Jacques S. Beckmann

Nili Avidan
Edna Ben Asher
Iris Kirstein
Tsviya Olender

Analysis of genetic variation as a tool for the development of personalized medicine

Department of Molecular Genetics

Tel. 972 8 934 4906 **Fax.** 972 8 934 4108
E-mail: jacqui.beckmann@weizmann.ac.il

Tracking genetic diseases

All traits, whether disease-related or not, have to some extent a genetic component. The recent developments in human genetics enabled the elucidation of the molecular etiology of a growing number of monogenic inherited disorders. These studies often involve the assignment of the disease locus to a defined chromosomal location, prior to its identification. These steps are but the beginning of the long quest: to understand the pathophysiology, to pave the way to better patient's management, and to develop efficient gene-based therapies. But they lead also to the development of objective and reliable diagnostic tools as well as to a better definition of disease nosology (as illustrated by the continuous elucidation and clarification of the limb girdle muscular dystrophies).

From Mendelian traits to pharmacogenetics

The so-called 'monogenic' diseases represent but a small fraction of all diseases. Thus, after these initial successes and those of the Human Genome Project, emphasis has now turned to the study of common traits. The latter are often called multifactorial, since they are also determined by non-genetic factors.

A given medication is rarely effective in all patients and can often cause adverse drug reactions (ADR) to some patients. It is generally thought that one's susceptibility to a given drug is, at least in part, genetically determined. This forms the rationale for (1) the recent emergence of pharmacogenetics, the study of the impact of heritable traits on pharmacology and toxicology, and (2) the promise of personalized medicine. Pharmacogenetics aims to pinpoint the extent to which genetic variation influences drug response. Such response may depend on one or more factors that can vary according to an individual's alleles (Fig. 1B).

In collaboration with Doron Lancet, we have started a multi-centric collaboration with TEVA and the Technion, to assess the genetic basis of the responses of Multiple Sclerosis (MS) patients participating in a clinical trial of an anti-MS drug (Cop1 or Copaxone). One of the outcomes of this trial will be the subdivision of the participants in this study into three major phenotypic classes: responders, non-responders and patients suffering from ADR. We hypothesize that specific DNA sequence variants near or within genes affecting the drug's mode of action, intake, distribution or metabolism will be more prevalent in one group than in the others (Fig. 1B). Following a candidate gene strategy, we search for the presence of genetic association between the phenotype and defined marker alleles in genes that are likely to affect drug response. Single Nucleotide Polymorphisms (SNPs) - i.e., DNA sites at which two distinct genomes differ from each other by one base pair (Fig. 1A) - are the major source of DNA polymorphism. SNPs are genotyped using Sequenom Massarray high-throughput mass spectrometry. Hopefully, such study will reveal the distinct SNP profiles more likely to be possessed by each phenotypic class, and eventually contribute to a better health policy.

Genetic diversity and proteomics

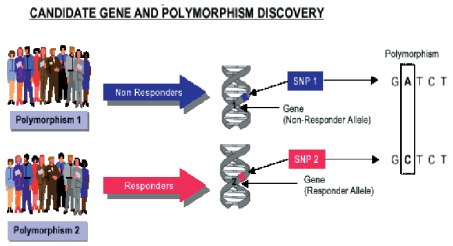
Genetic analyses will no doubt identify chromosomal regions containing susceptibility loci involved in defined traits. Being the most abundant source of genetic variation, SNPs may themselves cause the observed phenotypic differences. Pinpointing what SNP is however actually involved may be a much more challenging task. The trait-associated SNPs will fall essentially in two classes, those that affect quantitatively the spatio-temporal expression patterns and those that modify the protein's structure or function.

Given that there are somewhere between 3 to 10 million SNPs in the human genome, virtually all protein coding regions will have one or several SNPs. Thus, multiple naturally occurring variants will be found for each protein, a significant fraction of which will lead to an amino acid substitution (called cSNPs). The latter may modify structural or functional properties of the corresponding proteins, and occasionally result in pathological consequences (e.g. Fig. 1C), or affect response to medication. This research will lay the grounds for a systematic analysis of the impact of missense cSNPs on the corresponding protein's structure and function.

We analyze the molecular phenotypes of these cSNPs, namely their impacts on protein 3D structure, folding, binding

A: Single Nucleotide Polymorphism

B: Pharmacogenetics



C: 3D location of calpain 3 mutations

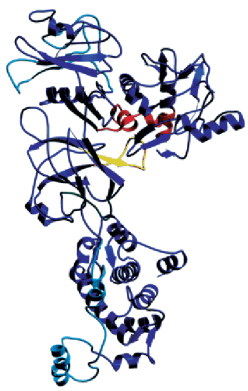


Fig. 1A+1B *Genetic (SNP) differences between individuals can cause large differences in drug effects. Stratification of patients into genotypes may increase the probability of drug efficancy and therapeutic window.*
Fig. 1C *Mutation clusters (red, yellow, green) in the calpain 3 model. the majority of mutations implicated in the development of LGMD2A are located at the domain interfaces (Jia et al., 2001).*

or stability, thereby providing insight on these proteins' function or dysfunction. This is done by a multidisciplinary approach, combining synergistically the expertise of genetics, structural biology and statistics. This research, performed in collaboration with Joel Sussman, relying on the comparison of two proteins (one with a known 3D structure and the second one identical - in sequence at least - to the first one, but for a single amino acid substitution), will contribute to a further understanding of the rules and principles governing protein folding. Furthermore, one additional outcome of this research is the capacity to predict and assess the potential pathologic character of cSNP variants, thereby hopefully, greatly facilitating the identification of genetic loci responsible for common diseases or differential drug responses. Hence, this type of studies may reveal new leads for diagnosis, prognosis, disease care and management, and eventually for the development and utilization of new therapeutic agents.

Selected Publications

Escary, J.L., Bottius, E., Prince, N., Reyes, C., Fiawoumo, Y., Caloustian, C., Bruls, T., Fujiyama, A., Cooper, R.S., Adeyemo, A.A., Lathrop, G.M., Weissenbach, J., Gyapay, G., Foglio, M. and Beckmann, J.S. (2000) A first high density map of 981 biallelic markers on human chromosome 14. Genomics 70,153-164.

Richard, I., Roudaut, C., Marchand, S., Baghdiguian, S., Herasse, M., Stockholm, D., Ono, Y., Suel, L., Bourg, N., Sorimachi, H., Lefranc, G., Fardeau, M., Sbille, A. and Beckmann, J.S. (2001) Loss of calpain 3 proteolytic activity leads to muscular dystrophy and to apoptosis-associated IkBa/Nuclear factor-kB pathway perturbation in mice. J. Cell Biol. 151, 1583-1590.

Jia, Z., Petrounevitch, V., Wong, A., Moldoveanu, T., Davies, P.L., Elce, J.S., Beckmann, J.S. (2001) Mutations in calpain 3 associated with limb girdle muscular dystrophy: analysis by molecular modeling and by mutation in m-calpain. Biophys J. 80, 2590-2596.

Eisenberg, I., Avidan, N., Potikha, T., Hochner, H., Chen, M., Olender T, Barash M, Shemesh M, Sadeh M, Grabov-Nardini, G., Shmilevich, I., Friedmann, A., Karpati, G., Bradley, WG., Baumbach, L., Lancet, D., Asher, E.B., Beckmann, J.S., Argov, Z., Mitrani-Rosenbaum, S. (2001) The UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase gene is mutated in recessive hereditary inclusion body myopathy. Nat. Genet. 29, 83-87.

Kaplan, J.C., Beckmann, J.S. and Fardeau, M. (2001) Limb-Girdle Muscular Dystrophies. In Disorders of Voluntary Muscle, 7th edition (Ed. Karpati G., Hilton-Jones D., Griggs R.C., in press).

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