Fall 11-28-2022

“What is the Environment Doing to Our Genes?”: A Pedigree Analysis of the Possible Genetic Basis of a Set of Familial Clinical Disorders

Emily N. Goodman
Eastern Kentucky University, emily_goodman5@mymail.eku.edu

EASTERN KENTUCKY UNIVERSITY

"What is the Environment Doing to Our Genes?": A Pedigree Analysis of the Possible Genetic Basis of a Set of Familial Clinical Disorders

Honors Thesis

Submitted

in Partial Fulfillment

of the

Requirements of HON 420

Fall 2022

By

Emily Goodman

Faculty Mentor

Dr. Pat Calie

Department of Biological Sciences

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Emily Goodman

Dr. Pat Calie, Department of Biological Sciences

Abstract: The exact contribution the environment plays in human health is unknown; however, it is estimated that 24% of the global disease burden and 23% of deaths are attributed to environmental factors (Remoundou and Koundouri 2009). With the statistics from previous studies drawing attention to the impact of the environment on humans, it makes sense why the number of clinical disorders is on the rise. Many are being impacted more frequently than in previous generations by clinical disorders. This study was conducted to investigate a personal pedigree to gain insight and analysis to determine a possible connection between the environment and medical situations. It appeared that somatic mutations were forming throughout the familial pedigree and the study aimed to find a possible explanation behind the rise of clinical disorders due to those mutations. Additionally, it utilized literature review to help explain the mutations and causes behind the clinical disorders in focus as well as the causative agents that can cause these mutations. The study, through interviewing techniques, generational construction, genetic testing, and the literature review revealed overlap in clinical disorders and the environment by highlighting the causative agents that stayed constant between the personal information given and the literature found. As a result, clinical disorders need to be of focus and the preventative measure we can take to help decrease the occurrence of clinical disorders affecting family’s needs to be prevalent.

Keywords and phrases: Genetics; Mutations; Pedigree Analysis; Cancer; Autoimmune Disorders; Alzheimer's Disease; Environmental implications

Table of Contents

Abstract…… ii

Keywords & Phrases……..iii

List of Figures……iv

Acknowledgements…….. v

Introduction……. .1

Interviews……. 3

Pedigree Analysis…….4

Genetic Testing…….6

Cancer(s)……..9

Mutations and Causes…….10

Environmental Implications……12

Stress……...12

Carcinogen Exposure……..13

Geographic Location……..14

Diet…….14

Autoimmune Disorders/Disease…….16

Mutations and Causes…….17

Environmental Implications……19

Stress……..19

Carcinogen Exposure…….21

Diet and Geographic Location……...21

Alzheimer's Disease……..22

Mutations and Causes……24

Environmental Implications……...26

Carcinogen Exposure and Geographic Location……26

Diet…….27

Results……28

Conclusion…….31

Bibliography……...33

List of Figures and Tables

Figure 1. The constructed genetic lineage……6

Figure 2. Genes screened from personal genetic testing……..8

Table 1. Prevalence of clinical disorders in the pedigree…….30

Acknowledgements

I would like to thank the EKU Honors college for their support and dedication to their students during the thesis process; as well as for giving students the opportunity to express themselves through personal and individual topics. I would like to give thanks to my mentor, Dr. Pat Calie, for holding me accountable to put my best foot forward and for helping me complete my thesis. I am thankful for Dr. Sweat and the Student Success Center for allowing me the opportunity to gain experience in my developmental, professional, and personal skills; as well as being one of the biggest supporters throughout my time at EKU. I would like to thank Dr. Coleman and all the Honor's professors I have had for giving me the skills and resources I would need to complete this thesis. I would like to thank my friends for being a support system and helping me relax during a stressful time. I want to give thanks to my family for taking part in my study and allowing me to learn more about our family history and clinical disorders. Finally, I want to give thanks to God for allowing me to gain knowledge and obtain the opportunities I have while at EKU.

Introduction

The exact contribution that the environment plays in human health is unknown; however, the estimate is that 24% of the global disease burden and 23% of deaths are attributed to the environment (Remoundou and Koundouri 2009). With data from previous studies drawing attention to the impact of the environment on humans, it is clear why the occurrence of clinical disorders is on the rise. The impact of a diagnosis of a clinical disorder is not just on the person who is affected, but it also affects the non-affected, the bystanders, the ones who share this disorder, and the rest of the family members who observe the disease progression. Clinical disorders are described as chronic diseases that affect daily activities such as work, energy levels, routines, etc. It is common for multiple people to have clinical disorders throughout their lifetime, but when they become overwhelming it raises concern. The concern of the study is when the clinical disorders start to take effect consecutively over short periods of time and when they start to arise in families with no explanation.

The focus of this research is a specific, personal familial lineage in which clinical disorders occur at a high frequency, at random, and with an external genetic cause – somatic mutations. It has become apparent that somatic mutations are occurring in the subjects' bodies rather than germline mutations. The hypothesis formulated from the research conducted is based on pedigree analysis. It states that the probable cause behind the development of most of the clinical disorders in this specific family lineage is due to somatic mutations induced through exposure to environmental compounds or agents. This paper will focus on the underlying environmental causes of these clinical disorders that are apparent in the familial lineage.

One factor influencing the development of somatic mutations leading to clinical disorders is geographical location. For example, families residing or that were raised in Kentucky have a higher chance of developing cancer due to Kentucky being ranked on a national level first in all-site cancer incidence and mortality. More specifically, 54 Kentucky counties are in the Appalachian region and in relation to the pedigree, the familial lineage resides in this group of counties. This region displays higher occurrences of cancers and autoimmune disorders (Rodriguez et al. 2018). As a result, geographic location will be one of the many pieces of evidence that represents the environmental/extrinsic factors.

The previous information about the specific clinical disorders in the familial lineage facilitated the development of the hypothesis, but it did not provide unequivocal support. Therefore, my specific research will include interviews on my family members that focus on their daily lifestyles: job, diet, living conditions, year of birth, clinical disorder(s) present, treatment for said disorder(s), and any new disorders that were not of focus or previously known. From the interviews a pedigree was developed, that is a genetic map was constructed with the clinical disorders indicated to highlight concordance between family members and their lifestyles and disorders. In addition, personal genetic testing of my DNA was completed to identify any specific gene(s) that could be causative agents of clinical disorders. The information gained about the clinical disorders and my family was examined for environmental implications to prompt predictions and to help support the hypothesis of somatic mutations in a family lineage. This family has multiple types of clinical disorders present, this study will focus on cancers (breast, chronic myeloid leukemia, lung, rectal, skin, and thyroid), autoimmune disorders/diseases (ADs) (colitis,

Crohn's, diabetes, Hashimoto thyroiditis, inflammatory bowel disease (IBD), lupus, and psoriasis), and Alzheimer's disease.

Interviews

Personal oral interviews were conducted with family members to obtain personal data on family members. The interview technique included a sample of questions that focused on the participants' aspects of their daily living. This included where they were born, when they were born, where their food came from growing up, their food source now, the clinical disorder(s) they have from the list and new ones that are recently diagnosed, the treatment they are undergoing or have had, their job, stress levels, geographical location, information about their parents, and if known cause of death and birthplace/year of their parents and grandparents. The interviews were conducted in a secluded setting where information could remain personal, and time was allotted for input and comments from the participants. The interview question list was presented to a sample of ten family members. These members were chosen based on their previous knowledge of their clinical disorders. Of the ten, eight were female, two were male, nine were genetically related, one was interviewed due to the immediate relation to me, and two control interviews were used. The two control interviews were that of my father and he was not of focus and my brother's interview because he did not present with any clinical disorders. The sample cohort provided a variety of disorders, backgrounds, types of treatments that either worked or did not work, and different genders to provide a diverse data set. The results of the interview revealed overlap in duplicate clinical disorders, but more importantly, it highlighted the overlap in environmental factors such as stress, occupation, carcinogen exposure, geographical location, and the type of diet my family

was eating and/or purchasing. Finally, it revealed new disorders that have affected the family here recently, subtypes of previously known disorders that were diagnosed and new disorders that were not previously known.

The main goal of the interviews was to find concordance between the living generations, but at the same time gain insight into the deceased generations of my family lineage. The information on the deceased members gave more proof per say in the support of the hypothesis at hand. The past generations displayed disorders that were not present in the living generations, old living styles, various sources of income and food, places of birth, cause of death, as well as geographical location and stress levels. It ultimately enhanced the hypothesis and allowed for the next step of the thesis to be conducted - a pedigree analysis.

Pedigree Analysis

The pedigree analysis detects, highlights, and focuses on the clinical disorders that were found throughout the interview process. The development of the pedigree directly came from oral interviews and previously known knowledge of the familial lineage. It covered five generations due to the knowledge and information I obtained from interviewing my family members. The design of the pedigree is shown in Figure One. Males are represented as ovals, females as squares. There are two control groups - a male sibling and my father. The control groups were chosen due to the sex of the participants and the answers to their interview questions. They do not demonstrate signs of clinical disorders or possess other unknown disorders. With the control groups in place, it gave relational evidence to the hypothesis and allowed me to highlight the overall abundance of clinical disorders affecting one side of my family.

Following the analyses of the control groups, the pedigree was constructed and labeled with the information on each family member - living and deceased. If deceased the pedigree had their date of birth, occupation, living quarters, exposures, medications, clinical disorder(s) and cause of death. If alive the information on the pedigree was date of birth, birthplace, if they have relocated, clinical disorder(s), treatment, diet, and occupation. This information and the design of the pedigree provided a guide to identify the shared disorders present in the family. The final pedigree contained-a total of 32 living and nonliving subjects. The proposed hypothesis, the probable cause behind the development of the clinical disorders in this specific familial lineage is due to somatic mutations induced through external environmental agents, received support based on the pedigree analysis.

Figure 1. Constructed Genetic Lineage

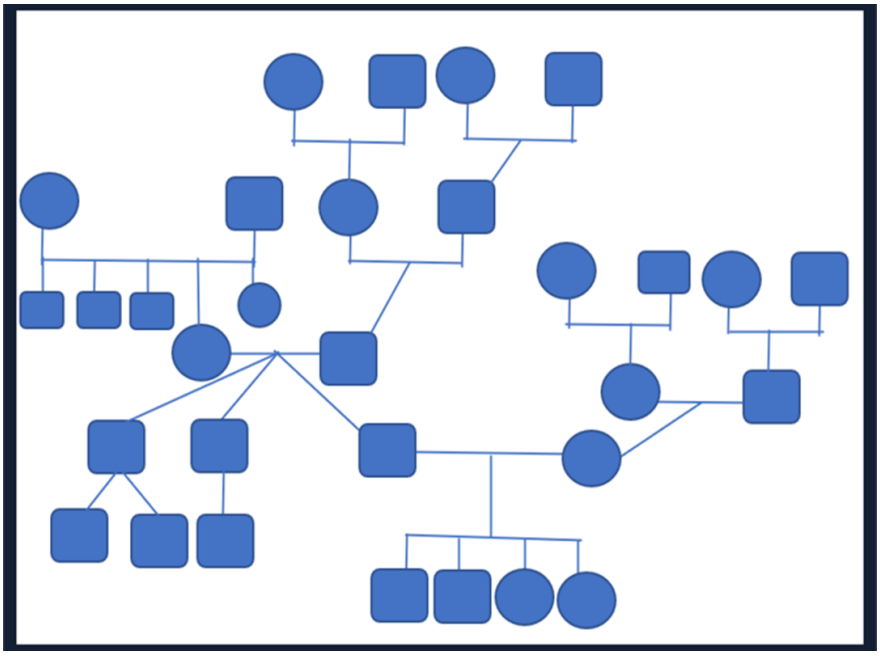

Figure 1. A constructed genetic lineage detailing the number of generations, participants, and sex in the following study.

Genetic Testing

After the interviews and the construction of the pedigree, the next step in this study was the personal genetic testing of specific alleles in my genome. The process began by finding a specific DNA testing kit that included some of the disorders that were prevalent in the pedigree. From there a saliva sample was sent to the facility, my results were read and processed, and then returned to me. The kit that was chosen screened my DNA for 147 genes for allelic variants that indicated an-increased risk of developing certain types of cancer, heart-related conditions, or other medical conditions. For example, the BRCA1 and BRCA2 genes (Figure 2A), CDK4 - a melanoma gene (Figure 2A), the BAP1 gene (Figure 2A), congenital heart disease (Figure 2B), Wilson's disease (Figure 2C) and many others that can be seen in Figure 2. Not only did the genetic testing screen for

certain disorders, but it also scanned for multiple genes that can cause the same disorder(s). The results from the genetic testing were negative. The test did not identify any genetic alleles that are currently recognized as clinically significant.

The personal genetic testing allowed certain conclusions to be drawn. These conclusions support the initial hypothesis of somatic mutations being the initial cause of the clinical disorders that appeared in this pedigree. Due to the manner in which genes are inherited if I, as a daughter, do not carry the alleles for the disorders screened and my father presents with no disorders, the obvious conclusion is that my maternal side does not carry the affected alleles either.

Figure 2: Genes screened in personal genetic testing

GENE	TRANSCRIPT	ASSOCIATED CONDITION(S)
SDHB	NM_003000.2	Endocrine, Gastric and Renal/Urinary Tract Cancer, Sarcoma
SDHC	NM_003001.3	Endocrine, Gastric and Renal/Urinary Tract Cancer, Sarcoma
SDHD	NM_003002.3	Endocrine, Gastric and Renal/Urinary Tract Cancer, Sarcoma
SMAD4	NM_005359.5	Colorectal, Gastric and Pancreatic Cancer
SMARCA4	NM_001128849.1	Gynecologic Cancer
SMARCB1	NM_003073.3	Nervous System/Brain and Renal/Urinary Tract Cancer
STK11	NM_000455.4	Breast, Colorectal, Gastric, Gynecologic and Pancreatic Cancer
TMEM127	NM_017849.3	Endocrine Cancer
TP53	NM_000546.5	Breast, Endocrine, Gastrointestinal, Genitourinary, Gynecologic, Hematologic, Nervous System/Brain and Skin Cancer, Sarcoma
TSC1	NM_000368.4	Nervous System/Brain, Pancreatic and Renal/Urinary Tract Cancer
TSC2	NM_000548.3	Nervous System/Brain, Pancreatic and Renal/Urinary Tract Cancer
VHL	NM_000551.3	Endocrine, Nervous System/Brain, Pancreatic and Renal/Urinary Tract Cancer
WT1	NM_024426.4	Renal/Urinary Tract Cancer

(A)

Cardiovascular-related genes

GENE	TRANSCRIPT	ASSOCIATED CONDITION(S)
ACTA2	NM_001613.2	Aortopathy
ACTC1	NM_005159.4	Cardiomyopathy, Congenital Heart Disease
ACTN2	NM_001103.3	Arrhythmia, Cardiomyopathy
ACVRL1	NM_000020.2	Hereditary Hemorrhagic Telangiectasia, Pulmonary Arterial Hypertension
APOB	NM_000384.2	Familial Hypercholesterolemia, Familial Hypobetalipoproteinemia
BAG3	NM_004281.3	Cardiomyopathy, Neuromuscular Condition
BMPR2	NM_001204.6	Pulmonary Arterial Hypertension
CACNA1C	NM_000719.6;NM_001129840.1	Arrhythmia, Cardiomyopathy, Congenital Heart Disease
CACNB2	NM_201590.2	Arrhythmia
CALM1	NM_006888.4	Arrhythmia
CALM2	NM_001743.4	Arrhythmia
CALM3	NM_005184.2	Arrhythmia
CASQ2	NM_001232.3	Arrhythmia, Includes Reporting of Carrier Status
CAV1	NM_001753.4	Pulmonary Arterial Hypertension
CAV3	NM_033337.2	Arrhythmia, Cardiomyopathy, Neuromuscular Condition
COL3A1	NM_000090.3	Aortopathy
CRYAB	NM_001885.2	Cardiomyopathy, Neuromuscular Condition
CSRP3	NM_003476.4	Cardiomyopathy
DES	NM_001927.3	Arrhythmia, Cardiomyopathy, Neuromuscular Condition
DMD	NM_004006.2	Cardiomyopathy, Neuromuscular Condition
DSC2	NM_024422.4	Arrhythmia, Cardiomyopathy
DSG2	NM_001943.3	Arrhythmia, Cardiomyopathy
DSP	NM_004415.2	Arrhythmia, Cardiomyopathy
EMD	NM_000117.2	Arrhythmia, Cardiomyopathy, Neuromuscular Condition
ENG	NM_000118.3	Hereditary Hemorrhagic Telangiectasia, Pulmonary Arterial Hypertension
F2*	NM_000506.3	Hereditary Thrombophilia
F5*	NM_000130.4	Hereditary Thrombophilia
F9	NM_000133.3	Hemophilia, Hereditary Thrombophilia
FBN1	NM_000138.4	Aortopathy
FHL1	NM_001449.4	Cardiomyopathy, Neuromuscular Condition
FLNC*	NM_001458.4	Cardiomyopathy, Neuromuscular Condition

GENE	TRANSCRIPT	ASSOCIATED CONDITION(S)
GDF2	NM_016204.2	Hereditary Hemorrhagic Telangiectasia
GLA	NM_000169.2	Cardiomyopathy, Lysosomal Storage Disease
GPD1L	NM_015141.3	Arrhythmia
HCN4	NM_005477.2	Arrhythmia, Cardiomyopathy
JUP	NM_002230.2	Arrhythmia, Cardiomyopathy
KCNE1	NM_000219.5	Arrhythmia
KCNE2	NM_172201.1	Arrhythmia
KCNH2	NM_000238.3	Arrhythmia
KCNJ2	NM_000891.2	Arrhythmia
KCNQ1	NM_000218.2	Arrhythmia
LAMP2	NM_002294.2	Cardiomyopathy, Glycogen Storage Disease
LDLR	NM_000527.4	Familial Hypercholesterolemia
LDLRAP1	NM_015627.2	Familial Hypercholesterolemia, Includes Reporting of Carrier Status
LMNA	NM_170707.3	Arrhythmia, Cardiomyopathy, Neuromuscular Condition
MYBPC3	NM_000256.3	Cardiomyopathy
MYH11	NM_001040113.1	Aortopathy
MYH7	NM_000257.3	Cardiomyopathy, Neuromuscular Condition
MYL2	NM_000432.3	Cardiomyopathy
MYL3	NM_000258.2	Cardiomyopathy
MYLK	NM_053025.3	Aortopathy
NKX2-5	NM_004387.3	Arrhythmia, Congenital Heart Disease
PCSK9	NM_174936.3	Familial Hypercholesterolemia
PKP2*	NM_004572.3	Arrhythmia, Cardiomyopathy
PLN	NM_002667.3	Arrhythmia, Cardiomyopathy
PRKAG2*	NM_016203.3	Arrhythmia, Cardiomyopathy
PRKG1	NM_006258.3	Aortopathy
PROC	NM_000312.3	Hereditary Thrombophilia
PROS1*	NM_000313.3	Hereditary Thrombophilia
RBM20	NM_001134363.2	Arrhythmia, Cardiomyopathy
RYR2	NM_001035.2	Arrhythmia, Cardiomyopathy
SCN5A	NM_198056.2	Arrhythmia, Cardiomyopathy
SERPINC1	NM_000488.3	Hereditary Thrombophilia
SGCD	NM_000337.5	Cardiomyopathy, Neuromuscular Condition
SMAD3	NM_005902.3	Aortopathy
SMAD4	NM_005359.5	Hereditary Hemorrhagic Telangiectasia

(B)

GENE	TRANSCRIPT	ASSOCIATED CONDITION(S)
TCAP	NM_003673.3	Cardiomyopathy, Neuromuscular Condition
TGFB2	NM_003238.3	Aortopathy
TGFB3	NM_003239.3	Aortopathy, Arrhythmia, Cardiomyopathy
TGFBR1	NM_004612.2	Aortopathy, Multiple Self-Healing Squamous Epithelioma
TGFBR2	NM_003242.5	Aortopathy
TMEM43	NM_024334.2	Arrhythmia, Cardiomyopathy
TNNC1	NM_003280.2	Cardiomyopathy
TNNI3	NM_000363.4	Arrhythmia, Cardiomyopathy
TNNT2	NM_001001430.2	Arrhythmia, Cardiomyopathy
TPM1	NM_001018005.1	Cardiomyopathy
VCL	NM_014000.2	Cardiomyopathy

Other genes

GENE	TRANSCRIPT	ASSOCIATED CONDITION(S)
ATP7B	NM_000053.3	Wilson Disease, Includes Reporting of Carrier Status
CACNA1S	NM_000069.2	Hypokalemic Periodic Paralysis, Malignant Hyperthermia Susceptibility
HAMP	NM_021175.2	Hereditary Hemochromatosis, Includes Reporting of Carrier Status
HFE	NM_000410.3	Hereditary Hemochromatosis, Includes Reporting of Carrier Status
HJV	NM_213653.3	Hereditary Hemochromatosis, Includes Reporting of Carrier Status

GENE	TRANSCRIPT	ASSOCIATED CONDITION(S)
OTC	NM_000531.5	Ornithine Transcarbamylase Deficiency
RYR1*	NM_000540.2	Malignant Hyperthermia Susceptibility, Neuromuscular Condition
SERPINA1	NM_000295.4	Alpha-1-Antitrypsin Deficiency, Includes Reporting of Carrier Status
SLC40A1	NM_014585.5	Hereditary Hemochromatosis
TFR2	NM_003227.3	Hereditary Hemochromatosis, Includes Reporting of Carrier Status

(C)

Figure 2. Genes for variants (genetic changes) screened for in the personal genetic testing: (A) Cancer related genes, (B) Cardiovascular related genes, and (C) Other genes.

With the information obtained from the interviews, the construction of the pedigree, the results from the genetic testing, and the development of the hypothesis, I completed outside research on the environmental factors that consecutively appeared in the interviews and that show effects on the development of the clinical disorders that are

prevalent in the pedigree. This supported the idea that somatic mutations are affecting my family. The first set of clinical disorders examined were cancers.

Cancer(s)

The topic of cancer is one focus because it is very prevalent in the pedigree. Cancer is defined as “the constant proliferation of cells that have managed to evade central endogenous control mechanisms” (Krieghoff-Henning et al 2017). This occurs due to DNA of a cell becoming damaged and then failing at cellular apoptosis. There are checkpoints in organisms that regulate cell proliferation and progression throughout the body, this ensures purity of cells with the understanding that there will be no pathologies forming. However cancerous or malignant cells surpass these checkpoints by having mutations that disable the checkpoints. For example, a cancerous cell can surpass the S-phase, go through G2 and then into mitosis as a damaged cell. For a cell to be coined as cancerous, one out of three preconditions must be met: (1) there must be variation within the cellular population; (2) this variation must be heritable; and (3) variation must affect survival and/or reproduction of altered cells (Abdallah et al 2013).

Uniquely, cancer cells have their own destiny, they proliferate at increased rates because of mutations in cell-cycle regulatory genes that immortalize the cancer cells. In addition, they can metastasize and migrate to different parts of an organism's body. For example, cells could find themselves in the blood vessels, lymphatic system, and blood stream allowing them to migrate throughout the body (IQWiG 2006).

Mutations and Causes

There are four main causes of cancer: (1) internal or external stress, (2) genetic and nongenetic variation, (3) genome replacement-based macroevolution, and (4) loss of systemic homeostasis (Abdallah et al. 2013). However, like previously mentioned, these four causes would not be possible without the genetic material becoming mutated. The most common mutations and the ones of focus in the study are that of chromosomal instability (CIN), tumor suppressor genes, and oncogenes. The mutations can occur for several reasons but the most common are external influences such as radiation or chemical agents. This relates directly to the hypothesis of somatic mutations affecting the pedigree in question.

The reasoning behind how these genetic conditions cause cancer is in the way they perform within an organism. For example, CIN is defined as the rate (cell to cell variability) of changed karyotypes of a given cell population (Abdallah et al. 2013). In other words, it is the translocation of segments of the chromosomes during mitosis - cell division- leading to structural or numerical chromosomal abnormalities. The difference between the two types of changes - structural and numerical- comes from how they rearrange and manipulate the chromosomes inside the body. Structural is gross chromosome rearrangements, such as amplifications and deletions of parts of chromosomes, and translocations between non-homologous chromosomes. This can be associated with replication stress, telomere dysfunction, and errors in the repair of double - strand breaks (DSB). Numerical is when there is complete loss or gain of entire or fractions of chromosomes, termed aneuploidy. The main effect CIN has is that it generates an altered karyotype of the person it is affecting by increasing or reducing non

clonal chromosomal aberrations (NCCAs) (Abdallah et al. 2013). In general, CIN is found in all cancers, but it is not the definite cause of all cancers. It is very dependent on external factors and how they affect the body in addition to other somatic changes. In this study, the focus is on type II CIN mechanisms; they do not have a molecular causative explanation and are more common (Abdallah et al. 2013)

In addition to CIN, as stated above, there are tumor suppressor genes and oncogenes. These two are remarkably similar because they both deal, when functioning correctly, with the development, maintenance, and growth of the cells. However, when influenced by an external factor they function incorrectly. Tumor suppressor genes do the opposite of allowing cells to grow, their main function being inhibiting normal cell proliferation and tumor formation. When mutated they lose this function by becoming inactivated or lost, removing the protection against cell proliferation, and allowing uncontrolled cellular growth (Cooper 2000). Oncogenes are the activated, mutated version of proto-oncogenes that everyone has in their bodies. When damaged by external factors such as carcinogen exposure, the proteins that these genes produce affect cell growth, proliferation, and survival - resulting in formation of malignant tumors (Eldridge 2022).

The reason these situations occur is not fully understood, and more specifically CIN is sometimes hard to measure. However, there is research that supports the hypothesis at hand and the claim that CIN, tumor suppressor genes, and oncogenes form due to environmental factors.

Environmental Implications

Environmental implications can include anything natural or man-made that causes harm to humans and their bodies. Smoking, diet, and pollutants play a role in most human cancers and the preference among age and gender is highlighting that of very young, and women - experiencing a heightened risk from exposure (Perera 1997). There is a prevalence in the pedigree with a gender preference for women. Most of the women in the pedigree have had a cancer diagnosis proven by a physician or a suspected diagnosis at a relatively early age. Genetics only applies to about 5% of cancers and the remainder is due to external environmental agents (Perera 1997). There are some environmental factors that are present in the answers from the participants that support the claim of environmental factors being the causative agent of most cancers. They include stress, carcinogen exposure, geographic location, and diet.

Stress

The external factors that humans deal with in their lives have a direct effect on their bodies. With direct relation to CINs is the "Stress-CIN-cancer evolution relationship." This states that with one of the four causative agents in effect stress induced genomic instability drives the dynamic variations of cancer. In the pedigree the participants interviewed each revealed their own levels of stress due to different factors. For example, one interviewee mentioned multiple jobs to support the family. Several other interviews emphasized their stress levels and that it was caused by an overlap in their other disorders. The hypothesis explains the unpredictability of NCCAs, i.e., nonrecurring chromosome alterations, and why many divergent molecular mechanisms can lead to

CIN. In addition, it explains why a large number of gene mutations are detected in a specific tumor yet have little overlap (Abdallah et al. 2013).

Multiple cancers can develop from CIN and in the pedigree Chronic Myeloid Leukemia exhibits this pattern. CML forms when chromosome 9 and chromosome 22 undergo reciprocal translocations by breaking parts of their chromosomes off and attaching those parts to the other chromosome. It forms an abnormal fusion gene known as BCR-ABL1 (Aitken 2021). Family occurrence is exceedingly rare with this disorder and is usually of the same kind - homogenous. This directly relates to it being a part of the somatic mutation cycle of cancer development.

Carcinogen Exposure

Carcinogen exposure is a major concern when examining the development of cancer. An article written in 1996 states that without the environmental factors, cancer incidence would be reduced by as much as 80%-90% (Perera 1996). A good indicator of carcinogen exposure is seen through molecular epidemiologic studies of biologic markers present in the cells. They can be chemically specific to indicate what type of carcinogen is affecting that cell. molecular epidemiology has the advantage of leaving a fingerprint when they bind to the DNA to form the cancer cell (Perera 1996).

Some of the main cancers that appear from carcinogen exposure are those of the skin and lung. One of the main carcinogenic exposures is arsenic when developing skin and lung cancer (Parsa 2012). The individual in the pedigree who experienced these cancers was exposed to arsenic through medications from previous diseases as well as electrical exposure from working on the Ford company line. Aside from arsenic, UV exposure is a

main causative agent of skin cancer. This individual, once retired, was exposed to solar radiation, possibly increasing the chance of getting cancer.

Geographic Location

The pedigree subjects have always been residents of Kentucky since the beginning of the pedigree. On a national level Kentucky ranks first in all-site cancer incidence and mortality (Rodriguez et al. 2018). But more specifically 54 counties of Kentucky are in the Appalachian region, this region showcases higher levels of cancer prevalence. My family is a part of this region, the past generations have been residents of this region. The answer as to why this region is so drastically impacted by this disease can be pinpointed to multiple reasons: underserved living areas that show decreased access to healthcare and decreased funds, carcinogen exposure due to the coal mining occupation, the rise in chemical plants within the area, and the disposal of toxic chemicals in the waterways leading to contamination.

Diet

The westernized diet is high in fat, high in salt, high in sugar, and high in protein. This type of diet is said to, with some biochemical processes, alter the hormone production, metabolism, or action at the cellular levels increasing the risk of certain cancers. One of the examples of the disorders that is in the pedigree that this is prevalent in is breast cancer. The two types of breast cancer found in the pedigree are hormone receptive (ER+ PR- and Her2+) and inflammatory breast cancer (IBC). Both types of breast cancer are prevalent in one person and with the help of the detailed constructed genetic lineage is a onetime occurrence. With the knowledge of the environmental factors presented above

and what was researched, each type of breast cancer that is known can evolve differently and independently from the next. For example, hormone receptive breast cancer, such as the one in the pedigree, can be a direct cause of participating in the westernized diet. This is occurring particularly in women due to the alteration of the hormones as well as the increased rates of hormone-dependent cancer (Adlercreutz et al. 1992).

The second breast cancer present in the individual is IBC. It evolved separately and does not relate or correlate to the first diagnosis of breast cancer. IBC is an aggressive breast cancer with decreased survival rates as well as higher risk for metastasis (Sizemore and Rudisill 2021). IBC is a type of triple negative breast cancer (TNBC). One study found that TNBC varies by age, focusing more so on the younger population. In addition, they saw a rise in the Appalachian area with individuals getting IBC. This demonstrates a direct correlation to the pedigree.

In addition to breast cancer, rectal cancer is also prevalent in the pedigree, and it can also be brought on by the diet one consumes. The previously mentioned information above prevalence to age and gender is reoccurring with this disorder as well. The individual diagnosed with rectal cancer was in her 20s and a female. Diets that are rich in red, processed, and grilled meats can lead to rectal cancer (Rattray et al. 2017).

Thyroid cancer can also be related to diet. A carbohydrate rich diet is a potential risk factor for the development of insulin resistance and the impairment of this might lead to a dysregulation of the PI3K/AKT pathway which is strongly related to thyroid cancer development (Nettore et al 2018). Hormone levels are also particularly important when considering the thyroid. The thyroid controls many hormones such as thyroid stimulating

hormone (TSH), triiodothyronine (T3), and thyroxine (T4), that if affected can release elevated levels of calcitonin resulting in a possible cancer diagnosis.

Furthermore, each cancer present in the pedigree has its own environmental causative agents that can coincide with a somatic mutation development. Thus, specific research was done on the types as well as subtypes of each disorder and the environmental implications, all with a specific focus on the pedigree.

Autoimmune Disorders/Disease (ADs)

Our immune systems are put in place to fight off bad disorders, diseases, viruses, and other pathologies that can cause problems. The main cells involved in this process are known as lymphocytes. These are white blood cells that are equipped with various antigen-detecting receptors and several physiological mechanisms of genetic recombination and somatic mutations. They can turn on pathways to initiate or defer cell growth in the presence of an antigen or foreign invader. This all happens because of T cell and B cell receptors (Alriyami and Polychronakos 2021).

With this background information on how the immune system works it directly leads into the next clinical disorder in focus with the pedigree. Autoimmune disorders/diseases occur when the immune system attacks the healthy cells in addition to attacking the foreign invaders inside the body. They are chronic conditions initiated by the loss of the immune system's tolerance to self-antigens (Cárdenas-Roldán et al. 2013). Due to the disorders being chronic they can cause morbidity as well as long term disability and with their complexity they have been studied as a separate disorder each time they appear rather than as a group of conditions (Jacobson 1997). The diseases differ greatly among

what organs they can affect, how they can affect them, and what the end result will be. The way that ADs form can be through genetics, epigenetics, and environmental factors. However, with the continuous rise of ADs in society it is difficult to associate these disorders with genetics revealing a somatic mutation stance.

Mutations and Causes

The main cause of ADs is not fully understood and is still ongoing, but there are some mutations and direct causes, namely the somatic-mutation hypothesis. The somatic-mutation hypothesis is associated with molecular signaling pathways that involve lymphocytes. It states that there is a range of effects on the immune system by affecting these pathways promoting proinflammatory signaling and cell survival of immune cells, ultimately disrupting the immune balance that is needed for recognition of antigens (Alriyami and Polychronakos 2021). One of the main examples that is presented with this hypothesis that highlights formation of an AD is when bone marrow generates many lymphocyte clones that carry autoreactive receptors. Normally these receptors are eliminated by checkpoints found within the body, however somatic mutations along with the receptors can lead to a bypass of the cellular checkpoints and potential accumulation of further mutations leading to autoimmunity (Alriyami and Polychronakos 2021).

In addition to the somatic-mutation hypothesis there are also specific alleles that are found at the HLA loci and non-loci that can highlight a predisposition to the disorder. ADs have a multifactorial inheritance of the disorder and may vary from one population or family to another, as well as within the same population. The genetic effect of the HLA loci and non-loci can be defined not just by genetic disposition but can be involved with the changing and defining relationship between the environmental factors associated

with ADs (Cruz-Tapias et al 2013). In addition, with the multifactorial inheritance, there is an overlap in disease correlation between autoimmune diseases and other clinical disorders. For example, there is a direct correlation between rheumatoid arthritis (RA) and cancer, both of which are in the pedigree. More specifically, there is a significantly increased risk of developing rheumatoid arthritis if Chronic Myeloid Leukemia is already present. This example is demonstrated in the pedigree with the individual being diagnosed with CML and then later with RA (Ehrenfeld 2013).

The last piece of evidence that coincides with the multifactorial inheritance is that ADs have other pathways for development, poly autoimmunity and familial autoimmunity. Poly autoimmunity is when there is a presence of two or more ADs in a single patient and familial autoimmunity is when relatives from a nuclear family present with different ADs (Cardenas-Roldan et al 2013). These two terms are prevalent in the pedigree and indicate that ADs have similar genetic, epigenetic, and environmental influences on them.

What was also gathered from the pedigree is that being a specific gender can be a cause of autoimmune disorders/disease. It is said that ADs affect middle-aged women more than they do men. The pedigree has two men that highlight an autoimmune disorder, but the cause is still unknown. The other autoimmune disorders in the pedigree are seen only in females. These mutations found in the body and the other mentioned causes above are not the only ones causing ADs in families and individuals.

Environmental Implications

The appearance of at least 50% of ADs have been attributed to unknown trigger factors (Ilchmann-Diounou and Menard 2020). Research is still underway to determine the main cause behind ADs. However, all the factors above indicate that there must be an external causative agent affecting the development and progression of these ADs. ADs do not begin the moment they become apparent or become evident, they can occur months or even years before indicating that there is likely an external factor causing the activation of the cells to bypass the checkpoints within the body. The most prevalent external factors that correlate with pedigree and the autoimmune disorders are the same as with cancers: stress, carcinogen exposure, geographic location, and diet.

Stress

Every human being in the world experiences stress differently and the outcome of this stress varies from person to person. High levels of unwanted constant stress can lead to an autoimmune disorder. If it is acute stress exposure it is more likely your body will react properly, however if it is chronic stress, it can be of greater concern. The participants' answers highlight high occurrences of stressors in their lifetime around the time of a diagnosis. For example, one individual noted that the appearance of their disorder came after their parents had decided to end their marriage. In addition, another appearance of an autoimmune disorder came after a cancer diagnosis. Many studies have observed that patients affected by ADs experience emotional stress before the disease rears its ugly face as well as exacerbating the disease.

A specific type of stress is not really known, but there are different studies that highlight psychological stress and how it can impair the intestinal barrier inside of the human body. The intestinal barrier affected can lead to gastrointestinal autoimmune disorders and irritable bowel syndrome (IBS) that are prevalent in the pedigree. This imbalance happens because of microbiota dysbiosis, intestinal hyperpermeability, and intestinal inflammation (Ilchmann-Diounou and Menard 2020). Coinciding with IBS and gastrointestinal disorders is the appearance of psoriasis as well as diabetes.

Psoriasis and diabetes are both prevalent within the pedigree, however diabetes only appears once while psoriasis is a recurring condition within the family. Coincidentally, both disorders are subtypes of IBS. IBS is used as a collective term for ADs such as ulcerative colitis and Crohn's disease. The percentage of patients with IBS and psoriasis is between 3% and 4% with higher levels seen in Crohn's (Hedin 2021). The individuals with the IBS are also the ones who present with psoriasis. They all are associated with vascular inflammation and studies have shown, as stated above, that stress can lead to this inflammation within the body.

One of the last disorders that stress can cause is Systemic Lupus Erythematosus (SLE). SLE is an AD that deals with inflammation that can lead to tissue damage in multiple organs. It is also found within the pedigree and the individual that presented with lupus was diagnosed right around the time of an immediate family member's cancer diagnosis and continues to have flare ups in stressful situations. Studies indicate that SLE can be exacerbated by ADs and even cause the disorder (Ilchmann-Diounou and Menard 2020).

Carcinogen Exposure

When referring to carcinogen exposure with autoimmune disorders/disease, it is the same types that were listed above with the cancers. It can be man-made such as cigarette smoke, but it can also be natural, which is the nature of most of the chemicals causing ADs. High levels of mercury, asbestos, silica, silver, gold, and iodine are seen to be increasing the rate of ADs. Focusing on iodine, it is directly related to causing Hashiomoto's thyroiditis. The disease is characterized by infiltration of the thyroid by T cells, B cells, and macrophages (Pollard et al. 2010). Our bodies do not produce iodine as we get older, rather the way we receive iodine is from our diet and what we eat. Hashimoto's is prevalent in the pedigree and the diet that this individual has indicates that it contains higher levels of iodine than one would expect.

Diet and Geographic Location

Diet and Geographic location are grouped together because the effect of diet on humans developing autoimmune disorders can be directly related to their location and where they reside as a population. As stated above in previous sections my family resides in eastern Kentucky, it is an underserved area where the food that is affordable and available is that of the westernized diet. A hypothesis titled "The Hygiene Hypothesis" indicates that the increase of individuals developing ADs is when they are migrating from a low incidence area - developing area- to a high incidence area -developed area- such as the westernized community because they are being exposed to factors and materials such as the westernized diet (Okada et al 2010).

The westernized diet once again is high in fat, high in salt, high in sugar, and high in protein. This type of diet directly affects the human gut microbiota, and this indirectly affects the development of ADs. The gut and its microbiota are vital to human health by having a direct influence on the immune system, and changes of this have been observed in multiple diseases such as Inflammatory Bowel Disease (IBD) (Jorg et al 2016). The gut is the main absorption in the body and when this is influenced by negative substances it can lead to dysbiosis and an immune imbalance between T regulatory and T effector cells an autoimmune disorder/disease. The incidence rate of ADs in Appalachia are still being researched, however there is probable cause to assume that they would be higher due to the exposure to certain food groups, socioeconomic status, and access to health care. Some studies indicate that some autoimmune disorders/diseases are accounting for a substantial number in Kentucky leading to other clinical disorders such as cardiovascular diseases (Nisiewicz et al. 2020).

Altogether, the autoimmune disorders/diseases prevalent in the pedigree overlap, but so do many autoimmune disorders/diseases. There is a direct correlation between ADs and their background on development. The ongoing research of ADs will allow new information to be brought to light, but with the knowledge gained from this research, the research, and the generational descent, the hypothesis is still holding true; somatic mutations due to environmental exposure is likely causing the overwhelming prevalence of clinical disorders.

Alzheimer's Disease

The last clinical disorder that will be of focus in this study is Alzheimer’s disease (AD). Alzheimer’s disease is a disease that affects cognitive thinking as well as activities

of someone's daily living. It is the most common form of dementia and as of 2021 over 6.2 million adults were affected by this disease (Khalid et al 2022). It is characterized by a decline in two or more cognitive domains including that of memory, language, personality, and behavior (Weller and Budson 2018). When living with Alzheimer's the person can experience the disease in different ways: it might affect them at different times of the day, it might be triggered by outside events, and sometimes they might be lucid, and other times they are less aware. It does cause degradation within the brain resulting in permanent damage. In the past Alzheimer's could only be diagnosed definitively after death, however with recent technological advancements such as lab techniques and imaging tests physicians can see biomarkers in a living person highlighting the biological signs of the disease.

The disorder itself can be split into two categories: early-onset Alzheimer's and late-onset Alzheimer's Disease. If the disease is developed before age 65 it is coined as early-onset Alzheimer's disease (EOAD) and in relation if the individual experiences symptoms after the age of 65 it is late-onset Alzheimer's disease (LOAD). There are other differences between the two rather than just the time of occurrence. These include the symptoms each person experiences; some studies say that in EOAD the behavioral and psychological symptoms are less severe or not as likely as they are in LOAD. Differently, apathy was more common in EOAD while delusions were in LOAD (Awada 2015).

Alzheimer's disease affects my family directly, an individual in direct relation to me was diagnosed and experienced the effects of LOAD that eventually lead to the end of her life. With the help of the interview answers as well as the built genetic lineage the

past generations on that same side of the family did not show any indication this should be a hereditary disease in our family pedigree. In addition the individual's child is experiencing symptoms of forgetfulness, memory loss and hallucinations. The diagnosis of Alzheimer's has not been placed but the signs are there. However, memory loss also comes with getting older, so it is hard to differentiate without a definite diagnosis. The appearance of the disease in the pedigree relates to the researched information that AD has been linked to age, female sex, and certain genes, as well as with other factors including education, poverty, lifestyle, and substance use (Khalid 2022).

Mutations and Causes

The linkage of the disease to certain genes and other disorders has been a research topic for many years. This research shows that the accumulation of the amyloid -**β** (A**β**) peptide – is a major component of amyloid plaques to initiate a cascade of pathogenesis that can lead to Alzheimer's Disease (Kim et al 2009). The most prominent genes that have been linked to the development of Alzheimer's is the APOE gene, amyloid precursor protein (APP), presenilin 1 (PSEN 1) and presenilin 2 (PSEN 2). Each of these genes differ in their formation and function, but there is some overlap between a couple of them.

The most common risk factor for Alzheimer's is the development of the APOE gene, allele E4 specifically. This gene has 3 different types that differ by several base pairs in their genetic sequences. E2 is associated with lower risk of developing AD, some studies even indicate that it can protect an individual from the disease. However, it is very rare in the general population. E3 is neutral in the sense that it does not protect or predispose an individual to the development of AD. E4, however, is the strongest genetic risk factor for

AD. If an individual only contains one allele of the E4 gene the risk of development is not as great, but if an individual has both alleles, the risk increases substantially (Kim et al. 2009). This allele is also associated with an earlier onset of the disease in addition to causing some conflicting views among populations and ethnic groups. Some studies show that this allele can have a weaker effect or no effect at all suggesting that other factors, such as the environment, may contribute to AD (Kim et al. 2009).

In addition to the APOE gene, there also is APP, PSEN1 and PSEN2. These three are more closely related to each other. APP is a transmembrane protein that is expressed at high levels in the brain, metabolized quickly, and overexpression works to control cell health and growth. However, the pathway that APP takes generates the A**β** peptide. When this pathway is mutated, the APP clusters around the γ-secretase cleavage site causing a change in the amino acid sequence leading to a production of a less soluble and more toxic A**β**42 (O'Brien and Wong 2011).

The presenilin genes coincide with the formation of APP. PSEN1 is the most common cause of familial AD. It cleaves the APP and two hypotheses have been proposed regarding its function. The first is known as the amyloid hypothesis and it infers that PSEN 1 mutations initiate disease pathogenesis by increasing the levels of the A**β**42. The second is the presenilin hypothesis and it consider the mutational effect that PSEN 1 exhibits and how it can cause a loss of essential presenilin functions in the brain, which in turn triggers neurodegeneration and dementia. PSEN2 is very similar to that of APP, it can enhance A**β** production and contribute to development of AD, however it plays less of a role than PSEN1 (Cai et al. 2015).

Environmental Implications

In addition to genetics being a factor in the development of AD, if an individual already presents with previous chronic disorders or they develop chronic disorders, including obesity, diabetes, respiratory diseases, depression, anxiety, sleep disorders, and many other factors that relate to the pedigree with the overlap in environmental exposure they are more likely to develop AD. This information leads to a postulate that even though this can be a genetic condition, perhaps it is brought on because of other diseases. To say AD is strictly an environmental clinical disorder would be wrong due to the development being partly from inherited genes. However, that is only around 10%-15% of genetic form that is inherited as an autosomal dominant fashion, meaning the remainder are possibly due to the environment. The environmental factors contributing to AD that was seen in the pedigree is that of carcinogen exposure, geographic location, and diet

Carcinogen exposure and Geographic location

Carcinogen exposure and geographic location are grouped together in this clinical disorder due to the exposure to specific heavy metals within the pedigree. There is some evidence that highlights environmental factors as a cause of Alzheimer's disease including the air quality that individuals live in, the exposure to toxic heavy metals, other trace elements, and then other miscellaneous environmental factors.

The air quality that is in focus causing AD is that of being exposed to high levels of nitrogen oxides, carbon monoxides, ozone and environmental tobacco smoke at home, work, and in other locations (Killin et al 2016). The toxic heavy metals that are in association with high levels of increased AD due to their chemical properties are arsenic,

lead, aluminum, calcium, cobalt, copper, and iron. Focusing on exposure to lead in direct relation to the pedigree is with the geographic location. Not only has most of the family resided in eastern Kentucky, but most, if not all of them, have visited or lived in the same household that was built around the 1940s. According to the report on the national survey of lead-based paint in housing, around 83% of privately owned homes built before 1980 have lead paint somewhere in the building. Over half of the lead-based paint is on the walls, ceilings, and floors (1995).

With this connection to the pedigree, it does not show direct causation, but it does indicate association. Even though association does not mean causation it does allow some insight into the cause behind Alzheimer's Disease if it is not presenting as a germline mutation.

Diet

The kind of diet a person participates in can affect almost all bodily functions including that of the mind. An excess of saturated fatty acids and simple sugars in the diet is an environmental factor leading to an increase in possible Alzheimer's disease development. The pedigree at hand, as stated above, participates in the westernized diet on average at least 10 times a week, whether this be eating fast food options or picking unhealthy items from the grocery store. The two cases of Alzheimer's disease in the family both participated in this type of lifestyle. Research shows that the westernized diet can enhance or induce AD pathological features in the brain. The effects on the acceleration of appearance or enhancement of brain amyloid accumulation and pathology indicate that dietary patterns can be an AD identifying factor in the brain. The westernized diet and impaired gut brain axis due to the high levels of salt, sugar, and fatty

acids can result in systemic inflammation, BBB deterioration and neuroinflammation. This is caused by increased levels of certain chemicals such as blood cytokines, chemokines, PPA, LPS, and bacterial amyloid (Wieckowska-Gacek et al 2021).

In the end, Alzheimer's disease pathology is undefined and will be a continuous topic of research until there is a cure, or if there ever is a cure. Alzheimer's disease is prevalent in my family history, and it is an informative disease when thinking about how the exposure to environmental factors as well as the diet we are consuming can affect the way our neurons work inside of the brain. The two cases are mother and daughter, but there is no for sure evidence that allows either standpoint to be proven.

Results

The results from the outside research, the interview answers, the building of the family lineage, and my genetic testing provide support for the hypothesis. In total, there were ten interviews conducted. 8 female and 2 males with the males being the control groups. From the interviews the disorders found included 1 female presenting with breast cancer and chronic myeloid leukemia, as well as rheumatoid arthritis. There was 1 male presenting with both lung cancer and skin cancer in addition to ulcerative colitis and rheumatoid arthritis. 1 female was said to have had ovarian cancer. 1 female experienced rectal cancer as well as Crohn's disease and 1 female experienced thyroid cancer, diabetes, and a possible Alzheimer's diagnosis. 1 female presented with Hashimoto's thyroiditis, and 1 female presented with vitiligo. 3 females presented with psoriasis but had other disorders as well; 1 with ulcerative colitis, 1 with psoriatic arthritis, and 1 mentioned previously with Crohn's and rectal cancer. Finally, 1 individual experienced Alzheimer's disease (Table 1). The disorders found throughout the study are listed below

in table 1 sorted by gender and type of clinical disorder. The table is set up in alphabetical order for reading to occur more easily. The Xs represent the number of individuals that exhibit the disorder.

Table 1. Prevalence of clinical disorders in the Pedigree.

Disorder	Female	Male
Cancer		
Breast Cancer	X	
Chronic Myeloid Leukemia	X	
Lung Cancer		X
Ovarian Cancer	X	
Rectal Cancer	X	
Skin Cancer		X
Thyroid Cancer	X	
Autoimmune Disorders		
Crohn's Disease	X	
Diabetes	X	
Hashimoto's Thyroiditis	X	
Inflammatory Bowel Disease	XX	
Lupus	X	
Psoriasis	XXX	
Psoriatic Arthritis	X	
Rheumatoid Arthritis	X	X
Ulcerative Colitis	X	X
Vitiligo	X	
Alzheimer's Disease	XX	

Table 1. The prevalence of clinical disorders throughout the family group by male and female with disorders identified from the research completed.

There were 18 total disorders found within the familial sample of 32 people. Around 56% of people interviewed and/or were present in the genetic lineage had showcased one or multiple disorders.

Conclusion

The number of clinical disorders present in the pedigree and the different types of disorders prevalent in the pedigree (Table 1) highlight the occurrence of clinical disorders in this specific family tree. It did reveal a gender preference among females that was supported with outside research and some overlap in disorders, specifically that of autoimmune disorders. However, based on the research, many autoimmune disorders are prevalent due to other disorders that are already seen. The environmental factors stated in the interviews were held constant throughout each participant indicating that they were from the same geographic location, ate the same diet, exposed to the same natural and man-made carcinogens, and experienced similar levels of stress. Most of the disorders were random and showed no direct concordance, but if there was a genetic basis it still was not appearing in every generation or even in every other generation.

The hypothesis stated that the probable cause behind the development of most of the clinical disorders in this specific family lineage is due to somatic mutations via environmental/external exposure. With the information obtained from the study including the personal genetic testing and the external factors that was prompted due to the significant appearance of them in the answers that in this pedigree there is some environmental influence playing a role in the development of clinical disorders.

The study itself does not show unequivocal proof for the conclusion reached, but it does demonstrate an association between two connections and can bring some questions

to play or help further research. There were several limitations to the study such as sample size, availability of information, statistical data, and memory from participants in past generations. However, with the information I did gain it helped answer some of the questions I had, and hopefully it can be used as a tool for future research. In addition, it can be used to answer other questions, understand how mutations form, bring awareness to the increasing rates of clinical disorders, and help implement preventative measure due to awareness of possible causative agents.

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