J, Kennedy JL, Farmer A, McGuffin P, Day R, Matthews K, Bakke P, Gulsvik A, Lucae S, Ising M, Brueckl T, Horstmann S, Wichmann HE, Rawal R, Dahmen N, Lamina C, Polasek O, Zgaga L, Huffman J, Campbell S, Kooner J, Chambers JC, Burnett MS, Devaney JM, Pichard AD, Kent KM, Satler L, Lindsay JM, Waksman R, Epstein S, Wilson JF, Wild SH, Campbell H, Vitart V, Reilly MP, Li M, Qu L, Wilensky R, Matthai W, Hakonarson HH, Rader DJ, Franke A, Wittig M, Schafer A, Uda M, Terracciano A, Xiao X, Busonero F, Scheet P, Schlessinger D, St Clair D, Rujescu D, Abecasis GR, Grabe HJ, Teumer A, Volzke H, Petersmann A, John U, Rudan I, Hayward C, Wright AF, Kolcic I, Wright BJ, Thompson JR, Balmforth AJ, Hall AS, Samani NJ, Anderson CA, Ahmad T, Mathew CG, Parkes M, Satsangi J, Caulfield M, Munroe PB, Farrall M, Dominiczak A, Worthington J, Thomson W, Eyre S, Barton A, Mooser V, Francks C, Marchini J. 2010. Meta-analysis and imputation refines the association of 15q25 with smoking quantity. Nat Genet 42(5):436–440.

Lumley T. 2009. rmeta: Meta-analysis, R package version 2.15.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81(3):559–575.

Rotimi CN, Jorde LB. 2010. Ancestry and disease in the age of genomic medicine. N Engl J Med 363(16):1551–1558.

Saccone NL, Saccone SF, Goate AM, Grucza RA, Hinrichs AL, Rice JP, Bierut LJ. 2008. In search of causal variants: refining disease association signals using cross-population contrasts. BMC Genet 9:58–68.

Saccone NL, Wang JC, Breslau N, Johnson EO, Hatsukami D, Saccone SF, Grucza RA, Sun L, Duan W, Budde J, Culverhouse RC, Fox L, Hinrichs AL, Steinbach JH, Wu M, Rice JP, Goate AM, Bierut LJ. 2009. The CHRNA5-CHRNA3-CHRNB4 nicotinic receptor subunit gene cluster affects risk for nicotine dependence in African-Americans and in European-Americans. Cancer Res 69(17):6848–6856.

Saccone NL, Culverhouse RC, Schwantes-An TH, Cannon DS, Chen X, Cichon S, Giegling I, Han S, Han Y, Keskitalo-Vuokko K, Kong X, Landi MT, Ma JZ, Short SE, Stephens SH, Stevens VL, Sun L, Wang Y, Wenzlaff AS, Aggen SH, Breslau N, Broderick P, Chatterjee N, Chen J, Heath AC, Heliovaara M, Hoft NR, Hunter DJ, Jensen MK, Martin NG, Montgomery GW, Niu T, Payne TJ, Peltonen L, Pergadia ML, Rice JP, Sherva R, Spitz MR, Sun J, Wang JC, Weiss RB, Wheeler W, Witt SH, Yang BZ, Caporaso NE, Ehringer MA, Eisen T, Gapstur SM, Gelemter J, Houlston R, Kaprio J, Kendler KS, Kraft P, Leppert MF, Li MD, Madden PA, Nothen MM, Pillai S, Rietschel M, Rujescu D, Schwartz A, Amos CI, Bierut LJ. 2010. Multiple independent loci at chromosome 15q25.1 affect smoking quantity: a meta-analysis and comparison with lung cancer and COPD. PLoS Genet 6(8).

Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K, Madden PA, Breslau N, Johnson EO, Hatsukami D, Pomerleau O, Swan GE, Goate AM, Rutter J, Bertelsen S, Fox L, Fugman D, Martin NG, Montgomery GW, Wang JC, Ballinger DG, Rice JP, Bierut LJ. 2007. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. Hum Mol Genet 16(1):36–49.

Schwartz AG, Wenzlaff AS, Bock CH, Ruterbusch JJ, Chen W, Cote ML, Artis AS, Van Dyke AL, Land SJ, Harris CC, Pine SR, Spitz MR, Amos CI, Levin AM, McKeigue PM. 2010. Admixture mapping of lung cancer in 1812 African-Americans. Carcinogenesis 32(3):312–317.

Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, Sirotkin K. 2001. dbSNP: the NCBI database of genetic variation. Nucleic Acids Res 29(1):308–311.

Sherva R, Wilhelmsen K, Pomerleau CS, Chasse SA, Rice JP, Snedecor SM, Bierut LJ, Neuman RJ, Pomerleau OF. 2008. Association of a single nucleotide polymorphism in neuronal acetylcholine receptor subunit alpha 5 (CHRNA5) with smoking status and with 'pleasurable buzz' during early experimentation with smoking. Addiction 103(9):1544–1552.

Shiraishi K, Kohno T, Kunitoh H, Watanabe S, Goto K, Nishiwaki Y, Shimada Y, Hirose H, Saito I, Kuchiba A, Yamamoto S, Yokota J. 2009. Contribution of nicotine acetylcholine receptor polymorphisms to lung cancer risk in a smoking-independent manner in the Japanese. Carcinogenesis 30(1):65–70.

Smith RM, Alachkar H, Papp AC, Wang D, Mash DC, Wang JC, Bierut LJ, Sadee W. 2010. Nicotinic alpha5 receptor subunit mRNA expression is associated with distant 5′ upstream polymorphisms. Eur J Hum Genet 19:76–83.

Stevens VL, Bierut LJ, Talbot JT, Wang JC, Sun J, Hinrichs AL, Thun MJ, Goate A, Calle EE. 2008. Nicotinic receptor gene variants influence susceptibility to heavy smoking. Cancer Epidemiol Biomarkers Prev 17(12):3517–3525.

TAG. 2010. Genome-wide meta-analyses identify multiple loci associated with smoking behavior. Nat Genet 42(5):441–447.

Thorgeirsson TE, Geller F, Sulem P, Rafnar T, Wiste A, Magnusson KP, Manolescu A, Thorleifsson G, Stefansson H, Ingason A, Stacey SN, Bergthorsson JT, Thorlacius S, Gudmundsson J, Jonsson T, Jakobsdotlir M, Saemundsdottir J, Olafsdotlir O, Gudmundsson LJ, Bjornsdotlir G, Kristjansson K, Skuladotlir H, Isaksson HJ, Gudbjartsson T, Jones GT, Mueller T, Gotlsater A, Flex A, Aben KKH, de Vegt F, Mulders PFA, Isla D, Vidal MJ, Asin L, Saez B, Murillo L, Blondal T, Kolbeinsson H, Stefansson JG, Hansdotlir I, Runarsdottir V, Pola R, Lindblad B, van Rij AM, Dieplinger B, Haltmayer M, Mayordomo JI, Kiemeney LA, Matthiasson SE, Oskarsson H, Tyrfingsson T, Gudbjartsson DF, Gulcher JR, Jonsson S, Thorsteinsdottir U, Kong A, Stefansson K. 2008. A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. Nature 452(3):638–642.

Thorgeirsson TE, Gudbjartsson DF, Surakka I, Vink JM, Amin N, Geller F, Sulem P, Rafnar T, Esko T, Walter S, Gieger C, Rawal R, Mangino M, Prokopenko I, Magi R, Keskitalo K, Gudjonsdotlir IH, Gretarsdottir S, Stefansson H, Thompson JR, Aulchenko YS, Nelis M, Aben KK, den Heijer M, Dirksen A, Ashraf H, Soranzo N, Valdes AM, Steves C, Uitterlinden AG, Hofman A, Tonjes A, Kovacs P, Hottenga JJ, Willemsen G, Vogelzangs N, Doring A, Dahmen N, Nitz B, Pergadia ML, Saez B, De Diego V, Lezcano V, GarciaPrats MD, Ripatti S, Perola M, Kettunen J, Hartikainen AL, Pouta A, Laitinen J, Isohanni M, Huei-Yi S, Allen M, Krestyaninova M, Hall AS, Jones GT, van Rij AM, Mueller T, Dieplinger B, Haltmayer M, Jonsson S, Matthiasson SE, Oskarsson H, Tyrfingsson T, Kiemeney LA, Mayordomo JI, Lindholt JS, Pedersen JH, Franklin WA, Wolf H, Montgomery GW, Heath AC, Martin NG, Madden PA, Giegling I, Rujescu D, Jarvelin MR, Salomaa V, Stumvoll M, Spector TD, Wichmann HE, Metspalu A, Samani NJ, Penninx BW, Oostra BA, Boomsma DI, Tiemeier H, van Duijn CM, Kaprio J, Gulcher JR, McCarthy MI, Peltonen L, Thorsteinsdotlir U, Stefansson K. 2010. Sequence variants at CHRNB3-CHRNA6 and CYP2A6 affect smoking behavior. Nat Genet 42(5):448–453.

Wang JC, Cruchaga C, Saccone NL, Bertelsen S, Liu P, Budde JP, Duan W, Fox L, Grucza RA, Kern J, Mayo K, Reyes O, Rice J, Saccone SF, Spiegel N, Steinbach JH, Stitzel JA, Anderson MW, You M, Stevens VL, Bierut LJ, Goate AM. 2009. Risk for nicotine dependence and lung cancer is conferred by mRNA expression levels and amino acid change in CHRNA5. Hum Mol Genet 19(6):3125–3135.

Weiss RB, Baker TB, Cannon DS, von Niederhausern A, Dunn DM, Matsunami N, Singh NA, Baird L, Coon H, McMahon WM, Piper ME, Fiore MC, Scholand MB, Connett JE, Kanner RE, Gahring LC, Rogers SW, Hoidal JR, Leppert MF. 2008. A candidate gene approach identifies the CHRNA5-A3-B4 region as a risk factor for age-dependent nicotine addiction. PLoS Genet 4(7):e1000125.

Wu C, Hu Z, Yu D, Huang L, Jin G, Liang J, Guo H, Tan W, Zhang M, Qian J, Lu D, Wu T, Lin D, Shen H. 2009. Genetic variants on chromosome 15q25 associated with lung cancer risk in Chinese populations. Cancer Res 69(12):5065–5072.

Zaitlen N, Pasaniuc B, Gur T, Ziv E, Halperin E. 2010. Leveraging genetic variability across populations for the identification of causal variants. Am J Hum Genet 86(1):23–33.

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Review

Genetic Polymorphisms Underlying Lung Cancer Susceptibility and Therapeutic Response

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Recent advances in genome analysis technologies have provided a detailed genome-wide view of cancerous and non-cancerous cells. Lung cancer is largely caused by tobacco smoking, but several studies have implicated inherited genetic factors in disease etiology. Genome-wide association studies (GWASs) using DNA chips have identified loci/genes with polymorphisms that underlie inter-individual differences in cancer susceptibility, including single nucleotide polymorphisms (SNPs). *CHRNA* (cholinergic receptor, nicotinic, alpha), *TERT* (telomerase reverse transcriptase) and *TP63* (tumor protein p63) loci have been linked to lung cancer susceptibility by GWASs. SNPs in *TERT* and *TP63* are preferentially associated with the risk of adenocarcinoma, the commonest histological type of lung cancer affecting both smokers and non-smokers, whereas those in *CHRNA* are associated with lung cancer risk irrespective of histological type. An association of functional polymorphisms in DNA repair/metabolic genes with the risk of squamous cell carcinoma, a major histological type developed in smokers, has been suggested, but it remains inconclusive. It was also suggested that an SNP in the *TP53* tumor suppressor gene influences the response to platinum-doublet chemotherapy in lung cancer patients. However, analyses have shown that only a subset of SNPs is involved in lung carcinogenesis/therapy. Further GWASs are needed to translate the information on genetic variations into cancer prevention and clinical practice by focusing on specific subtypes of lung cancers or therapeutic responses.

Key words: lung cancer, genome-wide association studies (GWASs), single nucleotide polymorphisms (SNPs), cancer susceptibility, DNA repair genes, metabolic genes

Introduction

Lung cancer is the leading cause of cancer mortality worldwide, with more than one million deaths each year. The different histological forms of lung cancer are typically divided into small cell lung cancer (SCLC, 20% in Japan) and non-small cell lung cancer (NSCLC, 80%), mainly adenocarcinoma (ADC, 40%) and squamous cell carcinoma (SQC, 30%) (1). Although lung cancer is largely caused by tobacco smoking, inherited genetic factors (i.e., genetic polymorphisms) may increase its risk according to recent genome-wide association studies (GWASs) using DNA chips, which allow the determination of genotypes for hundred thousands to millions of single nucleotide polymorphisms (SNPs) (2–7). Risk variants may result in different magnitudes of increased lung cancer risk depending on populations, smoking behavior, and histological types. Further studies of genetic factors will help to clarify the disease etiology and to identify high risk individuals for targeted screening and/or prevention. A recent study has also indicated that genetic polymorphisms also underlie inter-individual differences in response to cancer chemotherapy (8). Genetic polymorphisms provide a valuable tool for understanding the nature of human carcinogenesis and the outcomes of cancer therapy.

GWAS

Genetic polymorphisms responsible for cancer susceptibility have been investigated in case-control (association) studies where polymorphisms with different distributions between cancer cases and non-cancer controls have been identified (9) (Fig. 1A). Genetic factors involved in cancer susceptibility were previously studied in the polymorphisms of genes encoding proteins with the ability to metabolize carcinogens or suppress mutations induced by carcinogens. However, recent advances in molecular technology and knowledge of the distribution of genetic polymorphisms in the human genome have made it possible to identify genetic factors responsible for the development of common diseases using GWASs, including lung cancer (http://gwas.lifesciencedb.jp/cgi-bin/gwasdb/gwas_top.cgi) (10). Several cancer susceptibility genes/loci have been identified by GWASs. For example, gene polymorphisms in the *ABO* blood group gene (11) and the *ALDH2* (aldehyde dehydrogenase 2) gene (12) were associated with susceptibility to pancreat-

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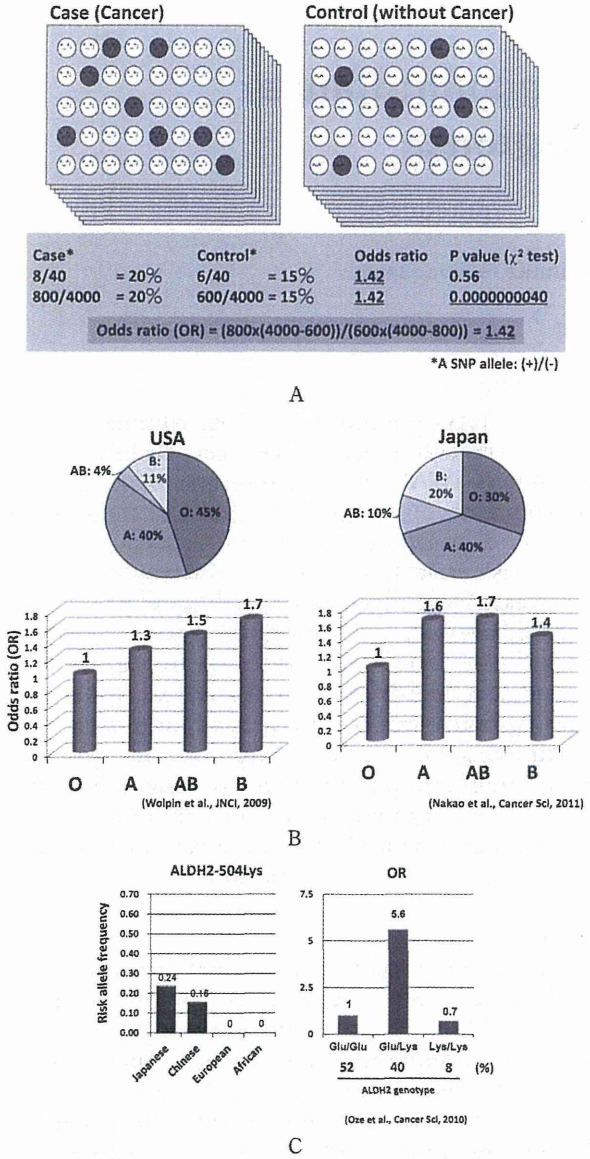

Fig. 1. GWAS. (A) Case-control study. The odds ratio (OR) is calculated as a measure of the effect size describing the strength of an association. OR is the ratio of the odds of an event occurring in one group (case) to the odds of it occurring in another group (control). Hundred thousands to millions of SNPs are examined in GWASs, which means that a very high number of case and control subjects must be tested to obtain statistically significant associations after correcting for the problem of multiple comparisons, e.g., using a Bonferroni correction. (B) ORs of individuals with each ABO blood type in the USA and Japan. Individuals in blood group O had a lower risk for pancreatic cancer than those in groups A, B or AB. The upper graphs show the ratio of ABO blood type in the USA and Japanese populations. (C) The left graph shows frequencies of chromosomes for risk allele in all chromosomes in each population. The frequencies were determined by the HapMap project. The right graph shows ORs of individuals with each *ALDH2* genotype in the Japanese population. Individuals with the *ALDH2 Glu/Lys* genotype had a higher risk for esophageal cancer than those with the *ALDH2 Glu/Glu* genotype.

ic and esophageal cancers, respectively. These results were consistent with earlier epidemiological evidence suggesting that blood group O subjects may have a lower risk of pancreatic cancer than groups A, B, or AB (13,14) (Fig. 1B), and that the *ALDH2 Glu/Lys* genotype confers a higher susceptibility to esophageal cancer than the *ALDH2 Glu/Glu* genotype due to the decreased elimination of acetaldehyde (15) (Fig. 1C).

GWASs of Lung Cancer Risk

Three chromosomal loci, 15q24-25.1, 5p15.33 and 6p21, were found to be associated with lung cancer risk in GWASs of European/American populations (2–4), while locus 3q28 was associated with lung ADC risk in a GWAS of Japanese/Korean populations (7). The chromosomal 15q24-25.1 region contains nicotinic acetylcholine receptor subunit genes, i.e., *CHRNA3* (cholinergic receptor, nicotinic, alpha 3) and *CHRNA5*, and their products are expressed in pulmonary epithelial cells and bind to nicotine, an addictive compound found in cigarette smoke, and nitrosamines, which are potential lung carcinogens in cigarette smoke and food (16,17). The associated lung cancer risk of intronic SNPs in 15q24-25.1 was replicated in Asian populations (18,19). However, the frequency of risk alleles was much lower than that in populations of European descent (Fig. 2A, left). Thus, the *CHRNA* risk alleles make a smaller subset of individuals more susceptible to lung cancer in Asians compared with European and American populations. Interestingly, the association with lung cancer risk was found irrespective of smoking and histological types in many studies, so *CHRNA* SNPs might contribute to the risk in a (active) smoking-independent manner (Fig. 2A, right). The involvement of other factors should be further investigated, such as food intake and passive smoking. On the other hand, the contribution of *CHRNA* SNPs to lung cancer risk via tobacco addiction has been also suggested (20). Thus, further studies need to investigate a cohort of subjects where detailed data are available on lung cancer development, food intake, active and passive smoking exposure, nicotine dependence, and the duration and intensity of smoking to elucidate how *CHRNA* SNPs contribute to lung cancer risk. The 5p15.33 region contains the *TERT* (telomerase reverse transcriptase) gene. TERT is known to function in telomere replication and maintenance, and it promotes epithelial cell proliferation (21). The risk allele frequency of the landmark SNP (rs2736100) is similar among ethnic populations (Fig. 2B, left), and associations have been detected in among Europeans, American and Asians (22,23) (Fig. 2B, right). Interestingly, this SNP is associated with the risk of ADC, but not SQC or SCLC (5,6,22–24), suggesting a preferential association of this locus with lung cancer risk in never-smokers (Fig. 2B, right). Risk associations of the

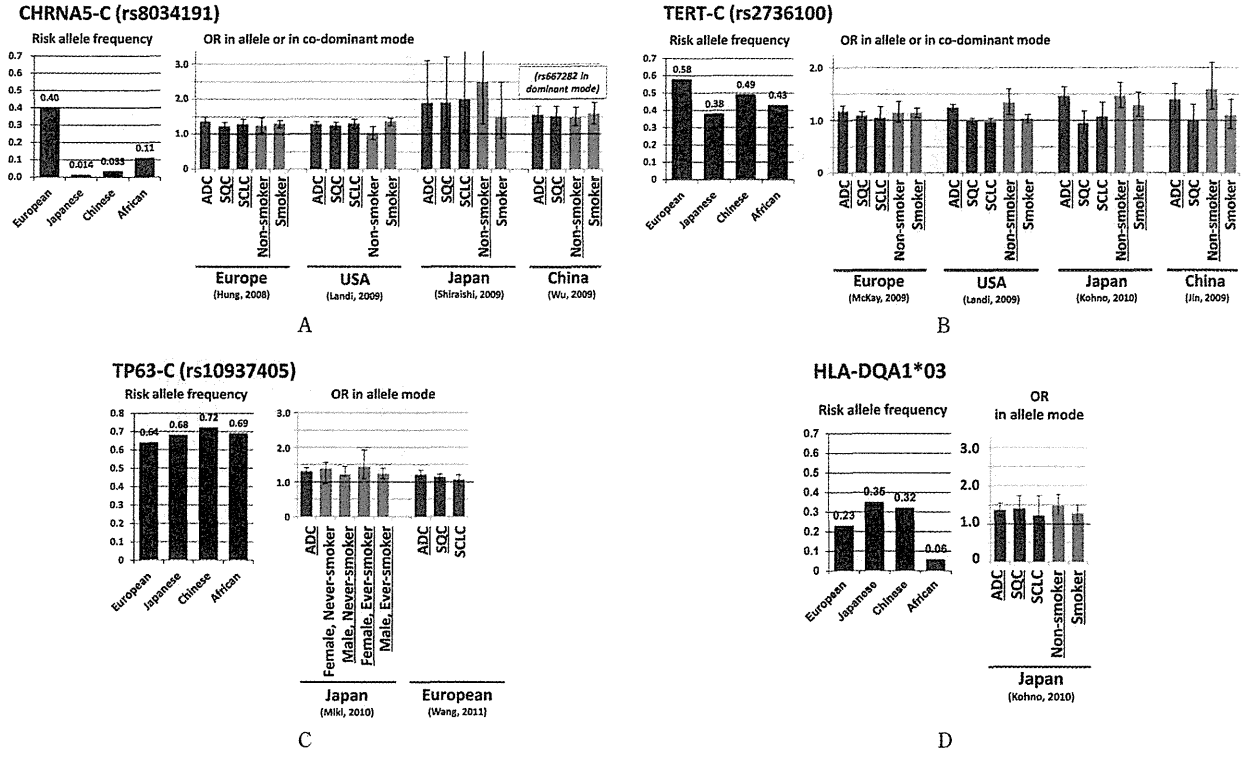

Fig. 2. Increased lung cancer risk with SNPs identified by GWASs according to populations, smoking behavior, and histological types. Categories where statistically significant association was observed are underlined. (A) rs8034191 (*CHRNA5*) at 15q24–25.1. (B) rs2736100 (*TERT*) at 5p15.33. (C) rs10937405 (*TP63*) at 3q28. (D) HLA-DQA1*03 (*HLA-DQA1*) at 6p21. The left graphs show frequencies of chromosomes for risk allele in all chromosomes in each population. Frequencies were determined by the HapMap project or the International Histocompatibility Working Group are shown on the left.

5p15.33 genotype have been detected in lung cancer and other types of cancers, including cancers of the brain, bladder, prostate, uterine cervix, and skin (25). The rs2736100 SNP is located in intron 2 of *TERT*, which is a putative regulatory region. It was previously suggested that risk alleles in the *TERT* gene are associated with shorter telomeres (25). Therefore, these variants may lead to an increase in the gradual shortening of telomeres over time, leading to genomic instability driving carcinogenesis in many organs. Thus, functional analyses of *TERT* SNPs are warranted. The 3q28 region contains the *TP63* gene that encodes a member of the tumor suppressor *TP53* (also known as p53) gene family, which is involved in development, differentiation, and the cellular stress response (26). The risk allele frequency of the landmark SNP (rs10937405) was similar among ethnic populations (Fig. 2C, left) and associations with lung ADC risk were detected in both Asians and Europeans (7,27) (Fig. 2C, right). However, the association appears to be stronger in Asians than Europeans (27). In Europeans, there is a weaker association with lung SQC risk, while the association with SQC risk is unknown in Asians. SNPs associated with lung cancer risk are located in intron 1 of *TP63* and it has been suggested that they have a functional role in the regulation of *TP63* gene expression. *TP63* is induced after the exposure of cells to DNA damage. Therefore, inter-individual differences in the accumulation of DNA damage and the response to genotoxic stress might contribute to lung cancer susceptibility. Associations of SNPs in the 6p21 region were found in a GWAS of Europeans (4). This region contains the *BAT3* (HLA-B associated transcript 3) and *MSH5* (mutS homolog 5) genes. The BAT3 protein complexes with a histone acetyltransferase, p300, which acetylates p53 protein in response to DNA damage, while MSH5 is involved in DNA mismatch repair. However, a recent pooled analysis by the International Lung Cancer Consortium failed to replicate the association of these SNPs with lung cancer risk (24). Our GWAS on Japanese lung ADC indicated that the HLA-DQA1 locus encoding a HLA (human leukocyte antigen)-class II protein (1 Mb proximal to the BAT3-MSH5 locus) is a significant region in 6p21. The *DQA1*03* allele of the *HLA-DQA1* gene was associated with an increased risk of the development of all major histological types of lung cancer (23) (Fig. 2D). It is possible that the *HLA-DQA1* polymorphism confers lung cancer susceptibility due to inter-individual

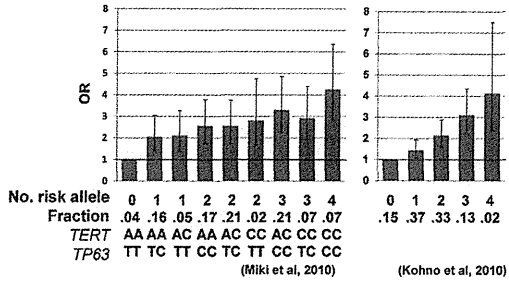

Fig. 3. Lung ADC risk of combined genotypes. Left: ORs of combined *TERT* (rs2736100) and *TP63* (rs10937405) genotypes. Right: ORs of combined *TERT* (rs2736100), *CHRNA3* (rs105173), and *HLA-DQA1* (*03) genotypes.

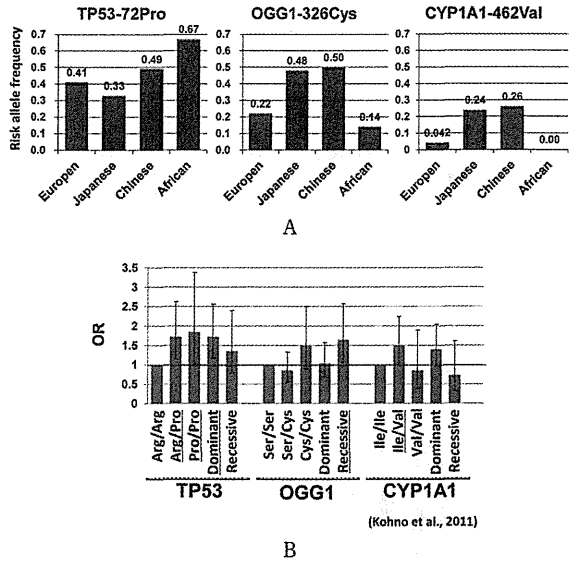

Fig. 4. Increased lung SQC risk with functional SNPs in the *TP53* (rs1042522), *OGG1* (rs1052133), and *CYP1A1* (rs1048943) genes. (A) Frequencies of risk alleles in each population determined by the HapMap project. (B) ORs of *TP53*, *OGG1*, and *CYP1A1* genotypes. *TP53-72Pro*, *OGG1-326Cys* and *CYP1A1-462Val* alleles were shown to be statistically significantly associated with lung SQC risk. Genotypes showing statistically significant association are underlined.

differences in the immune response against tumor cells. However, 6p21 is a highly polymorphic region containing major histocompatibility complex genes, therefore, the observed associations might have simply reflect differences in the population substructure (6). Further investigation of this region is warranted.

Lung ADC risk based on combined genotypes: Miki *et al.* and we estimated the risk of lung ADC caused by combined genotypes with multiple lung ADC susceptibility loci (7,23) (Fig. 3). These loci were suggested to independently confer risk, and carriers of all risk alleles, i.e., $<10\%$ of the population, had an odds ratio (OR) of >4.0 compared with those having no risk alleles. The results are significant when we consider that the OR of smoking for lung ADC risk is <2.0 in the Japanese population (28). The relative risk of carrying these variants should now be assessed in a cohort study to the relative ratios of these combined genotypes and to identify high risk individuals in near future.

Functional polymorphisms in DNA repair and metabolic genes: Studies of DNA adducts/damage, including that produced by tobacco carcinogens and their repair processes, have led to the identification of various metabolic and DNA repair genes with functional polymorphisms, which might possibly produce inter-individual differences in the rate of somatic mutation and the susceptibility to tobacco-related cancers (29). Representative SNPs in *TP53*, *OGG1*, and *CYP1A1* have been indicated to be associated with lung cancer risk for a long time (30–34) and the risk allele frequencies of those SNPs are shown to be different among ethnic populations. (Fig. 4A). The risk (72Pro) allele of the *TP53-Arg72Pro* SNP in the TP53 gene encodes a protein with a weaker apoptotic activity that better allows the survival of DNA-damaged cells compared with the 72Arg allele (35), while the risk (326Cys) allele of the Ser326Cys SNP in the *OGG1* gene encodes a DNA glycosylase with a weaker activity in repairing an oxidatively damaged promutagenic base produced by tobacco carcinogens, 8-hydroxyguanine, compared with the 326Ser allele (36,37). The risk (462Val) allele of the Ile462Val SNP in the *CYP1A1* gene encodes a metabolic protein with a higher activity in bio-activating the major tobacco procarcinogens, polycyclic aromatic hydrocarbons (PAHs), than the 462Ile allele (38). However, the associations of these functional polymorphisms were not investigated in previous GWASs due to the lack of probes for discriminating these polymorphisms in the platforms used by GWASs (http://www.ncbi.nlm.nih.gov/snp), so they remain unconfirmed. We previously demonstrated the association of these functional SNPs with lung SQC risk in a population where polymorphisms of the GWAS genes show associations (39). Genotypes for two DNA repair genes, *TP53* and *OGG1*, and a metabolic gene, *CYP1A1*, showed significant associations with SQC risk along with those for CHRNA3 and HLA-DQA1 (Fig. 4B). Based on these results, there is a need to analyze various functional polymorphisms together with millions of GWAS marker SNPs. This will provide a powerful method for analyzing these polymorphisms in populations that were tested in recent GWASs.

SNPs in DNA repair genes and therapeutic responses: Genetic polymorphisms underlie inter-individual differences in terms of susceptibility to disease and also the therapeutic response, although published association studies in this area lack sufficient case numbers (40). Agents that damage DNA or that disrupt

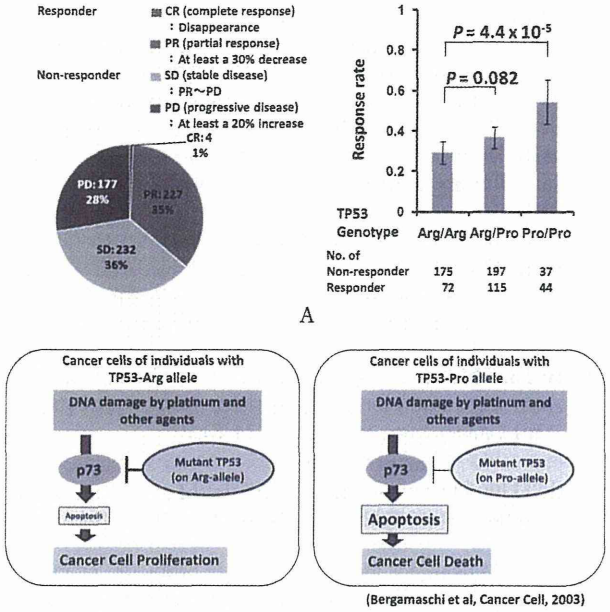

Fig. 5. Association of a *TP53* functional SNP (rs1042522) with the response to platinum-doublet therapy in 640 NSCLC patients. (A) Association result. The therapeutic response evaluated by the Response Evaluation Criteria in Solid Tumors (RECIST) was used as the primary end point of outcome to search for predictive factors. Patients were divided into two categories: responders were those with complete response and partial response, and non-responders were those with stable disease and progressive disease. ORs for the response (i.e., responder vs nonresponder) according to genotypes were calculated as a measure of difference in the response rate against therapy using an unconditional logistic regression analysis. The, the *TP53* SNP was defined as the one significantly associated with response. Left: Character of 640 NSCLC patients according to their response. Right: Difference in the response rate (i.e., fraction of responders) according to their *TP53* genotype. (B) Possible mechanism for the differential response. p73 is a p53-related protein that plays a role in apoptosis of cancer cells carrying *TP53* mutations via anticancer agents, although its function is abrogated by mutant p53 proteins. p53 mutants with a proline residue in codon 72 only weakly inhibit the function of p73 protein in NSCLC cells, so they efficiently induce the apoptosis of NSCLC cells treated with platinum and other anticancer agents.

chromosomal integrity are used in chemotherapy, so any variation in activities that repair DNA/chromosome damage might possibly influence the outcome for patients after chemotherapy (41). Thus, 640 patients with non-small-cell lung cancer (NSCLC) who received platinum-based doublet chemotherapy and whose responses were evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST) were evaluated in an association study to test for any link between their responses and the genotypes of 30 non-synonymous SNPs in 27 DNA repair genes (8). Homozygotes for the *TP53-72Pro* allele had a better response rate (54%) than those for the major allele *TP53-72Arg* (29%), irrespective of therapeutic regimens (Fig. 5A). The p53-72Arg protein has a greater activity in inducing apoptosis than the p53-72Pro protein, as described above (35), however, the opposite relationship was reported for mutant p53 proteins. p73 is a p53-related protein that plays a role in the apoptosis of cancer cells carrying *TP53* mutations via anticancer agents, and its function is abrogated by mutant p53 proteins. This abrogating activity is greater in mutant p53 proteins with an Arg residue at codon 72 compared with those with a Pro residue (42) and, therefore, the *TP53-72Pro* allele may lead to a better response to platinum-based doublet chemotherapy in NSCLC patients (Fig. 5B). This study indicates the potential utility of SNP as predictive markers for responses to chemotherapy.

Future Directions

Genetic factors affecting lung cancer risk have been identified by GWASs and other association studies. The results indicate that risk variants confer different magnitudes of increased risk in different populations for a variety of reasons, including differences in allele frequency and the genetic and environmental backgrounds that interact with the variants. Given the statistical power applied to the detection of associations in GWASs to date, there are unlikely to be many additional SNPs (tagged by commercially available DNA chips) with similar effects on alleles with high frequencies (>0.2) in populations of European ancestry (5,6). Thus, several different GWAS approaches are required to identify additional genetic factors underlying lung cancer risk. This should include GWASs of Asian populations. The fraction of lung ADC patients who are never-smokers and female is considerably higher in Asians than Europeans/Americans (43,44), suggesting that the former experience a greater or more distinct effect of genetic factors than the latter. Therefore, GWASs of Asians would be highly useful. In addition, GWASs focusing on specific lung cancer types would be worthwhile, because some genetic factors might be specifically associated with the risk of a specific type of lung cancer, such as SCLC, lung cancers in female never-smokers, or lung cancers with defined gene mutations. Lung ADC is known to develop via several carcinogenic pathways defined by oncogenic driver mutations in *EGFR*, *KRAS*, *HER2*, *ALK*, and *RET* genes, and the etiological factors are suggested to be different among those pathways (1,45,46). Finally, low frequency variants and common SNPs that have not been tagged by the DNA chips used in previous studies might also be involved in lung cancer risk (47,48). Thus, efforts to expand the scale of GWASs in terms of both sample size and SNP coverage, and to increase the number of SNPs taken forward to large-scale replication, may also lead to the identification of additional variants for lung cancer. Understanding the remaining genetic factors will help greatly in clarifying the disease etiology

and also in identifying high risk individuals for targeted screening and/or prevention. SNPs in DNA repair genes are associated with the response to platinum-doublet therapy. SNPs can be examined using blood cells and they may provide useful biomarkers in the clinical decision-making process for patients with advanced NSCLC who do not received surgery, which makes the molecular analysis of cancer cells difficult. No large-scale GWASs have been performed to investigate links with the response to lung cancer therapy or drug toxicities to the best of our knowledge, and these will also be useful for identifying biomarkers with clinical applications.

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References

1 Herbst RS, Heymach JV, Lippman SM. Lung cancer. N Engl J Med. 2008; 359: 1367–80.
2 Hung RJ, McKay JD, Gaborieau V, Boffetta P, Hashibe M, et al. A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. Nature, 2008; 452: 633–7.
3 McKay JD, Hung RJ, Gaborieau V, Boffetta P, Chabrier A, et al. Lung cancer susceptibility locus at 5p15.33. Nat Genet. 2008; 40: 1404–6.
4 Wang Y, Broderick P, Webb E, Wu X, Vijayakrishnan J, et al. Common 5p15.33 and 6p21.33 variants influence lung cancer risk. Nat Genet. 2008; 40: 1407–9.
5 Broderick P, Wang Y, Vijayakrishnan J, Matakidou A, Spitz MR, et al. Deciphering the impact of common genetic variation on lung cancer risk: a genome-wide association study. Cancer Res. 2009; 69: 6633–41.
6 Landi MT, Chatterjee N, Yu K, Goldin LR, Goldstein AM, Rotunno M, et al. A genome-wide association study of lung cancer identifies a region of chromosome 5p15 associated with risk for adenocarcinoma. Am J Hum Genet. 2009; 85: 679–91.
7 Miki D, Kubo M, Takahashi A, Yoon KA, Kim J, et al. Variation in TP63 is associated with lung adenocarcinoma susceptibility in Japanese and Korean populations. Nat Genet. 2010; 42: 893–6.
8 Shiraishi K, Kohno T, Tanai C, Goto Y, Kuchiba A, et al. Association of DNA repair gene polymorphisms with response to platinum-based doublet chemotherapy in patients with non-small-cell lung cancer. J Clin Oncol. 2010; 28: 4945–52.
9 Breslow NE, Day NE. Statistical methods in cancer research. Volume I—The analysis of case-control studies. IARC Sci Publ. 1980: 5–338.
10 Easton DF, Eeles RA. Genome-wide association studies in cancer. Hum Mol Genet. 2008; 17(R2): R109–15.
11 Amundadottir L, Kraft P, Stolzenberg-Solomon RZ, Fuchs CS, Petersen GM, et al. Genome-wide association study identifies variants in the ABO locus associated with susceptibility to pancreatic cancer. Nat Genet. 2009; 41: 986–90.
12 Cui R, Kamatani Y, Takahashi A, Usami M, Hosono N, et al. Functional variants in ADH1B and ALDH2 coupled with alcohol and smoking synergistically enhance esophageal cancer risk. Gastroenterology. 2009; 137: 1768–75.
13 Wolpin BM, Chan AT, Hartge P, Chanock SJ, Kraft P, et al. ABO blood group and the risk of pancreatic cancer. J Natl Cancer Inst. 2009; 101: 424–31.
14 Nakao M, Matsuo K, Hosono S, Ogata S, Ito H, et al. ABO blood group alleles and the risk of pancreatic cancer in a Japanese population. Cancer Sci. 2011; 102: 1076–80.
15 Oze I, Matsuo K, Hosono S, Ito H, Kawase T, et al. Comparison between self-reported facial flushing after alcohol consumption and ALDH2 Glu504Lys polymorphism for risk of upper aerodigestive tract cancer in a Japanese population. Cancer Sci. 2010; 101: 1875–80.
16 Minna JD. Nicotine exposure and bronchial epithelial cell nicotinic acetylcholine receptor expression in the pathogenesis of lung cancer. J Clin Invest. 2003; 111: 31–3.
17 Schuller HM. Nitrosamines as nicotinic receptor ligands. Life Sci. 2007; 80 (24–25): 2274–80.
18 Shiraishi K, Kohno T, Kunitoh H, Watanabe SI, Goto K, et al. Contribution of nicotine acetylcholine receptor polymorphisms to lung cancer risk in a smoking-independent manner in the Japanese. Carcinogenesis. 2009; 30: 65–70.
19 Wu C, Hu Z, Yu D, Huang L, Jin G, et al. Genetic variants on chromosome 15q25 associated with lung cancer risk in Chinese populations. Cancer Res. 2009; 69: 5065–72.
20 Thorgeirsson TE, Geller F, Sulem P, Rafnar T, Wiste A, et al. A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. Nature. 2008; 452: 638–42.
21 Baird DM. Variation at the TERT locus and predisposition for cancer. Expert Rev Mol Med. 2010; 12: e16.
22. Jin G, Xu L, Shu Y, Tian T, Liang J, et al. Common genetic variants on 5p15.33 contribute to risk of lung adenocarcinoma in a Chinese population. Carcinogenesis. 2009; 30: 987–90.
23 Kohno T, Kunitoh H, Shimada Y, Shiraishi K, Ishii Y, et al. Individuals susceptible to lung adenocarcinoma defined by combined HLA-DQA1 and TERT genotypes. Carcinogenesis. 2010; 31: 834–41.
24 Truong T, Hung RJ, Amos CI, Wu X, Bickeboller H, et al. Replication of lung cancer susceptibility loci at chromosomes 15q25, 5p15, and 6p21: a pooled analysis from the International Lung Cancer Consortium. J Natl Cancer Inst. 2010; 102: 959–71.
25 Rafnar T, Sulem P, Stacey SN, Geller F, Gudmundsson

J, Sigurdsson A, et al. Sequence variants at the TERT-CLPTM1L locus associate with many cancer types. Nat Genet. 2009; 41: 221–7.
26 Petitjean A, Hainaut P, Caron de Fromentel C. TP63 gene in stress response and carcinogenesis: a broader role than expected. Bull Cancer. 2006; 93: E126–35.
27 Wang Y, Broderick P, Matakidou A, Vijayakrishnan J, Eisen T, Houlston RS. Variation in TP63 is associated with lung adenocarcinoma in the UK population. Cancer Epidemiol Biomarkers Prev. 2011; 20: 1453–62.
28 Marugame T, Sobue T, Nakayama T, Suzuki T, Kuniyoshi H, et al. Filter cigarette smoking and lung cancer risk; a hospital-based case—control study in Japan. Br J Cancer. 2004; 90: 646–51.
29 Dong LM, Potter JD, White E, Ulrich CM, Cardon LR, Peters U. Genetic susceptibility to cancer: the role of polymorphisms in candidate genes. Jama. 2008; 299: 2423–36.
30 Sugimura H, Wakai K, Genka K, Nagura K, Igarashi H, et al. Association of Ile462Val (Exon 7) polymorphism of cytochrome P450 IA1 with lung cancer in the Asian population: further evidence from a case-control study in Okinawa. Cancer Epidemiol Biomarkers Prev. 1998; 7: 413–7.
31 Sugimura H, Kohno T, Wakai K, Nagura K, Genka K, et al. hOGG1 Ser326Cys polymorphism and lung cancer susceptibility. Cancer Epidemiol Biomarkers Prev. 1999; 8: 669–74.
32 Fan R, Wu MT, Miller D, Wain JC, Kelsey KT, et al. The p53 codon 72 polymorphism and lung cancer risk. Cancer Epidemiol Biomarkers Prev. 2000; 9: 1037–42.
33 Houlston RS. CYP1A1 polymorphisms and lung cancer risk: a meta-analysis. Pharmacogenetics. 2000; 10: 105–14.
34 Li H, Hao X, Zhang W, Wei Q, Chen K. The hOGG1 Ser326Cys polymorphism and lung cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev. 2008; 17: 1739–45.
35 Dumont P, Leu JI, Della Pietra AC, 3rd, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet. 2003; 33: 357–65.
36 Kohno T, Shinmura K, Tosaka M, Tani M, Kim SR, et al. Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. Oncogene. 1998; 16: 3219–25.
37 Yamane A, Kohno T, Ito K, Sunaga N, Aoki K, et al. Differential ability of polymorphic OGG1 proteins to suppress mutagenesis induced by 8-hydroxyguanine in human cell *in vivo*. Carcinogenesis 2004; 25: 1689–94.
38 Bartsch H, haugen A, Risch A, Shields P, Vineis P. Genetic susceptibility. In: Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC, editors. World health organization classification of tumors: Pathology and genetics, tumours of lung, pleura, thymus and heart. Lyon: IARC Press; 2004. p. 24–25.
39 Kohno T, Kunitoh H, Mimaki S, Shiraishi K, Kuchiba A, Yamamoto S, et al. Contribution of the TP53, OGG1, CHRNA3, and HLA-DQA1 genes to the risk for lung squamous cell carcinoma. J Thorac Oncol. 2011; 6: 813–7.
40 Camps C, Sirera R, Iranzo V, Taron M, Rosell R. Gene expression and polymorphisms of DNA repair enzymes: cancer susceptibility and response to chemotherapy. Clin Lung Cancer. 2007; 8: 369–75.
41 Olaussen KA, Dunant A, Fouret P, Brambilla E, Andre F, et al. DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med. 2006; 355: 983–91.
42 Bergamaschi D, Gasco M, Hiller L, Sullivan A, Syed N, et al. p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. Cancer Cell. 2003; 3: 387–402.
43 Subramanian J, Govindan R. Lung cancer in never smokers: a review. J Clin Oncol. 2007; 25: 561–70.
44 Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers--a different disease. Nat Rev Cancer. 2007; 7: 778–90.
45 Lovly CM, Carbone DP. Lung cancer in 2010: One size does not fit all. Nat Rev Clin Oncol. 2011; 8: 68–70.
46 Kohno T, Ichikawa H, Totoki Y, Yasuda K, Hiramoto M, Nammo T, et al. KIF5B-RET fusions in lung adenocarcinoma. Nat Med. 2012; 18: 375–7.
47 Bodmer W, Bonilla C. Common and rare variants in multifactorial susceptibility to common diseases. Nat Genet. 2008; 40: 695–701.
48 Cirulli ET, Goldstein DB. Uncovering the roles of rare variants in common disease through whole-genome sequencing. Nat Rev Genet. 2010; 11: 415–25.

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KIF5B-RET fusions in lung adenocarcinoma

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We identified in-frame fusion transcripts of *KIF5B* (the kinesin family 5B gene) and the *RET* oncogene, which are present in 1–2% of lung adenocarcinomas (LADCs) from people from Japan and the United States, using whole-transcriptome sequencing. The *KIF5B-RET* fusion leads to aberrant activation of RET kinase and is considered to be a new driver mutation of LADC because it segregates from mutations or fusions in *EGFR*, *KRAS*, *HER2* and *ALK*, and a RET tyrosine kinase inhibitor, vandetanib, suppresses the fusion-induced anchorage-independent growth activity of NIH3T3 cells.

A considerable proportion of LADCs, the most common histological type of lung cancer that comprises ~40% of the total cases, develops through activation of oncogenes, for example, somatic mutations in *EGFR* (10–50% of cases) or *KRAS* (10–30% of cases) or fusion of *ALK* (5% of cases), in a mutually exclusive manner[1–4]. Tyrosine kinase inhibitors (TKIs) targeting the EGFR and ALK proteins are effective in the treatment of LADCs that carry *EGFR* mutations and *ALK* fusions[1–3], respectively.

We performed whole-transcriptome sequencing (RNA sequencing)[5] of 30 LADC specimens from Japanese individuals to identify new chimeric fusion transcripts that could be targets for therapy[3,5,6]. These LADCs were 2 carcinomas with *EML4-ALK* fusions, 4 with *EGFR* or *KRAS* mutations and 24 without these fusions or mutations (**Supplementary Table 1**). Identifying candidate fusions represented by >20 paired-end reads and validation by Sanger sequencing of the RT-PCR products (**Supplementary Methods**) led to the identification of seven fusion transcripts, including *EML4-ALK* (**Supplementary Table 1**). We detected one of these fusions between *KIF5B* on chromosome 10p11.2 and *RET* on chromosome 10q11.2 in subject BR0020 (**Fig. 1** and **Supplementary Fig. 1a**). We then further investigated this fusion, as fusions between *RET* and genes other than *KIF5B* have previously been shown to drive papillary thyroid tumor formation[6,7].

RT-PCR and a Sanger sequencing analysis of 319 LADC specimens from Japanese individuals (**Supplementary Table 2**), including 30 that had been subjected to whole-transcriptome sequencing, revealed that 1.9% (6 out of 319) expressed *KIF5B-RET* fusion transcripts (**Fig. 1b** and **Supplementary Fig. 1b**). We identified four variants in these six tumors, and all of these variants were in frame (**Fig. 1a**).

A genomic PCR analysis of the six tumors that were positive for RET fusions revealed somatic fusions of the *KIF5B* introns 15, 16, 23 or 24 at chromosome 10p11.2 with the *RET* introns 7 or 11 at 10q11.2 (**Supplementary Fig. 1c,d**), indicating that a chromosomal inversion had occurred between the long and short arms in the centromeric region of chromosome 10 (**Supplementary Figs. 1e** and **2**). We verified this chromosomal inversion using fluorescence *in situ* hybridization, which revealed a split in the signals for the probes that flank the *RET* translocation sites in tumors positive for the *KIF5B-RET* fusion (**Supplementary Fig. 2**).

The tumors positive for the *KIF5B-RET* fusion were all well or moderately differentiated (**Table 1** and **Supplementary Fig. 3**). None of the subjects with these tumors had a history of thyroid cancer, and none showed abnormal findings in their thyroid tissues as determined by computed tomography or positron emission tomography before surgery for LADC. All five examined tumors with the *KIF5B-RET* fusion were positive for thyroid transcription factor 1 (TTF-1) and napsin A aspartic proteinase (Napsin A)[8] but were negative for thyroglobulin[9], indicating that they were of pulmonary origin (**Table 1** and **Supplementary Fig. 3**). The LADCs that were positive for the *KIF5B-RET* fusion showed twofold to 30-fold higher *RET* expression than non-cancerous lung tissues (**Fig. 1b** and **Supplementary Figs. 4** and **5**). An immunohistochemical analysis using an antibody against the C-terminal region of the RET protein detected positive cytoplasmic staining in the tumor cells of the fusion-positive LADCs (**Table 1** and **Supplementary Fig. 3b**) but did not detect this staining in any of the non-cancerous lung cells. A western blot analysis confirmed the expression of the fusion proteins in the LADCs (**Supplementary Fig. 6**).

To address the prevalence of *KIF5B-RET* fusions in LADCs from individuals of non-Asian ancestry, we examined LADCs in cohorts from the United States and Norway (**Supplementary Table 2**). We detected a fusion transcript in 1 of the 80 (1.3%) subjects from the

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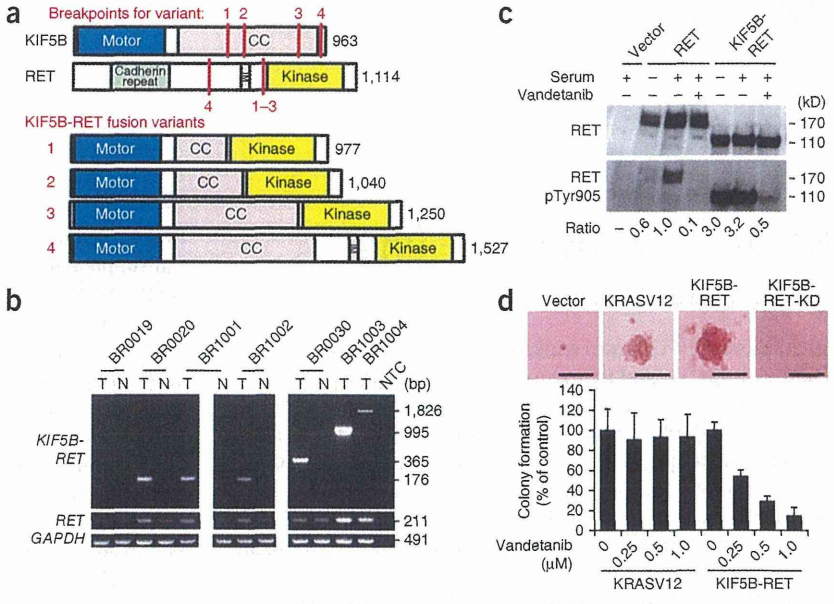

Figure 1 *KIF5B-RET* fusions in LADC. (**a**) Schematic representations of the wild-type KIF5B and RET proteins as well as the four fusion variants identified in this study. The breakpoints for each variant are indicated with red lines. CC, coiled coil; TM, transmembrane. (**b**) Detection of *KIF5B-RET* fusions by RT-PCR. RT-PCR products for the *RET* kinase domain (exons 12 and 13) and *GAPDH* are shown below. Six LADCs positive for *KIF5B-RET* fusions (T) are shown, with four corresponding non-cancerous lung tissues (N), a no-template control (NTC) and one LADC that was negative for the fusion (BR0019). (**c**) Activation of RET kinase activity in the KIF5B-RET protein and the suppression of this activity by vandetanib. H1299 lung cancer cells were transfected with an empty vector, wild-type RET (RET) or KIF5B-RET expression plasmids and treated either with DMSO (serum) or vandetanib, as indicated. The ratios of phosphorylated Tyr905 (pTyr905) RET to total RET signals with respect to wild-type RET after the serum treatment are listed below the gels. (**d**) Anchorage-independent growth of NIH3T3 cells expressing KIF5B-RET protein and the suppression of this growth by vandetanib. Representative pictures of colonies without vandetanib treatment (top). Scale bars, 50 μm. Bar graph showing the percentage (± s.d.) of colonies formed after treatment with the indicated amounts of vandetanib (average results of three independent experiments) with respect to those formed by DMSO-treated cells. The study was approved by the institutional review boards of institutions participating in this study.

United States (an individual of European ancestry) (**Supplementary Fig. 7**), but we detected no fusion transcripts in the 34 subjects from Norway (**Supplementary Table 3**); *KIF5B-RET* fusions occurred in 1–2% of LADCs in both Asians and non-Asians. The individual from the United States with the *RET* fusion was classified as an 'ever smoker', whereas the six individuals from Japan with the *RET* fusion were 'never smokers' (**Table 1**). Therefore, prevalence of LADC with regard to smoking status is unclear. We did not detect the *KIF5B-RET* fusion in other major subtypes of lung cancer, including 234 squamous-cell, 17 large-cell and 20 small-cell lung carcinomas (**Supplementary Table 3**). The fusion was also not present in other types of adenocarcinomas, including those of the ovary ($n = 100$) and colon ($n = 200$) (data not shown), suggesting that it is specific to LADC.

All seven subjects with LADC harboring the *KIF5B-RET* fusion were negative for *EGFR, KRAS* and *ALK* mutations or fusions and were negative for mutations in *HER2*, which is an additional driver mutation in LADC[10] (**Table 1** and **Supplementary Table 4**). The mutually exclusive nature of the *RET* fusions and other oncogenic alterations[1,2,11] suggests that the *KIF5B-RET* fusion is a driver mutation. All proteins encoded by the four *KIF5B-RET* fusion variants contained the KIF5B coiled-coil domain, which functions in protein dimerization[12], and retained the full RET kinase domain, similar to other types of oncogenic *RET* fusions observed in thyroid tumors (**Fig. 1a**)[13]. The KIF5B-RET proteins are likely to form a homodimer through the coiled-coil domain of KIF5B, causing an aberrant activation of the kinase function of RET in a manner similar to the *PTC-RET* and *KIF5B-ALK* fusions[7,14]. In fact, the N-terminal portion of the KIF5B coiled-coil region, which is retained in all variants, has been predicted to have the ability to dimerize through two coiled-coil structures[15]. Consistently, when the *KIF5B-RET* variant 1 was exogenously expressed in H1299 human lung cancer cells without wild-type or fusion *RET* expression, Tyr905, which is located in the activation loop of the RET kinase site[15,16], was phosphorylated in the absence of serum stimulation, indicating an aberrant activation of *RET* kinase[16,17] by fusion with *KIF5B* (**Fig. 1c**). This phosphorylation was suppressed by vandetanib, a TKI against RET (as well as other tyrosine kinases, including EGFR and VEGFR)[18] (**Fig. 1c** and **Supplementary Fig. 8**).

Expression of exogenous *KIF5B-RET*, but not *KIF5B-RET*-KD (a kinase-dead mutant corresponding to S765P in wild-type RET[17]), induced morphological transformation (**Supplementary Fig. 9**) and anchorage-independent growth of NIH3T3 fibroblasts in a way that was analogous to the induction caused by mutant *KRAS* (KRASV12) (**Fig. 1d**). Consistently, phosphorylation of Tyr905 was higher in the KIF5B-RET

Table 1 Characteristics of lung adenocarcinomas with the *KIF5B-RET* fusion

Sample	Country	Sex	Age[a]	Smoking	*KIF5B-RET* fusion[b]	Pathological stage	Pathological findings	RET staining	TTF-1 staining	Napsin A staining	Thyroglobulin staining
BR0020	Japan	Male	57	Never	K15; R12 (variant 1)	IIB	Moderately differentiated ADC	+	+	+	–
BR1001	Japan	Female	65	Never	K15; R12 (variant 1)	IB	Well differentiated ADC	+	+	+	–
BR1002	Japan	Female	64	Never	K15; R12 (variant 1)	IB	Well differentiated ADC	+	+	+	–
BR0030	Japan	Male	57	Never	K16; R12 (variant 2)	IA	Well differentiated ADC	+	+	+	–
BR1003	Japan	Male	28	Never	K23; R12 (variant 3)	IA	Well differentiated ADC	+	+	+	–
BR1004	Japan	Female	71	Never	K24; R8 (variant 4)	IA	Moderately differentiated ADC	NT	NT	NT	NT
NCI1580	USA	Male	63	Ever[c]	K15; R12 (variant 1)	II	Moderately differentiated ADC	NT	NT	NT	NT

[a]Age in years. [b]Fused exon numbers of *KIF5B* (K) and *RET* (R); and variant types (in parentheses) are shown. None of the subjects had oncogenic *EGFR*, *KRAS*, *HER2* or *ALK* mutations or fusions. [c]The number of pack years smoked for this subject is not known. NT, not tested.

protein than in the KIF5B-RET-KD protein. The anchorage-independent growth induced by *KIF5B-RET* was suppressed by treatment with vandetanib (<1 μM), whereas the growth induced by mutant *KRAS* was not (**Fig. 1d**). These results are similar to those observed for *RET* fusions in thyroid cancer[19]. We also detected phosphorylation of the KIF5B-RET protein at Tyr905 in fusion-positive LADC specimens (**Supplementary Fig. 6**). These results suggest that the *RET* fusions are a previously unidentified LADC driver mutation and a potential target for existing TKIs, including vandetanib, which has been recently approved by the US Food and Drug Administration for the treatment of thyroid cancer[18]. Further studies are warranted to promote molecular subtype diagnoses and personalized therapy options for LADC. For this purpose, both the clinical and biological features of this fusion are being investigated. For further information, please see the **Supplementary Note** and **Supplementary Tables 5** and **6**.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

RNA sequencing: H.I., K.Y., M.H., T.N. and H.S. Sequence data processing: Y.T., S.C. and I.Y. Molecular biological analyses: T.K., Y.S., R.I., H. Ogiwara, T.O., M.E., A.J.S., H. Okayama, A.H., Y.A. and S.O. Clinical and pathological analyses: K.T., K.F., V.S., S.W., I.S. and H.T. Manuscript writing: T.K., H.I. and T.S. Study design: T.K., H.I., C.C.H., T.Y., J.Y. and T.S.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Janku, F., Stewart, D.J. & Kurzrock, R. *Nat. Rev. Clin. Oncol.* **7**, 401–414 (2010).
2. Gerber, D.E. & Minna, J.D. *Cancer Cell* **18**, 548–551 (2010).
3. Lovly, C.M. & Carbone, D.P. *Nat. Rev. Clin. Oncol.* **8**, 68–70 (2011).
4. Soda, M. *et al. Nature* **448**, 561–566 (2007).
5. Meyerson, M., Gabriel, S. & Getz, G. *Nat. Rev. Genet.* **11**, 685–696 (2010).
6. Mani, R.S. & Chinnaiyan, A.M. *Nat. Rev. Genet.* **11**, 819–829 (2010).
7. Wells, S.A. Jr. & Santoro, M. *Clin. Cancer Res.* **15**, 7119–7123 (2009).
8. Bishop, J.A., Sharma, R. & Illei, P.B. *Hum. Pathol.* **41**, 20–25 (2010).
9. DeLellis, R.A., Shin, S.J. & Treaba, D.O. *Immunohistology of Endocrine Tumors* (Saunders, Philadelphia, Pennsylvania, USA, 2010).
10. Shigematsu, H. *et al. Cancer Res.* **65**, 1642–1646 (2005).
11. Herbst, R.S., Heymach, J.V. & Lippman, S.M. *N. Engl. J. Med.* **359**, 1367–1380 (2008).
12. Hirokawa, N., Noda, Y., Tanaka, Y. & Niwa, S. *Nat. Rev. Mol. Cell Biol.* **10**, 682–696 (2009).
13. Jhiang, S.M. *Oncogene* **19**, 5590–5597 (2000).
14. Takeuchi, K. *et al. Clin. Cancer Res.* **15**, 3143–3149 (2009).
15. Morii, H., Takenawa, T., Arisaka, F. & Shimizu, T. *Biochemistry* **36**, 1933–1942 (1997).
16. Vitagliano, D. *et al. Endocr. Relat. Cancer* **18**, 1–11 (2011).
17. Croyle, M. *et al. Cancer Res.* **68**, 4183–4191 (2008).
18. Commander, H., Whiteside, G. & Perry, C. *Drugs* **71**, 1355–1365 (2011).
19. Carlomagno, F. *et al. Cancer Res.* **62**, 7284–7290 (2002).

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特 集 家族性腫瘍に関する新知見

家族性胃癌

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Familial Gastric Cancer—An Update of Japanese Cases: Haruhiko Sugimura[*1], Hidetaka Yamada[*1], Hong Tao[*1], Kazuya Shinmura[*1], Moriya Iwaizumi[*2] and Masako Kasami[*3] ([*1]*Dept. of Tumor Pathology, and* [*2]*Dept. of Molecular Diagnostics, Hamamatsu University School of Medicine,* [*3]*Division of Laboratory Medicine, Iwata City Hospital*)

Summary

Since the international gastric cancer linkage consortium first proposed screening criteria for the detection of *CDH1* germline mutations in hereditary diffuse gastric cancer (HDGC), the low yields of previous attempts to identify patients with HDGC in Japan, where gastric cancer is endemic and mass screenings for it have been established, have made clinicians less enthusiastic about pursuing the genetic etiology of the peculiar occurrence of gastric cancer. A report published in 2011 described a case with a typical truncated mutation of *CDH1* and another with an exon 3 deletion of this gene. These findings have rekindled the curiosity of practicians and pathologists confronted with unusual gastric cancers of various types such as younger-onset, familial clustering, or the exhibition of a specific characteristic morphology. The status and history of the investigation of the genetic backgrounds of Japanese gastric cancers are reviewed, and the pathological features of the Japanese cases of HDGC are described. **Key words**: Hereditary diffuse gastric cancer, *CDH1*, Molecular epidemiology, Signet ring cell carcinoma, **Corresponding author**: Haruhiko Sugimura, Department of Tumor Pathology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, Shizuoka 431-3192, Japan

要旨 胃癌の遺伝連鎖解析国際コンソーシアムが，遺伝性びまん性胃癌（HDGC）の *CDH1* のスクリーニングをするための診断基準を提案して以来，胃癌の頻度が高く，また集団検診も確立されている本邦での探索は，実り多かったとはいえず，そのことが日常診療で胃癌の遺伝的要因への追求があまりなされなくなった理由なのではと思われる。2011年の，典型的な *CDH1* の欠損蛋白を生じるタイプの変異例とさらに *CDH1* のエクソン3の欠失例が本邦にも存在したという報告は，改めて，日常診療の上で若年発症，家族性発症，特異な組織像といったまれな胃癌例に遭遇する実地診療家や病理医の胃癌の遺伝的要因についての関心を呼び起こすと思われる。本邦における胃癌の遺伝的要因の歴史を概観し，本邦のHDGCの組織像を呈示する。

はじめに

胃癌研究会という，現在の日本胃癌学会の前身の組織が，当番世話人の浜松医科大学 故喜納勇教授（当時）によって開催され，家族集積性胃癌をテーマとして取り上げたのは1994年のことである。当時，遺伝性の大腸の原因遺伝子が続々と明らかになっているころであり，胃癌においても似た現象があるのであろうという着想であった。一方，連鎖解析をするほどの大家系などは，恐らく見つからないであろうという予想があり，集めてどうするのだという計画はあまり明確ではなかった。残念なことにそのプロジェクトは，喜納勇教授が急死されたこともあり，いったん止まったような形になったが，1998年に胃癌を多数発生するマオリ族の大家系がGuilfordらにより解析され，当時でも日本人の研究者にたいへんなじみのあったE-カドヘリン（CDH1）の生殖細胞系列の変異が見つかった[1]。現在では遺伝性びまん性胃癌 hereditary diffuse gastric carcinoma（HDGC）といわれる

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この疾患は大いに話題になった。すぐさま，この遺伝子*CDH1*の変異や遺伝性非ポリポーシス大腸癌（HNPCC）（本誌冨田の項参照）で重要となるreplication error（RER，現在はmicrosatellite instability，MSIという言葉のほうがよく使われる）などの指標が，当時国立がんセンターのYokota，Shinmuraらによって検索され，その結果は本邦の家族性胃癌についての決定版としてよく引用される[2]。家族性胃癌の原因には，p53[3]やSTK11[4]などもあり，また，分化型の胃癌の家族集積性も別個に議論されるが最近の総説に詳しい[5]ので，ここでは触れず，本稿では主にHDGCについて述べる。

過去を振り返る際に重要なのは，遺伝子検索の方法論，特にコストの変化である。家族歴からみて絶対に疑わしいという症例を選んで，少ない労力で有効な情報を返すというのは当然であり，あるいは対象集団での実態を把握することも重要である。本邦や韓国，ポルトガルのようにもともと胃癌頻度の高いところでは，頻度の低いところの家族集積と同じ基準を適用しても多くの非遺伝性の家族集積例が入ってしまい，遺伝子を原因として求める努力は徒労に終わる可能性もある。特に分化型の胃癌の家族集積についてはこれらの基準を胃癌低頻度国と胃癌高頻度国とで異なったものが提唱されている[6]。パーソナルゲノムの時代からは実感がわかないが，*CDH1*の遺伝子変化についてもまず，single strand conformation polymorphism（SSCP）というスクリーニング用の優れた方法でふるいに掛けてから塩基配列を決めるという方法が採用され，1999～2004年辺りまで，International Gastric Cancer Linkage Consortiumもどちらかというとやみくもな遺伝子検索をしないように種々の診断基準を呈示している[7,8]。時代とともに，あるいは症例が増えるたびに関連病歴の存在，たとえば乳腺の小葉癌や，大腸の印環細胞癌，前立腺癌といったものなども含めてきている。さらに，家族歴のないものなども報告されるようになっている[9]。

現時点で，ちょっと変な腫瘍があれば，体細胞にせよ生殖系列の細胞にせよ，そのゲノムの全ゲノムを解析するということは，少なくとも一般病院ではなされていない。多くの全ゲノム解析をしている先端的施設の研究者も，そのoutputを解析するバイオインフォマティシャンの必要性などいわゆるbig dataを扱う困難さ，あるいはコストについて極めて慎重なものいいをする場合が多い。特に大量の結果のうち真に患者の病態に関連するものはどれか，関連しないけれど深刻なものはどれか，そもそも全ゲノム解析の同意というのは，子孫の同意を取れないこともあり，絶対的なものではないのでどう対処すればよいのかといった，倫理的な問題への対処も必要となる。倫理委員会（Institutional Review Board，IRB）の対応も施設によって異なるようである。一方で，本邦の胃癌の診療レベルを考えると，明らかに遺伝的要因がある胃癌家系では予防的胃切除も含めたactionableな領域であり，手を拱いているだけだと将来不作為を問われかねない。

Ⅰ．診断基準の変化

2010年のupdate[10]では，1999年の診断基準をやや緩和することを勧めている。びまん型の胃癌に遭遇したら，①50歳以下のびまん性胃癌を含む2名以上の胃癌が家族にいるか，②年齢にかかわらず，3名以上のびまん性胃癌が1，2親等以内にいるか（法律的な用語と多少異なる），③家族歴にかかわらず40歳以下であるか，④本人あるいは家族にびまん性胃癌と小葉性乳癌があり少なくとも一方が50歳未満で診断されているものであるかである。

この四つの条件のどれかが当てはまったら，*CDH1*の遺伝子検査（後述のmultiplex ligation dependent probe amplification，MLPAを含む）を受ける同意を取り，予防的胃切除を含むフォローアップ体制に入るといった図が書かれている。上部消化管のサーベイランスのシステムの成否は保険診療制度も含めた内視鏡検査の普及度，場合によっては技量なども関係してくると思われる。予防的胃切除などは，いくら手術がうまい本邦の外科医でも慎重であるし，恐らくは内視鏡医の眼力も違うので，癌の存在を確かめてからになるのではと思う。最近，予防的胃切除例での粘膜内病変でのmappingが発表されていて[11]，内視鏡での検索の精度を上げるための基礎データという視点でまとめられている。

ここに述べた条件は，それまでの報告例で，従来の基準を満たさなくても*CDH1*の変異が見つかった例の臨床型などを徐々に含めていったものがあり，恐らくはまた変わるであろう。注目すべきは孤発でも40歳以下なら検索すべしという条件である。本誌にもあるLi-Fraumeni症候群あるいは生殖細胞系列のp53の変異などでも*de novo*の変化はあり[12]，すると*CDH1*の遺伝子解析の対象範囲は相当増えるだろう。胃癌の年齢分布を厚生労働省の統計でみても，また日常診療においても40歳以下の胃癌というのは著しくまれという印象をもっていない。“スキルス胃癌は若い女性に多い”などという，いい方は今でも多くのメディアやネット上で見受けられる。また，胃の疾患は症状の感じ方が主観的で，見つかるのが遅いと40代をわずかに超えることもあろう。そうなると40代前半くらいの，未分化系胃癌が全部対象になってしまう。

Ⅱ．方法論の変化

初期には，組織像の他に，とにかく家族歴と発症年齢が重要でスクリーニング方法としてSSCPが推奨されていたが，直接に全エクソンの塩基配列を決めることがコスト的にあるいは技術的にも容易になり，普及しだした。さらに，MLPAやcomparative genomic hybridization（CGH）で，大きなゲノムの再構成をみることを推奨している。方法論は，当然これから数年で大きく変わることはほぼ確実であるし，それによって*CDH1*以外の遺伝子の異常が明らかになる可能性がある。ゲノムの再構成などはサイズによっては現在普通にいわれている次世代シークエンスでは見つけるのが苦手な場合もあるという。次次世代といわれるようなあるいはさらに優れたシステムが登場した場合はその扱いも可能になるであろう。遺伝子検索への技術的，経済的障壁は今後ますます低くなっていくが，現在重要なのは，将来の遺伝子検査像も考えた上での同意の取り方，説明の仕方，検体の保存や将来の使用についての理解，データの扱いや情報の公開・共有と個人情報の秘密保持についての留意であると思う。全ゲノム解析をするとか，情報をweb上かつ研究者間で国際的に共有するといった内容は，深く理解した上での同意というのは一般の方にとっては現実的ではないのではないかという見方すらある。全ゲノム解析という言葉を含んだ同意書などが推奨されている。

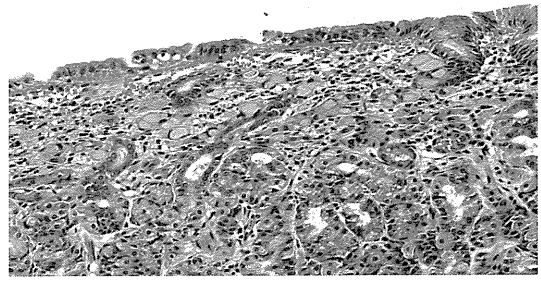

図1 粘膜内の印環細胞癌

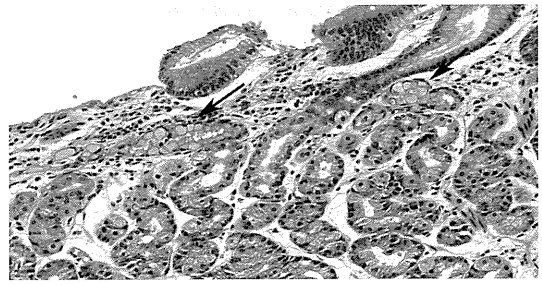

図2 基底膜内の上皮内印環細胞癌 signet ring cell carcinoma *in situ*（矢印）

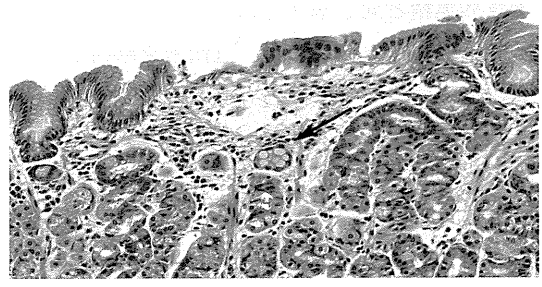

図3 基底膜内の上皮内印環細胞癌 signet ring cell carcinoma *in situ*（矢印）

Ⅲ．病理像

本邦の胃癌の病理については，症例数の多さと，胃の造影や胃カメラの発達と並行して発展してきた微小病変や，いわゆる早期病変の認識により，発表しているかどうかはさておき，蓄積されている知見，各医師の経験知は極めて多い。胃の早期病変の記述に始まる本邦胃癌の病理の研究は，術後胃を広範かつ詳細に検索する，つまり多数の切片をくまなく切りだし，具に検鏡するという極めてlabor intensiveな作業を伴うもので，この作業は胃癌の手術が行われていれば，通常の病理医の勤務する津々浦々のどんな病院でもroutine化する事態にまで発展した。その間，*Helicobacter*に類する細菌も“みていた”し，もちろんHDGCも“みていた”と思われる。遺伝的にHDGCと診断され，予防的胃切除を行われた検体が，病理学的に検索されるようになり，それらの報告をみると，本邦の胃癌取扱い規約の推奨する方法に近いが（向きは垂直であるが），多数の切片を切りだし，大弯びらきをした胃粘膜の図に癌の部位や深さをplotするという形で発表されている。これらのなかで，最も特徴的なのは印環細胞癌の巣が多発しているということである。いわゆるF lineに最も多いという論文もあるが，最近のものをみると分布はあまり特徴的ではない。比較的近位にplotされている。このような分布を前提とすると内視鏡でどこを探して取ってくるかというためには貴重な情報であろう。

自験例[13]は，長子が20代かつ胃癌で亡くなり，軽い胃の不調でも心配で来院した20代だが，すぐに内視鏡で（取り立てて，意識せず普通の所作で）印環細胞癌がでて手術となっている。遺伝子解析はその後に行われているので，米国の情況とはだいぶ異なる。ただ，国際的にはこのように内視鏡医の技量頼みというわけにいかないと思う。

前述のように，切除胃から多数切片を切りだすのは日本の病理医のお家芸であるから，複数の未分化系胃癌がみられた症例の報告はHDGCの報告以前からあるし，また，病理医の記憶にとどまっている。未発表の例も含めこれまで検索したかぎりでは本邦のHDGC例の組織像はほぼ，欧米からの報告と同様である。非常にaggressiveな組織像が死亡例などでみられる場合と，多発

病変がほとんど早期にとどまっているような場合とがあるが，それが発見時期のためなのか，*CDH1* の変異の種類によるのかは不明である。自験例の主たる粘膜内の腫瘍病変（図1），signet ring cell carcinoma *in situ* のようにみえる病変2か所（図2, 3）を呈示する。

今後，逆に組織像から遺伝的素因を疑わせるような特徴が記載されるであろう。なにせ，こんな病変は非特異的にみられると思っていても実際の病理診断の現場で遺伝子解析付きの検体をみているわけではない。かつて恩師の喜納勇教授は胃炎や腸上皮化生は日本人の胃ほとんどすべてにみられるので，重大な疾患とは考えていなかったが，北欧のヒトの胃粘膜をみて驚愕したと語っておられた。もちろん逆も真で，北欧の病理学者は日本人の胃粘膜は病気の宝庫であり，疾患研究の対象に大いになり得たのである。HDGC 例あるいは *CDH1* 変異キャリアーの胃粘膜の病理組織像をみても，その著者の恐らくは何千倍もの数のプレパラートをみているはずの本邦の病理医は，私も含め本当にこれが特異的な所見なのだろうかと疑い深くなっている。この意味で，今後，遺伝子型がわかった症例の胃粘膜の所見を詳細に観察する機会が増すことで，目から鱗が落ちるような体験をすることができるのではないかと楽しみである。

Ⅳ．ミスセンス変異の意義

CDH1 の機能解析は特にミスセンス変異の場合，その病的意義を主張するのには（あるいは論文にするには）機能的解析が必須であるとされる。collagen assay や invasion assay が推奨されている。もっとも，*in vitro* の人工的な環境下ばかりでなく，さらにヒト集団対照群（この定義がまた問題であるが）での頻度を確認することが重要かと思われる。近年，TaqMan[TR]として流通している定量的 PCR によって allele を区別する方法があり，ミスセンス変異の検体や，あるいは人工的に合成した鋳型を用いると数百の genotyping が短期間にでき，症例対照研究やコホート研究レベルの検体数について検討可能だという（山田英孝，私信）。今までの報告をまとめた論文などの表のなかのミスセンス変異の意義も今後さらに明らかになっていくと思われる。

おわりに

当教室では，喜納勇教授の時代に始まり，新村，山田と長年にわたり探索を続けている。労力の割になかなか典型的な HDGC が現れてくれなかったが，いったん症例が現れると，散発的ではあるが他施設からの問い合わせもある。未発表データなので断言はできないが，無視できない頻度で本邦にもあるのではと思われる。特に，詳細な胃癌全割標本の所見から想起するといった，現場の臨床医や病理医の興味が高まっている印象を受けている。

遺伝的要因の関係するかもしれない胃癌については，HDGC 以外にも多々ある。本邦では報告がまったくないわけではないが，あまりよくわかっていないというレベルの，本邦ではまだ，幻の遺伝性消化管腫瘍には MUTYH associated polyposis とか，過形成ポリポーシスを伴う遺伝性疾患などがあるが徐々に明らかになっていくと思われる。病理からみても genotype を伴った phenotype の解析が，われわれの目自体も進歩させていくのではないかと思われる。

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文　献

1) Guilford P, Hopkins J, Harraway J, *et al*: E-cadherin germline mutations in familial gastric cancer. *Nature* **392**(6674): 402-405, 1998.
2) Shinmura K, Kohno T, Takahashi M, *et al*: Familial gastric cancer: clinicopathological characteristics, RER phenotype and germline p53 and E-cadherin mutations. *Carcinogenesis* **20**(6): 1127-1131, 1999.
3) Yamada H, Shinmura K, Okudela K, *et al*: Identification and characterization of a novel germ line p53 mutation in familial gastric cancer in the Japanese population. *Carcinogenesis* **28**(9): 2013-2018, 2007.
4) Shinmura K, Goto M, Tao H, *et al*: A novel STK11 germline mutation in two siblings with Peutz-Jeghers syndrome complicated by primary gastric cancer. *Clin Genet* **67**(1): 81-86, 2005.
5) Kluijt I, Sijmons RH, Hoogerbrugge N, *et al*: Familial gastric cancer: guidelines for diagnosis, treatment and periodic surveillance. *Fam Cancer* **11**(3): 363-369, 2012.
6) Park JG, Yang HK, Kim WH, *et al*: Report on the first meeting of the International Collaborative Group on Hereditary Gastric Cancer. *J Natl Cancer Inst* **92**(21): 1781-1782, 2000.
7) Caldas C, Carneiro F, Lynch HT, *et al*: Familial gastric cancer: overview and guidelines for management. *J Med Genet* **36**(12): 873-880, 1999.
8) Brooks-Wilson AR, Kaurah P, Suriano G, *et al*: Germline E-cadherin mutations in hereditary diffuse gastric cancer: assessment of 42 new families and review of genetic screening criteria. *J Med Genet* **41**(7): 508-517, 2004.
9) Shah MA, Salo-Mullen E, Stadler Z, *et al*: De novo CDH1 mutation in a family presenting with early-onset diffuse gastric cancer. *Clin Genet* **82**(3): 283-287, 2012.
10) Fitzgerald RC, Hardwick R, Huntsman D, *et al*: Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research. *J Med Genet* **47**(7): 436-444, 2010.
11) Fujita H, Lennerz JK, Chung DC, *et al*: Endoscopic sur-

veillance of patients with hereditary diffuse gastric cancer: biopsy recommendations after topographic distribution of cancer foci in a series of 10 CDH1-mutated gastrectomies. *Am J Surg Pathol* **36**(11): 1709-1717, 2012.
12) Yamada H, Shinmura K, Yamamura Y, *et al*: Identification and characterization of a novel germline p53 mutation in a patient with glioblastoma and colon cancer. *Int J Cancer* **125**(4): 973-976, 2009.
13) Yamada H, Shinmura K, Ito H, *et al*: Germline alterations in the CDH1 gene in familial gastric cancer in the Japanese population. *Cancer Sci* **102**(10): 1782-1788, 2011.

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