

## NeuroChip, an updated version of the NeuroX genotyping platform to rapidly screen for variants associated with neurological diseases



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### ARTICLE INFO

#### Article history:

Received 8 March 2017

Received in revised form 8 May 2017

Accepted 8 May 2017

Available online 17 May 2017

### ABSTRACT

Genetics has proven to be a powerful approach in neurodegenerative diseases research, resulting in the identification of numerous causal and risk variants. Previously, we introduced the NeuroX Illumina genotyping array, a fast and efficient genotyping platform designed for the investigation of genetic variation in neurodegenerative diseases. Here, we present its updated version, named NeuroChip. The NeuroChip is a low-cost, custom-designed array containing a tagging variant backbone of about 306,670

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**Keywords:**  
Genotyping  
NeuroX  
NeuroChip  
Genetic screening  
Neurodegeneration

variants complemented with a manually curated custom content comprised of 179,467 variants implicated in diverse neurological diseases, including Alzheimer's disease, Parkinson's disease, Lewy body dementia, amyotrophic lateral sclerosis, frontotemporal dementia, progressive supranuclear palsy, corticobasal degeneration, and multiple system atrophy. The tagging backbone was chosen because of the low cost and good genome-wide resolution; the custom content can be combined with other backbones, like population or drug development arrays. Using the NeuroChip, we can accurately identify rare variants and impute over 5.3 million common SNPs from the latest release of the Haplotype Reference Consortium. In summary, we describe the design and usage of the NeuroChip array and show its capability for detecting rare pathogenic variants in numerous neurodegenerative diseases. The NeuroChip has a more comprehensive and improved content, which makes it a reliable, high-throughput, cost-effective screening tool for genetic research and molecular diagnostics in neurodegenerative diseases.

Published by Elsevier Inc.

## 1. Introduction

Neurodegenerative diseases are a major burden to the aging world population, and currently these diseases are incurable and irreversible. Common and rare genetic alterations in many genes have been identified as disease-causing or contributing to the development of neurodegeneration (Naj and Schellenberg, 2017; Singleton and Hardy, 2016). To date, there are 4 main uses of genetics: (1) to confirm a clinical diagnosis by identifying a causal mutation; (2) to identify risk variants and disease modifiers that influence risk for disease; (3) to increase knowledge of the molecular pathobiology of disease in the hopes of identifying therapeutic targets; and (4) to improve patient selection for pathway-specific clinical trial design. A reliable, high-throughput, and cost-effective platform that can rapidly conduct these functions could therefore be immensely valuable to the field.

Previously, we presented the NeuroX array, which was a collaborative effort with the objective of designing a genotyping platform that would allow rapid genetic characterization of samples in the context of genetic mutations and risk factors associated with common neurodegenerative diseases (Nalls et al., 2015). This was an exonic array (or exome chip) based on the Infinium HumanExome Beadchip v1.1 containing 242,901 exome-focused variants as well as 24,706 custom variants focusing on neurological diseases. The NeuroX array has already been successfully used in dozens of studies (Barber et al., 2017; Carrasquillo et al., 2016; Ghani et al., 2015; Nalls et al., 2016; Rosenthal et al., 2016). However, due to the backbone's focus on rare exonic variation, common nonexonic variants were largely missed, resulting in a modest genome-wide resolution and only partial capture of the known low frequency exonic variation. In addition, the number of genotype-phenotype associations and pathogenic variants keeps expanding, so there was a continued need for updating this useful platform.

Here, we report on an updated version of NeuroX, named NeuroChip. The NeuroChip backbone is based on a genome-wide genotyping array (Infinium HumanCore-24 v1.0) containing 306,670 tagging variants and a custom content that has been updated and extended with neurodegenerative disease-related custom content consisting of 179,467 variants. This backbone was chosen because of the low cost and good genome-wide resolution. This backbone is flexible and other arrays can be used with this custom content, such as population or drug development arrays (Infinium Multi-Ethnic, Infinium DrugDev). The NeuroChip allows to accurately identify rare neurodegenerative candidate variants and imputes over 5.3 million common variants. Its approximate cost of ~\$40 per sample is a fraction of the price of next-generation whole-exome or whole-genome sequencing, and therefore provides a valuable, high-throughput screening tool for loci and variants implicated in neurodegenerative diseases. Further, this array can be used as a tool to prioritize samples for more expensive genome sequencing approaches.

## 2. Methods

### 2.1. NeuroChip array design

The backbone of the array, the Infinium HumanCore-24 v1.0, contains 306,670 highly informative tagging SNPs which can be used for high-throughput and high-quality imputation of genome-wide variants across diverse populations (Illumina). In addition, the chip contains 179,467 custom disease-associated variants (Table 1) covering neurodegenerative diseases including: Alzheimer's disease (AD), Parkinson's disease (PD), Lewy body dementia, frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS), progressive supranuclear palsy, corticobasal degeneration, and multiple system atrophy. The custom-content has been curated by members of the International Parkinson's Disease Genomics Consortium (IPDGC) and the Comprehensive Unbiased Risk factor Assessment for Genetics and Environment in Parkinson disease (COURAGE-PD) consortium to include common variants and rare mutations implicated in neurological diseases as reported in the Human Gene Mutation Database (HGMD Professional, 2016.4, QJAGEN), the NHGRI GWAS Catalog ([www.ebi.ac.uk/gwas/](http://www.ebi.ac.uk/gwas/)), the Online Mendelian Inheritance in Man (OMIM) database ([www.ncbi.nlm.nih.gov/omim/](http://www.ncbi.nlm.nih.gov/omim/)), the Parkinson's Disease Mutation Database ([www.molgen.vib-ua.be/PDMutDB/](http://www.molgen.vib-ua.be/PDMutDB/)), the Alzheimer's Disease and Frontotemporal Dementia Database ([www.molgen.ua.ac.be/admutations/](http://www.molgen.ua.ac.be/admutations/)), and based on literature review as well as own data; particularly in the latter case, collaborators submitted variants that were identified in multiple ongoing (or completed) unpublished projects, including variants from genome-wide association (GWA), whole-exome, whole-genome, targeted sequencing studies, and systems biology studies. See [Supplementary Table 1](#) for the complete content of the NeuroChip array.

### 2.2. NeuroChip array genotyping

We genotyped a cohort of 273 controls as per the manufacturer's instructions (Illumina) to generate pilot NeuroChip data. These

**Table 1**  
Differences between NeuroX and the NeuroChip

Variant description	NeuroX	NeuroChip	Comparison
Total variants (pre-QC)	267,607	486,137	+218,530
Backbone	242,901	306,670	+63,769
Custom-content variants	24,706	179,467	+154,761
Indels	200	16,259	+16,059
Autosomal variants	261,477	473,442	+211,965
Coding variants	226,104	88,560	-137,544
Sex chromosomal variants	5906	11,840	+5934
Mitochondrial variants	219	160	-59
Variants with MAF <0.05	219,093	227,448	+8355
Variants with MAF <0.001	179,500	154,953	-24,547

Key: MAF, minor allele frequency.

samples have been collected by the North American Brain Expression Consortium (NABEC) and described elsewhere (Hernandez et al., 2012). In total, 183 males and 90 females were included. All samples were obtained from North American brain banks; included subjects reported European ancestry; and had no reported neurological disease. To assess the reproducibility of the NeuroChip, we genotyped 15 samples twice in separate experiments.

Raw data files were imported into GenomeStudio (version 2.0, Illumina). For initial quality control, we confirmed accurate, high-quality genotyping using a call rate threshold of >95%. We reclustered the samples using a GenCall threshold of 0.15 and recalled all variants. The genotyping cluster file based on ~3500 individuals of ongoing projects is available in the Supplementary Materials (Supplementary File 1). The mean call rate post-reclustering was 0.992 (range: 0.954–0.995). The data were exported from GenomeStudio using the Illumina-to-PLINK module 2.1.4 and imported into PLINK (version 1.90; Chang et al., 2015). Next, we checked individuals for discrepancies between reported sex and genotypic sex, cryptic relatedness (PIHAT <0.05), and heterogeneity contamination and found that no samples failed this quality control step.

### 2.3. NeuroChip content annotation

Annotation of the NeuroChip content was performed using ANNOVAR (Wang et al., 2010). For each variant, a gene-based annotation, *in silico* impact scores, and the frequencies from public databases were obtained. To predict the impact scores, the following algorithms were used: SIFT (Kumar et al., 2009), Polyphen-2 (Adzhubei et al., 2010), and CADD (Kircher et al., 2014). Population frequencies were obtained from the Exome Aggregation Consortium (version 0.3.1; <http://exac.broadinstitute.org/>) containing 60,706 individuals. In addition, all variants were investigated for their presence in the Human Gene Mutation Database (HGMD, accessed 20 December 2016). Variants associated with a common neurodegenerative syndrome (AD, ALS, FTD, and PD) were manually curated and are summarized in Supplementary Table 2.

### 2.4. NeuroChip content imputation

After confirming high-quality genotyping (call rate >95%) and European ancestry in all individuals (based on 1000Genomes clustering; Genomes Project et al., 2015), we performed imputation using the Michigan imputation server, according to established guidelines (<https://imputationserver.sph.umich.edu>; Das et al., 2016). In brief, genotypes were prepared for imputation using provided scripts (HRC-1000G-check-bim.pl), which compares variant ID, strand, and allele frequencies to the haplotype reference panel (HRC version r1.1, April 2016; McCarthy et al., 2016). A total of 332,015 autosomal SNPs were submitted to the Imputation Server using ShapeIT (v2.r790).

### 2.5. APOE allele genotyping

To determine the accuracy of APOE allele predictions, we performed Taqman genotyping of 2 non-synonymous APOE SNPs (rs7412 and rs429358) on an Applied Biosystems ViiA 7 Real-Time PCR System using an established protocol (Federoff et al., 2012). Two hundred seventy-two of 273 control samples had sufficient DNA for genotyping. Allelic discrimination was conducted using QuantStudio software (version 1.3, Thermo Fisher Scientific, Carlsbad, CA, USA). Taqman genotype results were then compared with the corresponding results for the same SNPs generated using the NeuroChip. Given the importance of APOE, NeuroChip was designed so that rs7412 is genotyped by 4 separate probes (3 of which

performed well: rs7412, seq-rs7412-B1, seq-rs7412-B3). Similarly, rs429358 was genotyped by 5 separate bead probes (2 of which performed well: seq-rs429358-T2, seq-rs429358-T3). This redundancy ensures accurate APOE genotyping by the NeuroChip platform.

## 3. Results

### 3.1. NeuroChip content overview

In total, the NeuroChip array contains 473,442 autosomal variants, 11,840 sex chromosomal variants, and 160 mitochondrial variants. In addition, 16,274 NeuroChip variants detect small insertions or deletions (Table 1). The overlap between NeuroX and NeuroChip is small ( $n = 19,289$  variants) due to the difference in the design of the backbone; the NeuroX array is focused on exonic content, whereas the NeuroChip is focused on genome-wide tagging content.

### 3.2. NeuroChip pathogenic variant content

In total, the NeuroChip harbors 8086 disease-associated variants that are included in HGMD, a professionally curated database of published genetic variants that have been linked to inherited human diseases (neurological and non-neurological). The NeuroChip HGMD content includes 1233 variants (1202 SNPs and 31 indels) linked to common neurodegenerative syndromes (see Supplementary Fig. 1 for a comparison between NeuroX and NeuroChip). In this content, after the manual curation, 601 variants are associated with ALS or FTD, 348 with PD, and 284 with AD. Fig. 1 shows the number of disease-associated variants per gene covered in common neurodegenerative syndromes based on the HGMD database content. Detailed, manually curated, and annotated variant lists for the abovementioned neurodegenerative disease categories are documented in Supplementary Table 2. These annotated lists can be used as filters to quickly screen for known mutations and risk variants.

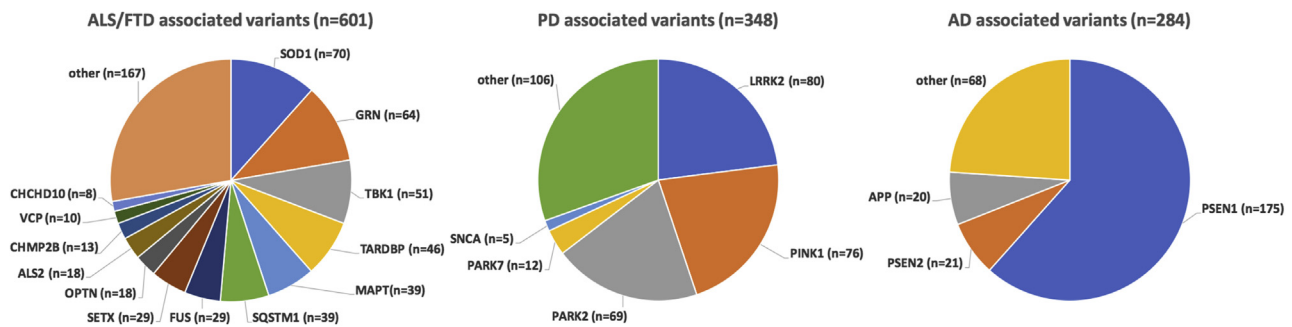
### 3.3. NeuroChip genotyping results

#### 3.3.1. Genotyping reproducibility

Of the 15 technical replicates, all samples yielded high quality, reproducible genotyping results. The mean concordance rate per technical replicate was 0.9996 (range = 0.9991–0.9999); on average, 190 variants (range = 27–435) differed per technical replicate (0.04% of the total included variants on the array). Across the 15 technical replicates, 1978 unique variants were discordant, of which 749 (37.9%) were from the backbone and 1229 (62.1%) were from the custom content (Supplementary Table 3).

#### 3.3.2. Imputation

Imputation of autosomal variants was performed on a series of 273 European descent individuals using the haplotype reference panel (McCarthy et al., 2016) containing 39,235,157 variants, all with an estimated minor allele count of  $\geq 5$  in 32,488 individuals. Initial preimputation filtering of the NeuroChip data (including removing duplicates and nonoverlapping variants, switch strands, and updating position) resulted in 332,015 variants. After imputation, 11,879,345 variants were obtained with an imputation  $R^2$  of >0.30. Filtering based on MAF >0.05, Hardy-Weinberg equilibrium  $p$ -value of  $>1e-6$  resulted in 5,316,028 variants. In this imputed data set, we successfully identified 22 of 26 PD risk alleles and 19 of the 21 AD GWA SNPs (Lambert et al., 2013; Nalls et al., 2014).



**Fig. 1.** Overview of the number of HGMD disease-associated variants that are present on the NeuroChip. Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia; PD, Parkinson's disease.

### 3.3.3. Genotype accuracy

GenTrain scores were calculated for all NeuroChip variants using GenomeStudio (version 2, Illumina). The GenTrain score is a statistical score based on the shapes of the different allelic clusters and their relative distance to each other (Illumina). Typically, GenTrain scores  $>0.7$  are considered high-quality genotypes. Previously, GenTrain scores of the NeuroX showed that genotyping quality in the custom content was lower compared with the backbone (Nalls et al., 2015). However, preliminary NeuroChip data from several ongoing projects (based on  $\sim 3500$  individuals) reveal that the backbone and the custom content have a high comparable average score (0.819 and 0.820, respectively), indicating high genotyping accuracy (Supplementary Figs. 2 and 3).

### 3.3.4. Validation of APOE genotyping

APOE alleles are important genetic risk factors for both AD and Lewy body dementia, but genotyping of this region is complicated by high GC content (Singleton et al., 2002; Strittmatter and Roses, 1996). For this reason, we chose to validate the accuracy of APOE allele genotyping by comparing Taqman results with genotype predictions from the NeuroChip (Supplementary Table 4). Taqman genotyping for rs7412 and rs429358 was successful in all 272 samples. NeuroChip genotyping for both SNPs was successful in 265 out of these 272 controls. Five samples were discordant for APOE allele genotyping between Taqman and NeuroChip due to relatively low-quality genotype calls in either the Taqman assay or the NeuroChip, representing 1.9% of our test cohort ( $n = 265$  samples). Overall, the performance of the NeuroChip for APOE genotyping was remarkably improved compared with the original NeuroX platform, which was unable to reliably detect rs7412 and rs429358 genotypes (Ghani et al., 2015; Nalls et al., 2015).

## 4. Discussion

The main goal was to develop a genotyping array that allows a rapid, high-throughput identification of common and rare single nucleotide variants in the human genome. Affordable screening of large cohorts for disease-associated variants allows for testing of polygenic inheritance that could explain the diversity of clinical and pathological characteristics of neurodegenerative diseases. The NeuroChip is estimated to cost  $\sim \$40$ /sample, which is currently less than  $\sim 10\%$  and  $\sim 5\%$  of the cost of whole-exome sequencing and whole-genome sequencing, respectively.

We have designed, implemented, and validated the NeuroChip array platform for high-throughput genotyping. However, it is important to recognize the limitations of this approach. Like all genotyping arrays, NeuroChip does not detect novel sequence changes. It is also not possible to genotype variants in complex genomic regions (e.g., due to pseudogenes) or to identify repeat

expansions due to the difficulty in designing reliable probes. Nevertheless, every effort was made to improve genotype calling in NeuroChip. For example, it was recognized that the APOE locus performed poorly on the original NeuroX platform (Ghani et al., 2015). Given the importance of this genomic region in neurodegeneration, the revised NeuroChip probe design included multiple probes for SNPs in this region. This led to reliable APOE allele calling with a concordance rate of 98.1% between NeuroChip and Taqman.

In conclusion, we describe the design and implementation of the NeuroChip array, which has a more comprehensive and improved content compared with NeuroX. This versatile genotyping platform provides the community with a novel tool that can be used in clinical and research settings. In a clinical setting, it is possible to rapidly screen patients for a large number of known pathogenic variants, and in a research setting, cost-effective and high-throughput detection of common and rare variants provides opportunities to perform several large-scale analyses, including GWAS, burden tests, and genetic risk scores calculations.

### Disclosure statement

Dr Mike A. Nalls' participation is supported by a consulting contract between Data Tecnica International LLC and the National Institute on Aging, NIH, Bethesda, MD, USA. Dr Nalls also consults for Illumina Inc, the Michael J. Fox Foundation and University of California Healthcare.

### Acknowledgements

The authors would like to thank all of the subjects who donated their time and biological samples to be a part of this study. NABEC control brain tissues were obtained from the Baltimore Longitudinal Study on Aging at the Johns Hopkins School of Medicine, and from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD. This work was supported in part by the Intramural Research Programs of the National Institute of Neurological Disorders and Stroke (NINDS), the National Institute on Aging (NIA), and the National Institute of Environmental Health Sciences both part of the National Institutes of Health, Department of Health and Human Services; project numbers Z01-AG000949-02, Z01-ES101986, 1Z1ANS003154. Faraz Faghri's contribution to this work has been supported in part through the grant 1U54GM114838 awarded by NIGMS through funds provided by the trans-NIH big data to Knowledge (BD2K) initiative ([www.bd2k.nih.gov/](http://www.bd2k.nih.gov/)). The project underlying this publication was funded by the German Federal Ministry of Education and Research under the support code 031 A 430 A. Responsibility for the content lies with the authors. The project was also supported

through the following funding organizations under the aegis of the EU Joint Programme for Neurodegenerative Disease Research (JPND; [www.jpnd.eu](http://www.jpnd.eu)). This study was also supported by Parkinson's UK (grants 8047 and J-0804) and the Medical Research Council (G0700943 and G1100643). Joshua M. Shulman is supported by the Huffington Foundation and a Career Award for Medical Scientists from the Burroughs Wellcome Fund.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2017.05.009>.

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