A Metabolic Mediator Gene "CanB" The Reason for Gain of Antibiotic Resistance in *Neisseria gonorroheae*

Abstract

In this review paper, I will be discussing the results of Daniel H.F. Rubin et al., about the study on the gain of function of antibiotic resistance in *Neisseria gonorrhoeae* (gonorrhea). First and foremost, the discoveries his group has made are important because of the resistance that *N. gonorrhoeae* has to most front-line antibiotics. Finding the cause of the infection and gaining the resistance could be the first step in finding a way to remove the resistance. His team found that the differences in *N. gonorrhoeae* strains and their strength against antibiotics could be appointed to a metabolic mediator gene *ngo2079* which the team named *canB* (β-carbonic anhydrase). This naming is due to it being parallel to the *can* gene in *Escherichia coli*. Working with two variants of the CanB gene: $CanB^{19E}$ and $CanB^{19G}$. They found that the 19E variant is necessary and sufficient for growth in absence of CO2, whereas the 19G variant confers CO2 dependency. And through their testing, the team found that 19G isolates were more likely to have resistance to ciprofloxacin. With those two varying strains, the team concluded that these differences in metabolic mediation are the reason for the gain of antibiotic resistance to fluoroquinolones.

Introduction

The purpose and reasoning behind choosing this paper are due to the potential future severity of gonorrhea. This infection continues to gain more antibiotic resistance to front-line antibiotics making it harder to treat. Due to this ongoing process, I wanted to shed light on those who are working in this field. To that end, Daniel H.F. Rubin et al. is also concerned with this issue facing society. In his paper, Rubin speaks on a discovery of a metabolic mediator gene *ngo2079* that codes for a β-carbonic anhydrase (CanB) in gonorrhea. This gene which the team named Canβ has two different variants $CanB^{19E}$ and $CanB^{19G}$. The results of this experiment showed that these two variants are drivers of CO2 dependency in gonorrhea. This CO2 dependency is directly related to the growth of gonorrhea within their specific niches. (Rubin 2023) The reason why Rubin and his team chose to pursue this is because of his past work, which will be discussed later.

Past Discoveries

The publication online of this paper was on January 5th, 2023. Due to this, there isn't recent progress to speak on, however, the information that they expanded upon is some of Rubin's past work. In 2020 Rubin had a paper published revolving around a discussion about past and present treatments of gonorrhea and potential treatments for the future, involving vaccination and

repurposing other medications in combination with treatments to fight gonorrhea. Specifically, gentamicin and azithromycin when used in conjunction had a 100% cure rate against the isolates chosen. (Rubin, D. H. F. 2020). Gentamicin was also tested by itself against ceftriaxone and was found to have not as much effectiveness against pharyngeal infections but a 94% effectivity rate against genital infections. (Rubin, D. H. F. 2020). The past work that Rubin has done created a solid framework for understanding his current publication on how CanB allows the gaining of antibiotic resistance to gonorrhea.

Other work by another team of researchers working with *Escherichia coli* conducted experimentation with the metabolic genes that confer antibiotic resistance. Allison J. Lopatkin et al. successfully found a noncanonical gene related to central carbon and energy metabolism (Lopatkin, A. J. 2020). These genes once found were knocked out of the chromosome of these clinical strains and then replaced with the wild type and mutant variants. Afterward, the team found a much higher metabolic rate of the antibiotics introduced to E. Coli. The discoveries made in this experiment referenced by Rubin in the paper at hand helped to show that it is possible to edit the infections genetically to make weaker strains that can be cured. The next step is to figure out how to get pathogens in non-lab settings to change their DNA.

A similar study conducted by Christophe Merlin, Millicent Masters, Sean McAteer, and Andrew Coulson in 2003 attempted to tackle the original issue of why carbonic anhydrases are important for E. Coli; Their work showed how carbonic anhydrase a gene called yadF (renamed to scan) is used to convert CO2 to bicarbonate for cellular functions. (Merlin 2003). This research was one of the first steps to figuring out how E Coli became dependent on carbonic anhydrases for survival, which led to later studies related to the causation of antibiotic resistance, paving the way for the research being conducted today.

Discussion

Coming down to the meaning of the results of the experiment, a few things were shown: A single gene mutation was necessary to confer dependence on CO2 into *N. gonorrhoeae*, that the $CanB^{19G}$ has reduced enzymatic activity compared with $CanB^{19E}$, $CanB^{19G}$ is associated with ciprofloxacin resistance in clinical isolates, and that $CanB^{19G}$ compensates for gyrase mutation-related fitness costs (Rubin 2023). The data presented in the 2023 paper is correct at this time, due to the relative newness of the paper. Because of this, there hasn't been ample time to rerun experimentation to double-check the work. Breaking down the course of the results, the team first found that they had to induce mutation through chemical reactions changing the Amino acid of G to E. Afterwards they observed the rate of oxygen consumption of these two strains and took the data of CO2 consumption efficiency. They found that the 19G variant, the more common lineage, doesn't grow very well in CO2-deficient environments. Whereas, the 19E variant can still grow just as well in a CO2-deficient environment. In both cases, when there was an abundance of CO2, both variants grew just as well as one another. This showed a clear dependence on CO2 for the 19G variant to grow. Another finding is concerning pH within the vaginal canal. A healthy pH is 4.5, and the viability for surviving in that niche was much higher for 19E. This is thought to be due to a lack of need for supplemental CO2 which allows the infection to better buffer its intracellular pH. The reason for the 19G association with ciprofloxacin resistance is due to the data analysis of a minimum inhibitory concentration (MIC) of ciprofloxacin which was four times higher than other variants (0.43 vs 0.10 $\mu g\ ml^{-1}$) (Rubin

2023). The other drug types mentioned were not as significant as the ciprofloxacin difference. This piece of data pointed to a considerable link between the 19G variant and incurring antibiotic resistance in the gonorrhea strain.

All of the past studies mentioned paved a road to the current day experimentation, from the inklings of the “can” gene being discovered in 2003 by Merlin et al. sparking questions about what it does for the E. Coli’s cellular function, to more recent discoveries by Lopatkin et al. in 2020. They were able to genetically weaken E. Coli by removing non-essential genes leading to easier-to-treat pathogens. Rubin’s interest with gonorrhea and possible roads of treatment, which he discussed in 2020 is a contributor to his current day work as well, with his use of combinations of antibiotics or the changing of the genome to neutralize the gonorrhea pathogen. With the recent finding of the canB gene by Rubin et al. he may have found an avenue for furthering the potential treatment options with genomic editing and antibiotic treatments.

Based on the results gathered it does seem to have a strong connection between the 19G variant and incurring antibiotic resistance. Although the researchers were quite sure there was no other carbonic anhydrase gene, this still does leave us with unanswered questions. Specifically, if we can truly, one hundred percent pinpoint this gene as the cause for the gain of antibiotic resistance in this pathogen, how can we disable it in the field and fight it? Another question in this vein that still needs to be answered is how the gyrase mutations alter the metabolic landscape of the cell (Rubin 2023). This question is due to gyrA mutations in the pathogen. Hopefully, in the future, we will be able to find the answers to these questions.

References

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