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Review

Gut Microbiome Impacts on the Progression and Treatment of Diabetes

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Abstract: Diabetes mellitus is an autoimmune disease that impacts millions of individuals worldwide. Diabetes is treatable and one can live a normal life with this treatment, yet there is still no cure and the exact mechanism through which it develops remains elusive. Diet, environment, genetics, and lifestyle are all factors contributing to the increasing number of diabetes cases, though genetics are being reconsidered as the primary factor. Instead, recent research has turned to the gut microbiome as both a cause of diabetes and a possible treatment and prevention option, with new data elevating the importance. However, this is controversial, because findings remain mixed and additional support is needed, as this manuscript will review. Presently, Type 1 diabetes (T1D) research has found that the microbiome can be used as a biomarker, because an increase in alpha- diversity is correlated to a decreased likelihood of developing diabetes. Research has also focused on using the gut microbiome to alleviate symptoms or prevent T1D. On the other hand, Type 2 diabetes (T2D) likely has a partial cause in gut microbiome dysbiosis due to increases in gut permeability and systemic inflammation leading to insulin resistance. However, an increase in beneficial bacteria through probiotics and other dietary changes could provide reversal of the disease state. Studies still need to be conducted to advance understanding of the exact mechanisms through which gut microbes can trigger or protect from diabetes, using further human clinical studies.

Diabetes mellitus is a chronic health condition that affects over 420 million people worldwide, in which the main feature of diabetes is higher than average blood glucose level. This is caused by either the pancreas not producing enough insulin or the body being unable to effectively use the insulin produced. High blood glucose, or hyperglycemia, can lead to skin conditions, nerve damage, vision loss, coma, and death. In addition, individuals with diabetes have a higher rate of heart attacks, strokes, and kidney failure. The rate of diabetes incidence worldwide is increasing, leading to a public health crisis as it approaches epidemic numbers (1).

There are three main types of diabetes, type 1, type 2, and gestational diabetes. Type 1 diabetes (T1D), also known as early-onset or juvenile diabetes, is an autoimmunedisorder where the pancreas produces insufficient levels of insulin, requiring affected individuals to supplement daily. This is directly caused by T-cell destruction of the pancreatic beta cells that produce insulin (2). Presently, the cause and prevention of T1D areunknown, but the occurrence is rising with approximately 86,000 children developing T1D each year worldwide (3). Type 2 diabetes (T2D) is the more common form of diabetesand typically affects adults. In contrast to T1D, T2D is characterized by insulin resistanceor the inability to effectively utilize insulin. Similarly, the exact mechanism behind the development is unknown, but obesity and inactivity, combined with genetics are three major risk factors (4). Gestational diabetes, which will not be discussed in this manuscript, occurs only in pregnant women who have not otherwise been diagnosed with diabetes. These women and unborn children are at higher risk of complications, but elevated bloodglucose typically disappears after delivery. However, women who have gestational diabetes are at a higher risk of developing T2D later in life (5).

The microbiome of the gastrointestinal (GI) tract is composed of bacteria, viruses, fungi, and protozoa that live in the digestive tract. These microorganisms can have many

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impacts on host biology beyond aiding in digestion, including enzyme synthesis, immune response, and interacting with the neuroendocrine system (6). As a result, it has become a research target for studying health and human disease. Compositional changes of the gut microbiome have been observed in both T1D and T2D (7), though the question of whetherthis is a cause or effect of the disease state remains to be determined. A perturbation of steady state in the microbiome, such as what is seen in diabetes, is referred to as dysbiosis. On its own, dysbiosis has been linked to increased immune response, inflammation, increased intestinal permeability, chronic infections, mental health issues, and many more. Of particular interest for T2D, inflammation and intestinal permeability are both associated with weight gain, one of the major risk factors (8). In addition, immuneresponses have been shown to be important for the development and progression of both T1D and T2D (9).

This manuscript seeks to contrast the state of the microbiome in T1D and T2D, by investigating microbial community composition in each disorder, as well as its potential use in diagnosis and treatment. The focuses of T1D research are children's health impacts, future treatments, and preventing the development of T1D. T1D is associated with changes in the gut microbiome, further affecting the overall health and immune responsesto the environment and diet. T1D is a lifelong disease, impacting overall wellbeing through both changes in one's diet and lifestyle. Though there is existing research that the gut microbiome is involved in the development of T1D, scientists are still investigating whether it can be utilized in treatment of the disease or in diagnosis as a biomarker. In contrast, researchers are beginning to believe that the gut microbiome has a causal relationship with the development of T2D. This is due to the fact that dysbiosis seen in T2D is also associated with inflammation and elevated gut permeability, both of which have also been further implicated in the development of insulin resistance. Therefore, present research focuses on the functional roles of the microbiota and shifting the microbiota to a healthy state to reverse the disease state. Determining whether dysbiosis associated with obesity and low activity level is a cause of T2D is an important step for reducing the incidence worldwide. The research presented here focuses mainly on human studies though clinical research, with additional support provided from research in mammalian models.

Type 1 Diabetes

Taxonomic Changes of Gut Microbiome T1D

In recent years, the rise of individuals with T1D under 15 years old has suggested that T1D may be less genetically based than the scientific community previously thought.This has led to research into the gut microbial diversity, showing variations in healthy individuals versus affected individuals, and thus implicating the role of the microbiome in T1D through these taxonomic changes. It has been shown that the overall microbial diversity goes down with diabetes and other autoimmune disorders and certain phyla vary greatly, namely Bacteroides and Firmicutes (7). These two phyla have inverse relationships in T1D versus control individuals, with Bacteroides increasing and Firmicutes decreasing in case samples (10).

There have also been specific taxa correlated with the development of T1D and specific changes in the taxonomy of the gut microbiome. In one study, the trajectories ofmicrobial diversity were explored in a cohort of 33 infants with T1D, showing that the species were highly variable among the cohort (11). In particular, there was an overabundance of *Blautia*, *Rikenellaceae*, and *Ruminococcus*. During this stage of development, the gut microbiome is still immature, thus easier to see taxonomic changesand possibly identify when T1D develops. In this study, 4 of the infants developed T1D while the study was being conducted. Through proper treatment of T1D, the individuals were stable throughout the study, which followed until the age of three (11). One additionalway of examining whether the microbial community can distinguish T1D disease state is through alpha diversity, which is also the species richness. This previous study found

that infants with T1D had a major drop of alpha diversity. The decrease of alpha diversity andthe increase in pathogenic bacteria resulted in a significant difference in gut microbiome between the infants with T1D and those unaffected. However, it was found that the gut microbiome needs more time to mature to reach its most optimal stage (11). Taxonomic changes in the microbial community have also been seen in older children with T1D. The first study to analyze this found that when comparing the microbiome of an individual with T1D and without, there is a vast difference in the number of *Bifidobacterium*, *Lactobacillus* and *Clostridium* (7), findings that have been confirmed in more recent studies. When compared to children without T1D, studies showed that the children with T1D had a much lower microbial diversity, accompaniedby increases in *Bacteroides*, *Blautia*, and *Streptococcus*, and decreases in *Bifidobacterium*, *Faecalibacterium*, and *Roseburia* (12). In addition, one of the largest andmost in depth longitudinal studies on the gut microbiome in the development of T1D is the TEDDY study, which collected 10,913 metagenomes from 783 children (13). This studyalso found differences between the T1D and unaffected disease states, though regional differences were crucial in their findings. All in all, there have been many confirmations that the gut microbiome is altered in a T1D disease state. However, further longitudinal studies are important to determine overall trends (13).

Use of the Gut Microbiome as a Biomarker

T1D can be diagnosed early in life, due the signs and symptoms heavily impacting daily life and the disease being relatively easy to test for (14). It has already beenestablished that individuals with lower counts of human leukocyte antigens (HLA) are at higher risk of developing T1D if not already affected (14). These are the antigens that regulate the body's immune response, and are one of the current biomarker tests used for T1D (15). However, since T1D is also associated with a change in microbiota, recent analysis has analyzed whether the microbial composition of the gut microbiome can beused as an additional biomarker of the disease state.

Using metagenomics, it has been suggested that the gut microbiome plays a functional role in T1D (14). Finally, the gut microbiota is also responsible for nutrients tothe host and can increase or decrease the host immune responses. Therefore, if the microbiome can be used as a biomarker for the T1D disease state, predisposed individuals could be monitored in order to prevent development of the disease (14) As discussed, the infant microbiome undergoes many taxonomic changes before reaching an adult-like state in the toddler years (10). The researchers realized that the children who eventually develop autoimmune disorders have a less diverse and stable microbiome thanhealthy children. As a result, this suggested that the microbiome in T1D was distinct fromhealthy toddlers, and that these bacterial markers could be used for early diagnosis. In addition, these researchers predicted that using negatively associated bacterial phyla as treatment could help prevent the development of T1D, if applied early enough (10).

The main study into the use of the microbiome as a biomarker investigated the changes of the microbiota and the association with T1D development (16). This study analyzed fecal, oral and vaginal samples from the non-obese diabetic (NOD) mice at different stages of their life. The main difference in the gut microbiota diverged betweeneight and ten weeks of age. At these ages the phylum and classes had deep contrasts; there were decreases of *Actinobacteria, Bacteroidetes, Proteobacteria,* and *Tenericutes*, and an increase in *Firmicutes* (16). This is important to note because most (61%) of the female mice in the study developed diabetes from 12-30 weeks of age, indicating that the establishment of the gut microbiome is most important in these earlier weeks. In addition,the fecal samples from the diabetic mice showed a significant reduction in diversity such that the alpha-diversity resembled immature 3-week old mice. The taxonomic changes showed the most at the genus level with the changes in *Coprobacillus, Staphylococcus* and *Escherichia*, correlated with increasing the likelihood of infection, disease and lower quality of health (16). As a result, some of the signs that a pre-diabetic NOD mouse willdevelop T1D is through

these taxonomic changes. If caught early enough, these signswould indicate a chance that the NOD mouse will not develop T1D with possible treatment. Hopefully, this research can form the basis of further studies in humans, inorder to decrease the rate of T1D occurrence worldwide.

Gut Microbiome as Treatment of T1D

The gastrointestinal microbiome is an ecosystem of its own and needs to be regulated and balanced to maintain a stable function. Studies have been conducted to see whether T1D can be controlled by changes in the gut microbiome, with multiple clinical trials in progress analyzing the effects of diet, probiotics, or even fecal transplants on disease state (17). At the time of publication of this review article, the focus of these in progress trials was largely on probiotics and prebiotics, and their ability to modulate glucose control and beta-cell function. However, as human studies can be hard to conduct, due to consent and control over confounding factors, there are a larger majorityof completed studies in mice. Therefore, it has been established that probiotics can prevent spontaneous autoimmune diabetes development in NOD mice, due to increased production of anti-inflammatory cytokines (18). Presently, human effectiveness is awaiting the results of the ongoing clinical trials.

A Chinese medicinal formula known as Danzhi Jiangtang Capsule (DJC) has been used to treat diabetes and, through unclear precise mechanisms, has been able to improvepancreatic functions. It is known that DJC reduces blood glucose and increases fasting plasma insulin (19). Due to these changes, the researchers saw a suppression of pancreatic beta-cell apoptosis, reducing the development of T1D in rats (19). Studies involving fecal transplantation (FMT) are more recent, where an individual with a classified healthy state, though healthy is still unclear, donates their fecal matter to be transplanted into another individual. The outcome is that the affected individual now has a healthier microbiome. The results of a very recent study showed that FMT reduces the endogenous insulin production and preserved residual beta cell function in individuals with T1D (20). Therefore, there has been moderate success in using the gut microbiome as treatment forT1D. All in all, by further understanding the role and changes of the microbiome in development of T1D, researchers hope to use treatments modulating this activity to decrease severity or prevalence of the disease state.

Type 2 Diabetes

Taxonomic Changes of Microbiome in T2D

Many studies have analyzed the differences in T2D disease state and healthyindividuals. In addition, those with prediabetes have been used as a secondary comparison. One of the first studies on the microbiome differences in T2D took place in Denmark (21), comparing 18 adult males with T2D and 18 control males. Using real-time quantitative PCR and pyrosequencing, the researchers found that there were significant differences at the phylum and class level. In this early study, the ratio of Bacteroides to Firmicutes was significantly positively associated with reduced glucose tolerance, due toa decrease in Firmicutes proportions in the diabetic group. This initial study represents the beginning of what is now a heavily researched division of T2D research. In fact, a review article from earlier this year analyzed the 42 observational studies on the microbial population in T2D (22). Overall, genera such as *Bifidobacterium*, *Bacteroides*, *Faecalibacterium*, *Akkermansia*, and *Roseburia* have been negatively associated with theT2D disease state, and *Ruminococcus*, *Fusobacterium*, and *Blautia* have been positively associated. However, this comprehensive analysis showed that *Lactobacillus* had contradictory results among studies. Therefore, as with T1D, there are observable differences between affected and unaffected individuals, though some trends need to be further elucidated.

Research has also shown that differences seen between these groups and unaffected individuals depend on the population. Given that different global populations have different core microbiomes due to genetics, immunities, and diet (23), it is

understandable that different cohorts would have different selection outcomes in the disease state (24). Analyzed the microbial composition of obese individuals with T2D andhealthy individuals in a Pakistani population, finding results that differed from previous studies on Indian populations. In this community, *Firmicutes*, *Clostridia*, and *Negativicutes* were among those increased, where *Bacteroides*, *Proteobacteria*, *Elusimicrobia*, and *Verrumicrobia* were decreased in T2D patients. In contrast, a study on an Indian population with T2D was dominated by *Proteobacteria* (25). Furthermore, a study in the United States comparing a control group, a pre-diabetes group, and a T2Dgroup found some genus and family level changes but were unable to confirm many previous results (26). Therefore, while the gut microbial community is clearly altered in T2D patients, there is also more research that needs to be done, especially at the familyand genus levels comparing different populations.

Finally, while these findings highlight potential hallmarks of T2D, it is important to point out that there are many factors in these individuals that could be affecting the gut microbiome. As discussed, obesity, low-activity level, and diet are all risk factors for developing T2D, yet these are also factors that will affect gut microbial composition. For example, Thingholm et al. (27) analyzed the microbial communities of lean non-diabetic individuals, obese non-diabetic individuals, and obese individuals with T2D in Germany. This group found that microbiome variation was more strongly associated with obesity asopposed to T2D occurrence, though there were still modest associations for T2D, with increases in *Escherichia* and *Shigella*. However, this manuscript also found that medications, such as antidiabetics, and dietary supplements were also sources of significant variation. As a result, the researchers emphasize the necessity of creating studies specifically to parse out these effects.

Gut Dysbiosis in Development of T2D

As mentioned, the microbiome of the GI tract has been implicated in inflammation, immune response, and increased gut permeability. Proposed mechanismsof insulin resistance include leakage of bacterial products into the bloodstream, which triggers the immune system (28), or leakage of inflammatory mediators, such as cytokines, directly into the circulatory system (29). Therefore, any increases in either inflammation or gut permeability can increase the risk of developing insulin resistance, a precursor for T2D. The following summarizes mechanisms through which gut dysbiosis can affect T2D, either through increasing harmful genera or decreasing beneficial genera (Figure 1).Specifically, the gut microbiome is of interest to explain how inflammation, immune response, gut permeability, and short-chain fatty acids can affect glucose metabolism and insulin resistance, promoting development of T2D.

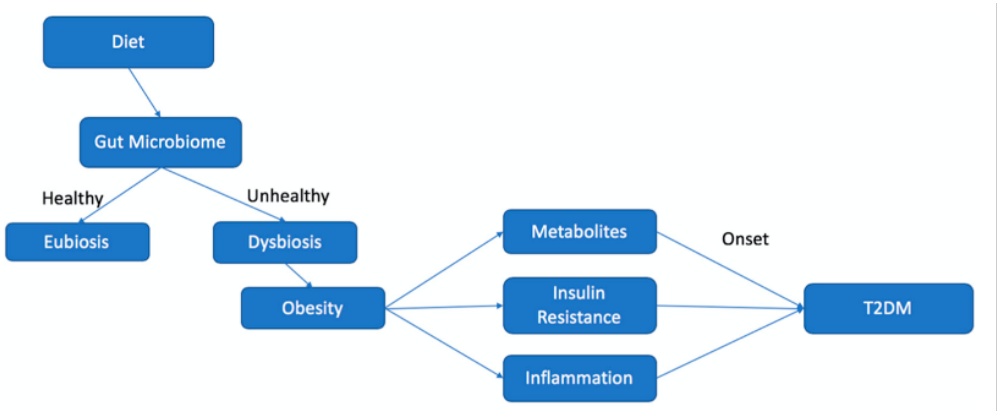

Figure 1. *Effect of diet on the microbiome and relationship leading to increased risk ofType 2 diabetes*. Through one's diet, the gut microbiome can either be in a state of eubiosis or dysbiosis. One possible outcome of dysbiosis is obesity, which through insulin resistance, inflammation, and bacterial metabolites can lead to development of T2D. Reprinted from (30).

First, permeability of the intestines is closely linked to inflammation and immune response, and studies have shown that these are possible mechanisms through which the gut microbiome can have a role in T2D development. Originally, it was established that T2D is associated with low-grade inflammation, though the mechanism was unclear. However, it has now been shown that changes in the gut microbiota induce metabolic endotoxemia and inflammation (31), both of which can be tied to elevated gut permeability and insulin resistance (28). As a result, this would explain why earlier studies showed lower levels of anti-inflammatory cytokines like IL-10 in individuals with T2D (32). Furthermore,these findings have been further supported by the confirmation that intestinal permeability is increased in humans with T2D (33) and an encroachment of bacteria on the inner mucous layer of the gut can lead to elevated glycated hemoglobin, or A1c (34). A1c isthe main test for individuals with diabetes and prediabetes, measuring average blood glucose over the past three months. Therefore, higher A1c indicates higher average bloodglucose levels. Overall, changes in the microbial composition of the intestines directly leads to changes in permeability and inflammation, and can promote T2D.

The second proposed mechanism by which gut leakage and inflammation can lead to T2D is through host immune response to increased levels of bacterial endotoxins in theblood. Lipopolysaccharides (LPS) are found in the outer membranes of Gram-negative bacteria, which enter the bloodstream through intestinal leakage, these endotoxins enterthe bloodstream. Here, they bind to TLR4 receptors, further activating proinflammatory pathways and initiating innate immune response (31). In turn, this pathway and elevated levels of LPS and TLR4 activation have been connected to insulin resistance (30,36). Therefore, with microbial changes increasing gut permeability to bacterial products, thehost immune response can lead to insulin resistance, raising the risk of developing T2D.

Additionally, linked to these findings is the decrease in SCFA-producing bacteria that has been established in T2D compositional changes (37). SCFAs like butyrate, acetate, and propionate have been implicated in overall gut health, however they have also been of recent interest in modulation of metabolism and energy homeostasis. A recent review specifically focused on communication between the gut and other tissues, along with how these molecules can impact insulin sensitivity (38). SCFA receptors have been found in skeletal muscle, adipose tissue, and the liver, implicating these peripheral tissues as being affected. Due to the adipose and liver tissues identification, increased levels of SCFAs have the potential to counteract obesity and insulin resistance (38). Thus, SCFA availability should be looked at in depth directly in regard to human T2D, in order to fully understand the impact of dysbiosis on the disease state.

Gut Microbiome and Treatment of T2D

Similar to other diseases that have been associated with dysbiosis, recent research has looked into using the gut microbiome to treat T2D. Unlike T1D, individuals who develop T2D are typically diagnosed with prediabetes prior to diagnosis of T2D, and both states have been shown to be reversible (39). Due to the discussed effects of dysbiosis associated with T2D, researchers have been attempting to shift the gut microbiome back to a eubiotic state, decreasing inflammation and supporting a healthy intestinal barrier, with the hopes of reversing T2D. However, at the same time, it is important to understandhow the current treatments for T2D are affecting the microbiome, in order to ensure that the dysbiosis is not exacerbated.

Current clinical trials focus on using probiotics, prebiotics, and other dietary supplements like fiber, in hopes of shifting the microbiota to a noninflammatory and "healthy" state, and have seen mixed results. Prebiotics have had conflicting success in alleviating symptoms of T2D. The first study into this analyzed the effect of 12 weeks ofprebiotic supplementation on the microbiota, endotoxemia, glucose tolerance, inflammatory markers, and intestinal permeability (40). This research study found that a galacto-oligosaccharide

mixture of prebiotic fiber had no significant effect on any of the categories, except for increasing bacterial diversity in the prebiotic group. However, theydid find that one of the common T2D treatments, metformin had a significant effect, which will be discussed further. A more recent study using a high-fiber diet, along with prebiotics and medicinal foods, increased a specific set of SCFA-producing strains (41). In turn, when these bacteria were present in higher numbers, patients also had lower A1c levels. Therefore, adding fiber to one's diet could help control T2D, though the researchers note that care should be taken to ensure that complex fibers are implemented.

Probiotic studies have mainly focused on *Lactobacillus* species, since they represent the largest proportion of available probiotics. Specifically, studies have looked at *L. casei* (42) and *L. reuteri* (43). Specifically, *L. reuteri* strains ADR-1 and ADR-3 showed beneficial effects on T2D patients. ADR-1 showed significant reductions in A1c and cholesterol levels, and heat-killed ADR-3 showed decreases in blood pressure after six months. However, most importantly, there were significant changes in the gut microbiota.When an eight-fold increase in fecal *L. reuteri* was seen, this corresponded with a significant decrease in A1c levels. Another intriguing finding was that *L. casei* was associated with significantly lower levels of blood bacteria. Returning to previously discussed topics, bacterial translocation has been associated with the development of insulin resistance. If this treatment was used in patients with prediabetes, there is a possibility that T2D could be prevented, since levels of blood bacteria would be reduced.

Finally, as mentioned above, current treatments can add confounding effects to any possible benefits of using probiotics and prebiotics. Metformin is one of the main T2D drugs, having been used for over 60 years. Metformin is widely prescribed because it is generally well tolerated and has few side effects, along with the ability to decrease A1c by 1.5 percentage points (44). One of the first analyses into the confounding effects oftreatment used 784 available metagenomes to parse out which changes were caused by metformin and which were caused by T2D (45), finding unique stratifications by metforminuse or nonuse. The changes in the microbiota caused by metformin can be seen as early as 24 hours after metformin administration, including decreased diversity, increased opportunistic pathogens like *Escherichia* and *Shigella*, and decreases in *Peptostreptococcaceae* and *Clostridiaceae_1* (46). On the other hand, another study found that in patients newly diagnosed with T2D, transfer of the metformin-treated microbiome to germ free mice improved glucose tolerance (47). Thus, more research is necessary to determine the exact effects of treatment on the gut microbiome, but it is important to control for treatment in further studies.

Conclusions and Future Directions

All in all, the gut microbiome is associated with compositional alterations in both T1D and T2D. Diabetes is unique because one can be diagnosed as prediabetic before being diagnosed as diabetic. The main purpose of identifying the differences in T1D is to determine whether the microbiome can be used as a biomarker for the disease state, since certain levels of bacteria such as *Bifidobacterium* and *Lactobacillus* can be markers in the progression of one's development of T1D. Research is still in its infancy to determine whether symptoms can be eased or prevented through modulating the microbiome composition, though it has shown to be relatively effective. On the other hand, the microbiome is thought to have a causal relationship with the development of T2D, due to the relationship of dysbiosis with inflammation, immune response, and gut permeability. Therefore, if these compositional changes can be identified and rectified early enough, the disease state can be prevented entirely. In addition, if T2D does develop, using treatment to alter the gut microbiome could reverse the disease state.

Future studies to understand T1D should include expanding taxonomic studies, researching mechanisms that cause processes such as deficient insulin metabolism, and human studies on using the microbiome as a biomarker. Factors such as diet and region can confound results in microbiome research, highlighting the need for additional

longitudinal studies. In addition, further conducted human clinical trials should be undertaken to determine whether probiotics and diet related treatments can alleviate symptoms or prevent the autoimmune response causing the disease. Since there is evidence that the gut microbiome may play a causal role in the development of T2D, further studies into the taxonomic changes are crucial for prevention. Specifically, longitudinal and multi-omics studies following individuals from the prediabetes state could help show exactly what taxonomic and functional changes are associated with the disease state. In addition, further studies into the exact mechanisms by which gut dysbiosis affects T2D development are necessary, such as SCFAs and immune response.Finally, some of the common T2D treatments can confound microbiome shifts, so furthercarefully designed studies are important for minimizing these effects. By doing this, the ultimate goal would be to use the gut microbiome as prevention of the disease state for both T1D and T2D, to decrease the rate of occurrence worldwide.

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