Analyses of Peripheral Blood Micro RNA Expression in Female Crack-Cocaine Dependents

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Abstracts will be considered for both poster and platform presentations

Disease biomarkers

Background Information

Drug dependency is a prevalent problem in modern society and cocaine is one of its the most devastating effectors, ruining the lives of millions of people around the globe. Current treatments for this condition are very expensive and not effective in preventing patients from relapsing. Clinical researchers have tried to use peripheral blood protein levels as biomarkers for drug dependency but could not reach a consensus. However, studies have shown that expression levels of several different Micro RNAs (miRs) might serve as useful biomarkers with potential for application in novel treatments of cocaine dependency. Pre-clinical studies on rodent models show that rats presenting compulsive-like cocaine taking behavior have abnormal miR expression in certain brain areas. The up or downregulation of certain miRs has been linked to inflammatory brain processes mediated by microglia and neuroplasticity mechanism in the reward system. These processes have been correlated with compulsive cocaine use and could help explain the development of hypersensitivity to cocaine-related cues, impulsiveness, and abnormal habit-like behaviors that are insensitive to adverse consequences. Based on the findings of pre-clinical trials we hypothesized that there would be a difference in the expression of certain miRs in the peripheral blood of the female crack-cocaine dependent group (n = 30) and the control group (n = 20). The targeted miRs were miR-212, miR-181, and miR-124. The housekeeping miRs were miR-24 and miR-126 which have been thoroughly validated as good standards for performing delta-delta CT in miRs.

Methodology

The research project was carried out as planned for the most part. There were only two diversions from the initial plan for the project. The first change was that initially the plan was to have 40 subjects in the female crack-cocaine dependent group, but due to low volume of blood 10 of the samples from the crack-cocaine group were discarded, leaving only 30 subjects. The second change was the inclusion of several new miRs in the project. A preliminary analyses of DNA methylation performed in collaboration with a lab from the University of Texas Health Science Center pointed miR-181 and miR-124 as possible biomarkers for crack-cocaine dependency. Therefore, we checked for alterations in the expression of miR-181 and miR-124 as well as miR-212.
RNA was extracted from the blood of the subjects using the PAXgene blood RNA extraction kit. Each sample of 200 ng of total RNA was converted to cDNA using miScript II RT HiFlex Buffer Kit (Qiagen). PCR was performed using the miScript II SyBr Green PCR Kit (Qiagen) and the respective primers for each miR (Qiagen). Delta-delta CT was performed to establish the level of expression of the targeted miRs in the crack-cocaine dependent and control groups. Non-parametrical statistical analyses (Mann Whitney U test) was performed to determine if levels of expression were significantly different in the patient and control group.

Results

The data indicated that miR-212 expression is not abnormally expressed in the peripheral blood

of crack-cocaine depends. In addition, the data indicate that miR-181 and miR-124 are upregulated in the peripheral blood of female crack-cocaine dependents. For miR-124 median latencies in control and crack-cocaine groups were 1.37 and 25.00; the distributions in the two groups differed significantly (Mann–Whitney $U = 449.00$, $P < 0.01$). For miR-181 median latencies in control and crack-cocaine groups were 096 and 6.25; the distributions in the two groups differed significantly (Mann–Whitney $U = 459.00$, $P < 0.01$). For miR-212 median latencies in control and crack-cocaine groups were 0.86 and 1.23; the distributions in the two groups was not significantly different (Mann–Whitney $U = 279.00$, $P > 0.05$). The findings of this study demonstrate that miR-124 and miR-181 might be promising biomarkers of the pathophysiology of crack-cocaine dependency. Analyses of the correlation between miR-124 and miR181 upregulation and clinical phenotypes for crack-cocaine dependency needs to be studied to establish the utility of these miRs as biomarkers. MiR-124 and miR-181 could help diagnose the presence and/or severity of a variety of clinical phenotypes in crack-cocaine dependency.