USING GENETIC AND MICROBIAL DATA FROM GENOME WIDE ASSOCIATION STUDIES TO PREDICT, PREVENT, AND TREAT DISEASE

By

Chloe Proctor
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Abstract

The understanding of the human microbiome is rapidly expanding as more people understand its magnitude. The review highlights the aspects of how genetic and microbial data can be used to predict, prevent, and treat disease. Genome-wide association studies (GWAS) can be used to collect this type of data, though caution must be used to avoid confounding variables. With this data, connections can be made between host genotype, microbial composition, and disease. In this review, some correlations between genetics and the microbiome, including the influence of sex chromosomes and specific mutations, will be discussed. Several important correlations have been found between genetics and the microbiome, but environmental influences have been shown to be more impactful than genetic ones. Through the analysis of studies of twins, this review discusses the impact of these various influences on the host microbiome. The presence of harmful microbes is usually an indicator of microbial dysbiosis, which can affect the host's metabolic and immune responses. A big unknown in this field of study is whether the dysbiosis or disease state came first, causing the other. This review discusses this question and focuses on how microbial dysbiosis affects leukemia patients. It has been shown that some microbes can help physicians predict a leukemia patient's risk of serious infection throughout the course of their chemotherapy treatment. This type of knowledge could be important in individualizing the treatment plan to increase a patient's chance of survival. The microbiome is vast, and the great impacts it has on the human body are becoming more evident. This area of study may shed light on patient symptoms and presentation and aid medical professionals in diagnostics, prevention, and treatment. With continued efforts, it may be possible to develop new treatments that are more effective and safer for patients.

Introduction

Using GWAS data, a person's genome and their relationship with their microbiome can be better understood, which ultimately can help in disease treatment and prevention. This review will begin by overviewing GWAS and how these studies can be helpful, then focus on the microbiome, how this is affected by genetics, and how this can be a factor in the development of various diseases. Finally, this review will discuss some common cancer types that are affected by the microbiome and how genetics ties into both. By exploring relevant literature, this review aims to bridge the gaps between the field's current understanding of how host genetics versus the microbiome may lead to disease, especially cancer.

GWAS are useful yet relatively simple studies to perform. They only require blood, urine, and/or fecal samples from the subjects, as well as filling out health questionnaires (Henshall, 2013). With this data, high throughput sequencing can be performed, followed by examination of the genomes for single nucleotide polymorphisms (SNPs), which are common mutations in a population (Gibbs and Singleton, 2006). SNPs are a type of mutation that are represented in greater than 1% of the population. They are usually caused by point mutations that replace the proper nucleotide with a different one (Brookes, 1999). SNPs are frequently found in the genome, but most are located outside of coding regions, which usually indicates that they are less harmful than those found within coding regions (Ni et al., 2022). The goal of GWAS is to correlate SNPs found in a person's genome to a phenotype or disease that they are presenting with (Henshall, 2013). With these established SNP-disease correlations, a person's sequenced genome can be examined to determine if they have an increased risk of a particular disease due to a specific SNP. GWAS are not typically capable of determining the causality of a SNP to a disease because human systems are far too nuanced for this type of generalization. Developing a

disease can be a long process and can be caused by many different factors, including genetic, microbial, lifestyle, and environmental. However, data from GWAS can be used to determine if a person has an increased risk for a particular disease, but it cannot usually be used to predict with certainty if they will develop the disease (Cano-Gamez and Trynka, 2020). Though GWAS is a very helpful tool, there are some challenges and limitations, including confounding variables and ethical issues, that will be discussed later in this review.

GWAS can also be used to gain information about an individual's microbiome. MicrobiomeGWAS is a computational tool used to help identify associations between host genetic variants and their microbiome, allowing for large-scale analysis of GWAS data (Hua et al., 2022). This information can help determine how a person's metabolic system interacts with certain microbes. Similar tools can also be used to study microbial composition. A sample can be collected from a specific body site to identify what microbes are residing there (saliva for oral microbiome, fecal matter for gut microbiome, etc.) (Yang et al., 2022). Understanding a person's microbiome composition and abundance can greatly help in the treatment and prevention of disease, especially as this field grows and researchers further understand the interactions between the microbes and antibiotics or other treatment interventions. This review focuses on GWAS serving to help garner genetic information and how that correlates with microbial information and possibly leads to disease.

The microbiome is a vast collection of all the microbes, both harmful and helpful, residing in or on a specific being. Humans have various microbiomes, including but not limited to oral, gut, skin, and vaginal. Each of these sites is slowly inhabited by microbes when an infant is born and grows (Lif Holgerson et al., 2011). The first exposure to microbes that humans experience is during vaginal birth when the infant is exposed to the mother's vaginal microbes (Lif Holgerson et al., 2011). In the case of Caesarian birth, it is more challenging to identify the first exposure, but the method of introducing the mother's vaginal microbes to the infant immediately after birth is being researched to better understand this process (Anthoulaki et al., 2023).

After the initial exposure, the infant is colonized by many of the environmental microbes that inhabit the air and surfaces, as well as the skin microbes from people that the child is in contact with. As far as the gut microbiome, the infant begins to be colonized by microbes in the mother's breast milk (Kordy et al., 2020; Ennis et al., 2024). Breast milk not only provides microbes to the infant but also provides some nutrients for the microbes to thrive in this new environment (Kordy et al., 2020). For instance, in human breast milk, there is a component that is undigestible by the infant and is thought to be solely for feeding the infant's gut microbes (Ennis et al., 2024). This relationship between human breast milk and gut microbe is an example of humans coevolving with microbes to benefit both organisms. As the child grows and experiences more, their microbiome, though somewhat genetically determined, is further molded to reflect the environment they are in and the people that surround them. This continues to change throughout their life as the child continues to experience and interact with new environments.

Microbes are generally portrayed as harmful, but it should be argued that the majority of microbes have either a helpful or neutral effect on their human hosts. A person's established microbiome works to fend off harmful microbes and keep them in a state of symbiosis (Li et al., 2008). When something goes wrong in the microbiome, maybe a harmful microbe takes over, the individual enters a state of dysbiosis where their microbiome is not necessarily working with them but against them (Peterson and Round, 2014). Many diseases correlate with this state of dysbiosis, though it is challenging to understand if the dysbiosis played a role in causing the disease or if the disease led to the dysbiosis (Zhang et al., 2015).

Ultimately, a healthy microbiome is necessary for a healthy life. An 'unhealthy' microbiome can lead to allergies, obesity, diabetes, and other chronic illnesses (Feehley et al., 2019). Humans need a certain level of interaction with the environment to build a healthy microbiome. Without this exposure, individuals are more susceptible to colonization by harmful microbes since they do not have to outcompete the good ones (Segura Munos et al., 2022). The unfortunate aspect of this is that there is no way for an average person going about their life to know if they are encountering mostly helpful or harmful microbes until it is too late. Preventative measures can be taken, including avoiding undercooked meat, practicing proper hygiene, and staying away from people who are sick. Despite best efforts to avoid harmful microbes, most individuals become colonized by some during their lives. Fortunately, infections usually remain acute, and the microbiome returns to normal eventually. In some cases, though, the harmful microbes can cause more severe problems and lead the individual into a state of chronic dysbiosis (Wilkins et al., 2019). Ideally, more research in this area will aid in the treatment of chronic dysbiosis and help alleviate patient's symptoms. This field is growing rapidly, and more links between the microbiome, especially in the gut, and how humans function are being discovered. As this field continues to expand, treatment or prevention plans will likely begin to shift to more microbial-focused. Understanding how a patient's microbiome interacts with them or with an intervention may help in treating the patient more effectively and efficiently.

A big unknown in this scope is understanding which harmful change came first and led to the other. There is insufficient research at this point to determine if the microbial composition change or the disease state occurred first. The patient's genetics (or environment) may cause a harmful shift in the microbiome, which begins the development of disease. On the other hand, maybe this microbial shift happens as a result of the disease progression. This review will continue to break this paradox down and work through it in different specific cases, as no real generalization can be made at this point.

Genome-Wide Association Studies and Mendelian Randomization

GWAS are helpful tools to learn more about possible correlations between genotype and phenotype. The GWAS workflow involves collection, sequencing, association testing, and meta-analysis (Ufflemann et al., 2021). The subjects of the study usually only have to fill out health questionnaires and provide blood, urine, and/or stool samples (Henshall, 2013). The DNA will then be extracted, and the subject's (or their microbiome's) genomes will be sequenced. In doing this, single nucleotide polymorphisms (SNPs) can be discovered that may be correlated to certain phenotypes or diseases the subject indicated on their health questionnaire (Uffelmann et al., 2021). This genomic data can also be used to predict the risk of developing various diseases. In the case of the gut microbiome,16S rRNA from the stool samples can be sequenced to determine what bacteria are colonizing the subject's gut at the time (Mas-Lloret et al., 2020). The subject's genome may also be correlated to the microbial colonizers (Hua et al., 2022). The computational tool discussed previously, MicrobiomeGWAS, can help advance the field and may be able to offer predictive data to help prevent disease before it progresses to harming the patient (Hua et al., 2022).

A major issue in these types of studies is confounding variables. These variables are a reason why the term correlation is used instead of causation in this type of research. In short, a confounding variable is a factor that makes the effects of the variables that are being studied less clear (Ewert and Sibthorp, 2009). An example of a confounding variable in GWAS is in a study investigating the microbial composition of children with autism; it was found that many autistic

children have less diverse gut microbiomes than non-autistic children (Yap et al., 2021). However, the generalization cannot be made that autism directly causes a less diverse microbiome because, usually, autistic children are more prone to picky eating, which is more likely what ultimately causes the lack of diversity (Yap et al., 2021). Confounding variables are common to GWAS and often prevent researchers from predicting causation. Another study found an increased risk of prostate cancer in people living further inland when compared to those living near the coast (Felici et al., 2024). However, further studies revealed that these groups have differing lifestyles (i.e., people living closer to the coast tend to be more active and eat healthier diets), which is more likely to be the cause of the increased risk than simply geographical distance to the coast (Felici et al., 2024). Had the researchers taken the data at face value and published it before further analyzing their populations, they could have led readers to believe that simply living by the coast has miraculous anticancer effects when it actually has more to do with lifestyle differences. These studies are prime examples of why it is crucial to consider other possible variables that may have affected the data after finding significant results. It is virtually impossible to fully eliminate confounding variables, so it is necessary to be aware of them and avoid making claims of causation when it is more likely a correlation (Veller et al., 2024). Because of this, more research is needed on many of the correlations identified by GWAS to further understand other variables and the possibility of causation.

Many GWAS studies use Mendelian Randomization (MR) to help eliminate the possibility of confounding variables. This test is typically performed after data has been collected from a GWAS to help identify if a correlating relationship is causal or not (Swerdlow et al., 2016). In this context, two-sample MR tests are often performed that use the data from two different GWAS so that SNPs can be divided into an exposure group and an outcome group (Ni et al., 2022; Hatcher et al., 2023). MR assumes that the SNPs in the data set are independent of confounding variables, and the associations are not due to any other factors (Ni et al., 2022). These assumptions can be somewhat subjective as confounders could be overlooked. A study that used MR to analyze the correlations between the gut microbiome and oral cancer found many causal relationships but later, through sensitivity analysis, found that this causality may have been due to unintentionally breaching the assumptions of MR (Zhang et al., 2024). Using Mendelian Randomization, a relationship was found between *Bifidobacterium* and colorectal cancer (CRC) risk; however, further studies uncovered other variables that may be involved, which led them to deny causality between these factors (Hatcher et al., 2023). These examples show how nuanced studying the microbiome can be because there are many different variables to consider, and determining causality can be challenging. That said, these types of studies are still relevant and important in determining if an individual has an increased risk of developing a disease. If this information is known before the person starts showing symptoms, the medical providers can try to instill preventative measures to avoid the disease. It may also help in treatment, knowing that the disease may have been primarily caused by genetics rather than some environmental variable. Finally, in diagnostics, if the individual presents with symptoms and medical professionals can obtain this genetic information, they can make a quicker and more accurate diagnosis.

Ethics must come into question when dealing with human subjects in a research study. Fortunately, GWAS typically do not cause many ethical concerns since the subjects are not being treated in any way (harmful or helpful), they are simply giving samples and personal health information. However, sometimes during a GWAS, it may be discovered that a subject has a known specific harmful SNP that predisposes them to a certain disease. There are some cases

where if the researchers discover a SNP in the genome that was previously unknown to the subject, they should report it to the individual so that preventative measures can be put in place. If this sort of information goes unreported to the individual, there may be harm to them later on if they develop a disease that could have been prevented. Another concern for GWAS is privacy. These studies obtain significant health information about many people, which should be dealt with carefully. Advanced technology is needed to handle this amount of data securely (Brauneck et al., 2024). It is especially challenging for the BioBanks that provide free open-source access to thousands of individual's genomic and phenotypic data via GWAS (Rothstein et al., 2016). The genomes must be maintained as completely anonymous, or the researchers could face serious consequences for breaching privacy. If these concerns are not carefully considered when performing GWAS, the subjects could be negatively affected by their participation.

Genetic Relationship with the Microbiome

It is well known that certain heritable genes predispose an individual to a specific cancer type. Not only are there certain genes that may predispose an individual to cancer, but studies are finding more genetic variants that have been passed down generations and continue to cause cancer (Carvalho et al., 2023). Moving forward, genetic testing to identify harmful variants in children in high-risk families would be beneficial to catch and treat cancers early. There is also research being done on the correlation between microbiome makeup and improving dietary choices (Manor et al., 2020). For example, if a person's microbiome is sequenced and it is discovered that they have a high abundance of a specific bacteria, that information can be used to suggest a personalized diet based on how those microbes thrive (Manor et al., 2020). A lot can be learned about an individual's predisposition for disease via genetic testing, but arguably, more can be learned through studying their microbiome either via genetic testing to understand specific links or through identifying their microbiome composition and further studying the effects these have on various host processes.

The microbiome makeup is mostly affected by environmental factors, including host lifestyle and diet, but studies have shown links between genes or genetic variants and the presence or abundance of certain bacteria. Using GWAS, links have been found between specific SNPs and *Ruminococcus* spp*., Coprococcus* spp., and *Bifidobacterium* spp. abundance (Hughes et al., 2020). Some SNPs have also been linked to specific bacteria deemed pathogenic due to their correlation with CRC (Xiang et al., 2023). *Ruminococcus* is a bacterial genus that has been associated with an increased risk of some cancers but has also been shown to be protective against other cancers, indicating that different species within a genus may have vastly different effects on various cancer types and more research needs to be done to discover the nuanced effects in different cases (Long et al., 2023). Studies have also shown that host genetic variants can functionally affect the microbe's genome (Zhernakova et al., 2024). This type of interaction can cause the microbes to behave differently than they may in another individual's gut based on resource availability or other physiological differences. For instance, a microbe relying on hormone production may behave differently in people with differing hormone levels, especially males versus females (Zhernakova et al., 2024). The microbiome link with genetic variants is not limited to the gut; studies have shown links between SNPs and the oral or nasal microbiome in cancer, though this is a bit outside the scope of this review (Yang et al., 2021; Liu et al., 2024). This information on correlations can be very helpful in treating cancers as more microbe-based therapies are being researched. It may be valuable to understand a person's genetic predispositions that may impact how their microbiome functions. The treatment regime can then

be more individualized based on the specific pathways the microbes are interfering with or interrupting to worsen the cancer.

The microbiome, though mostly environmentally influenced, does have some heritable components. As discussed previously, certain genetic variants can change the behavior or presence of some microbes (Zhernakova et al., 2024). As with most properties when discussing heritability, some microbes have been shown to be more heritable than others. A study on twins found a strong association between *Bifidobacterium* spp. abundance and variants in the *LCT* gene locus, both of which are involved in lactose metabolism (Goodrich et al., 2016). It was found that people who are lactose intolerant, on average, have higher levels of *Bifidobacterium* spp. in their gut than those who can tolerate lactose, likely due to the greater availability of lactose since the host cannot metabolize it independently (Goodrich et al., 2016; Schmidt et al., 2020). *Bifidobacterium longum* may even act helpfully to alleviate gastrointestinal symptoms in lactose-intolerant individuals (Vitellio et al., 2019). Though there is a strong association between bacterial abundance and lactose tolerance, it is possible that dietary preference is acting as a confounding variable and is responsible for the association (Goodrich et al., 2016). Another interesting aspect of this data that was not mentioned is the longevity. Those who are lactose-intolerant may eat less lactose since it causes negative symptoms. In this case, there would not be much competition between the host and bacteria for lactose. More studies should be done monitoring the *Bifidobacterium* spp. levels in those who are lactose intolerant who still consume lactose vs those who avoid lactose. If the abundance drops in people who do not consume lactose, this could be an area for further research as *Bifidobacterium* spp. are often important protective bacteria. This microbe will be discussed further in the following section of this review.

The gut microbiome has been found to have some heritable components, specifically in older primates rather than younger ones, indicating that after the establishment of the microbiome, the heritable aspects may become more evident (Grieneisen et al., 2021). Though this study was performed on non-human primates, there are several similarities to the human gut microbiome, so this may also be true for humans (Nagpal et al., 2018). Likely because of the comparative simplicity, many microbiome studies focus more on environmental influences than on genetic ones. However, it is important not to discount genetic influence on the gut microbiome as this information can aid in developing a more individualized treatment/prevention plan for the patient.

It is known that host genetics can influence the microbiome, which in turn can influence host phenotype in disease progression. There are several known SNPs that cause an increased risk of disease. Fucosyltransferase 2 (*FUT2*) homozygous loss-of-function mutation is known to cause an increased risk of inflammatory bowel disease (IBD), more specifically, Crohn's disease (Tong et al., 2014; Cheng et al., 2021). Individuals with this mutation are lacking in the H antigen, an important resource for intestinal bacteria, which is normally synthesized by FUT2 (Cheng et al., 2021). It has been found that individuals lacking this antigen due to the mutation have altered energy metabolism in their microbiome, leading to intestinal inflammation (Tong et al., 2014). This area should be further researched to see the effects that supplementation of the lacking molecule may have on affected patients. A link has also been found between a SNP in *NOD2* and Crohn's disease; the mutation in *NOD2* was correlated with a higher abundance of *Enterobacteriaceae* spp., which led to inflammation in the gut (Glas et al., 2010; Knights et al., 2014). These are only two of many examples on how an individual's genetics can shape and lead to disease through the microbiome. Cases like these are why it is critical to holistically understand what is causing a patient's symptoms instead of simply treating them.

Host genetics influencing the microbiome has also been linked to chronic non-disease states like obesity. Genetic mutations in the leptin signaling pathway have been correlated with obesity due to the impairment of its role in the modulation of inflammation and appetite (Bleckham et al., 2015). Obesity has been linked to specific microbes in both mice and humans, though the role of genetics in many cases is unclear at this point (Ley et al., 2005; Turnbaugh et al., 2009). Understanding these relationships is important in treating and preventing disease and should be a continued area of study.

The genetic link to cancer is much less challenging to study than the microbial link because there is less room for confounding variables. The idea that cancer can be heritable is certainly not a new field of research. It is well known that if a person has an immediate family member with cancer, they are more likely to develop that same type of cancer eventually (King et al., 2003). Of course, some types of cancer are more heritable than others. Some cases of cancer are sporadically or environmentally acquired and do not pose any increased risk to the family members (Carvalho et al., 2023). On the other side of the spectrum, inheriting a *BRCA1* or *BRCA2* mutation greatly increases an individual's risk of developing breast or ovarian cancer (King et al., 2003). In families that carry this mutation, there is usually a very high incidence of breast cancer (King et al., 2003). This is obviously a genetic factor in developing cancer, but epigenetic factors have also been shown to be important. Certain activities or exposures can cause harmful modifications to an individual's genome that can be harmful to their offspring. It has been shown that individuals who were exposed to tobacco during pregnancy have a greater likelihood of their children and even grandchildren developing cancer (Ortega-Garcia et al., 2010). The exposure during pregnancy affecting the fetus is obvious as it can directly modify the DNA. However, the effect on the individual's grandchild can only be explained by epigenetic modifications passed down the generations.

GWAS can be used to identify new possible correlations between genetic variants and risk of cancer. Often, when a person is diagnosed with cancer, genetic testing may be performed to further understand the cause of the cancer, which may help prevent and/or treat it in the future (Beltran et al., 2015; Hutchcraft et al., 2024). In further understanding SNPs and their relation to cancer, greater patient care can be provided (Seliem et al., 2024). A recent study proposes wider use of whole-exome germline sequencing of cancer patients to avoid missing any possibly important variants that could affect the patient or family members who may have inherited the variant as well (Beltran et al., 2015; Hutchcraft et al., 2024). It was found that in using guided sequencing, the common method now, over one-third of potentially cancer-causing variants are missed (Hutchcraft et al., 2024). Ideally, the medical field would have the time and resources to sequence every patient with this thoroughness to provide the best care possible, but unfortunately, the field is not advanced enough at this point to provide this high-level care to each patient in every situation. Even though the field is continually advancing, there are still many factors contributing to the type of care that a patient may receive, including but not limited to income, insurance, and location. This thorough whole-exome testing would usually only be done in specific, more challenging cases where the medical professionals are unsure of the best course of action.

Microbial Influence on the Host and Disease

As this field continues to grow, it is becoming more and more evident that the human microbiome is involved in many more processes and diseases than initially thought. Ties are being made to many physical and mental diseases/disorders (Duranti et al., 2020; Hong et al.,

2023). Each person's microbiome is unique and impacts their metabolic and immune systems uniquely. This unique impact likely boils down to a mixture of the person's environment and their genome. The environment has a huge impact on the microbiome and is a major predictor of the composition, but not necessarily the abundance of those colonizers (Gregory et al., 2016). Genetics may be a better indicator of microbial abundance rather than composition as certain people may have a mutation that makes them more (or less) hospitable for certain microbes than others, like in the case of the *LCT* gene and *Bifidobacterium* spp. that was previously discussed (Goodrich et al., 2016; Schmidt et al., 2020). Despite the various predictors, it is important to recognize that the human microbiome is constantly in flux and changes throughout a person's lifetime (Gerber, 2014). Studies have shown that the gut microbiome changes significantly even from season to season, but this may be due to different diets through the seasons based on food availability (Davenport et al., 2014). No matter the cause, this is a prime example of how human gut microbiomes are continually changing regardless of predisposition due to genetics or environmental factors.

Twin studies are a helpful tool in studying the heritable influences of the microbiome because co-housed twins can be compared to separated twins, and the effect of the environment versus genetics can become more evident (Bao et al., 2021; Brand et al., 2021). A study was conducted on pairs of twins where one has IBD and the other is unaffected. The healthy twins had components of their microbiome typical to IBD that usually do not show up in unaffected individuals (Brand et al., 2021). These IBD-like signatures were similar to their affected twin but also to other unrelated affected individuals, so there does not seem to be a strong correlation (Brand et al., 2021). People with family members affected by IBD (and other chronic diseases) are more likely to develop those diseases themselves, so the explanation for these similar IBD microbiome results is likely that the healthy twin is developing IBD (Gordon et al., 2015). This specific study on twins used a population aged 16 years and older and did not specify if the twins were co-housed or not, so it is challenging to make conclusions about any genetic or environmental factors (Brand et al., 2021). Another similar study looked at twins where one had food allergies and one was unaffected and found similar results that there were not any strong associations between the twins' microbiomes (Bao et al., 2021). In this study, the participants were all children, and it was not specified that they were co-housed, but it seemed to be implied (Bao et al., 2021). This kind of research opens doors to further studies aiming to understand where these microbiome differences in co-housed twin children come from.

Arguably, co-housed monozygotic twins should display the same disorders or allergies since they are exposed to the same environment and have the same genetics, especially in early age when they are consuming the exact same diet. Their identical genomes determine what can colonize them and how, and breast milk then begins feeding those early microbes (Gregory et al., 2016; Pannaraj et al., 2017). A component of breast milk, human milk oligosaccharides (HMO), is undigestible by the infant, and its sole purpose is to feed the infant's gut microbiome (Lawson et al., 2020). Along with this component, breast milk provides bacteria to colonize the infant from both the milk itself and the areolar skin (Nagpal et al., 2017). In real-world cases, despite their genetic and environmental similarity, identical twins often have differing microbiomes. There is no clear reason why this may be now, but studies have suggested the possibility of an epigenetic component (Yang et al., 2014). In a study where monozygotic twins were co-housed and fed the exact same diet, their microbiomes still responded differently, so it seems epigenetics may be the only answer (Yang et al., 2014). However, there is not much research to date on how or where these epigenetic variations may have stemmed from.

Mutational differences in the genome may also arise post-zygotically in monozygotic twins. Again, there is not much research on how or why these changes may have occurred, but these are major contributors to the differences in identical twins (Jonsson et al., 2021). As expected, twin age tends to positively correlate with the number of differing mutations in their genomes, and on average, 15% of twins have a significant number of differing mutations (Jonsson et al., 2021). An interesting distinction can be made between mutations that occur postnatally versus mutations that occur during development. Postnatal mutations arguably could be due to environmental differences, but mutations during development are more challenging to speculate the cause for. Most identical twins share a placenta, but in cases where they do not, it is possible that this placental difference could cause mutations at the developmental stage (Lewi et al., 2007). For the majority of monozygotic twins who do share a placenta, these mutational differences may be due to unequal sharing based on position in the womb, but more research is needed to identify other possibilities (Marceau et al., 2016). Generally, one twin is born smaller than the other due to a resource differential in the womb, and these size differences tend to remain relatively constant throughout life (Yokoyama et al., 2016). These differences could play a role in mutational differences as well, which ultimately could lead to differing microbial environments throughout life.

Another interesting genetic determinant of microbial composition is sex. A study conducted research on newborn twins' microbial diversity. The most significant finding was that in the case of dizygotic twin pairs, females and males had major differences in their microbiomes, with the females having a greater diversity than their male twin (Chen et al., 2021). This finding is interesting because it may be correlated with the statistic that female neonates have a greater chance of survival on average than males (Kent et al., 2012). Many factors could contribute to this statistic, but microbial differences likely play a part. Since it is known that the microbiome plays a protective role, it can be inferred that the lower diversity seen in males may be a part of their survival disadvantage (Li et al., 2008). Interestingly, no significant difference in mortality between male and female fetuses has been shown, yet male neonates and infants have a greater mortality rate than females (Zhao et al., 2017). This supports the idea that microbial differences play a role in neonate survival since fetuses do not encounter microbes until birth (Lif Holgerson et al., 2011). The genetic difference between the males and females is likely affecting microbial diversity and, ultimately, survival. This may be due to differences in hormone or cytokine production, but unfortunately, the mechanism of how these genetic differences cause lower microbial diversity in males is largely unknown to date (Cong et al., 2016). Another hypothesis is that the survival disparity is due to the chromosomal differences seen between the sexes since males only have one X chromosome and are, therefore, more susceptible to X-linked chromosomal disorders, which can have severe outcomes (Zhao et al., 2017). It is likely that both the microbial and chromosomal hypotheses contribute to the significant survival differences between male and female neonates.

The environmental effects on the microbiome can be seen in studies analyzing adult twin microbiomes who do not currently live together. Studies found that monozygotic twins had more similar microbiomes than dizygotic twins, who had more similar microbiomes than unrelated people (Xie et al., 2016). This is expected since it is known that there are some genetic components that affect the microbiome. It was also found that twins who lived together longer had more similar microbiomes than twins who moved away sooner (at about 16 years old), which is a good indicator that the teenage years shape the adult gut microbiome significantly and supports the hypothesis of the primate study discussed previously (Xie et al., 2016; Grieneisen et

al., 2021). Another study showed that living together is the best predictor for shared microbiomes (Tavalire et al., 2021). Siblings who did not live together (adopted at birth) were compared to unrelated people who do live together, and it was found that the unrelated people who do live together had more similar microbiomes than the related people living apart (Tavalire et al., 2021). This also supports the idea that the environment in earlier years of life is more influential in shaping the microbiome. An unrelated study on mice found that when two groups (one wildtype and one heterozygous for *Pax5,* a gene that they show to influence the microbiome) are cohoused, their microbiomes become similar regardless of genotype (Vicente-Dueñas et al., 2020). From what we know now, it can be assumed that the environment plays a larger role in shaping the microbiome than host genetics, though the latter's role cannot be discounted.

Normally, humans coexist with their microbiomes, and the relationship is symbiotic, both parties benefit. Humans provide a hospitable environment and nutrients for the microbe, and the microbes provide nutrients that humans cannot synthesize themselves and protection against harmful pathogens (Li et al., 2008). This relationship is critical to life, and problems occur when harmful bacteria begin outcompeting the helpful bacteria, leading to a state of dysbiosis (Zhang et al., 2015). Harmful microbes can cause inflammation and metabolic changes that may lead to acute or chronic disease (Zhang et al., 2015). *Bifidobacterium* spp. is an interesting genus often found in the gut and has made several appearances thus far in this review. As previously discussed, a bacterium is fed by a component in human breast milk that is undigestible to the infant; this bacterium is *Bifidobacterium* spp. (Ennis et al., 2024). *Bifidobacterium* spp. are early colonizers of the human gut and tend to stick around (Lawson et al., 2020). This is a bacterial genus containing gram-positive bacteria that are generally considered to have a positive effect on the human host (Gomes and Malcata, 1999). This genus is often used as a probiotic to help reduce a patient's negative microbial symptoms if they are in a state of dysbiosis. It has been shown to have anti-cancer properties and has been studied as a potential treatment and/or prevention method (Shang et al., 2024).

On the other hand, *Bifidobacterium* spp. have also been showing up in several GWAS as correlating with multiple types of cancers, though causality has not been determined (Hatcher et al., 2023; Long et al., 2023). This data is somewhat unexpected as this specific bacterium has been known to be a potential cancer therapy as it has many anti-cancer properties (Gomes and Malcata, 1999). For example, a recent study found that *Bifidobacterium longum* helped reduce CRC symptoms as well as slow the progression due to its modulating of the gut microbial environment, leading to decreased inflammation and a more effective host immune system (Shang et al., 2024). It was found that *B. longum* modulates the gut microbiome by reducing the abundance of cancer-promoting bacteria while allowing for greater anti-cancer bacteria colonization (Shang et al., 2024). This study is specific to *B. longum,* while the other studies discussed do not mention a specific species of the *Bifidobacterium* genus. If there truly is a causal relationship between the higher abundance of *Bifidobacterium* spp. and the development of various cancers, it is likely that certain species are cancer-promoting since studies have shown that other species have anti-cancer properties (Gomes and Malcata, 1999; Shang et al., 2024). Another possibility to explain the conflicting results is that the *Bifidobacterium* spp. that are present in higher quantities in those with cancer may be helping the patient rather than harming them. The specific mechanisms of influence of *Bifidobacterium* spp. on cancer risk are largely unknown to this day. Currently, it seems unlikely that *Bifidobacterium* spp. have a causal relationship with developing cancer since the majority of research indicates that this genus

provides more helpful than harmful effects in cancer patients (Ennis et al., 2024; Shang et al., 2024).

Aside from *Bifidobacterium* spp., other microbes can affect the host in ways that may increase or decrease the risk of developing and/or progressing cancer (Xu et al., 2023). There is not much research on specific microbes that are linked to cancer, but knowing the functions of various microbes can help hypothesize what may harm or help a patient. Any inflammatory pathogen, especially those that cause chronic inflammation, could lead to an increase in cancer risk (Singh et al., 2019). Some microbes affect host hormone, metabolic, or other physiological pathways that could either promote or reduce cancer risk depending on the specific situation (Xu et al., 2023). For example, *Marvinbryantia* (among others) contains a gene encoding ß-glucuronidase, which affects estrogen modulation, ultimately leading to an increased risk of the host developing breast cancer if dysregulated (Hong et al., 2023). There are numerous examples of microbes that may increase the host's risk of cancer, but this is a relatively new area of study and quite challenging to determine causality given the great potential of confounding variables, and it will likely take some time to make any claims.

Microbial Dysbiosis in Leukemia

Microbial dysbiosis may look different in cancer type as well as person to person, but for the sake of simplicity, this review will focus on leukemia to understand some more specific correlations. The pathogenesis of leukemia is often affected by dysbiosis in the gut microbiome, which can cause a weakening of the epithelial barriers in the intestines (Rashidi et al., 2019; Yu et al., 2021; Pötgens et al., 2023). This weakening of the gut barrier may be partially due to the lower abundance of *Odoribacter splanchnicus* caused by some common chemotherapy drugs, including daunorubicin (Pötgens et al., 2023). Overall gut microbial diversity tends to decline during the course of treatment (Lee et al., 2019; Rashidi et al., 2019). This is usually due to the intensity of the treatment since it is not specific and kills both the harmful and helpful cells/bacteria. Studies on mice have shown that this low gut microbial diversity can lead to not only the progression, but also the development of leukemia in genetically predisposed populations (Vicente-Dueñas et al., 2020). This study specifically treated the mice first with an antibiotic to reduce their microbial diversity, and this led to the development of leukemia in the genetically predisposed population (Vicente-Dueñas et al., 2020). If this knowledge also applies to humans, which is possible, it may be beneficial to monitor patients who receive antibiotic treatment to ensure their gut microbial diversity eventually returns to normal without first developing any other disease. It would also be beneficial to sequence the genomes of the patients to identify possible predispositions, but as previously discussed, this is not always an option due to a lack of resources. The use of antibiotics always poses a risk and should be monitored more closely.

Several studies have found *Streptococcus* spp. in higher abundance in patients with different types of leukemia as compared to controls. The authors mention that in other cancer types, this bacterial genus may be progressing the cancer via urea nitrogen recycling but that more research is needed to determine the role in leukemia progression (Yu et al., 2021). It has also been suggested that sugar fermentation is the leading factor in leukemia progression (van de Bogert et al., 2013). Either way, the bacteria is acting as an opportunistic pathogen because it is taking over when the host is weakened and many of the other beneficial bacteria cannot survive. Serious infection is common for leukemia patients as their immune system is severely weakened through chemotherapy, and pathogens can take advantage of this state (Galloway-Peña et al.,

2020). Once the infection begins, the host immune system cannot fight back effectively, and the pathogen can do serious damage to the patient. It may even worsen the cancer if it disrupts the balance between pro-inflammatory and anti-inflammatory cytokines (Binder et al., 2018). There may not be many options for treating these patients, but it has been shown possible to reverse serious infection in an acute myeloid leukemia (AML) patient through fecal microbiota transplant (FMT) (Lee et al., 2019). This has worked in the past because the newly introduced microbes begin to compete with the overtaking opportunistic pathogen, causing it to return to a more normal abundance (Lee et al., 2019; Malard et al., 2021). Fecal microbiota transplants can restore microbial symbiosis in patients with harmful microbes overtaking their microbiome (Malard et al., 2021). This type of treatment has been recently FDA-approved for *Clostridioides difficile* infection but is not researched enough to be common for leukemia patients who have acquired any infection during or after chemotherapy.

Understanding the patient's gut microbiome can be very influential in preventing infection and/or following the most effective and individualized treatment plan. A study found that AML patients with a higher abundance of *Porphyromonadaceae* spp. in their gut were more likely to avoid infection throughout chemotherapy, suggesting that this bacterial genus has a more protective role in the gut (Galloway-Peña et al., 2020). Many bacteria in the gut have protective roles, so it is interesting that this one in particular stands out in the case of AML patients undergoing chemotherapy. It has also been hypothesized that *O. splanchnicus* has a protective role as those with higher levels experience less gut barrier damage throughout the course of the disease (Pötgens et al., 2023). Supplementing with these protective bacteria should be an area for future studies to learn how to better prevent and/or treat infection during chemotherapy.

Current methods of treatment often require antibiotic administration, which can be very dangerous for cancer patients. For example, the administration of a specific type of antibiotic, carbapenems, has been associated with an increased risk of infection during the post-chemo recovery phase due to the antibiotic destroying the patient's microbiome (Ballo et al., 2020; Galloway-Peña et al., 2020). This poses a problem since carbapenems are especially good at treating severe infections, which are common from chemotherapy treatment (Papp-Wallace et al., 2011). Many strong antibiotics administered during chemotherapy will likely cause some harm due to the patient's weakened immune system. Medical professionals must make a choice between administering a weaker antibiotic and risking harm to the patient from the infection or administering carbapenems (or other strong antibiotics) and risking harm post-chemotherapy. Due to this dichotomy, it is important to understand a patient's microbiome and what may be causing or protecting against infection, especially since patients with reduced overall microbe abundance in their gut microbiome tend to develop more severe infections (Rashidi et al., 2019; Portlock et al., 2023).

The microbiome can also affect amino acid levels (Atasoglu et al., 1998). Differences in not only the composition of gut microbes but also amino acid levels due to these microbes have been found in AML patients when compared to control populations (Xu et al., 2023). Specifically, it was found that *Collinsella* spp. and *Coriobacteriaceae* spp. are linked to a higher expression of a few metabolites (Xu et al., 2023). This change in expression levels can alter various crucial pathways in the host involving cell survival and cell cycle progression, which can contribute to cancer development (Plati et al., 2011). AML patients have also been found to have an increased *Firmicutes* to *Bacteroidetes* ratio when compared to healthy individuals, which is not unexpected since this can be an indicator of dysbiosis (Koliada et al., 2017; Demirci et al.,

2020; Xu et al., 2023). All this microbial information, if gathered in the early phases of treatment, may help to determine the course of treatment to avoid serious infection as the chemotherapy progresses.

The *Firmicutes* to *Bacteroidetes* ratio is a frequently studied indicator of dysbiosis. Most studies show that an increased or decreased ratio correlates with some form of dysbiosis, whether that means obesity or a chronic disease in adults (Demirci et al., 2020). Several years ago, the research was trending toward this ratio being an indicator of Body Mass Index (BMI), but as this was studied further, the correlation became weaker as more confounding variables were discovered (Houtman et al., 2022). The reasoning behind this belief was due to the role that the *Firmicutes* and *Bacteroidetes* play in appetite regulation and fat storage (Sheepers et al., 2015; Zhang et al., 2020). An increased ratio was thought to be linked to obesity because this increased the short-chain fatty acid production in the body, which contributes to various processes, but mainly energy supply and appetite regulation (Sheepers et al., 2015; Zhang et al., 2020). Currently, more studies are being published showing that these correlations are not as clear as they were once thought to be. More research suggests that this correlation may only apply to individuals whose BMI is considerably high, not just borderline obese or overweight (Houtman et al., 2022). Early studies likely overlooked confounding variables in making their conclusions as many other factors could have influenced the apparent correlation, including diet, lifestyle, genetic predisposition, obesity level, and other comorbidities. That said, information about a person can still be obtained through analysis of their *Firmicutes* to *Bacteroidetes* ratio because of the context it provides into how their microbiome is interacting with their physiological processes.

If causality between a microbe and cancer has been determined, the question of which comes first remains. Did the microbe first colonize the patient and cause a change in metabolic, immune, or other host processes and ultimately led to cancer, or did the patient develop cancer, which then led to a different internal environment that was more hospitable for the microbe to colonize and take over? The answer to this question likely differs by disease and maybe even by individual, so no generalization can be made here. However, some studies are focusing on early signs of specific cancers to understand if the microbial colonization and abundance has already changed or not. More advanced precursors of CRC have been shown to have a further deviation from controls in microbial richness, though both early and advanced precursors have been shown to have a composition different than the control, with *Bacilli* spp. and *Gammaproteobacteria* spp. being enriched (Peters et al., 2016). This data could support either side of the question, depending on which angle it is looked at from. If the early precursors already deviate from control, this supports the idea that the microbes lead to disease. If this is the case, then the question becomes, what causes the change in microbial composition? These subjects may have always had a higher risk of developing the disease because of their microbiome. Then, this leads back to the host genetics. However, it could also be argued that the further deviation in the advanced precursors indicates the disease is leading to microbial change. Either way, more long-term studies are necessary in this area to find an answer to these questions. Studies should follow patients for a long period of time, routinely collecting stool samples to test how the microbiome changes throughout the patient's lifetime with or without health complications.

Using probiotics as a cancer therapy is a current area of study. This treatment option makes the most sense in the case of CRC, where the probiotics are more directly interacting with the cancer. The idea is that if the helpful bacteria is used as a supplement, it has an opportunity to change the metabolic, cellular, and immune processes that have gone awry in cancer (Fotiadis et

al., 2008). This is also an alternative to more aggressive treatments that may leave the patient more vulnerable to opportunistic pathogens (Galloway-Peña et al., 2020). Treating patients with a probiotic may help prevent infection during chemotherapy, which as discussed previously, can lead to serious outcomes, but this area requires more research before conclusions can be made (Rashidi et al., 2019). An older study found that *Bifidobacterium adolescentis,* which is already a commonly abundant member of the human gut microbiome*,* as a probiotic promoted immune cells that destroyed tumor cells (Lee et al., 2008). This species has been shown to have many properties that are beneficial to human health and longevity (Lee et al., 2008). There are several probiotics currently being studied for this purpose, and it is not strictly confined to the gut microbiome. There has been some success in halting lung tumor metastasis by treating the patient with an aerosolized probiotic (Le Noci et al., 2018). Though the simpler route is oral administration to affect the patient's gut microbiome, the idea of aerosolizing the probiotic opens doors to new methods of administering probiotics to have a greater positive effect on various human body microbiomes.

It was previously discussed that the microbiome is more involved in human processes than was once thought, and *B. adolescentis* is a prime example. Studies have shown that *B. adolescentis* is involved in the gut-brain axis by producing gamma-aminobutyric acid (GABA), which is an important neurotransmitter (Duranti et al., 2020). GABA, if dysregulated, can be an indicator of anxiety and depression (Kalueff and Nutt, 2007; Duranti et al., 2020). In the case of many cancers, the abundance of *B. adolescentis* is reduced, causing dysregulation of GABA, and therefore, can cause other health complications in the patient (Duranti et al., 2020). This shows medical professionals just how crucial a healthy microbiome is in the patient's overall health. Another example of a potential probiotic as a cancer therapy is *Lactobacillus reuteri,* which suppresses many pro-cancer cytokine pathways (Hemarajata et al., 2013). These are only a few examples of many being studied as cancer prevention and therapy options. Thus far, they have been shown to be effective mainly against CRC because of the more direct interaction, but probiotics given in conjunction with other cancer therapies or various methods of administration may be an option for other cancer types to avoid infection during or after treatment. Continuing studies are needed in this area of research to determine the best course of treatment.

Unfortunately, probiotics are usually sold as dietary supplements, meaning they do not require approval from the Food and Drug Administration (FDA) (Britton et al., 2021). This can be very dangerous as the companies that are making these products can very easily cut corners and add ingredients that they do not claim on the labels since they do not need approval to put these on the market. Another risk of probiotic use is antibiotic resistance (Merenstein et al., 2023). Antibiotic resistance is a growing problem globally, and regular probiotic use may worsen the problem through horizontal gene transfer of antibiotic-resistant genes from the probiotic to pathogens in the patient microbiome (Imperial and Ibana, 2016; Merenstein et al., 2023). It is unclear at this point how often horizontal gene transfers occur from probiotic to pathogen, so continued research is needed (Merenstein et al., 2023). Better regulation of probiotics may help prevent this issue if we ensure the bacteria in the supplement does not contain resistant genes. If probiotics are going to be a part of the future of cancer therapies, the FDA must start regulating their approval and requiring more research to ensure that these products are safe for both healthy people and cancer patients.

Conclusion

In this review, GWAS has been discussed, and how these studies can be used to obtain genetic and microbial data to help predict, prevent, and treat disease. This review aims to bridge the gaps between these fields and provide a more comprehensive view of using this type of data in conjunction. Though GWAS can provide vast amounts of information, caution must be used when presenting data because confounding variables are usually unavoidable and may skew data. That said, GWAS can be effective in identifying potential SNPs that may be correlated with an increased risk of disease. GWAS can also be used to correlate host genotypes with microbial composition and abundance. *Bifidobacterium* spp., in particular, is partially tied to the host genotype. This genus of bacteria has been shown to have anti-cancer properties and is being researched as a possible therapy for cancer patients. However, it has also been recognized in GWAS as more abundant in cancer patients than in healthy controls. At this time, it seems more likely that *Bifidobacterium* spp. are more helpful than harmful and potentially more abundant because they are playing a role against cancer. Continued research on these bacteria is needed to determine potential roles in cancer.

This review analyzed several twin studies to further understand the difference in environmental versus genetic impact on the microbiome. The current research supports the microbiome playing a larger role in that monozygotic twins tend to differ in microbiome composition and abundance if separated and tend to be more similar if co-housed. Studies show that unrelated people who live together tend to have more similar microbiomes than related people living apart (Tavalire et al., 2021). The environment arguably plays a larger role in microbiome composition than genetics, but the latter cannot be ignored. In the discussion of microbial dysbiosis in leukemia, it was shown that patients tend to decrease in microbial diversity throughout the course of chemotherapy treatment, leaving them more susceptible to infection (Lee et al., 2019; Rashidi et al., 2019). Some early predictors of serious infection during chemotherapy were also discussed, including the correlation of higher abundance of *Porphyromonadaceae* spp. with lower rates of bacterial infection (Galloway-Peña et al., 2020). Leukemia can be a very aggressive cancer, and more studies should be devoted to understanding these potential new mechanisms of prevention and treatment to offer more options for those afflicted.

These connections between genetics and the microbiome are important to understand so that medical professionals can have more methods to help their patients remain healthy. As this field is advancing, the technology to sequence patients is becoming cheaper and, ideally, can become more accessible to aid in prediction and prevention. If medical professionals can use this data to get ahead of the disease, maybe it can become less of a threat. Future studies should aim to understand how to correct the microbiome to bypass genetic risk factors. For example, if a genetic mutation is causing a depletion of some resource that is causing problems in the microbiome and leading to disease, maybe if the medical professionals prescribe a supplement of what the microbiome needs, that will help reduce (or prevent) disease. This manner of treating the root cause rather than the symptoms is more sustainable as it aims to completely irradicate the disease in a patient rather than just help the patient cope with the symptoms that they are experiencing. With more research being done, the magnitude of effects that the microbiome has on the human body are becoming more evident. If the field continues to grow at this rate, the knowledge gained may be used to create new types of treatments that are safer and more effective than previous methods.

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