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ABSTRACTS

Genes, chromosomes and disease

058: Phenome scan (genotype/dense phenotype association studies) of a comprehensive clinicopathological database derived from large numbers of autopsy cases

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[Background] Since most genes are expressed in more than one tissue and engaged in many functions, some genetic variant could be associated with unpredicted diseases with or without interactions with other genetic variants or environmental factors. Recently Pembrey and Jones proposed a new method, 'phenome scan' (analogous to 'genome-wide scan', but in an opposite direction), in which dense phenotypic information in human cohorts is scanned for associations with individual genetic variants. We attempted to apply this method to our comprehensive clinicopathological database derived from large numbers of autopsy cases. [Subjects] The subjects of this database derived from more than 2,000 consecutive autopsies from the patients died in a community-based geriatric general hospital during recent 12 years. Most of the cases were registered in a SNP database named 'A Database of Japanese Single Nucleotide Polymorphism for Geriatric Research (JG-SNP)' and freely accessible on the Internet at http://www.tmgh.metro.tokyo.jp/jg-snp/english/E_top.html. [Results] The average age of the patients was 80 years and male to female ratio was 1.2. The autopsy rate has declined from 46 to 21% during this period. The database contained patient information, histories of smoking and drinking, clinical diagnosis including 26 geriatric diseases, serum lipid data, 750 pathological findings, 42 major pathological diagnoses, atherosclerotic degrees of 10 major arteries, emphysematous degrees, and so on. Twenty-six geriatric diseases included 6 cardiovascular diseases, 3 neurological diseases, 5 metabolic and skeletal disease, 3 respiratory diseases, 4 malignancies and so on. Forty-two major pathological diagnoses included an extended and partially overlapping list of geriatric diseases. Both clinical and subclinical findings were registered among 750 pathological findings. DNA was extracted from unfixed renal tissue in all cases and used for molecular analysis. The results of application of this method to several genes such as CAR, NOS3 and PRKCH will be presented. [Conclusions] In contrast to physiological limitation of human genome (3 gigabase), human phenome is unlimited and difficult to define. Although the number of the subjects registered in this database and available medical information were limited, this database was still useful for the phenome scan to identify significant associations between genetic variant, especially for the genes of unknown functions, and geriatric diseases or pathological conditions.

059: Genome wide search for novel cancer gene in familial adenomatous polyposis (FAP) variant patients without detectable APC mutation

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Background and aim: FAP is an autosomal dominantly inherited form of colorectal cancer caused by a germline mutation in the adenomatous polyposis coli (APC) gene. We search for new cancer gene in a subgroup of APC mutation-negative FAP variant patients with autosomal dominant inheritance and typical adenomatous polyps (100s–1000) but have accelerated disease progression. Methods: We performed Whole Genome Sampling Assay on both the lymphocyte and polyp DNA of two affected individuals of a FAP variant family and lymphocyte DNA of 2 other affected, 4 unaffected members and 18 ethnicity-matched healthy controls using Affymetrix Genome-Wide Human single nucleotide polymorphism (SNP) Array 5.0. This SNP Array 5.0 enables genotyping of approximately 450 K SNPs and assaying of an additional 500 K non-polymorphic copy number (CN) probes, covering the entire genome with an average inter-probe distance of $\sim$10 kb. Results: By using the Copy Number Tool of Partek Genomic Suite Software, a 40 kb region consisting of 17 CN but no SNP probes showed gain of copy number in germline of all four affected members (mean CN = 4.20) compared to the four unaffected members as baseline. We are currently verifying this copy number gain and investigating the expression of a putative oncogene 18 kb downstream. Further, a 111 kb region on another chromosome, consisting of 44 CN probes, showed copy number loss in all eight polyp DNAs (mean CN = 0.68) compared to the matched lymphocyte DNAs of two affected individuals. Fragments chosen randomly from this

critical region for PCR analysis further confirmed the copy number loss. Data mining with NCBI Build 36.2, however, reveal no known gene but only one putative 2.2 kb transcript in melanoma tissue. Extensive RT-PCR analysis indicated that this transcript is probably non-existent in lymphocyte and colonic mucosa tissues. Conclusion: Structural variations may contribute to polyposis in FAP variant patients without detectable APC germline mutation.

060: Modelling potential CNV effects on human fertility by manipulating meiosis in mice

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Male and female fertility requires completion of meiosis to produce the haploid gametes. Meiosis is a complex process in which the two genomes in the diploid cell have to be aligned with single base pair accuracy and linked by crossover events to ensure correct segregation at the cell divisions needed to produce haploid cells. In humans fertility is highly variable and a substantial part of that variation is genetic. The phenotype is hard to measure and involves input from two individuals making whole genome association studies difficult if not impossible. Candidate gene approaches based on a knowledge of the underlying processes of gametogenesis have had limited success. This is a reflection of the large number of genes thought to be required, particularly for sperm production, and since the majority of mutations are likely to be recessive, homozygosity at any one locus for such a mutation is likely to be rare. It is possible that more common processes such as copy number variation may be involved. The level of proteins in the cell may be critical of function especially when forming structural complexes with multiple other proteins. We have asked if compound heterozygosity at genes encoding proteins required for meiosis can affect the process. SYCE1 and SYCE2 are proteins of the meiotic Synaptonemal Complex. We have shown that SYCE2 is essential for male and female fertility in mice and here we show that this is also true for SYCE1. Heterozygous null animals are fertile in the case of both genes. Male mice heterozygous for both null alleles are also fertile but have an increased rate of XY asynapsis and reduced sperm count showing that compound hemizygosity in genes involved in the same pathway can have a significant impact. Since these proteins form a complex with others to generate the Synaptonemal Complex without which cross-overs can not complete it is likely that the defects we observe result from changes in protein levels. Copy number variation could result in similar effects in human populations.

061: Identification of a novel chromosomal locus in a Belgian FTLD-MND family

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Frontotemporal lobar degeneration (FTLD) is a neurodegenerative brain disorder with a prevalence similar to that of Alzheimer's disease in the population below age 65 years. In some patients FTLD symptoms are accompanied by signs of motor neuron disease (MND). A positive family history is observed in up to 50% of FTLD patients indicating a significant contribution of genetics to the etiology of FTLD. A high degree of genetic heterogeneity has been observed with different mutations in the genes encoding the microtubule-associated protein (MAPT), progranulin (PGRN), charged multivesicular body protein 2B (CHMP2B) and valosin containing protein (VCP). Further, two loci on chromosome 9 at 9q21–q22 and 9p13–21 were implicated in FTLD with MND (FTLD-MND). In Belgian familial FTLD patients 65% remained unexplained by mutations in known FTLD genes. Of one patient diagnosed with FTLD-MND, we collected DNA of relatives and performed a genome-wide scan. We identified and finemapped a novel chromosomal locus that was not previously linked to FTLD and/or MND. Haplotype analysis identified a risk haplotype of 23 cM that co-segregated with disease. Further reduction of the candidate region and mutation analyses of positional and functional candidate genes are ongoing. Identification of the mutation in the underlying disease gene will significantly contribute to the understanding of neurodegenerative disease mechanisms in FTLD.

062: Genotype-phenotype correlations for CNVs identified in patients with mental retardation; increased array resolution refines detected CNVs, selects candidate genes and diagnoses known syndromes

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Submicroscopic copy number variants (CNVs) identified by array genome hybridization (AGH) are now known to cause mental retardation (MR) as frequently as cytogenetically detectable genetic defects. We screened 100 trios each comprising a child with idiopathic MR and both normal parents using Affymetrix 500 K GeneChip® Human mapping assays and detected causative de novo CNVs in 16 cases. Two patients carried overlapping deletions of 9p11.2p13.3; one a ~11 Mb deletion from 33.7 to 44.7 Mb, and the other a ~4.6 Mb deletion from 34.1 to 38.7 Mb. The distal non-overlapping portion (from 38.7 to 44.7 Mb) is highly enriched in low copy repeat (LCR) elements and poorly assayed by Affymetrix 500 K SNP probes. We re-screened both patients using Affymetrix 6.0 GeneChips® which have greater than threefold higher coverage of the region and include non-SNP probes which show more robust performance for regions involved in LCR. On this platform we found the single deletion in the first case to be composed of two smaller deletions (a ~6 Mb deletion from 33.4 to 39.3 Mb and a 0.7 Mb deletion from 44.1 to 44.8 Mb) as well as an additional small deletion (of 0.6 Mb from 44.2 to 44.8 Mb) in the second patient previously undetected. Choosing the appropriate platform is important when assaying genomic regions involved in complex genomic architecture.

We found a ~10.8 Mb deletion of 7p15.3 in another patient. Our patient's CNV overlaps with five other deletion and duplication CNVs reported in the DECIPHER database, suggesting the identification of a novel region that is prone to disease causing variation. A 6.9 Mb duplication of 8q12.1 in another case involves the CHD7 gene, which causes CHARGE syndrome when deleted. This patient's phenotypes include defects in organ systems (cardiac, ocular, auditory and genital) affected in CHARGE syndrome. In another patient we identified

a rare whole chromosome uni-parental disomy (UPD) for chromosome 16 that we believe is pathogenic. This heterodisomic UPD would not have been detected by a non-SNP probe AGH platform.

We also screened patients who were normal on Affymetrix 100 K GeneChip® AGH with the higher resolution 500 K platform and detected causative CNVs in two patients. A 4p16.3 deletion in a patient subsequently diagnosed with mild Wolf-Hirschorn syndrome and a novel 1.5 Mb duplication of 8q22.3 in another case. These data highlight the usefulness of higher resolution AGH and demonstrate the varying benefits of different probe sets.

063: Systematic resequencing of the coding exons of the X chromosome in X-linked mental retardation

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Mental retardation (MR) affects 1–3% of live births and has both genetic and non-genetic causes. A proportion of cases with genetic abnormalities are attributable to mutations of genes on the X chromosome. Although many X-linked MR (XLMR) genes have been reported, identification of more by conventional approaches has become problematic. To address this, we have implemented a strategy in which the coding exons of 719 X chromosome genes have been systematically resequenced for disease-causing variants in 206 XLMR families. This approach has yielded at least 10 new XLMR genes with several more to be validated. Many families still remain to be explained. The study has revealed the pattern of haplotypic coding sequence variation on the X chromosome and indicates that loss of function of $\sim$1% of X chromosome genes is compatible with apparently normal existence. This work has also highlighted issues that may be faced in the future by whole genome screens for rare disease-causing variants in other complex phenotypes.