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Editorial Open Access

Functional Dissection of Novel Genes on Complicated Diseases

Youming Zhang[1*] **and Yun Zhang**[2]

[1]*Genomic Medicine Section, Division of Respiratory Sciences, National Heart and Lung Institute, Imperial College London, London, UK*

[2]*Department of Biological and Medical Sciences, Faculty of Health and Life Sciences, Oxford Brookes University, Oxford, UK*

[*]**Corresponding author:** Youming Zhang, Genomic Medicine Section, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, UK, Tel: 44-0-20-7352-8; E-mail: y.zhang@imperial.ac.uk

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Editorial

Human complicated diseases such as asthma, diabetes, rheumatoid arthritis and hypertension are caused by the combinations of environmental and genetic factors. Genetic and genomic approaches for the complicated disease have been successful due to powerful Genome-Wide Association Studies [GWASs] and positional cloning studies. More than 2,000 robust associations with more than 300 complex diseases and traits were identified in recent years [1]. These associations either have been identified previously as candidate genes for the diseases or novel genes that the pathophysiologic mechanisms to the diseases are unknown. Genetic approaches on essential hypertension have identified more than 130 genes that have roles on the disease [2]. One of the strengths of GWAS and positional cloning is that many novel genes that were never suspected to have roles in the diseases were identified. The classic example is ORMDL3 gene on human chromosome 17q21. It was not regarded as an asthma candidate gene until the polymorphisms of ORMDL3 were found to have association with asthma in a first-generation GWAS of approximate 1000 children with asthma and 1000 controls [3]. Since then, the association has been replicated in many populations. The consistency of this association is far greater than for any other asthma locus previously identified, indicating that understanding the function of the ORMDL3 is of major importance to the understanding of asthma aetiology as well as the potential of improved therapies and treatments [4]. For post genomic era, dissection of the functional roles of novel genes identified by GWAS and positional cloning becomes increasing importance. It will eventually provide therapeutic means for the complicated diseases. The development of platforms for genetic and genomic research makes it possible for systematically investigating the novel genes in several approaches.

Cellular Models for Studying Novel Genes Function

Gene silencing or gene knockdown is a power tool for decoding the networks and pathways that contain the novel gene of interest. With the gene specific siRNA transfection into the cells, the gene will dramatically decrease the expression. One of the approaches for gene silencing is time series study on cells. It applies stimulators of the cells at different time points and examines the phenotypes in silenced cells and control cells. This approach is particularly useful when the gene's production is involved in inflammatory diseases. The most used stimulators for epithelium cells are IL1B, TNFA, poly IC, alternaria and oxidative stress. Cytokines changes normally will be observed if the genes are involved in inflammatory response. Gene over-expression in cells can be completed by expression vectors that are transfected in the cells. The interested gene is often linked with express tag such as Green Fluorescent Protein [GFP] as a reporter of the expression. This approach is not only very helpful to study the location of the gene expression, but also possible show the dynamic movement of the interested gene products after stimulation. The cellular models can also be used for global gene expression profiling and metabolites screening.

Pathway Identification and Interacting Molecules

The advantage of high-throughput microarray technologies is that they offer a new opportunity to gain insight into the global gene expression, leading to the identification of new pathways that the novel gene regulates. Microarray analyses now allow the simultaneous examination of the expression of all known human genes from particular cells or tissues. The measurements at multiple time pointscan be used for the systematic identification of altered gene expression in response to particular stimuli. Furthermore, statistical analyses with clustering algorithms permit the identification of genes that are co-regulated in response to the stimulus. Metabolites such as lipoproteins and lipids can work as disease modulators and risk factors. Novel technologies permit the determination of a broad spectrum of metabolites simultaneously at high resolution. Metabolite screening can also be applied in the cell level and in animal studies. It not only provides new insight of the metabolism of the gene's product but also possible identifies new biomarkers for the diseases. Pharmacologic approaches include screening the inhibitors of the pathways that the genes products are involved in and to examine the phenotypes of the cells after inhibition. Global gene expression profiling can also be integrated in pharmacologic approaches for the dissection of the gene's functions. Co-immunoprecipitation with mass spectrum analysis is also likely to reveal the working partners with the new molecules. Proteomic approach includes liquid chromatography and tandem mass spectrometry [MS/MS], it can be used comprehensively for proteins and their biological functions [5].

Chromatin immunoprecipitation coupled with microarrays [ChIP–chip] is the standard technique for identifying the locations and biochemical modifications of bound proteins genome-wide. Recent advances in ChIP methodology have overcome some of the limitations of the 'standard' ChIP experiment, and the development of complementary assays and analyses have expanded the number, types and resolution of protein–DNA interactions that have been discovered [6]. Approaches for characterizing DNA-protein interactions has been developed in three-dimensional chromatin capture (3C) and chromtatin interaction analysis with paired –end tag sequencing [ChIA-PET] [7,8].

Epigenetic effects are mediated gene expression through mechanisms by molecules that bind DNA other than sequence variations. The environment, diet and aging are all the factors that can influence epigenome. Epigenetic effects are another possible cause of complicated disease. The dramatic increase in the prevalence,

incidence of complicated diseases in recent decade can be explained by genetic and environmental effect. The patterns of gene expression that determine cellular type and function become stably restricted during development, partly through methylation of CpG sequences and gene silencing. Islands of CpG sequences are positioned at the 5' UTRs of many human genes. It is important to recognise that age, sex, genetic polymorphism and environmental factors have all been strongly associated with altered methylation at selected loci [9]. The relative contribution of these factors to methylation at loci genome-wide is not known, and it is not certain to what extent true epigenetic inheritance with transmission between generations takes place. These factors will have to be taken into account if methylation changes at individual loci are to help understand complex diseases.

The Metagenomic Approaches

The genetic studies have shown the central importance on complicated diseases, but a worthwhile understanding of the causes of the diseases needs to reconcile consistent epidemiological indications of the importance of the microbiome to the diseases [10]. These include the protection afforded by a rich microbial environment in early life. For asthma, observations that the bronchial tree contains a characteristic flora that is disturbed by the presence of pathogens such as Haemophilus influenzae [11]. Birth cohort studies showed that the presence of the same pathogens in throat swabs and predicts the later development of asthma [12]. Ninety percent of the cells in the human body are microorganisms including bacteria, parasites and archaea [13]. These microorganisms are commensal on body surfaces exposed to the external environment including the gut, respiratory tract and skin. Although most bacteria are not cultivable with standard methods, the membership of complex microbial communities can be quantified and classified by DNA sequencing of the conserved bacterial 16S rRNA gene. Bacteria are classified by these sequences into Operational Taxonomic Units [OTUs]. Sequences of other regions may be necessary for precise discrimination at the species level. The study of the role of the microbiome of complicated diseases is in its infancy. Even at this early stage. However there are a number of questions that suggest a structured set of experiments that will elucidate how the microbiome operates in health and diseases.

Animal Models for the Novel Genes

Mouse models provide valuable means to understand the functions of novel genes. A knockout mouse is a genetically engineered mouse in which one or more genes have been made inoperative and is the one of the most use in gene targeting. A conditional knockout approach allows researchers to delete the gene of interest in a time- and space-dependent manner. The technique of conditional gene expression applies site-specific DNA recombinase systems in mouse genome. The development of Zinc Finger Nuclease (ZFN) system offers another means for genomic editing. ZFN has specifications by linking a DNA-binding domain of a versatile class of eukaryotic transcription factor Zinc Finger Proteins (ZFPs) with the nuclease domain of the FokI restriction enzyme. Zinc finger domains can be engineered to target desired DNA sequences and this enables zinc-finger nucleases to target unique sequences within complex genomes [14]. ENU is a chemical mutagen that randomly induces germ-line point mutations into DNA. Panels of mutagenized mice may be screened for mutations in a gene of interest or for the appearance of particular phenotypes. Both gene-driven and phenotype driven can be used for studying the functions of novel disease genes [15].

In general, recent robust genetic and genomic approaches have identified many novel genes that increase the risk of developing complicated diseases such as asthma, diabetes and hypertension. A next phase of endeavour is to understand the function of these genes and how they interact with the environment. The advantage technologies including NextGen sequencing, transcriptome (RNA-Seq) studies, pathway analysis, global methylation studies, metabolomics and metagenomics analysis will greatly transform our knowledge to the complicated diseases. The progressive and systematic use of cellular and animal models is an important bridge to developing new therapies that will improve clinical management of the complicated diseases in the future.

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