Any sample-based medical study can create biobanks. Within this broad category, however, we can classify biobanks by other criteria, i.e. target population, timeframe, and the health status of the population.

At the Organisation for Economic Co-operation and Development (OECD) meeting for 'Human Genetic Research Databases (HGRD): Issues of Privacy and Security' in Tokyo in February 2004, Dr Bartha Knoppers classified biobanks depending on the target population. There are basically four types: regional- or province-based biobanks, biobanks based on national populations, ethnic-based databases, and biobanks based on international/regional endeavours. Using a timeframe is another way of classifying biobanks. The biobank can be fixed at one point in time (cross-sectional), or it can include a follow-up research programme (longitudinal or cohort). Finally, biobanks can also focus on the health status of the target populations.

The OECD meeting used HGRD as an equivalent term for 'biobank'. The OECD report contains much information on the different types of biobanks in a global context (OECD 2006). In this chapter, however, I will discuss only the domestic issues of national biobanks.

Public and private biobanks

We can classify human resource banking systems into private and public ones. 'Public' here does not mean that the banking system is government-funded. Rather, 'public' refers to the function of biobanks in medical research. If a biobank distributes its collection to qualified researchers using an open and fair evaluation system, we call the biobank 'public'.

A 'private' biobanking system belongs to a specific organisation or person and supports specific researchers. Its biobanking policies govern the function of the organisation. The collection is not open to the public, and distribution of material and information is only for a collaborative or commercial purpose. Private biobanks can be supported by public funds. Alternatively, a publicly funded research project can create a private biobank, not open to qualified researchers.

During the OECD meeting in Tokyo, the following issues were discussed. When using genetic material and information, it is important for the biobank to be *public*, because (1) health data form part of a national heritage, (2) genetic data are essentially public and shared, (3) health data are recorded at public expense, and therefore, constitute a national resource, (4) it is essential that this resource remains open for public research, and (5) a public biobank avoids duplication of a collection. On the other hand, arguments for building a *private* biobank include: (1) health data are a commodity; (2) there is no inherent difference with traditional clinical trial data (data owned by the sponsor of clinical trials, data embodied in a drug master file for legislative purposes, and data exclusivity); (3) *de novo* collections require additional funds; and (4) the 'market place is always right' (OECD 2006). In addition it could be argued that a private biobank could serve the protection of sample donors well, as a private

banking system could tightly control the use of human material and information. In practice, a public banking system would not be able to limit the use of data efficiently.

In the context of this discussion, even deCODE Genetics, which is a representative private biobank, was discussed in Iceland in a 'public' light:

> Due to the nature of the data and their origin, they cannot be subject to ownership in the usual sense, neither by institutions, companies nor individuals. It is, however, both fair and a duty to utilise the data in the interests of the health sciences and public health. This can best be done by the government authorising the creation and operation of a single centralised database, which has the following benefits: (1) acquisition of new knowledge of health or disease, (2) improved quality and economy in the health system, (3) development of high-technology industry and employment in Iceland, and (4) potential for attracting business to Iceland (OECD 2006).

Therefore, deCODE Genetics works under the guidance of the government and represents national benefits even though it initiated the commercial operation (Masui 2002b; Rose 2006). In this sense, the deCODE case is a quasi-public activity. This discussion clearly demonstrates the public nature of human genome material and data.

In any case, a biobank should maintain a public policy over data and sample collection. Another reason for public concern over biobanks lies in the way they are used and whom they benefit. It is important for the users of a biobank to maintain their public status and act accordingly. This leads us to the issue of the need for benefit sharing, though this discussion, as I will explain below, has great difficulty in resolving the problems of maintaining anonymity and the incompatibility of the benefactor and the beneficiary. To complicate the discussion on the nature of 'public' even further, it has to be pointed out that the concept has different implications depending on the context. This point will become clear when comparing the Japanese and British biobanks in the latter part of this chapter.

There is another reason for not regarding biobanking as a private commercial activity. Research resource banking systems require a large amount of 'dead stock'. Dead stock refers to potentially useful stock of data and/or materials that are not necessarily in constant use. This is important, for if a biobank collects only currently useful 'valuable' stock, the biobank would be out of date as research trends shift. Collections should not be too narrow and must be prepared for possible changes in the future. In this respect, the biobank does not fit the requirements of a private commercial activity, because a commercial activity seeks efficiency and does not tolerate 'dead stock'. This discussion raises issues of funding for biobank systems associated with their role in society.

Japanese genome research

In 2000, the Japanese government started the Millennium Project, a five-year target for science and technology in seven commercially promising areas (Government Office 2006). The project involved research on the human genome and required the creation of guidelines.

Coinciding with the planning of the Millennium Project in 1999, three cases of genome analysis were reported without the specific informed consent of the donors (METI 2006). And because genetic/genome information in Japan is regarded as the ultimate form of private information (Nukaga and Tsutani 2006), the mass media were eager to report misconduct in genome research. The three cases helped the government and academia to establish a consensus for the need of official guidelines on genomic research. The first ethical guidelines on genome analysis were issued on 28 April 2000 (MHLW 2006). The Ministry of Health, Labour and Welfare (MHLW) published 'the Millennium Guidelines'(*Idenshi Kaiseki Kenkyu ni fuzuisuru Rinrimondai tou ni taiou surutame no Rinrishishin*) for the regulation of millennium genome projects.

Independent of the guidelines, the Bioethics Committee, the Council for Science and Technology (BC, CST) (*Kagaku Gijutsu Kaigi, Seimei Rinri Iinkai*) issued the 'Fundamental Principles of Research on the Human Genome' (*Hito Genomu Kenkyu nikansuru Kihongensoku*) (CST 2000), on 14 June 2000. 'The Millennium Guidelines' were the standard operational protocol for researchers. The principles were influenced by 'the Universal Declaration on the Human Genome and Human Rights' of UNESCO 1997 (UNESCO 1997). These principles focused on a conceptual framework for genome research and were not intended as practical guidelines for researchers.

Other ministries have been funding human genome research projects, so the government decided to establish the guidelines in collaboration with three ministries: the MHLW, the Ministry of Education, Culture, Sports and Technology (MEXT), and the Ministry of Economy, Trade and Industry (METI). 'The Ethical Guidelines for Analytical Research on Human Genome/Genes' (*Hito Genomu/Idenshi Kaiseki Kenkyu ni kansuru Rinrishishin*) were issued on 29 March 2001 (MHLW, MEXT, METI 2001). The new guidelines replaced the old guidelines from the MHLW, merging the trends from the old guidelines, but covering a wider range and in some areas loosening the regulatory framework (issues of anonymity, surrogate decision-making process, and existing holdings).

From 2003, the Data Protection Bill (*Kojin Jyouhou Hogo Houan*) was discussed intensively, and the public became interested in the discussions regarding data protection. However, the use of genome information in research was not closely examined, which may have occurred because academic research was exempt from the Act (Article 50) and academic areas already had the guidelines. Of course, modifications to the guidelines were necessary according to the regulatory framework of the 2003 Act (*Kojin* 2003).

According to the Act, the MHLW drafted the 'Guidelines for Appropriate

Handling of Personal Data in Medical and Care Services' (*Iryou/Kaigo kankei Jigousha ni okeru Kojinjouhou no Tekisetsu na Toriatsukai no tameni*), issued on 24 December 2004 (MHLW 2004). The guidelines established due process for data processing in medical practice and care. However, the guidelines provided very little information about how to transfer medical information from the medical field to research.

Since the Data Protection Act (*Kojin* 2003) became effective on 1 April 2005, the guidelines for medical research had to be adjusted to comply with the Act. The revision process was completed in December 2004, and became effective in April 2005 (MHLW, MEXT, METI 2004). The revisions focused on the area of data protection and the principles of safeguarding data, i.e. systematic, personnel, physical, and technical safeguards, and other adjustments according to OECD data protection principles (OECD 1980) that are incorporated into the Act (*Kojin* 2003).

The government also tightened the regulatory consistency between the explanations of the research purposes when obtaining informed consent from sample donors and the actual use of materials and information in medical research. As the new guidelines require the assurance of explicit consent, the range of research applications after informed consent is limited. These requirements in general are not suitable for biobank activities, because biobanks, in principle, serve future, still unknown research purposes.

Genomic research has generated several biobanks in Japan. I will describe two of them: the Pharma SNP Consortium,[2] and Biobank Japan.[3]

The Pharma Single Nucleotide Polymorphism Project

The Pharma SNP Consortium (PSC) was established in September 2000, and started its collection in February 2001, just before the three ministries jointly issued the guidelines for analytical research on human genome/genes (MHLW, MEXT, METI 2001). The Consortium gathered forty-three pharmaceutical companies, and collected samples from about 1200 'common' Japanese people. It collected the blood of donors, blood test results and data from questionnaires. The samples were completely anonymous and processed as unlinked data sets of DNA, blood, and sample information. The project was conducted at the Tokyo Women's Medical University, under the leadership of Professor Naoyuki Kamatani, and Professor Yusuke Nakamura's group of researchers at Riken[4] analysed the single nucleotide polymorphisms (SNPs). The project analyzed the frequency of SNPs of about 170 genes of drug-metabolising enzymes in a population of ordinary Japanese. The results are expected to serve as the normal control for pharmaceutical research projects.

The samples were donated with informed consent, but with two conditions: the permission for use by the PSC and the guaranteed donation of the samples to a public bank after the completion of PSC's project. The people who agreed to these two conditions participated as volunteers. The PSC processed the donated blood not only to purify the DNA for its own project, but also to make

immortalised cell lines by infecting the B-lymphocyte cell population of blood with the Epstein-Barr (EB) virus. Artificial infection of human B-lymphocyte with the EB virus causes an extended culture period or immortalisation without much alteration of individual genome integrity. Therefore, the EB virus transforms a limited resource from a donor to an almost unlimited resource of human DNA. After the completion of the project, the cell lines were donated to the Human Science Research Resource Bank,[5] and to the Japanese Collection of Research Biologicals, JCRB Cellbank.[6] These banks collaborate under the guidance of the MHLW.

Although this research plan received partial funding from the MHLW, it was initiated and primarily funded by consortium members of industry and was not part of the Millennium Project. In 2000, there were no specific guidelines applicable to the PSC genome project. Therefore, following publication of the Millennium Project Guidelines, the Consortium published its own guidelines.

This PSC genome project constitutes the first major collection of genome material for research by industry in Japan. As the collection of genome material and data was still a publicly sensitive matter, PSC maintained a cautious attitude during the whole period. The data was released in February 2004, and just after two months the data had been made accessible to the consortium members. As in other countries, the industrial initiative requires stricter standards than those of academia because journalists and the public watch their activities much more carefully than those of academics. The public policy was applied not only to the operation, but also to the collection of data, and the Consortium opens all its activities on its website. There may be other reasons for establishing public policy for PSC activities. It might be difficult to establish a consistent plan for the distribution of benefits among the members of the Consortium without it. Therefore, if a policy was devised for allocating certain periods of exclusive use of the data by members of the Consortium, the members would have to accept the policy of making the acquired data public. The other issue might be the continuation of funding. The generation of research data does not require much effort and funding after collection. On the other hand, the collected and immortalised materials demand care and back-up funding for long-term maintenance. The best use of publicly collected material might be considered as an ethical use of donated materials under the appropriate informed consent. This situation may have pushed the Consortium to establish its own public policy: all the established cell lines were donated to public cell banks. In this way, the collection became a useful tool in the public domain.

At the time of the donation of materials to the cell banks, the accompanying sample data from the cell lines remained at Tokyo Women's Medical University. In this way, the data and materials were managed independently, maintaining a high level of security. Professor Naoyuki Kamatani at the Women's Medical University was responsible for providing the available data upon request. The Consortium only lasted three years, from September 2000 to May 2003, but the collection has become a valuable public resource in Japan.

The data generated from the project is also accessible to the public. Those who wish to access PSC materials are asked to obtain authorisation for their project plans from their research ethics committee. This process is enforced because public policy stipulates the ethical justification for the use of donated materials and information. More importantly, donors were informed at the time of giving consent that a research ethics committee would authorise the future use of their materials from the public cell banks.

In the beginning, public biobanks discussed the international distribution of PSC materials. However, since many Japanese pharmaceutical companies are international, public biobanks decided to distribute the materials and information internationally as well as in Japan. In accordance with public policy lines, the PSC completely publicises its activities, protocols, proceedings of research ethics committees, research results, and materials and information. PSC, however, is an exceptional case in Japanese research, especially the 'public status' of the bank and its political transparency.

Biobank Japan

In the official Japanese papers, the concept of the 'biobank plan' appeared in a report from the Subcommittee on Science Project Evaluation and Life Science, Genome Research Working Party (*Genomu Kenkyu Ryouiki Shou-Iinkai*) on 20 March 2002.[7] The report included recommendations for a policy on genomic research in Japan. The report defined the 'biobank' as follows:

> a government supported collection of, preferably, immortalised and propagable EB virus transformed B-cell lines derived from patients and their family members, patients that have experienced adverse drug reactions, and volunteers. The collection should be voluntarily initiated and organized by researchers, medical doctors, and the pharmaceutical industry. The samples should be maintained and managed by the biobank and provided to qualified researchers upon request [translated by the author].

This description of the biobank reflects the practice of PSC described above. At this stage, the report recommended the collection of samples from 360,000 people, including 20,000 'normal' volunteers.

After the publication of the report, the Evaluation Subcommittee for Research Planning (*Kenkyu Keikaku Hyouka Bunkakai*), a subordinate of the Science, Technology and Academia Committee of the MEXT, issued the 'Promotion Strategy of Research and Development of Life Science' (MEXT 2002). In the report, the subcommittee recommended 'establishing the infrastructure for the management of resources and technology to realise personalised medicine (tailor-made medicine)' [translated by author]. On 24 July 2002, the Cabinet Committee of Science and Technology (*Sougou Kagaku Gijutsu Kaigi*) issued the 'Estimation of Science and Technology related Budget in 2003' (*Heisei 15 nendo no kagakugijutsu yosan no gaisanyoukyu ni*

mukete). The suggested budget was settled for the next fiscal year under the project title, 'The Realization of Tailor-made Medicine on the Genetic Characteristics of Individuals' (*Kojin no identokusei ni oujita teirameido iryou no jitsugen*), earmarking an estimated 40 billion yen (US$330 million) over five years (in a recent presentation, US$180million, October 2006). This plan became known as the Biobank Japan Project.

Biobank Japan aims to collect data from 300,000 patients (or cases) with over forty major diseases, in cooperation with over forty cooperating hospitals (Ohnishi and Nakamura 2005). This is primarily a five-year research project for collecting samples and information, with the supplementary function as a biobank. The study design includes the preparation of controls from the samples of patients with other diseases. The project defines the biobank as:

> A collection of DNA and serum of patients with certain diseases and patients with effectual and adverse drug reactions. Its infrastructure facilitates the analysis of SNPs and the creation of order-made ('made to order'; 'personalised') medicine [translated by the author].

The Biobank Japan Project started in the fiscal year of 2003 with the project title 'Realization of Order-made Medicine (*Ooda-ameido Iryou Jitsugen ka Purojekuto*)'.

Although the term 'order-made' is not English, its meaning is significant to the project. Professor Nakamura, its leader, favours the use of the term 'order-made', though the documents from the MEXT stick to the 'tailor-made' medicine. Professor Nakamura expressed his view that the concept of 'tailor-made' smacks of a class society, and said that genome science should provide benefits to the general public, not to a privileged elite. Therefore, if the research outcome only benefits the wealthy or is distributed along class lines, it would not be consistent with what Biobank Japan intends to offer the public. The spirit in which Biobank Japan was established, then, would have to be in agreement with the purpose of the public medical insurance system in Japan, which to a great extent has accomplished equality and solidarity with the financially underprivileged.

Biobank Japan is a large, case-control study and does not intend to perform follow-up studies. The project compares the genome–environment interactions of patients with a certain disease with those of carriers of another disease. For example, patients with diabetes might be studied in comparison with those with cancer. In addition, the secondary use of the collected material and information is part of the project plan. Biobank Japan announced that it provides collected material to qualified researchers, who have authorisation from the ethics committees of their institutes and meet the required qualifications of the biobank. However, Biobank Japan provides the DNA samples, but not the data, to the researcher. In the original plan, the research project which had received DNA material would provide the analytical results of the DNA to the biobank, and the data analysis centre of the biobank would analyse the DNA data with

other medical and lifestyle data and return the final results. As I will describe below, the governance of material and information by Biobank Japan is very different from that of UK Biobank, which plans to provide data, but not distribute DNA material.

The British plan for genome research[8]

The UK House of Commons' Science and Technology Committee issued the key report 'Human Genetics: Science and Its Consequences' in 1995. This report provided the starting point for research policies on genome science and its application in the UK. Although, the committee criticised UK Biobank in its report 'The Work of the Medical Research Council [MRC]' in 2003 (House of Commons 2003), the 1995 report paved the way for the establishment of UK Biobank.

In 1998, two important events occurred in the field of genomic research. One was the participation of Celera Genomics in the human genome project, and the other was that the Icelandic parliament passed the Icelandic Health Sector Database Act. Although the Icelandic Health Sector Database was not successful for various reasons as I mentioned earlier, the Icelandic controversy stimulated discussion on the value of human genetic material and data.

In the planning process of UK Biobank, the MRC at the same time discussed a policy for the governance of materials and produced the interim guidance document 'Human Tissue and Biological Samples for Use in Research – Operational and Ethical Guidelines' 2001 (MRC 2001). An important point of these guidelines was to discuss the public status of human tissue collections and the governance of the funding body handling the collection.

UK Biobank

The author has been following the developments of UK Biobank from its inception (Masui 2002b, 2003, 2004b; Masui and Takada 2005). It is clear that UK Biobank cannot be regarded as a research project *per se*. Its aim is to collect biomaterials (blood and urine) and information (medical information, lifestyle questionnaires, and measurements) from 500,000 UK citizens, ranging in age from 40 to 69 years old, and to conduct a follow-up in 20–30 years.[9] Its aim is:

> UK Biobank project will enable scientists to gain a unique insight into the genetic and environmental causal factors associated with a wide range of debilitating diseases, providing vital information needed to work on future preventative and curative measures (Wellcome Trust 2006a).

The UK Biobank project is expected to provide research resources to qualified researchers. It is conceivable that the core concept of the UK Biobank project

is that of a social experiment set up to obtain the best possible outcome from human genome research in the context of the medical health sector in the UK. For this reason, UK Biobank serves as a custodian of the resources of UK citizens.

The UK started discussions on a population biomedical collection in late 1998. In May 1999, the MRC and Wellcome Trust (WT) held a workshop entitled 'UK Population Biomedical Collections', at which it reviewed major genome cohort projects funded by them. These funding bodies jointly announced that they would fund the preparatory steps of the UK Population Biomedical Collection in June 1999. In October and November of the same year, the WT held two consecutive meetings, the 'Workshop on Pharmacogenetics' and the 'Workshop on Human Biomedical Collections'. Moreover, in November 1999, the MRC issued the interim guidance entitled 'Human Tissue and Biological Samples for Use in Research'. These activities followed and strengthened the joint announcement in June. In the ethical guidelines, the MRC proposed a material governance policy and discussed public collection and custodianship because the fundamental question of a large biobank concerns its impact on not only individuals, but also society at large. For this reason, the MRC and the WT have maintained a policy of engagement in public debate on the public nature of the collection. These discussions focus in particular on the collection of UK Biobank and the National DNA banking network (Rawle 2003).

During the preparatory stage of UK Biobank, the MRC and the WT surveyed public perspectives on genome research and biobanks. They attached great value to obtaining public trust to facilitate progress in the biobank (PSP 2002). The acceptance rate for enrolment was estimated at around a quarter or less. It was therefore important for the success of UK Biobank that people in the locations for recruitment and in that particular age range were favourably aware of the aims and the process of the project. This meant that UK Biobank attached value to its public status and was motivated to survey the public perception of UK Biobank (PSP 2002). At the same time, they studied the perceptions of professionals in UK Biobank (Hapgood *et al.* 2006). Even though there was continuing criticism of the UK Biobank project (Giles 2006), the funding bodies and the biobank have come to feel confident and comfortable with its status.

In the spring of 2003, the funding bodies, consisting of the MRC, the WT, and the Department of Health, appointed a CEO and started practical moves for the project. UK Biobank was registered as a limited company and as a charity. In 2005, a new CEO was appointed. In my interview with Professor Rory Collins, the CEO of UK Biobank (February 2006), Professor Collins said that the project was planning to recruit people independently from general practitioners, and that UK Biobank had applied to the Patient Information Advisory Group (PIAG),[10] and obtained authorisation from the Minister of Health to survey potential participants without their consent. The terms of the PIAG are as follows:

> Section 60 of the Health and Social Care Act 2001 provides the power to ensure that identifiable patient information needed to support essential NHS activity can be used without the consent of patients. The power can only be used to support medical purposes that are in the interests of patients or the wider public, where consent is not a practicable alternative and where anonymous information will not suffice.[10]

In short, although UK Biobank itself does not have any specific legal powers, its activities are framed and supported by the Data Protection Act of 1998 and the Human Tissue Act of 2004.

Differences between Japanese and UK biobanks

As mentioned above, definitions of biobanks differ in a variety of respects. Although the biobanks in Japan and the UK are planned to be public, their different conceptualisations of public, risks, benefit, and trust affect their 'public' status. The scope of the differences reveals important points of consideration in the concept of collecting human material and information.

Preparing Biobank Japan and UK Biobank

The implementation of the plans for Biobank Japan started in the fiscal year of 2003. Until then, the Millennium genome studies (which had commenced in 2001) had experienced two years of practice, during which its guidelines had already been revised once in 2001 (MLHW, MEXT, METI 2001). The planning committee for the project believed that Japan was prepared for Biobank Japan, and that there would be no great need for further preparation (Ohnishi and Nakamura 2005). In fact, the project was believed to be a large and expensive version of the Millennium Project. The Japanese government did not regard this project as something new, so it had no notion of having to deal with the particular challenges of a social experiment.

The PSC project had been organised as an industrial initiative and had collected about 1200 blood samples from 'ordinary' Japanese people. It had immortalised the blood samples and donated them to the public banks. As described earlier, the success of the PSC project may have had a strong impact on Biobank Japan, because the practice of PSC had influenced the definition of 'biobank' used in the March 2002 report 'Genome Research Working Party' (*Genomu Kenkyu Ryouiki Shou-Iinkai*), chaired by Dr Yoshiyuki Sakaki. Biobank Japan established the Ethical, Legal, and Social Issues (ELSI) Working Group in 2003. And in 2004, the MEXT, the funder of the bank, established an ELSI Committee independently from the project.[11] Its task was to monitor and to advise the biobank project.

For UK Biobank, the draft of the Ethics and Governance Framework was open to public comments from September 2003, and public comments were summarised and published in May 2004. Then, the Ethics and Governance

Committee (EGC) first met in November 2004.[12] They discussed the issues of supervision and ethics and governance of UK Biobank. Preparatory activities for UK Biobank differed substantially from those of Biobank Japan, especially regarding the relationship between biobank and society. In 1995, the Science and Technology Committee of the House of Commons reported its views in 'Human Genetics: Science and its Consequences'. And in 1999, the Human Genetics Commission (HGC) was established to create long-term science and ethical policies on human genomic research.[13] Until the establishment of the Committee, there had been no initiative to create long-term policy recommendations. Since creating and implementing such a policy for genome research would require the cooperation of multiple ministerial departments, a central committee was regarded as indispensable.

In addition to this top-down infrastructure, the Department of Health and the Department of Trade and Industry decided to jointly fund the Genetics Knowledge Parks (GKP) for five years, from 2002 until 2007.[14] This created a bottom-up movement in the field. In 2004, the Human Tissue Act 2004 was passed,[15] and in 2005 the Human Tissue Authority was established to address the issues of human tissue,[16] the essential resource of genome information. This would provide the legal framework for the use of human tissue in genome research as a whole.

These elements of the policy of the House of Commons and the HGC, the engagement of GKP and the Ethics and Governance Committee (EGC, see below) and the legal framework, together with UK Biobank, give an edge to the UK in the area of human genome research. Moreover, these movements indicate that the UK government has seriously considered indirect human experimentation. This may be due partly to the UK tradition of epidemiology, which involves collecting medical data from UK citizens. The use of genome information is regarded as central to its future success. The UK policy, then, was a determination to build a robust trust based upon the dialogue between stakeholders. These activities were regarded as necessary for a mature society to consider and accept the possible outcomes of genome research.

Differences in the origin of researchers in UK and Japan

The academic background of researchers in biobank projects is an important factor in their development. In Japan, genetic research of single-gene disorders started in the 1980s. These studies were successful, and their outcomes created excitement among citizens and funding bodies. For this reason, genetic research based on DNA science has acquired a good reputation and much funding. The conclusion of the Human Genome Project changed the interests in genetics research from single-gene disorders to genome research of multifactorial diseases. In Japan, genetic researchers of DNA science who had been working on single-gene disorders were the first to move into the area of biobanks.

In comparison, in the UK, it was epidemiologists, by incorporating genome

information into their epidemiological analytical processes, who have led UK Biobank and existing genome epidemiology projects. Genome information would be a powerful tool in stratifying human populations, but the contribution of genetic factors is much smaller in common multifactorial diseases. Careful handling and examination of other human environmental information is essential to the study of multifactorial disorders of the human body. Epidemiology, which traditionally pursued the association of diseases with various biological and environmental factors, also provides methods for the study of multifactorial diseases.

The study of single-gene disorders has an exceptional position in the study of human diseases, focusing on the causative relationship between genetic information and disease. However, epidemiologists have struggled to obtain comparable data from multifactorial phenomena. In multifactorial research, a genomic factor does not necessarily constitute a major factor in causing a common disease. The study of the interaction between the genome and the environment requires skill in the collection of non-genomic information. In this sense, epidemiologists have an advantage in their academic training of collecting information on the human body in a comparative context.

Obtaining disease data is not without its complications, because individual doctors have different styles of diagnosis, and to study human disease requires standardisation of the description of each disease. Researchers then require standardised data on patients. Thus, data from blood tests seem suitable and useful, but they show only a one-time section of a patient and have limited value.

Therefore, obtaining human data may benefit from the experience of epidemiologists. Due to the difference in academic origins of biobanking in Japan and in the UK, then, the epistemological backgrounds of the biobanks in Japan and the UK are quite different.

Regulation of sampling

In Japan, clinical technicians draw most of the blood samples. However, a clinical technician can only draw blood up to 20 ml under a MHLW Notice.[17] PSC collected 30 ml (10 ml for biochemical analysis, 10 ml for DNA isolation, 10 ml for immortalisation of B-cell fraction), and Biobank Japan collects 20 ml (6 ml for biomedical analysis, 7 ml for DNA and plasma isolation, and 7 ml for serum isolation). The 20 ml limitation of the MHLW's Notice may seem to set a limit for sample collection.

There is another limiting factor in Japan. In ethical discussions, informed consent is of primary value in medical research planning. In Japan, the philosophy of informed consent strictly regulates the amount that can be sampled and the use to which it may be put. Consequently, medical researchers are very careful not to take 'unnecessary' or 'excess' blood when sampling. This trend may also limit the sample size.

Ten millilitres of blood yields about 200 μg of DNA. Thus, in Japan, the

immortalisation of samples is favoured because the immortalised cell line produces unlimited amounts of DNA samples from each individual. So the biobank report included an immortalisation plan.[18] However, the immortalisation process for blood is expensive and causes an increase in the chance of cross-contamination of samples. Therefore, the immortalisation of samples requires great care and a large budget.

As for sampling regulation in the UK, UK Biobank issued 'Sample handling and the storage, subgroup protocol and recommendations, version 1.0' on 7 July 2004 (UK Biobank 2004). It stated that the Biobank collects 40 ml blood (30 ml for DNA and plasma, 10 ml for serum). According to this sampling schedule, UK Biobank takes more than four times the samples for DNA analysis compared to Biobank Japan. Moreover, UK Biobank seems to be planning to save a limited amount of samples by conducting genome analysis within the biobank. In this way, they can recover leftover samples from the genome analysis. However, Biobank Japan has decided to provide a 5 µg aliquot of DNA and a 0.5 ml aliquot of serum from individual samples to a qualified researcher.[19] This illustrates a different strategy in governing samples.

On the distribution policy of data samples, UK Biobank plans to provide sample information to researchers, but Biobank Japan does not give the sample information to the researcher. In summary, the biobanks of Japan and the UK have greatly contrasting policies for governing genetic material and information.

Enrolment focus – differences in follow-up and risk management[20]

The UK Biobank project is a cohort study for up to thirty years, and enrolment will focus on 'ordinary' UK citizens. The bank follows the health status of participants during this period. The considerable follow-up process requires referencing and collecting samples and data from individuals. This requires long-term collaboration between researchers and participants. Although, in the original plan, the biobank itself does not perform the research, custodianship requires a well-prepared strategy and structure for supporting a dialogue among stakeholders in cooperation with the EGC.

In contrast, Biobank Japan aims to collect samples and information from patients, and perform research of their own, and does not include a follow-up programme. Patients are generally more amenable to research enrolment than volunteers. In the stage of research planning, one disease is compared to another disease as the control group. The collection schedule for Biobank Japan is of lower risk than that of UK Biobank because it does not contain long-term collaboration with research subjects and society.

The various scandals of the 1990s in the UK may have stimulated careful risk management of the biobank planning process. In particular, the public sector had lost credibility after scandals concerning BSE, GMO, and human tissue retention without appropriate consent. Programme officers from UK Biobank and the funding bodies were very careful about publicity, until the funding

bodies officially announced the establishment of the UK Biobank project in April 2001. Though the project requires enthusiastic participants, expectations that are too high may mislead people and result in the collapse of the project.

The idea was that 'too high of an expectation kills all'. Until they were ready to respond to the public or opponents, they wanted to keep UK Biobank low profile. Another aspect to the UK risk management strategy concerns the nature of the benefit of UK Biobank. The programme officers stated that the benefit of UK Biobank lies in the increase in knowledge. This basic statement is essential because it shows UK Biobank's intention to support competitive research projects without making any promises in principle. However, this is a difficult task.

If researchers only raise 'appropriate' expectations, they may not obtain sufficient support from the non-expert population, and funding bodies might lose interest in their projects. Therefore, researchers tend to exaggerate the promises of their research programme. Programme officers of UK Biobank, during my interviews with them, often complained about researchers who are promising too much. It is conceivable that the researchers felt that they should obtain the support of citizens and motivate funding bodies in the biobank project. This might be the cause for the behaviour of researchers during the incubation period of a research programme.

While transferring the knowledge on hypothetical and uncertain scientific research to non-experts, the possible failure of experimentation should be made explicit and discussed rationally and calmly. However, research results from studies in scientific communication revealed that the transfer of the hypothetical nature of science knowledge to non-scientists and even to scientists is not unproblematic (Shamos 1995).

Biobank Japan uses a very different risk management strategy. Biobank Japan operates in the name of the 'Realisation of "order-made" medicine', and it emphasises the practical benefits from the beginning. In Japan, among scientists there are many complaints about the poor acceptance of clinical research. There are various reasons for this, though the phenomenon is regarded as a reflection of a poor understanding of human experimentation. Next, it reflects the Japanese misunderstanding of science, i.e. science and technology are seen as the tamed servants for the well-being of humans. Emphasising the practical benefits of a research project is important for obtaining funds and the support of citizens, but this strategy increases the risks of a misunderstanding of the nature of science. In order to avoid the risks, the projects must guarantee substantial outcomes. It is said that this sequence of providing prospects beneficial to society is important in promoting research and it was praised in the third 'Science and Technology Basic Plan (*Kagaku Gijutsu Kihon Keikaku*)' of the Council for Science and Technology Policy.[21] However, this positive-reward cycle, i.e. positive outcome cultivates support of science, cannot absorb the uncertainty and hypothetical nature of scientific research, and might increase the risks associated with medical research and create a poor understanding of science.

Balancing risk and benefit

In medical practice, a patient and a doctor focus on the risks and benefits of a treatment to the patient. In the clinical setting, then, the subject is singular, and the focus is on the improvement of the condition of the patient, i.e. the well-being of the patient is the primary concern. By contrast, in the context of biomedical research involving human subjects, medical doctors and researchers cannot focus only on the benefits to a particular participant. The situation is described in the Declaration of Helsinki section 7, 'In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens'. Of course, the participant's welfare is the primary concern in research planning. Therefore, the Declaration states that: 'In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society (section 5)'. However, medical research is also concerned with the due course of scientific human experimentation. Since medical research needs to obtain scientific and comparable results, the experiments require randomised trials and placebos. The World Medical Association has spent much time discussing the 'placebo-controlled trial' and incorporated notes of paragraph 29 and 30 in the Declaration. Certainly, following due procedure places 'risks and burdens' on the participants.

Medical research of this kind therefore basically aims to bridge the participants' current risks and the burdens and benefits of the next generation. This idea is a central point of departure in medical experimentation. Thus, if we take into account the differences in medical research on living human beings and indirect medical research using genetic material and data, the latter could substantially reduce the risks and burdens of a participant in medical research experimentation. It is imperative that indirect medical research supported by biobanks is done as efficiently as possible so as to reduce the risks and burdens on human beings. In this sense, biobanks could have indispensable value to medical development and to the welfare of research participants and should be promoted.

Though benefits to the public are also an important issue for biobanks, the UK and Japan take the same stance of not paying the donors for participation. Since medical research produces benefits that are not meant for the participants themselves, but for the next generation, the donors expect that their tissues, cells, and information will contribute to the health of future generations. The policy requires the biobank to have public status and responsibility.

If we think of benefit sharing as a form of reciprocity, the ethical burden lies with the researcher's use of human material and data. As medical research may result in commercial benefit, academic honours, and public reputation, researchers should be aware of the public status of research resources. What it means in reality varies in each case. However, researchers must pay considerable attention to donors and society, as their cooperation enables them to study human beings and to perform indirect human experimentation.

No direct personal benefit to participants

Both the biobanks in Japan and the UK are careful to deny the personal gain, not only monetary but medical, of individual participants. The public has difficulties in accepting this.

In Japan, the idea of order-made or personalised medicine has been very popular, since it was thought that genome research would lead to a miracle cure for the diseases of individual patients. This idea seems to derive from the images of the workings of antibiotics against infectious agents. Or sometimes participants misunderstood how the genome research could benefit their own health. However, it takes a long time for research outcomes to produce a practical cure.

The idea was put forward that 'personalised medicine' resembles traditional medical practice focusing on individual patients; diagnostic and therapeutic information created by genome research of multifactorial diseases would not work differently from traditional medical knowledge in the essential sense. It was stressed that research results of population studies could be adapted to an individual patient at the clinical phase. In these aspects, medical research and treatment are different.

These ideas should be familiar to the general public. However, ordinary citizens do not see them in a favourable light, since direct and substantial benefit is easy to understand. Therefore, research domains should explain the consequences of medical research to make the participants and public understand them.

It is interesting that UK Biobank suffered a similar problem concerning the benefit to participants. In the public comments on the Ethics and Governance Framework 2003 (UK Biobank 2003), there were a few queries on what could be regarded as a substantial benefit to participants. As a policy, UK Biobank clearly denied the benefit to participants. The Ethics and Governance Committee, at the first meeting (November 2004), took the initiative in denying any substantial benefit or data return to participants and reconfirmed the voluntary status of the participants, so as not to raise unrealistic expectations (EGC 2006).

Although the biobank seems to be a natural consequence of post-sequence biomedical science in the genome era, we are not sufficiently prepared yet to accept this type of 'indirectly invasive' activity. Only if we build on the basis of trust can the idea of a biobank survive the many problems to come. Since the idea of research is not a promise in the future, in some cases 'unconditional' support should be necessary.

What is the role of trust in the participation in medical research?

In his memorial essay on human experimentation, the philosopher Hans Jonas claimed that in the course of medical research we should not depend on 'trust' because trust is not based on an equal relationship and on independence (Jonas

1974). Therefore, the trust relationship in principle cannot call for voluntary donations.

His claim is fundamentally important and correct, but I believe that in medical research it would not be practical and in some sense wrong. There are never entirely equal relationships between medical doctors or researchers and patients or participants in medical research. Medical research now involves non-medical researchers and often involves commercial interests. In practice, the researchers propose a research project to patients or healthy volunteers and try to obtain motivated participation. Of course, the motivation of the researcher not only focuses on the public good, but also on their personal and research interests. In the dialogue with research participants, the research domain encourages them with openness and a sense of responsibility to understand the intended results of the medical research project, which are intrinsically uncertain and can only be revealed in the (sometimes faraway) future. Without a responsible attitude, the participants cannot be encouraged to support medical research in the long run. If a project cheats its participants, medical research generally loses credibility and cannot build a trustworthy partnership between researchers and participants. Though the described process seems essential, a project needs to collect a certain number of participants within a certain period and cannot often tolerate the burden. The situation is painful, therefore, and the professionals in medical research should develop norms on medical research.

In this sense, the informed consent process of participants is important to researchers. This process gives researchers a chance to reflect on what they are doing with and to the participants. The process is indispensable in developing a norm for medical researchers. We should try and seek to establish professionalism in medical research. Professionalism is traditionally established in medical, legal, and religious areas. I believe that in the era of human experimentation we need to consider the issue seriously. In doing so, the words of Richard Shryock might be helpful to understand and make up our minds to take the nature of medical science of human diseases and its difference from 'science' *per se* (Shryock 1974).

> Physicians were the only scientists who, because they were also practitioners of a vital art, were constantly being pushed to hasty and careless conclusions. Other research men, uncertain in the face of new problems, could suspend judgment and proceed with due caution. Practitioners confronted with dying patients did not dare to wait; they must act quickly and, if necessary, 'take chance.' Even during hours stolen for research, they were still under pressure to get practical results *as soon as possible*.

Shyrock originally wrote this about 70 years ago, and the situation with current medical research does not seem to have changed much. We have to keep this in mind and maintain the dialogue between the various stakeholders and among

medical researchers and doctors. In this way, we can judge ourselves better in the coming era of human experimentation.

Reflections

Though the biobank projects discussed in this chapter may have differences, a minimum requirement for all biobanks should be the acquisition and maintenance of public trust. How can medical research, including genome research, build or acquire trust? Among policy-makers and related people, it is generally believed now that public engagement is essential. However, it is far from clear if public engagement is truly succeeding in engaging the public (Wellcome Trust 2006b).

To control the risks of genome research, scientists, medical doctors, and funding bodies should promote a dialogue with citizens to make them familiar with the uncertainties of science, rather than with exaggerated promises. However, it has been reported that the general public and even researchers, medical doctors, and policy-makers have a difficult time understanding the hypothetical nature of science. Moreover, we still have very few clues about the use of genome information-based research and biomedical research and the resulting medical practices.

Observations of the Japan and UK biobanks made me aware of the dynamism of dialogue among stakeholders. A philosophy of monitoring and compliance will never bring about an era of amenable human experimentation in research, since the system is essentially based on outside ruling that contrasts with the cultivation of voluntariness of research domain and public. If the system could build on benefit-exchange of the research and the participants, 'volunatriness' would not be necessary. However, it seems not easy to understand indirect and/or remote exchange of benefit. As discussed, medical research involved a huge amount of indirect connection or exchange among generations involving the research participants, benefactors, and the future patients and beneficiaries. Such a philosophy based on remote benefit will not motivate the public to participate in important medical research. And the trend to demand direct benefits of science and medical research will weaken and damage the trust in medical research, because most results of 'advanced' research, including genome research to date, have not been benefiting the present patients in practice. At this moment we have to 'believe' that we should cultivate the cooperation of motivated stakeholders and stimulate dialogue, and then we may be able to construct a system of supporting medical research. This process in itself could realise the best use of genome research based on the human material and information of individuals, such as biobanks can support. As a beginning, we need to strengthen our role and position in the indirect and remote processes involved in genome and medical research.

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Notes

1 July 2002 at the JMA.
2 Available at: http://www.jpma.or.jp/psc/frame-j.html, in Japanese. http://www.jpma.or.jp/psc/frame-e.html, in English, last visited on 21 November 2006.
3 Available at: http://www.biobankjp.org/index.html, last visited on 21 November 2006.
4 Riken is a governmental institution of comprehensive research on science and technology. http://www.riken.jp/engn/r-world/riken_menu/index.html, last visited on 21 November 2006.
5 Available at: http://www.jhsf.or.jp/index_b.html, last visited on 21 November 2006.
6 Available at: http://cellbank.nibio.go.jp/cellbank.html, last visited on 21 November 2006.
7 The Subcommittee on Science Project Evaluation and Life Science, Genome Research Working Party (*Genomu Kenkyu Ryouiki Shou-Iinkai*) was chaired by Dr Yoshiyuki Sakaki. It is located under the Committee of Science and Technology, and Academia (*Kagaku Gijutsu Gakujutsu Shingikai*) of the MEXT.
8 For a thorough review of the regulatory frameworks of Japanese and British genome research, see Porter, P. (2004) 'The regulation of human genetic databases in Japan', SCRIPT-ed http://www.law.ed.ac.uk/ahrb/script-ed/issue3/japan.pdf). He made an extensive comparison between the Japanese guidelines (2001 version) and the Ethics and Governance Framework of UK Biobank. The Japanese genome research guidelines have been revised, though the basic framework has not changed.
9 Available at: http://www.ukbiobank.ac.uk/, last visited on 21 November 2006.
10 Available at: http://www.advisorybodies.doh.gov.uk/piag/, last visited on 21 November 2006.
11 Available at: http://biobankjp.org/plan/elsi_com.html, last visited on 21 November 2006.
12 Available at: http://www.egcukbiobank.org.uk/, last visited on 21 November 2006.
13 Available at: http://www.hgc.gov.uk/Client/index.asp?ContentId=1, last visited on 21 November 2006.
14 Available at: http://www.dh.gov.uk/assetRoot/04/02/08/05/04020805.pdf, last visited on 21 November 2006.

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6．細胞培養の倫理問題，特許

増井 徹

培養細胞と社会をめぐり2つの問題を取り上げる．人体に由来する培養細胞の倫理問題を配慮することは重要である．また，培養細胞を含むリサーチツールの特許問題は研究政策の重要な課題となっている．倫理問題と特許問題は互いに関係のない問題のようでありながら，社会で行われている研究という問題を考える重要な入り口である．

はじめに

培養細胞で倫理問題が生じるのは，それが尊厳を有する人（由来者）に由来するからである．通常は「提供者」と表現されるが，提供組織が由来する個人という意味で，本文では「由来者」と表現する．

重要な点は，由来者の研究協力の意思が社会の中で生かされることだと考えている．そして由来者の意思が生かされることによって科学が阻害されない研究環境が醸成されていくと考えている．また，由来者個人との関係のみでなく，社会の中での研究という位置づけからも倫理問題は重要性をもつ．この個人と社会という両側面から倫理問題が培養細胞の取り扱いで顔を出す．この点については，ヒトES細胞とiPS細胞の議論のところでくわしく触れる．

また，最後に述べるが2002年の知的財産基本法の成立から，特許の問題も遺伝子改変動植物および遺伝子改変細胞などにとって大きな意味をもつようになってきた．

このような背景から，培養細胞の研究利用も，由来者があり，社会で行われる科学研究に利用されるという問題を抜きにしては語れなくなった．そしてこのような事情の故に，倫理問題や特許問題が医学・生物学研究において重要な位置を占めるようになってきたのである．

ヒト細胞の個別識別の問題

尊厳と基本的権利をもつ人を特別と考えても，顕微鏡で細胞の形を見ただけでは，ヒトかマウスかを見分けることすらできない．実際に細胞バンクに寄託される細胞で，ヒトといわれたものがマウスだったり，その逆だったりということがある．

この動物種の取り違えの問題は実際には見分けることができる．古典的な方法として細胞バンクでは，アイソザイムを調べることによって由来動物の識別を行っている．電気泳動における酵素タンパク質の移動の違いを検出することによって，動物種を識別する方法である．

ヒト由来試料に関しては，short tandem repeats（STR）と呼ばれるヒトDNAの中に存在する多型マーカーを用いて個体識別をする方法を用いて細胞の由来者の識別を行うことができる．この方法が利用できるのは培養細胞が由来者の刻印をもち続けるためである．この個体識別情報の取り扱いに関する議論は盛んである．特に，この情報が警察捜査に用いられる情報

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であるという側面からの議論が多い．すなわち，細胞の個別識別に用いる情報自体はかなり機微に触れる性質を有するのである．とはいえ，この情報は細胞の取り違いを調べる必須な情報である．細胞の取り違えのあるところで，科学的な研究は困難である*1．そこで，細胞バンクとしては個別識別情報について注意深い取り扱いを行っている*2, *3．

*1 培養細胞の「クロスコンタミネーション」および「ミスアイディンティフィケーション」に関する警告：http://cellbank.nibio.go.jp/cellbank/qualitycontrol/crosscontami01.html（2008年8月）

*2 http://cellbank.nibio.go.jp/cellbank.html

*3 水澤 博，増井 徹，田辺秀之：科学，71：1601-1608，2001

培養細胞と研究利用における注意

生物の最小単位として細胞を考えるようになったのは，それほど古いことではない．最初は機能というよりも，構造単位・建築ブロックとしての細胞（小部屋）のイメージが創られた．この時期は，顕微鏡の発達，マクロ解剖から組織学へという動き，それに生化学的研究の始まりという生物学上の重要な時期と重なっている．

初めて動物細胞が体外で培養できるようになった時，多くの研究者が，生命の本質へ一歩近づいたと感じた．そして，培養された一つひとつの細胞の加算的総合としての生体という考え方，ひとつの細胞それ自身を生命の本質とみる考え方が生まれた．

そして，個体のクローン技術の進歩は，一つの培養細胞の核から，個体を発生させる可能性を示し，由来者との関係が疎遠になってはいるが，体外で培養されている細胞の遺伝的総体は由来者のそれであることを思い出させることとなった．

由来者と由来する培養細胞の間を結ぶ情報は大きく2種類ある．一つはゲノム情報，もう一つは試料情報と呼ばれるその由来者や提供試料にまつわる記載である．試料情報は個人情報，健康情報，医療情報など取り扱いに注意を要する情報を含み，採取，処理，保存，利用，廃棄に関して，倫理的問題が生じる．それらは，現時点では，研究倫理指針と呼ばれるもので規制されている．

ゲノム情報も試料情報も個人情報である．そこで個人情報保護法が重要な役目を果たすと考えられている．しかし，学術分野が適応除外されているので，一群の研究倫理指針による規制が行われているわけである*4．

これらの指針は改正を繰り返しており，規制について考えるときは最新の指針に従う必要がある．

*4 文部科学省：生命倫理・安全に対する取組 http://www.mext.go.jp/a_menu/shinkou/seimei/main.htm#section2

厚生労働省：医学研究に関する指針一覧 http://www.mhlw.go.jp/general/seido/kousei/i-kenkyu/index.html

ヒトES細胞とヒトiPS細胞についての最近の話題

培養細胞の中で特に規制が厳しいヒトES細胞の指針では，2つのことが重要となる．一つは人の萌芽であるヒト胚の滅失を伴うこと，もう一つはES細胞の多能性である．マウスES細胞の場合には，後者の問題は個体作出の能力で検証できる．しかし，人の場合には個体作出は法律と指針により禁止されているので，全能性を検証することはできない．ヒトの場合には人個体を作出する可能性という表現，あるいは多能性を用いるのが適当であろう．

2006年のマウスiPS細胞作製の成功は素晴らしい研究成果である．従来ES細胞の多能性・全能性の起源はマターナルファクターと呼ばれている卵に由来する因子が重要であろうと考えられてきた．それが4個の遺伝子を導入することで可能であるということを示した．そして2007年にヒトiPS細胞の作製に成功し，これは科学的というよりも社会的にも大きなインパクトをもつ．

ヒト由来のES細胞とiPS細胞を比較すると，著しい違いは「ヒト胚の滅失」を伴うか，伴わないかという問題である．それゆえに，ヒトiPS細胞は倫理的問題の少ない，そして患者にとって拒絶反応のない移植可能な細胞の供給源として期待がもたれている．

しかし，ヒトiPS細胞の医療応用の課題は，人体への移植というだけにとどまらない．この技術は遺伝子治療の範囲に入る．すなわち，組織を摘出して培養細胞を培養しそこに複数の遺伝子を導入して身体に戻すという技術である．そして，日本では新しい遺伝子治療の試みは大変に少なくなっている．このような状況から，遺伝子導入によらず体細胞をiPS化する技術が模索されているのが現状である．

また，細胞の性質から考えたときに，体細胞に多能性を与えることは，もともとの細胞がもつ安定性の破壊を意味する．不安定であるからいろいろな細胞に分化するのである．それゆえにいろいろな細胞に分化する可能性が生まれる．そして，ヒトES細胞が最低限の人間の技術関与を意味するならば，iPS細胞は最大限の技術関与を意味する．現在の治療用細胞製品の考え方では，技術的な関与が大きくなるほど，その安全性を示すことが難しいと考えられている．それゆえに，日本の医療技術が現在のように安全性に固着している中で，ヒトES細胞でさえ試せない移植技術をヒトiPS細胞で試すことはさらに困難が伴うと考えられる．ということは，ヒトiPS細胞の利点を生かすためには，まずはヒトES細胞の移植治療での問題の解決が必須となると考えられるのである．

また，ヒトES細胞の樹立と使用において重要な点は社会的な視点である．すなわち，通常の培養細胞の場合は由来者の研究利用に関する承諾が重要な位置を占めるが，ヒトES細胞の場合には，その元となる胚について両親あるいは母親の承諾があれば処分することができるものかについて，諸説ある．例えば，先に述べたSTRを用いた個体識別法を考えると，ヒトES細胞については由来者が存在しないのである．すなわち，胚の遺伝子構成は両親の組み合わせではあるのだが，生きている人間の誰のものでもないのである．そのため，ヒトES細胞の個別識別情報を公開したところで，その両親の可能性は多くあり，胚の側から親を特定する情報を得られる可能性はごく少ない．このあたりに，ヒトES細胞は由来者を想定できる通常培養細胞と異なった性質をもつ．

もう一つ気になることがヒトiPS細胞についてある．それは，新聞や政府の研究費支給においてヒトiPS細胞がもつ夢のような可能性を喧伝しすぎたために，例えば患者からiPS細胞樹立のために組織をもらうときに問題が生じることである．患者にとって，新聞の記事が喧伝してきたiPS細胞による治療の可能性は大きな希望である．しかし，その希望に反して実際はまだまだ研究が必要な分野であることが理解されていない．それゆえに例えば自分の治療にそのiPS細胞が使えないのかと詰め寄られた研究者の誠実な説明が大変に不誠実に見えるという懸念がある．科学技術の可能性を論じるのはよいのだが，それが現実離れしていると，現実と夢の間の齟齬が大きな問題を生む．ヒトES細胞の時はその提供者（由来者でない点に注意）にとってもそこから由来したES細胞は拒絶反応が起こる可能性があったが，iPS細胞は由来者の細胞として本来治療に使える可能性が高いというのが売りであり，研究の現段階の困難さを訴える以外に研究者が「患者の訴え」に応える合理的な説明はない．科学の発展の中で，社会的な問題，患者の心情を考えるとこのような大きな倫理的問題が生まれる．そしてこの問題は培養細胞は誰のものかという権利問題，ひいては特許問題へとつながっていく．

リサーチツールと特許問題

1 検証の場を提供するリサーチツールとしての培養細胞

前述したように，培養細胞には一定の条件で増え続け，多くの研究者が同じ細胞を用いて研究が行えるという大きな利点がある．この後に述べる‘リサーチツール’として有用性の高いものである．

科学の本質は，誰も最終的決定権をもたない，そして，誰も個人的権威をもたないということであると思う．その逆を考えるとわかりやすいが，「私が言っているのだから正しい」というのが一番非科学的なのである．そこで，科学では検証ということが重要となる．培養細胞は，その検証の場を提供する道具であり，同じ細胞を使って同じ条件で実験をすれば，同じ結果が得られるはずである．それと同時に，同じ細胞を異なった実験系において，同じ結論を示す，異なった結果を得るということも重要である．

2 リサーチツールの共有と特許・知的所有権

未来の研究に長期にわたって用いることを見越して，研究者は培養細胞の樹立に労力を惜しまない．そうして樹立された培養細胞が多くの研究者に，予想もできない新しい使われ方をすることによって，その真価を発揮できるという側面も存在する．そして，これらの培養細胞を用いた科学の検証を支えるためには，例えば細胞バンクによる品質管理が重要な役割を果たすのである（1章4，5参照）．と同時に，このような科学の重要性と必要性を提供者，社会に理解してもらうことが重要となる．

ここで述べた共有という問題を考えた時，特許は相反するように思われる．しかし，特許の一側面は，情

報を公開し共有する見返りに，その労力に相当する対価を発明者に保証することともいえる．共有は英語でshareであるが，“Give me my fair share” といえば「正当な分け前をよこせ」である．例えば，従来の研究者社会では，培養細胞の樹立者への敬意を，謝辞として表し，原論文を引用することで保証してきた．この伝統が単純に特許というもので置き換わるものではない点を意識しながら，培養細胞の恩恵を受ける者として，特許・知的所有権について現在進行中の議論が，科学を貫徹しにくくなる方向へ進まないように注意することが大切である．

特許の最近の動き

知的財産という言葉は昔から使われていたが，政府の主導のもとに2002年に「知的財産戦略会議」が設置され，その夏には，「知的財産戦略大綱」が公表され，2002年の末に「知的財産基本法」の成立，2003年の施行と同時の「知的財産戦略本部」の設置という動きによって，知的財産という言葉は，研究開発のメインストリームでの合言葉になった感がある．多くの提言，報告，指針などが公開されている．全国の大学および公的研究機関（以下，「大学等」）でもTLO（技術移転機関）が作られ，自分たちの知的財産の管理と活用を推進しようという機運が著しい．それらの動きは，もちろん広範な科学研究や開発に関わり，企業のみでなく大学に対しても積極的に活動することを求めるものである．知的財産立国という言葉が行きかう中，大きなうねりが生まれていることは事実であるが，これが定着するのにはまだ時間がかかるように思われる．

ここでは医学・生物研究の基盤となる培養細胞の作成と利用の問題を視野に入れ，特許問題の最近の動きについて概説する．

企業と大学，営利と非営利

1 特許権の効力範囲

企業にとって特許戦略はその命脈を司るものであり，多くの労力をその活用に使っている．また，知的財産基本法の中でも，第7条「大学等の責務等」，第12条「研究開発の促進」，第13条「研究成果の移転の促進等」の中で，大学等について述べている．

大学等にいると，「特許」というと企業の問題，営利活動の問題であり，「われわれアカデミアには関係ない話」と考えがちである．ところが，2004年11月の「特許発明の円滑な使用に係る諸問題について」（http://www.jpo.go.jp/shiryou/toushin/shingikai/pdf/strategy_wg_prob/00.pdf）の中で産業構造審議会知的財産政策部会特許制度小委員会特許戦略計画関連問題ワーキンググループは，大学等と企業，および営利と非営利の活動と特許権の効力の問題について検討を加え以下のように述べている．「大学等での研究活動については，わが国の特許法が営利又は非営利目的により他者の特許発明の実施に区別を設けていないことをかんがみると，実施者が企業（営利機関）か大学等（非営利機関）であるかの相違によって特許権の効力が及ぶ範囲が異なるものではない．これまで非営利機関である大学等を訴える利益に乏しかったこと等のさまざまな配慮により，実際に大学等が特許侵害により訴えられることはほとんどなかったが，今後産学官連携が進み活発化していけば，大学等が訴訟当事者となる場合も想定されることから，第69条第1項について正しい認識が求められる．（36～37ページ）」と述べている．

この見解の根拠として，この報告書は広範な諸外国（米国，英国，ドイツ，フランス，欧州，台湾，韓国，中国，インド，シンガポール）における「試験または研究」の例外について検討をし，特許技術の改良を目的とした研究開発のみを「試験または研究」の例外とすることが妥当であると結論している．ということは，特許技術を使用した研究開発はすべて特許権の効力の及ぶ範囲であるということである．

また2006年に公開された「大学等における政府資金を原資とする研究開発から生じた知的財産権についての研究ライセンスに関する指針」（http://www8.cao.go.jp/cstp/output/iken060523_2.pdf）において総合科学技術会議も，その注3では，産業構造審議会の2004年の意見を引き「非営利目的の研究であっても，特許権の侵害を問われ，研究が差止めの対象となる可能性も否定できない」と述べている．しかし同時に，「大学等の試験研究に対して特許権が及ぶか否かについての判決は（日本の裁判所からは）出ておらず，本規程（第69条第1項）についての判例は確立していない」と述べている．そして，指針の本文では多くの意見を踏まえて，総合科学技術会議の姿勢としては，政府資

金を原資とする非営利目的の大学等の研究開発については，「知の創造拠点である大学等の役割や大学等における研究の自由度の確保の重要性を踏まえ」ることを求めている．

2 リサーチツール特許のロイヤルティ

また，2007年に公表した「ライフサイエンス分野におけるリサーチツール特許の使用の円滑化に関する指針」(http://www8.cao.go.jp/cstp/output/iken070301.pdf)の中では，汎用性が高く代替性の低い上流技術として，ライフサイエンス分野における遺伝子改変動植物やスクリーニング方法のようなリサーチツール特許を取り上げ，その広範な流通の促進を訴えている．この中では，「大学等の間でのライセンス供与の場合は，大学等の学術振興の観点から，無償（有体物提供等に伴う実費を除く）とすることが望ましい」と述べている．しかし，同時に，「ライセンス供与にあたり，対価以外の妥当なライセンス条件が付与されることを妨げるものではないと」と述べている．これは，リーチスルークレーム（開示された発明に基づいて，将来なされるであろう発明に対するロイヤルティに対するクレーム）を認めるものである．

それと対応するように，2007年度の文部科学省大学知的財産本部調整事業の調査研究報告書「リサーチツール特許使用の円滑化に係る調査研究」(http://ipw.naist.jp/cast/_research/reasercht.pdf）においては，三極合意におけるリーチスルークレームが例外的なケースを除いて認められなくなったという状況にもかかわらず，大学等に対するアンケート調査においては，約20％が「リサーチツールの成果から，開発段階に応じたマイルストーンあるいは成果の売り上げに応じたロイヤルティを得ることについて『当然いただくべき』」と述べている．

また，少し古いアンケートになるが，2004年1月に公表された「ライフサイエンス分野におけるリサーチツール特許の使用に関するアンケート調査結果」（日本製薬工業協会，JBA，http://www.jpo.go.jp/shiryou/toushin/shingikai/pdf/strategy_wg06/paper06_b1.pdf）によると，企業でも成功するかわからないリサーチツールについて，「精査の上，交渉に入る」が半数を占め，そのほかは「ある程度成果が出るまで無視して実施する」あるいは「研究目的であり，権利侵害にあたらないと判断して実施する」という対応が続いている．

また，この調査結果は，リサーチツール特許のロイヤルティにおいて，「最終製品の売り上げに関する権利」を要求される場合が，「一時金」の要求に続いて2番目に多いことを示している．

3 日本における特許侵害に対する動き

日本における特許に関する文書の中で，自分たちの特許権の防衛や権利については声高に主張されているが，それは翻れば，自分たちが他人の特許権の使用に忠実にロイヤルティを払うのかという問題を無視しているように見える．この後者の自分たちに請求される特許料の問題について，多くの報告書や指針は述べていない．企業にとって特許戦略は単純に金のやり取りの問題であると言われる．多額の費用を使って侵害訴訟に勝つよりも，一時金として支払いをしたほうが得な場合はそれをとるのが常識であろう．例えば国際特許侵害で米国や欧州で訴訟を起こされれば数億単位の裁判資金が必要である．負けた場合にはさらに多額の費用を払う必要がある．とするならば数億程度の要求なら飲んだほうが得だという判断をするという．

大学等が「政府資金を原資とする」研究について侵害訴訟を受けた時にどのような対応が取れるだろうか．ただ，金で解決をするだけの資金もなく，ましてや裁判費用を捻出をすることもできない状況となれば，どうなるのだろうか．その点で，2007年度に行われた「リサーチツール特許使用の円滑化に係る調査研究」において，大学がどれほどの特許侵害の警告を受けたかの調査がないのは残念である．

特許法の精神は科学技術の進歩のために，技術を公開するかわりにその技術に特許権を与えるというものだとすると，自分たちが特許侵害をする可能性について考えることが重要となるだろう．例えば，大学等が「研究使用のみ」という条件の下に技術供与，有体供与を受けて研究・開発を始めたとしてそれが成功して，企業と組んで特許申請をしたとすると，この時点で先の「研究使用のみ」という条項から外れるということは当然であろう．

「これまで非営利機関である大学等を訴える利益に乏しかったこと」が大学等が特許侵害訴訟を訴えられない理由とされている．しかし，それ以外にも，特許技術の価値を高めるためには，それが利用されて産業的な意味で良い成果が出ることが重要であり，大学等での特許技術の利用は，特許を保有する機関にしてみ

れば，無償で研究開発をして当該特許技術の価値を高めてくれるという点で重要であると考えられているという．これがもし成り立つならば，果実が育つまで待っておいしいところを手に入れようという話である．そして，出口ではリサーチツールの成果物に応じたロイヤルティの支払いが成り立つ必要がある．

培養細胞の話からはかなり外れたように考えられるかもしれない．ただし，市販の発現ベクターを利用して細胞機能を改変し，アッセイ系を作り成功したとする．このときに，市販のベクターについているResearch Use Onlyの範囲を超える前には，立ち止まって考える必要があるということである．

4 海外での事例

海外においても大学が特許侵害で訴えられたケースは少ない．しかし，米国では2002年に有名なDuke大学が訴えられ，敗訴した．ここでは大学が特許技術を利用する行為がその「組織の正当な業務の遂行のためであって，『娯楽のため，単なる好奇心を満たすため，又は厳密に哲学的な探求のため』といえない場合には，『試験的使用の例外』は適応されない」〔Madley v. Duke University, 307 F.3d 1351（Fed. Cir. 2002)〕とされたという．これは国際的にみてもかなり極端であると言われるが，米国企業が例えば日本の大学等の特許侵犯行為を米国で訴える時には，日本の大学等にとっては不利なものとなりかねない．

リサーチツールとしての培養細胞と培養技術

培養細胞や培養技術はリサーチツールであり，それが特許化されていることが，あるいは自ら特許化しようとするときに，先行特許などについて，また，そのときの特許動向について，また国による違いについて知っている必要があると考えられる時代になった．ただ，特許の専門家の中には，大学等の先生はそんなことを考えず，ともかく新しいことを発見発明すればよいという意見がある．しかし，ここには大きな前提がある．それは，それらを代行してくれる特許部門が充実していればの話である．先のアンケート調査「リサーチツール特許使用の円滑化に係る調査研究」においては，ある開発にあたっての先行特許調査は国内のみしか実施していないが75％を占める．この状況では，せっかく開発したものが論文になったとしても先行特許のため企業化へは結びつかないということが起きる．一方で企業を対象とした「ライフサイエンス分野におけるリサーチツール特許の使用に関するアンケート調査結果」では，探索研究に着手する前に出願・公開された特許まですべてを調査することが半数以上の企業で行われている．

おわりに

リサーチツール特許の動きについて述べてきたが，世界的動向，また日本国内での知財熱の中で，好むと好まざるとにかかわらずここで述べた特許の問題に巻き込まれる可能性がある．個人の姿勢の問題ではとても片付かない大きな問題であることは確かである．

倫理問題と特許問題は全く異なった領域のようではあるが，実は社会の中での科学技術の問題として一つのくくりで論じることができることは，ES細胞とiPS細胞を論じた部分で感じてもらえたことと思う．われわれが行っている研究が，基礎研究であったとしても社会の中で行われ，特に政府資金を原資にして行われているということを注意深く考える必要があり，制度設計を間違えるととんでもないところに至ってしまう可能性があることを強く感じる．研究者の皆様も，安穏と研究をしているだけで済む時代でなくなったことを理解していただく必要があるように思う．私自身が「他人の役に立つような研究をするなんてみっともない」という古典的な理学部の中で育ったことを考えると時代の移り代わりの速さと厳しさを感じる．

Impact of alcohol consumption with polymorphisms in alcohol-metabolizing enzymes on pancreatic cancer risk in Japanese

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The putative impact of alcohol on pancreatic cancer (PC) risk remains controversial. Here, we conducted a case-control study in Japanese to assess the impact of alcohol in conjunction with polymorphisms in alcohol-metabolizing enzymes. Cases were 160 patients with pancreatic cancer at Aichi Cancer Center, Nagoya, Japan. Two control groups of 800 age- and sex-matched non-cancer subjects each were independently selected. The impact of alcohol and polymorphisms in *aldehyde dehydrogenase 2* (*ALDH2*) Glu504Lys, *alcohol dehydrogenase* (*ADH*) *1B* His48Arg, and *ADH1C* Arg272Gln on PC risk was examined with multivariate analysis adjusted for potential confounders to estimate odds ratios (ORs) and 95% confidence intervals (CIs). Results showed no independent impact of alcohol or genotype on PC risk except former drinking. To avoid reverse causation, former drinkers were excluded in further analyses. In the analysis of the combined effects of alcohol consumption and genotype, significant impact of alcohol was seen for those subjects with *ALDH2* Lys+ allele, *ADH1B* His/His, or *ADH1C* Arg/Arg (trend $P = 0.077$, 0.003, or 0.020, respectively), each of which is associated with a high concentration or rapid production of acetaldehyde. Analysis of genotype combinations showed that 'ever drinking' with both *ADH1B* His/His and *ALDH2* Lys + was the most potent risk factor for PC relative to 'never drinkers' with both *ADH1B* His/His and *ALDH2* Glu/Glu [OR (95% CI); 4.09 (1.30–12.85)]. These results indicate that alcohol has an impact on PC risk when the effects of alcohol consumption and metabolism are combined. Acetaldehyde may be involved in the mechanisms underlying PC development. (*Cancer Sci* 2009; 100: 296–302)

The mortality of pancreatic cancer (PC) in Japan is increasing, and is now the sixth leading cause of cancer death. The age-adjusted incidence rates and mortality of PC are 9.1 and 8.4 for men and 5.3 and 4.9 for women, respectively.[1] Because of the difficulty in detecting this cancer in the early operable stage and lack of any curative treatment apart from complete surgical removal, 5-year relative survival rate is only 5.5%.[2] Epidemiological research of PC risk should therefore play an important role in both prevention and decreasing the number of PC deaths.

Lifestyle and other risk factors known to affect the incidence of PC include age, smoking, obesity, diabetes mellitus, chronic pancreatitis, and family history of PC.[3–7] The effect of alcohol consumption on risk has also been investigated in many case-control or cohort studies, but results have been inconsistent.[7–14] In many studies, the impact of alcohol disappeared after adjustment for potential confounders, particularly smoking habits,[12–14] while several groups found a significant impact of alcohol even after adjustment for confounders.[10,11] In our previous report, an impact of alcohol was seen only among former drinkers, and not among current drinkers.[7]

Alcohol is first oxidized to acetaldehyde by the alcohol dehydrogenase (ADH) enzymes, particularly ADH1B and ADH1C. Acetaldehyde is further oxidized to acetate by aldehyde dehydrogenase (ALDH) enzymes, to which ALDH2 is the major contributor. Encoding genes display polymorphisms that modulate individual differences in alcohol-oxidizing capability.[15,16] Regarding ADH1B His48Arg (rs1229984), the 48His allele represents a superactive subunit of ADH1B which has about a ×40 higher maximum velocity (Vmax) than the less active *ADH1B* Arg/Arg form of ADH1B.[15,16] The *ADH1C* 272Arg allele represents a superactive subunit of ADH1C which has a ×2–3 higher Vmax than the *ADH1C* 272Gln allele (rs1693482).[15,16] The *ADH1B* and *ADH1C* genes are located close together in the short arm of chromosome 4, and *ADH1B* His48Arg and *ADH1C* Arg272Gln polymorphisms are considered to be in linkage disequilibrium.[17–19] However, *ADH1B* does not necessarily predict the *ADH1C* locus among Japanese.[20] As for the *ALDH2* Glu504Lys polymorphism (rs671), the 504Lys allele encodes a catalytically inactive subunit.[15,16] Individuals with the *ALDH2* Glu/Lys genotype have only 6.25% of normal ALDH2 504Glu protein, indicating a dominant negative effect of *ALDH2* 504Lys.[21] The *ADH1B* 48His, *ADH1C* 272Arg, and *ALDH2* 504Lys alleles, associated with higher accumulation or rapid production of acetaldehyde, are clustered in Asian populations such as Japanese.[20,22–24] Therefore, these three genetic polymorphisms modify toxic acetaldehyde exposure and are expected to affect cancer risk, especially in Asian populations in whom minor alleles are common.

Here, we conducted an age- and sex-matched case-control study to explore the impact of alcohol consumption in conjunction with genetic polymorphisms in alcohol-metabolizing enzymes on PC risk among Japanese.

Materials and Methods

Study subjects. Cases were 160 PC patients with no prior history of cancer who were diagnosed at Aichi Cancer Center Hospital (ACCH), Nagoya, Japan, between January 2001 and November 2005. To avoid spurious associations, we used two independent non-cancer control groups [control 1 (C1), $n = 800$; control 2 (C2), $n = 800$] to give an overall case : control ratio of 1:5. Sex- and age-matched (±2 years) C1 and C2 subjects were independently selected from outpatients who visited ACCH during the same period without a history of any cancer. When results from C1 and C2 were consistent, we pooled controls (C1 + C2) for analysis.

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All subjects were enrolled at first visit to ACCH in the hospital-based Epidemiological Research Program II at ACCH (HERPACC-II) between January 2001 and November 2005. The framework of HERPACC-II has been described elsewhere.[25,26] Briefly, all first-visit outpatients at ACCH aged 20–79 years were asked to fill out a self-administered questionnaire regarding lifestyle items before the development of current symptoms, which was then checked by trained interviewers. Outpatients were also asked to provide a 7-mL blood sample. Approximately 95% of eligible subjects completed the questionnaire and 50% provided blood samples. All data were loaded into the HERPACC database and linked periodically with the hospital cancer registry system to update the data on cancer incidence. Our previous study showed that the lifestyle patterns of first-visit outpatients were in accordance with those in a general population randomly selected from Nagoya, confirming external validity for the study.[27] This study was approved by the Ethics Committee of Aichi Cancer Center Institute. Informed consent was obtained at first visit from all participants.

Genotyping of *ALDH2*, *ADH1B*, and *ADH1C*. DNA of each sample was extracted from the buffy coat fraction using a BioRobot EZ1 with an EZ1 DNA blood 350 µL kit or QIAamp DNA blood mini kit (Qiagen K.K., Tokyo, Japan). Polymorphisms of alcohol-metabolizing enzymes *ALDH2* Glu504Lys, *ADH1B* His48Arg, and *ADH1C* Arg272Gln were examined based on TaqMan assays by Applied Biosystems (Foster City, CA, USA). The principle of the TaqMan real-time polymerase chain reaction (PCR) assay system using fluorogenic probes and 5′ nuclease has been described by Livak.[28] All of the assays were done in 96-well PCR plates using a 7500 Fast Real-Time PCR System (Applied Biosystems) coupled with the 7500 Fast System SDS software. Amplification reactions (5 µL) were done in duplicate with 30 ng of template DNA, 2× TaqMan Universal Master Mix buffer (Applied Biosystems), 20× primer and probe mix (Applied Biosystems). Thermal cycling was initiated with a first denaturation step of 20 s at 95°C, and then by 40 cycles of 3 s at 95°C and 30 s at 62°C. Genotyping quality was statistically assessed using the Hardy–Weinberg test in our laboratory; when allelic distributions for controls departed from the Hardy–Weinberg frequency, genotyping was assessed using another method.

Assessment of exposure. Daily alcohol consumption in grams was determined by summing the pure alcohol amount in the average daily consumption of Japanese sake (rice wine), shochu (distilled spirit), beer, wine and whiskey, with one cup of Japanese sake (180 mL) considered equivalent to 25 g of ethanol; one large bottle of beer (720 mL) to 25 g; one glass of wine (80 mL) to 10 g; and one shot of whiskey (28.5 mL) to 12.5 g. One drink of shochu, which contains 25% ethanol, was estimated at 108 mL and 27 g of ethanol. Cumulative smoking exposure was evaluated as pack-years, the product of the average number of packs per day and the number of years of smoking. Height and body weight at baseline and weight at age 20 years were self-reported. Current body mass index (BMI) and BMI at age 20 were calculated as current weight and weight at age 20 divided by height squared, respectively, and expressed as kg/m^2. Family history of pancreatic cancer was considered positive when at least one parent or sibling had a history of pancreatic cancer.

Statistical analysis. All statistical analyses were performed using Stata version 10 (Stata Corp., College Station, TX, US). A *P*-value <0.05 was considered statistically significant. Accordance with the Hardy–Weinberg equilibrium among controls was checked with the χ^2-test to assess discrepancies between expected and observed genotype and allele frequencies. Differences in characteristics between cases and controls were compared with the χ^2-test, and the Mann–Whitney test was used to compare age distribution between two groups. Frequency of alcohol consumption was categorized into the five levels of never, rare, 1–2 times, 3–4 times, and 5–7 times per week. Drinking status was categorized into the three groups of never drinkers, former drinkers, and current drinkers. Drinking experience was categorized into the two groups of never drinkers and ever drinkers. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using an unconditional logistic regression model adjusted for potential confounders. Potential confounders considered in multivariate analysis were age as a continuous variable, sex (male or female), pack-years of smoking (0, 1–20, 21–40, ≥41), current BMI (<20.0, 20.0–22.4, 22.5–24.9, 25–29.9, ≥30.0 kg/m^2), BMI at age 20 years (<20.0, 20.0–22.4, 22.5–24.9, 25–29.9, ≥30.0 kg/m^2), history of diabetes (yes or no), and family history of PC (yes or no). Among *ALDH2*, *ADH1B*, and *ADH1C* polymorphisms, the two polymorphisms other than that under evaluation were included as confounders when appropriate.

Trends in alcohol impact were assessed using a score test for average daily ethanol consumption, with scores of 0, none; 1, <30 g; and 2, ≥30 g. Gene–environmental interactions were assessed by the logistic model, which included interaction terms between ethanol consumption and genes with scores of 0, homozygote in major alleles; and 1, others.

Results

Table 1 shows baseline characteristics of case subjects and the two independent control groups. Men accounted for 70.6% of all subjects. Findings were consistent across both control groups. A significant prevalence of more than 40 pack-years of smoking was seen among case subjects. A history of diabetes was also significantly common in cases as compared to each control group. A current BMI <22.5 was more prevalent in cases, whereas the distribution of BMI at 20 years did not significantly differ between cases and the two controls.

Distributions for alcohol-related characteristics and their adjusted ORs with 95% CIs for PC are shown in Table 2. A significantly increased risk of PC was seen in former drinkers [pooled controls: adjusted OR (95% CI), 4.71 (2.74–8.08)] but not in current drinkers [pooled controls: 1.18 (0.79–1.78)] relative to never drinkers. Adjusted ORs (95% CIs) for frequency of alcohol consumption per week relative to never drinkers among pooled controls were 1.37 (0.70–2.71) for less than once per week (rare drinkers), 0.93 (0.44–1.98) for 1–2 times, 1.99 (1.14–3.45) for 3–4 times, and 1.61 (1.04–2.49) for 5–7 times, showing an increase in OR with drinking frequency (trend $P = 0.026$). To further analyze the impact of alcohol on PC risk, we categorized drinkers into two groups according to average alcohol consumption per day, calculated as the product of pure alcohol consumption of reported alcoholic drinks per day and drinking frequency. We defined drinkers with an intake of <30 g alcohol/day as 'moderate' drinkers and those with an intake of 30 g or more as 'heavy' drinkers. Overall, an impact of alcohol consumption on PC risk was observed among heavy drinkers [pooled controls: adjusted ORs (95% CIs) for alcohol consumption: 1.44 (0.96–2.15) for moderate and 1.92 (1.14–3.21) for heavy drinkers relative to never drinkers], and PC risk increased with alcohol consumption (trend $P = 0.012$). However, if former drinkers were excluded, the impact of alcohol among heavy drinkers disappeared [pooled controls: adjusted OR (95% CI), HR 1.39 (0.79–2.45)].

Adjusted ORs (95% CIs) for PC by genotype distributions of *ALDH2*, *ADH1B*, and *ADH1C* genotype are shown in Table 3. The distribution of these three genotypes was in accordance with expected values according to the Hardy–Weinberg equilibrium. Adjusted ORs (95% CIs) of *ALDH2* Glu/Lys and Lys/Lys were 1.29 (0.91–1.81) and 0.65 (0.32–1.34) relative to *ALDH2* Glu/Glu. In addition to *ALDH2*, the *ADH1B* and *ADH1C* genotypes were also not found to be independent risk factors.

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Table 1. Characteristics of case and control subjects

		Cases (%)	Control 1 (%)		Control 2 (%)	
		(n = 160)	(n = 800)	P-value	(n = 800)	P-value
Age	Median (range)	60 (28–78)	60 (27–79)	0.927	60 (26–79)	0.881
Sex						
	Men	113 (70.6)	565 (70.6)	1.000	565 (70.6)	1.000
	Women	47 (29.4)	235 (29.4)		235 (29.4)	
Pack-years of smoking						
	0	56 (35.0)	340 (42.5)	0.013	350 (43.8)	0.009
	1–20	21 (13.1)	132 (16.5)		125 (15.6)	
	21–40	31 (19.4)	155 (19.4)		152 (19.0)	
	≥41	51 (31.9)	159 (19.9)		157 (19.6)	
	Unknown	1 (0.6)	14 (1.8)		16 (2.0)	
History of diabetes						
	No	126 (78.8)	734 (91.8)	<0.001	737 (92.1)	<0.001
	Yes	34 (21.3)	66 (8.3)		63 (7.9)	
Current BMI (kg/m^2)						
	<20.0	33 (20.6)	118 (14.8)	0.051	115 (14.4)	0.075
	20.0–22.4	51 (31.9)	215 (26.9)		207 (25.9)	
	22.5–24.9	42 (26.3)	265 (33.1)		274 (34.3)	
	25.0–29.9	30 (18.8)	188 (23.5)		179 (22.4)	
	≥30.0	4 (2.5)	8 (1.0)		18 (2.3)	
	unknown	0 (0.0)	6 (0.8)		7 (0.9)	
BMI at age 20 years (kg/m^2)						
	<20.0	50 (31.3)	264 (33.0)	0.129	244 (30.5)	0.109
	20.0–22.4	58 (36.3)	330 (41.3)		368 (46.0)	
	22.5–24.9	33 (20.6)	146 (18.3)		130 (16.3)	
	25.0–29.9	11 (6.9)	38 (4.8)		38 (4.8)	
	≥30.0	2 (1.3)	1 (0.1)		3 (0.4)	
	Unknown	6 (3.8)	21 (2.6)		17 (2.1)	
Family history of pancreatic cancer						
	No	152 (95.0)	770 (96.3)	0.459	770 (96.3)	0.459
	Yes	8 (5.0)	30 (3.8)		30 (3.8)	

Table 2. Odds ratios (ORs) of pancreatic cancer by alcohol-related characteristics

	Cases	Pooled controls		Control 1		Control 2	
	n	n	ORs[†] (95% CI)	n	ORs[†] (95% CI)	n	ORs[†] (95% CI)
Drinking status							
Never drinkers	47	602	1.00 (reference)	304	1.00 (reference)	298	1.00 (reference)
Former drinkers	33	75	4.71 (2.74–8.08)	43	4.29 (2.39–7.72)	32	5.24 (2.84–9.64)
Current drinkers	80	923	1.18 (0.79–1.78)	453	1.25 (0.81–1.92)	470	1.11 (0.73–1.70)
			Trend P = 0.755		Trend P = 0.572		Trend P = 0.977
Drinking frequency per week							
None	47	602	1.00 (reference)	304	1.00 (reference)	298	1.00 (reference)
<1 time	12	119	1.37 (0.70–2.71)	62	1.39 (0.68–2.85)	57	1.32 (0.64–2.70)
1–2 times	9	126	0.93 (0.44–1.98)	69	0.87 (0.40–1.90)	57	0.96 (0.43–2.13)
3–4 times	23	160	1.99 (1.14–3.45)	70	2.28 (1.26–4.16)	90	1.67 (0.93–2.98)
≥5 times	69	589	1.61 (1.04–2.49)	295	1.69 (1.07–2.69)	294	1.56 (0.99–2.45)
Unknown	0	4	NA[‡]	0	NA[‡]	4	NA[‡]
			Trend P = 0.026		Trend P = 0.013		Trend P = 0.074
Alcohol consumption per day							
0 g	47	602	1.00 (reference)	304	1.00 (reference)	298	1.00 (reference)
<30 g	77	745	1.44 (0.96–2.15)	371	1.50 (0.98–2.31)	374	1.37 (0.90–2.08)
≥30 g	36	254	1.92 (1.14–3.21)	125	1.99 (1.15–3.46)	129	1.79 (1.04–3.08)
			Trend P = 0.012		Trend P = 0.012		Trend P = 0.032

[†]ORs were adjusted for age, sex, pack-years of smoking, history of diabetes, current BMI, BMI at 20 years, and family history of pancreatic cancer.
[‡]NA indicates not available because of the absence of subjects in this category.

As shown in Tables 2 and 3, ORs and trends for alcohol-related characteristics and genotype distributions were consistent across the two control groups. We therefore pooled data for the two control groups in later analyses. In addition, to exclude possibility of reverse causation, former drinkers were excluded in these later analyses on the assumption that alcohol-related diseases due to long exposure of alcohol, such as alcoholic pancreatitis, might influence the reporting of drinking status.

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Table 3. Odd ratios (ORs) of pancreatic cancer by genotype distribution of *ALDH2*, *ADH1B*, and *ADH1C* genotypes

		Cases	Pooled controls		Control 1		Control 2	
		n	*n*	ORs[†] (95% CI)	*n*	ORs[†] (95% CI)	*n*	ORs[†] (95% CI)
ALDH2								
	Glu/Glu	74	790	1.00 (reference)	404	1.00 (reference)	386	1.00 (reference)
	Glu/Lys	77	653	1.29 (0.91–1.81)	325	1.35 (0.94–1.94)	328	1.22 (0.85–1.75)
	Lys/Lys	9	157	0.65 (0.32–1.34)	71	0.79 (0.37–1.67)	86	0.55 (0.26–1.17)
ADH1B								
	His/His	101	975	1.00 (reference)	482	1.00 (reference)	493	1.00 (reference)
	His/Arg	55	551	1.00 (0.70–1.43)	274	0.99 (0.68–1.43)	277	1.00 (0.69–1.45)
	Arg/Arg	4	74	0.51 (0.18–1.45)	44	0.47 (0.16–1.35)	30	0.55 (0.18–1.63)
ADH1C								
	Arg/Arg	140	1428	1.00 (reference)	711	1.00 (reference)	717	1.00 (reference)
	Arg/Gln	20	169	1.22 (0.73–2.01)	86	1.20 (0.71–2.05)	83	1.28 (0.75–2.19)
	Gln/Gln	0	3	NA[‡]	3	NA[‡]	0	NA[‡]

[†]ORs were adjusted for age, sex, pack-years of smoking, history of diabetes, current BMI, BMI at 20 years, and family history of pancreatic cancer.
[‡]NA indicates not available because of the absence of subjects in this category.
ALDH2, aldehyde dehydrogenase 2; *ADH1B*, alcohol dehydrogenase 1B; *ADH1C*, alcohol dehydrogenase 1C.

Table 4. Combined impact of alcohol consumption with *ALDH2*, *ADH1B*, or *ADH1C* genotypes on pancreatic cancer risk among case and control subjects, excluding former drinkers

Alcohol consumption/day	Cases *n*	Pooled controls *n*	Genotype ORs[†]	(95% CI)	*P*-value	Cases *n*	Pooled controls *n*	Genotype ORs[†]	(95% CI)	*P*-value	Interaction *P*[‡]
ALDH2			Glu/Glu (normal)					Lys+ (weak)			
0 g	8	143	1.00	(reference)		39	459	1.67	(0.75–3.75)	0.211	
<30 g	27	423	1.35	(0.58–3.18)	0.485	28	268	2.26	(0.93–5.46)	0.072	
≥30 g	18	184	1.98	(0.77–5.13)	0.159	7	48	3.27	(1.03–10.44)	0.045	
			Trend *P* = 0.284					Trend *P* = 0.077			0.920
ADH1B			His/His (rapid)					Arg+ (slow)			
0 g	28	392	1.00	(reference)		19	210	1.17	(0.61–2.21)	0.640	
<30 g	32	422	1.44	(0.80–2.57)	0.221	23	269	1.42	(0.75–2.70)	0.286	
≥30 g	16	114	2.99	(1.39–6.44)	0.005	9	118	1.30	(0.54–3.17)	0.558	
			Trend *P* = 0.003					Trend *P* = 0.722			0.096
ADH1C			Arg/Arg (rapid)					Gln + (slow)			
0 g	41	550	1.00	(reference)		6	52	1.59	(0.61–4.12)	0.341	
<30 g	45	613	1.31	(0.80–2.15)	0.289	10	78	2.52	(1.11–5.69)	0.026	
≥30 g	21	200	2.07	(1.05–4.06)	0.035	4	32	2.47	(0.74–8.25)	0.141	
			Trend *P* = 0.020					Trend *P* = 0.644			0.774

[†]Odd ratios (ORs) were adjusted for age, sex, pack-years of smoking, history of diabetes, current BMI, BMI at 20 years, family history of pancreatic cancer and nonevaluated two polymorphisms.
[‡]Interactions evaluated in the model included age, sex, pack-years of smoking, history of diabetes, current BMI, BMI at 20 years, family history of pancreatic cancer, alcohol consumption by score (none: 0, <30 g: 1 and ≥30 g: 2), *ALDH2*, *ADH1B*, or *ADH1C* by score (*ALDH2* Glu/Glu: 0, Lys+ : 1, *ADH1B* His/His: 0, Arg+ : 1 and *ADH1C* Arg/Arg:0, Gln+ : 1), and the cross-product of scores.
ALDH2, aldehyde dehydrogenase 2; *ADH1B*, alcohol dehydrogenase 1B; *ADH1C*, alcohol dehydrogenase 1C.

To assess the influence of alcohol and acetaldehyde metabolism in PC risk, we evaluated the impact of the combination of *ALDH2*, *ADH1B*, or *ADH1C* genotypes with daily alcohol consumption (Table 4). Overall, no impact of alcohol consumption on PC risk was observed among cases and controls. However, on combination of *ALDH2* genotype and alcohol consumption, adjusted ORs (95% CIs) of moderate and heavy drinkers with the *ALDH2* Glu/Glu or Lys + allele relative to never drinkers with *ALDH2* Glu/Glu were 1.35 (0.58–3.18) and 1.98 (0.77–5.13) for those with *ALDH2* Glu/Glu, and 2.26 (0.93–5.46) and 3.27 (1.03–10.44) for those with *ALDH2* Lys+, and alcohol consumption showed a borderline trend to increased PC risk in *ALDH2* Lys+ (*ALDH2* Glu/Glu, trend-$P = 0.284$, *ALDH2* Lys+, trend-$P = 0.077$). Among those with the *ADH1B* His/His genotype, adjusted ORs (95% CIs) for PC with alcohol consumption were 1.44 (0.80–2.57) for moderate and 2.99 (1.39–6.44) for heavy drinkers, compared with never drinkers, with this trend being significant (trend $P = 0.003$). In contrast, the trend was not significant among those with *ADH1B* Arg+ allele (trend $P = 0.722$). With regard to the *ADH1C* genotype, trends were similar to those for the *ADH1B* genotype (*ADH1C* Arg/Arg, trend $P = 0.020$, *ADH1C* Gln+, trend $P = 0.644$). Interaction of the *ADH1B* genotype with alcohol consumption was marginally significant, suggesting the existence of a gene–environment association between alcohol consumption and alcohol metabolizing enzymes (interaction $P = 0.096$).

The combined impact of *ALDH2* genotype with either *ADH1B* or *ADH1C* genotype and drinking experience on PC risk is further explored in Table 5. Adjusted ORs (95% CIs) of current drinkers with both *ADH1B* His/His and *ALDH2* Glu/Glu or Lys+, relative

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Table 5. Combined impact of *ALDH2* with *ADH1B* or *ADH1C* genotypes and drinking experience on pancreatic cancer risk among case and control subjects

	ALDH2 Glu/Glu (normal)			*ALDH2* Lys+ (weak)		
	Cases *n*	Pooled controls *n*	ORs† (95% CI)	Cases *n*	Pooled controls *n*	ORs† (95% CI)
ADH1B His/His (rapid)						
Never drinkers	4	94	1.00 (reference)	24	298	2.09 (0.70–6.29)
Current drinkers	24	367	1.78 (0.58–5.45)	24	169	4.09 (1.30–12.85)
ADH1B Arg + (slow)						
Never drinkers	4	49	1.88 (0.44–7.97)	15	161	2.21 (0.69–7.09)
Current drinkers	21	240	2.15 (0.68–6.80)	11	147	1.84 (0.54–6.33)
ADH1C Arg/Arg (rapid)						
Never drinkers	8	133	1.00 (reference)	33	417	1.48 (0.65–3.36)
Current drinkers	35	540	1.27 (0.55–2.95)	31	273	2.29 (0.95–5.51)
ADH1C Gln + (slow)						
Never drinkers	0	10	NA‡	6	42	2.69 (0.84–8.58)
Current drinkers	10	67	3.09 (1.07–8.90)	4	43	2.07 (0.55–7.78)

†Odds ratios (ORs) were adjusted for age, sex, pack-years of smoking, history of diabetes, current BMI, BMI at 20 years, family history of pancreatic cancer, and nonevaluated polymorphisms.
‡NA indicates not available because of the absence of subjects in this category.
ALDH2, aldehyde dehydrogenase 2; *ADH1B*, alcohol dehydrogenase 1B; *ADH1C*, alcohol dehydrogenase 1C.

to never drinkers with both *ADH1B* His/His and *ALDH2* Glu/Glu were 1.78 (0.58–5.45) and 4.09 (1.30–12.85), respectively. While adjusted ORs (95% CIs) of current drinkers with *ADH1B* Arg+ and *ALDH2* Glu/Glu or Lys+, relative to never drinkers with both *ADH1B* His/His and *ALDH2* Glu/Glu were 2.15 (0.68–6.80) and 1.84 (0.54–6.33), respectively. These findings show that the combination of *ADH1B* His/His with *ALDH2* Lys+ for current drinkers was the most potent risk factor for PC. With regard to combinations of *ADH1C* and *ALDH2* genotypes, PC risk among current drinkers with the combination of *ADH1C* Arg/Arg with *ALDH2* Lys+ allele relative to never drinkers with both *ADH1C* Arg/Arg and *ALDH2* Glu/Glu was marginally significant [adjusted ORs (95% CIs): 2.29 (0.95–5.51)]. The OR (95% CI) for current drinkers with the combination of *ADH1C* Gln+ with *ALDH2* Glu/Glu allele relative to never drinkers with both *ADH1C* Arg/Arg and *ALDH2* Glu/Glu was 3.09 (1.07–8.90).

Discussion

Here, we found that the risk of PC was increased with alcohol consumption in subjects with the *ALDH2* Lys+ allele, or *ADH1B* His/His or *ADH*1 Arg/Arg genotypes, but not in those with the *ALDH2* Glu/Glu genotype, or *ADH1B* Arg+ or *ADH1C* Gln+ alleles. Combined analysis of *ALDH2* with the *ADH1B* or *ADH1C* genotypes demonstrated a significant impact of alcohol in subjects with both *ALDH2* Lys+ and *ADH1B* His/His relative to those with both *ALDH2* Glu/Glu and *ADH1B* His/His. To our knowledge, this is the first study to examine the combined significance of alcohol consumption and each of the *ADH1B*, *ADH1C*, and *ALDH2* genotypes in PC risk.

The carcinogenic effect of acetaldehyde in various types of cancer has been shown in experimental studies.[29,30] Given that the metabolisms of alcohol and acetaldehyde are strongly influenced by genetic polymorphisms of alcohol-metabolizing enzymes, namely *ALDH2* Glu504Lys, *ADH1B* His48Arg, and *ADH1C* Arg272Gln, evaluation of the effect of these polymorphisms on cancer risk in combination with alcohol consumption is worthwhile. Consistent with previous reports,[12–14] we found that an impact of alcohol consumption on PC risk was not recognized if genotypes of *ALDH2*, *ADH1B*, and *ADH1C* were not taken into consideration. However, stratification of analyses by the respective genotypes revealed a significant or marginally significant impact of alcohol consumption on PC risk among subjects with *ALDH2* Lys+, *ADH1B* His/His, and *ADH1C* Arg/Arg. These findings suggest that among populations in which any of these three genotypes is prevalent, the association between alcohol and PC may be null unless the genotype is included as a potential confounder.

ALDH2 of the 504Lys allele has been shown to be an inactive form exerting a dominant negative effect on alcohol-metabolizing activity *in vitro*.[15,16] Peng *et al.* validated this negative effect in human studies, showing that among subjects with the *ALDH2* Glu/Lys genotype, the peak in acetaldehyde blood concentration and area under the curve (AUC) for acetaldehyde after the intake of 0.5 g/kg ethanol were about 20 and 30 times higher than respective values in subjects with *ALDH2* Glu/Glu.[31] On this basis, pancreatic cells in individuals with the *ALDH2* Glu/Lys genotype would be exposed to a considerably larger amount of acetaldehyde after ingestion of alcohol. This striking difference in acetaldehyde metabolism would also explain why the impact of alcohol was observed only among subjects with the *ALDH2* Lys+ allele. In contrast, among subjects with *ALDH2* Glu/Glu, acetaldehyde peak and AUC were not statistically different between those with *ADH1B* His/His and *ADH1B* Arg/Arg,[31] although the *ADH1B* 48His allele represents a superactive subunit of ADH1B with a higher Vmax than the other allele.[15,16] Considering these previous and present results, we speculate that the rapid production and exposure of acetaldehyde within pancreatic cells exposed to alcohol influences the risk of PC with regard to *ADH1B* and *ADH1C*.

In the additional analyses of the combined impact of *ADH1B* or *ADH1C* with the *ALDH2* genotype, a particular increase in risk was seen among current drinkers who had both *ADH1B* His/His and *ALDH2* Lys+ or both *ADH1C* Arg/Arg and *ALDH2* Lys+. This finding supports our hypothesis that acetaldehyde may be involved in the underlying mechanisms of PC development. This analysis also demonstrated that PC risk among current drinkers with the combination of *ADH1C* Gln+ with the *ALDH2* Glu/Glu allele was significantly increased compared with never drinkers with both *ADH1C* Arg/Arg and *ALDH2* Glu/Glu, which is not consistent with our hypothesis. However, the number of control subjects in our study population with both *ADH1C* Gln+ and *ALDH2* Glu/Glu was too small to rule out chance association. Validation of these results will require studies in a larger number of subjects in other populations.

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In the present study, we separately assessed the impact of *ADH1B* and *ADH1C* on PC risk. Although several reports have found linkage disequilibrium between *ADH1B* rs1229984 and *ADH1C* rs1693482,[17–19] results from a recent large-scale study conducted in Europe strongly support the independent impact of these two loci on aerodigestive tract cancer risk, regardless of linkage disequilibrium.[32] This finding reflects those of our previous study on drinking behavior.[20] Reasonable assessment can therefore be carried out on the individual effects of *ADH1B* and *ADH1C*.

Chronic pancreatitis has been suggested as contributing to PC risk,[5] and several studies have shown that the *ADH1B* His + allele increases the risk of pancreatitis in heavy drinkers.[33,34] With regard to the mechanism of pancreatic cancer, acetaldehyde exposure may increase PC risk by inducing a state of chronic pancreatitis. Information regarding past history of pancreatitis may aid in further understanding the mechanisms underlying pancreatic cancer.

In a previous case-control study, Miyasaka *et al.* reported that although the *ALDH2* Lys+-allele was found to be a risk factor for PC among subjects with both drinking and smoking habits, frequency of drinking habit did not differ significantly between patients and controls, regardless of presence or absence of *ALDH2* Glu/Lys polymorphisms.[35] These findings suggested possible effect modification by smoking in the impact of *ALDH2* polymorphism. However, in our analyses stratified by drinking and smoking, we did not observe this effect (data not shown). This inconsistency may have arisen by chance due to the small sample size of both studies, or may be attributed to residual confounding by smoking or other factors. Another explanation may be due to the selection of controls from different population bases, which might in turn affect allele frequencies as well as prevalence of drinking or smoking habit. Cases and controls in our study were all sampled from the same population base at Aichi Cancer Center. In contrast, the study by Miyasaka *et al.* investigated cases from National Kyushu Cancer Center and sampled controls from a comprehensive population-based longitudinal study conducted in rural areas in Aichi prefecture. Future studies should employ an appropriate study design, possibly a prospective one, with appropriate confounders and a sufficient number of subjects to sustain stratification by smoking and drinking as well as multiple genotypes.

With regard to the methodological background of our study, one important factor was selection of the control base population. We used non-cancer patients at the ACCH for this purpose on the basis that our subjects arose within this population, thereby warranting internal validity. We have previously confirmed the similarity of this population to the general population in terms of a range of exposures of interest, in this case alcohol consumption, thereby warranting external validity.[27] Further, genotype distribution of the *ALDH2*, *ADH1B*, and *ADH1C* polymorphisms in our controls was similar to that in the general population.[36] A second potential source of bias was the medical background of the controls. However, our previous study in women demonstrated that this had only limited impact: more than 66% of non-cancer outpatients at ACCH have no specific medical condition, while the remaining 34% have specific diseases such as benign tumors, non-neoplastic polyps or both (13.1%); mastitis (7.5%); gastrointestinal disease (4.1%); or benign gynecologic disease (4.1%).[37] The situation for men is comparable. Bias from this issue, if present, therefore appears limited. Furthermore, in contrast to standard hospital-based studies, the HERPACC system is less prone to information bias because all data are collected prior to diagnosis. Lastly, we did not apply an adjustment of multiple comparisons in the analysis because we have an *a priori* hypothesis in the present study. Therefore, our findings need to be interpreted cautiously.

In conclusion, alcohol intake has an impact on PC risk when alcohol consumption and genotype polymorphisms of alcohol-metabolizing enzymes are combined. Our finding that the impact of alcohol on PC risk was observed among individuals with *ALDH2* Lys+, *ADH1B* His/His, or *ADH1C* Arg/Arg, associated with a rapid production or high accumulation of acetaldehyde, indicates that acetaldehyde may play a substantial role in the underlying mechanism of PC.

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Meta-analyses of the methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and risk of head and neck and lung cancer

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ABSTRACT

Authors report the results of four meta-analyses of studies that examined the association between methylenetetrahydrofolate reductase (*MTHFR*) *C677T* and *A1298C* polymorphisms and head and neck cancer (nine studies, 2076 cases and 4834 controls for *C677T*; four studies, 1439 cases and 3941 controls for *A1298C*), and lung cancer (ten studies, 5274 cases and 7435 controls for *C677T*; seven studies, 5098 cases and 6243 controls for *A1298C*). The summary odds ratio (OR) of head and neck cancer was 0.92 (95% CI: 0.76–1.11) for *MTHFR* 677 TT and 0.68 (95% CI: 0.37–1.26) for *MTHFR* 1298 CC. The OR of lung cancer was 1.22 [95% confidence interval (CI): 0.95–1.55] for *MTHFR* 677 TT and 1.07 (95% CI: 0.83–1.38) for *MTHFR* 1298 CC. Results from the meta-analysis of three studies on *C677T* stratified according to dietary folate intake showed an increased risk for individuals with low folate intake (OR = 1.37, 95% CI: 0.92–2.06 for head and neck and OR = 1.28, 95% CI: 0.97–1.68 for lung) *versus* high folate intake (OR = 0.85, 95% CI: 0.63–1.16 for head and neck, and OR = 0.94, 95% CI: 0.79–1.12 for lung). Despite the lack of formal statistical significance, these findings are consistent with the hypothesis that folate play a role in lung and head/neck carcinogenesis, and show the need to incorporate data on folate intake when interpreting results of *MTHFR* polymorphisms in relation to cancer risk.

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1. Introduction

The potential protective effect of folate on cancer risk has been of research interests in the last decade [1]. In humans, folate plays the fundamental role of providing methyl groups for de novo deoxynucleoside synthesis and for intracellular methylation reactions [2], and low folate levels was shown to lead to uracil misincorporation during DNA synthesis, chromosomal damage, DNA strand breaks, impaired DNA repair, and DNA hypomethylation [3]. Sequence variants in genes coding for key enzymes in the folate metabolism, such as methylenetetrahydrofolate reductase (MTHFR), were suggested to be associated with folate levels and DNA methylation [4,5].

MTHFR irreversibly catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulating form of folate, thus playing a central role in balancing DNA synthesis (which involves 5,10-methylenetetrahydrofolate), and DNA methylation (which involves 5-methyltetrahydrofolate). Two common polymorphisms associated with lower enzyme activity and reported to exist in negative linkage disequilibrium have been described: C677T in exon 4 and A1298C in exon 7 [6–9]. Individuals who are homozygous TT for *MTHFR*

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