2016

BOIS 412/812: Human Genetics—A Peer Review of Teaching Project Benchmark Portfolio

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Course Portfolio for BIOS 412/812 Human Genetics: Synthetic Review Paper

Abstract: This portfolio focuses on Human Genetics, an upper-division course taken primarily by biology majors to fulfill elective credit in their degree. This course studies the genetic basis for human variation, with the goal of placing this variation in the context of human evolutionary history and the consequences of this variation for medical understanding and treatments. In Human Genetics, students complete an original synthetic research paper on a human genetic disorder. Through writing this paper, students are expected to learn how to navigate electronic databases and online resources on human genetic diseases, and to read and synthesize the primary scientific literature. This portfolio describes the teaching methods used to guide students through this process. The information and concepts to be taught in Human Genetics are expected to be useful for students going on to do research in a biological field, for those intending to pursue medical and health-related professions, and in general in producing informed and critical citizens who are empowered to make scientifically sound decisions.

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1. Objectives of the Portfolio

The objective of this Peer Review Course Portfolio is to develop and document the curriculum for BIOS412/812, Human Genetics. This course has been historically taught by a faculty member who is retiring, and so I took over this responsibility and taught the class for the first time in the Spring Semester of 2016.

One goal of this portfolio was to make the learning objectives for this course explicit and design performance-based assessments. Through the workshops and retreats in the Peer Review of Teaching Program, these goals were developed and ultimately incorporated as a list of specific learning objectives (included in the course syllabus) and just-in-time-teaching (JiTT) weekly assignments.

Because BIOS412 is an ACE10 class, it is expected provide the students with an educational experience that both teaches and assesses information collection, synthesis, interpretation, presentation, and reflection. Thus, a second goal of this portfolio was to develop a research paper project that fulfills these goals. Documents provided to the students to guide their research and paper-writing and examples of student papers are included in the appendices. I used this portfolio to help me develop this new (to me) course, and will include it as documentation of teaching in my promotion and tenure packages.

2. Description of the Course

Human Genetics focuses on advanced concepts in genetics, with an emphasis on human biology and applications of this knowledge to medical diagnosis and treatment. This includes mechanisms of inheritance, the nature of genetic variation, how this variation is expressed, and how this variation exists in populations and over evolutionary time. General Genetics (BIOS-206) is a prerequisite for Human Genetics; it is therefore assumed that students will enter the class at least familiar with the molecular and cellular basis for biological inheritance, mechanisms and control of gene expression, the basic structure of the human genome, and the segregation of alleles in populations.

Human Genetics is designated as an ACE10 course. To fulfill the ACE10 learning outcome ("generate a creative or scholarly product that requires broad knowledge, appropriate technical proficiency, information collection, synthesis, interpretation, presentation, and reflection") students write a synthetic review paper on a human genetic disease over the course of the semester.

The major learning objectives in Human Genetics include (see Appendix A for syllabus):
 1) understand the current, state-of-the-art methods used to identify genetic variation in humans and the approaches used to connect genetic variation to phenotypic variation, particularly disease phenotypes;

2) understand how human genetic variation informs our understanding of disease and guides and constrains health care decisions

3) develop the critical skills required to comprehend, interpret, and critique the primary scientific literature, with the ultimate goal of empowering students to independently approach the scientific literature

4) synthesize a body of primary scientific literature on a human genetic disease and write a review of the disease, describing its genetic underpinnings, its phenotypic outcomes, and current and future approaches for diagnosis and treatment.

Student learning of these course goals was assessed using weekly short answer problem sets based around the textbook or assigned reading; three midterm exams; and a research paper synthesizing the scientific literature on a human genetic disorder.

In the spring semester of 2016, Human Genetics had an enrollment of 22 students in the undergraduate section (BIOS-412) and one student in the graduate section (BIOS-812). Of the 22 students enrolled in BIOS-412, 15 were seniors, five were juniors, and two were graduate students. Majors included biological sciences (15 students), psychology (1), forestry (1), microbiology (1), biochemistry (1) and plant biology (1).

3. Teaching methods

Classroom instruction and assignments: The primary mode of in-class instruction in Human Genetics was canonical lecture. I utilized both the white-board and projected presentations - concepts and details were loosely transcribed from prepared notes on to the white board, while the presentation slides were mainly used to provide the students with figures and tables from the textbook or primary literature. I wrote on the board, rather than presenting the students with pre-written text on slides, to slow down my presentation of the material and provide students time to take their own notes and assimilate the material.

At least once during most classes I presented the students with a multiple-choice question and solicited student answers via clickers. If a majority of the answers were incorrect, the students were given the opportunity to discuss the question amongst themselves and then I posed the question again. In almost every case the class converged on the correct answer. After presenting the correct answer, I asked students to explain what exactly was wrong with the incorrect answers. Often the question was posed at the very beginning of class, as a way of focusing the students' attention and setting the atmosphere for the rest of the class period.

Outside of class, the students were expected to read the textbook (*Genetics and Genomics in Medicine,* Strachan et al. 2015) and papers from the primary scientific literature. They were also required to complete short weekly assignments through Blackboard based on the assigned reading. These assignments consisted of 3-5 short-answer questions, and

were assigned at the end of the week and due Monday at 11:00 (24 hours prior to class on Tuesday). The assignments were then graded before class on Tuesday and the students' answers were used to guide both the lecture topics and in-class questions (Just-in-Time-Teaching). Frequently the in-class question would repeat or re-state the assigned question that seemed to provide the students with the most difficulty. Students were given full credit on the assignments for simply providing a reasonable answer, but I awarded more points (that did not go towards their final grades) for correct answers. These points allowed the students to see whether their answers were correct and helped guide their studying for the midterm exams, since some assigned questions were repeated verbatim or only slightly modified on the exams.

Human Disease Review Paper: The major assignment outside of class was the review paper, which constituted 20% of the final grade for the semester. The project was carried out in stages throughout the semester, with each stage requiring an assignment to be turned in that contributed to part of the 20% of the grade associated with the whole project. At most stages the students got full credit for simply completing that part of the assignment on time; but the rough draft and final draft of the paper were graded on the merits of the work. The rationale for dividing the assignment into stages was 1) to spread the workload out over the semester and prevent students from procrastinating; 2) allow me to provide feedback, guidance, and course corrections throughout the process. The stages of the research paper assignment are described below:

Stage 1. Students were first introduced to the disease project with a document (Appendix B.1) and an in-class presentation on the internet resources available for researching these diseases. The presentation focused on two databases hosted by the National Institutes of Health (NIH): Online Mendelian Inheritance in Man (OMIM) and the Genetics Home Reference. These databases provide extensive information regarding human genetic diseases. OMIM is more technical and extensively references the scientific literature; it is equivalent to hundreds of review articles in a database format. The Genetics Home Reference site is more user friendly, presents information that is more accessible to the layman, and provides information on disease advocacy and support organizations. The presentation also introduced the class to websites and search engines for investigating the scientific literature, especially Google Scholar and Pubmed (hosted by NIH). I also encouraged the judicious use of Wikipedia, as a repository of basic and non-controversial information (although it may not always be apparent what entries on Wikipedia belong to this category). Finally, students were given a list of 15 human genetic diseases and encouraged to briefly research the diseases on the list, choose one that would be their focus of their paper, and write a brief summary of their chosen disease's characteristics. With my permission, two students chose diseases not originally on the list; these additions will be incorporated in the next iteration of the class. Students were required to convey their choice as one of the assigned homework questions due the second Tuesday class of the semester.

Stage 2. The second stage of the project required the students to find a peer-reviewed primary research paper (not a review) from the literature on their chosen disease and provide a summary of this paper. The structure of this summary was provided to the

students in the document included in Appendix B.2. These assignments allowed me to ensure that the students were discovering appropriate scientific literature and provided them with a framework for critically reading the scientific literature, and for restating the often technical language in journal articles in their own words. These assignments were due the third (choosing an article) and fourth (summarizing the article) Tuesday classes of the semester.

Stage 3. The third stage in the project was a list of 10 references on the students' chosen disease topic that would form the basis for their research. This assignment allowed me to ensure that students were accessing recent, relevant, peer-reviewed scientific literature for use in their research. This assignment was due in the sixth Tuesday class of the semester.

Stage 4. The next stage of the project was an outline of the paper, following the structure provided in Appendix B.3 as a guide. This document provided the students with a description of the major sections their paper could contain, and specific questions their research should answer. The outline was due Thursday of the eighth week of the semester.

Stage 5. The fifth stage was a draft of the final paper. The draft was to be complete, with proper formatting, citations, and polished spelling and grammar. These drafts were carefully graded and commented to provide the students with the necessary feedback to write a high-quality final draft, and if all the comments were addressed, the final draft received a high grade. The drafts were due on Thursday the 14th week of the semester.

Stage 6. The final stage in the project was the completed research paper, due the Tuesday of finals week.

4. Analysis of student learning

Gender effects on student performance: Student grades were comprised of homework assignments, the stages of the research paper, and three in-class exams; the majority of class points were assigned to the exams (60%). Exams consisted of a mix of multiple true/false, short answer, and problem-solving questions.

Unexpectedly, a stark and statistically significant effect of gender was observed on the distribution of class grades (Figure 1). This effect was most pronounced for the three exams, as the grade distribution was the widest (greatest variance) for this component of the class, but was also observed to a lesser extent in the distribution of final grades that included homework assignments and the research paper. In all cases, the nine female students significantly outperformed their 14 male counterparts. This effect was strongest for exam #1, where the median female student score was 92, while the median male student score was 78.5 (P-value = 0.001). On each exam, the top three, two, and five scores all belonged to female students (exams 1,2, and 3 respectively); and the top five final grade scores were all given to female students.
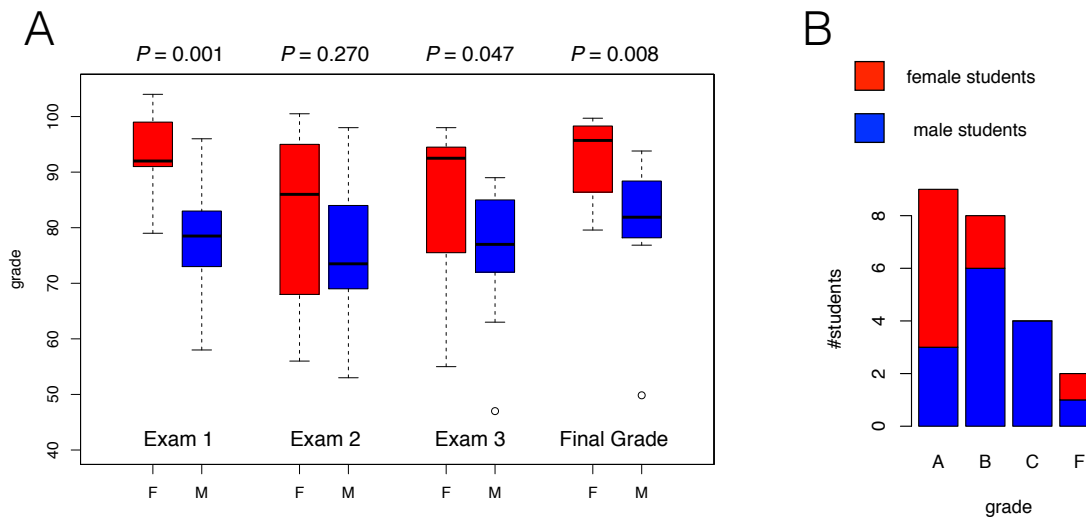
Figure 1. (A) Boxplots of grades for three in-class Exams and the Final Grade (%) for the semester, separated by student gender. Female student distributions are indicated in red; male students in blue. Outlier points are indicated as circles. *P*-values from a Mann-Whitney test of the difference between median exam scores for female and male students are indicated above the boxplots. The grades for Exams 1 and 2 were scaled by adding four and eight points to each score, respectively, which resulted in some (female) students earning > 100 points for the exam. One female student who stopped attending the class was excluded from the plot for Exam 3 and the Final Grade, as she did not take the exam or finish the final project. (B) Distribution of final letter grades separated by gender.

As there is currently only data from one year of this course, it remains to be seen whether this result reflects a general trend among students choosing to enroll in Human Genetics, or whether this was merely a one-time effect.

Human Disease Review Paper: At all stages of the review paper project, feedback was provided to the students.

Most students correctly identified relevant primary literature sources for their first paper (Stage 2). The most common error at this stage was choosing a review paper that did not present new data or experiments (four students initially chose review papers). This error was unsurprising, since the distinction between review articles and articles that present new results is most likely unknown to students who have not had extensive experience with the scientific literature, and not always easy to discern (although three of the chosen review articles did explicitly state that they were review articles in the abstract). Other inappropriate choices included a study that focused very narrowly on a treatment of one specific manifestation of the disease, rather than the underlying genetic basis or more general therapeutic or surgical treatments. Students were provided with this corrective

feedback over email, and all students selected appropriate papers for their summaries on the second try.

Student summaries of the first paper were largely acceptable, suggesting that the template provided in Appendix B.2 provided them with enough structure to allow them to dissect the primary literature.

Student lists of 10 relevant peer-reviewed publications (Stage 3) were also all acceptable, suggesting that the instruction provided in Stage 1 and the feedback on the first paper choices sufficiently enabled the students to navigate the scientific literature.

Outlines of the research paper (Stage 4) were assigned mainly to encourage the students to make substantial progress on their research well in advance of the deadline for completing the final product, and thus students were given full credit for completing the assignment on time, and minimal feedback was provided on the structure or content of the outline. Outlines ranged from minimal (Appendix C.1) to virtually completed final papers (Appendix C.2).

The first drafts of the research papers, predictably, showed a wide range of quality, from polished, completed papers that required minimal revision before submitting the final version, to drafts that were incomplete, poorly structured, and replete with grammatical errors. By far the most common errors involved the use of citations, particularly their formatting. Many students used a numbered citation format, but failed to order the citations by their appearance in their paper. Other students used an (author, year) citation format but failed to include the year in the in-text citation. Some students repeatedly cited the same source in multiple consecutive sentences, rather than including a single citation at the end of the section. Multiple students failed to adequately cite the literature throughout their paper; two students did not include any in-text citations at all, and one student failed to provide a bibliography of their references. These errors were unsurprising, given that most of these students likely had little or no experience with the citation style in scientific publications, and all errors of this kind were readily corrected for the final version of the paper.

Nine examples of both the first and final draft papers are provided in the appendix. Comments and suggestions for improvement were provided on hard copies of the first drafts and returned to the students. The first three examples (Appendix D.1, Appendix D.2, and Appendix D.3) represent the best submissions - cases where the first draft was virtually complete and required minimal edits for the final draft and students received perfect grades on both drafts.

The next three examples (Appendix D.4, Appendix D.5, and Appendix D.6) represent average student submissions. These first drafts all required significant modification before the final version and were all scored at 70%. In addition to formatting and grammatical errors, the first drafts in this group required extension and elaboration of one or more sections of the paper. These three students varied in the success with which they

improved their paper for the final version, earning a 95%, 97%, and 92% on the final draft (Students 4, 5 & 6 respectively).

The final three examples (Appendix D.7, Appendix D.8, and Appendix D.9) represent low-quality student papers. The first drafts were scored at 60% (Students 7 & 8) and 80% (Student 9). The first drafts from students 7 & 8 were all incomplete in some way and required extensive modification. The first draft from student 9 was largely complete and required only minor edits and some changes to formatting and citations, but many of these changes were not incorporated in the final version, leading to only a mild improvement in score for the final draft (90%). Both students 7 & 8 did improve their papers for the final version, scoring a 83% and 87% respectively, but these final papers were still of poorer quality than the average paper in the class.

5. Planned Changes

I felt that the mix of projected slides and writing on the board worked well and allowed the students time to take careful notes, while also enabling me to use relevant figures and tables from the textbook or the literature to illustrate relevant concepts. The exam structure also succeeded in identifying what concepts the students did and did not learn. On each of the first two exams, the students did very poorly on one question (overall scoring 33% and 22% of the total possible points); the points corresponding to these questions were then added back each student's exam. In future classes, I will either make an extra effort to convey the concepts assessed by these questions, or rewrite the exam questions to try and better match what was taught.

In future iterations of this class, I intend to increase the time spent both in and outside of class on reading and interpreting the scientific literature. The assigned readings and in-class discussion of the papers clearly engaged the students and conveyed to them both scientific knowledge but also how that knowledge is obtained - something that is not as readily apparent in textbooks. Ideally, the class would have no textbook but would rely solely on primary scientific literature, review articles, and some science writing for the lay public; but it may be a few more iterations before that becomes possible.

Many aspects of the review paper project went well. In the future I intend to incorporate two changes to this project. First, the students needed more practice restating complex and technical scientific language in their own words. This was clearly a challenge for most of the students; a few approached this problem by extensively quoting the articles they read in their paper, which, although ethically defensible, neither encourages synthesis of the information nor makes for very readable text. This will be a significant challenge, given how extremely technical and detailed the relevant literature is; but nonetheless worth attempting. I think doing this together as a class for one paper, before each student summarizes a paper on their chosen disease topic, would be the simplest way to provide the class with this practice. Second, I intend to provide more detailed feedback at the outline stage of the project. I would like to give the students freedom to focus on the aspects of their research topic that interest them the most, and would like to

avoid constraining them too much with prescribed templates and formats; there is already plenty of that in Appendix B.3. This motivated using the outline solely as a guard against procrastination, and not critiquing the content of student outlines. However, I think this is outweighed by the benefits of being able to provide students with feedback regarding which aspects of their papers could be more or less detailed, and encouraging them to find more or different source articles early in the writing process. Requiring both a

"draft" and final version of the outline could also allow me to set a higher bar for the final draft of the outline, aspiring for all students to produce a document on par with the best outlines submitted this semester (Appendix C.2).

6. Summary and Synthesis

The first iteration of the human disease review paper, although not without flaws, seemed to be largely a success. Even the lowest-scored review papers were substantial works of research, and students clearly learned many concepts and details regarding their chosen disease. Informal student feedback indicated that students enjoyed the project, and found the staged structure helpful in guiding their research and writing and preventing procrastination, as was intended. Although it may be modified, I intend to include this project in future iterations of the class.

The Peer Review of Teaching Project was unquestionably useful in helping me to develop Human Genetics and design the human disease project. But I feel its benefits go beyond this specific course. By facilitating discussion of pedagogy and encouraging instructors to approach teaching as if it were research (designing experiments and collecting data on student learning), this project helped me to think more critically and carefully about my teaching; about what my expectations of student learning are and how to match my teaching to help students meet those goals; and empowered me to actively and effectively work to improve my teaching.

Appendices

BIOS 412/812: Human Genetics
Spring 2016 syllabus

Class meets Tuesday and Thursday 11:00-12:15 PM, Beadle Center 176

Instructor: Dr. Colin Meiklejohn, Assistant Professor, School of Biological Sciences
Office: 302A Manter Hall
Office hours: scheduled by appointment
email: cmeiklejohn2@unl.edu

Textbook: *Genetics and Genomics in Medicine*. Strachan, Goodship and Chinnery
(the digital version is a cheaper option)
Clickers are required

Course description and learning objectives: This course focuses on advanced concepts in genetics, with a focus on human biology and applications of this knowledge to medical diagnosis and treatment. This includes mechanisms of inheritance, the nature of genetic variation, how this variation is expressed, and how this variation exists in populations and over evolutionary time. In fulfillment of the ACE10 learning outcome ("generate a creative or scholarly product that requires broad knowledge, appropriate technical proficiency, information collection, synthesis, interpretation, presentation, and reflection") you will write a synthetic review paper on a human genetic disease. Through this project you will learn to search scientific literature; how to read and interpret the scientific literature; and how to write a short review of recent scientific publications on the disease.

Expectations:
BIOS206 (General Genetics) is a prerequisite for Human Genetics. I therefore assume that students are familiar with the molecular and cellular basis for biological inheritance, mechanisms and control of gene expression, the basic structure of the human genome, and the segregation of alleles in populations.

For each lecture topic, you will have assigned reading and questions about the reading. The questions will be posted on Blackboard and are due by noon the day before class. These assignments are due the day before class so that we can spend class time focused on the concepts that are challenging to the most students; I need the time to adjust the next day's lecture based on your answers. Completing these assignments is part of your grade; but you will get full credit so long as you make an honest effort to answer the questions to the best of your ability.

I expect that you will come to class, read the textbook and assigned papers from the scientific literature, do the assigned homework problems, think, have fun and use logic over memorization. Please take advantage of office hours if you are struggling with the material, sooner rather than later.

Learning in groups is encouraged, but this is only effective if all group members are contributing. The reading assignments may be done together with other students, but if someone else is doing most of the work, you will likely get poor exam grades. Copying someone else's homework and turning it in as your own work is not only a violation of the Student Code of Conduct (for both students), it is an ineffective way of learning the material and preparing for the exams.

Evaluation and grading: Your course grade is based on three midterm exams, homework assignments and a synthetic review paper investigating a human genetic disease. Each of these 5 components (3 midterms, homework, research paper) comprises 20% of the final grade. Depending on the results, midterm exam scores may be scaled. Final grades will be determined as follows (out of 500 points):

495 (99%) A+	440 (88%) B+	390 (78%) C+	340 (68%) D+
460 (92%) A	410 (82%) B	360 (72%) C	310 (62%) D
450 (90%) A-	400 (80%) B-	350 (70%) C-	300 (60%) D-

Academic integrity: Students are expected to adhere to guidelines concerning academic dishonesty outlined in Section 4.2 of University's Student Code of Conduct. Violations of this policy will result in a minimum sanction of a grade of zero being given for the graded item on which the violation occurred, and may include other actions, such as an F for the course. All incidents will be reported to the Dean of Students. Behavior and remarks that disparage persons or activities in the course, or detract from a serious learning environment are not tolerated.

Email policy: Students are encouraged to contact the professor via email; however, please be respectful in your use of email. Many details about the course can be found in this syllabus, and conceptual questions are better discussed in lecture, office hours, and study sessions. Please do not fill the instructors' inboxes with every question that may arise regarding course material. Email is a good way to schedule appointments and conduct course-related business. When you do email, it should be considered a formal means of communication, and messages must be structured accordingly. This means that each email must include an informative subject header, an appropriate salutation, complete sentences with accurate spelling and punctuation, and an appropriate closing and signature. Emojis are acceptable. Emails that do not follow this structure may or may not be answered. These rules will help prepare you for virtually any future employment, as email is a ubiquitous form of professional communication.

Students are strongly encouraged to send email through Blackboard or from @huskers or @unl email address. In the past I have experienced problems receiving email from services such as yahoo and rocketmail, and so messages sent from these accounts may not be answered.

Students with Disabilities: Students with disabilities are encouraged to contact me, or a teaching assistant, for a confidential discussion of their individual needs for academic

accommodation. It is the policy of the University of Nebraska-Lincoln to provide flexible and individualized accommodations to students with documented disabilities that may affect their ability to participate fully in course activities or to meet course requirements. To receive accommodation services, students must be registered with the Services for Students with Disabilities (SSD) office, 132 Canfield Administration, 472-3787.

Learning Goals	*Strachan et al* reading
Background material • Explain the relationship between nucleotides, DNA, genes, chromatin, nucleosomes, chromosomes • Describe DNA replication and transcription • Describe the processes of genome replication and cell division • Compare and contrast mitosis and meiosis • Explain homolog pairing and recombination in meiosis • Describe the difference between introns and exons and explain splicing • Explain the genetic code and the role of RNA molecules in gene regulation, including transcription and translation. • Describe the structure of the human genome, explain gene families and repetitive DNA	Chapters 1 & 2
• Explain and diagram DNA cloning, PCR, and DNA sequencing • Explain the principles and uses of nucleic acid hybridization	Chapter 3
• Explain the process of mutation and the generation of genetic variation • Describe the structure of genetic variation in human populations • Explain the signatures and consequences of natural selection acting in human populations	Chapter 4
• Perform genetic inference based on segregation of genotypes and phenotypes in families and populations • Calculate genotype frequencies from allele frequencies under Hardy-Weinberg equilibrium	Chapter 5
• List the classes of *cis*-regulatory sequences, *trans*-regulatory proteins, and small regulatory RNAs and how they regulate gene expression (both transcriptional and post-transcriptional regulation) • Explain how chromatin modification regulates gene expression • Describe the role of epigenetics in human diseases	Chapter 6
• Describe how genetic variation results in disease • Describe the role of repetitive DNA in disease • Predict transmission patterns of chromosomal abnormalities	Chapter 7
• Describe genetic studies used to understand disease inheritance	Chapter 8
• Describe genetic treatments and genetic interactions with drug treatments	Chapter 9
• Describe the genetics and progression of cancer • Explain the advances derived from genomic studies of cancer	Chapter 10
• Compare and contrast technical and ethical aspects of genetic testing	Chapter 11

Course Calendar

January 19	Identify human disease topic
January 26	Identify recent scientific publication on chosen disease
February 11	Synopsis of recent publication due
February 11	<u>Exam I</u>
February 25	List of citable publications
March 15	Review outline
March 17	<u>Exam II</u>
April 5	Review rough draft
April 28	<u>Exam III</u>
May 3	Review paper

This semester you will write a synthetic review paper on a human genetic disease. Through this project you will learn to search scientific literature; how to read and interpret the scientific literature; and how to write a short review of recent scientific publications on the disease.

The first step is to choose from one of the human genetic disorders listed below. These disorders vary in their severity, quality of life, genetic basis, and currently available treatments. You can learn about each of these conditions from many online sources, which you will also use to find scientific articles about the condition you choose to research:

Google (search, news, scholar)
Wikipedia
*Genetics Home Reference (http://ghr.nlm.nih.gov/)
*Online Mendelian Inheritance in Man (OMIM - omim.org)
Web of Science: used to find primary (journal) articles and review articles in biology and other scientific disciplines
PubMed: provides access to Medline, used to find primary (journal) articles and review articles in the biomedical sciences
EurekAlert!: features science, health and technology news; sponsored by the American Association for the Advancement of Science (http://www.eurekalert.org/)
Science Daily: breaking science news stories and feature articles (http://www.sciencedaily.com/)

Human disease topics
Angelman syndrome
Beckwith-Wiedemann syndrome
Beta thalassemia
Celiac disease
Cystic fibrosis
Duchenne and Becker muscular dystrophy
Hutchison-Gilford syndrome
Leigh syndrome
Lesch-Nyhan syndrome
Marfan syndrome
Parkinson disease
Prader-Willi syndrome
Sickle cell disease
Tay-Sachs disease
Werner syndrome

This assignment is worth 2% of your course grade, and is due at the beginning of class on Tuesday, February 9.

The second step in this project is to write a summary of the primary literature article you have selected on a human disease. The goal of this exercise is to help familiarize you with the structure of a scientific paper and how to extract information from them. The following sections of your synopsis correspond to the sections of a typical scientific article, although not all articles will indicate each section with an explicit header. For each section, write a few sentences or a short paragraph addressing the prompts listed below. Given the technical nature of these articles, describing the methods and results in your own words may be significantly challenging; however this challenge is precisely the purpose of this assignment. Copying a sentence verbatim from the article is not acceptable.

Abstract
Write 1-2 sentences that re-state, *in your own words*, a result highlighted in the paper's abstract.

Introduction
What aspect of the disease (genetic basis, phenotypic manifestation, treatment options) is addressed in this paper?

Methods
Summarize <u>one</u> of the main experiments in the paper as follows:
       - Describe the authors' hypothesis or hypotheses
       - Describe at least one experiment that they do to test their hypotheses
       - What is the logic behind this experiment?

Results
What type of data did the authors collect?
What is the main result to come out of this experiment?
What conclusions do the authors draw from their results?

Future directions
What are the implications of this work for this particular disease? How will the research (eventually) lead to improved quality of life for individuals with this disease?
Describe one experiment that could be done as a follow-up to the experiments described in the paper.

Human Genetic Disease Research Project

The following outline provides a recommended structure for your disease research paper. Your paper should include most or all of these points, assuming that they are applicable to your chosen disease. These recommendations may also help you identify interesting primary literature articles that directly address one or more of these topics.

Provide an introduction to the disease
- Give a brief summary of the disease, including but not limited to: what defines the syndrome, phenotypic range, physiological effects, prognosis, and quality of life.
- What is the epidemiology of the disease? Or, how often does the disease occur, in which populations, how many births? Discuss details about higher incidence in certain cultures, geographical areas, or other boundaries.
- What is the inheritance pattern? (autosomal, sex-linked, dominant, recessive, etc.) Does this have a relation with the epidemiology?
- Provide a brief historical perspective of the disease. Who discovered it and when? Who now supports research of it? Is the disease in the news? What advocacy groups exist for the disease?

Define the genetic component of the disease
- What gene(s) underlie this disease, and how was the genetic component discovered?
- What would the karyotype of an affected individual look like (if different from normal)?
- What is the wild-type role of the gene?
- What are the most commonly reported mutations?
- What are the population genetics of the disease alleles? How frequent are disease alleles, and are there differences in frequencies between populations? What selective pressures are hypothesized to act on genotypes with one or two copies of the disease allele(s)?

Describe the molecular biology and biochemistry of the disease
- What is the phenotype of the disease at the cellular level? What cells or tissues are involved?
- Does the gene(s) encode a protein? What does the protein do? Is the protein involved in a known pathway?
- How does the mutant form of the gene affect the cell?
- Find the homolog of the protein in rhesus monkey, mice, and Drosophila. Is the mutation in a conserved region?

Clinical aspects and organismal phenotypes
- Diagnostic tests
- Variability in phenotype: penetrance and expressivity
- Role of the genetic element in other diseases
- Animal models

Issues for the affected individual and treatments/prospects
- What are the major challenges faced by individuals with the disease?
- What is the current state-of-the-art in detection, treatment, or avoidance (e.g. screening pre-implantation embryos)?
- What are some potential treatments that have been proposed or are in research and development (e.g. clinical trials)?

Celiac Disease Outline

1. Introduction
    a. What is Celiac disease
2. Cause
    a. Diet
    b. Risk modifiers
3. Genetics
    a. HLA-DQ2 and HLA-DQ8 alleles
    b. Gene inheritance
4. Diagnosis
    a. Blood tests
    b. Endoscopy
    c. Pathology
    d. Other tests
    e. Gluten withdrawal
5. Treatment
    a. Diet
6. Future Research Directions
    a. Reduce diet restrictions

BIOS 412
Review Paper Outline

Topic: Hutchinson-Gilford Progeria Syndrome

i.   Introduction to HGPS
- a. A rare premature aging disease that begins to manifest and is generally diagnosed within the first year or two of life. [4,7,8,9]
  - i. Characterized by rapid progression of phenotypes associated with aging
    1. Stunted growth→Both height and weight
    2. Loss of hair and subcutaneous fat (lipodystrophy)
    3. Skeletal abnormalities→ joint abnormalities and mobility, mandibular and cranial abnormalities, and finger-tip tufting
    4. Prominent eyes and a beaked nose
    5. Skin discoloration, mottling, and prominent vasculature (specifically on scalp)
  - ii. Often suffer from arteriosclerosis and atherosclerosis [4,8,9]
    1. Generally die around 13 years of age on average due to related problems→heart attack or stroke
- b. Affects about 1 in 4 million live births annually [8]
- c. Sporadic, Non-inherited autosomal dominant point mutation→90% of cases [4,11]
- d. History
  - i. First described by Dr. Jonathan Hutchinson in 1886 and Hastings Gilford in 1897 but genetic basis was not uncovered until 2003 [6]

ii.   Genetic Component of HGPS
- a. Mutation is located in the lamin A/C gene (LMNA) located on chromosome 1 [4,8,9,11]
- b. Normal function of LMNA is to produce two structural proteins that belong to the Type V class of intermediate filaments→ lamin A and lamin C [4,7,11]
  - i. Both proteins are made in majority of differentiated somatic cells [4,7,11]
  - ii. Have nearly identical sequence, except lamin A is slightly longer than lamin C→ formed by alternative splicing [4,11]
    1. lamin A is first produced as prelamin A and must undergo additional modification and processing steps before becoming mature lamin A
  - iii. Components of the nuclear lamina, which is a mesh-like scaffold that binds to the inner membrane of the nuclear envelope supporting its structure as well as maintains chromosomal compartmentalization within the nucleus [8,11]
    1. Also believed to play a role in various nuclear activities such as regulating movement of molecules in and out of the nucleus and the gene activity of certain genes [11]
    2. Known to interact with various components within the nucleus including dsDNA, transcriptional regulators, nuclear membrane associated proteins, and nuclear pore complexes [8,11]
- c. HGPS is most commonly caused by a sporadic synonymous point mutation located in exon 11 of LMNA in the third position of codon 608 (positin1842; C>T)→ GGC to GGT [4,8,9,11]
  - i. Although this mutation encodes for the same amino acid (glycine) it activates a cryptic splice donor site within the exon resulting in a 150 nucleotide (50 amino acid) deletion [4,8,11]

1. Within this deleted region resides and important cleavage site, ZMPSTE24, that is necessary for one of the later processing steps in which a farnesyl modification is removed from the prelamin generating the mature lamin A protein [4,7,11]
   a. This farnesyl modification is used to bind the prelamin A to the inner membrane and when cleaved releases the lamin A protein from the inner membrane allowing it to properly function in the nuclear lamina [11]

ii. As a result→ a mutated form of prelamin A, called progerin, is produced
   1. Since progerin does not contain the necessary cleavage site it retains the farnesyl modification and remains bound to the inner membrane [9,11]
      a. As progerin accumulates this causes abnormal nuclear structure (blebbing), instability, and other complications
      b. Also reduces lamin B1 production→further disrupts lamina [10]

d. This is a dominant negative mutation because one mutated allele is enough to cause nuclear blebbing and other HGPS phenotypes essentially inhibiting the normal allele [9]
   i. Phenotypes are not due to haploinsufficiency because mice without production of lamin A did not have nuclear abnormalities or HGPS associated phenotypes [9]

e. This gene is conserved and has homologs in various species
   i. Drosophila have two lamin genes (lamC: A-type and Dm0:B-type)→ genetic analysis indicates lamin genes came from same ancestor as vertebrate lamin genes [1]
      1. However, A-type lamins in drosophila are not farnesylated so cannot mutate lamC to cause progerin production
   ii. Mice and primates (Rhesus Monkey) both have LMNA gene that produces A-type lamins
      1. Mice have been widely used to model this disease and exhibit some of the same type of HGPS phenotypes following a C>T point mutation in exon 11 of the LMNA gene to produce progerin→ position 1827 (codon 609) in mice [11]

iii. How progerin affects normal cellular function
   a. LMNA is expressed in nearly all differentiated somatic cells through-out the body so most cells can be affected by a mutation in this gene [4,7,11]
      i. Especially→ Bone cells and fibroblasts in skin, muscle, and vascular cells [9]
      ii. However, cells of the nervous system express LMNA at lower levels so the production of progerin in these cells is minimal→ generally HGPS do not have neural deficits [4,9,11]
         1. Also no increased incidence of cancer [4]
   b. Abnormal and unstable nuclear structure [4,7,8,9,11]
      i. caused by permanent attachment of progerin to the inner membrane→ as progerin accumulates nuclear blebbing occurs
   c. Reduction of lamin B1 [10]
      i. Affects DNA replication and decreases activity of RNA pol II
   d. Epigenetic Alterations [8,10]
      i. Normal lamin A interacts with dsDNA, specifically at heterochromatic regions, and potentially controls gene expression through these interactions [8]
      ii. According to the findings of McCord, progerin interacts differently than normal lamin A, resulting in altered patterns of repressive histone marks [8]
         1. Specifically those associated with facultative heterochromatin, H3K27me3

2. Occurs before nuclear blebbing indicating they arise very early on in the progression of this disease and potentially responsible for rapid phenotypic manifestation
    iii. Disease related alterations were determined to have occurred at several promoter regions resulting in altered gene expression→ incidences of upregulation and downregulation were both observed [8]
    iv. These alterations to the pattern of heterochromatin were also found to disrupt chromatin-lamina associations resulting in loss of proper chromosomal compartmentalization within the nucleus [8]
    v. Also control protein-chromatin interactions→ also alters gene expression [10]
        1. Believed to control the binding and release of lamin-associated proteins from the nuclear periphery [10]
        2. Proteins most likely "regulate gene silencing and activation of developmentally regulated genes"[10]
e. Cell Metabolism/Mitochondrial dysfunction [11]
    i. According to a proteomic analysis done by Rivera, HGPS fibroblasts show a dramatic metabolic alterations indicating a switch from oxidative to glycolytic metabolism due to defective oxidative phosphorylation (mitochondrial dysfunction)→contributes to premature organ decline in HGPS patients
        1. Upregulated→glycolytic proteins
        2. Downregulated→oxidative phosphorylation proteins (ATP synthase complex V) and translational apparatus proteins (ribosomal proteins)
    ii. These same results were observed in progeroid heterozygous and homozygous mice models and in homozygous ZMPSTE24-/- mice models
        1. The ZMPSTE24-/- mice models produce prelamin A proteins that are unable to mature (retain the farnesyl modification), but do not contain the same deletion that causes the production of progerin
            a. These results showed that accumulation of prelamin A has the same mitochondrial effects as the accumulation of progerin.
f. Telomere Shortening [4]
    i. According to a study done by Decker, overall telomere lengths in HGPS fibroblast cells did not follow the same chromosome specific pattern observed in normal cells
        1. Indicating a more random chromatin arrangement in HGPS cells
    ii. On average, telomere lengths in HGPS cells were shorter than those in normal age-matched cells
        1. Also all HGPS cells analyzed possessed at least one chromosome with telomeres that were too short to be detected, however, this was not the case in normal cells
    iii. Premature senescence due to these shortened telomeres is a potential cause or contributing factor of the arterial changes that result in heart attack and strokes of HGPS individuals
g. Mitotic chromosome miss-segregation and binucleation [2]
    i. Progerin forms insoluble cytoplasmic aggregates that associate with membrane-like structures, with retained farnesyl group, throughout the mitotic cytoplasm
        1. Lamin A is normally in an unpolymerized state during mitosis→ aggregates disrupt normal membrane morphogenesis during mitosis
        2. Chromosome lagging during anaphase was also observed
h. Shortage of mesenchymal stem cells (MSCs) [12]
    i. MSCs are needed for tissue replenishment

        ii.   Shortage results from reduction in specific differentiated cell types due to increased hypoxia sensitivity due to progerin

        iii.   Affects multipotency and differentiation [6]

iv.   Clinical aspects and organismal phenotypes of HGPS

   a.   Normal aging process

       i.   The accumulation of progerin are believed to be a factor in the normal aging process for the same reasons listed above→ higher levels of both have been found in cells of normal individuals of advanced ages and shown to increase in levels in an age dependent manner, however, still present in much lower amounts than HGPS cells [4,7,8,11]

           1.   Telomere shortening due to replication damage is a common occurrence during cell divisions so telomeres get shorter and shorter as an individual ages until the cell is no longer able to divide (senescence) [4]

                a.   In normal individuals of older age it is believed that these shorter telomeres and down regulation of ZMPSTE24 induces the production of progerin→potentially feeding into the normal aging process by accelerating cell senescence [4,7,11]

                b.   This production of progerin with age would also contribute to the nuclear abnormalities, mitochondrial dysfunction, and reduced transcription/DNA repair often observed in these normal cells [7,11]

   b.   Variance in expressivity and penetrance

       i.   Most individuals are heterozygotes so no data comparing phenotypic severities due to two mutant alleles vs. one in humans was found.

        ii.   However, mouse models used in Rivera study indicate that the phenotypic effects, specifically with regards to mitochondrial dysfunction, are time and dose dependent [11]

           1.   The homozygous progeroid mice had more severe and earlier onset phenotypic symptoms than the heterozygous progeroid mice models (dose-dependence)

           2.   The young heterozygous mice (10 weeks) also had less severe phenotypes and mitochondrial dysfunction than the old heterozygous mice (32-weeks)→time-dependence based on accumulation of progerin

   c.   Other diseases associated with LMNA mutations

       i.   Other mutations in the LMNA gene that disrupt the processing of prelamin A cause a group of 12 diseases known as laminopathies [7,11]

           1.   Each has distinct clinical phenotypes associated with various tissues (muscle, peripheral nerve, adipose, bone, and skin) and features (myopathy, cardiomyopathy, lipodystrophy, neuropathy, and premature aging) [7]

   d.   Diagnosis generally occurs prior to two years of age and is initially based on the observable phenotypes such as stunted growth, lipodystrophy, and other characteristics associated with rapid aging. [8,9]

       i.   It can then be confirmed with genetic testing to determine presence of the associated LMNA mutation

v.   Health and treatments

   a.   Health Implications

     i. Despite joint abnormalities and vascular issues, individuals had generally good mobility→ testing in the high mobility range [9]

     ii. Hearing loss [9]

     iii. Hyperopia [9]

     iv. Cardiovascular complications

        1. Studies showed drop out of vascular smooth muscle cells, reduced elasticity and thickening of arterial walls, and reduced vascular compliance resulting in high blood pressure [9]

        2. Most individuals die due to these complications

b. Current treatments and/or clinical trials

     i. Growth Hormones→ increased overall growth (height and weight) [9]

     ii. Farnesyl transferase inhibitors [5,9]

        1. Clinical trials showed increased weight gain and fat tissue, improved vascular stiffness, and bone mineralization resulting in reduced fracture rate

           a. However, only occurred in some individuals [5]

           b. Extended life of treated individuals by 1.6 to 2.4 years [5]

        2. Works by preventing the addition of the farnesyl group to prelamin A so the mutated proteins don't remain bound to the inner membrane restoring nuclear structure [9]

     iii. Isoprenylation inhibitors [11]

        1. Preventing progerin isoprenylation was shown to increase ATP synthesis and increased mitochondrial function in mouse models→ currently being evaluated through clinical trials

        2. Possible combination with farnesyl transferase inhibitors also in question

     iv. Rapamycin [3]

        1. Major target→ mammalian target of rapamycin (mTOR)

           a. Central regulator of cell growth and various cellular process

        2. However, can also promote macroautophagy→ natural process the cell uses to get rid of damaged or unnecessary components through lysosomal degradation

        3. Studies showed significant decrease in progerin accumulation in treated cell→ rid cells of progerin by inducing autophagy and increased solubility of progerin cytoplasmic aggregates

           a. Improved nuclear blebbing and was even able to reverse nuclear blebbing

           b. Restored H3K27me3 modifications

           c. Extended cellular lifespan by at least 30 days

        4. Concluded that it improves nuclear phenotypes, postpones cellular senescence, and increases rate of progerin turn over

c. Potential treatments [6]

     i. RNA Therapy

        1. Removal or inhibition of the alternative splice site (mutation site)

     ii. Cell Replacement Therapies

        1. Using matched normal cells or tissue stem cells from HGPS-iPS cells that have been genetically corrected

     iii. Gene Therapy

        1. Targeted gene therapy→ mutated gene is repaired outside of the body and then reintroduced into the body

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4/11/2016

BIOS 412: Human Genetics

Celiac Disease Review Rough Draft

Celiac disease belongs to the class of autoimmune disorders in which an immune response is mediated upon the body's own healthy tissues, causing damage and inflammation. The genomic region that has been associated with several autoimmune diseases is the human leukocyte antigen (HLA) or major histocompatibility (MHC) region on chromosome 6. Upon the ingestion of foods containing the gluten protein, patients with celiac disease trigger an immune response that results in a range of enteropathies that are most commonly characterized by mucosal damage and villous destruction. Traditionally, a gluten-free diet has been the most effective treatment, but emerging research has presented innovative treatments that work at the molecular level, by binding and blocking the binding site of the antigen-presenting molecule HLA-DQ2 and/or HLA-DQ8 (Deilli-Crimi et al.).

The immune system is extremely polymorphic; its genetic variation has been under positive selection in order to maximize the number and types of proteins (MHC proteins and others) that bind specific foreign molecules that might be pathogenic. As a result, fully understanding the exact genetic components that contribute to an autoimmune disease is often quite challenging. To further establish the complexity of an autoimmune disease, it is worth mentioning that genes within the HLA region are packed into a small region amounting to only about 2% of chromosome 6. And, as a result, a haplotype of this nature (where genes are within close proximity to one another) tends to be clustered in genes that are inherited intact as the chance of the genes being separated by recombination is low (Strachan 107-112).

The oldest historical records describing celiac disease come from the Greek physician Aretaeus of Cappadocia between the 1st and 2nd centuries AD. The symptoms were reported, but the exact cause was not. It was not until the 19th century that British physician Samuel Gee would propose the idea that celiac disease is linked to diet, which was later confirmed in the 1950's by Dicke and colleagues who established that foods containing gluten, including wheat, barley, and rye, are the cause of symptoms (Losowski). *— you should include the publication year in the in-text citation*

Current consensus indicates that the worldwide prevalence of celiac disease is at about 1% of the general population, with higher incidences among females. However, some regions show increased prevalence, such as Western Sahara, while other regions *where?* have had no reported cases (Deilli-Crimi et al.). Celiac disease is a polygenic disorder that follows a complex, non-Mendelian inheritance pattern that is centered about the HLA region in which certain alleles associated with the disease show strong linkage disequilibrium, or nonrandom association (Medrano et al.). In addition, recent findings confirmed the differential expression of certain genes in children patients as opposed to adult patients, suggesting an age effect (Pascual et al.).

There are several key molecular interactions that underlie the symptoms of celiac disease. The pivotal molecules are HLA-DQ2 and/or HLA-DQ8 antigens and T-cells, which interact directly to generate the destructive autoimmune response. HLA-DQ2/HLA-DQ8 heterodimers that are specific for gluten binding use a T cell repertoire (TCR) that binds gluten-derived peptide antigens and present them to T-cells which then secrete inflammatory cytokines in response. The unusual molecular structure of gluten proteins, which are nearly half composed of proline and glutamine residues, ~~which~~ makes them more difficult to digest in the intestinal tract, ultimately result*ing* in the formation of many small peptide fragments that are susceptible to chemical modification by tissue transglutaminase and subsequent HLA-DQ binding (Koning).

There are many candidate genes at multiple loci in several chromosomes in HLA and non-HLA regions that are suspected of contributing to the genetic risk of pathogenesis. But the main alleles associated with the disorder exist in the HLA region that are located on chromosome 6 (Deilli-Crimi et al.). The allele that shows the strongest association with celiac disease is HLA-DQ2 (Araya et al.). Furthermore, a dosage effect has been observed. Children who inherit a copy of HLA-BQB1*02 (one of the alleles that encode for HLA-DQ2) from both parents were found to be at a higher risk of developing celiac disease than those who inherited the allele from only one parent (Deilli-Crimi et al.).

It is important to note that the vast majority of people who carry HLA-DQ2 or HLA-DQ8 predisposing alleles will not manifest celiac disease. It is only the majority of celiac patients that happen to carry the HLA-DQ2/DQ8 allele. This is a good indicator of other non-MHC allele factors contributing to pathogenesis, such as epigenetics and the environment (diet). Surprisingly, recent evidence uncovered by genome-wide association studies (GWAS) suggest that the vast majority of SNP's that are associated with disease occur in non-coding regions of the genome, including regulatory sequences (promoters, enhancers, etc…) and non-coding RNA (ncRNA) genes (Deilli-Crimi et al.). A special class of noncoding RNA's, microRNA, was found to be differentially upregulated as revealed by biopsies of celiac disease patients and controls. Specifically, a miRNA known as miR-449 was found to be the most upregulated. That miRNA also happens to be the one that targets mRNA's that code for the translation of the NOTCH1 inflammation regulator. In concordance with the finding, NOTCH1 was discovered to be at lower concentrations in the small intestines of patients with celiac disease. Overall, however, not much data have yet been gathered in support of the role of ncRNA's in celiac disease (Iaffaldano et al.).

Another epigenetic mechanism ~~in suspicion of~~ *suspected to* contribut**e** ~~ing~~ to increased risk of celiac disease is allele specific methylation, which also occurs in noncoding sequences. Using microarray screening technologies, Hutchinson and others reported finding allele-specific methylation at several expression quantitative trait loci, one of which was ~~of a~~ *the* long non-coding RNA DLEU1 that has been associated with celiac disease.

In order to obtain a good understanding of the disease and its mechanism, the focus of discussion will shift to the actual site of action: epithelial cells of the small intestines. To start off, the CD4 T cells that are specific for gluten peptides bound to HLA-DQ2/DQ8 have only been detected in celiac disease patients (Abadie et al.). Upon examination, T-cell receptor usage was found to be biased among patients as opposed to controls, and a key non-germline encoded arginine residue was discovered as part of the T-cell receptor structure and determined to be essential in the binding of T cell receptors to HLA-DQ-gluten peptide complex (Broughton et al.). On its own, a gluten-derived peptide consisting mostly of glutamine and proline residues is actually weakly bound to HLA-DQ2/DQ8 heterodimers and therefore will only trigger a weak response from T cells. To induce high affinity, the action of tissue transglutaminase is needed to deamidate glutamine into glutamate, essentially converting an electrically neutral amino acid into a negatively-charged amino acid. Upon tissue damage, transglutaminase enzyme is recruited and acts to immediately modify gluten peptides, causing greater binding affinity between HLA-DQ2/DQ8 and the gluten peptides, which ultimately leads to an elevated T cell response, increasing intestinal tissue damage. Fundamentally, the T-cell mediated response is not triggered on a full scale upon the encounter ~~of~~ *with* the first gluten peptide, but in a snowball fashion (Wal et al.).

Arguably, the most fascinating aspect about the molecular basis of the disease is the root of the problem itself: why do gluten-derived peptides cause an immune response to begin with? The answer lies in the pathogenic world. On the surface of small intestines, the extracellular matrix is a major barrier between our cells and all sorts of other foreign microbes. But a common theme in evolutionary biology is that host and parasites coevolve. In order to gain access to host tissues, a parasite will secrete proteases that break down the extracellular matrix of the host. As it turns out, like gluten, the extracellular matrix is unusually high in proline residues. Therefore, the presence of high levels of prolines can be interpreted by the immune system as an attempt by parasites to degrade the extracellular matrix and will respond accordingly. It is the upregulated expression of the tissue transglutaminase in celiac disease patients that has been suggested to induce an autoimmune response in individuals who carry certain HLA-DQ alleles (Ostensson et al.).

Although the majority of the complexity of celiac disease is contributed to its polygenic and epigenetic nature, the environment remains a critical factor in the onset of disease. And since the primary site of action in the disease are the small intestines, the role of the patient's intestinal microbiome was interrogated. A study by Nistal el al., among other works, confirms the observation that fecal samples from celiac disease patients display different microbiota compositions than healthy individuals. They've also reported a significant difference in presence

If this is a scientific name it should be italicized

of Bifidobacterium bifidum between untreated celiac disease patients and healthy adults. A

also

reduction in the diversity of B. bifidum was observed in treated celiac disease patients and healthy adults but not in untreated patients.

Other findings point to the possibility of certain genotypes influencing the composition of the microbiome. The most relevant genotype here, HLA-DQ, has been found to be in association

with reduced B. bifidum (Olivares et al.). This bacterial species is worthy of special and careful attention because of the anti-inflammatory effects it induces. Upon the encounter of a gluten-derived peptide bound to an HLA-DQ heterodimer, T cells produce pro-inflammatory cytokines such as TNF-α and IL-1β. It was discovered that B. bifidum induces an inhibitory effect on the secreted cytokines, and different strains of the bacterium correspond to varying inhibitory strength. The proline-rich structure of gluten proteins render them more difficult to degradation by the action of the body's own proteases, allowing the proline-rich peptides to reach the lamina propria of small intestines. Interestingly, the B. bifida breaks down gluten peptides further by proteolysis during digestion such that the gluten-derived peptides that reach the intestinal epithelia are of different structural patterns than those (less degraded) normally bound by HLA-DQ antigens. This ultimately leads to a reduced or inhibited pro-inflammatory secretions by T cells (Laparra and Sanz). *So ultimately celiac disease increases the presence/abundance of B. bifidum; but the bacteria then makes the disease less severe by reducing inflammation. Is that right?*

This paragraph uses the subjunctive (hypothetical) case — "would be to" — but the diagnostics you describe are actually what is used. Just state, for example, "an accurate diagnostic tool is"

Since this is an autoimmune disorder, an accurate diagnostic tool would be to measure the concentration of modified gluten antibodies in patient serum before and after the implementation of a gluten-free diet. If a significant drop in gluten-specific antibodies levels is observed after introducing a gluten-free diet as opposed to before, then one can conclude with significant certainty that ~~they are encountered with a case of~~ *a patient has* celiac disease (Koning). Another highly effective diagnostic method would be to perform serological tests on potentially affected individuals while they're on gluten-containing diet and measure the ratio of IgA antibodies to tissue transglutaminase. If the results are positive, a duodenal biopsy may be performed to confirm the results. Samples from the duodenum of the small intestines are examined for signs of villous damage or other features in the lamina propria that would confirm the results. Moreover, a genetic test can also be used to confirm results or rule out the possibility of a celiac disease. An

individual who is negative for both HLA-DQ2 and HLA-DQ8 is a very poor candidate for celiac disease (Kochlar et al.).

There are several major challenges celiac disease patients have to manage besides adhering to a strict gluten-free diet. Celiac disease is associated with numerous other enteric malignancies and autoimmune diseases that can afflict multiple body organs and systems including endocrine, hepatic tissue, and the nervous system. The most common associated disease is dermatitis herpetiformis, characterized by the formation of itchy skin lesions and erythematous papules. Individuals affected with this disorder are also likely to ~~be predisposed to~~ carry HLA-DQ2/DQ8. Overall, patients can expect to endure more complications than those that result directly from celiac disease, such as diarrhea and abdominal pain (Kochlar et al.).

This paragraph could come earlier in the paper

The gluten-free diet has been the traditional method of treatment of celiac disease, and perhaps the most effective since patients experience symptom relief and reversal of intestinal tissue damage. Living in the western hemisphere where most cuisines have historically been high in gluten-containing foods, it can be a challenge for some patients to completely rule out gluten from dietary intake. However, keeping the patients well-informed about the seriousness of the disorder may boost their motivation to abstain from gluten. There has been research efforts aimed at developing effective treatment strategies, working at the molecular level, to block the binding sites of HLA-DQ2/DQ8 that would otherwise bind gluten peptides. The trick here is to design peptide analogs that bind HLA-DQ's with higher affinity than gluten-derived peptides (Juse et al.). From the previous discussion of the impact of an individual's intestinal microbiome and the role B. bifida bacteria play in reducing inflammation, one can ponder about the potential alternative treatment plans using microorganisms.

If the references are presented in this section in the order in which they occur in the text, then ~~text~~ they should be numbered here[8] and the in-text citation should be a number— [1], [2], etc. If you keep the in-text author citation format, then the references should be alphabetized here.

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10/10

Overall, this draft is quite complete and well-constructed. I think a few suggestions could improve the organization of the paper →

- It would make sense for some of the descriptions of the phenotypic and clinical manifestations of celiac disease to come at the beginning. Introduce the reader to the disease before you jump into the genetics.

- Some information is presented multiple places in the paper — think about where these facts fit best and cut out extra repetition from the paper

- ~~In a few places, the first sentence of a paragraph doesn't match the rest of the paragraph~~

██████████

5/1/2016

BIOS 412: Human Genetics

Celiac Disease Review Final Draft

Celiac disease belongs to the class of autoimmune disorders in which an immune

response is mediated upon the body's own healthy tissues, causing tissue damage and

inflammation. The genomic region that has been associated with several autoimmune diseases is

the human leukocyte antigen (HLA) or major histocompatibility (MHC) region on chromosome

6. Upon the ingestion of foods containing the gluten protein, patients with celiac disease trigger

an immune response that results in a range of enteropathies that are most commonly

characterized by mucosal damage and villous destruction. Traditionally, a gluten-free diet has

been the most effective treatment, but emerging research has presented innovative treatments

that work at the molecular level, by binding and blocking the binding site of the antigen-

presenting molecule HLA-DQ2 and/or HLA-DQ8 [1].

The immune system is extremely polymorphic; its genetic variation has been under

positive selection in order to maximize the number and types of proteins (MHC proteins and

others) that bind specific foreign molecules that might be pathogenic. As a result, fully

understanding the exact genetic components that contribute to an autoimmune disease is often

quite challenging. To further establish the complexity of an autoimmune disease, it is worth

mentioning that genes within the HLA region are packed into a small region amounting to only

about 2% of chromosome 6. And, as a result, a haplotype of this nature (where genes are within

close proximity to one another) tends to be clustered in genes that are inherited together as the

chance of the genes being separated by recombination is low [2].

There are several major challenges celiac disease patients have to manage besides adhering to a strict gluten-free diet. Celiac disease is associated with numerous other enteric malignancies and autoimmune diseases that can afflict multiple body organs and systems including endocrine, hepatic tissue, and the nervous system. The most common associated disease is dermatitis herpetiformis, characterized by the formation of itchy skin lesions and erythematous papules. Individuals affected with this disorder are also likely to carry HLA-DQ2/DQ8 alleles. Overall, patients can expect to endure more complications than those resulting directly from celiac disease, such as diarrhea and abdominal pain [3].

The oldest historical records describing celiac disease come from the Greek physician Aretaeus of Cappadocia between the 1st and 2nd centuries AD. The symptoms were reported, but the exact cause was not. It was not until the 19th century that British physician Samuel Gee would propose the idea that celiac disease is linked to diet, which was later confirmed in the 1950's by Dicke and colleagues who established that foods containing gluten, including wheat, barley, and rye, are the cause of symptoms [4].

Current consensus indicates that the worldwide prevalence of celiac disease is at about 1% of the general population, with higher incidences among females. However, some regions show increased prevalence, such as Western Sahara, while other countries like Burkina Foso have had no reported cases [1]. Celiac disease is a polygenic disorder that follows a complex, non-Mendelian inheritance pattern that is centered about the HLA region in which certain alleles associated with the disease show strong linkage disequilibrium, or nonrandom association [5]. In addition, recent findings confirmed the differential expression of certain genes in children patients as opposed to adult patients, suggesting an age effect [6].

There are several key molecular interactions that underlie the symptoms of celiac disease. The pivotal molecules are HLA-DQ2 and/or HLA-DQ8 antigens and T-cells, which interact directly to generate the destructive autoimmune response. HLA-DQ2/HLA-DQ8 heterodimers that are specific for gluten binding use a T cell repertoire (TCR) that binds gluten-derived peptide antigens and present them to T-cells which then secrete inflammatory cytokines in response. The unusual molecular structure of gluten proteins, which are nearly half composed of proline and glutamine residues, which makes them more difficult to digest in the intestinal tract, ultimately resulting in the formation of many small peptide fragments that are susceptible to chemical modification by tissue transglutaminase and subsequent HLA-DQ binding [7].

There are many candidate genes at multiple loci in several chromosomes in HLA and non-HLA regions that are suspected of contributing to the genetic risk of pathogenesis [1]. The allele that shows the strongest association with celiac disease is HLA-DQ2 [8]. Furthermore, a dosage effect has been observed. Children who inherit a copy of HLA-BQB1*02 (one of the alleles that encode for HLA-DQ2) from both parents were found to be at a higher risk of developing celiac disease than those who inherited the allele from only one parent [1].

It is important to note that the vast majority of people who carry the HLA-DQ2 or HLA-DQ8 predisposing alleles will not manifest celiac disease. It is only the majority of those who are already affected with celiac disease that have been found to carry the HLA-DQ2/DQ8 allele. This is a good indicator of other non-MHC allele factors contributing to pathogenesis, such as epigenetics and the environment (diet). Surprisingly, recent evidence uncovered by genome-wide association studies (GWAS) suggest that the vast majority of SNP's that are associated with disease occur in non-coding regions of the genome, including regulatory sequences (promoters, enhancers, etc…) and non-coding RNA (ncRNA) genes [1]. A special class of noncoding

RNA's, microRNA, was found to be differentially upregulated as revealed by biopsies of celiac disease patients and controls. Specifically, a miRNA known as miR-449 was found to be the most upregulated. That miRNA also happens to be the one that targets mRNA's that code for the translation of the NOTCH1 inflammation regulator. In concordance with the finding, NOTCH1 was discovered to be at lower concentrations in the small intestines of patients with celiac disease. Overall, however, not much data have yet been gathered in support of the role of ncRNA's in celiac disease [9].

Another epigenetic mechanism suspected to contribute to increased risk of celiac disease is allele specific methylation, which also occurs in noncoding sequences. Using microarray screening technologies, Hutchinson and others reported finding allele-specific methylation at several expression quantitative trait loci, one of which was the long non-coding RNA DLEU1 that has been associated with celiac disease [10].

In order to obtain a good understanding of the disease and its mechanism, the focus of discussion will shift to the actual site of action: epithelial cells of the small intestines. To start off, the CD4 T cells that are specific for gluten peptides bound to HLA-DQ2/DQ8 have only been detected in celiac disease patients [11]. Upon examination, T-cell receptor usage was found to be biased among patients as opposed to controls, and a key non-germline encoded arginine residue was discovered as part of the T-cell receptor structure and determined to be essential in the binding of T cell receptors to HLA-DQ-gluten peptide complex [12]. On its own, a gluten-derived peptide consisting mostly of glutamine and proline residues is actually weakly bound to HLA-DQ2/DQ8 heterodimers and therefore will only trigger a weak response from T cells. To induce high affinity, the action of tissue transglutaminase is needed to deamidate glutamine into glutamate, essentially converting an electrically neutral amino acid into a negatively-charged

amino acid. Upon tissue damage, transglutaminase enzyme is recruited and acts to immediately modify gluten peptides, causing greater binding affinity between HLA-DQ2/DQ8 and the gluten peptides, which ultimately leads to an elevated T cell response, increasing intestinal tissue damage. Fundamentally, the T-cell mediated response is not triggered on a full scale upon the encounter with the first gluten peptide, but rather in a snowball fashion [13].

Arguably, the most fascinating aspect about the molecular basis of the disease is the root of the problem itself: why do gluten-derived peptides cause an immune response to begin with? The answer lies in the pathogenic world. On the surface of small intestines, the extracellular matrix is a major barrier between our cells and all sorts of other foreign microbes. But a common theme in evolutionary biology is that host and parasites coevolve. In order to gain access to host tissues, a parasite will secrete proteases that break down the extracellular matrix of the host. As it turns out, like gluten, the extracellular matrix is unusually high in proline residues. Therefore, the presence of high levels of prolines can be interpreted by the immune system as an attempt by parasites to degrade the extracellular matrix and will respond accordingly [14].

Although the majority of the complexity of celiac disease is contributed to its polygenic and epigenetic nature, the environment remains a critical factor in the onset of disease. And since the primary site of action in the disease are the small intestines, the role of the patient's intestinal microbiome was interrogated. A study by Nistal el al., among other works, confirms the observation that fecal samples from celiac disease patients display different microbiota compositions than healthy individuals. They've also reported a significant difference in presence of *Bifidobacterium bifidum* between untreated celiac disease patients and healthy adults. A reduction in the diversity of *B. bifidum* was observed in treated celiac disease patients and healthy adults but not in untreated patients [15].

Other findings point to the possibility of certain genotypes influencing the composition of the microbiome. The most relevant genotype here, HLA-DQ, has been found to be in association with reduced *B. bifidum* [16]. This bacterial species is worthy of special and careful attention because of the anti-inflammatory effects it induces. Upon the encounter with a gluten-derived peptide bound to an HLA-DQ heterodimer, T cells produce pro-inflammatory cytokines such as TNF-α and IL-1β. It was discovered that *B. bifidum* induces an inhibitory effect on the secreted cytokines, and different strains of the bacterium correspond to varying inhibitory strength. The proline-rich structure of gluten proteins render them more difficult to degradation by the action of the body's own proteases, allowing the proline-rich peptides to reach the lamina propria of small intestines. Interestingly, the *B. bifidum* breaks down gluten peptides further by proteolysis during digestion such that the gluten-derived peptides that reach the intestinal epithelia are of different structural patterns than the other less degraded gluten peptides normally bound by HLA-DQ antigens. This ultimately leads to a reduced or inhibited pro-inflammatory secretions by T cells [17]. The fact that gut *B. bifidum* alleviates the automimmune inflammatory response could potentially shed some light on alternative treatment methods.

An accurate diagnostic tool is to measure the concentration of modified gluten antibodies in patient serum before and after the implementation of a gluten-free diet. If a significant drop in gluten-specific antibodies levels is observed after introducing a gluten-free diet as opposed to before, then one can conclude with significant certainty that the patient has celiac disease [7]. Another highly effective diagnostic method would be to perform serological tests on potentially affected individuals while they're on gluten-containing diet and measure the ratio of IgA antibodies to tissue transglutaminase. If the results are positive, a duodenal biopsy may be performed to confirm the results. Samples from the duodenum of the small intestines are

examined for signs of villous damage or other features in the lamina propria that would confirm the results. Moreover, a genetic test can also be used to confirm results or rule out the possibility of a celiac disease. An individual who is negative for both HLA-DQ2 and HLA-DQ8 is a very poor candidate for celiac disease [3].

The gluten-free diet has been the traditional method of treatment of celiac disease, and perhaps the most effective since patients experience symptom relief and reversal of intestinal tissue damage. Living in the western hemisphere where most cuisines have historically been high in gluten-containing foods, it can be a challenge for some patients to completely rule out gluten from dietary intake. However, keeping the patients well-informed about the seriousness of the disorder may boost their motivation to abstain from gluten. There has been research efforts aimed at developing effective treatment strategies, working at the molecular level, to block the binding sites of HLA-DQ2/DQ8 that would otherwise bind gluten peptides. The trick here is to design peptide analogs that bind HLA-DQ's with higher affinity than gluten-derived peptides [18]. Alternative and modern treatment strategies seem to be promising and theoretically effective.

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BIOS 412
Review Paper Rough Draft

Hutchinson-Gilford Progeria Syndrome

<u>Introduction</u>

[handwritten: Numbered references should be ordered based on first appearance in the paper — this should be [1,2,3,4]]

Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare premature aging disorder that begins to manifest within the first year or two of life. Individuals with HGPS exhibit characteristic phenotypes of aging, such as lipodystrophy, hair loss, skin discoloration or mottling, prominent vasculature, and a beaked nose [4,7,8,9]. Due to the rapid and early manifestation of these phenotypes, these individuals also have stunted growth in both height and weight, skeletal abnormalities of the cranium, mandible, joints, and fingers (tufting), and cardiovascular complications [4,7,8,9]. Specifically, these individuals often suffer from arteriosclerosis and atherosclerosis, generally resulting in death around age 13 on average due to heart attack or stroke [4,8,9].

HGPS affects 1 in 4 million live births each year [8]. It was first described in 1886 by Dr. Jonathan Hutchinson and in 1897 by Hastings Gilford, but it was not until 2003 that the genetic basis was discovered [6]. Studies found that in 90% of HGPS cases, the disorder is caused by a *de novo*, non-inherited autosomal dominant (C>T) point mutation [4,11]. *[handwritten: in what gene/genes?]* Due to this sporadic nature, no frequency differences between cultural populations have been observed or documented. Current research has been primarily focused on the cellular effects of progerin, how these effects lead to rapid phenotypic aging, and the utilization of this knowledge in developing potential treatments. The continuation of HGPS research is further advocated for due to the potential knowledge that can be gained about the normal aging process.

Genetic Component

The point mutation responsible for majority of HGPS cases is located on Chromosome 1 within exon 11 of the lamin A/C gene (LMNA) [4,8,9,11]. The normal function of this gene is to produce A and C type lamin proteins [4,7,11]. These, along with type B lamin proteins, are intermediate filaments that come together to form the nuclear lamina, a mesh-like protein scaffold underlying the nuclear membrane [8,11]. The nuclear lamina supports the overall structure of the nucleus and interacts with chromatin, membrane associated proteins, transcriptional regulators, and nuclear pore complexes [8,11]. Through these interactions, the nuclear lamina has been shown to play a role in chromosomal compartmentalization and various nuclear activities such as gene expression and the movement of molecules in and out of the nucleus [11].

Lamins A and C are formed from the same gene through alternative splicing and have nearly identical protein sequences, but differ in length [4,11]. In addition, prelamin A must go through several post-translational modification steps before becoming mature lamin A, whereas, lamin C does not require these additional processing steps [4, 7,11]. The C>T point mutation that causes HGPS is a synonymous mutation located in the third position of codon 608 (position 1842) of the LMNA gene [4,8,9,11]. Even though this mutation does not alter the amino acid sequence, it activates a cryptic splice donor site that interferes with normal splicing and results in a 150 nucleotide, 50 amino acid, deletion in prelamin A molecules [4,8,11]. Within the deleted region is a cleavage site, ZMPSTE24, necessary for the removal of the c-terminal farnesyl group and conversion prelamin A to mature lamin A [11]. As a result, these mutated prelamin A proteins, called progerin, retain the c-terminal farnesyl groups, which cause the progerin proteins to remain attached to the inner nuclear membrane disrupting nuclear structure [9,11]. As progerin accumulates, the nuclear structure becomes increasingly abnormal resulting in characteristic nuclear blebbing and instability [9,11].

Due to the important role that the nuclear lamina plays in overall nuclear stability, lamin genes are not just present in the genome of humans, but are also present in many other organisms, such as *Drosophila* and mice species. *Drosophila* have two different lamin genes, lamC that produces A type lamins and DmO that produces B type lamins [1]. However, the lamin A proteins produced in *drosophila* are not farnesylated like in humans. This prevents the use *drosophila* as model organisms for HGPS because the lamC gene cannot be mutated to produce progerin [1]. Despite these differences, genetic analysis has revealed that homologous lamin genes in *drosophila* and vertebrates actually came from the same ancestor, indicating a high level of conservation [1]. This conservation is further illustrated by the presence of LMNA genes in mice and primates.

Mice have been widely used as model organisms for HGPS because following induction of a similar C>T point mutation in the LMNA genes of mice, they begin to produce progerin and exhibit some of the same phenotypes as individuals with HGPS [11]. In fact, analysis of knock mice models revealed that HGPS disease phenotypes were not be caused by haploinsufficiency because even though these mice produced no lamin A, they did not have any nuclear abnormalities or other HGPS associated phenotypes [9]. Based on these results, it was determined that the HGPS mutation works instead in a dominant negative fashion since only one mutant allele is required for disease phenotypes and appears to out compete any sort of potential compensation by the normal allele [9]. However, these mice do not exhibit all of the HGPS phenotypes present in humans and cannot be used as complete models of this disease.

Effect on Cellular Function

LMNA is expressed in nearly all differentiated somatic cells through-out the body so any mutation in this gene can affect the function of many different cell types, especially bone cells and fibroblasts of skin, muscle, and vascular tissue [4,7,11]. In contrast, cells of the nervous system have

been found to express LMNA at substantially lower levels than other cells of the body [4,9,11]. As a result, the production of progerin in nerve cells is minimal, which may explain why HGPS individuals show no indication of neural deficits [4,9,11].

As previously mentioned, progerin causes abnormal nuclear structure because it remains attached to the inner nuclear membrane by the retained farnesyl group. This disruption to the nuclear structure is further intensified as progerin accumulates within the cell because progerin molecules come together to form insoluble cytoplasmic aggregates that bind to the nuclear membrane and cause the nuclear blebbing observed later on in the progression of the disease [2]. These progerin aggregates also interfere with proper cell division because lamin A is normally present in an unpolymerized state during mitosis [2]. As a result, this abnormal membrane morphogenesis can cause chromosome misalignment during mitosis and disrupt proper nuclear division, which can lead to improper chromosome separation and binucleation [2]. Mitosis was also observed to be further disturbed by chromosome lagging during anaphase in HGPS cells [2].

Normal lamin A is known to interact directly with chromatin at specific heterochromatic regions. These interactions promote proper chromosomal compartmentalization within the nucleus as well as play a potential role in gene expression [8]. In a study done by McCord, progerin was found to interact differently with chromatin than normal lamin A proteins causing an altered pattern of H3K27me3 at numerous chromatin locations [8]. This histone modification is a repressive mark associated with facultative heterochromatin commonly used in the regulation of gene expression [8]. Further analysis revealed that these disease-related epigenetic alterations were present at several promoter sites, resulting in deviations, both higher and lower, from normal levels of expression in corresponding genes [8]. These histone alterations were also shown to result in loss of normal chromosomal compartmentalization because they prevented normal overall association of chromatin with the nuclear

lamina [8]. The nuclear lamina is also believed to control the binding and release of lamin-associated proteins from the nuclear periphery, which most likely regulate the expression of developmentally regulated genes by either silencing or activating transcription [10]. Progerin was also shown to affect these protein-chromatin interactions altering gene expression [10]. McCord further concluded that these modifications to H3K27me3 occurred well before nuclear blebbing in HGPS cells indicating that these alterations arise very early on and may be a potential cause for the rapid phenotypic manifestation of this disease [8].

In a proteomic study, Rivera found dramatic differences in the level of certain proteins found in HGPS fibroblasts compared to control fibroblasts [11]. An upregulation of glycolytic proteins and a downregulation of proteins involved in oxidative phosphorylation, ATP synthase (complex V), and translational apparatus proteins, ribosomal proteins, was found in HGPS fibroblasts [11]. These proteomic alterations in HGPS cells indicate an evident switch from oxidative to glycolytic metabolism due to defective oxidative phosphorylation. This defect in cell metabolism is called mitochondrial dysfunction and is believed to contribute to the premature organ decline in HGPS individuals [11]. These same results were also found through proteomic analysis of both heterozygous and homozygous progeriod mouse models and ZMPTSTE24-/- mice models [11]. The ZMPTSTE24-/- mice do not produce progerin, but do lack the cleavage site necessary for the removal of the farnesyl group so are unable to convert prelamin A into mature lamin A. Using these mice, it was illustrated that build-up of prelamin A has similar effects on cellular metabolism as the build-up of progerin [11].

Upon analysis, Decker found that the overall telomere lengths in HGPS cells did not follow the same chromosome specific pattern observed in normal cells, suggesting a more random arrangement of chromatin in HGPS cells [4]. One such deviation discovered was that on average the telomeres were shorter in HGPS cells and at least one chromosome in every HGPS cell analyzed had telomeres shorter

than the threshold for detection, which was not observed in any of the control cells [4]. However, the chromosome in which these extremely short telomeres occurred was not consistent and varied between HGPS cells [4]. Since telomeres become shorter with each replication and cell division, telomere length is a critical factor in how many times a cell can divide. Therefore, it is believed that the overall shorter telomeres in HGPS cells lead to the premature senescence that cause arterial changes and eventual heart attack or stroke of HGPS individuals [4]. It is not yet clear why HGPS cells possess shorter telomeres, but it has been found through studies of the normal aging process that shorter telomeres may actually induce the production of progerin in normal cells later in life [7]. This gives insight into a potential mechanism behind the very rapid accumulation of progerin and subsequent phenotypic manifestation of HGPS.

Progerin was also found to negatively affect the multipotency and differentiation of mesenchymal stem cells (MSCs) by increasing the sensitivity of MSCs to hypoxia [12]. This causes an overall shortage of mesenchymal stem cells in HGPS patients, which are necessary for tissue replenishment, leading to an overall reduction in the amount of certain differentiated cell types [12]. This same affect was seen on vascular smooth muscle cells, which presented with increased DNA damage and nuclear abnormalities in HGPS cells [12]. These defects are, therefore, likely contributors to the serious cardiovascular complications associated with HGPS.

Clinical Aspects and Associated Diseases

This disease is part of a group of 12 diseases known as laminopathies, each one resulting from a different mutation in the LMNA gene that affects the proper processing of prelamin A [7,11]. Despite this commonality, these diseases all present with a distinct set of clinical phenotypes associated with various tissues including myopathy, cardiomyopathy, lipodystrophy, neuropathy, and premature aging [7]. HGPS is generally diagnosed prior to two years of life based on stunted growth, lipodystrophy, and

overall failure to thrive [8,9]. These initial signs are rapidly followed by the onset of other characteristic phenotypes associated with aging [9]. Genetic testing for the associated LMNA mutation can then be performed to confirm the visual diagnosis.

HGPS exhibits complete penetrance. Due to the dominant negative affect of this mutation, the presence of only one mutated allele is necessary to cause HGPS and will always result in HGPS [9]. Potentially due to the sporadic origin of this mutation, most individuals with HGPS are heterozygous for the mutated allele [11]. This makes comparison of phenotypic severity between heterozygote and homozygote individuals extremely difficult to achieve. However, using both heterozygous and homozygous progeriod mouse models, Rivera was able to reveal that the effects of progerin, with regard to mitochondrial dysfunction, is both dose and time dependent [11]. Phenotypic comparison between heterozygous and homozygous progeriod mice revealed that homozygous mice exhibited disease phenotypes at an earlier age and with increased severity than heterozygous mice [11]. Younger heterozygous mice, 10 weeks old, also showed less severe phenotypes and mitochondrial dysfunction than older heterozygous mice, 32 weeks old [11]. Together these results show how the amount of progerin affects the phenotypic severity of the disease.

Progerin has been found in cells of individuals of advanced ages and therefore believed to play a role in the normal aging process [7]. In a study done by McClintock analyzing the aging process of normal skin cells compared to HGPS cells, progerin was not only found in normal skin cells, but also determined to increase in an age-dependent manner just as in HGPS cells [7]. However, the level of progerin was still substantially lower in normal cells of even the most advanced ages compared to the level of progerin in HGPS cells [7]. Although, the effects may not be as drastic in normal cells as they are in HGPS cells, studies regarding HGPS have given incredible insight into the process of aging. Telomeres have been shown to shorten over the lifetime of an individual and act as a sort of molecular clock

controlling the lifespan of cells and subsequently of individuals as a whole [4]. As previously mentioned,

it is believed that the shortening of telomeres due to replicative damage and cellular division may

actually cause the activation of the cryptic splice site in certain cells leading to the production of

progerin in normal cells at advanced ages [4,7,11]. This progerin production and accumulation would in

turn cause the same cellular affects observed in HGPS cells to occur in normal cells, thereby serving as a

potential explanation for the increased incidence of nuclear abnormalities, mitochondrial dysfunction,

and reduction in transcription/DNA repair seen in the normal cells of older individuals [7,11].

Health Issues and Treatments

The prognosis for individuals with HGPS is very bleak, with a 100% fatality rate [9]. As of now,

there is no cure for this disease and most of the treatments are geared towards pacifying the age

associated ailments caused by this disease. Studies have shown that HGPS individuals experience drop

out of smooth vascular muscle cells, reduced elasticity and thickening of arterial walls, and reduced

vascular compliance resulting in high blood pressure [9]. As a result, a great deal of emphasis is placed

on treating these cardiovascular complications, however, almost all individuals eventually die during

their adolescent years from heart attack or stroke [9]. Growth hormones are also administered in

attempt at increasing the overall height and weight of these individuals [9]. This treatment has proven

to be successful to some extent, but a large gap in overall growth still remains in comparison to healthy

children of the same developmental age [9]. Other ailments commonly exhibited by individuals with

HGPS include hearing loss, hyperopia, and joint abnormalities. These individuals remain surprisingly

mobile, however, despite joint abnormalities and cardiovascular issues, with majority testing within the

average mobility range for their age [9].

There are several treatments currently undergoing clinical trials aimed at combating different

molecular components of the disease. One such treatment focuses on the use farnesyl transferase

inhibitors, which inhibit the addition of the farnesyl group to prelamin A molecules during the early post-translational modification steps [9]. By inhibiting the addition of the farnesyl group, the mutated prelamin A proteins will no longer remain permanently attached to the inner membrane restoring nuclear structure [9]. Clinical trials for this type of drug showed increase weight gain and fat tissue, improved vascular stiffness, and bone mineralization resulting in reduced fracture rate [5,9]. The administration of this drug also extended the lifespan of individuals on average by about 1.6 to 2.4 years [5]. However, these outcomes only occurred in some treated individuals, but not all [5]. Another treatment undergoing clinical trials is the use of isoprenylation inhibitors. In mice models, it was shown that preventing progerin isoprenylation increased ATP synthesis and reduced overall mitochondrial dysfunction [11]. The potential use of isoprenylation inhibitors in combination with farnesyl transferase inhibitors has also been proposed and shown to enhance the beneficial outcomes of farnesyl transferase inhibitors in early trial stages [11]. However, the use of isoprenylation inhibitors individually and in combination with farnesyl transferase inhibitors is still under evaluation.

Rapamycin is another proposed drug currently undergoing clinical trials and evaluation. The major target of rapamycin in the body is a central regulator of cell growth and various other cell process known as the mammalian target of rapamycin (mTOR) [3]. However, rapamycin has also shown to induce macroautophagy, which is the normal process used by cells to get rid of damaged or unnecessary components through lysosomal degradation [3]. Rapamycin has also been shown to increase the solubility of progerin that has formed cytoplasmic aggregates [3]. By promoting autophagy and dissolution of progerin aggregates, rapamycin has proven successful in decreasing the amount of progerin accumulation in HGPS cells. This increase in progerin turn over has allowed for improvement and even reversal of nuclear blebbing, restored H3K27me3 modifications, and extended the cellular lifespan by an average of 30 days thereby successfully postponing cellular senescence [3].

In addition to the treatments currently undergoing evaluation, there are several more prospective treatments not yet ready for actual application or clinical trials due to an increased level complexity [6]. These treatments include RNA, gene, and cell replacement therapies. RNA therapy would involve the removal or inhibition of the alternative splice site activated by the mutation whereas targeted gene therapy would involve the actual repair of the mutated gene outside of the body followed by its reintroduction into the body [6]. Cell replacement therapies would focus primarily on the use of matched normal cells or stem cells from HGPS-iPS cell that have been genetically corrected [6]. Although different in methodology, all present steep obstacles that will take a great deal of effort and time to overcome prior to actual application. However, these treatments may hold the key to more a permanent or even complete remedy to HGPS.

Very nice work- fix the citations/references #'s and the few small edits and this is ready to go.

10/10

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BIOS 412
Review Paper

Hutchinson-Gilford Progeria Syndrome

Introduction

Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare premature aging disorder that begins to manifest within the first year or two of life. Individuals with HGPS exhibit characteristic phenotypes of aging, such as lipodystrophy, hair loss, skin discoloration or mottling, prominent vasculature, and a beaked nose [1,2,3,4]. Due to the rapid and early manifestation of these phenotypes, these individuals also have stunted growth in both height and weight, skeletal abnormalities of the cranium, mandible, joints, and fingers (tufting), and cardiovascular complications [1,2,3,4]. Specifically, these individuals often suffer from arteriosclerosis and atherosclerosis, generally resulting in death around age 13 on average due to heart attack or stroke [1,3,4].

HGPS affects 1 in 4 million live births each year [3]. It was first described in 1886 by Dr. Jonathan Hutchinson and in 1897 by Hastings Gilford, but it was not until 2003 that the genetic basis was discovered [5]. Studies found that in 90% of HGPS cases, the disorder is caused by a *de novo*, non-inherited autosomal dominant point mutation in the lamin A/C gene [1,6]. Due to this sporadic nature, no frequency differences between cultural populations have been observed or documented. Current research has been primarily focused on the cellular effects of this mutation, how these effects lead to rapid phenotypic aging, and the utilization of this knowledge in developing potential treatments. The continuation of HGPS research is further advocated for due to the potential knowledge that can be gained about the normal aging process.

Genetic Component

The lamin A/C gene (LMNA) is located on chromosome 1 and normally functions to produce A and C type lamin proteins [1,2,6]. These, along with type B lamin proteins, are intermediate filaments that come together to form the nuclear lamina, a mesh-like protein scaffold underlying the nuclear membrane [3,6]. The nuclear lamina supports the overall structure of the nucleus and interacts with chromatin, membrane associated proteins, transcriptional regulators, and nuclear pore complexes [3,6]. Through these interactions, the nuclear lamina has been shown to play a role in chromosomal compartmentalization and various nuclear activities such as gene expression and the movement of molecules in and out of the nucleus [6]. Lamins A and C are both formed from LMNA through alternative splicing and have nearly identical protein sequences, but differ in length [1,6]. In addition, prelamin A must go through several post-translational modification steps before becoming mature lamin A, whereas, lamin C does not require these additional processing steps [1, 2,6].

The C>T point mutation that causes the majority of HGPS cases is a synonymous mutation located in the third position of codon 608 (position 1842) within exon 11 of the LMNA gene [1,3,4,6]. Even though this mutation does not alter the amino acid sequence, it activates a cryptic splice donor site that interferes with normal splicing and results in a 150 nucleotide, 50 amino acid, deletion in prelamin A molecules [1,3,6]. Within the deleted region is a cleavage site, ZMPSTE24, necessary for the removal of the c-terminal farnesyl group and conversion of prelamin A to mature lamin A [6]. As a result, these mutated prelamin A proteins, called progerin, retain the c-terminal farnesyl groups, which cause the progerin proteins to remain attached to the inner nuclear membrane disrupting nuclear structure [4,6]. As progerin accumulates, the nuclear structure becomes increasingly abnormal resulting in characteristic nuclear blebbing and instability [4,6].

Due to the important role that the nuclear lamina plays in overall nuclear stability, lamin genes are not just present in the genome of humans, but are also present in many other organisms, such as *Drosophila* and mice species. *Drosophila* have two different lamin genes, lamC that produces A type lamins and DmO that produces B type lamins [7]. However, the lamin A proteins produced in *Drosophila* are not farnesylated like in humans. This prevents the use *Drosophila* as model organisms for HGPS because the lamC gene cannot be mutated to produce progerin [7]. Despite these differences, genetic analysis has revealed that homologous lamin genes in *Drosophila* and vertebrates actually came from a common ancestor, indicating a high level of conservation [7]. This conservation is further illustrated by the presence of LMNA genes in mice and primates.

Mice have been widely used as model organisms for HGPS because following induction of a similar C>T point mutation in the LMNA genes of mice, they begin to produce progerin and exhibit some of the same phenotypes as individuals with HGPS [6]. In fact, analysis of knock-out mice models revealed that HGPS disease phenotypes are not caused by haploinsufficiency because even though these mice produced no lamin A, they did not have any nuclear abnormalities or other HGPS associated phenotypes [4]. Based on these results, it was determined that the HGPS mutation works instead in a dominant negative fashion since only one mutant allele is required for disease phenotypes and appears to out compete or inhibit any sort of potential compensation by the normal allele [4]. However, these mice do not exhibit all of the HGPS phenotypes present in humans and cannot be used as complete models of this disease.

Effect on Cellular Function

LMNA is expressed in nearly all differentiated somatic cells through-out the body so any mutation in this gene can affect the function of many different cell types, especially bone cells and fibroblasts of skin, muscle, and vascular tissue [1,2,6]. In contrast, cells of the nervous system have been

found to express LMNA at substantially lower levels than other cells of the body [1,4,6]. As a result, the production of progerin in nerve cells is minimal, which may explain why HGPS individuals show no indication of neural deficits [1,4,6].

As previously mentioned, progerin causes abnormal nuclear structure because it remains farnesylated and subsequently attached to the inner nuclear membrane. This disruption to the nuclear structure is further intensified as progerin accumulates within the cell because progerin molecules come together to form insoluble cytoplasmic aggregates that bind to the nuclear membrane and cause the nuclear blebbing observed later on in the progression of the disease [8]. These progerin aggregates also interfere with proper cell division because lamin A is normally present in an unpolymerized state during mitosis [8]. As a result, this abnormal membrane morphogenesis can cause chromosome misalignment during mitosis as well as disrupt proper nuclear division, which can lead to improper chromosome separation and binucleation [8]. Mitosis was also observed to be further disturbed by chromosome lagging during anaphase in HGPS cells [8].

Normal lamin A is known to interact directly with chromatin at specific heterochromatic regions. These interactions promote proper chromosomal compartmentalization within the nucleus as well as play a potential role in gene expression [3]. In a study done by McCord, progerin was found to interact differently with chromatin than normal lamin A proteins causing an altered pattern of H3K27me3 at numerous chromatin locations [3]. This histone modification is a repressive mark associated with facultative heterochromatin commonly used in the regulation of gene expression [3]. Further analysis revealed that these disease-related epigenetic alterations were present at several promoter sites, resulting in deviations, both higher and lower, from normal levels of expression in corresponding genes [3]. These histone alterations were also shown to result in loss of normal chromosomal compartmentalization because they prevented normal overall association of chromatin with the nuclear

lamina [3]. The nuclear lamina is also believed to control the binding and release of lamin-associated proteins from the nuclear periphery, which most likely regulate the expression of developmentally regulated genes by either silencing or activating transcription [9]. Progerin was also shown to affect these protein-chromatin interactions altering gene expression [9]. McCord further concluded that these modifications to H3K27me3 occurred well before nuclear blebbing in HGPS cells indicating that these alterations arise very early on and may be a potential cause for the rapid phenotypic manifestation of this disease [3].

In a proteomic study, Rivera found dramatic differences in the level of certain proteins found in HGPS fibroblasts compared to control fibroblasts [6]. An upregulation of glycolytic proteins and a downregulation of proteins involved in oxidative phosphorylation, ATP synthase (complex V), and translational apparatus proteins, ribosomal proteins, was found in HGPS fibroblasts [6]. These proteomic alterations in HGPS cells indicate an evident switch from oxidative to glycolytic metabolism due to defective oxidative phosphorylation. This defect in cell metabolism is called mitochondrial dysfunction and is believed to contribute to the premature organ decline in HGPS individuals [6]. These same results were also found through proteomic analysis of both heterozygous and homozygous progeriod mouse models and ZMPTSTE24-/- mice models [6]. The ZMPTSTE24-/- mice do not produce progerin, but do lack the cleavage site necessary for the removal of the farnesyl group so are unable to convert prelamin A into mature lamin A. Using these mice, it was illustrated that build-up of prelamin A has similar effects on cellular metabolism as the build-up of progerin [6].

Upon analysis, Decker found that the overall telomere lengths in HGPS cells did not follow the same chromosome specific pattern observed in normal cells, suggesting a more random arrangement of chromatin in HGPS cells [1]. One such deviation discovered was that on average the telomeres were shorter in HGPS cells and at least one chromosome in every HGPS cell analyzed had telomeres shorter

than the threshold for detection, which was not observed in any of the control cells [1]. However, the chromosome in which these extremely short telomeres occurred was not consistent and varied between HGPS cells [1]. Since telomeres become shorter with each replication and cell division, telomere length is a critical factor in how many times a cell can divide. Therefore, it is believed that the overall shorter telomeres in HGPS cells lead to the premature senescence that cause arterial changes and eventual heart attack or stroke of HGPS individuals [1]. It is not yet clear why HGPS cells possess shorter telomeres, but it has been found through studies of the normal aging process that shorter telomeres may actually induce the production of progerin in normal cells later in life [2]. This gives insight into a potential mechanism behind the very rapid accumulation of progerin and subsequent phenotypic manifestation of HGPS.

Progerin was also found to negatively affect the multipotency and differentiation of mesenchymal stem cells (MSCs) by increasing the sensitivity of MSCs to hypoxia [10]. This causes an overall shortage of mesenchymal stem cells in HGPS patients, which are necessary for tissue replenishment, leading to an overall reduction in the amount of certain differentiated cell types [10]. This same affect was seen on vascular smooth muscle cells, which presented with increased DNA damage and nuclear abnormalities in HGPS cells [10]. These defects are, therefore, likely contributors to the serious cardiovascular complications associated with HGPS.

Clinical Aspects and Associated Diseases

This disease is part of a group of 12 diseases known as laminopathies, each one resulting from a different mutation in the LMNA gene that affects the proper processing of prelamin A [2,6]. Despite this commonality, these diseases all present with a distinct set of clinical phenotypes associated with various tissues including myopathy, cardiomyopathy, lipodystrophy, neuropathy, and premature aging [2]. HGPS is generally diagnosed prior to two years of life based on stunted growth, lipodystrophy, and overall

failure to thrive [3,4]. These initial signs are rapidly followed by the onset of other characteristic phenotypes associated with aging [4]. Genetic testing for the associated LMNA mutation can then be performed to confirm the visual diagnosis.

HGPS exhibits complete penetrance. Due to the dominant negative affect of this mutation, the presence of only one mutated allele is necessary to cause HGPS and will always result in HGPS [4]. Potentially due to the sporadic origin of this mutation, most individuals with HGPS are heterozygous for the mutated allele [6]. This makes comparison of phenotypic severity between heterozygous and homozygous individuals extremely difficult to achieve. However, using both heterozygous and homozygous progeriod mouse models, Rivera was able to reveal that the effects of progerin, with regard to mitochondrial dysfunction, is both dose and time dependent [6]. Phenotypic comparison between heterozygous and homozygous progeriod mice revealed that homozygous mice exhibited disease phenotypes at an earlier age and with increased severity than heterozygous mice [6]. Younger heterozygous mice, 10 weeks old, also showed less severe phenotypes and mitochondrial dysfunction than older heterozygous mice, 32 weeks old [6]. Together these results show how the amount of progerin affects the phenotypic severity of the disease.

Progerin has been found in cells of individuals of advanced ages and therefore is believed to play a role in the normal aging process [2]. In a study done by McClintock analyzing the aging process of normal skin cells compared to HGPS cells, progerin was not only found in normal skin cells, but also determined to increase in an age-dependent manner just as in HGPS cells [2]. However, the level of progerin was still substantially lower in normal cells of even the most advanced ages compared to the level of progerin in HGPS cells [2]. Although, the effects may not be as drastic in normal cells as they are in HGPS cells, studies regarding HGPS have given incredible insight into the process of aging. Telomeres have been shown to shorten over the lifetime of an individual and act as a sort of molecular clock

controlling the lifespan of cells and of individuals as a whole [1]. As previously mentioned, it is believed that the shortening of telomeres due to replicative damage and cellular division may actually cause the activation of the cryptic splice site in certain cells leading to the production of progerin in normal cells at advanced ages [1,2,6]. This progerin production and accumulation would in turn cause the same cellular affects observed in HGPS cells to occur in normal cells, thereby serving as a potential explanation for the increased incidence of nuclear abnormalities, mitochondrial dysfunction, and reduction in transcription/DNA repair seen in the normal cells of older individuals [2,6].

Health Issues and Treatments

The prognosis for individuals with HGPS is very bleak, with a 100% fatality rate [4]. As of now, there is no cure for this disease and most of the treatments are geared towards pacifying the age associated ailments caused by this disease. Studies have shown that HGPS individuals experience drop out of smooth vascular muscle cells, reduced elasticity and thickening of arterial walls, and reduced vascular compliance resulting in high blood pressure [4]. As a result, a great deal of emphasis is placed on treating these cardiovascular complications, however, almost all individuals eventually die during their adolescent years from heart attack or stroke [4]. Growth hormones are also administered in attempt at increasing the overall height and weight of these individuals [4]. This treatment has proven to be successful to some extent, but a large gap in overall growth still remains in comparison to healthy children of the same developmental age [4]. Other ailments commonly exhibited by individuals with HGPS include hearing loss, hyperopia, and joint abnormalities. These individuals remain surprisingly mobile, however, despite joint abnormalities and cardiovascular issues, with majority testing within the average mobility range for their age [4].

There are several treatments currently undergoing clinical trials aimed at combating different molecular components of the disease. One such treatment focuses on the use farnesyl transferase

inhibitors, which inhibit the addition of the farnesyl group to prelamin A molecules during the early post-translational modification steps [4]. By inhibiting the addition of the farnesyl group, the mutated prelamin A proteins will no longer remain permanently attached to the inner membrane allowing for the restoration of nuclear structure [4]. Clinical trials for this type of drug showed increase weight gain and fat tissue, improved vascular stiffness, and bone mineralization resulting in reduced fracture rate [4,11]. The administration of this drug also extended the lifespan of individuals on average by about 1.6 to 2.4 years [11]. However, these outcomes only occurred in some treated individuals, but not all [11]. Another treatment undergoing clinical trials is the use of isoprenylation inhibitors. In mice models, it was shown that preventing progerin isoprenylation increased ATP synthesis and reduced overall mitochondrial dysfunction [6]. The potential use of isoprenylation inhibitors in combination with farnesyl transferase inhibitors has also been proposed and shown to enhance the beneficial outcomes of farnesyl transferase inhibitors in early trial stages [6]. However, the use of isoprenylation inhibitors individually and in combination with farnesyl transferase inhibitors is still under evaluation.

Rapamycin is another proposed drug currently undergoing clinical trials and evaluation. The major target of rapamycin in the body is a central regulator of cell growth and various other cell processes known as the mammalian target of rapamycin (mTOR) [12]. However, rapamycin has also shown to induce macroautophagy, which is the normal process used by cells to get rid of damaged or unnecessary components through lysosomal degradation [12]. Rapamycin has also been shown to increase the solubility of progerin that has formed cytoplasmic aggregates [12]. By promoting autophagy and dissolution of progerin aggregates, rapamycin has proven successful in decreasing the amount of progerin accumulation in HGPS cells. This increase in progerin turn over has allowed for improvement and even reversal of nuclear blebbing, restored H3K27me3 modifications, and extended the cellular lifespan by an average of 30 days thereby successfully postponing cellular senescence [12].

In addition to the treatments currently undergoing evaluation, there are several more prospective treatments not yet ready for actual application or clinical trials due to an increased level complexity [5]. These treatments include RNA, gene, and cell replacement therapies. RNA therapy would involve the removal or inhibition of the alternative splice site activated by the mutation whereas targeted gene therapy would involve the actual repair of the mutated gene outside of the body followed by its reintroduction into the body [5]. Cell replacement therapies would focus primarily on the use of matched normal cells or stem cells from HGPS-iPS cell that have been genetically corrected [5]. Although different in methodology, all present steep obstacles that will take a great deal of effort and time to overcome prior to actual application. However, these treatments may hold the key to a more permanent or even complete remedy to HGPS.

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Human Genetics
Synthesis Paper
Topic: Lesch-Nyhan Disease

Lesch-Nyhan Syndrome (LNS) was not an officially diagnosed disease until 1964 in the United States of America when two physicians, Lesch and Nyhan, recognized a pattern of abnormal neurological function, self-mutilating behavior, and exceptionally high levels of uric acid in the urine of two young brothers (Lesch & Nyhan, 1964). It is not surprising that the recognition of LNS did not come until the 1960s. The disease is an "Orphan Disease", meaning it is exceptionally rare. For example, in the United Kingdom, a country with fifty-six million people, there is an average of only four new cases per year (McCarthy, 2006). Overall, one out of eight hundred thousand live births will be affected by Lesch-Nyhan (Hall, 2001). In none of the research was it mentioned any specific population or geographical region being more prone to or having higher incidence rates of LND. This could be due to the large percentage of mutations leading to LND being de novo mutations instead of mutations being passed though families as will be discussed later in this paper.

Soon after Dr. Lesch and Dr. Nyhan published their work describing the syndrome, more cases were reported and studied. From the fifth family to be studied with a history of the syndrome, the largest family being studied in that time period, a pedigree was created. Combining the information from the five families, a team at the University of Virginia School of Medicine was able to deduce that the inheritance pattern in LNS was X-linked recessive (Shapiro, 1966). This was determined as only boys were affected and the ratio matched what was predicted, one in four boys affected from unaffected parents. Females thought to be carriers due to having an affected son showed slightly higher levels of uric acid in urine (Shapiro, 1966); this

supports the hypothesis of X-linked transmission as the phenotype of heterozygotes is slightly altered.

Male to male dominant transmission as a pattern was eliminated as a possible hypothesis because of the evidence of female carriers having differing levels of uric acid than average and because most boys with LNS at the time of this research were institutionalized very early in childhood or died in infancy (Shapiro, 1966), leaving them unable to pass on genetic information. Male to male dominant transmission could also be eliminated as a potential hypothesis due to the presence of rare affected females. In one such case, an affected female had a monozygotic twin who was unaffected by the disease. It was able to be determined through fibroblast study that in the affected twin, the wild type allele has been silenced throughout the body while in the unaffected twin the mutant allele had undergone the X-inactivation (Gregorio, 2005).

With the mode of inheritance determined through pedigree analysis and confirmed later with the study of affected females, scientists began to search for the cause of this disease. Due to the excessive amounts of uric acid produced by patients manifesting symptoms of LNS, it was assumed by a team headed by J. Edwin Seegmiller that the disease must be caused by an enzyme involved in purine metabolism losing its function (Seegmiller, 1967). The enzyme responsible was indeed involved in purine metabolism: hypoxanthine-guanine phosphoribosyltransferase (Seegmiller, 1967).

We know today that the gene that codes for this enzyme is quite large, forty-four kb, and contains nine exons in humans (Lapucci, 2006). Because LNS is caused by a single gene mutation, an affected individual does not show a karyotype different than an unaffected individual. This gene leading to LNS when mutated is called the HPRT gene, and it is located on

the long arm of the X chromosome at Xq26 (Torres, 2012). In the year 2003, over two hundred mutations had been observed within the HPRT gene leading to the symptoms of LNS, and it was found that thirty percent of all mutations in the HPRT gene are de novo (Ohdoi, 2002). In 2015, twenty-seven new mutations at the HPRT locus were identified in a single study, bringing the total of known HPRT mutations leading to LNS to over four hundred (Ceballos-Picot, 2015), which further emphasizes how this region of the X chromosome is prone to new mutations or, if it is mutating at the same rate as the rest of the genome, the mutations must be occurring in areas where the affects produce a noticeable phenotype.

When the mutation at the HPRT gene is large scale, such as the deletion or a frame shift, the phenotype associated with LNS will always be fully displayed in males who only possess the mutant copy or in females who have the wild-type copy inactivated. Some of these large scale mutations can be caused by incorrect splicing regulation as was the case in the twin study discussed previously (Gregorio, 2005). The phenotype is always displayed because these mutations lead to the production of hypoxanthine-guanine phosphoribosyltransferase enzymes that have completely lost their function (Ceballos-Picot, 2015). Without a functional hypoxanthine-guanine phosphoribosyltransferase enzyme, the patient is not able to properly metabolize purines, leading to the build up of uric acid (Lapucci, 2006). This build up of uric acid was a part of the original observations by Lesch and Nyhan which also included "hyperuricema, mental retardation, choreoathetosis and self-destructive biting" (Lesch & Nyhan, 1964). Other phenotypes now associated with LNS are severe muscular dystonia and any form of self-mutilation in addition to biting (Gregorio, 2005).

Clinically, it can be difficult to diagnose the forms of self-mutilation in LNS compared to self mutilation that occurs with other neurological abnormalities such as autism. An important

study was done to shed light on this aspect of LNS. The study, preformed by a team led by Scott Hall in 2001, showed that in in patients with LNS the self-mutilation is almost exclusively sudden and violent, even initially when the behavior is first occurring. In patients with autism, however, the self-mutilating behaviors unusally increase gradually (Hall, 2001). It was also found in this study that episodes of self-mutilation correlated with the child being left alone without interaction with a caretaker (Hall, 2001), which the authors believe could be a sign that some of the LNS phenotype may be tied to environmental factors.

In some individuals that exhibit the phenotype for LNS there is no mutation within the HPRT gene. Using Real time PCR it was discovered that there was a mutation in the regulatory sequence, and this mutation prevented the HPRT gene from being transcribed (Torres, 2012) . After testing both parents, the mother was determined to be a carrier for this regulatory mutation (Torres, 2012). Studies such as this further confirm that LND is is transmitted though X-linked inheritance.

It is likely that this wide array of phenotypes affecting the muscular, renal, and neurological functions of the body occur when the HPRT gene is no longer coding for a working hypoxanthine-guanine phosphoribosyltransferase enzyme because purines are essential in all tissues of the body and not limited to one specific cell or tissue type (McCarthy, 2006). For example, patients with LND have been shown to have significant decreases in their white and gray brain matter (Schretlen, 2013). Regions especially affected where the basal ganglia, frototemporal, and the limbic system. These decreases in brain matter could imply that deficient purine metabolism leading to the buildup of incorrect cellular products could affect brain growth and development, especially in the regions previously mentioned (Schretlen, 2013).

In Individuals who have a mutation in the HPRT gene that does not lead to complete loss of hypoxanthine-guanine phosphoribosyltransferase enzyme function show a less severe phenotype known as Lesch-Nyhan Variants (Ceballos-Picot, 2015). These mutations cause a phenotype that has lower levels of cognitive impairment and no self-mutilating behaviors. Because these phenotypes belong to a different disorder than LNS, it can be concluded that the phenotype range for LNS is very small and without all of the symptoms, an individual does not actually have LNS but instead a less severe variant disorder.

Currently, there is no cure for the phenotypes associated with LNS. Instead, efforts are made by caregivers to manage physical and behavioral symptoms according to lesch-nyhan,org. One of main symptoms that leads to a diagnosis of LND, the overproduction of uric acid, can be managed with the use of a drug called allopurinol (Torres P. P., 2007). Allopurinol inhibits the enzyme xanthine oxidase to prevent the body from synthesizing excessive amounts of uric acid. The study preformed by Torres, Prior, and Puig showed that even after long term use of allopurinol, patients did not have any adverse side effects and the drug maintained its effectiveness. It was found that with the use of this drug, patients were able to consistently have seventy-four percent less uric acid excretion compared to their baseline levels. Managing the uric acid levels in patients and returning them closer to the normal range does not lessen the other symptoms of the disease such as self-mutilation, however (Hall, 2001).

Currently, behavioral therapy is the only method that can be used in an attempt to control the self mutilating behavior found in LNS, according to lesch-nyhan.com. Pharmaceuticals, such as s-adenosylmethionine, have been shown to provide little or even worsened behavior in LND patients (Dolcetta, 2013). In another study, 5-hydroxytryptophan in combination with a peripheral decarboxylase inhibitor carbidopa and imipramine was used in an attempt to control

the self-mutilating behavior is LNS (Nyhan, 1980). This treatment appeared to be successful initially, but after three months each of the patients became immune to the pharmacological effects, rending the treatment ineffective.

When behavioral therapy is used, it is important that it does not involve negative punishment. Studies have shown that when negative punishment, such as an electric shock, follow the self-mutilating behavior, the frequency of the self-mutilating behavior increases (Zilli, 2008). The authors of the study suggest that early in the progression of LNS, individuals accidently injure themselves due to the lack of motor coordination associated with muscular degeneration. After the accidently injury, improper dopamine signals in the basal ganglia reinforces these behaviors, leading to individuals with LNS to begin to regularly self-mutilate (Zilli, 2008). Therefore, when a negative punishment is applied following self-mutilation, it acts as a reinforcement of the behavior because the improper dopamine signals are further stimulated.

In a review article published in 2000, restraint, in the form of splits, wheelchairs, and keeping the patient from any sharp objects was the most common form of behavioral modification (Olson, 2000). In fact, the removal of these restraints often lead to noticeable distress in patients with LNS and increased levels of self-mutilating behavior. Treatments including extinction, systematic desensitization, and play therapy used in conjunction were shown to decrease the self-injurious behavior in several separate studies to the point where the child no longer needed to be restrained (Olson, 2000). Hall found in his research that positive and continued interactions with caregivers could reduce the frequency of self-mutilating behaviors (Hall, 2001). Even though there are some behavioral treatments shown to be effective, many children still have all of their teeth extracted to prevent lip biting, cheek biting, and finger biting (Hall, 2001).

There was a significant study published in 2015 by a team lead by Dr. Piedimonte that shows potential to permanently treat the self-mutilating behavior associated with LNS. In this case study, the team of doctors applied deep brain stimulation consistently to a twenty-nine-year-old patient via a bilateral electrode implant. The patient showed progressive improvement and soon did not need his hands tied to prevent self-mutilation. Caretakers also reported a better overall mood and willingness to help with his own needs. Five years later, there were no adverse side affects and the self-mutilation behavior had been totally eliminated in the patient (Piedimonte, 2015).

Due to the severe neurological impairments, self-mutilating behaviors, and stress on the renal system due to the high levels of uric acid being excreted, it is not surprising that individuals with LNS do not have a normal life expectancy; most individuals only survive into the second or third decade of their life, and this lifespan length is only possible with diligent treatment and therapy by the patient's family (Nyhan O. J., 2014).

Because of the severe phenotypes, burden of care on family members, and short life expectancy with low qualify of life by people suffering from LNS, many families with a history of LNS undergo genetic testing in an attempt to prevent having children with this disease. Initially, embryos with LNS were identified during pregnancy by culturing amniotic fluids (Halley, 1977). The amniotic fluids were cultured on a coverslip and the autoradiographic analysis was available a week later. By observing the karyotypes and the autoradiograohic analysis, a unaffected male, affected male, and unaffected female were all correctly identified (Halley, 1977). However, the amniotic fluids were not received until the mothers were in approximately their twentieth weeks of pregnancy, and then it took almost two weeks to get the

results back. This led the mother with the affected male fetus to have an abortion at twenty-two

weeks (Halley, 1977).

Today, there is technology available to completely avoid a pregnancy with a fetus

affected by LND instead of terminating an affected pregnancy, a practice that is much more

ethically accepted in current culture. This is done using in vitro fertilization (IVF). In a study

published in 1999, researchers were able to create blastocysts using the egg and sperm of parents

who already had one affected son using standard IVF procedure (Ray, 1999). After the

blastocysts had been dividing for three days, the healthiest were chosen to be genetically tested.

This genetic test was a single cell PCR amplification of DNA from a biopsied cell from the

healthy blastocysts. The PCR primers were designed to anneal to the region just before exon 8 in

the HPRT gene as the mother and affected son had both been previously found to have a deletion

in exon 8 (Ray, 1999). After five rounds of IVF treatment, a successful pregnancy and delivery

of an unaffected infant occurred (Ray, 1999). However, in all of the cycles there were always

several blastocysts that did not survive the biopsy procedure, making this method potentially

very difficult to preform successfully without patients who are willing to undergo multiple cycles

of IVF like the couple in the study. By 2003, this problem seemed to have been somewhat

reduced as a team in Australia were able to fertilize and biopsy five blastocysts with no

complications and implant two with the highest chances of surviving. This lead to the delivery of

a healthy, non-carrier, baby girl (Cram, 2003).

When studying LNS and the HPRT gene, it is possible to use animal models because the

HPRT gene is in a highly conserved region. According to ncbi.gov, the gene is conserved

between humans, Rhesus monkeys, chimpanzees, dogs, cows, mice, chickens, zebra fishes, and

frogs. 156 other organism contain an ortholog of the human HPRT gene. The animal model most

widely used is the mouse. In a review article published in 2016, it is pointed out that the animal models of Lesch-Nyhan have some limitation due to mice having slightly different purine metabolism pathways than humans. The models can only study one aspect of the disease at a time, either the dysfunction in purine metabolism or the neurological problems leading to self-mutilation (Knapp, 2016). The mouse models have been useful for studying the importance of the neurotoxins and depleted dopamine in the brain at different points in development. It has been found that when young mice are exposed to the neurological conditions associated with LNS that cause damage to the basal ganglia, they develop severe self-mutilating behavior similar to the human phenotype (Knapp, 2016). When the mice are not exposed to this until they are adolescents, however, they do not develop self-mutilating behavior despite similar levels of damage caused to the basal ganglia (Knapp, 2016). Further research on this topic could greatly increase scientists understanding of brain development in babies and children with a deficient HPRT gene.

Research being tested in clinical trials is very slow for LNS; on the government website for clinical trials, clinicaltrials.gov., there have been only four studies since 2003. One of these studies was not able to be completed and one did not have results. The two studies that attained results were for the drug Ecopipam. Ecopipam was a drug meant to reduce self-mutilation. Both of these clinical trials were sponsored by a pharmaceutical company. In the original clinical trial, the majority of the participants showed some improvements with mild side affects such as nausea and respiratory infection. In the second trial when Ecopipam was compared to the placebo, however, the study had to be terminated due to serious side affects experienced by the group receiving Ecopipam such as dystonic crisis and unusual somnolence.

The slowness of current research is most likely due to the limited number of individuals in the population affected with LNS. The amount of government funding on LNS is not enough to be recorded as an independent category, meaning less than a million dollars were provided to study the disease. Without government funding, all of the money for research has to come from independent organizations.

In summary, LNS is an inherited X-linked recessive disorder. It is always expressed in affected males and rarely in carrier females. The phenotype is caused by a large scale mutation in the HPRT gene, leading to a lack of the enzyme hypoxanthine-guanine phosphoribosyltransferase. Without this enzyme, individuals are not able to correctly metabolize purines. This leads to severe phenotypes of self-mutilation, neurological conditions, dystonia, and high levels of uric acid in the body. These symptoms will be expressed in the first years of life. Thus far, research has been primarily based on genetic testing and IVF treatments to prevent couples from having affected children. There is no cure for LNS, but improvements have been made in managing the symptoms of the disease.

This is an excellent paper. ~~If you have more results you~~ **10/10**
one
~~These~~ minor point~~s~~ that it might be interesting to comment on:
~ Is there any discussion of gene therapy? Putting back a functional HPRT gene would presumably solve the problem...
otherwise this is ~~complete~~ fine for the final draft.

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Human Genetics
Synthesis Paper
Topic: Lesch-Nyhan Disease

Lesch-Nyhan Syndrome (LNS) was not an officially diagnosed disease until 1964 in the United States of America when two physicians, Lesch and Nyhan, recognized a pattern of abnormal neurological function, self-mutilating behavior, and exceptionally high levels of uric acid in the urine of two young brothers (Lesch & Nyhan, 1964). It is not surprising that the recognition of LNS did not come until the 1960s. The disease is an "Orphan Disease", meaning it is exceptionally rare. For example, in the United Kingdom, a country with fifty-six million people, there is an average of only four new cases per year (McCarthy, 2006). Overall, one out of eight hundred thousand live births will be affected by Lesch-Nyhan (Hall, 2001). In none of the research was it mentioned any specific population or geographical region being more prone to or having higher incidence rates of LND. This could be due to the large percentage of mutations leading to LND being de novo mutations instead of mutations being passed though families as will be discussed later in this paper.

Soon after Dr. Lesch and Dr. Nyhan published their work describing the syndrome, more cases were reported and studied. From the fifth family to be studied with a history of the syndrome, the largest family being studied in that time period, a pedigree was created. Combining the information from the five families, a team at the University of Virginia School of Medicine was able to deduce that the inheritance pattern in LNS was X-linked recessive (Shapiro, 1966). This was determined as only boys were affected and the ratio matched what was predicted, one in four boys affected from unaffected parents. Females thought to be carriers due to having an affected son showed slightly higher levels of uric acid in urine (Shapiro, 1966); this

supports the hypothesis of X-linked transmission as the phenotype of heterozygotes is slightly altered.

Male to male dominant transmission as a pattern of inheritance was eliminated as a possible hypothesis because of the evidence of female carriers having differing levels of uric acid than average and because most boys with LNS at the time of this research were institutionalized very early in childhood or died in infancy (Shapiro, 1966), leaving them unable to pass on genetic information. Male to male dominant transmission could also be eliminated as a potential hypothesis due to the presence of rare affected females. In one such case, an affected female had a monozygotic twin who was unaffected by the disease. It was able to be determined through fibroblast study that in the affected twin, the wild type allele has been silenced throughout the body while in the unaffected twin the mutant allele had undergone the X-inactivation (Gregorio, 2005).

With the mode of inheritance determined through pedigree analysis and confirmed later with the study of affected females, scientists began to search for the cause of this disease. Due to the excessive amounts of uric acid produced by patients manifesting symptoms of LNS, it was assumed by a team headed by J. Edwin Seegmiller that the disease must be caused by an enzyme involved in purine metabolism losing its function (Seegmiller, 1967). The enzyme responsible was indeed involved in purine metabolism: hypoxanthine-guanine phosphoribosyltransferase (Seegmiller, 1967).

We know today that the gene that codes for this enzyme is quite large, forty-four kb, and contains nine exons in humans (Lapucci, 2006). Because LNS is caused by a single gene mutation, an affected individual does not show a karyotype different than an unaffected individual. This gene leading to LNS when mutated is called the HPRT gene, and it is located on

the long arm of the X chromosome at Xq26 (Torres G. P., 2012). In the year 2003, over two hundred mutations had been observed within the HPRT gene leading to the symptoms of LNS, and it was found that thirty percent of all mutations in the HPRT gene are de novo (Ohdoi, 2002). In 2015, twenty-seven new mutations at the HPRT locus were identified in a single study, bringing the total of known HPRT mutations leading to LNS to over four hundred (Ceballos-Picot, 2015), which further emphasizes how this region of the X chromosome is prone to new mutations or, if it is mutating at the same rate as the rest of the genome, the mutations are occurring in areas where the affects produce a noticeable phenotype.

When the mutation at the HPRT gene is large scale, such as the deletion or a frame shift, the phenotype associated with LNS will always be fully displayed in males who only possess the mutant copy or in females who have the wild-type copy inactivated. Some of these large scale mutations can be caused by incorrect splicing regulation as was the case in the twin study discussed previously (Gregorio, 2005). The phenotype is always displayed because these mutations lead to the production of hypoxanthine-guanine phosphoribosyltransferase enzymes that have completely lost their function (Ceballos-Picot, 2015). Without a functional hypoxanthine-guanine phosphoribosyltransferase enzyme, the patient is not able to properly metabolize purines, leading to the build up of uric acid (Lapucci, 2006). This build up of uric acid was a part of the original observations by Lesch and Nyhan which also included "hyperuricemia, mental retardation, choreoathetosis and self-destructive biting" (Lesch & Nyhan, 1964). Other phenotypes now associated with LNS are severe muscular dystonia and any form of self-mutilation in addition to biting (Gregorio, 2005).

Clinically, it can be difficult to diagnose the forms of self-mutilation in LNS compared to self mutilation that occurs with other neurological abnormalities such as autism. An important

study was done to shed light on this aspect of LNS. The study, preformed by a team led by Scott Hall in 2001, showed that in in patients with LNS the self-mutilation is almost exclusively sudden and violent, even initially when the behavior is first occurring. In patients with autism, however, the self-mutilating behaviors usually increase gradually (Hall, 2001). It was also found in this study that episodes of self-mutilation correlated with the child being left alone without interaction with a caretaker (Hall, 2001), which the authors believe could be a sign that some of the self-mutilating LNS phenotype may be tied to environmental factors.

In some individuals that exhibit the phenotype for LNS there is no mutation within the HPRT gene. Using Real time PCR it was discovered that there was a mutation in the regulatory sequence, and this mutation prevented the HPRT gene from being transcribed (Torres G. P., 2012) . After testing both parents, the mother was determined to be a carrier for this regulatory mutation (Torres G. P., 2012). Studies such as this further confirm that LND is is transmitted though X-linked inheritance.

It is likely that this wide array of phenotypes affecting the muscular, renal, and neurological functions of the body occur when the HPRT gene is no longer coding for a working hypoxanthine-guanine phosphoribosyltransferase enzyme because purines are essential in all tissues of the body and not limited to one specific cell or tissue type (McCarthy, 2006). For example, patients with LND have been shown to have significant decreases in their white and gray brain matter (Schretlen, 2013). Regions especially affected where the basal ganglia, frototemporal, and the limbic system. These decreases in brain matter could imply that deficient purine metabolism leading to the buildup of incorrect cellular products could affect brain growth and development, especially in the regions previously mentioned (Schretlen, 2013).

Individuals who have a mutation in the HPRT gene that does not lead to complete loss of hypoxanthine-guanine phosphoribosyltransferase enzyme function show a less severe phenotype known as Lesch-Nyhan Variants (Ceballos-Picot, 2015). These mutations cause a phenotype that has lower levels of cognitive impairment and no self-mutilating behaviors. Because these phenotypes belong to a different disorder than LNS, it can be concluded that the phenotype range for LNS is very small and without all of the symptoms, an individual does not actually have LNS but instead a less severe variant disorder.

Currently, there is no cure for the phenotypes associated with LNS. Instead, efforts are made by caregivers to manage physical and behavioral symptoms according to lesch-nyhan,org. One of main symptoms that leads to a diagnosis of LND, the overproduction of uric acid, can be managed with the use of a drug called allopurinol (Torres P. P., 2007). Allopurinol inhibits the enzyme xanthine oxidase to prevent the body from synthesizing excessive amounts of uric acid. The study preformed by Torres, Prior, and Puig showed that even after long term use of allopurinol, patients did not have any adverse side effects, and the drug maintained its effectiveness. It was found that with the use of this drug, patients were able to consistently have seventy-four percent less uric acid excretion compared to their baseline levels. Managing the uric acid levels in patients and returning them closer to the normal range does not lessen the other symptoms of the disease such as self-mutilation, however (Hall, 2001).

Currently, behavioral therapy is the only method that can be used in an attempt to control the self mutilating behavior found in LNS, according to lesch-nyhan.com. Pharmaceuticals, such as s-adenosylmethionine, have been shown to provide little improvement or even worsened behavior in LND patients (Dolcetta, 2013). In another study, 5-hydroxytryptophan in combination with a peripheral decarboxylase inhibitor carbidopa and imipramine was used in an

attempt to control the self-mutilating behavior is LNS (Nyhan J. K., 1980). This treatment appeared to be successful initially, but after three months each of the patients became immune to the pharmacological effects, rending the treatment ineffective.

When behavioral therapy is used, it is important that it does not involve negative punishment. Studies have shown that when negative punishment, such as an electric shock, follow the self-mutilating behavior, the frequency of the self-mutilating behavior increases (Zilli, 2008). The authors of the study suggest that early in the progression of LNS, individuals accidently injure themselves due to the lack of motor coordination associated with muscular degeneration. After the accidently injury, improper dopamine signals in the basal ganglia reinforces these behaviors, leading to individuals with LNS to begin to regularly self-mutilate (Zilli, 2008). Therefore, when a negative punishment is applied following self-mutilation, it acts as a reinforcement of the behavior because the improper dopamine signals are further stimulated.

In a review article published in 2000, restraint, in the form of splits, wheelchairs, and keeping the patient from any sharp objects was the most common form of behavioral modification (Olson, 2000). In fact, the removal of these restraints often lead to noticeable distress in patients with LNS and increased levels of self-mutilating behavior. Treatments including extinction, systematic desensitization, and play therapy used in conjunction were shown to decrease the self-injurious behavior in several separate studies to the point where the child no longer needed to be restrained (Olson, 2000). Hall found in his research that positive and continued interactions with caregivers could also reduce the frequency of self-mutilating behaviors (Hall, 2001). Even though there are some behavioral treatments shown to be effective, many children still have all of their teeth extracted to prevent lip biting, cheek biting, and finger biting (Hall, 2001).

There was a significant study published in 2015 by a team lead by Dr. Piedimonte that shows potential to permanently treat the self-mutilating behavior associated with LNS. In this case study, the team of doctors applied deep brain stimulation consistently to a twenty-nine-year-old patient via a bilateral electrode implant. The patient showed progressive improvement and soon did not need his hands tied to prevent self-mutilation. Caretakers also reported a better overall mood and willingness to help with his own needs. Five years later, there were no adverse side affects and the self-mutilation behavior had been totally eliminated in the patient (Piedimonte, 2015).

Due to the severe neurological impairments, self-mutilating behaviors, and stress on the renal system due to the high levels of uric acid being excreted, it is not surprising that individuals with LNS do not have a normal life expectancy; most individuals only survive into the second or third decade of their life, and this lifespan length is only possible with diligent treatment and therapy by the patient's family (Nyhan O. J., 2014).

Because of the severe phenotypes, burden of care on family members, and short life expectancy with low qualify of life by people suffering from LNS, many families with a history of LNS undergo genetic testing in an attempt to avoid having children with this disease. Initially, embryos with LNS were identified during pregnancy by culturing amniotic fluids (Halley, 1977). The amniotic fluids were cultured on a coverslip and the autoradiographic analysis was available a week later. By observing the karyotypes and the autoradiograohic analysis, a unaffected male, affected male, and unaffected female were all correctly identified (Halley, 1977). However, the amniotic fluids were not received until the mothers were in approximately their twentieth weeks of pregnancy, and then it took almost two weeks to get the results back. This led the mother with the affected male fetus to have an abortion at twenty-two weeks (Halley, 1977).

Today, there is technology available to completely avoid a pregnancy with a fetus affected by LND instead of terminating an affected pregnancy, a practice that is much more ethically accepted in current culture. This is done using in vitro fertilization (IVF). In a study published in 1999, researchers were able to create blastocysts using the egg and sperm of parents who already had one affected son using standard IVF procedure (Ray, 1999). After the blastocysts had been dividing for three days, the healthiest were chosen to be genetically tested. This genetic test was a single cell PCR amplification of DNA from a biopsied cell from the healthy blastocysts. The PCR primers were designed to anneal to the region just before exon 8 in the HPRT gene as the mother and affected son had both been previously found to have a deletion in exon 8 (Ray, 1999). After five rounds of IVF treatment, a successful pregnancy and delivery of an unaffected infant occurred (Ray, 1999). However, in all of the cycles there were always several blastocysts that did not survive the biopsy procedure, making this method potentially very difficult to preform successfully without patients who are willing to undergo multiple cycles of IVF like the couple in the study. By 2003, this problem seemed to have been somewhat reduced as a team in Australia were able to fertilize and biopsy five blastocysts with no complications and implant two with the highest chances of surviving. This lead to the delivery of a healthy, non-carrier, baby girl (Cram, 2003).

When studying LNS and the HPRT gene, it is possible to use animal models because the HPRT gene is in a highly conserved region. According to ncbi.gov, the gene is conserved between humans, Rhesus monkeys, chimpanzees, dogs, cows, mice, chickens, zebra fishes, and frogs. 156 other organism contain an ortholog of the human HPRT gene. The animal model most widely used is the mouse. In a review article published in 2016, it is pointed out that the animal models of Lesch-Nyhan have some limitation due to mice having slightly different purine

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Because the mouse models are not completely representative of the human disease as discussed above, research is now being done on human embryonic stem cells (Urbach, Schuldiner, & Benvenisty, 2004). By using human cells that have been mutated to have the HPRT mutation that appears in LNS, scientists are hopeful that they will be able to learn even more about this disease. This knowledge could lead to a better understanding and future areas for treatment for individuals affected with LNS.

There has also been a recent push for gene therapy as a cure for LNS as it is a disease caused by a single gene mutation. However, gene therapy for the HPRT gene is not as easy as it sounds. Because LNS causes severe neurological problems, the HPRT gene would have to be replaced in brain tissue. This is currently not practical due to the blood-brain barrier; scientists do not have a vector for inserting the HPRT gene that can cross this barrier (Cattelan, Dolcetta, Hladnik, & Fortunati, 2013). In 2013, however, scientists did have success with replacing the

HPRT enzyme in the body instead of replacing the gene (Cattelan, Dolcetta, Hladnik, & Fortunati, 2013). They did this by attaching the enzyme that was created in the lab to a protein that had the ability to pass through biological membranes, which allowed it to enter deficient cells. This was all done in vivo, meaning the patient would have to return for continued therapy (Cattelan, Dolcetta, Hladnik, & Fortunati, 2013). This makes enzyme therapy a less appealing option than gene therapy, but until a vector can be created or discovered to cross the blood brain barrier, enzyme replacement therapy will continue to be as close to gene therapy as scientists can get.

Research being tested in clinical trials is very slow for LNS; on the government website for clinical trials, clinicaltrials.gov., there have been only four studies since 2003. One of these studies was not able to be completed and one did not have results. The two studies that attained results were for the drug Ecopipam. Ecopipam was a drug meant to reduce self-mutilation. Both of these clinical trials were sponsored by a pharmaceutical company. In the original clinical trial, the majority of the participants showed some improvements with mild side affects such as nausea and respiratory infection. In the second trial when Ecopipam was compared to the placebo, however, the study had to be terminated due to serious side affects experienced by the group receiving Ecopipam such as dystonic crisis and unusual somnolence.

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Marfan syndrome was first described in 1896 by French physician Antoine Marfan, who observed one of the characteristic symptoms, disproportionately long limbs, in a young girl who he was treating. The genetic basis of the syndrome was discovered nearly sixty years later in a study of patients with Marfan syndrome and their families. In 1991, it was found that mutation of the *FBN1* gene on chromosome 15 is responsible for the symptoms that characterize Marfan syndrome.

The *FBN1* gene encodes fibrillin-1, which is a large protein with several functions. Fibrillin-1 is exported from the cell into the extracellular matrix, which is made up of molecules outside of the cell whose primary function is to connect cells to each other. One of the main functions of fibrillin-1 in the extracellular matrix is as a structural component of microfibrils. Microfibrils are important parts of two types of fibers in the extracellular matrix: elastic and oxytalan fibers. These fibers provide stability and some elasticity to tissue. Along with their structural function, these fibers also play an important role in the regulation of growth factor concentration.

Marfan syndrome is caused by the mutation of the *FBN1* gene. Over 1300 different mutations of the *FBN1* gene have been observed, varying in the number of nucleotides changed and the severity of symptoms they ~~lead to~~ cause. The mutation [*the most common one? Or all Marfan mutations?*] causes a misformed fibrillin-1 protein to be created. The misformed protein will not be able to fold properly, leading to improper association with the other components of the microfibrils. Since the fibrillin-1 is unable to enter the proper conformation, the strength of the microfibrils is weakened. This also greatly harms the ability for transforming growth factor beta (TGF-β) to bind to the fibers.

The lack of TGF-β binding to fibers would suggest lower concentrations of TGF-β. Why is this not the case? Do fibers sequester &or degrade TGF-β?

The effects of excess TGF-β are where much of the new research on Marfan syndrome is ~~investigating.~~ focused High levels of TGF-β cause lower muscle mass and increase the level of elastin degradation. Elastin is another component of the elastic fibers. Degradation of elastin combined with a misformed fibrillin-1causes further weakening of connective tissue. Some have proposed that it is the high levels of TGF-β that cause many of the symptoms of Marfan syndrome rather than the misformed fibrillin-1.

Marfan syndrome is an autosomal dominant disorder, which means that an affected parent has a 50% chance of passing the disorder onto each child they have. While most patients with the disorder have a parent or other close family member with the disorder, it is estimated that anywhere from 15-30% of the cases are caused by a new mutation that was not present in either parent. The disease affects males and females equally and does not seem to be more prevalent in any one race or ethnicity. About 1 in 5000 people has Marfan syndrome. A similar syndrome has been observed in mice with mutations to the homolog to the *FBN1* gene in that species.

The symptoms of Marfan syndrome are highly variable. This is thought to be the case for two main reasons. The first is the nature of the mutation of the *FBN1* gene. While most of the mutations that cause Marfan syndrome are caused by the change of just one base, it is thought that the specific base or bases that are mutated can lead to somewhat different symptoms. It is also thought that alleles of other genes could cause interactions which may also cause different symptoms.

The most prominent symptoms of Marfan syndrome can be found in the skeletal system, eyes, and cardiovascular system. This correlates with where elastic fibers are found in the

greatest abundance in the body. The symptoms affecting the skeletal system are the most visible symptoms of Marfan syndrome. Individuals with Marfan syndrome often grow to be taller than average and have long and thin arms, legs, and fingers. Individuals with Marfan syndrome often have highly flexible joints due to the lessened strength and elasticity of their ligaments.

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Due to the discovery of other disorders that share many symptoms with Marfan syndrome and the decreasing cost of genetic testing, the diagnostic criteria for Marfan syndrome has been

revised to place more of an emphasis on the *FBN1* gene. The other two other criteria are lens

dislocation/myopia and aortic root Z-score, which is a measurement taken with an

electrocardiogram. If the Z score shows an aortic enlargement or possible dissection along with

an another of the two criteria, the individual is diagnosed with Marfan syndrome.

Thirty years ago, the life expectancy of those with Marfan syndrome was only 30-40

years due to problems with diagnosing the disease and lack of treatment options. Though Marfan

syndrome cannot be cured, there are treatments available today that have increased the life

expectancy by more than 30 years. One of the most common treatments is the use of beta

blockers which slow the heart rate and lower blood pressure and decrease stress on the aorta.

Another promising therapy is the use of angiotensin II receptor antagonists. Not only do these

antagonists reduce blood pressure, but they also reduce the levels of TGF-β. Even with these

medications, aortic dissections are possible. If a dissection does occur, surgery is generally

undertaken to repair the tear with a graft.

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You need to include in-text citations — a number or the author + year (Lee et al. 1999) indicating the source of each fact/concept. You can follow examples in any of the primary literature you have read.

~~text~~ you This paper is well written and provides a thorough description of the symptoms and cause of the disease. I think you could expand on the genetics a little bit → can you provide a bit more detail on the relationship between the different mutations that cause Marfan and the phenotypic effects? Are missense mutations less/more severe than nonsense mutations? Etc. Also, are there any prospects for genetic treatments/gene therapy, or do drugs that ~~attenuate~~ lower blood pressure and TGF-β levels sufficient? Do individuals with Marfan typically undergo genetic testing of
↳ or ever
embryos to try and ensure their children do not inherit their allele?

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Marfan Syndrome

BIOS 412

5/2/2016

Marfan syndrome was first described in 1896 by French physician Antoine Marfan, who observed one of the characteristic symptoms, disproportionately long limbs, in a young girl who he was treating. The genetic basis of the syndrome was discovered nearly sixty years later in a study of patients with Marfan syndrome and their families. In 1991 (Dietz et al, 1991), it was found that mutation of the *FBN1* gene on chromosome 15 is responsible for the symptoms that characterize Marfan syndrome.

The *FBN1* gene encodes fibrillin-1, which is a large protein with several functions (Dietz et al., 2005). Fibrillin-1 is exported from the cell into the extracellular matrix, which is made up of molecules outside of the cell whose primary function is to connect cells to each other. One of the main functions of fibrillin-1 in the extracellular matrix is as a structural component of microfibrils. Microfibrils are important parts of two types of fibers in the extracellular matrix: elastic and oxytalan fibers. These fibers provide stability and some elasticity to tissue. Along with their structural function, these fibers also play an important role in the regulation of growth factor concentration (Bonetti, 2009).

Marfan syndrome is caused by the mutation of the *FBN1* gene (Dietz et al., 1991). Over 600 different Marfan-causing mutations of the *FBN1* gene have been observed, varying in the number of nucleotides changed and the severity of symptoms they cause. These mutations take the form of frameshifts, splice errors, nonsense mutations, and missense mutations, which form the majority of mutations found in the *FBN1* gene (Hilhorst-Hofstee et al., 2010). While the exact type of mutation may vary, the mutations that cause Marfan syndrome cause a misformed fibrillin-1 protein to be created. The misformed protein will not be able to fold properly, leading to improper association with the other components of the microfibrils (Robinson & Godfreyb, 2000). Since the fibrillin-1 is unable to enter the proper conformation, the strength of the

microfibrils is weakened. This also greatly harms the ability for transforming growth factor beta (TGF-β) to bind to the fibers (Bonetti, 2009).

The inability of TGF-β to bind to microfibrils causes an excess amount of free TGF-β in the body. The effects of excess TGF-β are where much of the new research on Marfan syndrome is investigating. High levels of TGF-β cause lower muscle mass and increase the level of elastin degradation. Elastin is another component of the elastic fibers. Degradation of elastin combined with a misformed fibrillin-1causes further weakening of connective tissue. Some have proposed that it is the high levels of TGF-β that cause many of the symptoms of Marfan syndrome rather than the misformed fibrillin-1 (Benke et al., 2013).

Marfan syndrome is an autosomal dominant disorder, which means that an affected parent has a 50% chance of passing the disorder onto each child they have. While most patients with the disorder have a parent or other close family member with the disorder, it is estimated that anywhere from 15-30% of the cases are caused by a new mutation that was not present in either parent. The disease affects males and females equally and does not seem to be more prevalent in any one race or ethnicity (Dietz et al., 2005). About 1 in 5000 people has Marfan syndrome. A similar syndrome has been observed in mice with mutations to the homolog to the *FBN1* gene in that species (Pereira et al., 1999).

The symptoms of Marfan syndrome are highly variable. This is thought to be the case for two main reasons. The first is the nature of the mutation of the *FBN1* gene. While most of the mutations that cause Marfan syndrome are caused by the change of just one base, it is thought that the base or bases that are mutated can lead to somewhat different symptoms (Aoyama et al., 1995). The exact correlation between genotype and phenotype is not well understood in Marfan

syndrome. The phenotype is variable even in patients who have had complete deletions of the *FBN1* gene (Hilhorst-Hofstee et al., 2010) If a complete deletion of the gene also leads to variable symptoms, it seems clear that environmental, genetic, or epigenetic factors may be having some sort of effect on the phenotype as well. This would seem to make studying the differential effects of mutations on phenotype very difficult and would make results of such studies unreliable at best. Perhaps the most well understood correlation between genotype and phenotype in Marfan syndrome comes in those who have deletions in both copies of the *FBN1* gene. These cases are highly rare as they require both parents to have Marfan syndrome and pass the affected allele onto the child or for two *de novo* mutations to occur in the child. These cases have shown that being homozygous for Marfan-causing *FBN1* mutations is likely to be lethal before birth and if the child is born they are unlikely to survive more than a few months(Capotorti et al., 1959, Chemke et al. 1984, Schollin, Bjarke, & Gustavson, 1988). This would seem to point to some phenotypic effect of the unaffected allele. Perhaps some of the variation in phenotype is due to level of expression of the unaffected allele.

The most prominent symptoms of Marfan syndrome can be found in the skeletal system, eyes, and cardiovascular system. This correlates with where elastic fibers are found in the greatest abundance in the body. The symptoms affecting the skeletal system are the most visible symptoms of Marfan syndrome. Individuals with Marfan syndrome often grow to be taller than average and have long and thin arms, legs, and fingers (Loeys et al., 2010). Individuals with Marfan syndrome often have highly flexible joints due to the lessened strength and elasticity of their ligaments (McKusick, 1991).

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Due to the discovery of other disorders that share many symptoms with Marfan syndrome and the decreasing cost of genetic testing, the diagnostic criteria for Marfan syndrome has been revised to place more of an emphasis on the *FBN1* gene. The other two other criteria are lens dislocation/myopia and aortic root Z-score, which is a measurement taken with an electrocardiogram. If the Z score shows an aortic enlargement or possible dissection along with an another of the two criteria, the individual is diagnosed with Marfan syndrome (Loeys et al., 2010, Chubb & Simpson, 2012).

As with many dominant disorders, Marfan syndrome presents a more difficult problem. In a recessive disorder, typically the disease phenotype is caused by the lack of a certain protein or metabolite. However, in the case of Marfan syndrome the disease phenotype is caused by the presence of a fibrillin-1 protein that is misfolded and does not function properly. In light of this, it has been proposed that an RNA based therapy could be effective in treating Marfan syndrome. The goal of such treatment would be to suppress the mutated allele that causes Marfan syndrome while allowing the wild-type allele to be expressed (Kilpatrick & Phylactou, 1998). However, as the study by Hilhorst et al. (2010) showed, Marfan syndrome could also be caused by haploinsuficiency. So, if this is indeed the case, it would seem that down-regulating the mutated allele would not be enough to fully treat the disease. To fully treat the disease the wild-type allele would have to be up-regulated in order to give the patient the correct gene dosage.

Thirty years ago, the life expectancy of those with Marfan syndrome was only 30-40 years due to problems with diagnosing the disease and lack of treatment options. Though Marfan syndrome cannot be cured, there are treatments available today that have increased the life expectancy by more than 30 years (Fusar-Poli et al, 2008). One of the most common treatments is the use of beta blockers which slow the heart rate and lower blood pressure and decrease stress on the aorta. Another promising therapy is the use of angiotensin II receptor antagonists. Not only do these antagonists reduce blood pressure, but they also reduce the levels of TGF-β (Keane & Pyeritz, 2008). Even with these medications, aortic dissections are possible. If a dissection does occur, surgery is generally undertaken to repair the tear with a graft (Castellano et al., 2013).

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Primary Ciliary Dyskinesia

14 April 2016

Human Genetics

Primary Ciliary Dyskinesia (PCD) is a congenital, heterogeneous disease affecting the ciliary structure and function [1]. PCD is present in all human races [1], and affects one out of every 16,000 individuals [16]. PCD is characterized by the immotility or ineffective beating of all cilia present in the body [1]. This includes cilia of the upper and lower respiratory tracts, as

well as spermatozoa flagellae [4]. Situs inversus occurs in 50% of PCD cases [1]. This is due to the inability of embryonic cilia to shift the heart to the left side [1]. When situs inversus is present, PCD is called Kartagener syndrome [4]. Patients have a 50% chance to have situs inversus because without normal cilia, there is an equal chance of normal and reversed visceral transportation [1].

PCD is a heterogeneous disease, with many different genes affecting the disease phenotype [1]. However, patients in different subgroups of PCD, such as those having different morphological defects as caused by different genes, appear to have the same clinical symptoms [1]. Physiological effects of PCD can include chronic sinusitis, due to decreased mucociliary transport in the paranasal sinuses [1]. This can lead to anosmia [1] and the development of nasal polyps [4]. Patients may also develop bronchiectasis, of by age two or three years, due to inadequate mucociliary clearance of the lungs and chronic bronchitis [1]. Because of the inadequate mucociliary clearance, the patient must cough to transport secretions within the respiratory tract [1]. PCD patients also have a higher risk of otitis, otosalpingitis, and conductive hearing loss due to the inadequate mucociliary clearance of the Eustachian tubes and the middle ear [1]. In some cases, an IgA deficiency and underdevelopment of the frontal sinuses are also seen in PCD patients [1]. The immune system is often normal in examined patients [1]. Daily airway clearance is necessary to try to preserve lung function [13].

There is no formal registry to collect life expectancy data, but most papers say that life expectancy of PCD patients is "near normal" [13]. There is also no formal way to assess the average age at which disability greatly affects quality of life [13]. There are three periods in which the quality of life of a PCD patient may change. There are often several symptoms during the initial diagnosis period, which can lead to a poorer quality of life [13]. There is often then a Cinderella period, in which there are few symptoms and the disease is easily managed [13]. Finally, symptoms will become more prevalent and serious after the Cinderella period [13].

[handwritten note: I'd never heard of this... the symptoms return at midnight? ☺]

PCD has a relatively high prevalence because it is a heterogeneous disease [1]. It affects one out of every 16,000 individuals [10], and is present in all human races [1]. The prevalence of PCD is relatively high, considering that nearly all males are sterile and females have possibly decreased fertility [1]. It is common in parts of Sweden that are isolated or sparsely populated, as this region is known as the "inbred island" [1]. PCD is also highly prevalent in two isolated populations of Polynesians, however the disease is not associated with Kartagener syndrome in this area [4].

It is likely that the inheritance pattern of PCD is an autosomal recessive pattern, as determined from pedigrees [1]. One study found that the *[handwritten: odds (?)]* ratio between affected and healthy sibs is close to 1:3 [1]. PCD and Kartagener syndrome can also be seen in monozygotic twins [4]. The first molecular demonstration of autosomal recessive inheritance of lateralization defects was found when it was observed that in some patients with PCD, the proper body asymmetry is the results of random determination of left-right asymmetry [4]. In two sibships, compound heterozygous mutations resulted in PCD with or without left-right inversion [4]. It has been difficult for researchers to detect linkage in PCD, because it is a highly heterogeneous autosomal recessive condition [4]. The parents of affected persons are relatives ~~in a percentage that is than~~ *[handwritten: more likely to be]*

than

higher ~~than~~ average [1]. There have also been studies looking to see if PCD is a result of a

a

Founder's effect or a recurrent mutation event. There have also been studies looking into

alternative inheritance patterns for PCD, such as autosomal dominant or X-linked patterns [8].

Research is supported by NID-sponsored Genetic Disorders of Mucociliary Clearance

Consortium (CGMCC) [13]. Advocacy groups for patients include the PCD Foundation [13] and

the PCD Family Support Group [14].

There are several genes that may contribute to PCD. The first gene with mutations ~~to e~~

found to be associated with PCD was isolated from *Chlamydomonas reinhardtii* [4]. Loss of

function mutations in the human gene related to the *Chlamydomonas reinhardtii* dynein IC78

result in PCD [10]. *DNAI1* is also a possible contributor to PCD. Dynein intermediate gene

DNAI1 is localized on 9p13-p21 and is composed of 20 exons encoding a 699 amino acid protein

[4]. Axonemal dynein intermediate-chain gene 1 (*DNAI1*) had been found to be responsible for

some cases of PCD without situs inversus [4]. Compound mutation heterozygosity in *DNAI1*

results in PCD with situs solitus or situs inversus [4]. This may be due to a splicing defect [4].

Cilia contain over 100 different polypeptides, therefore 100 different genes may be involved [1].

At least five types of ciliary defects can be distinguished morphologically [1]. Axonemal

ultrastructure is highly conserved throughout evolution [4]. Transient ciliary abnormalities may

be produced by environmental toxins or infections agents [8]. No chromosomal defects were

found as the underlying causes of PCD in a study [8].

This fits better up here

Many ultrastructural subtypes of PCD seen by electron microscopy, but they all produce

the same clinical features [1]. PCD and Kartagener syndrome are associated with missing or

abnormal dynein arms, abnormal radial spokes, and missing central pair of microtubules [4].

Disease cilia had absent or abnormally short dynein arms or the spokes were short or absent [1]. A reduction in number of dynein arms and in their length was most common abnormality in a study [1]. Dyneins are microtubule stimulated ATPases that induce movement towards the minus end of microtubules [8]. Cilia were also found to have random orientation [1]. In some cases, cilia had no ultrastructural defects, but lacked a fixed orientation [1].

Homologs for the involved genes and proteins have been studied in rhesus monkies, mice, and *Drosophila*. An example is the *iv* mice strain kept by Layton [8], as well as the *hpy* mouse mutant [8].

italicize.

Diagnostic tests for PCD include examining the ciliary ultrastructure through transmission electron microscopy [1], as well as measuring the nitric oxide levels as a screening process [13]. Chest x-rays may also be used [13]. The major challenges for patients with PCD include maintain air way clearance, dealing with chronic upper and lower respiratory infections, and chronic otitis media [13]. In order to monitor airway health, sputum cultures may be studied, and pulmonary function tests, imaging, and bronchoscopies may be performed [13]. The most important thing for patients with PCD to do is to maintain airway clearance [13]. This can be done through chest physiotherapy, vest therapy, acapella, flutter, or quake devices, mucus-thinning drugs, bronchodilators, antibiotics and steroids [13].

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- This is a good start - what you have is well-written and clearly presented. I think you should add/elaborate on a couple of topics →

7/10

- Can you go into a little detail on ciliary structure? What are dyneins and what are their roles in cilia?

- what are the prospects for gene therapy? Is anyone working on a cure or are people/doctors focused just on treating the symptoms? This could go in the now (very short) paragraph on research and ~~advocacy~~ groups.
advocacy

Primary Ciliary Dyskinesia

████████████

4 May 2016

Human Genetics

Primary Ciliary Dyskinesia (PCD) is a congenital, heterogeneous disease affecting the ciliary structure and function [1]. It was first described in 1933, and the cellular basis of PCD was described in 1976 [2]. PCD is present in all human races [1], and affects one out of every 16,000 individuals [3]. PCD is characterized by the immotility or ineffective beating of all cilia present in the body [1]. This includes cilia of the upper and lower respiratory tracts, as well as spermatozoa flagellae [4]. Situs inversus occurs in 50% of PCD cases. This is due to the inability of embryonic cilia to shift the heart to the left side [1]. When situs inversus is present, PCD is called Kartagener syndrome [4]. Patients have a 50% chance of developing situs inversus because without normal cilia, there is an equal chance of normal and reversed visceral transportation [1].

PCD is a heterogeneous disease, with many different genes affecting the disease phenotype. However, patients in different subgroups of PCD, such as those having different morphological defects as caused by different genes, appear to have the same clinical symptoms. Physiological effects of PCD can include chronic sinusitis, due to decreased mucociliary transport in the paranasal sinuses [1]. This can lead to anosmia [1] and the development of nasal polyps [4]. Patients may also develop bronchiectasis by age two or three years, due to inadequate mucociliary clearance of the lungs and chronic bronchitis. Because of the inadequate mucociliary clearance, the patient must cough to transport secretions within the respiratory tract. PCD patients also have a higher risk of otitis, otosalpingitis, and conductive hearing loss due to the inadequate mucociliary clearance of the Eustachian tubes and the middle ear. In some cases, an IgA deficiency and underdevelopment of the frontal sinuses are also seen in PCD patients. The immune system is often normal in examined patients [1]. Daily airway clearance is necessary to try to preserve lung function [5].

There is no formal registry to collect life expectancy data, but most papers say that life expectancy of PCD patients is "near normal." There is also no formal way to assess the average age at which disability greatly affects quality of life. PCD has a wide range of symptom severity, and therefore may affect patients' quality of life in varying degrees. There are three general periods in which the quality of life of a PCD patient may change. There are often several symptoms during the initial diagnosis period, such as chronic lung infections and the need for daily airway clearance and treatment with antibiotics. A patient may next experience a "Cinderella period" during their adolescence and early childhood. It is named thus, because while symptoms are still present, they are often well managed and treatment is required less often. Finally, symptoms will become more prevalent and serious after the Cinderella period during the third or fourth decade of life. Bronchiectasis that is already present may spread, making lung infections more common and severe. Supplemental oxygen may be required, and lung transplants may be considered. By the fifth decade of life, many patients report disability due to lung impairment [5].

PCD has a relatively high prevalence because it is a heterogeneous disease [1]. It affects one out of every 16,000 individuals [6], and is present in all human races [1]. The prevalence of PCD is relatively high, considering that nearly all males are sterile and females have possibly decreased fertility. It is common in parts of Sweden that are isolated or sparsely populated, as this region is known as the "inbred island" [1]. PCD is also highly prevalent in two isolated populations of Polynesians, however the disease is not associated with Kartagener syndrome in this area [4].

It is likely that the inheritance pattern of PCD is an autosomal recessive pattern, as determined from pedigrees [1]. One study found that the odds ratio between affected and healthy

sibs is close to 1:3 [1]. PCD and Kartagener syndrome have also been observed in monozygotic twins, suggesting that Kartagener syndrome is a part of PCD. However, there has not been a large-serious study performed to confirm this. The first molecular demonstration of autosomal recessive inheritance of lateralization defects was found when it was observed that in some patients with PCD, the proper body asymmetry is the result of random determination of left-right asymmetry. In two sibships, compound heterozygous mutations resulted in PCD with or without left-right inversion.

It has been difficult for researchers to detect linkage in PCD, as it is a highly heterogeneous autosomal recessive condition [4]. Studies can show that the parents of affected persons are more likely to be relatives than average [1]. Based on this information, studies have been looking into the question of if PCD is a result of a founder's effect or rather a recurrent mutation event. There have also been additional studies looking into alternative inheritance patterns for PCD, such as autosomal dominant or X-linked patterns. In one such study, a mother had five affected sons with three different fathers, which caused the researched to wonder if another inheritance pattern is possible. Because PCD is a heterogeneous disease, it is possible that there are different types of inheritance at play that all lead to dynein dysmorphology or dysfunction [2]. In general, however, it is accepted that the inheritance pattern of PCD is autosomal recessive.

PCD is the result of anomalies in the ciliary structure, leading to immotile or ineffective cilia. Cilia are made up of nine outer doublet microtubules encircling two inner singlet microtubules. This structural bundle is known as the axoneme. The characteristic "9+2" microtubule arrangement is seen in a cross section of the axoneme using electron microscopy. The axoneme is approximately 0.25 μm in diameter, and can vary in length depending on the cell

type and organism. Within the axoneme, inner and outer rows of dynein arms are attached to the A tubule of each doublet microtubule, and the dynein arms extend out to the B tubule of the neighboring double microtubule [7].

Cilia and flagella beat, with bends in the hair-like structures propagating from the axoneme base. Ciliary and flagellar movement is due to the sliding between pairs of the outer doublet microtubules within the axoneme. The dynein arms are the motor proteins in axonemes and are responsible for movement. Formation and breakage of cross-bridges formed between the dynein arm and the B tubule, and the binding and hydrolysis of ATP, help produce the force for active sliding. Axonemal dyneins are composed up heavy, intermediate, and light chains. The heavy chains can hydrolyze ATP, while the intermediate and light chains help secure the dynein arm to the A tubule and may help regulate dynein activity. The dynein arms are active at different times, based on their location, so that the axoneme can bend in two directions. Actively sliding in one half of the axoneme will cause the cilia to bend towards one side, while sliding in the other half will produce bending on the opposite side. Through this pattern, the cilia and flagella are able to actively beat. Studies have found that the central singlet microtubules and radial spokes, which link the central singlets to the A tubules of the outer doublets, may play a role in controlling the bend of flagella [7].

Variations in the "9+2" pattern, seen in some protozoans, have demonstrated that the central singlet microtubules are not necessary for axonemal beating, and that motility is still present when there are less than 9 outer doublet microtubules, but with a lower frequency [7].

There are several genes that may contribute to PCD. The first gene with mutations found to be associated with PCD was isolated from *Chlamydomonas reinhardtii*. *Chlamydomonas*

reinhardtii is an unicellular alga with two flagellum that have similar axonemal structure to human respiratory cilia and sperm tails [4]. Loss of function mutations in the human gene related to the *Chlamydomonas reinhardtii* dynein IC78 result in PCD [6]. *DNAI1* is also a possible contributor to PCD. Axonemal dynein intermediate gene 1 *DNAI1* is localized on 9p13-p21 and is composed of 20 exons encoding a 699 amino acid protein. Axonemal ultrastructure is highly conserved throughout evolution, which indicates that mutations to the genes responsible for this structure are not favorable and therefore not selected for. *DNAI1* had been found to be responsible for some cases of PCD without situs inversus. Compound mutation heterozygosity in *DNAI1* results in PCD with situs solitus or situs inversus. This may be due to a splicing defect. Mutations in *DNAH5*, a dynein heavy chain gene, have also been associated with PCD. Mutations in the dynein heavy chain pseudogene *DNAH7p* have also been associated with PCD. Dynein heavy chains are encoded by multiple genes, and therefore mutations to any of these other genes may also play a role in PCD [4]. Mutations in *DNAI1* and *DNAH5* account for almost 30 percent of all PCD cases. Cilia contain over 100 different polypeptides, therefore over 100 different genes may be involved. In many cases, the exact cause of PCD is unknown. At least five types of ciliary defects can be distinguished morphologically, and these defects may each have several genetic causes [1]. Transient ciliary abnormalities may also be produced by environmental toxins or infections agents. No chromosomal defects have been found as the underlying causes of PCD [2].

Homologs for the involved genes and proteins have been studied in rhesus monkeys, mice, and *Drosophila*. An example is the *iv* mice strain kept by Layton, which was used to study situs inversus, as well as the *hpy* mouse mutant, which was used to study male sterility in PCD due to sperm immotility [2]. Another study with mouse mutants who were defective in genes

required for cilia growth (*Hfh4*) or beating (*Kif3B* and *Kif3A*) demonstrated a link between cilia motility and lateralization [4].

Many ultrastructural subtypes of PCD seen by electron microscopy, but they all produce the same clinical features [1]. PCD and Kartagener syndrome are associated with missing or abnormal dynein arms, abnormal radial spokes, and missing central pair of microtubules [4]. Disease cilia had absent or abnormally short dynein arms or the spokes were short or absent. A reduction in number of dynein arms and in their length was most common abnormality found in studies [1]. In some cases of PCD, cilia were also found to have random orientation. In such cases, cilia had no ultrastructural defects, but lacked a fixed orientation [1].

Diagnostic tests for PCD include examining the ciliary ultrastructure through transmission electron microscopy [1], as well as measuring the nitric oxide levels as a screening process [5]. Dynein and microtubule structural defects viewed using election microscopy are the main diagnostic clues that a patient has PCD. Nitric oxide levels are chronically reduced in PCD. This is notable because nitric oxide production is upregulated in the respiratory epithelium during infection. Therefore, it would be expected for an individual with chronic lung infections to have elevated nitric oxide levels. If the levels are actually reduced, it is a sign that the patient may have PCD. Chest x-rays may also be used to diagnose PCD [5].

The major challenges for patients with PCD include maintaining air way clearance, and living with chronic upper and lower respiratory infections and chronic otitis media. Currently, efforts are focused in treatments that slow the progression of the disease and help manage symptoms. In order to monitor airway health, sputum cultures may be studied, and pulmonary function tests, imaging, and bronchoscopies may be performed. These procedures may be done

during a period of patient health to provide a "baseline" for later comparison. It is recommended that routine sputum cultures are taken every six to twelve months, as well as during infection. Pulmonary function tests have been historically used for patients with cystic fibrosis, asthma, and chronic obstructive pulmonary disease. Studies are not clear on the value of pulmonary function tests in patients with PCD, but it is still recommended patients have these tests every three to six months. Baseline CT scans are important when looking for structural changes of the lungs, such as the presence and progression of bronchiectasis. CT scans should be limited, however, due to the radiation exposure. X-rays can also be useful when looking for pneumonia, but it is more difficult to see structural changes, like bronchiectasis, on an x-ray. Bronchoscopy allows for the physician to look directly into the passageways of the patients' lungs, as well as for the removal of mucus plugs or collecting tissue samples [5].

The most important thing for patients with PCD to do is to maintain airway clearance. This helps preserve lung function, as well as prevent future infections. This can be done through chest physiotherapy, vest therapy, acapella, flutter, or quake devices, mucus-thinning drugs, bronchodilators, antibiotics and steroids. Chest physiotherapy and vest therapy are also used for cystic fibrosis patients and they both work to physically loosen and move mucus secretions out of the lungs and the airway. Acapella, flutter, and quake devices are positive expiratory pressure devices, and help open up the airways, and in some cases vibrate the airways, in order to help the patient cough mucus up an out of the respiratory system. Physical exercise also helps to open up the airways and move mucus out of the lungs. Mucus thinning drugs help thin secretions in order to make them easier to remove. Bronchodilators act to open and relax the airways, which helps to move mucus out. Antibiotics and steroids can be used on a case-by-case basis to treat infection, or as a preventative measure. Myringotomy tubes and hearing aids may also be necessary when

chronic otitis media is present. Usually a combination of these physical and pharmaceutical treatments is used to treat PCD [5].

Further research into the mechanism and treatment of PCD is supported by NID-sponsored Genetic Disorders of Mucociliary Clearance Consortium (CGMCC). The clinical studies available are focused on understanding PCD rather than trying gene therapies. Currently, clinical studies include comparing the inflammatory and microbiologic marker in sputum in cystic fibrosis and PCD patients, studying the long-term lung function and disease progression in children with early onset PCD, studying pathogens of PCD lung disease, and studying dyskinesia, heterotaxy, and congenital heart disease [5]. Advocacy groups for patients with PCD include the PCD Foundation [5] in North America and the PCD Family Support Group [8] in the United Kingdom.

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PARKINSON'S DISEASE

By

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Bios 412

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ROUGH DRAFT –PARKINSON'S DISEASE

with numbered citations, the number should correspond to the order in which the citation appears in the paper

Parkinson's disease (PD) is a nervous system progressive disorder due to basal ganglia degeneration and a decrease of dopamine [7]. This means that there is a defect in genes where proteins cannot produce enough dopamine that lead to loss of control of precise motor movements. Parkinson's disease (PD) mainly affects middle aged or elderly people [7]. This is due to "wear and tear" on the body; however it can arise in all ages, however this is usually rare. Common symptoms of PD include tremor, dyskinesia, rigid muscles, as well as less accurate movement or muscles [1]. The disease is progressive and is often times not fatal [1]. Meaning it gets worse over time) The saying with PD is "you will die with Parkinson's, not from it [1]". There are medical options that help control your body by helping the body cope with the defect. Currently, PD has no cure.

what are "less accurate muscles"? misleading loss neural loss of motor control.

PD

make these one sentence

Medical treatments to help control muscles by dopaminergic therapy can help improve quality of life [8]. This is crucial because there are millions of people in the US and around the world that suffer from PD. The demographic breakdown of the disease is as follows: PD occurs in about 1-2% of people at age 60, 1 million in the US, and 4 million worldwide [1]; PD affects 1.7% of the Chinese population [10]; 60,000 people get diagnosed every year in the US with PD [1]. It is inherited and usually has two possible ways of inheritance. PD follows an autosomal dominant or autosomal recessive inheritance pattern, depending on what gene(s) are affected [2].

what is this?

order

Some background on Parkinson's relies on many studies on neurotransmitters because this lies at the heart of the disease. Torquato Torqauti

Not sure what you are trying to say here

was this research in the context of PD, or some other context?

first isolated dopamine in 1910 [7]. The disease was actually discovered before ~ *link them / connect to PD*

1910. James Parkinson discovered the disease in 1817 [7]. The discovery of the

disease coupled with the discovery of neurotransmitters, especially dopamine

greatly helped the further studies on PD and how it works. The first identified *OMIM*

mutation in PD was a missense mutation of A53T in alpha synuclein gene [4]. A big

foundation is the Michael J. Fox foundation. He is a celebrity that suffers from PD

and he greatly supports the research to help patients. The National Institute of

Neurological Disorders and Stroke (NINDS) support PD. ~~Lets move from the~~ *This kind of narrative is usually absent from research papers*

~~background to see how the genetic differences affect individuals that suffer from PD.~~

PD can result from a mutation in LRRK2, PARK2, PINK1, PARK7 and the *(6) (1) / parkin / DJ1 Gene*

SNCA gene [1]. The most common genes affected come from the PARK gene family *These two sentences seem contradictory...*

[1]. However, the most common risk factor for PD is a mutation in the *alpha synuclease.*

Did this study really show this? Or did you read this fact in paper [5] but another study showed it?

glucocerebrosidase or GBA gene [5]. The gene PARK1 associated with PD was first *(4)*

linked to PD in 1995 [4]. This finding led to other studies to see how gene defects

leads to defective proteins and how it decreases dopamine, which is needed for fine-

tuning of motor skills. *parkin*

The important point here should be the kinds of mutations that give rise to PD— this suggests they are point mutations or small indels, not gross chromosomal rearrangements

Although there are mutations in some genes, a person with PD does not have

a different karyotype than a normal person [4]. ~~Meaning~~ a patient with PD will have *Don't start sentences with "Meaning"*

23 chromosomes, however, it may be missing parts or segments of chromosomes,

some could be noticeable, whereas some are not. Mutations in the PARK2 and *what does PARK7 encode?*

PARK7 genes are the most frequent mutations for people with PD [4]. PARK2

encodes parkin, a protein that helps degrade unneeded proteins by tagging proteins

that are damaged or in excess and help maintain mitochondria [4]. PARK gene

Is the parkin protein located in mitochondria?

deletions and point mutations occur worldwide in diverse ethnic groups [4].

PD is the second most frequent neuro-degenerative disease behind Alzheimer's disease, since it occurs more than 1% after the age of 65 years [2]. There are many similarities and differences between these two diseases, which will be covered later. PD is found in 1 million people in the US, while 60,000 people are diagnosed every year (US) and 4 million people worldwide [1]. So, with the frequency of this disease, what causes PD?

PD is often caused by genetic predisposition and exposure to certain toxins such as chronic exposure to herbicides and pesticides may cause mutations in PD causing genes [4]. These chemicals cause a disruption in genes by entering the genome. These genes code for proteins that produce neurotransmitters. The brain ages slow and progresses slowly, which will result in defective cognitive, memory, and motor functions, so aging is a major factor for PD [3]. During aging, increases in metabolic or oxidative stress can lead to neuronal defects and cell death [3]. Now that we have seen the genetic component, lets dive into the molecular biology of this disease.

see end ✳

There are many mutations that lead to a defect in PARK genes. PD causing genes such as PARK2 could be deleted, duplicated or contain a mutation that codes for a different amino acid (missense) [1]. A different coding sequence leads to defective genes and proteins that affect the nervous system and the cells that make up this system. The main cells affected are neurons and the nervous system, which cause a defect in the ubiquitin-proteasome system as well as a loss of function of parkin that cannot control the release of dopamine, which is needed for control of

muscles [4]. The neurons cannot create action potentials to stimulate the nervous system to control motor movements, which is why the phenotype corresponds with the problems that lye [lie] at the genetic level of this disease. So if there is a defect in this pathway, it cannot fulfill the pathway and thus, a product cannot be formed. The product is dopamine, and this leads to a severe decrease in Dopamine. PARK genes encode for a protein called parkin, which is expressed in the brain or substantia nigra [4]. Parkin is a vital protein coded by the PARK genes.

Parkin is a 465 ~~long~~ [long protein] amino acid located at the PARK 2 locus on [the long arm of] chromosome six, ~~the long arm~~ [8]. *Parkin* functions as an ubiquitin ligase that will attach ubiquitin molecules to wrongly folded proteins to flag them so they can be [by the] proteasome processed [8]. Parkin targets damaged proteins and degrade [s] them to regulate the release of dopamine [4]. Parkin is found in the ubiquitin-proteasome pathway that degrades proteins, and without this function it is lethal to cells [4]. Therefore if Parkin cannot degrade defective proteins, the cells will have degraded proteins that will not be able to produce proteins. From a broad view cells from this defect often degrade due to abnormal leading to ineffective and inefficient cells that ultimately lead to cell death.

[Mutations in] PARK2 leads to degradation and cell death of the substantia nigra [5]. Nerve cells in this region produce dopamine, and this tissue death leads to an inability to produce dopamine [5]. Loss of PARKIN function leads to abnormal mitochondrial structure and function [9]. Mitochondria functions in energy production and is vital to powering protein ~~productions that are used~~ [synthesis] in the nervous system [9]. Dopamine [and all cells]

relays messages that control body movement, and a lack of dopamine severely hurts

this ability [7]. Dopamine ~~is~~ has a very important physiological role controlled by parkin

and is conserved ~~in~~ across mammal~~ian genetics~~s [7]. Since this gene is ~~vital to~~ Conserved in mammals,

~~various~~ animal models can ~~look at~~ be used to study these genes ~~at a~~ more in-depth ~~level with manipulation~~ in an experimental setting.

~~and other variables.~~

(margin right) Is the connection between PARKIN and dopamine really this direct?

(margin left) You've already said that PARKIN is conserved

The Parkin gene is conserved, and is deemed very important in mammals.

This is why a defect in this gene causes abnormal phenotypes. The most noticeable

being uncontrollable shaking due to loss of motor skills. In order to treat this

disease, animal models are used since PD genes are conserved. Mouse models are also

frequently used to study PD because ~~of the~~ in mice the substantia nigra degeneration es due to

mutations ~~genes that are conserved~~ in Parkin/PARK2 (or whatever) [7]. The model is that mice ~~will~~ are get injected to *(margin: with what?)*

decrease dopamine production and degrade the substantia nigra so that it

resembles a human that suffers from PD. So, from these defects, lets see how this

affects the phenotypes and how this also affects the clinical side and treatment of

this disease.

The diagnostic test used to see if someone as PD is a neurological

examination performed by the doctor as well as medical history examination [2].

Family history and family trees ~~is a~~ are very good indicator of ~~diseases~~ PD specifically?. It can give you a

probability of inheriting the disease and can help determine if your kids are at risk

as well. This is vital since it has inheritance patterns as stated previously. This is

commonly used to diagnose people to see if they have PD or not. → Is family history used to diagnose a patient? or

The actual phenotype depends on what gene is affected in one's genome. to predict whether someone will get PD?

Different mutations can lead to different symptoms and defective proteins based on what [which] gene is affected [8]. Heterozygous patients with PD experience less severe symptoms than those with homozygous mutations of PD genes [8]. Alzheimer's has very similar symptoms because it is a neurodegenerative disease and is a result of the aging process [8]. Alzheimer's is a mutation [disease] that results in [from] gene defects that code [are important in] for a different part of the brain, the hippocampus and the entorhinal cortex [8]. Dopamine is not able to transfer the message[s] from this part of the brain, which often results in memory loss and not motor loss, due to the nature of the hippocampus [7]. In order to treat this disease, different *[different between PD & Alzheimer's?]* animal models are used to study PD.

Mice are widely used as an animal model for researching PD [3]. To do this, mice are injected with toxic chemicals that cause loss of function in PD causing genes such as the SNpc region [3]. Motor function decreases in Mice [mice] as they age [3]. Age associates genes that most affected mice can be used to look at in humans with PD [3]. From the in depth study on mice models, newer treatments could arise to help patients cope with PD.

PD patients are faced with inability to control motor functions [8]. PD patients also face pain and *a reduced?* ability to swallow since it takes neurons to power movements [8]. Through medical history examination and a neurological test, PD can be detected [8]. *Future.* Stem cells in embryos can be directed to make more dopamine which can help avoid PD before birth [8]. However this has to be done at a very early age, and even in the womb, but sometimes it is hard to detect PD in a baby before birth. There are several treatments and drugs used to help cope with PD.

Carbidopa-levodopa is the most effective medication since it is a natural

[Margin notes: "Clearly state that, mutations that have accumulated"; "What do you mean by age-associated genes?"; "People are doing this?!? Or this is a hypothetical, proposed treatment?"]

chemical that can enter the brain since it can pass through the blood brain barrier [4]. This drug is effective because it can be converted to dopamine to help increase dopamine levels in the body [4]. ~~By having a drug that can be turned into dopamine will greatly help.~~ Another effective drug is called a MAO-B inhibitor. MAO-B inhibitors help prevent the breakdown of the brain dopamine by inhibiting enzyme monoamine oxidase B, which metabolizes dopamine [4]. This will allow dopamine to buildup and not be degraded, which can help increase levels of dopamine in the brain. Another great treatment, although controversial is Deep Brain Stimulation. (DBS) can help stimulate parts of the brain that are used to control motor movement and help activate parts of the brain that are not frequently actvated due to defective genes [8]. It is controversial because many people think deep brain stimulation can be harmful to normal individuals. However, it is being tested further and will hopefully improve life of those suffering with PD.

Is DBS really part of reference 8?

Parkinson's Disease has no cure and most of the problems are due to defective genes that cannot code for the parkin protein that get's rid of damaged proteins that make dopamine. Most treatments attack the problem of low levels of dopamine in the body and find different ways to increase dopamine in the brain. Future studies and advancement in technology to identify more pathways associated with PD will greatly improve the chances of curing PD. As for now, only treatments can help cope with PD. If further studies can find how to fix gene defects so that they can produce the correct amino acid sequence in order to produce parkin proteins, dopamine levels will rise. However, the complex nature of the

pathways coupled with the genome will create big challenges in order to cure this

disease.

7/10

⊛ The paper in general, and this paragraph in particular, could use some reorganization. ~~Sentences~~ This paragraph contains facts on many topics that should be separated into distinct paragraphs that each focus on a different topic ⟶

Genetic component
_____

Types of PARK mutations

Molecular biology

ubiquitin pathway ⎤
Dopamine production ⎦)

how are these related?

Physiology / Phenotype
_____

Nervous system
Loss of action potentials

One suggestion for how to arrange the paper:

- How do mutations in PARK2, PARK7, PINK, etc. disrupt the protein
- How does a disrupted protein affect cellular function?
- How does disrupted cellular function affect the body / individual?

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PARKINSON'S DISEASE

By

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Bios 412

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PARKINSON'S DISEASE

Parkinson's disease (PD) is a nervous system progressive disorder due to basal ganglia degeneration and a decrease of dopamine [1]. PD is the second most frequent neuro-degenerative disease behind Alzheimer's disease, since it occurs more than 1% after the age of 65 years [2]. There are many similarities and differences between these two diseases, which will be covered later. PD is found in 1 million people in the US, while 60,000 people are diagnosed every year (US) and 4 million people worldwide [3]. The demographic breakdown of the disease is as follows: PD occurs in about 1-2% of people at age 60, 1 million in the US, and 4 million worldwide [3]; PD affects 1.7% of the Chinese population [4]; 60,000 people get diagnosed every year in the US with PD [3]. It is inherited and usually has two possible ways of inheritance. PD follows an autosomal dominant or autosomal recessive inheritance pattern, depending on what gene(s) are affected [2]. So, with the frequency of this disease, what causes PD?

James Parkinson discovered the disease in 1817 [1]. Parkinson's relies on the mechanisms and pathways of neurotransmitters. The neurotransmitter dopamine is defected or at abnormal levels in a patient that suffers form PD. Torquato Torqauti first isolated dopamine in 1910 [1]. The discovery of the disease coupled with the discovery of neurotransmitters, especially dopamine greatly helped the further studies on PD and how it works. The discovery of Neurotransmitters paved the way for further PD studies.

PD is often caused by genetic predisposition and exposure to certain toxins such as chronic exposure to herbicides and pesticides may cause mutations in PD causing genes [5]. These chemicals cause a disruption in genes by entering the genome. These genes code

for proteins that produce neurotransmitters. The brain ages slow and progresses slowly, which will result in defective cognitive, memory, and motor functions, so aging is a major factor for PD [6]. During aging, increases in metabolic or oxidative stress can lead to neuronal defects and cell death [6].

Dopamine is affected in patients that suffer from PD. This is due to defects in genes where proteins cannot produce enough dopamine. A major effect of PD is that it leads to a loss of neural transmitters, which ultimately causes loss of motor control. Parkinson's disease (PD) mainly affects middle aged or elderly people [1]. This is due to "wear and tear" on the body; PD can arise in all ages, however this is usually rare. Common Symptoms of PD include tremor, dyskinesia, rigid muscles, as well as less accurate movement or muscles [3]. The disease is progressive, is often times not fatal but PD gets worse over time [3]. The saying with PD is "you will die with Parkinson's, not from it [3]". Essentially PD does not harm your organs just the response of the nervous system and it's ability to send neurotransmitters. There are medical options that help control your body by helping the body cope with the defect, but as of now PD has no cure. A big foundation to finding a cure is the Michael J. Fox foundation. He is a celebrity that suffers from PD and he greatly supports the research to help patients. The National Institute of Neurological Disorders and Stroke (NINDS) is also researching new cures and coping mechanisms for PD. What genes or mutations are these research institutes looking at?

The first identified mutation in PD was a missense mutation of A53T in alpha synuclein gene [7]. PD commonly results from a mutation in LRRK2, PARK2, PINK1, PARK7 and the SNCA gene [3]. The most common genes affected are PARK1, PARK2 and PARK7 genes [3]. The gene PARK1 associated with PD was first linked to PD in 1995 [5]. PARK1 is

located on chromosome 4 and codes for alpha synuclein [5]. PARK 2 is located on

chromosome 6 and codes for the protein *parkin* [5]. PARK7 is found on chromosome 1 and

codes for protein deglycase or DJ-1 [5]. This finding led to other studies to see how gene

defects leads to defective proteins such as *parkin* and how it decreases dopamine, which is

needed for motor skills.

Although there are mutations in some genes, a person with PD does not have a

different karyotype than a normal person [5]. A patient with PD will have 23 chromosomes,

however, it may be missing parts or segments of chromosomes, some could be noticeable,

whereas some are not. Gross chromosomal rearrangements may also lead to deletions or

defective genes that can cause PD. Mutations in the PARK2 and PARK7 genes are the most

frequent mutations for people with PD [5]. PARK2 encodes *parkin*, which is found in the

mitochondria [5]. *Parkin* helps degrade unneeded proteins by tagging proteins that are

damaged or in excess and help maintain mitochondria [5]. *Parkin* is expressed in the brain

or substantia nigra [5]. Parkin is a vital protein coded by the PARK genes. PARK7 encodes a

protein of the peptidase C56 family [5]. PARK mutations occur worldwide in diverse ethnic

groups [5].

There are many mutations that lead to a defect in PARK genes. PD causing genes

such as PARK2 could be deleted, duplicated or contain a mutation that codes for a different

amino acid (missense) [3]. A different coding sequence leads to defective genes and

proteins that affect the nervous system and the cells that make up this system. A mutation

will kill cells which lead to no dopamine production. This leads to a severe decrease in

Dopamine.

The main cells affected are neurons and the nervous system, which cause a defect in the ubiquitin-proteasome system as well as a loss of function of parkin that cannot control the release of dopamine, which is needed for control of muscles [5]. *Parkin* is a 46 amino acid long protein located at the PARK 2 locus on the long arm of chromosome six [8]. *Parkin* functions as an ubiquitin ligase that will attach ubiquitin molecules to wrongly folded proteins to flag them so they can be processed by the proteasome [8]. Parkin targets damaged proteins and degrades them to regulate the release of dopamine [5]. *Parkin* is found in the ubiquitin-proteasome pathway that degrades proteins, and without this function it is lethal to cells [5]. *Parkin* kills damaged cells and is how the body reacts to the damaged cells. Therefore if *parkin* cannot degrade defective proteins, the cells will have degraded proteins that will not be able to produce proteins, which ultimately leads to cell death. PD patients are able to survive because they still produce some dopamine which allows them to move, however it may be uncontrollable.

Mutations in PARK2 lead to degradation and cell death of the substantia nigra [5]. Nerve cells in this region produce dopamine, and this tissue death leads to an inability to produce dopamine [9]. Nerve cells produce dopamine by signaling molecules at junction gap receptors **[9].** These receptors produce action potentials that release dopamine [9]. Loss of PARKIN function leads to abnormal mitochondrial structure and function [10]. Mitochondria functions in energy production and is vital to powering protein productions that are used in all cells [10]. Dopamine relays messages that control body movement, and a lack of dopamine severely hurts this ability [1]. The actual phenotype depends on what gene is affected in one's genome. Different mutations can lead to different symptoms and

defective proteins based on what gene is affected [8]. Heterozygous patients with PD

experience less sever symptoms than those with homozygous mutations of PD genes [8].

The neurons cannot create action potentials to stimulate the nervous system to control

motor movements. The most noticeable phenotype is uncontrollable shaking and posture

instability due to loss of motor skills. Another symptom is the reduced ability to swallow

since neurons power muscles needed to swallow. Dopamine is a very important

physiological role controlled by *parkin* and is conserved in mammalian genetics [1]. Since

this gene is vital to mammals, various animal models can look at genes at a more in depth

level with manipulation and other variables.

In order to research this disease mouse models are frequently used to study PD

because of the similarity of PD with other neuro-degenerative diseases such as Alzheimer's

[3]. It is crucial to see the differences between the two diseases. Alzheimer's has very

similar symptoms because it is a neurodegenerative disease and is a result of the aging

process and accumulation of mutations [8]. Alzheimer's is a disease that results from gene

defects that are important in a different part of the brain, the hippocampus and the

entorhinal cortex [8]. Dopamine is not able to transfer messages from this part of the brain,

which often results in memory loss and not motor loss, due to the nature of the

hippocampus [1]. Since these two diseases are very correlated, different animal models are

used to study PD. Mice are widely used as an animal model for researching PD [6]. To do

this, mice are injected with toxic chemicals that cause loss of function in PD causing genes

such as the SNpc region [6]. Motor function decreases in mice as they age [6]. Age

associates genes that most affected mice can be used to look at in humans with PD [6]. Age

associated genes are prone to mutations because they have an accumulation of mutations over time, not because of the age itself. From the in depth study on mice models, newer treatments could arise to help patients cope with PD. Mice are also used because in mice the substantia nigra also degenerates due to mutations in PARK2 and PARK7 [1]. The model is that mice will get injected to decrease dopamine production and degrade the substantia nigra so that it resembles a human that suffers from PD.

The diagnostic test used to see if someone as PD is a neurological examination performed by the doctor as well as medical history examination [2]. Family history and family trees is a very good indicator of PD. It can give you a probability of inheriting the disease and can help determine if your kids are at risk as well. This is vital since it has inheritance patterns as stated previously. This is commonly used to diagnose people to see if they have PD or not. PD can be predicted to see a child's risk or can be used to see a patient's likelihood of contracting the disease. Through medical history examination and a neurological test, PD can be detected [8]. Stem cells in embryos can be directed to make more dopamine, which can help avoid PD before birth [8]. However this has to be done at a very early age, and even in the womb, but sometimes it is hard to detect PD in a baby before birth. Most of the stem cell research is proposed and is not being used because of the ethical dilemma. There are several treatments and drugs used to help cope with PD.

Carbidopa-levodopa is the most effective medication since it is a natural chemical that can enter the brain since it can pass through the blood brain barrier [5]. This drug is effective because it can be converted to dopamine to help increase dopamine levels in the body [5]. By having a drug that can be turned into dopamine will greatly help. Another

effective drug is called a MAO-B inhibitor. MAO-B inhibitors help prevent the breakdown of the brain dopamine by inhibiting enzyme monoamine oxidase B, which metabolizes dopamine [5]. This will allow dopamine to buildup and not be degraded, which can help increase levels of dopamine in the brain. Another great treatment, although controversial is Deep Brain Stimulation (DBS) can help stimulate parts of the brain that are used to control motor movement and help activate parts of the brain that are not frequently activated due to defective genes [11]. It is controversial because many people think deep brain stimulation can be harmful to normal individuals. However, it is being tested further and will hopefully improve life of those suffering with PD.

Parkinson's Disease has no cure and most of the problems are due to defective genes that cannot code for the *parkin* protein that get's rid of damaged proteins that make dopamine. Most treatments attack the problem of low levels of dopamine in the body and find different ways to increase dopamine in the brain. Medical treatments to help control muscles by dopaminergic therapy can help improve quality of life [8]. Dopaminergic therapy is adding in dopamine neurotransmitters to the right tissues in the brain. Since there are no cures, therapy to help patients cope with PD can help patients live a longer life. This is crucial because there are millions of people in the US and around the world that suffer from PD.

Future studies and advancement in technology to identify more pathways associated with PD will greatly improve the chances of curing PD. As for now, only treatments can help cope with PD. If further studies can find how to fix gene defects so that they can produce the correct amino acid sequence in order to produce parkin proteins,

dopamine levels will rise. However, the complex nature of the pathways coupled with the

genome will create big challenges in order to cure this disease.

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First Draft

<center>**Review Paper: Warner Syndrome**</center>

Introduction to Warner Syndrome

Werner Syndrome (WS) is ~~considered to be~~ one of the segmental progeroid syndromes diseases manifesting ~~the~~ accelerated aging that affect multiple organs and tissues (Martin and Oshima 2000). The disease is named after the German scientist, Otto Werner who discovered and identified the syndrome from four siblings who ~~have~~ *had* the symptom of premature aging in 1904. WS ~~is~~ *was* discovered to ~~be~~ result from *from* a rare autosomal recessive ~~inheritance with the~~ *alleles of* mutations at the WRN gene on chromosome 8, which encode*s* the RecQ DNA helicase (Yu et al. 1996). Either sex can be affected and also with the higher chance between brother and sisters from the same carrier or infected parents (Takemoto et al. 2013).

this ref is not in your references

↳ what does this mean? Just that it runs in families?

The global incidence rate ~~that the individual will be born with the~~ *of* WS disease is less than 1 in 100,000 live births (Hasty et al. 2003). However, in Japan and Sardinia, they tend to have ~~the~~ *a* higher rate of ~~affecting individual of~~ 1 in 20,000-40,000 and 1 in 50,000, respectively (Masala et al. 2007). The approximated lifespan for these WS patients is around 50 years old globally (Hasty et al. 2003), 47-48 in Japan, and 54 years in Sardinia (Masala et al. 2007).

In between 2009-2011, a nationwide epidemiology study in Japan ~~is~~ *was* conducted with 6921 surveys sent to hospitals and clinical*s* experienc*ed* with WS (Takemoto et al. 2013). They found that more than 90% incidence were recorded as cardinal signs with many criteria. WS patients are usually observed as *having a* normal phenotype early in life until they reach *the* late 20s to early 30s, where the first ~~sign of~~ symptom*s* begin. The first clinical sign of WS ~~patients after the~~ *is a* lack of early

↳ what does this mean?

First Draft

adolescent spurt, which ~~are leading~~ to short stature, ~~losing~~ body weight, ~~suffering from~~ skin atrophy, ~~losing of~~ regional subcutaneous fat, and graying of hair. The loss of fat is usually related to the ulcerations in the regions such as elbows and ankles. In addition, the limb ulcers are influenced by neuropathy and vascular insufficiency, which ~~they~~ may require amputation later on. Others symptoms include type 2 diabetes mellitus, osteoporosis, bilateral ocular cataracts, several forms of arteriosclerosis, alopecia, malignancies, telangiectasia, and peripheral neuropathy (Leistritz et al. 2007).

The severity of patients with Werner syndromes can be explained by the different ~~in~~ functions of WRN gene, which plays a very important role in DNA replication, repair, and recombination. One ~~of~~ WS characteristic is ~~known for~~ increasing ~~in~~ genomic instability due to many mutations, which likely is the cause of cancer and many more diseases that normally ~~occur~~ late onset ~~for most~~ unaffected individuals (Crabbe et al. 2007). However, the common cause of death is usually related to myocardial infarction or cancers (Huang et al. 2006)

Genetic component of the disease

After the discovery of the Werner Syndrome in 1904 as part of ~~the~~ Otto Werner's dissertation research in Germany, many researchers and geneticists have been both accidently and directly ~~working on~~ identifying ~~this~~ WRN gene and its relative functions leading to the diseases and the different ~~in~~ phenotype ~~of the~~ affected individuals. Furthermore, between 1934 and 1941, two scientists from New York, Oppenheimer and Kugel, named the term "Werner Syndrome" igniting the popularity on this research topic. However, not until 1981 that the real genetic

First Draft

component, the WRN gene on chromosome 8, was discovered for the first time after centuries of study by many geneticists (Goto 2004).

Really? More than 100 years?

Werner Syndrome ~~in general~~ is ~~as mentioned,~~ caused by mutation of the WRN gene on chromosome 8. The approximate size ~~distances~~ of this gene is ~~about~~ 250 kb, containing about 35 exons, which ~~which~~ 34 of ~~those~~ are ~~the~~ protein coding ~~sequences~~ (Yu et al. 1996). The WRN protein ~~gene~~ is ~~also encoded for~~ a 180-kb multifunctional nuclear protein that belongs to ~~considered being in~~ the RecQ family of helicases (Grey et al. 1997). Among ~~the~~ WS affected individuals, their karyotypes usually differ from the normal population karyotype due to the abnormal expression of the mutated WRN gene. This difference in WRN expression can cause the loss of a ~~is one possibility of losing~~ telomere at the end of chromosome, which ~~as a~~ can consequently ~~creating a covalently~~ fusions between chromosomes or sister chromatids, reciprocal translocations, ~~and is also~~ leading to a change in karyotype (Crabbe et al. 2007).

One main Role of WRN ~~is involved in the RecQ family includes~~ requires an N-terminal domain that codes for exonuclease activity (Huang et al. 1998) and also where a domain for single strand-DNA annealing activity is happened to be located (Muftuoglu et al. 2008). Within this ~~region of~~ N-terminal domain with a single strand-DNA annealing activity of helicase and exonuclease, the main wild type functions are thought to be related to DNA repair, DNA recombination, and DNA replication of the chromosome (Brosh et al. 2006; Friedrich et al. 2010). Furthermore, the proteins produced from WRN gene are ~~is~~ also involved in telomere maintenance (Crabbe et al. 2007).

Are these the same domain or 2 different domains?

First Draft

The mutation in WRN create malfunction or ~~the absent~~ [absence] of the proteins. There are ~~recently~~ more than 70 WRN gene mutations found in human, such as nonsense mutations, missense mutation, substitutions at splice junction, and insertion/deletions have been showed in WS patients (Aydoğan et al. 2015). These different in mutations however are possible to cause some slightly different in the disease symptom.

[Handwritten margin note: Can you elaborate on this point? Are insertions/deletions more severe than missense mutations. Is there one mutation that is very common?]

Molecular biology and biochemistry of the disease

Study of Laure Crabbe in 2006 has proven that the cause of genomic instability in WS cells has the direct effect on the telomere dysfunction. Since human linear chromosomes undergo terminal sequence loss during cell replication and eventually the telomere become critically short (Crabbe et al. 2007).

The phenotype of the disease at the cellular level.. Cell and tissues involved are...

The WRN proteins involve with helicase and exonuclease activity (Yu et al. 1996). The main function of the proteins produced from WRN gene is required for cell growth and proliferation (Li et al. 2014). The cellular pathways involved of these WRN proteins that have been studying implied that it monitor and maintain DNA integrity (Rossi et al. 2010)

The mutant form of the gene affect the cell by..

There are known homolog of the protein in mice and also the mutation is in a conserved region of mice (Li et al. 2014).

First Draft

Clinical aspects and organismal phenotypes

Diagnostic test... Next-generation sequencing analysis of the WRN gene could be performed by looking for the WRN Frameshift mutation (Yang et al. 2015).

Variability in phenotype of WS is highly a penetrant gene at least for disease like cancer (Yang et al. 2015).

Role of the genetic element in other diseases....

Animal model is often performed with mice (Li et al. 2014).

Issues for the affected individual and treatments prospects

The major challenges faced by individuals with the disease....

The current state-of-the-art in detection, treatment, or avoidance...

(screening pre-imlantation embryos)

Some potential treatments that have been proposed or are in research and development... (Clinical trials?)

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synd/892 at Who Named It?

This is a good start, but you need to finish the paper—
turn the last two sections from outline form to ~~text~~ paragraph text.
Without this text, it is difficult for me to evaluate whether these
sections of the paper are adequate. If you can complete these sections
by ~~the end of the week,~~ Monday I will look at them and give you comments
before you turn in your final draft.

6/10

Introduction to Werner Syndrome

Werner Syndrome (WS) is one of the segmental progeroid syndromes diseases manifesting accelerated aging that affect multiple organs and tissues (Martin and Oshima 2000). The disease is named after the German scientist, Otto Werner who discovered and identified the syndrome from four siblings who had the symptom of premature aging in 1904. WS was discovered to result from rare autosomal recessive alleles of mutations at the WRN gene on chromosome 8, which encodes the RecQ DNA helicase (Yu et al. 1996). There is also the known chance that the disease mutations tend to run in families. Both sexes can be affected and also with even higher change between brother and sisters from the same carrier or infected parents since (Takemoto et al. 2013).

The global incidence rate of Werner Syndrome disease is less than 1 in 100,000 live births (Hasty et al. 2003). However, in Japan and Sardinia, they tend to have a higher rate of 1 in 20,000-40,000 and 1in 50,000, respectively (Masala et al. 2007). The approximate Lifespan for these WS patients is around 50 years old globally (Hasty et al. 2003), 47-48 in Japan, and 54 years in Sardinia (Masala et al. 2007).

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a lack of early adolescent growth spurt, which leads to short stature, loss of body weight, skin atrophy, Loss of regional subcutaneous fat, and graying of hair. The loss of fat is usually related to the ulcerations in the regions such as elbows and ankles. In addition, the limb ulcers are influenced by neuropathy and vascular insufficiency, which may require amputation later on. Others symptoms include type 2 diabetes mellitus, osteoporosis, bilateral ocular cataracts, several forms of arteriosclerosis, alopecia, malignancies, telangiectasia, and peripheral neuropathy (Leistritz et al. 2007).

The severity of patients with Werner syndromes can be explained by the different functions of WRN gene, which plays a very important role in DNA replication, repair, and recombination. One WS characteristic is increasing genomic instability due to many mutations, which likely is the cause of cancer and many more disease that normally have a late onset in unaffected individuals (Crabbe et al. 2007). However, the common cause of death among WS patients is usually related to myocardial infarction or cancers (Huang et al. 2006).

Genetic component of the disease

After the discovery of the Werner Syndrome in 1904 as part of Otto Werner's dissertation research in Germany, many researchers and geneticists have been both accidently and directly identified the WRN gene and its relative functions leading to the diseases and the different phenotypes between affected individuals. Later in 1934 to 1941, two scientists from New York, Oppenheimer and Kugel, named the term "Werner Syndrome" igniting the popularity on this research topic. However, it

was not until 1981 that the real genetic component, the WRN gene on chromosome 8, was discovered for the first time after centuries of study by many geneticists (Goto 2004).

Werner Syndrome is caused by mutation of the WRN gene on chromosome 8. The approximate size of this gene is 250 kb, containing about 35 exons, 34 of which are protein coding (Yu et al. 1996). The WRN protein is a 180-kb multifunctional nuclear protein that belongs to the RecQ family of helicases (Grey et al. 1997). Among WS affected individuals, their karyotypes usually differ from the normal population karyotype due to the abnormal expression of the mutated WRN gene. This difference in WRN expression can cause the loss of a telomere at the end of chromosome, which can consequently create fusions between chromosomes or sister chromatids, reciprocal translocations, leading to a change in karyotype (Crabbe et al. 2007).

One main role of WRN requires an N-terminal domain that codes for exonuclease activity (Huang et al. 1998) and also where a domain for single strand-DNA annealing activity is happened to be located (Muftuoglu et al. 2008). Within this N-terminal domain with single strand-DNA annealing activity of helicase and exonuclease, the main wild type functions are thought to be related to DNA repair, DNA recombination, and DNA replication of the chromosome (Brosh et al. 2006; Friedrich et al. 2010). Furthermore, the proteins produced from WRN gene are also involved in telomere maintenance (Crabbe et al. 2007).

The mutations in WRN create malfunction or absence of the proteins. There are more than 70 WRN gene mutations found in human, such as nonsense

mutations, missense mutation, substitutions at splice junction, and insertion/deletions have been showed in WS patients (Aydogan et al. 2015). These different in mutations however are possible to cause some slightly different in the disease symptom.

Molecular biology and biochemistry of the disease

Study of Laure Crabbe in 2006 has proven that the cause of genomic instability in WS cells has the direct effect on the telomere dysfunction. Since human linear chromosomes undergo terminal sequence loss during cell replication and eventually the telomere become critically short (Crabbe et al. 2007). This is one important concept because one of telomere main function is to maintain the chromosome stability. Abnormalities phenotype or shortening of the telomere region on the chromosome may eventually leads to complication like chromosomal breakage and translocation (Friedrich et al. 2010).

The phenotype of the disease at the cellular level is usually observed by visualizing the telomere region, repeated sequences at both ends of the chromosomes. Since the mutation in the WRN gene is proven to have the direct affect on shortening the telomere on the chromosomes, different phenotypes at the cellular may be able to identify. Among these differences, the chromosome breakage and translocation of chromosomes, rejoined region of two different chromosomes may be able to visualized in the karyotype as well (Friedrich et al. 2010).

There are many cell types and tissues involved with the Werner Syndrome. Because of the mutations within the WRN gene that is normally function as the

growing stimulator and telomere maintaining mechanism. Mutations in WRN gene then is possible to interrupt the normal function of the gene, causing the change in different types of protein productions, which leads to the abnormal development of the effected cells and tissues. These cells and tissues abnormalities tend to manifest the old person age phenotype, which again leads from the malfunction of the proteins produced from the mutant WRN gene. Many obvious examples are greying of hair color from the effected hair cells, and early ulcerations of the skin from the effected skin cells and tissues.

The WRN proteins involve with helicase and exonuclease activity (Yu et al. 1996). The main function of the Werner protein produced from WRN gene is required for cell growth and proliferation (Li et al. 2014). The cellular pathways involved WRN protein that has been studied implied that it monitor and maintain DNA integrity (Rossi et al. 2010). Other known tasks of this protein are maintenance and DNA repair. The protein also supports the process of DNA replication as a preparation for cell division.

Mutations in the WRN gene lead to the production of an abnormally short and nonfunctional WRN protein. This protein is also believed to broken down very easy and unable to transport to the cell's nucleus, where it normally interact with DNA. However, we are not yet fully understood how the mutations in WRN gene lead to WS signs and symptoms. One assumption would be that cells with mutant WRN protein tend to divide more slowly and stop dividing earlier compared to the normal cells, which affect growing process of the individuals carrying the disease. In addition, the nonfunctional WRN protein also allows the DNA damage to increase,

which disrupts the normal cells functions and the cells will usually be associated with diseases, cancers (Aggarwal et al. 2011).

The WRN gene also found to be highly conserved within mice population. Many scientists discovered known homolog of the proteins in mice and also the mutation is in a conserved in the region of WRN gene in mice (Li et al. 2014). The study shows that the mutations in the mouse WRN gene also cause the early death and many complication age-related disorders, similarly to human.

Clinical aspects and organismal phenotypes

The most popular diagnostic test for Werner is the Next-generation sequencing analysis of the WRN gene, looking for the WRN mutations, especially Frame shift mutation (Yang et al. 2015). However, there are different levels of the diagnostic options. For example, the level of biochemical genetic test likes protein analysis. Another level is molecular genetics tests, which is for mutations like deletion/duplication analysis, and sequencing analysis of the entire coding region is also fall into this group of diagnostic.

Variability in phenotype of WS is highly a penetrant gene at least for disease like cancer (Yang et al. 2015). Many individuals could become a carrier that the disease alleles are penetrant. However, WS phenotype could be extremely expressivity when the individuals carry two mutant alleles in the WRN gene, which lead to the manifestation of the normal aging-related diseases.

Role of the genetic element in other disease like cancer is very well studying right now. According to the Liu Yang study in 2015 identified that novel WRN

frameshift mutation was identified in three cancer patients and one in the youngest

unaffected daughter, carrier. In the other disease involving cardinal muscle is also

highly possible to be affected by the genetic element abnormalities of the WS.

Issues for the affected individual and treatments prospects

To detect the disease early on, the family members of the infected individual

with the Werner Syndrome are required to have the sequencing analysis tested for

the mutant WRN gene. The couples consulting for sequencing analysis test before

having children can also be performed to avoid having the infected children with the

WS.

Many treatments or technics for several symptoms of Werner Syndrome

have found to be very effective. For example, treatment for skin ulcers with

standard or novel techniques is proven to help reducing the causative of the

symptom (Yeong & Yang 2004). Another treatment option for skin ulcers is the

Bosentan, digital ulcers treatment, which it is proven to be very effective in people

with Werner syndrome having complication with the skin ulcers (Matucci-Cerinic et

al 2011). Scanning for type 2 diabetes mellitus and consulting with the physician for

controlling the symptom may also help. Acceptable results have been reported with

use of pioglitazone to treat for the insulin resistance in-patient with WS (Imano et al

1997). Regular treatment for malignancies in WS is also one of the options to treat

for tumors and cancer related symptoms.

The major challenges faced by individuals with the disease is always

involving maintaining the healthy conditions, since many abnormalities both

genotype and phenotype of the infected individuals are constantly increase

overtime. Since it is very difficult to treat or reduce the symptoms of the WS.

Individuals could prevent secondary complications by adjusting their Lifestyle. For

example, smoking avoidance, regular exercise, and weight control to reduce

atherosclerosis risk are extremely recommended by the physicians. Excellent skin

care, trauma avoidance, and examination to treat problems early may also help.

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Numbered citations should correspond to the order of first appearance in the paper (so this should be ref [1])

Sickle Cell Disease

The foundation of sickle cell disease is built on the polymerization of deoxy

in what? Not like a physical deformity...

sickle hemoglobin (HbS) causing deformity and complications to arise when vasoocclusion and

follows suit... but what do you mean by that?

hemolytic anemia occur [3]. This disease follows suite and individuals benefits from phenotypic

↳ benefit how?

heterogeneity. Those that are homozygous dominant are completely unaffected while those that

for wild-type alleles

are homozygous recessive typically suffer from anemic disorders. Heterozygous individuals are

→ what? The fact that heterozygous individuals are unaffected? Isn't that because disease alleles are recessive?

often unaffected by the downfalls of the disease, but maintain some beneficial aspects. This can

be explained by genetic variability of fetal hemoglobin gene expression and co-inheritance of α

thalassemia [3].

what does this mean?

Sickle cell disease is not limited to Mendelian genetics, genotypically, there are a

variations of homozygosity. The HbS mutation is the most common and well-versed form of this

disease. Other genotypes are apparent when heterozygosity is produced via HbS and other

hemoglobin variants such as HbC, HbE, and HbD [3]. The β-globin genotype is most

concentrated in most of all sickle-cell based genetic studies. However, HbS-β thalassemia can

play a significant role in heterozygosity.

has   *through confering*

Heterozygosity has seen benefits in resistance to malaria. Innate resistance represents the

what is the significance of this statement.

mechanisms of resistance that work in the early stages of diseases or infections. This precedes

the immune response that adapts in order to take over after the course of a few days [1].

Resistance to human malaria was first genetically demonstrated in 1954 when sickle-cell

heterozygous individuals responded significantly less to *Plasmodium falciparum* infections [1].

Continued research including genome-wide associations has identified the HBB locus to be the

source of this resistance. Populations living near the coasts Kenya and Lake Victoria ~~illustrate~~ [have]

higher frequencies of sickle-cell heterozygotes than those in the highlands where malaria is

uncommonly transmitted [1]. This follows the concept of positive selection. ~~With~~ [In] populations

living in areas where malaria is high, there has been a genetic shift ~~in genotypic frequencies of~~ [towards an]

[in] increase~~d~~ heterozygotes because these individuals show greater fitness. This explains the absence

of a selective sweep involving the removal of the sickle cell allele. This is supported by studies

of *P. falciparum* transmission into individuals, (AS, AA, SS). Sickle-cell heterozygotes (AS)

survive better than those with normal hemoglobin, homozygous dominant (AA) [1].

Homozygous recessive (SS) have the highest mortality rate due to the symptoms of the deformed

red blood cells.

The carrier form of HbS has been found to be negatively associated with all potentially

lethal forms of falciparum malaria; however, HbC and α-thalassemia were limited to cerebral [do you mean protection against malaria?]

malaria and severe anemia, respectively [1]. HbS red blood cells are assumed to have specific

physical or biochemical properties of which affect the invasion, growth, and development of *P.*

falciparum parasites. [6].When such cells undergo in vitro, parasite-infected HbAS red blood

cells also tend to sickle. In Sudan, the immune system's recognition of *P. falciparum* infected red

blood cells was greater in children in addition to the up-regulation of malaria-specific cell-

mediated immune responses [10]. A variety of studies have shown that malaria resistance is

enhanced in younger patients. It is suggested that if malaria protection by HbS in AS individuals

was an innate resistance, it should be independent age [1].

Chronic intravascular hyper-hemolysis has been found to characterize a new, specific

type of sickle cell anemia subphenotype. Identification of this may highlight the finding of

disease modifying factors in order to develop new therapies [7]. This novel subphenotype has

shown signs of reduced vasoocclusive pain but sooner mortality rates as well as increased reticulocytosis which is a physiological response to extravascular and intravascular red blood cell destruction [7]. Lactic dehydrogenase (LDH) can be distributed in order to measure hemolysis levels and would be a possible identifier of this subphenotype. Association of lower HbF levels has been found in relation to decreased α thalassemia, a genetic factor that has been found to reduce anemia and hemolysis [7]. This subphenotype has illustrated increased severity as high levels of HbF are associated with reduced pain, acute chest pain, and mortality as HbF has the ability to inhibit polyermization of HbS. In turn, this decreased vasoocclusion and hemolysis [7].

Another common symptom of sickle disease is hypoxia which is especially common in children. This has been associated with increased severity of the disease characterized by low levels of HbF resulting in cases of abnormal lung function [4]. This can only partially be explained by anemia as one study found that it represented only 5% of arterial oxygen desaturation. [4]. One factor that potentially explains the diminished affinity of HbS for oxygen is the increased concentration of erythrocyte 2,3-biphosphoglycerate. This decreased the life span of an erythrocyte via apoptosis due to increased Ca (2+) activity [5].

A major cause of death in patients with sickle cell disease may be pulmonary hypertension. Frequent reports of sudden death in adults with sickle cell disease in the absence of coronary artery disease has been associated with hemolysis [4]. Despite having lower pulmonary-artery pressures and higher cardiac outputs than patients with primary pulmonary hypertension, patients with sickle cell disease and pulmonary hypertension had a significantly higher mortality rate than did patients with sickle cell disease who did not have pulmonary hypertension. [2] Chronic hemolysis releases hemoglobin into plasma which can catalyze the

reaction between oxygen and nitrogen resulting in acute and chronic pulmonary vasoconstriction by increasing the transcriptional up-regulation of adhesive molecules [2].

Echocardiography can be used to identify at-risk patients through the noninvasive measurement of tricuspid regurgitant jet velocity. This prognostic tool can provide additional data alongside the transcranial carotid Doppler flow-velocity assessment, which is used to predict the risk of stroke in children with sickle cell disease. [2] Research has found hydroxyurea increases levels of HbF, therefore, decreasing morbidity from vasoocclusive complications in patients [8]. A more extreme form of treatment is the transplantation of hematopoietic stem cells. Unfortunately, patients with hemoglobin-based diseases, such as sickle cell disease, have been found to have transplant rejection more frequently than other blood disorders including aplastic anemia, severe combined immune deficiency, and malignant conditions [9]. The common downfall results from iron overload leading to liver disease. Patients with β-thalassemia who undergo transplantation early have seen a higher success rate [9].

It is vital to continue research on not only treatments, but the fundamental aspects of the disease. By understanding the biochemical breakdown of sickle cell disease, therapeutic options can be enhanced in addition to the potential for a cure. The variations discovered thus far have demonstrated the complexity of the disease as well as an opportunity to work from a genetic basis to a physiological understanding. Sickle-cell disease still contains many unknowns and maintains an unpredictable course; however the benefits of heterozygosity provide justification of continued research.

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The description of the symptoms and complications of sickle-cell are very thorough, if technical. But the description of the genetic component needs to be clarified and re-organized. I suggest the following structure →

What is the gene or genes that are mutated in sickle-cell anemia?

How many alleles are there and how do they interact to cause disease?

What goes wrong at the cellular, tissue, or whole-body level in affected individuals? (Complications as well...)

What are the current and future treatment options?

Malaria resistance could go either place

You have many of these ideas already in sentences in the paper, it's a matter of putting them together in the right way.

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<div align="center">Sickle Cell Disease</div>

The foundation of sickle cell disease is built on the β-globin variant found on the short arm of chromosome 11 [1]. Hemoglobin composition changes throughout development. Fetal hemoglobin (HbF) is composed of two α-globin proteins and two γ-globin protein [2]. Adult hemoglobin (HbA) will have two β-globin proteins adjoining two α-globin proteins [1,2]. At birth, the infant has roughly 80% HbF and 20% HbA. This transition is complete around 6 months with HbA being the homozygous dominant, or wild-type [2]. Deformity of red blood cells into a crescent-shape is due to variation in the β-globin amino acid chain. At position 6, valine replaces glutamic acid causing the polymerization of deoxy sickle hemoglobin (HbS). This leads to the formation of long protein chains causing complications such as vasoocclusion and hemolytic anemia [1,3].

With this disease, those that are homozygous for wild-type alleles (HbA) are completely unaffected while those that are homozygous recessive typically suffer from anemic disorders. Recessive homozygosity can result from two copies of HbS or one copy of HbS and another β-globin variant such as HbC, HbE, and HbD [1,3]. In addition, recessive variations include α thalassemia and β thalassemia [3]. Thalassemia is a Greek word referring to autosomal inheritance of blood disorders. Alpha-thalassemia results from a mutation on chromosome 16 causing a deficiency in α-globin proteins which in turn produces excess β-globin proteins. Because this mutation occurs on different chromosome, another gene separate from the one for sickle cell disease, it does not affect the genotype. However, it has shown to have phenotypic implications [4]. Beta-thalassemia is caused by the deficiency of β-globin proteins leading to

additional α-proteins, and the mutation is found on chromosome 11 [2]. Beta-thalassemia

mutations are also allelic variations of the globin genes that can be passed on to the offspring [1].

There are two types of β-thalassemia, β^0 and β^+. Both cause a reduction in β-globin; however, β^0

causes complete absence of hemoglobin synthesis leaving those individuals with phenotype

produced by the other copy present [2]. If they are HbS/ β^0 thalassemia, they would be

phenotypically similar to those that are HbS/HbS. Most patients with β^+ have shown milder

forms of the disease as it still produces some normal hemoglobin [3].

Heterozygous individuals are often unaffected by the downfalls of the disease, but

maintain an additional beneficial aspect not seen in wild-type individuals. Heterozygosity has

benefits through conferring resistance to malaria without the harmful side effects [3]. This is due

to production of HbS from only one allele. Because the other allele for heterozygotes (HbA)

produces normal hemoglobin, the symptoms such as vasooclusion and anemia to not develop, yet

these individuals are still protected from malaria [1,3]. Resistance to human malaria was first

genetically demonstrated in 1954 when sickle-cell heterozygous individuals responded

significantly less to *Plasmodium falciparum* infections [4]. Continued research including

genome-wide associations has identified the HBB locus to be the source of this resistance.

Populations living near the coasts Kenya and Lake Victoria have higher frequencies of sickle-

cell heterozygotes than those in the highlands where malaria is uncommonly transmitted [4].

This follows the concept of positive selection. In populations living in areas where malaria is

high, there has been a genetic shift toward an increase in heterozygotes because these individuals

show greater fitness. This explains the absence of a selective sweep involving the removal of the

sickle cell allele. This is supported by studies of *P. falciparum* transmission into individuals,

(HbA/HbS, HbA/HbA, HbS/HbS). Sickle-cell heterozygotes (HbA/HbS) survive better than

those with normal hemoglobin, homozygous dominant (HbA/HbA) [4]. Homozygous recessive (HbS/HbS) have the highest mortality rate due to the symptoms of the deformed red blood cells.

The carrier form of HbS has been found to be negatively associated with all potentially lethal forms of falciparum malaria; however, HbC and α-thalassemia have not shown protection against malaria and are limited to conditions of cerebral malaria and severe anemia, respectively [4]. HbS red blood cells are assumed to have specific physical or biochemical properties which affect the invasion, growth, and development of *P. falciparum* parasites. [5].When such cells undergo in vitro, parasite-infected HbA/HbS red blood cells also tend to sickle. In Sudan, the immune system's recognition of *P. falciparum* infected red blood cells was greater in children in addition to the up-regulation of malaria-specific cell-mediated immune responses [6]. A variety of studies have shown that malaria resistance is enhanced in younger patients. At first it was thought that the resistance is innate in that the mechanisms of resistance work in the early stages of diseases or infections. This precedes the immune response that adapts in order to take over after the course of a few days [4]. However, it is suggested that if malaria protection by HbS in HbA/HbS individuals was an innate resistance, it should be independent of age which is not consistent with the findings of the study in Sudan [4].

Chronic intravascular hyper-hemolysis has been found to characterize a new, specific type of sickle cell anemia subphenotype. Identification of this may highlight the finding of disease modifying factors in order to develop new therapies [7]. This novel subphenotype has shown signs of reduced vasoocclusive pain but sooner mortality rates as well as increased reticulocytosis which is a physiological response to extravascular and intravascular red blood cell destruction [7]. Lactic dehydrogenase (LDH) can be distributed in order to measure hemolysis levels and would be a possible identifier of this subphenotype. Association of lower HbF levels

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Analyzing the Genetic and Clinical Aspects of the Mutation in the Cystic Fibrosis Transmembrane Regulator Gene

Dr. Colin Meiklejohn
Human Genetic Disease Paper

Abstract

caused by

✓ *CF is* ~~An~~ *a* extensively studied disorder. Cystic fibrosis ~~is~~ a monogenic inherited disease. This
results from a

disease ~~is a~~ mutation in the cystic fibrosis transmembrane regulator gene. *(CFTR)* ~~The~~ CFTR ~~encoded~~ *encodes* for

a complex protein that is vital to normal functioning. This research paper compiles information

over the disorder and examines life with the disease from a clinical, molecular, and genetic

viewpoint. The method for writing included looking over various scientific articles. Both review

papers and peer reviewed articles were considered. — *FYI, most review papers are peer-reviewed.*
The distinction between ~~new~~ review articles and
primary literature is whether new data is
presented in the paper (no for review papers)

Introduction

Cystic fibrosis is a fairly common autosomal recessive disorder in European populations.
by
It is characterized ~~as~~ thick and sticky fluid in the cells that produce mucus, sweat and digestive

juices. Symptoms include pain in the abdomen, burning sensation in the chest, coughing,

wheezing, and gastrointestinal problems. Patients with CF will have salty-tasting skin, frequent

lung infections, poor growth and weight gain, frequent greasy, bulky stool or problems with

bowel movements, and even infertility in males. The secretions of the cell aren't thin and

slippery as in normal people, so instead of acting as a lubricant, the secretions plug up tubes,

ducts, and passageways. The buildup of mucus traps bacteria, which increases the risk for

infection. This leads to lung damage and eventually respiratory failure. The mucus buildup

prevents the release of digestive enzymes from the pancreas, making the break down and

Absorbance of food difficult. The disorder required daily attention but those diagnosed with it can live fairly normal lives with an average life expectancy in the 20s and 30s.

Cystic fibrosis has a prevalence of 1 in 2500 European populations. Cystic fibrosis occurs in roughly 1 in 2000 births in northern European population. Approximately 30,000 people in the US are living with cystic fibrosis. Every year there are 1,000 new cases. The majority of people with CF are diagnosed by two years old.

It is speculated that cystic fibrosis has been around since 3,000 BC, although the first paper wasn't published about it until 1938. Dorothy Hansine Andersen published "Cystic Fibrosis of the Pancreas and its Relation to Celiac Disease: A clinical and Pathological Study," in the *American Journal of Disease of Children*. It is a complex disorder and the symptoms and severity of the symptoms vary from case to case. The information presented in the Introduction needs to be cited, like you do for other information later on in the paper

Genetic Component

People with cystic fibrosis have inherited two copies of the defective gene. Each copy must come from a parent meaning that both parents must be carriers of at least one copy of the gene for their offspring to inherit the disease. Carriers of the disease only have one copy and do not show any of the symptoms.

In 1989 cystic fibrosis was found to be caused by mutations in a previously unstudied gene. The novel cystic fibrosis transmembrane regulator (CFTR) gene was predicted to regulate transmembrane conductance, and was subsequently shown to function as a channel that allows chloride ions to pass through the cell membrane. A great deal was discovered about aspects of CFTR biology that would inform diverse fields such as protein trafficking and membrane

Transport. According to the Cystic Fibrosis Mutation Database there are currently 1,991 mutations CFTR mutations. The most common mutation, F508 is a deletion of three nucleotides. This results in a loss of the amino acid phenylalanine (F) at the 508th position on the protein. The gene (deletion) F508 on chromosome 7 has been found in approximately 70% of gene carriers for Cystic Fibrosis and provides an opportunity to offer detection of carrier state both to members of the general populations and to members of families whom cystic fibrosis has been diagnosed (Knight *et al.* 1990). This mutation alone accounts for two-thirds of the mutations in Cystic Fibrosis worldwide.

If you were to look at the karyotype of an affected individual you would not be able to see a difference. In other words the karyotype of an affected in individual looks the same as a normal individual. This is because the CF mutation is too small to be seen on a karyotype.

It's fine to include this point but it's not particularly interesting in the case of CFTR. Instead, can you say something more about the mutations that cause CF? In addition to F508, how many other mutations are there? Are they indels, missense, nonsense, etc

The product of the CFTR gene is a chloride ion channel important in creating sweat, digestive juices, and mucus. When there is a mutation in this gene it prevents normal secretion from the chloride ion channels. The absence of this gene function is what cause the thick mucus in patients with CF.

This isn't right...
CFTR protein?

The CFTR gene was cloned and identified to produce the protein cAMP which regulates chlorine ion channels. This seemed to be the cause of the thickening of mucus. It was believed that lack of this gene is what caused the hyper-mucosity. Further research has found that the mechanism for this is complex and varies from organ to organ with lungs being the most complex. It is now hypothesized that is actually a gene downstream to this gene that causes the symptoms that we see in the people with the disease. The mucus hyper-production that typifies CF does not appear to be a direct cause of a defective CFTR but, rather, to be a downstream

Consequence. In organs like the lung, up-regulation of mucin gene expression by inflammation results from chronic infection; however, in other instances and organs, the inflammation may have a non-infectious origin (Kreda, 2016). Since there are many variations of the disease it is hard to pinpoint the exact cause and it may not be the same for all mutations.

Molecular Biology and Biochemistry of disease

Mutations in the ~~cystic fibrosis transmembrane conductance regulator~~ (CFTR) gene affect multiple organs, including the intestine, sweat glands, pancreas and the reproductive system, but cystic fibrosis lung disease causes most morbidity and leads to premature mortality in cystic fibrosis patients (Griesenbach et al., 2016). CF mostly affects the epithelium cells of the lungs, pancreas, liver, and kidneys. The mutation causes either loss of function or dysfunction. The reason that death from lung infection is so predominant is due to the dehydration of liquid on the surface of airways. This prevents ciliary action of the cells that normally move mucous away. Another alternative is that disorder causes inflammation in the host. Patients with CF are susceptible to opportunistic bacteria, most notably Pseudomonas aeruginosa (Zemanick et al. 2010). This theory that inflammation is what causes eventual lung breakdown is less widely accepted. The role of CFTR expression in inflammatory cells such as neutrophils, macrophages, and more recently T cells, has been widely, and so far, inconclusively debated, but studies overall appear to suggest a potential defect in adaptive immune responses in patients with CF, which may explain the exaggerated pulmonary inflammatory responses that have been generally observed, an area that requires further studies (Griesenbach *et al.*, 2015).

CFTR ~~gene~~ is found at the q31.2 locus of chromosome 7. It is 230,000 base pairs long, and encodes for a protein that is 1,480 amino acids long. While there are approximately 2,000

do you mean different alleles?

variations of diseases in the CFTR gene, there is no evidence that all these cause the disease phenotype. CF allele distribution varies by population. It is hypothesized that having CF

Can you expand on this point? How would this benefit occur? Is the benefit hypothesized to be associated with the CF disease, or might heterozygous carriers get this benefit?

benefited from resistance to Cholera and other causes of diarrhea. A study done by Patrick Sosnay at John Hopkins University looked at genotype and phenotype data for 39,696 affected people in clinics and registries and clinics in North America and Europe. In these individuals, 159 CFTR variants had an allele frequency of 0.01%. These variants were evaluated for both clinical severity and functional consequence, with 127 (80%) meeting both clinical and functional criteria consistent with disease. Assessment of disease penetrance in 2,188 fathers of individuals with cystic fibrosis enabled assignment of 12 of the remaining 32 variants as neutral, whereas the other 20 variants remained of indeterminate effect (Sosnay et al., 2013). Those with a single working copy are carriers, but otherwise function normally. The most current theory on how cellular defect and cause clinical effects is that defective ion transport leads to dehydration in the airway epithelia, and therefore thickening mucus. Population based studies of CF are becoming more popular, with 1.2 million people subjects in the US alone. Genetic testing is highly recommended for people who are showing symptoms and for couples who might be carriers for the gene.

Citations for this paragraph?

Is there a broader point to be made from this study, or is it just showing that there are lots of different CFTR mutations that cause CF?

Clinical Aspects and organismal phenotype

Screening included _for CF_ testing for immunoreactive trypsinogen, then confirmation through a scan for CFTR mutations. Most children are diagnosed by six months of age. Diabetes is the most common non-pulmonary complication, but lung disease and infertility are also issues.

There is a wide variability in the disease and most treatments are tailored to the individual. Most treatment options are aimed at reducing symptoms of the disease rather than the cause of the disease. There are a variety of different therapies that patients can go through. Airway clearance helps to get rid of thick mucus in the lungs. This therapy may require a respiratory therapist. An inflatable vest vibrates the chest at high frequencies to help loosen and thin mucus. Inhaled medicines can also aid in opening air passageways. A liquid medicine is usually made into a mist or aerosol. The medicine is then inhaled via a nebulizer. Pancreatic enzyme supplement capsules can also be taken with every meal to help facilitate the absorption of important nutrients. Antibiotics include Piperacillin, Azithromycin, Aztreonam, Ciprofloxacin, and Tobramycin. → _why are CF patients given these antibiotics?_

CF is Equally prevalent in males and females, but females have a slightly shorter life expectancy. The reason for this is unknown but the gap is decreasing as healthcare becomes better and more accessible. Similar "gender gaps" _what is this?_ have been seen in Asthma and COPD. Researchers have speculated that sex hormones play a role in this gap. More research needs to be done as there are many factors affecting life span such as exercise, nutrition, socioeconomics, and environment.

People suffering from CF are often given a special diet or a list of recommended foods. This diet is high in fat and calories. Since the mucus is so thick in the intestines, it makes it hard

for ? fat-soluble

For nutrients like fats vitamins to be absorbed. That is why teenagers suffering from this disorder are usually short and underweight for their age. This also makes them more susceptible to illness as their bodies can't fight off infections very well. Consuming extra calories aids in fighting off infections as well as keeping their lungs strong. Even if the patient isn't feeling hungry it is necessary to keep consuming food to fight off malnutrition. Just like anyone else they need a well-balanced diet. It is recommended that they consume 2,900-4,500 calories per day. Just like any other factor of the disease, everyone person has their own nutritional needs. Patients are able to work with a CF nutritionist to develop a diet plan for weight gain.

Issue for affected individuals and treatments

There is no cure for CF and it is a chronic illness. It puts a great deal of emotional stress on a person as well as their family. People with CF need constant care, expensive medication, and are more prone to depression or anxiety. The disorder is lifelong and also shortens the lifespan of the patient. Those suffering from CF are more prone to depression only due to the amount of stress they are under combined with the decrease in quality of life. Exercise can be beneficial to those suffering because it releases endorphins as well as strengthening the lungs capacity. People with CF shouldn't smoke or it will increase their chances of lung disease. Despite requiring daily care, CF doesn't stop people from performing daily tasks such as working or going to school. Patients are required to check in with a doctor every three months and the better treatment they receive the longer lifespan they will have.

Treatment includes antibiotics, chest physiotherapy, and pancreatic enzyme replacement for those with pancreatic insufficiency. Lung transplants are also an option. Gene therapy has

b
Been explored as a potential cure for CF. It is focused around trying to put a functional copy of

the CFTR gene into affected cells. Gene transfer has been attempted multiple times, ~~such as~~ with

liposomes and viral vectors in animal models and in clinical trials. Unfortunately these methods

were inefficient. This is because the very few of the cells take up the vector and express the gene.

Pulmonary gene therapy is especially difficult because the lung is a complex organ designed to

keep out foreign bacteria and viruses. Some gene therapy may not completely fox CFTR gene _fix?_

function but even a little bit can improve lung disease. The discovery that even a slight improvement

in gene function can improve the disease has renewed interest in gene therapy as a form of

treatment. Extensive antimicrobial treatment can be used to eradicate infections in the lungs.

Unfortunately ~~the resistant nature of bacteria~~ this gives rise to resistant strains. of bacteria

   The US food and Drug administration approved the first drug that targeted a cause of

cystic fibrosis in 2012. This drug, ivacaftor, helps to reopen chloride channels that are closed by

G551D mutation. Until then most drugs were only administered to treat symptoms of the

disorder. The FDA approved the second drug to target the root cause of CF in 2015. These CFTR

modulators are a breakthrough in treatment and can add decades to the life of CF patients.

   The possibility of a lung transplant can improve the lives of patients. In this surgical

procedure the unhealthy lung is removed and replaced with a healthy one. A lung transplant

requires a lot of planning and is fairly expensive. Sometimes a transplant called living donor

lobar lung transplant is performed where only the lower lobe of the lung is transplanted. The

major risk of this procedure is rejection of the organ or infection. It is only recommended in

people who have extensive lung damage. It is important to note that the transplant does not cure

How many patients have recieved this type of drug? How are they doing? It is probably too early to say that these drugs add decades to CF patient life since they were approved in 2012 & 2015.

the

~~The~~ problem of CF and the new lung will also be affected by the thickening of the mucus. That is why this procedure is less common.

Conclusion

In some countries, newborns are screened for this disorder. The knowledge about the disease is expanding and many new treatment*s* have finished clinical testing and being acclimated into mainstream treatment. Our understanding of cystic fibrosis pathophysiology and genetics is constantly expanding and being refined, leading to improved management of the disease and increased life expectancy in affected individuals (Griesenbach *et al.*, 2016). There is still much that is not known about CF such as what other modifier genes affect disease progression. There are other challenges like adequate healthcare. Most drugs for CF are expensive and not everyone can afford them. Even more important a lot of countries don't have access to sufficient health care. Developing drugs is a long and costly process. While scientists may be close to finding a permanent cure, it will be years before the drug is put on the market. Current medicine has greatly improved the quality of life for people living with CF. Hopefully patients will see a breakthrough in treating the cause of the disease. Either way one thing is for certain, more research needs to be done.

You need to include a bibliography with the references you cite at the end of the paper. You also need to cite the sources of your information throughout the paper, not just in the few places where you have done so. Otherwise, you have made a good start with this draft.   8/10

Analyzing the Genetic and Clinical Aspects of the Mutation in the Cystic Fibrosis Transmembrane Regulator Gene

BIOS 412
03/29/16
Dr. Colin Meiklejohn
Human Genetic Disease Paper

Abstract

Cystic fibrosis is an extensively studied disorder caused by a monogenic inherited disease. This disease results from mutation in the cystic fibrosis transmembrane regulator gene. CFTR codes for a complex protein that is vital to normal functioning. This research paper compiles information over the disorder and examines life with the disease from a clinical, molecular, and genetic viewpoint. The method for writing included looking over various scientific articles.

Introduction

Cystic fibrosis is a fairly common autosomal recessive disorder in European populations. It is characterized by thick and sticky fluid in the cells that produce mucus, sweat, and digestive juices. Symptoms include pain in the abdomen, burning sensation in the chest, coughing, wheezing, and gastrointestinal problems. Patients with CF will have salty-tasting skin, frequent lung infections, poor growth and weight gain, frequent greasy, bulky stool or problems with bowel movements, and even infertility in males. The secretions of the cell aren't thin and slippery as in normal people, so instead of acting as a lubricant, the secretions plug up tubes, ducts, and passageways. The buildup of mucus traps bacteria, which increases the risk for infection. This leads to lung damage and eventually respiratory failure. The mucus buildup prevents the release of digestive enzymes from the pancreas, making the break down and

absorbance of food difficult. The disorder requires daily attention but those diagnosed with it can live fairly normal lives with an average life expectancy in the 20s and 30s.

Cystic fibrosis has a prevalence of 1 in 2500 European populations. Cystic fibrosis occurs in roughly 1 in 2000 births in northern European population (Zemanick et al. 2010). According to the National Institute of Health approximately 30,000 people in the US are living with cystic fibrosis. Every year there are 1,000 new cases. The majority of people with CF are diagnosed by two years old.

It is speculated that cystic fibrosis has been around since 3,000 BC (Busch 1989). Although the first paper about CF wasn't published until 1938. Dorothy Hansine Andersen published "Cystic Fibrosis of the Pancreas and its Relation to Celiac Disease: A clinical and Pathological Study," in the *American Journal of Disease of Children* (Andersen 2009). It is a complex disorder and the symptoms and severity of the symptoms vary from case to case.

Genetic Component

People with cystic fibrosis have inherited two copies of the defective gene. Each copy must come from a parent meaning that both parents must be carriers of at least one copy of the gene for their offspring to inherit the disease. Carriers of the disease only have one copy and do not show any of the symptoms.

In 1989 cystic fibrosis was found to be caused by mutations in a previously unstudied gene. The novel cystic fibrosis transmembrane regulator (CFTR) gene was predicted to regulate transmembrane conductance, and was subsequently shown to function as a channel that allows chloride ions to pass through the cell membrane. A great deal was discovered about aspects of

CFTR biology that would inform diverse fields such as protein trafficking and membrane transport. According to the Cystic Fibrosis Mutation Database there are currently 1,991 CFTR mutations. The most common mutation, F508 is a deletion of three nucleotides. This results in a loss of the amino acid phenylalanine (F) at the 508th position on the protein (Griesenbach et al., 2016). The F508 deletion on chromosome 7 has been found in approximately 70% of gene carriers for cystic fibrosis and provides an opportunity to offer detection of carrier state both to members of the general populations and to members of families whom cystic fibrosis has been diagnosed (Knight *et al.,* 1990). This mutation alone, accounts for two-thirds of the mutations in cystic fibrosis worldwide.

The different mutations that cause CF can be divided into four classes. This first class of mutations affect protein production and include the nonsense mutation G542X. There are mutations throughout the CFTR gene that produce premature termination signals because of splice site abnormalities, frameshifts due to insertions or deletions, or nonsense mutations (Tsui, 1992). The second class of mutations affect processing, where proteins fail to progress through the biosynthetic pathway. This includes the most common mutation, deletion of phenylalanine at residue 508 (F508). The third class is defined by problems in protein regulation. An example of this is the G551D mutation. Although missense mutations have been reported in the regulatory domain, there appear to be fewer mutations than in other parts of the protein (Welsh and Smith, 1993). The fourth class of mutations causing CF result from defective conducting, where the rate of ion flow through a single open channel is reduced. The missense mutation R117H in the first membrane-spanning domain falls into this class.

The product of the CFTR gene is a chloride ion channel important in creating sweat, digestive juices, and mucus. When there is a mutation in this gene it prevents normal secretion from the chloride ion channels. The absence of this gene function is what cause the thick mucus in patients with CF.

The CFTR gene was cloned and identified to produce a protein, the CFTR protein that regulates chlorine ion channels. This seemed to be the cause of the thickening of mucus. It was believed that lack of this gene is what caused the hyper-mucosity. Further research has found that the mechanism for this is complex and varies from organ to organ with lungs being the most complex. It is now hypothesized that it is actually a gene downstream to this gene that causes the symptoms that we see in the people with the disease. The mucus hyper production that typifies CF does not appear to be a direct cause of a defective CFTR but, rather, to be a downstream consequence. In organs like the lung, up-regulation of mucin gene expression by inflammation results from chronic infection; however, in other instances and organs, the inflammation may have a non-infectious origin (Kreda, 2016). Since there are many variations of the disease it is hard to pinpoint the exact cause and it may not be the same for all mutations.

Molecular Biology and Biochemistry of disease

Mutations in the CFTR gene affect multiple organs, including the intestine, sweat glands, pancreas and the reproductive system, but cystic fibrosis lung disease causes most morbidity and leads to premature mortality in cystic fibrosis patients (Griesenbach et al., 2016). CF mostly affects the epithelium cells of the lungs, pancreas, liver, and kidneys. The mutation causes either loss of function or dysfunction. The reason that death from lung infection is so predominant is

due to the dehydration of liquid on the surface of airways. This prevents ciliary action of the cells that normally move mucous away. Another alternative is that the disorder causes inflammation in the host. Patients with CF are susceptible to opportunistic bacteria, most notably *Pseudomonas aeruginosa* (Zemanick et al. 2010). This theory that inflammation is what causes eventual lung breakdown is less widely accepted. The role of CFTR expression in inflammatory cells such as neutrophils, macrophages, and more recently T cells, has been widely, and so far, inconclusively debated, but studies overall appear to suggest a potential defect in adaptive immune responses in patients with CF, which may explain the exaggerated pulmonary inflammatory responses that have been generally observed, an area that requires further studies (Griesenbach *et al.*, 2015).

CFTR is found at the q31.2 locus of chromosome 7 (Zengerling, 1987). It is 230,000 base pairs long, and encodes for a protein that is 1,480 amino acids long. While there are approximately 2,000 different alleles in the CFTR gene associated with the diseases, there is no evidence that all these cause the disease phenotype. CF allele distribution varies by population. It is hypothesized that having CF benefited from resistance to cholera and other causes of diarrhea. That is heterozygous individuals would be less susceptible to cholera. The selection of the CF mutation and its cellular consequences present evidence which suggests that resistance to cholera may have been the environmental factor which selected CF heterozygotes over their 'normal' homozygote cohort (Rodman 1991). A study done by Patrick Sosnay at John Hopkins University looked at genotype and phenotype data for 39,696 affected people in clinics and registries and clinics in North America and Europe. In these individuals, 159 CFTR variants had an allele frequency of 0.01%. These variants were evaluated for both clinical severity and functional consequence, with 127 (80%) meeting both clinical and functional criteria consistent

with disease. Assessment of disease penetrance in 2,188 fathers of individuals with cystic fibrosis enabled assignment of 12 of the remaining 32 variants as neutral, whereas the other 20 variants remained of indeterminate effect (Sosnay *et al.,* 2013). This study is significant because it shows that there are a lot of different mutations in the CFTR gene that give rise to CF. Those with a single working copy are carriers, but otherwise function normally. The most current theory on how cellular defect can cause clinical effects is that defective ion transport leads to dehydration in the airway epithelia, and therefore thickening mucus. Population based studies of CF are becoming more popular, with 1.2 million people subjects in the US alone. Genetic testing is highly recommended for people who are showing symptoms and for couples who might be carriers for the gene.

Clinical aspects and organismal phenotype

Screening for CF includes testing for immunoreactive trypsinogen, then confirmation through a scan for CFTR mutations. Most children are diagnosed by six months of age. Diabetes is the most common non-pulmonary complication, but lung disease and infertility are also issues.

There is a wide variability in the disease and most treatments are tailored to the individual. Most treatment options are aimed at reducing symptoms of the disease rather than the cause of the disease. There are a variety of different therapies that patients can go through. Airway clearance helps to get rid of thick mucus in the lungs. This therapy may require a respiratory therapist. An inflatable vest vibrates the chest at high frequencies to help loosen and thin mucus. Inhaled medicines can also aid in opening air passageways. A liquid medicine is usually made into a mist or aerosol. The medicine is then inhaled via a nebulizer. Pancreatic

enzyme supplement capsules can also be taken with every meal to help facilitate the absorption of important nutrients. Antibiotics include Piperacillin, Azithromycin, Aztreonam, Ciprofloxacin, and Tobramycin. All of these drugs are designed to treat bacterial infections. Tobramycin is specially designed to treat lung infections.

CF is equally prevalent in males and females, but females have a slightly shorter life expectancy. The reason for this is unknown but the gap is decreasing as healthcare becomes better and more accessible. Similar "gender gaps" have been seen in asthma and COPD (chronic obstructive pulmonary disease). Researchers have speculated that sex hormones play a role in this gap. More research needs to be done as there are many factors affecting life span such as exercise, nutrition, socioeconomics, and environment.

People suffering from CF are often given a special diet or a list of recommended foods. This diet is high in fat and calories. Since the mucus is so thick in the intestines, it makes it hard for nutrients like fats and vitamins to be absorbed. That is why teenagers suffering from this disorder are usually short and underweight for their age. This also makes them more susceptible to illness as their bodies can't fight off infections very well. Consuming extra calories aids in fighting off infections as well as keeping their lungs strong. Even if the patient isn't feeling hungry it is necessary to keep consuming food to fight off malnutrition. Just like anyone else they need a well-balanced diet. It is recommended that they consume 2,900-4,500 calories per day. Just like any other factor of the disease, everyone person has their own nutritional needs. Patients are able to work with a CF nutritionist to develop a diet plan for weight gain.

Issue for affected individuals and treatments

   There is no cure for CF and it is a chronic illness. It puts a great deal of emotional stress on a

person as well as their family. People with CF need constant care, expensive medication, and are

more prone to depression or anxiety. The disorder is lifelong and also shortens the lifespan of the

patient. Those suffering from CF are more prone to depression only due to the amount of stress

they are under combined with the decrease in quality of life. Exercise can be beneficial to those

suffering because it releases endorphins as well as strengthening the lungs capacity. People with

CF shouldn't smoke or it will increase their chances of lung disease. Despite requiring daily care,

CF doesn't stop people from performing daily tasks such as working or going to school. Patients

are required to check in with a doctor every three months and the better treatment they receive

the longer lifespan they will have.

        Treatment includes antibiotics, chest physiotherapy, and pancreatic enzyme replacement

for those with pancreatic insufficiency. Lung transplants are also an option. Gene therapy has

been explored as a potential cure for CF. It is focused around trying to put a functional copy of

the CFTR gene into affected cells. Gene transfer has been attempted multiple times, with

liposomes and viral vectors in animal models and in clinical trials. Unfortunately these methods

were inefficient. This is because very few of the cells take up the vector and express the gene.

Pulmonary gene therapy is especially difficult because the lung is a complex organ designed to

keep out foreign bacteria and viruses. Some gene therapy may not completely fix CFTR gene

function but even a little bit can improve lung disease. The discovery that even a slight

improvement in gene function can improve the disease has renewed interest in gene therapy as a

form of treatment. Extensive antimicrobial treatment can be used to eradicate infections in the lungs. Unfortunately this gives rise to resistant strains of bacteria.

The US food and Drug administration approved the first drug that targeted the cause of cystic fibrosis in 2012. This drug, ivacaftor, helps to reopen chloride channels that are closed by G551D mutation. According to the FDA website, two 48 week clinical studies were conducted with 213 patients. In both studies, patients taking ivacaftor showed significant and sustained improvement in lung function. Before 2012 most drugs were only administered to treat symptoms of the disorder. The FDA approved the second drug to target the root cause of CF in 2015. These CFTR modulators are a breakthrough in treatment and have the potential to add decades to the lives of CF patients.

The possibility of a lung transplant can improve the lives of patients. In this surgical procedure the unhealthy lung is removed and replaced with a healthy one. A lung transplant requires a lot of planning and is fairly expensive. Sometimes a transplant called living donor lobar lung transplant is performed where only the lower lobe of the lung is transplanted. The major risk of this procedure is rejection of the organ or infection. It is only recommended in people who have extensive lung damage. It is important to note that the transplant does not cure the problem of CF and the new lung will also be affected by the thickening of the mucus. That is why this procedure is less common.

Conclusion

In some countries, newborns are screened for this disorder. The knowledge about the disease is expanding and many new treatments have finished clinical testing and are being acclimated

into mainstream treatment. Our understanding of cystic fibrosis pathophysiology and genetics is constantly expanding and being refined, leading to improved management of the disease and increased life expectancy in affected individuals (Griesenbach *et al*., 2016). There is still much that is not known about CF such as what other modifier genes affect disease progression. There are other challenges like adequate healthcare. Most drugs for CF are expensive and not everyone can afford them. Even more important a lot of countries don't have access to sufficient health care. Developing drugs is a long and costly process. While scientists may be close to finding a permanent cure, it will be years before the drug is put on the market. Current medicine has greatly improved the quality of life for people living with CF. Hopefully patients will see a breakthrough in treating the cause of the disease. Either way one thing is for certain, more research needs to be done.

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