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Investigation of heart proteome of different consomic mouse strains. Testing the effect of polymorphisms on the proteome-wide trans-variation of proteins

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ABSTRACT

We investigated to which extent polymorphisms of an individual affect the proteomic network. Consomic mouse strains (CS) were used to study the trans-effect of the cis-variant (polymorphic) proteins of the strain PWD/Ph on the proteins of the host strain C57BL/6J. The cardiac proteome of ten CSs was analyzed by 2-DE and MS. Cis-variant PWD proteins altered a high number of C57BL/6J proteins, but the number of trans-variant proteins differed considerably between different CSs. Cardiac hypertrophy was induced in CSs. We found that high variability of the proteome, as induced by polymorphisms in CS14, acts protective against the complex disease.

Abbreviations: SNP, single nucleotide polymorphisms; CS, consomic strain; Chr, chromosome; GWAS, genome-wide association studies; QTL, quantitative trait loci; MGI, mouse genome informatics; TAC, transversal aortic constriction; IVS, intraventricular septum; LVID, left ventricular end-diastolic diameter; LV PW, left ventricular posterior wall; LVM, left ventricular mass; BW, body weight; EF, ejection fraction; FS, fractional shortening; 2-DE, two dimensional gel electrophoresis.

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1. Introduction

Common multifactorial diseases are caused by genetic and/or environmental perturbations. Moreover, disease associated modifier genes are involved which influence the individual severity of the disease. For example, familial hypertrophic cardiomyopathy (FHC) is caused by a mutation in a gene encoding the sarcomeric β-myosin binding protein [1]. In order to investigate the modifier effect, 26 patients, all carriers of the same mutation in the sarcomeric gene MyBP-C, were investigated with regard to particular polymorphisms [2,3]. In spite of the presence of the same mutation, the severity of the disease differed among the 26 family members considerably, and this was found to be due to distinct polymorphisms, apparently acting as disease modifiers.

Recent developments in DNA analysis have led to comprehensive genome-wide association studies (GWAS) aimed at detecting genes/single nucleotide polymorphisms (SNPs) that modify the expression of a disease caused by genetic or environmental factors. In these studies a general observation is that many SNPs associated with a disease can be detected, but the vast majority of effect sizes contributing to the disease phenotype are small [4]. For example, 47 distinct genetic variants associated with blood pressure and hypertension were found to explain collectively only a few percent of the heritability of blood pressure [5]. It has been concluded that the complete genetic architecture of blood pressure involves possibly hundreds of genetic variants [6], but that current GWAS results have identified only a subset of this architecture [5].

Chromosomal substitution strains (consomic strains, CS) of the rat have been used as an animal model to screen the chromosomes separately for disease modifier loci acting as quantitative trait loci (QTL). Two strains (BN and SS) of the rat differing in sensitivity against hypertension have been studied in this way [7–9]. The different chromosomes were screened for QTLs that show association with high blood pressure. QTLs related to blood pressure were identified, but surprisingly on almost every chromosome [8]. The results suggested that global responses to an initial stimulus of blood flow, such as salt intake, change the expression of genes throughout the genome [10].

The results considered show that the phenotypic expression of a disease in response to a genetic variant or external stimulus involves many genes, but with different consequences for each individual due to the polymorphisms [4]. This suggests behind the action of a particular disease gene a global acting disease modifier effect [11]. The global modifier would result from the cellular proteomic network and the connected regulatory system. The efficiency of the functional network in compensating the deleterious effect of the stimulus would depend on the number, type and composition of the interacting individual variants that form the genetic background of each individual.

It is a particular feature of the genetic system, that the genome of each individual is composed of two sets of alleles, that from the mother and that from the father, which become combined under heterozygote conditions arbitrarily. Recently the genome of one person was analyzed by next generation sequencing and the maternal genome was compared with the paternal genome with respect to polymorphisms [12]. The two genomes differed by about 11,000 SNPs resulting in non-synonymous amino acid exchanges in coding genes. In addition, 178,616 SNPs were found in non-coding regions up to 10 kb upstream of genes and 6599 in transcription factor binding sites. The arbitrary combination of the alleles of so many genes in an individual may alter fundamental characteristics of biological systems such as the robustness against perturbations. This was defined as a property that allows a system to maintain its function even under internal (genetic) or external (environment) perturbations [13,14]. In a cell the proteomic network of interaction and expression is highly coordinated and tightly controlled [15]. Therefore, this system must be maintained by adapting the effect of the different polymorphisms (and other mutations) of each individual [16]. This is a prerequisite for normal development and differentiation of cells and tissues. But adapting the polymorphisms to the proteomic system, i.e. to the network of proteins including expression, regulation, interaction and distribution of proteins, may lead to conditions not necessarily "perfect" [17] in each case. In consequence, the proteomic system may differ between individuals in robustness against perturbations [18]. Robustness against perturbations and its limitation has been studied in various molecular biological systems [13,14,19–21]. Moreover, mathematical models have been proposed to define robustness [14,16,17,21].

The aim of our investigation was to define in the proteome-wide expression of proteins a criterion that may indicate the capacity of an individual proteome to react robustly against perturbations. As an animal model CSs of the mouse were used, and the proteome of the heart was investigated by large-gel 2-dimensional electrophoresis (2-DE) [22] and mass spectrometry (MS). The consomic mouse model used consists of 20 C57BL/6J (B6) strains, each strain differing from each other by one chromosome (the two homozygotes) that has been replaced by the corresponding chromosome from another strain, the PWD/Ph (PWD) mouse strain [23]. Therefore, the large number of polymorphisms which exist between B6 and PWD was reduced in each strain to the polymorphisms (alleles) of one single chromosome, the PWD-Chr. This allows us to test the extent of the trans-effect of the different sets of variants encoded by the PWD-Chr of each CS on the variability of the B6 proteome (Fig. 1). According to our hypothesis high variability in a CS (compared to B6 to detect the effect of the PWD-Chr) may reflect high elasticity of the proteomic network, i.e. high buffer capacity of proteins in up- and down-regulation and high flexibility in using alternative pathways in protein–protein interaction. High variability might be an indication of high robustness of the proteomic network of this CS against perturbations. In another CS, however, the particular composition of the trans-acting polymorphisms may confine the robustness of the proteome. The frequency of trans-variant proteins of a CS induced by the cis-variant proteins (the polymorphic proteins of the consomic chromosome PWD) by protein-protein interaction was taken as a parameter to compare the robustness of the proteomic network between the CSs. If the trans-effect induced in the proteomic network by the cis-variant genes/proteins is high, this would indicate a high potential of this CS to establish homeostasis in the proteomic network even in case of perturbation (high robustness).