.org/10.1086/519795

Genotyping was performed Cardiff University: https://www.cardiff.ac.uk/mrc-centre-neuropsychiatric-genetics-genomics

Quality Control and Principle Component Analysis

4 Perform standard quality control procedures in PLINK v1.9: https://www.cog-genomics.org/plink/

5 Remove individuals:

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5.1 With low overall genotyping rates (< 98%), related individuals (Identity-By-Descent PIHAT > 0.1) and heterozygosity outliers (> 2 SDs away from the mean)

5.2 Whose clinically reported biological sex did not match the genetically determined sex.

6 Conduct principle component analysis on a linkage disequilibrium (LD) pruned set of variants (removing SNPs with an r2 > 0.05 in a 50kb sliding window shifting 5 SNPs at a time) after merging with European samples from the HapMap reference panel.

7 Remove:

7.1 Individuals who are > 6 SDs away from the mean of any of the first 10 principal components.

7.2 Variants if they had a low genotyping rate (< 99%), Hardy-Weinberg Equilibrium p-value < 1 x 10-5, and minor allele frequency < 1%.

1,000 Genomes Project and APOE Genotyping

8 Following quality control, input genotypes separately to the 1,000 Genomes Project reference panel (phase 3 release 5) using the Michigan Imputation Server (https://imputationserver.sph.umich.edu).

9 Retain only variants with imputation quality >0.8, to keep only high quality calls to merge across the cohorts.

10 Tracking Parkinson's data was lifted over to genome build hg38 using liftOver (https://genome.ucsc.edu/cgi-bin/hgLiftOver).

Twenty genetic principal components are generated from a linkage-pruned SNP set (removing SNPs with an r2 > 0.02 in a 1000kb sliding window shifting 10 SNPs at a time).

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Extreme outliers are removed according to the first 5 principal components (> 6 SDs away from the mean).

11 Recalculate the genetic principal components after removing outliers, as extreme outliers can substantially affect the calculation of genetic principal components.

11.1 These first 5 new principal components are included as covariates in future GWAS to adjust for population substructure

11.2 Additional outliers who are > 6 SDs away from the mean of any of the first 5 principal components are excluded.

12 Restrict genotyping of APOE to samples and SNPs that survive these QC procedures based on two common SNPs of the APOE gene: 388 T > C (rs429358) and 526C > T (rs7412).

13 The three haplotypes (ε2(388 T–526 T), ε3(388 T-526C), ε4(388C-526C)) and six genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, ε4/ε4) formed by these SNPs are then used to determine ε4 status in each sample.

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