Comparison of Gene Expression in the Kidneys of Male and Female Mice Using the DNA MicroArray Mgu74Av2

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The kidney is the principal excretory organ of the body, with males and females differing in their ability to excrete many potentially toxic compounds through the kidneys. This is particularly true of compounds that are organic anions, which in several instances are excreted at a more rapid rate in males than in females. Many commonly prescribed drugs are organic anions, suggesting that dosing of these drugs may need to be substantially different for males and females. The underlying genetic mechanisms for these differences are poorly understood. Our general hypothesis was that differences in renal gene expression underlie the physiological differences between males and females in the processing of drugs and other xenobiotics. Accordingly, we measured gene expression using the DNA MicroArray Mgu74Av2, comparing three male and three female mice of approximately 11 weeks of age. Microarray experiments yielded large amounts of data that was analyzed (using GeneSifter™) to identify genes whose expression appeared to be different in males and females.

Our results showed that genes involved in antioxidant activity showed a slight increase in expression in male mice, whereas genes involved in chaperone activity showed a decrease. Among transporter genes, ion transport and organic acid transport showed an overall increase in males, while oxygen and lipid transporter genes showed a decrease. Further experimentation will be needed to clarify the physiological and pharmacological significance of these results.

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Introduction

It has long been known that males and females differ in their ability to process (metabolize and/or excrete) various xenobiotics, including many pharmaceuticals. The genetic underpinnings for these differences remain largely unexplored. We hypothesized that differences exist in renal gene expression between male and female mice that potentially underlie the physiological differences between the two sexes, including drug-handling ability. The purpose of our experiment was to measure gene expression in male and female kidneys, in an effort to identify functional categories that showed either an increase or decrease in expression between the two genders.

Methodology

The DNA MicroChip Mgu74Av2 was used to test expression levels in the six mice. The GeneChip probe array is a novel technology that combines photolithography and combinatorial chemistry. Thousands of oligonucleotide probes are synthesized to each array. Oligonucleotides are genetic sequences, and these sequences are clustered in specific areas called probe cells. Each probe contains millions of copies of each gene sequence, and is manufactured such that an oligonucleotide's probe is in a known location, and each probe is packed into a cartridge. Procedures for tissue sampling consist of converting RNA to tagged, complementary RNA (crane). Biotin-labeled crane, referred to as the "target," is hybridized in a hybridization mix (a simple crane solution), which includes the fragmented target, BSA, probe array controls, and herring sperm DNA. It is adhered, or "hybridized," to the MicroArray probe in a 16-hour incubation period. The hybridized MicroArray probe is stained with a streptavidin phycoerythrin conjugate and scanned by the GeneChip® Scanner 3000, or the GeneArray® Scanner. The amount of light emitted at 570 nm is proportional to the bound target at each probe array. This means that pixel intensity, or brightness, is directly proportional to expression levels. As mentioned before, the oligonucleotides are clustered in specific areas. The cRNA is tagged with Biotin (a fluorescent dye) and hybridizes to the oligonucleotides. If initial quantities of the complementary nucleic acid are high, a large amount of cRNA will hybridize to its complementary strand on the DNA MicroChip. When scanned using either the GeneChip® Scanner 3000 or GeneArray® Scanner, the hybridized strands appear "bright." This brightness correlates to pixel intensity, which in turn correlates to the amount of cRNA transcript hybridized. Through generic physiological processes, the quantity of a specific nucleic acid correlates to the amount of peptide translated and produced. MicroArray Suite 5.0® was used to quantify expression values from the DNA MicroChip. Results were tabulated in an Excel™ spreadsheet, with signal-log ratios, and the perceived general change (between wildtype females and males).

Data Analysis

The GeneSifter™ analysis program was used to analyze the raw data from the GeneChip. A pairwise analysis was

conducted using two experimental groups; group one comprised wildtype females, while group two comprised wildtype males. Members of group two were compared to members of group one. The results reflected changes in the male samples; the males showed either an increase or decrease. A threshold of 2 (a gene had to show at least a two-fold change) was set, and samples were normalized to the median. Student's *t* test was performed on each group, and a Benjamini and Hochberg False Discovery Rate test was run on the data obtained. The resulting genes were separated by ontogeny into their respective categories on the basis of biological process, cellular component, and molecular function. Statistical significance (based on percentage of change) was calculated from the data, and conclusions were made from the results.

RESULTS

From results obtained from the Mgu74Av2 GeneChip and analyzed using GeneSifter™, 141 genes were shown to be up-regulated whereas 193 genes were shown to be down-regulated.

Our analysis was based on the formula: percentage of up- or down-regulated genes that fall into a particular ontogeny divided by the percentage of that ontogeny represented on the chip. We compared the two values—the numbers procured from up-regulation and the numbers from down-regulation. Using this formula, we hoped to find the categories that were over-represented in a specific change. If a category showed consistent changes (over-representation in up-regulation and under-representation in down-regulation, or vice versa) it was considered significant. From our analysis, the significant changes in molecular function were seen in the families of antioxidant activity, which showed an over-representation in the up-regulated category in males, and chaperone activity, which showed an over-representation in down-regulation.

The primary goal of our experiment was to analyze the change in transporter activity. We followed the same formula used for the analysis of molecular function, to calculate over-representation of transporter activity. From our analysis, the families of ion transport and organic acid transport showed an over-representation of up-regulation in males, whereas lipid transport and oxygen transport showed over-representation of down-regulation.

CONCLUSION

From the analysis of our results of molecular function, we conclude that family of antioxidant activity and ion and organic acid transport showed increase in overall expression in males, whereas the family of chaperone activity and lipid and oxygen transport showed decrease.

The central goal of our experiment was to analyze transporter expression and, although transport activity did not show significant variation in molecular function, families within transport activity did show change. In transporter activity, the families of ion transport and organic acid transport showed an overall increase in males, while the families of lipid transport and oxygen transport showed decrease. This is significant because the families of ion transport and organic acid transport are involved in drug processing and it has been hypothesized that variation in transporter expression is directly responsible for an individual's ability to handle certain medication. Further experimentation, including qPCR (quantitative polymerase chain reaction) is required to verify results.

Our research identifies the genetic mechanisms that underlie physiological differences between males and females and, in turn, presents further research into the differences in pharmacokinetics between the two sexes.

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