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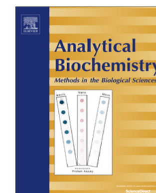

The relations between metabolic variations and genetic evolution of different species

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ABSTRACT

Metabonomics has been applied in many bio-related scientific fields. Nevertheless, some animal research works are shown to fail when they are extended to humans. Therefore, it is essential to figure out suitable animal modeling to mimic human metabolism so that animal findings can serve humans. In this study, two kinds of commonly selected body fluids, serum and urine, from humans and various experimental animals were characterized by integration of nuclear magnetic resonance (NMR) spectroscopy with multivariate statistical analysis to identify the interspecies metabolic differences and similarities at a baseline physiological status. Our results highlight that the dairy cow and pig may be an optimal choice for transportation and biodistribution studies of drugs and that the Kunming (KM) mouse model may be the most effective for excretion studies of drugs, whereas the Sprague–Dawley (SD) rat could be the most suitable candidate for animal modeling under overall considerations. The biochemical pathways analyses further provide an interconnection between genetic evolution and metabolic variations, where species evolution most strongly affects microbial biodiversity and, consequently, has effects on the species-specific biological substances of biosynthesis and corresponding biological activities. Knowledge of the metabolic effects from species difference will enable the construction of better models for disease diagnosis, drug metabolism, and toxicology research.

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With increasing frequency of application of metabonomics in the bio-related fields such as early disease detection, drug metabolism, and toxicology research [1–3], it is necessary and important to characterize and compare the metabolic similarities and dissimilarities between humans and various laboratory animals in the control and baseline statuses so that the results from animal models can serve humans. Animal modeling does help humans to understand the mechanism of disease development, drug screening, and some other biological functions before the research can be extended to clinical applications. Among a great number of animal models, some are successful and represent excellent agreement with human studies and, thus, exert a positive impact on the human community [4,5]. Unfortunately, it is a fact that some animal models fail when their results are used in human bodies. Therefore, it is vital to select the proper types of experimental animals to improve the chance of success for extension of the findings from animals to humans.

Usually, the primary concerns about the animal selections focus on the experimental budget, research objects, the availability of the animals, and some other reality factors such as environment facility control [6] in traditional animal experiments. Furthermore, some researchers may consider the similarity of some certain immune reaction between animals and humans from the genetic level and analyze the animal order when considering animal selection [7]. But these considerations often lack the fundamental basis from the metabolite level and further experimental validation, although it is very important in metabonomic-based studies. Commonly, the objective animals in the modeling construction will be among mice, rats, rabbits, pigs, guinea pigs, fish, canines, felines, and rhesus monkeys [8]. The animal species should be chosen according to the purpose of mimicking drug stimulation, disease processes, and gene function well in humans, and it is necessary to characterize the baseline physiological variations and ensure the boundaries of metabolic normality between humans and different animal species in order to be confident of and accurately understand drug- or disease-induced responses. Considering the above facts and the fact that serum and urine samples are the most

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used biological samples in metabonomic studies, we collected serum samples from various species, including human, dairy cow, guinea pig, KM (Kun Ming)[1] mouse, pig, and SD (Sprague–Dawley) rat, and collected urine samples from human, Balb/c mouse, KM mouse, rabbit, SD rat, and Wistar rat to characterize and interpret the metabolic and biochemical correlations of interspecies by nuclear magnetic resonance (NMR) spectroscopy.

The NMR spectrum of a biological sample enables the simultaneous identification and monitoring of hundreds of low-molecular-weight endogenous metabolites, thereby providing a biochemical profile of an organism [9]. However, the NMR resonance of some metabolites may be overlapped with each other, which will lead to difficulty in analyzing the metabolite of interest. Multivariate statistical analysis facilitates the comparison of NMR spectra of serum and/or urine of humans and animals, enabling the establishment of variations in metabolic profiles and highlighting their metabolic relations at the metabolite level [10]. Thus, the metabonomic method based on information-rich NMR spectroscopic data can be used to evaluate normal physiological variations and similarity and to further understand the evolution relations between humans and laboratory animals [11]. Therefore, in this study we tried to compare the metabolic similarity and dissimilarity between humans and these commonly used experimental animals to study their metabolic and evolution relations at the baseline status by using NMR-based metabonomics. The purpose of this study was to identify the most suitable animal model for the bio-related and especially metabonomic-related research community.

Materials and methods

Animals and biofluid sample collections

The animal study protocol was in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Chinese Animal Welfare Committee and was approved by the Ethical and Research Committee of Xiamen University. The healthy experimental animals, including rat, mouse, guinea pig, and rabbit, were obtained from the Xiamen University Laboratory Animal Center, and pig and dairy cow were obtained from Shanghai Biotree Biotechnology Co. Ltd. Animals were allowed to acclimate for at least 1 week before initiating the experiment. As two characteristic biofluids that are easily collected and most frequently used in metabonomics analysis, serum and urine were collected from human and laboratory animals, respectively. The serum samples were collected from dairy cow, guinea pig, KM mouse [3], pig, and SD rat ($n = 8$), respectively, and the urine samples were collected from Balb/c mouse, KM mouse, rabbit, SD rat, and Wistar rat ($n = 8$), respectively. The serum and urine samples of human were selected from our previous work [12]. The animals and human were fasted overnight before collecting serum samples, and all of the urine samples were collected between 8 and 12 am to avoid metabolic changes caused by the circadian rhythms.

Preparation of biofluid samples

Serum samples were prepared by mixing 400 μl of serum with 200 μl of a 90-mM phosphate buffer (pH 7.4) in 0.9% deuterated saline solution. The serum–buffer mixture was centrifuged at 10,000 rpm at 4 °C for 10 min, and then 550 μl of the supernatant was transferred into a 5-mm NMR tube.

Urine samples were prepared by mixing 400 μl of urine with 200 μl of 1.5 M deuterated phosphate buffer (NaH_2PO_4 and K_2HPO_4 containing 0.05% TSP [sodium 3-(trimethylsilyl)-2,2,3,3-$^2H_4$ propionate], pH 7.4). The urine–buffer mixture was left to stand for 5 min at room temperature and then centrifuged at 10,000 rpm at 4 °C for 10 min to remove suspended debris. The supernatant (550 μl) was then transferred into a 5-mm NMR tube.

^1H NMR spectroscopic analysis

The serum and urine samples were analyzed randomly using proton (^1H) NMR spectroscopy. All spectra of the serum samples were acquired at 298 K on a Varian INOVA-600 NMR spectrometer (operating at 599.92 MHz for ^1H) equipped with an inverse detection probe with a shielded Z-gradient, and all spectra of the urine samples were analyzed at 298 K using a Bruker AMX-600 spectrometer equipped with a TXI CryoProbe operating at 600.13 MHz.

NMR spectra of the serum samples were acquired using a water-suppressed CPMG (Carr–Purcell–Meiboom–Gill) spin–echo pulse sequence (cpmgt2pr) (RD-90°-(τ-180°-τ)$_n$-Acq). Spin–echo loop time ($2n\tau$) of 70 ms was applied with a relaxation delay of 2.0 s. A total of 96 transients were collected into 16,384 points over a spectral width of 8000 Hz. A standard one-dimensional spectrum using the first increment of the NOESY (nuclear Overhauser effect spectroscopy) pulse sequence (RD-90°-t_1-90°-t_m-90°-Acq) with water suppression (noesypr1d) was acquired for the urine samples. The spectra were acquired with a relaxation delay of 2.0 s at a fixed interval t_1 of 4 μs. The water resonance is irradiated during relaxation delay and the mixing time t_m of 100 ms. A total of 96 transients were collected into 16,384 data points with a spectral width of 12,000 Hz.

NMR spectral processing

The NMR spectra were processed by using MestReNova (version 7.1). All free induction decays (FIDs) were multiplied by an exponential weighting function with a 1.0-Hz line broadening factor prior to Fourier transformation. All of the ^1H NMR spectra were then phased by manual correction and baseline corrected by using the Bernstein polynomial fit method with a respective polynomial order for the purpose of best baseline correction effect. ^1H NMR spectra of serum and urine samples were referenced to the internal lactate CH_3 resonance at 1.33 ppm and the single peak of TSP at 0 ppm, respectively. After the above operation, the chemical shift ranges including the resonances from water and urea were manually removed to eliminate baseline effects of imperfect water signal and urea signal. The spectra over the ranges of 0.5 to 9.0 ppm for serum samples and 0.5 to 10.0 ppm for urine samples were integrated automatically into regions with an equal width of 0.005 ppm. The integrated data were finally normalized to the total area of the NMR spectrum to compensate for the overall concentration differences before statistical analysis.

Multivariate statistical analysis

Multivariate statistical analyses were performed by using SIMCA-P+ software (version 11.0.0.0, Umetrics, Umeå, Sweden). Principal component analysis (PCA) was initially carried out by using a par-centered approach for both serum and urine samples to examine class clustering and find possible outliers. The samples were visualized by using the principal component (PC) score plots, where each point represented an individual sample.

After PCA, orthogonal partial least squares discriminant analysis (OPLS–DA) was separately applied to the samples from human and

[1] *Abbreviations used:* KM, Kun Ming; SD, Sprague–Dawley; NMR, nuclear magnetic resonance; PCA, principal component analysis; PC, principal component; OPLS–DA, orthogonal partial least squares discriminant analysis; 2D, two-dimensional; 3D, three-dimensional; aminoacyl-tRNA, aminoacyl-transfer RNA; ABC transporter, ATP-binding cassette transporter.

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