Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-231-2825.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2011 - 12/31/2014

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100054865

6. **Project Number and Title of Research Project:** 10. *Investigation of Molecular Contributors to Early Onset Parkinson's Disease in Identical Twins*

7. **Start and End Date of Research Project:** 5/29/2013 – 12/31/2014

8. **Name of Principal Investigator for the Research Project:** Kent E. Vrana, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $320,606

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
None			

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Tekin, Izel	Graduate Assistant	50
Lewis, Mechelle M.	Assistant Professor	1
Huang, Xuemei	Professor	1
Vrana, Kent E.	Professor	1

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
Quant Studio	Purchase of this equipment upgraded and updated the ability of the institution to perform high-throughput genetic and genomic analyses. The equipment resides in the Genome Sciences Core Facility and provides College of Medicine investigators with this state-of-the-art technology (real-time PCR and SNP Genotyping).	$205,772.71

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes____X_____ No_____

If yes, please indicate the source and amount of other funds:

These funds were supplemented with endowment funds to the principal investigator, Kent E. Vrana (Elliot S. Vesell endowed professor).

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No____X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds awarded:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans:

We plan on submission of an NIH R01 application for the June 6th deadline.

12. Future of Research Project. What are the future plans for this research project?

We were able to identify multiple genetic targets that may be related to different Parkinson's disease characteristics. Using these data, we aim to: 1) expand these observations in larger subject populations; 2) investigate the relevance of novel genetic targets in the larger populations; and 3) study the functional effects of genetic variants on protein function.

13. **New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes____X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female			1	
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	
Black				
Asian				
Other				
Unknown				
Total			**1**	

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No_____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

Purchase of QuantStudio upgraded and updated the ability of the institution to perform high-

throughput genetic and genomic analyses. The equipment resides in the Genome Sciences Core Facility and provides College of Medicine investigators with this state-of-the-art technology (real-time PCR and SNP Genotyping).

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No_____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable

performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

The main goal of this project is to identify novel genetic causes for Parkinson's disease. In order to achieve this goal, we will perform Next Generation Sequencing that will allow the sequencing of the whole genome of selected subjects. The subjects will be pairs of identical twins, with one twin in each pair having been diagnosed with early-onset Parkinson's disease and the other being healthy. Our approach will provide us with enough sequencing depth to discover any acquired variations in the genomes of the affected individuals. The subjects are currently being followed by a movement disorder specialist who is part of this project; hence we have myriad clinical data regarding our subjects. As these subjects are also part of an ongoing project, their blood samples are currently stored. The genomic DNA will then be sequenced by deep sequencing. The automated Apollo 324 library preparation system and Illumina MiSeq platform will be employed for sequencing. Several bioinformatic platforms will be utilized for the analysis of the genomic sequences (Galaxy, BaseSpace).

Methods

The subjects that were involved in this study are also a part of Dr. Xuemei Huang's longitudinal study, and they have already been consented for genetic analyses. The blood samples from each subject were processed, and the genomic DNA was isolated by a commercially available kit (QIAGEN QIAamp DNA Blood Mini Kit). The genomic DNA was then sheared and Next Generation libraries were prepared via the automated Apollo 324 platform. The libraries were then clustered using the Sequence by Synthesis Kit from Illumina and they were sequenced by the HiSeq instrument (via paired end reads) at the Genomics Core Facility at Penn State College of Medicine Institute for Personalized Medicine. Data were streamed to an Illumina Compute server to generate FastQ sequence files. We have utilized commercially available bioinformatics software (CLC Genomics Workbench, QIAGEN) to analyze the FastQ files, omit low-quality base reads, align them to the reference genome sequence (hg19) and call variant. Algorithms that were pre-installed in the CLC Genomics Workbench were used to filter and sort variants that were different among twin pairs.

Results

We have obtained more variants for each discordant twin than we had initially expected. Hence, we are currently conducting further steps to filter and eliminate false positives. Once the variants are identified, they will be validated using Sanger sequencing. We have screened several genes that have previously been reported to lie within pathways that lose function in PD and have been singularly searched to identify variants. None of the known risk factors contained mutations specific to the twin that has PD (SNCA: alpha synuclein, LRRK2: leucine rich repeat kinase, GBA: glucocerebrosidase). We have identified an insertion/deletion within a gene called the GRB10 interacting GYF protein 2 (GIGYF2) in the PD twin of two out of the three discordant twin pairs that have been sequenced to date (Table 1).

Table 1: Presence of GIGYF2 variants among identical discordant PD twins

Pair #1	**Pair #2**	**Pair #3**
Pro1232_Gln1232 delInsPro [HET]	delPro1231fs delGln1211fs [HET]	Intronic indel

We are in the process of sequencing the fourth twin pair, and the pipeline is in place for the analysis of their sequence. In addition, we will confirm the aforementioned variants with Sanger sequencing, and identified further targets. We anticipate at least thirty mutations among each twin pair. However, follow-up work will be required to understand their relevance to PD.

Additional goals and results

In parallel with the whole genome sequencing of identical discordant PD twins, we have conducted experiments on sporadic PD patients, investigating genetic contributors to PD symptoms. The sections below describe the experiments that have been conducted and the results that were obtained.

Project Goals, Objectives and Specific Aims

To identify genetic variants within the coding region of PD genomes that will contribute to different PD characteristics.

Methods

We have selected subjects whose age of onset is significantly far apart (Figure 1, Table 2). This separation in the phenotype allows us to attribute the genetic data to the patient outcome. For this particular experiment, we have selected 11 early (age of onset 40-55) and 10 late onset (age of onset 60-75) patients.

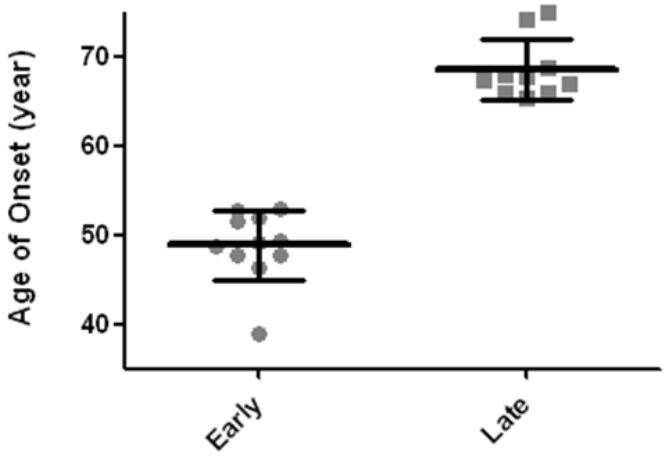

Figure 1: Grouped representation of subjects selected for exome sequencing with different ages of PD onset.

We performed whole exome sequencing using Nextera Exome Enrichment Kit (Illumina). This kit utilizes transposons to cut genomic DNA into smaller pieces. These are then tagged with unique barcodes and combined to obtain genomic libraries. These libraries were then pulled down with hybridization probes designed against the coding region of the human genome. These exome libraries were then sequenced using the HighSeq instrument. The resulting FastQ files were streamlined to Illumina's Compute server as described above. Multiple sample variant calling was utilized to obtain the different variants within each group. Identification of novel variants that underlie age of onset awaits completion of the association studies. CLC Genomics Workbench was employed (with the similar pipeline utilized for the twin subjects) again for a pathway focused analysis and investigation of the exome data, which will be described below in more detail.

Table 2: Mean ages with standard deviations for subject used for age of onset analysis

Subjects	**Mean ± Standard Deviation**
Early	48.8 ± 3.9
Late	68.5 ± 3.4

In parallel to the deep sequencing studies for discordant twins and PD subjects with different ages of onset, we have also investigated whether changes in the monoaminergic signaling pathways will contribute to different disease progression. These candidate genes were obtained upon investigation of particular genes within the whole exome data. The overall alignment and variant calling were performed as described previously in this report. The targets that seemed valuable were then confirmed with Sanger sequencing. In addition, to investigate the effect of variants within the components of monoaminergic signaling pathway, logical genes and their variants were tested in large sample groups. The sequencing of larger subject populations was performed using TaqMan SNP Genotyping Assay Kit (Life Technologies) with the QuantStudio for amplification.

Results

After we were able to obtain all the variants within the monoaminergic signaling pathway, we calculated the p-values for each variant using Fischer's Exact test to compare variant presence in each group. Then we took the –log(p-value) for each variant and we were able to obtain a plot that was easier to compare the slight differences between p-values for each variant (Figure 2). This allowed to us to further investigate variants that were more pronounced in one group vs the other. From this list of targets, we found that a previously reported single nucleotide

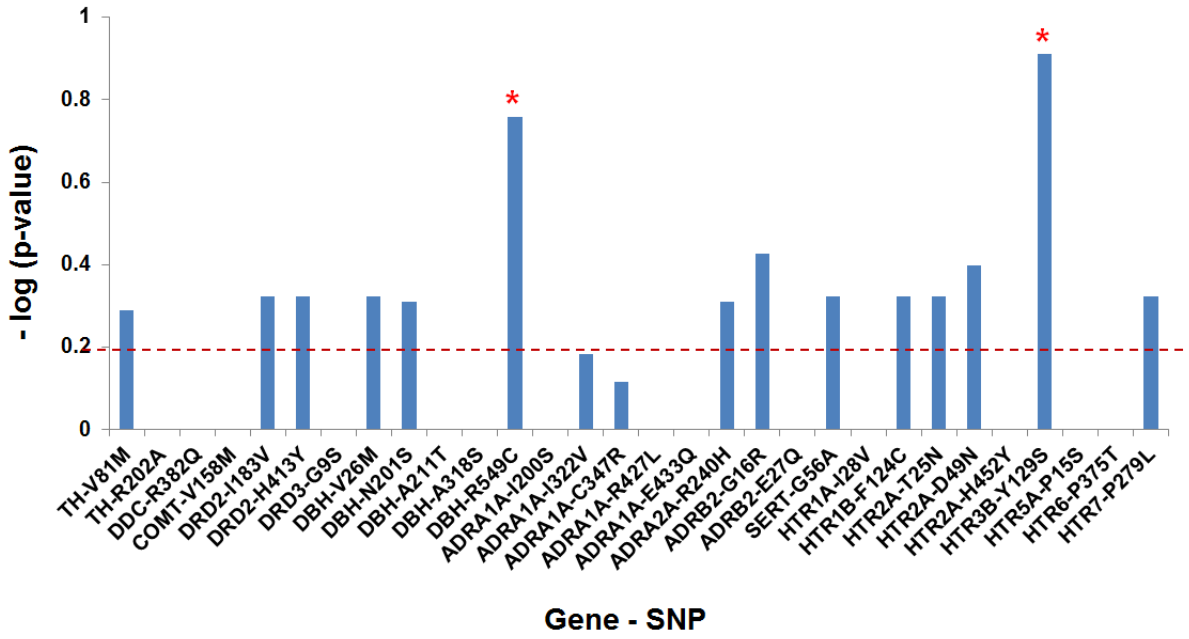

Figure 2: Mathematical comparison of the p-values obtained for each SNP within the monoaminergic signaling pathway using the Fischer's Exact test.

polymorphism (SNP) in the coding region of the dopamine beta hydroxylase (DBH) gene was represented more in the late onset group (conversion of an arginine at position 549 to a cysteine; R549C, Table 3). We have also confirmed the existence of this SNP using a well-established sequencing technique; Sanger sequencing, and we were able to obtain the same genotypes for these subjects (Figure 3).

Table 3: Allelic frequencies of the DBH-R549C SNP in each of the age of onset groups (MAF: Minor Allele Frequency)

Early (n=11)				**Late** (n=10)			
CC	**CT**	**TT**	**MAF**	**CC**	**CT**	**TT**	**MAF**
10	1	0	0.05	6	4	0	0.20

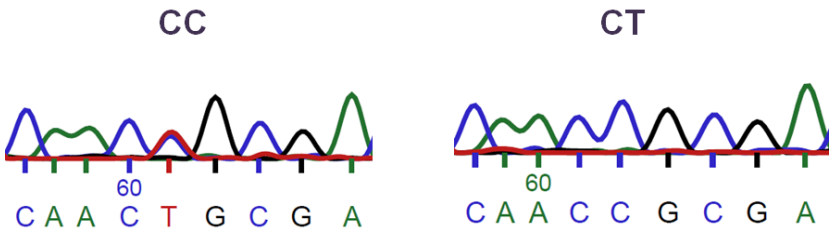

Figure 3: Representative Sanger chromatograms for DBH-R549C wild type (CC) and heterozygous (CT) genomes.

After observing the disproportionate representation of this SNP, we wanted to evaluate its importance by sequencing more subjects and analyzing its contribution to PD age of onset. We have sequenced 52 PD subjects and 32 controls for the DBH-R549C SNP.

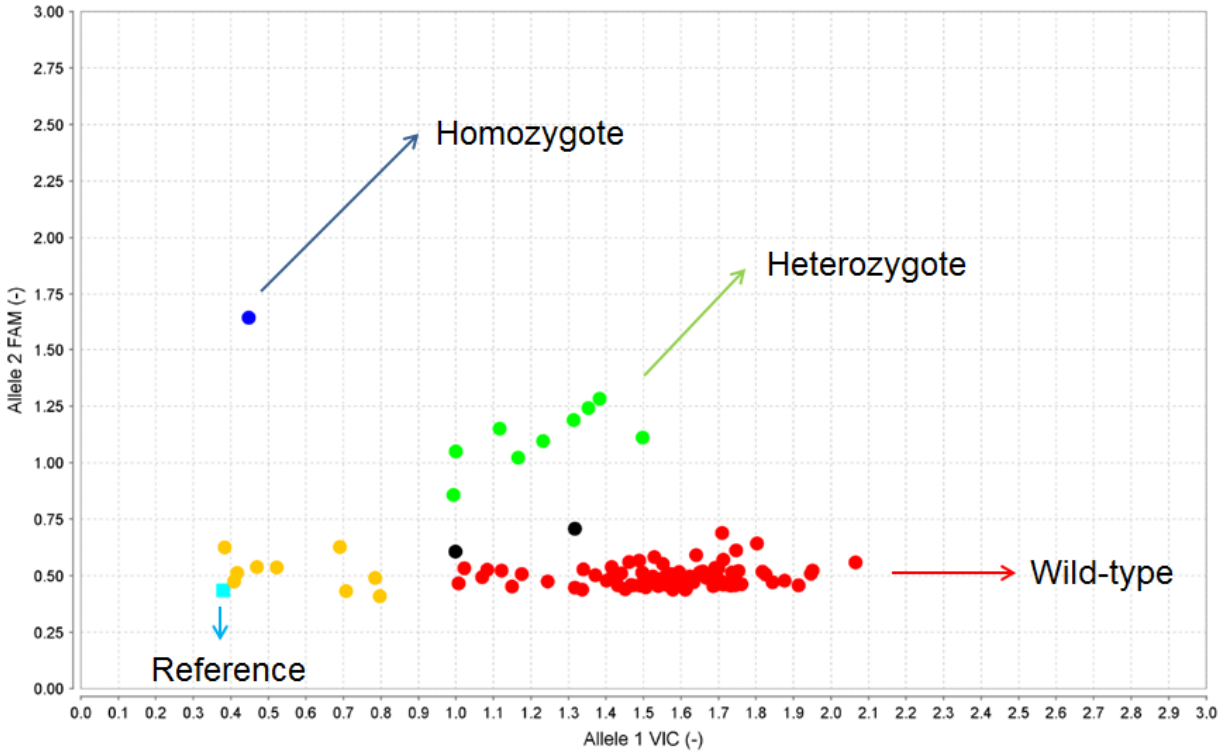

Figure 4: Representative graph depicting the TaqMan SNP Genotyping results.

We were able to rapidly generate genomic data with the TaqMan SNP Genotyping Assay (Figure 4). When we sequenced more subjects, we did not see a significant association with DBH-R549C SNP and age of onset in PD (Figure 5).

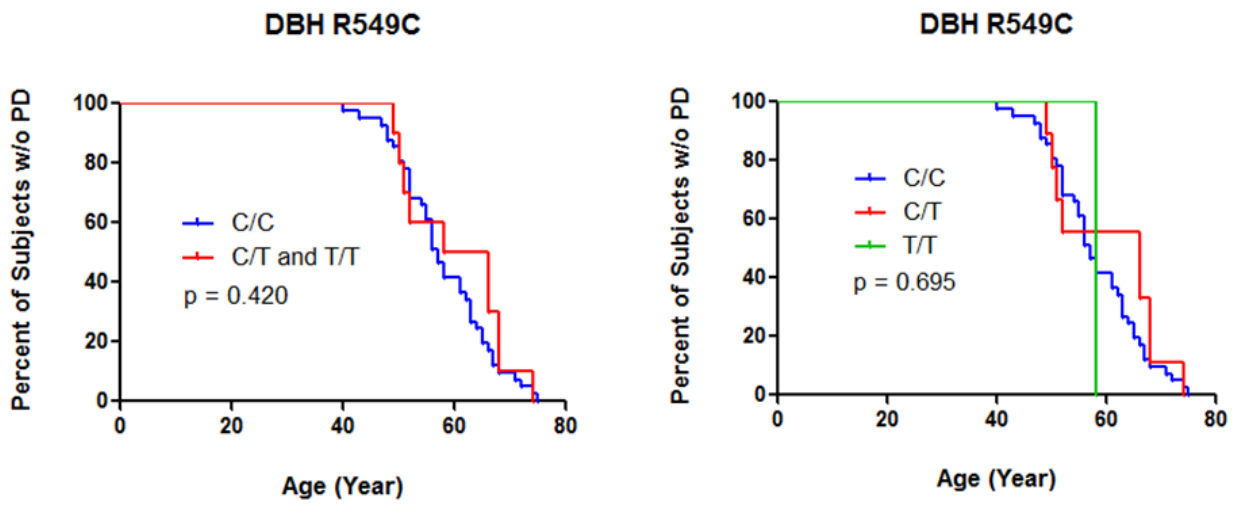

Figure 5: Kaplan-Meier curves showing the association between DBH-R549C genotypes. On the left, the analysis was performed after grouping the heterozygous and homozygous subjects together. The graph on the right shows the analysis performed with all the genotypes analyzed separately (there was only one homozygous subject).

The allelic frequencies of each group for this polymorphism are shown in Table 4. Interestingly, the PD group had a significantly higher allelic frequency for this SNP (7-fold higher representation in PD patients; Figure 6). This finding would suggest that DBH-R549C polymorphism may be a risk factor for PD. We are currently sequencing 100 more PD subjects and controls to see if we will be able to replicate this finding with more subjects.

Table 4: Allelic frequencies of PD and control subjects for the DBH-R549C polymorphism

PD (n=52)				**Control (n=32)**			
WT	**HET**	**HOM**	**MAF**	**WT**	**HET**	**HOM**	**MAF**
42	9	1	0.106	31	1	0	0.015

p=0.028 with Chi-square test.

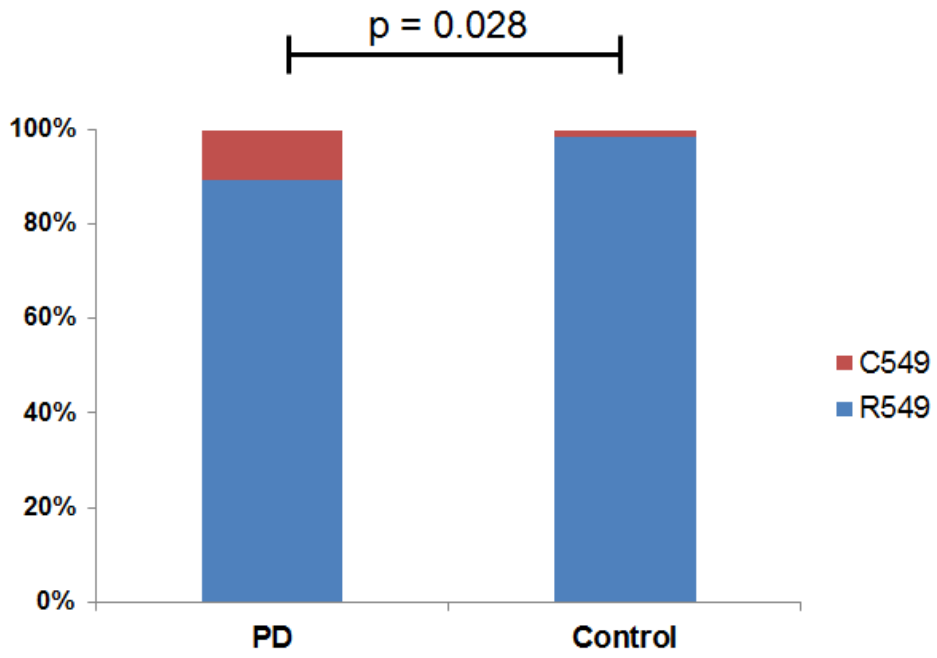

Figure 6: Percentage of each allele in PD and control groups

Published Abstracts, Posters and Presentations

Tekin, I., Carkaci-Salli, N., Lewis, M.M., Mailman, R.B., Huang, X., Vrana, K.E. Tyrosine Hydroxylase as a Genetic Contributor to Parkinson's Disease Progression, Session Number: 694, Abstract Control Number: 17522, 2014 Annual Society for Neuroscience Conference, Washington, DC

Tekin, I., Carkaci-Salli, N., Lewis, M.M., Mailman, R.B., Huang, X., Vrana, K.E. Genetics of Tyrosine Hydroxylase and Parkinson's Disease, 2014 Pennsylvania State University 29th Annual Graduate Exhibition, University Park, PA

Tekin, I., Carkaci-Salli, N., Lewis, M.M., Mailman, R.B., Huang, X., Vrana, K.E. Genetics of Tyrosine Hydroxylase and Parkinson's Disease, 2014 Penn State Institute of Neuroscience Annual Research Retreat, Hershey, PA

Tekin, I., Carkaci-Salli, N., Lewis, M.M., Mailman, R.B., Huang, X., Vrana, K.E. Genetics of Tyrosine Hydroxylase and Parkinson's Disease, 2014 Pennsylvania State University 4th Annual Center for Medical Genomics Retreat, Hershey, PA

Tekin, I., Carkaci-Salli, N., Lewis, M.M., Mailman, R.B., Huang, X., Vrana, K.E. Genetics of Tyrosine Hydroxylase and Parkinson's Disease, 2014 Penn State College of Medicine Student Research Forum, Hershey, PA

Tekin, I., Carkaci-Salli, N., Lewis, M.M., Mailman, R.B., Huang, X., Vrana, K.E. The V81M (G241A) Polymorphism of Human Tyrosine Hydroxylase Increases the Michaelis-Menten Constant for Tyrosine and May Accelerate Progression of Parkinson's Disease, 2013 Penn State College of Medicine Student Research Forum, Hershey, PA

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one

publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Caveat emptor: SNP reporting in pharmacogenomics	Tekin, I., Vrana, K.E.	Pharmacology	October, 2013	☐Submitted ☐Accepted ☒Published
2. Complex molecular regulation of tyrosine hydroxylase	Tekin, I., Roskoski, R, Jr, Carkaci-Salli, N., Vrana, K.E.	Journal of Neural Transmission	April 2014	☐Submitted ☐Accepted ☒Published
3. The V81M variant of tyrosine hydroxylase contributes to freezing of gait in Parkinson's disease	Tekin, I., Carkaci-Salli, N., Lewis, M.M., Mailman, R.B., Huang, X., Vrana, K.E.	Parkinsonism and Related Disorders	December 2014	☒Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans:

The extended sequencing from the comprehensive exome and twin data will be investigated further and the resulting data will be published.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If

there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No_____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No ___

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

NAME Kent E. Vrana	POSITION TITLE Elliot S. Vesell Professor & Chair
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Iowa, Iowa City, IA	B.S.	05/78	Biochemistry
LSU Medical Center, New Orleans, LA	Ph.D.	05/83	Biochemistry
Carnegie Inst. of Washington, Baltimore, MD	Postdoc	06/86	Embryology & Mol Biol

A. Personal Statement

Dr. Vrana has over thirty years of experience in genetics, genomics, transcriptomics, and proteomics. Notably, he has been actively involved in the neuroenzymology and neurotoxicology aspects of dopamine synthesis since a graduate student in 1979. Over the years, his laboratory was the first to isolate (and disseminate) a full-length cDNA clone for tyrosine hydroxylase and to characterize the role of catecholamine-derived quinones in neuronal toxicity. He has a long history of examining the functional genomics and proteomics of substance abuse (cocaine, alcohol, and heroin). He has studied the proteomic differences between embryonic stem cells and mesenchymal stem cells. His laboratory is currently using next-generation sequencing technologies to explore the roles of naturally-occurring sequence variants in the progression and severity of Parkinson's disease. He also serves as the director of the Penn State College of Medicine Drug Discovery, Development and Delivery (D^4) Core Facility and serves as the chair of the oversight committee for the Genome Sciences core facility (under the direction of a pharmacology faculty member). Dr. Vrana also has twenty-five years of education development and mentoring activities. He has personally mentored over 28 graduate students and postdoctoral fellows that have gone on to occupy leadership positions in academia, industry and philanthropic organizations. He has been active in education locally, nationally, and internationally. Indeed, in 2009, he was named an honorary professor of the School of Medicine of the Peruvian University of Applied Science in Lima, Peru, and was inducted into the Society of Distinguished Educators at Penn State College of Medicine.

B. Positions and Honors

1983-1986	NIH Postdoctoral Fellow, Dept. Embryology, Carnegie Institution of Washington, Baltimore, MD
1986-1991	Assistant Professor and Director of Graduate Studies, Dept. Biochemistry, West Virginia University Health Sciences Center, Morgantown, West Virginia
1991-2003	Assistant Professor/Associate Professor/Professor and Director of Graduate Studies, Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC
2004-pres.	Elliot S. Vesell Professor and Chair, Department of Pharmacology, Milton S. Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, PA

C. Selected Publications (since 2008; from a total of over 160)

1. Kuntz KL, Patel KM, Grigson PS, Freeman WM, Vrana KE. Heroin self-administration II. CNS gene expression following withdrawal and cue-induced drug-seeking behavior. **Pharmacol Biochem Behav** 2008; 90(3):349-56.
2. VanGuilder HD, Vrana KE, Freeman WM. Twenty-five years of quantitative PCR for gene expression analysis. **Biotechniques** 2008; 44(5):619-26. *The final two authors contributed equally.*
3. Lull ME, Erwin MS, Morgan D, Roberts DCS, Vrana KE*, Freeman WM*. Persistent proteomic alterations in mPFC with abstinence from cocaine self-administration. **Proteomics – Clin Apps** 2009,3(4):462-472. PMCID: PMC2742427 *Final two authors contributed equally.*
4. Kuntz-Melcavage KL, Brucklacher RM, Grigson PS, Freeman WM, Vrana KE. Gene expression changes following extinction testing in a heroin behavioral incubation model. **BMC Neurosci** 2009; 10:95. PMCID: PMC2733140
5. Freeman WM, Vrana KE. Future prospects for biomarkers of alcohol consumption and alcohol-induced disorders. **Alcohol Clin Exp Res** 2010; 34(6):946-54. PMCID: PMC2900387
6. Freeman WM, Salzberg AC, Gonzales SW, Grant KA, Vrana KE. Classification of alcohol abuse by plasma protein biomarkers. **Biol Psychiatry 2010**; 68(3):219-22. PMCID: PMC2890298
7. Lull ME, Freeman WM, VanGuilder HD, Vrana KE. The use of neuroproteomics in drug abuse research. **Drug Alcohol Depend**; 2010; 107(1):11-22.
8. Freeman WM, Vanguilder HD, Guidone E, Krystal JH, Grant KA, Vrana KE. Plasma proteomic alterations in non-human primates and humans after chronic alcohol self-administration. **Int J Neuropsychopharmacol** 2011:1-13. PMCID: PMC3107900
9. Helms CM, Messaoudi I, Jeng S, Freeman WM, Vrana KE, Grant KA. A longitudinal analysis of circulating stress-related proteins and chronic ethanol self-administration in cynomolgus macaques. **Alcohol Clin Exp Res**. 2012;36(6):995-1003. PMCID: PMC3324628.
10. Torrente MP, Freeman WM, Vrana KE. Protein biomarkers of alcohol abuse. **Expert Rev Proteomics**. 2012;9(4):425-36. PMCID: PMC3535006.
11. Vrana KE. “Making it real” time. **BioTechniques** 2013; 54:312-313. PMID: 23905171
12. Tekin I, Vrana KE. Caveat emptor: SNP reporting in pharmacogenomics. **Pharmacology**. 2013;92(5-6):319-23. Epub 2013 Dec 12. PMID: 24356117
13. Carkaci-Salli N, Salli U, Tekin I, Hengst JA, Zhao MK, Gilman TL, Andrews AM, Vrana KE. Functional Characterization Of The S41Y (C2755a) Polymorphism Of Tryptophan Hydroxylase 2. **J Neurochem** 2014 Jun 4. doi: 10.1111/jnc.12779. [Epub ahead of print] PMID: 24899127
14. Sathyendra V, Donahue HJ, Vrana KE, Berg A, Fryzel D, Gandhi J, Reid JS. Single Nucleotide Polymorphisms in Osteogenic Genes in Atrophic Delayed Fracture-Healing: A Preliminary Investigation. **J Bone Joint Surg Am** 2014;96(15):1242-8. PMID: 25100770
15. Tekin I, Roskoski R, Jr., Carkaci-Salli N, Vrana KE. Complex molecular regulation of tyrosine hydroxylase. **J Neural Transm** 2014 May 28. [Epub ahead of print] PMID: 24866693.

BIOGRAPHICAL SKETCH

NAME Xuemei Huang	POSITION TITLE Associate Professor of Neurology, Neurosurgery, Radiology, Pharmacology, and Kinesiology
eRA COMMONS USER NAME Xuemei	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education,*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Beijing Medical Univ. Beijing, P.R.C.	M. D.	1987	Medicine
Cancer Research Institute, Beijing, P.R.C.	Internship	1987-88	Nuclear Medicine
Purdue University, West Lafayette, IN	Ph.D.	1989-94	Neuropharmacology
Univ. of Colorado Health Sci. Center, Denver	Post-doc	1994-96	Clin. Pharmacol.
University of Washington, Seattle	Internship	1996-97	Medicine
Univ. of North Carolina, Chapel Hill	Residency	1997-2000	Neurology
Emory University/UNC School of Medicine	Fellowship	2001-02	Movement Dis./CRC

A. Personal Statement

I am a board-certified research neurologist whose clinical practice and scholarly interests are focused on neurodegenerative disorders, particularly Parkinson's disease (PD). Although I do human-related research, my very strong basic science training has been a major influence on both my clinical medicine, and on my strong interest in what is now called translational neuroscience. For example, while I was a fellow at Emory University in 2001, I led the effort that designed a clinical safety protocol that ultimately permitted the resurrection of the only full dopamine D_1 full agonist now available for human experimentation. This paved the way for several investigator-initiated studies on cognitive deficits, including a large trial currently sponsored by the NIMH. I have been NIH funded since 2003, just after I began my first faculty position. My major focus has the use of novel imaging modalities and designs to elucidate neurocircuitry underling various signs and symptoms of PD, and the development of imaging biomarkers for early detection and progression of PD. It is hard to be a PD clinician or researcher, and not think about the interactive role of genetic and environmental factors in PD etiology, and it is a small jump to consider other types of CNS dysfunction. Our group has also been contributing to understand the role both genetic and environmental factors in PD etiology and progression. I have track record of working synergistically in multidisciplinary efforts, both as a contributor and as a leader. I am pleased to provide clinical expertise and consultations for the study that Dr. Kent Vrana is proposing. I also believe that our ongoing PD cohort study and large movement disorder clinic will provide rich patients population to explore the clinical relevance of the genetic mutations.

B. Positions and Honors.

Positions and Employment

2012-present Vice Chair for Research, Department of Neurology, Penn State Hershey Medical Center.

2008-present Associate Professor (Tenured), Departments of Neurology, Neurosurgery, Radiology, Pharmacology. Kinesiology & Bioengineering, Penn State University and Penn State Hershey Medical Center.

2008-present	Director, Hershey Brain Analysis Research Laboratory for Neurodegenerative Disorders, Penn State Hershey Medical Center
2008-present	Adjunct Prof., Dept. of Neurology, University of North Carolina School of Medicine (UNC SOM), Chapel Hill, NC
2005-2008	Acting Chief, Division of Movement Disorders, Department of Neurology, UNC SOM
2003-2008	Medical Director, National Parkinson's Foundation Center of Excellence, UNC SOM
2002-2008	Assistant Professor (Tenure-Track), Department of Neurology, UNC SOM
2001-2002	Clinical Fellow/Instructor, Dept. of Neurology, UNC SOM

Additional recent publications of importance to the field (in reverse chronological order)

1. Du G, Lewis MM, Sen S, Wang J, Shaffer ML, Styner M, Yang QX, **Huang X**. Imaging nigral pathology and clinical progression in Parkinson's disease. Mov Disord. 2012;27(13):1636-43. PMCID: PMC3510346.
2. Lewis MM, Du G, Kidacki M, Patel N, Shaffer ML, Mailman RB, **Huang X.** 2012. Higher Iron in the Red Nucleus Marks Parkinson's dyskinesia. *Neurobiol. Aging*. published online 23 November 2012.
3. Du G, Lewis MM, Styner M, Shaffer ML, Sen S, Yang QX, **Huang X.** Combined R2* and diffusion tensor imaging changes in the substantia nigra in Parkinson's disease. Mov Disord. 2011;26(9):1627-32. PMCID: PMC3154471.
4. Sen S, Flynn MR, Du G, Troster AI, An H, **Huang X**. Manganese accumulation in the olfactory bulbs and other brain regions of "asymptomatic" welders. Toxicol Sci. 2011;121(1):160-7. PMCID: PMC3080190.
5. Chen H, **Huang X**, Guo X, Mailman RB, Park Y, Kamel F, Umbach DM, Xu Q, Hollenbeck A, Schatzkin A, Blair A. Smoking duration, intensity, and risk of Parkinson disease. Neurology. 2010;74(11):878-84. PMCID: PMC2836869.
6. Chen H, Mosley TH, Alonso A, **Huang X**. Plasma urate and Parkinson's disease in the Atherosclerosis Risk in Communities (ARIC) study. Am J Epidemiol. 2009;169(9):1064-9. PMCID: PMC2727240.
7. Lewis MM, Slagle CG, Smith DB, Truong Y, Bai P, McKeown M, Mailman RB, Belger A, **Huang X**. 2007. Task specific influences of Parkinson's disease on the striato-thalamo-cortical and cerebello-thalamo-cortical motor circuitries. *Neurosci*. 147(1):224-35. PMID: 17499933.
8. **Huang X**, Chen, H, Miller W, Mailman R, Woodard J, Chen P, Xiang D, Murrow R, Wang, Y.-Z, Poole C. 2007 Lower LDL-cholesterol associated with Parkinson's disease. *Mov. Dis.*22:377-381. PMID: 17177184
9. McKeown MJ, Uthama A, Abugharbieh R, Palmer S, Lewis M, **Huang X**. Shape (but not volume) changes in the thalami in Parkinson disease. BMC Neurol. 2008;8:8. PMCID: PMC2386499.
10. **Huang X** Abott R, Petrovitch H, Mailman R and Ross W. 2008. Low LDL cholesterol and increased risk of Parkinson's disease: prospective results from Honolulu-Asia Aging Study. *Mov Disord.* 23(7):1013-8 PMID: 18381649

BIOGRAPHICAL SKETCH

NAME Mechelle Mayleben Lewis	POSITION TITLE Assistant Professor, Neurology and Pharmacology
eRA COMMONS USER NAME mmlewis5	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education,*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Creighton University, Omaha, NE	BA	05/89	Psychology
Emporia State University, Emporia, KS	MS	05/91	Experimental Psych
University of North Carolina, Chapel Hill, NC	PhD	05/97	Neurobiology

A. Personal Statement

I am a neuroscientist with a broad background and with specific training in key research areas (Parkinson's disease, dopamine synthesis) pertinent to this application. Although I currently do human subject related research, my strong training in basic research positions me well to work on this translational project. For example, my dissertation research focused on analyzing the effects of dopamine agonists at the D_1 receptor and their propensity for causing tolerance, as well as mapping the D_1 and D_5 pharmacophores. I have worked on all aspects of several clinical projects (design; subject recruitment; imaging data collection, management, and analysis; and interpretation) and have a keen interest in both normal and dysfunctional brain processes, as well as the development of markers to help us better understand brain function. I also have a successful history of working with both basic scientists and clinicians and am a co-investigator on two current NIH-funded R01 projects and a recently awarded U01 grant, two of which are focused on development of biomarkers in Parkinson's disease and the other on understanding the role of environmental neurotoxicants on the development of neurobehavioral disorders. I am the central liason for all three projects and assist the PI in coordinating these multi-disciplined, collaborative projects. I have a track record of working synergsitcally with multidisclined teams, as evidenced by my publications. I am pleased to provide my scientific expertise to the study that Dr. Kent Vrana proposes and I look forward to working with a superb group of investigators to address the important research questions posed in the application.

B. Positions and Honors

Positions and Employment

1997-1999	Postdoctoral Fellow, Molecular Pharmacology, University of North Carolina, Chapel Hill, NC
1999-2001	Research Associate, Neuroscience Center, University of North Carolina School of Medicine, Chapel Hill, NC
2001-2004	Senior Research Scientist, DarPharma, Inc., Chapel Hill, NC
2005-2006	Fellow, Neurology Department, University of North Carolina School of Medicine, Chapel Hill, NC

2006-2008	Research Associate, Neurology Department, University of North Carolina School of Medicine, Chapel Hill, NC
March 2008-June 2008	Assistant Professor of Research, Neurology Department, University of North Carolina School of Medicine, Chapel Hill, NC
July 2008-Present	Assistant Professor, Departments of Neurology and Pharmacology, Penn State University College of Medicine, Hershey, PA

C. Selected Peer-reviewed Publications (Selected from over 50 peer reviewed original research papers)

Five most relevant to the current application

1. **Lewis, M.M.**, Du, G., Kidacki, M., Patel, N., Shaffer, M.L., Mailman, R.B., and Huang, X. Higher iron in the red nucleus marks Parkinson's dyskinesia. Neurobiol Aging Nov 21, 2012. PMID: 23177595, NIHMS: NIHMS418498 (no PMCID available).
2. Du, G., **Lewis, M.M.**, Sen, S., Wang, J., Shaffer, M.L., Styner, S., Yang, Q.X., and Huang, X. Imaging nigral pathology and clinical progression in Parkinson's disease. Mov Dis, Nov; 27(13): 1636-1643, 2012. PMID: 23008179, PMCID: 3510346.
3. Du, G, **Lewis, MM**, Shaffer, ML, Chen, H, Yang, QX, Mailman, RB, and Huang, X. Serum cholesterol and nigrostriatal R2* values in Parkinson's disease. 2012 PLoS ONE (epub April 17, 2012) PMID: 22530017; PMCID: 3328461.
4. **Lewis MM**, Du G, Sen S, Kawaguchi A, Truong Y, Lee S, Mailman RB, Huang X. Differential involvement of striato- and cerebello-thalamo-cortical pathways in tremor- and akinetic/rigid-predominant Parkinson's disease" Neurosci. Mar;17;177:230-9, 2011. PMID: 21211551: PMCID: 3049982.
5. Du G, **Lewis MM**, Styner M, Shaffer ML, Sen S, Yang QX, Huang X. 2011. Combined R2* and diffusion tenser imaging changes in the substantia nigra in Parkinson disease. Mov Dis, Aug 1, 26(9): 1627-1632, 2011. PMID: 21618607; PMCID: 3154471.

Additional publications of importance to the field (in reverse chronological order)

6. Sen, S., Kawaguchi, A, Truong, Y., **Lewis, MM**, and Huang, X. Dynamic changes in cerbello-thalamo-cortical motor circuitry during progression of Parkinson's disease, Neuroscience, Mar 17, 166(2): 712-719, 2010. PMID: 20034546: PMCID: 2852615.
7. **Lewis, MM**, Smith, AB, Styner, M, Gu, H, Poole, R, Zhu, H, Li, Y, Barbero, X, Gouttard, S, McKeown, MJ, Mailman, RB, and Huang, X. Asymmetrical lateral ventricular enlargement in Parkinson's disease. Eur J Neurol 16(4): 475-481, 2009. PMID: 19187264; PMCID: 2908273.
8. Park, J., Wu, Y-H., **Lewis, M.M.**, Huang, X., and Latash, M.L. Changes in multi-finger interaction and coordination in Parkinson's disease. J Neurophysiology 2012 (in press).
9. McKeown, MJ, Uthama, A, Abugharbieh, R, Palmer, S, **Lewis, MM**, and Huang, X. Shape (But Not Volume) Changes in the Thalami in Parkinson Disease. BMC Neurology: 2008 8:8. PMID: 18412976; PMCID: 2836499.
10. **Lewis, MM**, Slagle, CG, Smith, AB, Truong, Y, Bai, P, McKeown, MJ, Mailman, RB, Belger, A and Huang, X. Task specific influences of Parkinson's disease on the striato-thalamo-cortical and cerebello-thalamo-cortical motor circuitries. Neuroscience 147(1): 224-235, 2007. PMID: 17499933; PMCID 1939944.

BIOGRAPHICAL SKETCH

NAME Izel Tekin	POSITION TITLE Graduate Assistant
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.*)

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Yeditepe University, Istanbul, Turkey	B.S.	06/10	Genetics and Bioengineering
Penn State College of Medicine, Hershey, PA	Ph.D.	08/15 (exp.)	Pharmacology

A. Personal Statement

Ms. Tekin completed her undergraduate studies in the field of genetics and bioengineering in Istanbul, Turkey. She has since been training in the field of pharmacology, biochemistry, genetics and neuroscience under the supervision of Dr. Kent E. Vrana. Dr. Vrana has over thirty years of experience in genetics, genomics, transcriptomics, and proteomics. Specifically, he has been actively involved in the neuroenzymology and neurotoxicology aspects of dopamine synthesis for more than 30 years. He has also trained and mentored several successful scientists that moved into careers in different fields. Ms. Tekin has already published several papers on her work with the neurogenetics of Parkinson's disease. She is currently expected to complete her doctorate dissertation within the next 6 months. She has also spent one year in the Movement Disorders clinic shadowing Dr. Xuemei Huang. This experience has allowed her to interact with patients and better understand the clinical properties of Parkinson's disease. She has brought her short, but in depth, experiences to her work and will apply her knowledge to this project. The current proposal is a good fit for her basic science and clinical backgrounds.

B. Positions and Honors

2010-2015 Graduate Assistant, Department of Pharmacology, Penn State College of Medicine, Hershey, PA

C. Publications

Journal Articles:

1. **Tekin, I.**, Carkaci-Salli, N., Bewley, M.C., Sung, S.S., Vrana, K.E. A novel polymorphism (A378T) in human tryptophan 2 disrupts enzyme activity by preventing binding of molecular iron, *Biochemical and Biophysical Research Communications*, (In Preparation), 2015.
2. **Tekin, I.**, Vgontzas, A., Lewis, M.M., Kothori, S., Kong, L., Lu, Y., Vrana, K.E., Huang, X. Previous limb injury with chronic pain affects the side of onset in Parkinson's disease. *European Journal of Neurology* (In Preparation), 2014.

3. **Tekin, I.**, Carkaci-Salli, N., Lewis, M.M., Mailman, R.B., Huang, X., Vrana, K.E. The V81M variant of tyrosine hydroxylase contributes to freezing of gait in Parkinson's disease, *Parkinsonism and Related Disorders*, (Submitted), 2014.
4. Carkaci-Salli, N., Salli, U., **Tekin, I**., Hengst, J.A., Zhao, M.K., Gilman, T.L., Andrews, A.M., Vrana, K.E. Functional characterization of the S41Y (C2755A) polymorphism of tryptophan hydroxylase 2. *Journal of Neurochemistry*, 130(6): 748-758, 2014.
5. **Tekin, I.**, Roskoski, R, Jr, Carkaci-Salli, N., Vrana, K.E. Complex molecular regulation of tyrosine hydroxylase (Review), *Journal of Neural Transmission*, Epub, doi: 10.1007/s00702-014-1238-7, 2014.
6. **Tekin, I.**, Vrana, K.E. Caveat emptor: SNP reporting in pharmacogenomics (Commentary), *Pharmacology*, 92: 319-323, 2013.
7. Carkaci-Salli, N., Salli U., Kuntz-Melcavage K.L., Pennock M.M., Ozgen H., **Tekin I**., Freeman W.M., Vrana K.E. TPH2 in the ventral tegmental area of the male rat brain. *Brain Research Bulletin*, 84(6): 376-80, 2011.