# Application of Massive Parallel Sequencing in Forensic Psychiatry and Behavioral Science Using Custom Panels including Markers Linked to Human Behavioral Traits

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## INTRODUCTION

Behavior depends on multiple neural pathways and is affected by two factors: genetics and the environment. Although genetics is only one factor in the development of behavior, knowledge of its influences can provide insight on the etiology of certain types of behavior. Oxytocin (OXT) and serotonin (5-HT) are two neurotransmitters that have been correlated with social behavior (1). Beginning to understand the influence of OXT and 5-HT on social behavior may help explain underlying causes for aggressive and antisocial behaviors. These behaviors have become a major problem as the United States currently has the largest incarceration rate in the world (2).

OXT is a neuropeptide synthesized in the hypothalamus. Its function is restricted to the peripheral reproductive tissue and central nervous system (3). Although OXT is a key component in the birthing process, equal concentrations are found in the posterior pituitary and plasma in both men and women (4). 5-HT is found in the midline of the brain stem and in the gastrointestinal tract. It plays a role in both physiological systems and psychiatric disorders (5).

Genes associated with OXT and 5-HT contain polymorphic sites that can be studied in order to relate or link to certain behavioral traits. Single nucleotide polymorphisms (SNPs) are single base variations found at a specific location on the genome and considered to be the most abundant type of polymorphism in humans (6). While some associations between SNPs and behavior have been reported, previous studies have been limited on the number of SNPs using conventional methods. Massive parallel sequencing (MPS) is a promising technology that provides an opportunity to analyze a large number of markers simultaneously (7). A custom primer panel can be comprehensively designed in order to include markers targeted for specific biochemical pathways.

The purpose of this research was to determine if there is an association between SNPs and behavior, including criminal, drug, and antisocial behavior. This study analyzed two SNPs located within the intron region of the OXT gene (rs877172 and rs4813625) and three SNPs located within the serotonin transporter gene (5-HTT) (rs25531, rs6314 exonic, and rs6311) using single base extension (SBE) and MPS with a custom designed panel of SNPs linked or related to genes of these neurotransmitters. A student sample set (N=100) was genotyped and individuals participated in a survey designed to assess behavioral traits. This study also explored the use of MPS in behavioral genetics. Forty-eight SNPs were analyzed using MPS. It is expected that these SNP markers will serve as a large panel used to analyze multiple behaviors.

## RESULTS

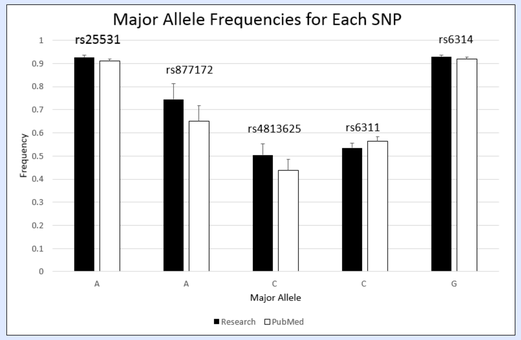


Figure 1: Major allele frequencies for each SNP.

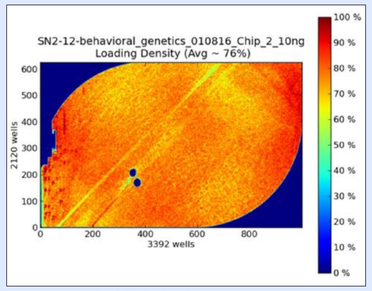


Figure 2: Example of chip loading density.

| Chip # | ISP Loading | Final Library | Number of Reads |
|---|---|---|---|
| 1 | 79% | 86% | 3,477,977 |
| 2 | 76% | 85% | 3,200,510 |
| 3 | 88% | 92% | 3,751,862 |
| 4 | 79% | 77% | 2,894,485 |

Table 1: ISP loading, library, and number of reads for all four chips.

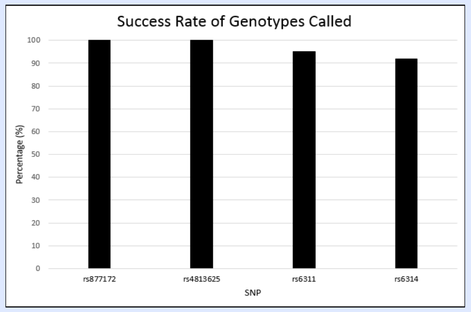


Figure 3:Success rate for genotypes called using MPS.

| Locus | He | Ho |
|---|---|---|
| rs6314 | 0.1452 | 0.0833 |
| rs6311 | 0.5138 | 0.4651 |
| All | 0.3295 | 0.2742 |

Table 2: Expected versus observed Hardy-Weinberg Equilibrium for each locus.

| Markers | |
|---|---|
| Hardy-Weinberg Equilibrium | p value |
| rs25531 | 1.0000 |
| rs6314 | 0.0006* |
| rs6311 | 0.0063* |
| rs877172 | 0.0481 |
| rs4813625 | 0.4244 |
| Linkage Disequilibrium | p value |
| rs25531/rs6314 | 1.0000 |
| rs25531/rs6311 | 0.2891 |
| rs25531/rs877172 | 0.7447 |
| rs25531/rs4813625 | 0.5134 |
| rs6314/rs6311 | 0.0675 |
| rs6314/rs877172 | 0.4463 |
| rs6314/rs4813625 | 0.6950 |
| rs6314/rs877172 | 0.8578 |
| rs6311/rs4813625 | 0.0434 |
| rs877172/rs4813625 | 0.0000* |

*p<0.001

Table 3: Hardy-Weinberg Equilibrium and linkage disequilibrium analysis.

rs25531

| Behavior | p value | b | SE | Exp (B) |
|---|---|---|---|---|
| Drug 2 + Antisocial Behavior | 0.006 | 1.658 | 0.604 | 5.250 |
| Drug 1 + Antisocial Behavior | 0.015 | 1.418 | 0.582 | 4.127 |

Table 4: Logistic regression analysis for rs25531.

rs877172

| Behavior | p value | b | SE | Exp (B) |
|---|---|---|---|---|
| Property Crime + Antisocial Behavior | 0.017 | -1.109 | 0.465 | 0.330 |

Table 5: Logistic regression analysis for rs877172.

## DISCUSSION & CONCLUSIONS

- Allele/genotype frequencies were compared with previously reported Caucasian populations published in PubMed
- SNPs analyzed using both methods were compared and all alleles called were concordant between the two methods
- Custom primer panel may be used to assess a large panel of behavioral markers
- Significant associations were found for SNPs rs25531 and rs877172
- Results provide some evidence that OXT and 5-HT influence behaviors including drug use/distribution, property crime, and antisocial behavior

## MATERIALS AND METHODS

**Sample Collection:** Buccal swabs were collected from Sam Houston State University students who agreed to participate in a survey aimed at collecting information about the students' behavior. The survey consisted of 31 sections, including psychopathy, empathy, and antisocial behavior. All protocols used were approved by the Institutional Review Board at Sam Houston State University.

**DNA Extraction and Quantification:** DNA was extracted on the QIAcube (Qiagen, Hilden, Germany) using the QIAamp® DNA Investigator Kit (Qiagen) as per manufacturer's instructions. DNA quantitation was performed on a 7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA), using SYBR® Green Master Mix (Thermo Fisher Scientific) and D21S11 primers (Integrated DNA Technologies, Coralville, Iowa).

**Single Base Extension:** PCR primers were designed using the software Primer3Plus and AutoDimer. Samples were amplified using the Type-IT® Microsatellite PCR Kit (Qiagen) with a DNA target of 0.2ng. PCR amplification was performed on the GeneAmp® PCR System 9700 (Thermo Fisher Scientific). SBE was performed using the SNaPshot® Multiplex Kit (Thermo Fisher Scientific) according to manufacturer's instructions. Purified SBE products were run on a 3500 Genetic Analyzer (Thermo Fisher Scientific). Allele calling was performed using GeneMapper® Software v4.1 (Thermo Fisher Scientific).

**Massive Parallel Sequencing:** Samples (N=92) were prepared using a DNA target of 10ng. A custom primer panel was designed using Ion Ampliseq (Thermo Fisher Scientific). Amplification, adapter ligation, purification, and library quantification were performed according to manufacturer's protocols for custom panels (Thermo Fisher Scientific). Libraries were pooled and loaded onto the Ion Chef and sequencing was performed on the Ion PGM according to manufacturer's protocol. Samples were analyzed using the Ion Torrent Suite v4.6 with a custom bed file.

**DNA Analysis:** Allele and genotypic frequencies were compared to those published in PubMed. Hardy Weinberg Equilibrium, linkage disequilibrium, and heterozygosity observed (Ho) and expected (He) were analyzed using Genetic Data Analysis Software. Null allele analysis was performed using the Genepop software v4. Logistic and linear regression analysis was performed using SPSS® Software (IBM).

## REFERENCES

1. Pagani J, Williams Avram S, Cui Z, Song J, Mezey E, Senerth J, et al. Raphe serotonin neuron-specific oxytocin receptor knockout reduces aggression without affecting anxiety-like behavior in male mice only. Genes, Brain, and Behavior. 2015;14(2):17-76.
2. Sherman Lw. Reducing incarceration rates. The promise of experimental criminology. Crime Delinq. 2000; 46(3):299-314.
3. Ross HE, LJ. Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. Front Neuroendocrinol. 2009;30(4):534-47.
4. Gimpl G, Fahrenholz F. The Oxytocin Receptor System: Structure, Function, and Regulation, Physiological Reviews. 2001;81(2):629-83.
5. Lucki I. The spectrum of behaviors influenced by serotonin. Biol Psychiatry. 1998;44(3):151-62.
6. Butler JM. Afvanced Topics in Forensic DNA Typing: Methodology. Massachusetts. Waltham. 2011.
7. Berglund EC, Kiialainen A, Syvanen A-C. Next generation sequencing technologies and applications for human genetic history and forensics. Investig Genet. 2011;2:23.

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