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「応用ゲノム」
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予稿集
Proceedings

International Symposium on Applied Genomics 2007

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Tokai University, Japan

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Lap-Chee Tsui ●Vice-Chancellor and President, The University of Hong Kong

Lessons learned from studying a single-gene disorder

The Human Genome Project and the advances in genomic technologies have made the cloning of the genes for single-gene disorders relatively easy. There are, however, a few lessons that can be learned from our study of cystic fibrosis, which is a common single gene disorder in the Caucasian population. The gene was mapped by family analysis to the long arm of human chromosome 7 and isolated on the basis of its chromosome localization. It is now well established that CFTR functions as a cAMP-regulated chloride channel in the secretory epithelium but more recent studies show that it may have additional activities. Over 1,300 mutations have been identified in the CFTR gene. Although the most frequent CFTR mutation accounting for 70% of the mutant alleles, comprehensive mutation analysis fail to identify the mutations in all CF patients. In addition, while there is good correlation between certain clinical presentation and CFTR genotype, severity of CF disease could not be predicted by CFTR genotype alone. It is thought that modifier genes and environmental factors are important. While attempts are being made to define the molecular bases of the genetic modifiers, including the use of animal models, it is also found that mutations in CFTR could cause other diseases, which are now sometimes referred as “CF-related diseases”. Taken altogether, it can be said that there is really no simple ‘single gene’ disorders. In addition, our experience re-emphasizes the importance of patient sample and clinical data collection, both of which are crucial to success of study. It is also essential to combine basic science and clinical research, and, to engage in interdisciplinary collaborations. While working on CF, it occurred to me and my colleague, Dr Steve Scherer, that the genomic resources and technologies accumulated in my laboratory could be used for large-scale genome analysis and study of other diseases. This latter work will be discussed in the context of the Toronto Center for Applied Genomics.

http://www.hku.hk/vcoffice/menu/biography.htm

Sunyoung Kim ●Seoul National University, Korea

Ken-ichi Arai ●Founding President, Asia-Pacific IMBN; Special Professor, LSBM, RCAST & Professor Emeritus, University of Tokyo; President and CEO, SBI Biotech Co., Ltd.

Building Asia Pacific R & D Highway for Genomic Medicine and Clinical Development

In the old paradigm where "necessity triggers invention", the frontier race had been the competition for limited resources such as land and raw materials. In the new paradigm where "innovation creates necessity", the frontier race has been the competition for resources of unlimited value such as science & technology through discovery & innovation. This is achieved through collaborative network of researchers. Translation of discovery into new products by creating new industry is an important agenda. In 21st century, Asia become the focal point of growth in biotechnology and new centers of biomedical research as well as bioindustry emerged throughout Asia. I would like to discuss several issues such as;

1. Network Bio-research Centers in Asia such as BioPolis of Singapore, BioNexus of Malaysia, BIOTEC of Thailand, SIBS of Shanghai, BioMax of Seoul, Academia Sinica/NHRI of Taiwan, OIST of Japan etc.
2. Build Common International Hub in Asia by collaboration of A-IMBN, eIMBL, EMBO, ICGEB and FAOBMB/IUBMB and to create A-IMBL (Asian EMBL), and to launch Nature A-IMBN Research News
3. Share Common Biotechnology Platform in Asia by building translational research centers for genomic medicine. Networking biomedical centers for multinational clinical trials.
4. Build R & D Highway in Asia by building support system for bioventures (VCs, incubators) and by promoting university-industry collaboration
5. Organize Regulatory Environment & Infrastructure in Asia to deal with IP issues, safety evaluation by building FDA/CDC like network in Asia.

I would like to share the view of Ms. Keiko Oishi (CMIC Co., Ltd) regarding "Opportunities for Asian collaboration and perspectives of developing multinational clinical trials in Asia."

Eugene Berezikov ●Hubrecht Institute, Utrecht, The Netherlands

Evolution of microRNAs

MicroRNAs are a large class of non-coding regulatory RNAs that are involved in almost every aspect of a cell life, from cell proliferation to differentiation to apoptosis. The roles of miRNAs in development and disease are increasingly recognized. We have used massively parallel sequencing technology for discovery and expression profiling of miRNAs in various model organisms, including nematodes, fish, human and primates. In all the investigated species we found numerous novel miRNA candidate genes that often are not phylogenetically conserved and usually expressed at lower levels compared to conserved miRNAs. We also discovered that vertebrate genomes contain mirtrons - miRNAs derived from introns and processed by splicing machinery instead of Drosha excision. Similar to canonical miRNAs some mirtrons are well-conserved while others are species-specific. Our findings suggest that along with highly conserved ancient miRNAs there are many evolving miRNA candidate genes. We propose that such miRNAs may contribute to evolutionary novelty through diversification of gene regulatory networks. We also hypothesize that miRNA candidate genes that have not yet evolved a function, when disregulated, may still be associated with disease like cancer.

http://www.niob.knaw.nl/researchpages/berezikov

Nikolaus Rajewsky ●Max Delbruck Center for Molecular Medicine, Germany

Prediction of microRNA targets

Carlo M. Croce M.D. ●The John W. Wolfe Chair in Human Cancer Genetics
Department of Molecular Virology, Immunology and Medical Genetics, The Ohio State University, Comprehensive Cancer Center

microRNAs in Human Cancer

MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B

MicroRNAs (miRNAs) are small, noncoding RNAs that regulate expression of many genes. Recent studies suggest roles of miRNAs in carcinogenesis. We and others have shown that expression profiles of miRNAs are different in lung cancer vs. normal lung, although the significance of this aberrant expression is poorly understood. Among the reported down-regulated miRNAs in lung cancer, the miRNA (miR)-29 family (29a, 29b and 29c) has intriguing complementarities to the 3'-UTRs of DNA methyltransferase (DNMT)3A and -3B (*de novo* methyltransferases), two key enzymes involved in DNA methylation, that are frequently up-regulated in lung cancer and associated with poor prognosis. We investigated whether miR-29s could target DNMT3A and -B and whether restoration of miR-29s could normalize aberrant patterns of methylation in non-small-cell lung cancer. We show that expression of miR-29s is inversely correlated to DNMT3A and -3B in lung cancer tissues, and that mir-29s directly target both DNMT3A and -3B. The enforced expression of miR-29s in lung cancer cell lines restores normal patterns of DNA methylation, induces re-expression of methylation-silenced tumor suppressor genes, such as FHIT and WWOX, and inhibits tumorigenicity *in vitro* and *in vivo*. These findings support a role of miR-29s in epigenetic normalization of NSCLC, providing a rationale for the development of miRNA-based strategies for the treatment of lung cancer.

http://medicine.osu.edu/mvimg

Dec.20

Mikiko C.Siomi
●Institute for Genome Research
University of Tokushima

piRNA/rasiRNA: a novel class of functional non-coding RNAs

Gene silencing pathways triggered by small RNAs are generically called RNA silencing. RNA interference (RNAi) triggered by short-interfering RNA (siRNA) of 21- to 23- nucleotide (nt) long is a representative of it. Extensive studies on RNA silencing mechanisms revealed that members of the Argonaute family play important roles in the pathways. In *Drosophila*, the Argoanute family, defined by the presence of PAZ and PIWI domains, consists of five members, which includes AGO1, AGO2, AGO3, Piwi, and Aubergine. One of our goals is to understand the functional differences among the fly Argonaute members in RNA silencing. Previously we have shown that AGO1 and AGO2 in *Drosophila* function in gene silencing through specific binding with miRNA and siRNA, respectively. siRNA-loaded AGO2 functions in RNAi as Silcer, directly responsible for cleaving a target completely complementary to siRNA. miRNA-associated AGO1 is thought to repress translation of target mRNAs without cleaving them, but AGO1 also possesses Slicer activity as does AGO2. Piwi, Aubergine, and AGO3 (the PIWI proteins) are specifically associated with a subset of endogenous small RNA, termed Piwi-interacting RNAs (piRNAs). Unlike miRNAs, piRNAs are 24-30 nt long, only expressed in gonads, and function in genome surveillance through association with the PIWI proteins by silencing mobile elements that have sufficient potency to invade genome by inserting themselves into the DNA elements. Recently, a model for piRNA biogenesis in *Drosophila* was proposed. In addition, 3' end modification of piRNAs in fly ovaries was determined as methylation by mass spectrometric analysis. However, many aspects of piRNA biogenesis and modification remain unclear. Our current work is concerning the mechanisms of piRNA biogenesis and modification and the identification of factor(s) required for these processes.

References
1) Okamura K et al. Distinct roles for Argonate proteins in small RNA-directed RNA cleavage pathways. ***Genes Dev.*** 18: 1655-66. 2004
2) Miyoshi K et al. Sicer function of *Drosophila* Argonautes and its involvement in RISC formation. ***Genes Dev.*** 19: 2837-2848. 2005
3) Saito K et al. Specific association of Piwi with rasiRNAs derived from retrotransposon and heterochromatic regions in the Drosophila genome. ***Genes Dev.*** 20: 2214-2222. 2006
4) Gunawardane LS et al. A Slicer-mediated mechanism for rasiRNA 5' end formation in Drosophila. ***Science*** 315: 1587-1590. 2007
5) Saito K et al. Pimet, the *Drosophila* homolog of HEN1, mediates 2'-O methylation of Piwi-interacting RNAs at their 3' ends. ***Genes Dev.*** 21: 1603-1608. 2007
6) Nishida K.M et al. Gene silencing mechanisms mediated by Aubergine-piRNA complexes in Drosophila mal gonad. ***RNA*** 13: 1911-1922. 2007

http://www.genome.tokushima-u.ac.jp/dgfa/index.html

Yukio Kawahara, MD, PhD
●The Wistar Institute

The regulation of microRNA function by RNA editing

MicroRNAs (miRNAs) mediate translational repression or degradation of their target mRNA by RNA interference (RNAi), and an increasing line of evidence indicated that miRNA genes are tightly connected with the pathogenesis of certain human diseases. The levels of individual miRNAs vary under different developmental, cellular, or pathological condition, thus the elucidation of their regulatory mechanisms is essential to the use of these small RNA molecules as future targets for drug development as well as for the diagnosis of certain human diseases.

Although miRNAs are single-stranded, 19- to 22-nucleotide molecules, each is generated from a long primary transcript (pri-miRNA) that consists of an imperfect short dsRNA region and a loop. Pri-miRNAs are sequentially processed by the nuclear Drosha-DGCR8 complex to ~60-70nt intermediates (pre-miRNAs) and then by the cytoplasmic Dicer-TRBP complex to mature miRNAs.

We and other groups have recently provided a line of evidence that certain pri-miRNAs are subject to RNA editing that converts adenosine to inosine in dsRNA regions, and highlighted the multiple roles of this modification in the regulation of expression levels and functions of certain miRNAs. RNA editing can alter the fold-back dsRNA structure of miRNA precursors, thereby affecting their subsequent processing steps. For instance, the editing of two specific sites of pri-miR-142 RNAs completely suppresses its cleavage by the Drosha-DGCR8 complex, while the editing of pri-miR-151 RNAs results in complete blockage of its cleavage by Dicer-TRBP complex and accumulation of edited pre-miR-151 RNAs. By contrast, the editing of certain pri-miRNAs results in the expression of edited mature miRNAs. We found that two adenosine residues both positioned in the 5'-proximal half "seed sequence" of miR-376 RNAs are highly edited in select tissues. The seed sequence is critical for formation of the RNA duplex between target mRNA and miRNA. As expected, the edited miR-376 RNAs indeed silenced a set of genes different from those targeted by the unedited miR-376 RNAs both in vivo and in vitro, revealing a distinct role of RNA editing in miRNA-mediated gene silencing.

We recently conducted a survey of RNA editing sites in about 250 human pri-miRNAs, revealing that more than 20% of these pri-miRNAs are edited significantly in vivo. These results indicate that RNA editing has a big impact on miRNA function and could be a potential therapeutic target for miRNA-associated diseases.

Augustine Kong ●deCODE Genetics, Sturlugata 8, IS-101 Reykjavik, Iceland

Recent Discoveries and New Challenges

Through large scale genome-wide association studies, susceptibility variants that confer risks to complex diseases are identified at a pace that is unprecedented. These successes often raised new questions and lead to novel challenges. Examples in cancer, diabetes, cardiovascular diseases, atrial fibrillation, restless legs syndrome, pigmentation, glaucoma and other complex traits, will be used to highlight some of the interesting results and problems.

Josephine Hoh, Ph.D. ●Associate Professor,
Yale University,
Department of Epidemiology and Public Health

Genome-wide association (GWA) approach has become a norm to map the genetic variants that predispose humans to common, complex diseases. With the advance of genotyping technologies and the reduction of genotyping cost, GWA studies using 500K to 1.8m markers (or SNPs) are underway. In this talk, I focus on designs for the optimal GWA studies. When time permits, I will present a latest work of applying a GWA case-control study to a rare disease.

Risch and Zhang [1] have demonstrated the substantial increase in power obtained by selecting the affected sibling who expresses the higher end of a distribution of a quantitative trait and the unaffected sibling having the lower end in the trait spectrum. The principle of extreme discordant sibpair analysis was extended in the AMD GWA study for case-control selection [2, 3]. Drusen size was used as a quantitative trait measure for which all cases had the extreme size of drusen and controls none or only a few small drusen. Controls also were matched to cases as much as possible for known risk factors as siblings presumably share the same environment. This strategy was estimated to reduce genotyping by 10 to 40 fold [1].

To date, the most efficient use of controls is reported in a large GWA study on seven diseases in that each case group was contrasted to the same control group [4]. The GWA studies conducted in Iceland by DeCode Genetics have also used various subsets selected from one big population control cohort. Such practices may be expected to be adopted in most future GWA studies.

1. Risch, N. and H. Zhang, *Extreme discordant sib pairs for mapping quantitative trait loci in humans*. Science, 1995. **268** (5217): p. 1584-9.
2. Klein, R.J., et al., *Complement factor H polymorphism in age-related macular degeneration*. Science, 2005. **308** (5720): p.385-9.
3. Dewan, A., et al., *HTRA1 promoter polymorphism in wet age-related macular degeneration*. Science, 2006. **314** (5801): p.989-92.
4. Consortium, W.T.C.C., *Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls*. Nature, 2007. **447** (7145): p.661-78.
5. Williams, S.M., et al., *Problems with genome-wide association studies*. Science, 2007. **316** (5833): p.1840-2.
6. Easton, D.F., et al., *Genome-wide association study identifies novel breast cancer susceptibility loci*. Nature, 2007. **447** (7148): p.1087-93.
7. Gudbjartsson, D.F., et al., *Variants conferring risk of atrial fibrillation on chromosome 4q25*. Nature, 2007. **448** (7151): p.353-7.
8. Hakonarson, H., et al., *A genome-wide association study identifies KIAA0350 as a type 1 diabetes gene*. Nature, 2007. **448** (7153): p.591-4.
9. Moffatt, M.F., et al., *Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma*. Nature, 2007. **448** (7152): p.470-3.
10. Sladek, R., et al., *A genome-wide association study identifies novel risk loci for type 2 diabetes*. Nature, 2007. **445** (7130): p.881-5.
11. Thorleifsson, G., et al., *Common sequence variants in the LOXL1 gene confer susceptibility to exfoliation glaucoma*. Science, 2007. **317** (5843): p. 1397-400.
12. Shriner, D., et al., *Problems with genome-wide association studies*. Science, 2007. **316** (5833): p.1840-2.
13. Couzin, J. and J. Kaiser, *Genome-wide association. Closing the net on common disease genes*. Science, 2007. **316** (5826): p.820-

2.
14. McPherson, R., et al., *A common allele on chromosome 9 associated with coronary heart disease*. Science, 2007. **316** (5830): p.1488-91.
15. Zeggini, E., et al., *Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes*. Science, 2007. **316** (5829): p.1336-41.
16. Scott, L.J., et al., *A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants*. Science, 2007. **316** (5829): p.1341-5.
17. Saxena, R., et al., *Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels*. Science, 2007. **316** (5829): p.1331-6.
18. Frayling, T.M., et al., *A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity*. Science, 2007. **316** (5826): p.889-94.
19. Duerr, R.H., et al., *A genome-wide association study identifies IL23R as an inflammatory bowel disease gene*. Science, 2006. **314** (5804): p.1461-3.

Katsushi Tokunaga ●Department of Human Genetics, Graduate School of Medicine, University of Tokyo

Genome-wide association studies in Japanese with special reference to narcolepsy

Genome-wide association studies (GWAS) using new high-throughput SNP typing technologies have successfully identified susceptibility genes to several common diseases. We have been participating in several GWAS efforts in the Japanese population. First, we typed 389 unrelated healthy Japanese by GeneChip Human Mapping 500K Array and investigated the data cleaning criteria by means of log QQ p-value plot in quasi-case-control studies. The preferable criteria were found to be: SNP call rate > 95%, confidence score = 0.5 in BRLMM algorism, Hardy-Weinberg's equilibrium test p-value > 0.001, and minor allele frequency > 5% or 1%.

Human narcolepsy is a typical sleep disorder with an incidence of about 0.15% in Japanese and affected by multiple genetic and environmental factors. A HLA class II gene (*DQB1*0602*) is a well-established susceptibility gene, whereas the other genes are yet to be identified. We previously carried out a GWAS for narcolepsy using 23,000 microsatellite markers, and reported several candidate regions as well as a new resistance gene (Kawashima *et al*. Am. J. Hum. Genet. 2006). Now, we performed GWAS using the 500K Array in 222 narcoleptics and 389 controls, and detected some 30 candidate SNPs. Then, the second screening was performed using a different sample set with 159 narcoleptics and 190 controls. At least one SNP showed a replicated association with a combined odds ratio of 1.79 ($p = 4.4 \times 10^{-7}$). The subsequent high-density association mapping surrounding the SNP showed that this SNP is primarily associated with the disease. This SNP is located closely to two genes and the risk allele was associated with lower mRNA levels of the genes. Interestingly, both genes were reported to have certain functions relevant to sleep regulation.

We are also responsible for Human SNP Typing Center for the Applied Genomics Initiatives supported by MEXT, Japan. Thus, we established the 500K Array database of 450 healthy Japanese and completed GWAS typing for multiple system atrophy and panic disorder. At present, we are producing the control data using GeneChip Array 6.0 and performing the GWAS typing for other diseases.

Another effort in our typing center is to establish a new multiplex SNP typing technology, which is suitable for high-density association studies for narrowing-down analyses of candidate regions. We developed a new method named DigiTag2 (Nishida *et al*. Anal. Biochem. 2007), which enables us to perform 32-plex or 96-plex SNP typing with high conversion rates and accuracy without specific instruments.

http://www.humgenet.m.u-tokyo.ac.jp/

Mark McCarthy
●Oxford Centre for Diabetes, Endocrinology and Metabolism; and the Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK

Sweet dreams: finding genes for diabetes and obesity

Until recently, progress in identification of the genetic variants influencing predisposition to common forms of diabetes and obesity has been slow. This is in marked contrast to the large number of genes implicated in rare monogenic forms of both conditions. However, recent advances in genetics, genomics and informatics have transformed the situation, and researchers are now able to undertake well-powered scans designed to detect association signals across the entire genome.

For type 2 diabetes, the six genome-wide association studies so far performed (all in samples of North European origin) have extended the number of loci harbouring common variants implicated in diabetes-susceptibility into double figures. Amongst the novel loci identified by this process include variants in/around genes which encode beta-cell zinc transporters (*SLC30A8*) and putative regulators of beta-cell mass (*CDKN2A/B* and *CDKAL1*). One of these new-found loci, mapping to the fat mass and diabetes associated (*FTO*) gene, influences individual risk of type 2 diabetes through a primary effect on fat mass, making this the first common variant known to influence weight and individual risk of obesity.

These findings offer two main avenues for clinical translation. First, the identification of new pathways involved in disease predisposition - for example, those influencing zinc transport and islet regeneration in the case of type 2 diabetes - offers opportunities for development of novel therapeutic and preventative approaches. Second, with continuing efforts to identify additional genetic variants, it may become possible to use patterns of predisposition to tailor individual management of these conditions.

However, it is important to realise that the variants so far identified explain only a small proportion of overall susceptibility to these conditions (well below 10 percent), and cannot explain the degree of familial aggregation which is routinely seen in epidemiological studies. A range of complementary approaches is currently being adopted, by our own group and others, to identify additional type 2 diabetes susceptibility loci, and to characterise those already found. To find further loci implicated in diabetes and obesity, we are: (a) combining data from multiple genome-wide scans, and performing very large-scale replication studies, so as to increase the power to detect variants of smaller effect; (b) undertaking genome-wide surveys of structural variants; and (c) initiating targeted deep resequencing efforts to trap low frequency, intermediate penetrance variants that may have escaped attention by classical linkage and association approaches. To characterise the loci already found, we are resequencing the genomic intervals of interest in large patient cohorts, and planning large-scale fine-mapping. Finally, we have begun to translate these new discoveries into information of relevance to improved clinical management of diabetes and obesity, by focusing on the epidemiological, physiological and pharmacological consequences of the susceptibility variants.

Masato Kasuga
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Multistage genome-wide association study of type 2 diabetes mellitus in Japanese population.

Diabetes mellitus is one of the main threats to human health in the twenty-first century. The total number of people with diabetes worldwide was estimated at between 151 million and 171 million in 2000 and is projected to increase to 221 million in 2010 and to 366 million in 2030. Needless to say, the increase in the number of people with diabetes will be accompanied by an increase in the number of those with diabetic complications such as nephropathy, retinopathy, neuropathy, and atherosclerosis. Type 2 diabetes accounts for more than 90% of cases of diabetes worldwide. The development of this common form of diabetes results from complex interactions between environmental and genetic factors. It is important to identify the susceptibility genes to type 2 diabetes, to understand the pathogenesis of this disease and to develop new approaches to its prevention and treatment.

The recent progress in human genomic science and technology has allowed the undertaking of genome-wide association studies with comprehensive single nucleotide polymorphism (SNP) typing. As part of the Millennium Genome Project of Japan, we performed a multistage genome-wide association study of the common form of type 2 diabetes mellitus with a total of 1612 cases and 1424 controls and with 100,000 SNPs from the standard Japanese polymorphisms (JSNP) database (http://snp.ims.u-tokyo.ac.jp/index_ja.html) generated by this project. Successful genotyping results were obtained for 82,343 autosomal SNPs by the multiplex PCR-based Invader assay, and 10 SNPs in seven genes showed significant association ($P < 0.05$). Most of these genes previously have not been associated with diabetes in any population. The lowest P value of 2.2×10^{-12} was obtained for a SNP in "Gene A". Given that three of the 10 positive SNPs resided in this gene, we examined additional known polymorphisms as well as those revealed by sequencing of the gene in 24 Japanese subjects. We found that a SNP in "Gene A" showed the lowest P value of 6.7×10^{-13} (odds ratio = 1.49; 95% confidence interval = 1.34 to 1.66). Among control subjects, the homeostasis model assessment of β cell function (HOMA-β) was significantly lower ($P = 0.0024$) in homozygotes for the risk allele of this polymorphism than in individuals with the other two genotypes, suggesting that this allele may be associated with impairment of insulin secretion.

Our data thus implicated "Gene A" as a susceptibility gene for type 2 diabetes. The identification of this genes, as well as other 6 genes in our study may provide insight into the pathogenesis of type 2 diabetes as well as a basis for the development of new therapeutic agents.

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Large-Scale Identification And Characterization Of Developmental Brain Disease Genes

Schizophrenia (SCZ) and autism (AUT) are common, devastating and poorly treated mental disorders. Converging evidence suggests that genetically disrupted synaptogenesis and synaptic plasticity during development may underlie the pathogenesis of such brain disorders. We therefore hypothesize that a significant fraction of SCZ and AUT cases are a result of frequent novel mutations in many different genes involved in synapse formation and function. Our strategy is to identify the underlying synaptic genetic factors predisposing to SCZ and AUT by using a new, two-step strategy: first the direct re-sequencing of 1000 genes coding for proteins which regulate an entire brain-specific “machine”, the synapse, in a large number of patients, and secondly evaluating the functional effects of disease-related variants of these genes in several animal models. This will allow us to identify the most promising mutations for future functional genomics analyses, including transgenic mouse models. In contrast to the all too few mutations discovered to date, we expect to identify a significant number of genes directly causing or increasing the susceptibility to SCZ and AUT.

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Causes of regulatory variation in the human genome

The recent comparative analysis of the human genome has revealed a large fraction of functionally constrained non-coding DNA in mammalian genomes. However, our understanding of the function of non-coding DNA is very limited. In this talk I will present recent analysis in my group and collaborators that aims at the identification of functionally variable regulatory regions in the human genome by correlating SNPs and copy number variants with gene expression data. I will also be presenting some analysis on inference of trans regulatory interactions and evolutionary consequences of gene expression variation.

www.sanger.ac.uk/Teams/Team16

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The Cancer Genome Atlas

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Array-based high-throughput sequencing of leukemia genome

Somatic mutations in the genome of cancer cells likely play an essential role in the malignant transformation mechanism, as is already evident in acute myeloid leukemia (AML) cases with the activating mutations in *RUNX1, NPM1, KIT*, or *NRAS* gene. Since a normal karyotype without apparent chromosome abnormalities is identified in the leukemic blasts for >50% of AML cases, sequence alterations in such genome are presumably yet to be identified.

To discover cancer-promoting mutations in the AML genome, we here developed a large-scale re-sequencing "wafer" in a collaboration with Perlegen Sciences Inc., which enables the sequence determination at >nine million nucleotides. Oligonucleotide probes on the wafer were designed to analyze coding regions as well as splicing signals for the exon/intron boundary among ~5,600 human genes, (1) which are already known to be mutated in human cancers, and (2) the products of which are involved in DNA repair system, chromosome regulation, redox regulation, intracellular signal transduction, protein kinases, transcriptional factors, cell cycle regulation, and apoptosis.

For the detection of somatic mutations in AML, we prepared, from a total of twenty AML cases, CD34-positive hematopoietic progenitors (“leukemic fraction”) as well as paired CD4-positive normal T-cells (“control fraction”). As the first phase sequencing, genomic DNA of each leukemic fraction was hybridized with the sequencing wafer, leading to the discovery of a total of >9,000 non-synonymous mutations among >3000 genes on the wafer. We then proceeded to the second phase of the project; another sequencing wafer was constructed only to determine such non-synonymous mutations, and was subjected to hybridization with a leukemic fraction or with a paired control fraction. Surprisingly, most of the “mutations” identified in the analysis of leukemic fractions were also present on the genome of the control fractions. Therefore, most of such "mutations" are likely rare polymorphism in the AML genome.

One of the *bona fide* mutations discovered in the second phase project turned out to lead to a novel amino acid substitution in a non-receptor-type protein tyrosine kinase. Interestingly, this mutated tyrosine kinase was shown to acquire a transforming potential. A forced expression of this mutated kinase abrogates cytokine-dependency from a hematopoietic cell line, and also substantially inhibit differentiation of blood cells.

Our strategy is thus potent in discovering somatic mutations in cancer genome in a high-throughput manner.