Undergraduate Research in the Brzustowicz Laboratory
Human Genetics Institute
Department of Genetics
Rutgers University

Genetics plays an important role in the development of many of the major psychiatric illnesses. Our laboratory works on trying to understand exactly how changes at the level of DNA can increase the risk for developing such an illness. This is a challenging area of research, requiring the use of varying analytic techniques and the cooperation of a team of researchers with differing areas of expertise.

Disorders Studied:
Our laboratory primarily studies schizophrenia and autism. Autism is a serious neurodevelopmental disorder characterized by deficits in communication, abnormal social interactions, and rigid or repetitive interests and behaviors. Autism and related disorders manifest during the first three years of life may affect up to 1% of the population, being four times more common in males than females. Schizophrenia is a serious neuropsychiatric illness characterized by withdrawal from reality, illogical patterns of thinking, delusions, and hallucinations, and accompanied in varying degrees by other emotional, behavioral, or intellectual disturbances. Schizophrenia is estimated to affect approximately 1% of the general population. Men typically develop the disorder in their late teens to early twenties, and women in their mid-twenties to early thirties.

Study Types:
To study the potential role of genetics in these disorders, our laboratory studies DNA sequences from individuals with and without these illnesses. We commonly conduct several general types of studies.

1. *Genetic linkage studies* compare DNA from family members with and without an illness to search for chromosome segments that have been inherited in common by the individuals with the illness. These studies are typically conducted by examining DNA 'marker' sequences spaced at regular intervals across the each of the chromosomes. Once a linkage signal is detected in a region, *fine-mapping studies* using a denser set of markers may be used to narrow the size of the shared risk-increasing DNA segment.

2. *Association studies* also compare DNA sequences from individuals with and without the illness. These may be people within the same family (*family-based association study*) or people with the illness and controls from the same general genetic population (*case-control study*). Candidate association studies may examine a single gene at a time or several adjacent genes or several functionally related genes may be studied simultaneously. Several types of DNA variations may be examined for these studies, including single base changes that may alter protein structure or patterns of gene expression. Genome-Wide Association Studies (GWAS) will examine on the order of a million markers spread across all the chromosomes to search the entire genome for evidence of association.

3. *Post-mortem studies* in our lab use RNA or protein extracted from the brains of deceased individuals with the illness and from psychiatrically normal controls to look for differences in gene expression. These expression differences can also be compared to differences in the DNA of these different individuals. These studies may examine the expression of a single gene or a set of genes.

4. *Cell culture model system studies* are studies that use cells from humans, grown in culture, as model systems to study gene expression. Recently, we have begun to use induced pluripotent stem cells (iPSCs) and neural stem cells derived from iPSCs in our lab. These can be generated from individuals with specific illnesses and from control individuals for comparison. The cells can be characterized in a variety of ways (e.g. RNA expression), and the results of experimental manipulations (e.g. drug exposures) can be tested.

5. *Reporter system assays* are studies that examine potential function of a specific DNA variation. Once we have identified a specific sequence variation as potentially important in regulating gene expression (through association and/or post-mortem studies), we will clone the allelic variants of the sequence into a plasmid that will produce a protein product we can easily express and assay in a cell-culture system. We then compare the levels of expression of the different variants to see if there is a significant difference.

Analytic Methods: A growing part of our research is computational in nature or composed primarily of data analysis. For example, we do not do the genotyping of genome-wide SNP chips in our lab, nor do we do genome wide DNA sequencing in our lab. The lab work for these experiments is done in a large core facility and we receive the resulting data (often on 2 or 3 TB hard drives) for analysis. Data management skills well beyond Excel are needed to work with this type of data. Methods employed generally fall into one of three general categories:

1. *Use of existing software for phenotypic data analysis.* We have clinical data of many different types available on many human subjects, all participants in genetic studies. Some of our projects involve examining these data for patterns and correlations just within the phenotypic data, or to relevant genetic data. Analysis of these data can sometimes be conducted using the statistical functions of Excel or often with other popular statistical analysis programs (e.g. SPSS). Students interested in working on phenotypic data projects should be comfortable with general statistics (descriptive statistics and basic inferential statistics).

2. *Use of existing software for genetic data analysis.* We use a large number of existing programs for our data analysis. While a few of the programs have web interfaces, most run from the command line or have only a very simple GUI. While some run in the Windows environment, some require Linux. While programming knowledge is not strictly required to run these programs, some basic knowledge of scripting is very helpful as there is a frequent need for small scripts to alter data formats or automate routine processes. Students interested

in working in this area are strongly encouraged to take 447:302, Quantitative Biology and Bioinformatics, if they don't already have programming experience.

3. *Development of new analysis software.* We have projects that involve developing new computer programs for genetic data analysis. These programs are generally based on the programming language Python. Students interested in working in this area must have some prior programming experience. 447:302, Quantitative Biology and Bioinformatics, is a great place to get a good introduction to Python.

Laboratory Methods: The amount of bench work conducted in our lab is steadily declining as more of our work is shifting to the analysis of large scale data that is generated for us by core facilities. I anticipate having bench research project available for undergraduates only through AY 2016-2017. We utilize a number of specific techniques to carry out these studies.

1. *PCR* (Polymerase Chain Reaction) is the most commonly used technique in our laboratory. Since virtually every project we do requires PCR at some stage, learning to successfully perform this technique is critical for success in any bench project.

2. *Genotyping* is another very common procedure in our laboratory. We currently utilize several different genotyping methods or platforms, however there are a number of common experimental elements across genotyping methods.

3. *Cell culture* is an important part of several of our research projects that use human cells as a model system for studying gene expression.

4. *Cloning* of specific DNA sequences for further analysis, such as in the reporter assays, can be a part of some projects.

5. *Statistical analysis* of data is a key part of every bench experiment. While we do not expect undergraduate students to become experts in every aspect of the analyses, we do expect them to learn the basic ideas behind the analysis of their data and to learn to run the appropriate Windows-based analysis software.

Writing: Good writing is a critical, but sometimes underappreciated, skill in science. Research in Genetics requires a paper at the end of each semester, and these papers will be graded both on scientific content and quality of writing. Students who demonstrate weak general writing ability may be required to enroll in 355:096 (The Writing Center) as a condition of continuing in our laboratory. In more extreme cases, students may be required to enroll in 355:312 (Writing for Biology) or 355:302 (Scientific and Technical Writing) as a condition of continuing. Please remember that the research requirement for the Genetics major requires two semesters in the same laboratory; students who are unsure of their writing ability should be sure they will have room in their schedule for a writing course during the second semester of research, if necessary.

What we don't do:

1. We do only a limited amount of direct work with human subjects. For students who are especially interested in patient research, it is possible to arrange some limited human subject experience as part of our autism project.

2. We do not currently do any animal-based work in our laboratory. On occasion we may extract DNA, RNA, or protein from animal organs that are provided to us by another laboratory.

3. We do not currently have any projects that work with radioactive materials in the laboratory. While we try to limit our use of other types of dangerous or toxic materials, many of the chemicals in the laboratory can be harmful if improperly handled.

Typical Undergraduate Projects:
Undergraduate students typically team up with a research faculty member, graduate student, post-doctoral fellow, or technician to work on a specific aspect of an ongoing project. The degree of independence of the student project is determined by the student's background, amount of time spent in the laboratory, and type of research experience (e.g. honor's thesis). We are not generally able to accommodate students who wish to pursue a research project of entirely their own design that does not match our funded research program. Typical undergraduate projects might involve analyzing a clinical data set for correlations between specific phenotypic traits and DNA variants in a candidate gene of interest, writing a computer program to find errors within a large dataset, or measuring the levels of RNA of specific genes of interest from a series of cell lines grown in culture.

Eligibility and Expectations:
1. Only under very unusual circumstances do we accept students who are not Genetics majors into the laboratory. This is because research is a requirement for the Genetics major and so we are obligated to offer our laboratory slots first to Genetics majors. Our laboratory is popular, so we typically cannot accommodate non-majors.

2. Due to the amount of supervision required for our research projects, we are limited in the number of students we can accept each semester. Our laboratory typically has 6-8 undergraduates working in it at any given time. Due to the increasing size of the Genetics major and so increasing demand for Research in Genetics, students will be accepted into the Research in Genetics for only two semesters at a time. Students with excellent performance and those planning to do an Honors Thesis will be invited back for the following year. Since priority is given to these returning students, we may only have a few available slots for a given semester.

3. Students are expected to spend some time in the laboratory prior to registering for credit. Depending on the student's interest and the availability of project spots, students may do some introductory work in the wet lab or some introductory data analytic or programming work. The purpose of this trial is to give students a chance to see what work in our lab is like, and to give us a chance to see if a given student has the appropriate background for the proposed project.

4. Students working in the wet lab must also attend the REHS Laboratory Safety training prior to commencing research in the lab. The schedule is available at: http://rehs.rutgers.edu/rehs_train.html.

5. Students are expected to spend the minimum of 3 hours in the laboratory per registered credit. This is a minimum; students spending less time may have their grade impacted. Students should understand that laboratory work often does not run on a predicted schedule, so they need to be willing to be flexible in their scheduling and time spent in the lab. Students should plan to spend as many days per week in the lab as possible. Typically, planning to schedule one's entire laboratory time into just one or two days a week is not productive and will not be approved. For safety reasons, students are not allowed to work alone in the laboratory. Any work during evenings or weekends must be coordinated with your direct research supervisor to ensure appropriate supervision.

6. Students are expected to be "good lab citizens". This means working in a cooperative fashion with other in the lab, obeying all safety regulations, following our standard lab practices, and helping out with some general tasks that are important to the efficient running of the laboratory.

7. Students are required to submit a paper at the end of each semester which conforms to the requirements for Research in Genetics. This paper will be graded for both scientific content/accuracy (50% of paper grade) and general quality of writing (50% of paper grade). Please see the writing evaluation form in the appendix for details on the areas to be evaluated.

8. Students are expected to attend the 90 minute weekly lab meeting (Tuesdays from 12-1:30 PM) and give a 20 minute PowerPoint presentation about their research project at the end of each semester. It is not unusual for the first presentation (and paper) to focus mainly on the background of the project, as frequently the first semester in the lab is primarily spent learning techniques. Students who are registered for Research in Genetics should plan their class schedules so that they can attend lab meeting. Only unavoidable conflicts with courses required for the Genetics major will be considered a valid reason for not being able to attend lab meeting.

9. Grading is based on attendance and performance in lab (40%) as well as the final paper (40%) and lab presentation (20%). We occasionally have quizzes for the undergraduates in the lab, reviewing some of the basic principles that you will be learning about; these count as part of your lab grade. Please note that research in genetics should not be considered any "easy A". While most

students do receive an A, it is because they have worked hard in lab and taken the requirements seriously (not necessarily because their experiments have all worked, but because they have put in serious effort in trying). Over the past few years, about 25% of the research grades I have given out have been less than an A. The major reasons for grades less than an A are poorly written research papers and/or failure to spend the required time in lab.

10. Students may also want to consider registering for 447:410 Research in Genetics – Writing Intensive, which fulfils the SAS requirements for a writing course. While the laboratory component of this course is the same as regular Research in Genetics, this version requires more frequent, structured writing assignments with feedback and the opportunity to revise and resubmit portions of the research paper over the full semester. While this course will address problems in writing at all levels, the intended focus is to help students with the specifics of writing research papers within the discipline of genetics. Students with very weak writing fundamentals are urged to seek assistance with those aspects of writing through the Writing Center.

Appendix – Areas Considered for Evaluation of Papers

Part 1: Content
Scientific accuracy:
Comprehensiveness of background:
Appropriate description of methods:
Appropriate presentation and interpretation of results:
Appropriate use of technical language:
Appropriate use of citations:

Part 2: Writing
Overall structure:
Paragraph structure:
Sentence structure:
Grammar and spelling:
Use and format of tables and figures:
Format of references: