amplification. Whatever the case, the findings might provide clues to molecular mechanisms of neuroblastoma development.

In summary, the present study showed that CIMP is present specifically in neuroblastomas with poor prognosis and that can be sensitively detected by focusing on *PCDHB* methylation. CIMP seems to be a promising new prognostic marker, and its evaluation and investigations into the mechanisms underlying CIMP in neuroblastomas seem warranted.

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Tutorial

New uses for computer in medical education, clinical practice, and patient safety in the US and Japan

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ABSTRACT
There has been a rapid expansion of computer use in medicine recently in the US and Japan. The reasons are availability of high speed and wireless connections, decreasing cost, demands for increased quality of care and documentation, and improving medical education. On the other hand, there are disadvantages which are extra time and effort needed, vulnerability to viruses, breaches of patient confidentiality, and high cost at start-up.
One way to decide if the advantages of computers in medicine overcome the disadvantages to show physicians computer programs that may be useful to them. There are many such programs in Japanese as well as in English which are discussed in this paper.
A major difference between the US and Japan is the high use of personalized digital assistants (PDAs) by US physicians as compared to Japanese physicians. PDAs can decrease medical errors due to rapid information access while improving efficiency.
Although the market for the PDAs is currently decreasing in Japan, the coming merger of the cell phone and the PDA into the "smart phone" is likely to stimulate Japanese physicians' interest in PDAs for medical use, especially considering the widespread popularity of cellular phones in Japan.

KEYWORDS
Computer, personal digital assistance (PDA), medical education, clinical practice, patient safety, smart phone

1 Introduction

1.1 Computerization in the field of medicine in Japan

The history of computerization in medicine started in 70s. [1] At that time, the main purpose of computerization was labor-saving for the process of insurance claim and the scope was limited only within administrative section in medical institutions. The physician order entry system (POES) appeared in 80s by a centralized system of a host computer and based on the computerization of clinical laboratory and pharmacy. [2] The POES contributed reducing patient's waiting time in clinical institutions and also making the process of insurance claim efficient. The growth of networking, especially the Internet in 90s enhanced cooperation among clinical professionals or clinical institutions. [3] Also, the electronic medical record (EMR) came into realistic and a hospital in the west of Japan implemented EMR and got rid of paper first in 1999. [4]

In 2001, Japanese government established e-Japan policy, and health care and social welfare is one of the main target fields. [5] Then, the ministry of health labor and welfare (MHLW) published "IT ground design for healthcare system" in the end of 2001. It focused on EMR and the national standard software for electronic process of insurance claim. It made target to implement by the end of 2006; over 60% of institutions which has more than 400 beds should install EMR and over 70% of institutions should install the national standard software for electronic process of insurance claim. [6] According to the survey by the MHLW in 2002, [7] only 1.3% out of total

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8,023 hospitals have EMR and 15.3% have the POES. It is also only 2.3% that the percentage of hospitals installed the national standard software for electronic process of insurance claim. However, such numbers are dramatically increasing recently.

As overall, computers are very popular among Japanese people and international survey in 2003 [8] showed that 38% had laptop or desktop computers in 2002 and 68% had a mobile phone in 2003 in Japan. The corresponding numbers for the US were 66% and 54%. The percentage of the Internet users were 45% for Japan and 55% for the US in 2002 (ITU Telecommunication Indicators).

1.2 Computerization in the field of medicine in the US

There has been a rapid expansion of computer use in medicine recently in the US for a number of uses including medical education at all levels, point of service medical information (especially diagnostic, treatment, and medications), medical research, EMRs, electronic billing, electronic prescribing, and the collection of data to determine quality of care and quality of medical education.

Some possible reasons why computers are increasingly used in US medical care are availability of high speed connections, availability of personal digital assistants (PDAs), availability of wireless connections, decreasing cost of hardware and software, public and government demands for increased quality of care and documentation of that quality, too much information to process without electronic help.

Wireless LANs are much more common today in hospitals than in doctors' offices. Only about 8 percent of physician practices have gone wireless. By comparison, 61 percent of integrated delivery networks and 36 percent of stand-alone hospitals have some wireless capability in the US. [9]

In terms of security, wireless network should be protected, at least, by a combination of wireless-specific ways such as WPA/EAP according to IEEE802.1X with IPSec/VPN technologies. In addition, a separation of traffic by creating VLANs, and installation of a firewall between wired and wireless networks tightened the security of the WPA/EAP-equipped wireless networks. [10]

2 The advantages and disadvantages of using the computer in medicine

How can computers improve quality of care and document that quality? They can avoid illegible handwriting, can be programmed to find errors in dosage, medication name, medication interactions, and identifying allergic patients or the wrong patient, computerized records can be backed up and are less likely to be lost or unavailable, computerized records can more easily be transferred even over long distances, more easily collect data such as mortality or number of patients seen or types of diagnosis seen.

How can computers improve medical education? They can decrease the amount of class time where there is information transfer without interaction, increase the amount of class time available to answer questions and concentrate on confusing or difficult topics, teach medical students and residents how to efficiently get the most accurate, useful, and up to date information through computer programs. They can then use this technique for the rest of their career. Computers can decrease the amount of information needed to be memorized and reduce the chance of error due to faulty memory. Finally they can decrease the amount of time needed to read journals and books while still maintain high quality knowledge.

What are the disadvantages of computer use in medicine? They can be less useful for those physicians who cannot type quickly, take extra time and effort to get used to, create psychological discomfort with a new way of practicing medicine, be vulnerable to viruses and technical problems that risk lost of data unless backed up, be vulnerable to breaches of patient confidentiality, sometimes increase the amount of time needed to get work done, create fear that computerized data can be used by the legal system against doctors and hospitals, create the fear of making the interaction between the patient and doctor seem less personal and have high cost at start-up

3 Useful websites for medicine

3.1 Japanese language websites for laptops or desktops

One way to decide if the advantages to computers in medicine overcome the disadvantages to show physicians computer programs that may be useful to them. Some of the useful programs for free of charge are as follows:

3.1.1 Clinical guideline

There are about 30,000 new Japanese language medical articles published monthly. [11] It is difficult to catch up with up-to-date clinical evidence. Medical Information Network Distribution Service (Minds) http://minds.jcqhc.or.jp which is shown in Fig.1 is run by Japan Council for Quality Health Care (JCQHC). [12] There are several clinical guidelines; such as asthma, lung cancer, diabetes, etc. These guidelines were provided some grants from the MHLW and developed by way of Evidence Based Medicine (EBM). There are two types of guideline for each disease; one is for clinical professionals and the other is for lay people.

3.1.2 Drug information

Drug reference is the most frequently asked among clinical professionals and even asked by patients. Even in Japan, 1 drug on average is approved for use each week. [13] The Pharmaceuticals and Medical Devices Agency (PMDA) http://www.info.pmda.go.jp/ which is shown in Fig.2 provides electronic insert packages for prescription drugs and also rug safety information. [14] It used to be an affiliation of MHLW, however, it was incorporated since 2004.

3.2 English language websites for laptops or desktops

In terms of medical education, http://www.healcentral.org is a U.S. non-profit group dedicated to promoting on-line medical education and is available to medical educators world-wide. [15] This organization gathers programs from many medical schools which are primarily in the US. Their site is in English although there are some multi-lingual areas such as patient instructions written in Japanese. Healcentral.org which is shown in Fig.3 has over 30,000 images plus videos and interactive programs. Heal information is not copy-righted and available to anyone. There are two new projects being undertaken by healcentral.org.

The first project is called heal/local and information is available at healcentral.org. It is a trial project available to any medical school or professor in the world which will personalized the heal information for that particular school or professor. More information is available at their website.

The second project was announced in June, 2005 and is a merger of healcentral.org with a large non-profit medical education on-line learning organization in England which will add a large amount of additional content to healcentral.

In terms of clinical care, the most frequent uses of computers and PDAs by US physicians is for information gathering and, more specifically, to find drug information. A very popular but expensive program written in English is www.uptodate.com which takes no money from any outside organization and therefore claims to be free of bias. [16] It is a commercial company which hires outside experts to update its information every six months. It is a searchable data-base with information by disease or by drug. In addition, there is a program to detect drug interactions and an area for the latest developments in a specialty. It is limited to Internal Medicine and most of its subspecialties, Pediatrics, Obstetrics and Gynecology, and is adding Neurology soon. There is a PDA version of this information. An on-line introduction for up-to-date which is shown in Fig.4 is available at http://www.uptodate.com/subscribers/tutorial/index.html.

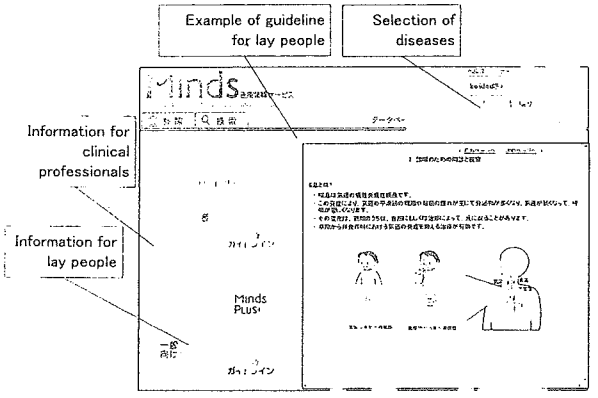

Fig.1 The guideline for asthma as an example for the site of Minds.

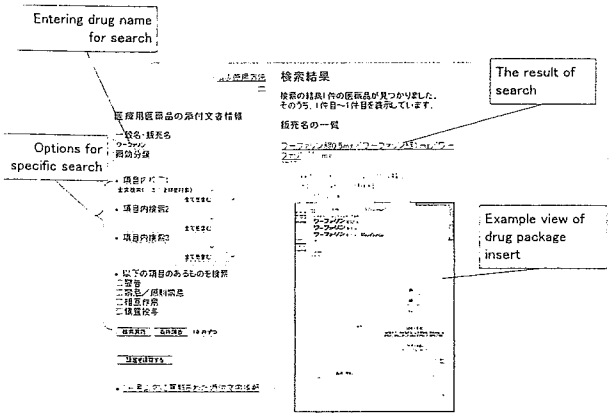

Fig.2 The webpage of PMDA and example of drug package insert.

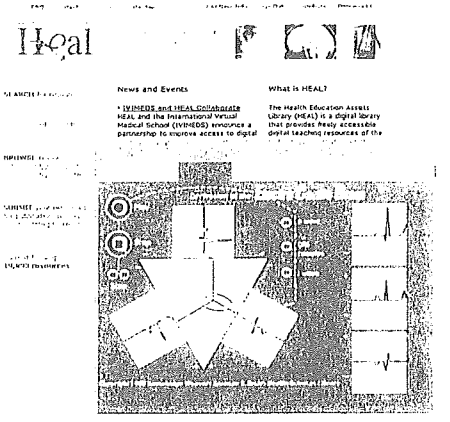

Fig.3 The webpage of Heal and an example of animation for Electro Cardio Gram.

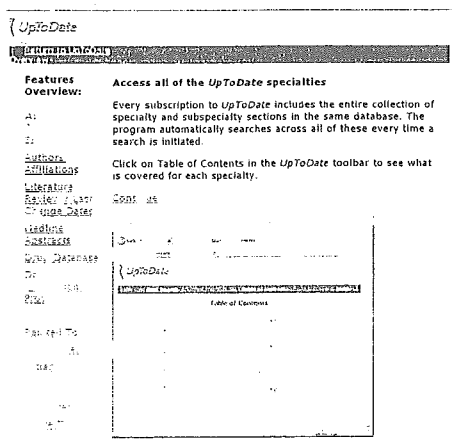

Fig.4 The webpage of introduction for Up-To-Date.

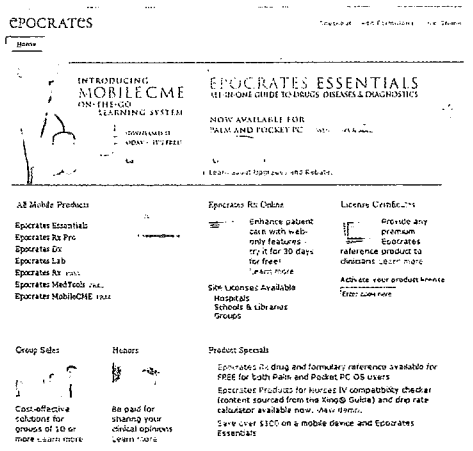

Fig.5 The webpage of epocrates.

Another program which claims to be the most popular computer program in the US is www.epocrates.com as shown in Fig.5. [17] This program is either for computer or PDA use. It is a commercial program that does receive financial support from the drug industry and therefore is very reasonably priced but at risk of bias. The key feature that distinguishes epocrates from other programs is its easy to use "multi-check" function which can find drug interactions for up to 30 medications including non-prescription medications and alternative medications such as herbs. This program is written in English only and the company does not have any immediate plans to convert it to the Japanese language. Japanese physicians were surprised to find out that there is a warning in both the epocrates and up-to-date program concerning the consumption of green tea and the use of blood thinners such a warfarin since green tea also has an anti-coagulation effect. This is an example of information that would be very difficult and time consuming to get from a paper-based product.

4 Utilization of the PDA for medicine

4.1 PDA for Japanese medical professionals

Comparing to the US, people do not use the PDAs so much in Japan. The PDAs are relatively expensive in Japan, and have the difficulty of entering Japanese characters. The cell phones are more popular than PDAs in Japan. These issues seem the reasons for minor use of PDAs in Japan. The Statistics Bureau in the ministry of internal affairs and communications in Japan published the survey of household economy of 2004, [18] and items related to information shows that the percentage of PDA ownership is 1.7%. The similar figures among medical professionals can not be available. According to Japanese proceeding of Joint conference on Medical Informatics in 2004, there are only two articles about PDA out of 568. [19]

The most usage of PDA in the medical fields is for patient safety. [20] PDA with bar code reader scans ID code on a name tag of medical staff, ID code of a patient's wrist band, and bar code on a bag of intravenous drip or blood transfusion. It enables to identify what was done, who did it, when it was done, whom it was done to, why it was done, and how it was done. In other words, it can keep track of clinical process. If any mistake occurs, such as wrong blood type, wrong medication, or misidentify a patient, PDA shows warning.

In addition to patient safety, PDAs can be useful for enhancing clinical care and medical education. PDAs can be available for drug information, reference to clinical guidelines, medical dictionary, or calculations of creatininine clearance and pediatric dosage, etc. The example is shown in Fig.6. According to the information from a PDA software company, the number of downloading trial version of software from March 2005 through June 2005 are about 5,000 for drug information and clinical guidelines respectively, and about 2,000 for pediatric dosage calculation and concise medical dictionary for abbreviations. However, the number of purchase is not obtainable. The approximately 95% of the users are medical professionals and the rest of users are medical representative at pharmaceutical companies.

4.2 PDAs for english medical professionals

The major difference noted by the second author, who is an American physician, is the high use of mobile computer technology by U.S physicians as compared to Japanese physicians. The most commonly used mobile device in U.S medicine is PDA. For example, approximately 40% of U.S doctors use PDAs in

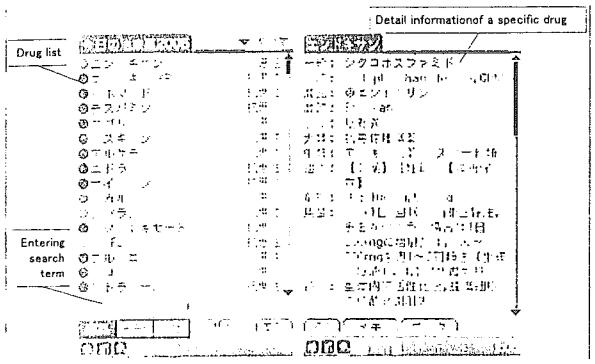

Fig.6 Drug information as an example of software for PDA.

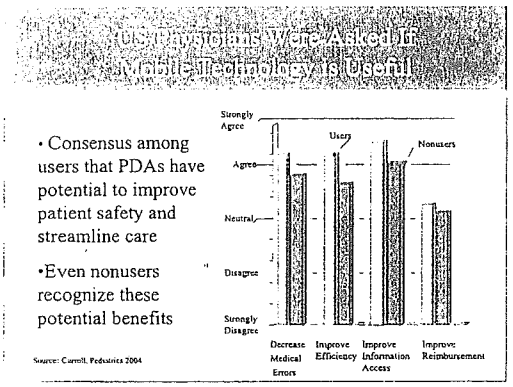

Fig. 7 Physicians' recognition about usefulness of mobile technology in the US.

2003 which was a 50% increase since 2001. This is expected to grown to >75% of all US physicians by 2007. Half of all members of the American College of Physicians (Specialty of Internal Medicine) were using PDAs in 2001. Specialist use of PDAs is highest among cardiologists, anesthesiologists, psychiatrists, and oncologists.

A survey of physicians using PDAs in 2002 showed that approximately 70% used them for drug reference and approximately 40% used them for scheduling. The remaining uses such as dictation, electronic prescribing, hospital interface, and other uses were approximately 20% or lower. [21]

When US physicians were asked if mobile technology is useful, the majority of users and non-users agreed that they decrease medical errors, improve efficiency, and improve information access. The result is shown in Fig.7.

In an article in The Journal of the American Medical Informatics Association in 2001, Bates reported that physicians using PDAs reported that these devices prevented an average of two adverse events. [22]

Why do PDAs improve patient safety? Insufficient drug and drug interaction information are the most common causes of serious errors and is easier to obtain quickly from a PDA. PDAs can be updated frequently so their drug information is more up to date. Multiple drug interactions are extremely difficult to determine without computer technology. PDAs can send wireless alerts with physiologic, laboratory, and medication information. PDAs can manage the transition between house staff with less chance for error. PDAs can reduce handwriting errors.

Japanese physicians may be reassured that pharmacies have computer technologies to detect drug incompatibility but errors detected after the prescription is written require extra time to correct for the pharmacist, patient, and physician. In addition, patients may sometimes go to more than one pharmacy so that interactions may not be detected.

What is the proof that PDAs improve patient safety? There is only limited information in this new field but initial reports are encouraging. For example, improvement in compliance to US national asthma guidelines in patient evaluation and drug therapy occurred after the introduction of PDAs containing these guidelines. [23] Improved documentation and patient follow-ups by emergency medicine residents was noted after the introduction of the PDA. [24] An interesting study showing that patient use of PDAs improved health was reported by Szynal in 2001 in the journal, Health Data Management. [25] The outcome measurement was improved admission condition when used by patients awaiting transplant surgery

Why do US physicians think that mobile computing improves their efficiency? When desktop computers are shared, there is extra time required for each physician to log in. This is not a problem with a personal PDA even if there is a once a day log in. In addition, PDAs do not involve the physician leaving the patient to find a computer. Furthermore, diagnostic and treatment help is available within seconds and may save lives or reduce complications if seconds are critical in patient care. Finally, PDAs are more up to date than paper books so physician does not have to look for multiple texts to get the most up to date information.

Do PDAs help promote good medical education? There is also limited data since this field is so new. One study showed use of PDAs improved learning of evidence-based medicine. [26]

5 The future-probable merger of cell phone and the PDA in near future in both US and Japan

Many companies providing the PDAs have been withdrawing from Japanese market, for example Handspring, IBM Japan, Palm Computing, and Sony. But recently, a new type of merging cell phone and the PDA has become a real product and its market is

increasing in the world. Such product is called "smart phone" and some companies, for example Palm, Nokia, NTT DoCoMo have already produced such smart phones. Major OS companies for PDA are Microsoft and Palm. Furthermore, Bill Gates, chairman and chief software architect of Microsoft announced the release to manufacturing of windows mobile 5.0. [27]

Some people may be concerned about the electromagnetic interference between cell phones and medical devices. As recent information, patients, visitors and staff may use cell phones in any area of the hospital, as long as they are at least 1 meter from operating medical devices. [28]

Taking it into consideration that popularity of cell phone in Japan and the PDAs in the US, it seems that the future of smart phone is promising even in medicine. Also there is a business chance to expand mobile computer technology in medicine.

6 Conclusion

There has been a rapid expansion of computer use in medicine recently along with the diffusion of information technology in the US and Japan. There are a lot of pros and cons for computer use. Since governments promote computerization in medicine, if medical staff recognizes more benefits of computers, they can use them for improving safety and efficiency in medicine.

The use of mobile computer technology in medicine is much less prevalent in Japan as compared to the US. There is still a possibility to enhance medical education, clinical practice and patient safety by using such technology in Japan. In order to implement such mobile computer technology into medicine, more collaboration is necessary for both medical institutions and computer companies.

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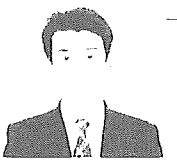

Daisuke KOIDE

Daisuke KOIDE has been an associate professor at the University of Tokyo since 2003. He received B.A. in pharmaceutical science from Tokyo University of Science in 1991, and Master and Ph.D. in Health Science from the University of Tokyo in 1993 and 1996 respectively. His research interests are medical informatics and pharmacoepidemiology. He is also a director of the Drug Safety Research Unit and a councilor of Japanese Society for Pharmacoepidemiology. He was awarded a bronze medal at the 11th triennial congress Medinfo2004 in San Francisco; the international meeting for the medical informatics in 2004.

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Appendix 1

ComputerApplications For Teaching and Clinical Care - Japanese

Desktop applications

Electronic clinical guidelines
http://minds.jcqhc.or.jp/to/index.aspx Medical Information Network Distribution Service (Minds)
http://www.mnc.toho-u.ac.jp/mmc/guideline/index.htm Library, Toho Medical University

Electronic textbooks
http://www.banyu.co.jp/merck/index.html Merck Manuals
http://www.ibaraisikai.or.jp/treasure/chisivew/chisiki.html Knowledge of Internal Medicine
http://www.kanazawa-med.ac.jp/~hasumura/ A Physical Examination Skills
http://next.bml.co.jp/diagnosis/contents.html diagnosis on Web / Contents
http://www.kanazawa-med.ac.jp/~htogkmu/msnote.html Ob/Gyn HyperText for Medical Students
http://www.qent.med.kyushu-u.ac.jp/virtual.html Virtual Otolaryngology Hospital
http://www2.kpu-m.ac.jp/~picu/ Pediatric ICU Manual
http://cancerinfo.tri-kobe.org/ Cancer Information Japan (PDQ,etc)
http://www.srl.info/index.html Clinical laboratory
http://www.hamt.or.jp/KENSA/MSTAFF/ECG/ecg.html Electrocardiogram ECG
http://www.jichi.ac.jp/usr/cpth/us./us.home.html Ultrasound
http://web.kanazawa-u.ac.jp/~med23/NMImageConf.html Nuclear Medicine Imaging Conference
http://web.sc.itc.keio.ac.jp/anatomy/anatomy/anatomy.html anatomy
http://www.lib.kobe-u.ac.jp/products/anatomy/index.html anatomy
http://bme.ahs.kitasato-u.ac.jp/qrs/phy/index.html Physiology
http://pharma1.med.osaka-u.ac.jp/textbook/Pharm-Textbook.html Pharmacology 1
http://pharma1.med.osaka-u.ac.jp/textbook2/Pharm-Textbook2.html Pharmacology 2

Electronic journals
http://e-medicine.sumitomopharm.co.jp/e-medicine/index2.html Journal Watch
http://www.nankodo.co.jp/yosyo/user/html/ New England Journal of Medicine

Drug information
http://www.info.pmda.go.jp/ Pharmaceutical and Medical Devices Information (PMDA)
http://mid.cc.kumamoto-u.ac.jp/top.html Drug Package Insert
http://www.nihs.go.jp/dig/jindex.html Drug Info Guide
http://bme.ahs.kitasato-u.ac.jp:8080/docs/ts/html/note/index.html Drug Information for Patients
http://www.jah.ne.jp/~kako/ Emergency Drug information
http://www.j-poison-ic.or.jp/homepage.nsf Japan Poison Information Center
http://center.umin.ac.jp/cgi-open-bin/hanyou/lookup/search.cgi?parm=POISON UMIN Poison Database

Medical laws
http://law.e-gov.go.jp/cgi-bin/idxsearch.cgi Database of laws and regulations
http://web.kyoto-inet.or.jp/org/kanpo/3W/houki/houritu.html Medical laws, etc.

Medical writing
http://www.sunmedia.co.jp/kitei.html#A Instructions for Authors Domestic
http://www.toukoukitei.net/index.html Instructions for Authors Oversea
http://www.biwa.ne.jp/~fumika/eiyou.htm English writing support
http://mhlw-grants.niph.go.jp/ MHLW Grants System

Micellaneous
http://s2001.medic.mie-u.ac.jp/icd/ ICD 10 and DPC (Diagnosis Procedure Combination) coding

http://lsd.pharm.kyoto-u.ac.jp/ja/index.html Medical and Pharmaceutical dictionaries
http://www.sam.hi-ho.ne.jp/tootake/yougo2.htm Medical and Pharmaceutical dictionaries
http://di.m-pharma.co.jp/foreign/index.html Foreign Language Conversation in Hospital and Pharmacy
http://food.tokyo.jst.go.jp/index.html Food Composition Database
http://www.geocities.jp/kazu_hiro/index.html Nursing Site

PDA sites

Software
http://www.medicalview.co.jp/stedman/stedman03.shtml STEDMAN'S Medical Dictionary
http://www.m2plus.com/mproducts/05pda/pda.html Drug information 2005 for PDA
http://www.m2plus.com/mproducts/resident/resident.html The Resident
http://www.m2plus.com/mproducts/migiude/migi1-2.html Mr. Reliable Vol. 1/2 (Introduction)
http://www.geocities.jp/palmpro/HOW/HOWDRUG.HTM DoseSpeed Ver.8.0
http://www.geocities.jp/palmpro/HOW/HOWMEDUNIT.HTM Medical Unit Converter
http://www.nextftp.com/moritaro/Develop/Dev-Acd.html Acid-Base for Palm
http://www006.upp.so-net.ne.jp/kono/BSACalc.htm BSA Calculator

Link sites
http://www5.ocn.ne.jp/~palm-med/ Palm Med & Pharma
http://unoubeya.main.jp/ Right Brain ^^ Room
http://www.lab.toho-u.ac.jp/med/peds/link2/ Palm user's links for medical professionals

Appendix 2

ComputerApplications For Teaching and Clinical Care -English

Desktop applications

http://www.healcentral.org Central data site for many multimedia programs some with voice and thousands of images for medical student education in the basic sciences and clinical areas. Site is run by three US medical school with a government grant and is free and open to the public including international medical schools. There are a few Japanese language patient education documents. Excellent site for medical education, and the best I have seen so far.

Heal Local System for Individual Medical School-in testing phase-available sometime in 2005. contact bas@mednet.ucla.edu. if interested in participating. Will have any information needed from "heal" site plus local University information with local control of the site. It is being created to fit into international computing standards. University of Tokyo could consider running such a site and possibly sharing it with other medical schools. Alternatively, Japan Ministry of Education could consider running such a site for all Japanese medical schools with a mixture of English language and Japanese language programs.

www.nlm.nih.gov National Library of Medicine has medline plus which is English and Spanish Language Patient Information including animation and voice

http://www.umassmed.edu/strokestop/module_one/module_fr.html This is the free program from the University of Massachusetts that Dr. Peskin demonstrated to teach neuro-anatomy and neuro-physiology. It is not a protected document and you are welcome to use it

http://www.med-ed-online.org An American on-line medical education journal. Go to resources for multimedia educational programs

http://www.msu.edu/user/inetproj/homepage5.html American med-school with a number of on-line curricula including how to use the internet for medical education

http://www.epocratesonline.com on line US drug reference with excellent drug interaction feature, and including non-prescription drugs, and herbal medications. There is also a pill identifier for patients using an unknown medication, tables, alerts on drug adverse reactions or major medical news of clinical importance. It is updated weekly. You may try it free for one month and is $US59 per year. Problem for Japanese physicians is that it is US medications. Dr. Peskin contacted them and they have no plans for a Japanese language version soon. Japan could benefit greatly from a program like this with the program for drug interactions being most useful.

http://mycourses.med.harvard.edu/vp_view.asp?frame=Y&tracking=Y&case_id={B517ED16-BFB1-4856-B207-12F9623B539D}

continuing medical education/ available for anyone but not free

http://cme.nejm.org/

continuing medical education based on the New England Journal of Medicine articles

PDA(PERSONAL DIGITAL ASSISTANT) APPLICATIONS

-FOR THOSE JAPANESE PHYSICIANS WHO WOULD LIKE TO START USING PDAs NOW

-FOR THOSE JAPNESE PHYSICIANS WHO WILL WAIT AND BUY A CELL PHONE-PDA COMBINATION DEVICE, AND WILL NEED

THIS INFORMATION WHEN THEY MAKE THAT PURCHASE

WHICH PDA TO BUY, YOU MUST FIRST DECIDE-Which operating system (software system that runs the machine) to use (current choices):

Palm OS-runs on devices made by companies such as Palm and Handspring Advantages: small, lightweight, comfortable to carry with you. Less Expensive, Lots of Medical Software. Long battery life. Disadvantages: Does not easily merge with all Microsoft desktop applications. Is losing market share and medical applications may not be as plentiful and well supported in the future. For example, Sony currently makes these but is stopping production for the Japanese market.

Pocket PC (being discontinued and replaced by Mobile 5 so you can buy a current Pocket PC or wait a short time for Japanese mobile 5 PDAs) Runs on devices made by Hewlett Packard, Compaq, and Casio. Advantages: Larger with bright color screens so easier to see, coordinates with Windows functions very well, Disadvantages: More Costly, Larger size makes them less comfortable to carry with you, less medical software available but quickly catching up with PalmOS devices.

Mobile 5-Sharp and Samsung are coming out with devices soon for the Japanese market. Will be used to combine cell phones and PDA. This may be what is needed to get large numbers of Japanese physicians to use PDAs for medical applications.

PDA Buyer's Guide: www.epinions.com; www.barginpda.com, www.cnet.com

WHERE TO GET MEDICAL PROGRAMS THAT YOU WANT TO USE ON YOUR PDA

- www.handango.com: leader in medical software
- http://pbrain.hypermart.net/: Great source for medical PDA info, software, news
- www.zdnet.com site with software & hardware reviews, comparison buying, more

Utilities: (only needed for some programs. in my opinion, only useful for advanced users)

Adobe Acrobat: http://www.adobe.com/products/acrobat/readerforpalm.html

Medical Calculator: http://www.doctorsgadgets.com/software/free-pda-drug-reference.htm

Treatment Guidelines/References:

Am Coll. of Cardiology Guidelines: http://www.acc.org/clinical/palm_downloadstep1.htm

Asthma NHLBI Guideline: http://www.aafp.org/x28143.xml

Breast Cancer Prediction Tool: http://smi-web.stanford.edu/people/pcheng/breastca/

Cholesterol ATP III Calculator: http://hin.nhlbi.nih.gov/atpiii/atp3palm.htm

Current Clinical Strategies (Book/PDA Out & In-Pt, Medicine, HIV, CCU, Psychiatry, Peds) http://ccspublishing.com/ccs/ ($15-25/book, $50 for all in the series)

Evidence Based Pearls: www.handango.com; search "Evidence Based Practice" ($19.95/year)
(Dr. Frank Domino, Associate Professor at the University of Massachusetts, who helped prepare some of the PDA information in this handout, has a financial interest in Evidence Based Pearls)

Immunizations: http://www.immunizationed.org/anypage.aspx?pagename=shotshome

Medical Decision Tools (MedRules): http://pbrain.hypermart.net/medrules.html

OB Wheel: http://www.fppda.com/timobppc.htm

Drug Databases

Epocrates Drug Database: http://www2.epocrates.com/products/os/rx_subs.html
Three choices:

"Essentials" Most expensive and complete. ($US139/year)
Most popular drug reference in the US. Dr. Peskin's favorite. Includes information on diagnosis of a large number of diseases, commonly used tables, US used medications including prescription, nonprescription, and herbal medications. Most useful is ability to check for up to 30 drug interactions. Also has infectious disease information, lab information, automatic alerts for warnings about drugs or very important news concerning medical issues. Updated weekly. Is inexpensive relative to "Up To Date" partly due to this private company taking money from drug companies. Dr. Peskin finds it reliable but there is the potential for conflict of interest.
Problem for Japanese physicians is that it is based on US drugs. In Dr. Peskin's opinion, a PDA version in Japan is very likely to reduce medication errors and drug interactions, and to lead to more correct diagnoses.

"RxPro" (just Drug and Diagnosis Information: $59/year) Excludes the laboratory information.

"Rx" (just Drug information. Free. Great for medical students and others who need to limit their expenses

PDA Resource Pages

Ectopic Brain: http://pbrain.hypermart.net/

Medical Student PDA Café: http://www.medstudentcafe.com/pdacenter.htm

Text-Like Apps

InfoRetriever—EBM Abstracts, Cochrane, POEMS, Practice Guidelines (Pocket PC) $250.00
- www.infopoems.com ($249.00 per year)
- HanDBase—database application; download free applets or write your own; On-Call; $30.00 www.handango.com
- UpToDate—full database of web version, free with subscription ($400-500) www.uptodate.com (In Dr. Peskin's opinion, too expensive, and the drug information is not as useful as Epocrates for US Physicians. The drug information has even less usefulness for Japanese physicians. Diagnostic information may be useful for residents in specialties where they are in and out of operating room and do not have easy access to desk-top computers.)
- SkyScape : 5 Minute Clinical Consult, FerriGuide, etc. Abridged Textbooks (part of Epocrates Essential) www.handango.com

Electronic Medical Records

- Patient Tracker—www.handheldmed.com; Intuitive, FREE, desktop for $300
- PatientKeepter—Biggest, $35-40; Enterprise Option (busy for me)
- Handbase Patient Tracker Applet—Free, Simplistic

Web Resources on PDA's

Ectopic Brain: http://pbrain.hypermart.net/: Great source for medical PDA info, apps, news

UT HSC SA: http://www.library.uthscsa.edu/internet/pda.cfm good links

—Counseling Tools:
Smoking Cessation: http://www.smokefree.gov/hp-hcsit.html
Preventive Services: http://pda.ahrq.gov/index.html

Laboratory Investigation (2005) 85, 895–907

www.laboratoryinvestigation.org

Loss of blood group A antigen expression in bladder cancer caused by allelic loss and/or methylation of the *ABO* gene

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Loss of ABO blood group antigen expression has been reported in transitional cell carcinoma (TCC) of the bladder. Synthesis of the ABO blood group antigen was genetically determined by allelic variants of the *ABO* gene assigned on 9q34.1. We analyzed loss of heterozygosity (LOH) and promoter hypermethylation of the *ABO* gene in TCC and compared them with alterations of A antigen expression in TCC, dysplasia and normal urothelium. A total of 81 samples of TCC of the bladder obtained from transurethral resection (TUR) ($n=44$) and radical cystectomy ($n=37$) were examined. Expression of the A antigen was evaluated by immunohistochemical staining (IHC) using anti-A antigen monoclonal antibody. LOH of the *ABO* gene locus was examined by blunt-end single-strand DNA conformational polymorphism (SSCP) analysis using flouresence-based auto sequencer. Promoter hypermethylation of the *ABO* gene were examined by bisulfite PCR-SSCP (BiPS) analysis and/or methylation-specific PCR (MSP). Loss of A allele and/or hypermethylation were significantly associated with abnormal expression of the A antigen in cases undergoing TUR ($P=0.02$) and radical cystectomy ($P=0.0005$). For the analysis of the concomitant dysplasia in 23 cases with TCC of the bladder, the expression of the A antigen was maintained, regardless of the A allelic loss or methylation status in the tumor. In conclusion, A allelic loss and hypermethylation in the promoter region of the *ABO* gene showed significant correlation with reduction of A antigen expression in TCC, while the expression of the A antigen is maintained in concomitant dysplasia or normal urothelium, suggesting that loss of the *ABO* gene and/or its promoter hypermethylation is a specific marker for TCC.
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Keywords: bladder cancer; *ABO* gene; LOH; promoter hypermethylation; dysplasia

Superficial bladder cancers often show multifocal occurrences or metachronous recurrence after transurethral resection (TUR), and eventually develop into invasive bladder cancer. Allelic loss on chromosome 9 is the most frequent genetic event in transitional cell carcinomas of the bladder,[1–4] that is observed in 70% of invasive bladder cancers and even in 50% of superficial bladder cancers at Stage G1.[4] Whether or not loss on chromosome 9 arises in urothelial lesions such as dysplasia is crucial to the understanding of early genetic events in bladder carcinogenesis. Some authors have reported on the allelic loss of chromosome 9 that occurs in the small urothelial lesions and normal bladder urothelium in their attempts to trace genetic alterations using microsatellite markers.[5,6] However, it is still difficult to analyze allelic status in small epithelial regions obtained from formalin-fixed, paraffin-embedded tissues, and a few data have been reported regarding early genetic alterations in bladder dysplasia.[3,7] ABO (H) blood group antigens are constitutively expressed on epithelial cells such as those found in the gastrointestinal tract and urothelium. A reduction in blood-group A antigen (GalNAcα1-3[Fucα1-2]Galβ1-3GlcNAc-R) expression was reported in transitional cell carcinoma (TCC) of the bladder

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and showed significant correlation with an invasive phenotype.[8–11] Orntoft and Wolf[12] examined the correlation between blood-group antigen expression and the activity of glycosyltransferases in TCC of the bladder and reported that the activity of A glycosyl transferase was severely reduced in tumors showing loss of A antigen expression. This phenomenon drew our attention, due to the fact that the determinant of the ABO blood-group antigen is synthesized by the action of the *ABO* gene encoding ABO glycosyltransferase assigned to chromosome 9q34.1, where loss of heterozygosity (LOH) was frequently reported in bladder cancer.[1–4] The *ABO* gene is composed of seven exons and six introns and encodes ABO glycosyltransferase, of which substrate specificity is determined by genetic polymorphisms in exons 6 and 7 (Figure 1).[13,14] Blood-group A antigen is synthesized by α-*N*-acetylgalactosaminyltransferase (A-GalNAc transferase), which catalyzes the transfer of *N*-acetylgalactosamine to the subterminal β-galactosyl residue of the blood-group H carbohydrate chain. Blood-group B-antigen is synthesized by B-galactosyl transferase, which catalyzes the transfer of galactose to the subterminal β-galactosyl residue of the blood-group H carbohydrate chain. The *ABO* gene in blood-group O donors lacks glycosyltransferase activity, for it has a deletion on a guanine residue at the nucleotide position 261 in exon 6, causing protein truncation at codon 117.[13–16] Immunohistochemistry using anti-A monoclonal antibody in bladder cancer may be useful to evaluate the allelic status of the *ABO* gene locus at 9q34.1 in those who are heterozygous for ABO genotypes. Expression of blood-group A antigen is stable enough even in formalin-fixed paraffin-embedded specimens, and this could be applicable in the analysis of small lesions that are too small to be examined by genetic analysis. Two papers were so far reported as to the correlation between reduced expression of A antigen and A allelic loss in TCCs of the bladder.[17,18] Meldgaard *et al*[17] analyzed 22 bladder tumors for LOH of the 9q allele by PCR-restriction fragment length polymorphism (RFLP) analysis of the *ABO* locus at 9q34. Seven tumors from heterozygous informative individuals were sorted by flowcytometry. LOHs were detected in the most aneuploid subpopulation of cells in two cases, but both cases were losing O-alleles. No LOHs were detected in analysis of the low aneuploid subpopulation. As all tumors showed loss of blood group ABH antigen expression, they concluded that LOH of the *ABO* locus on chromosome 9q34 is not the cause of loss of blood group ABH expression in human bladder cancer.[17] Orlow *et al*[18] analyzed 19 patients with bladder cancer serologically typed as blood group A. Expression of A antigen was maintained in 14 samples in normal urothelium, while it was reduced in nine tumors. PCR-RFLP analysis showed loss of the A allele in one tumor sample showing reduced expression of the A antigen. They indicated that the lack of the A antigen expression in certain bladder tumors is due to the allelic loss of the *ABO* gene and that in some of these tumors, the loss involved the surrounding chromosomal region at 9q34.1–4.[18] These two reports did not support the correlation between A-allelic loss and the reduced expression of the A antigen in the majority of bladder cancers. Recent advance in cancer epigenetics shed light on the reduced expression of A antigen in malignant cells. Kominato *et al*[19,20] reported that hypermethylation of the promoter region of the *ABO* gene induced *ABO* gene silencing in their study using a human stomach carcinoma cell line. Iwamoto *et al*[21] established subclones with positive or negative expression of the A antigen from parental colonic cancer cell lines and reported a distinct difference in the methylation pattern of the CpG island of the promoter region of the ABO glycosyltransferase, that is densely methylated in a subclone lacking the expression of the A antigen. Gao *et al*[22] examined 30 oral squamous carcinomas for expression of the A and B antigens and A/B glycosyltransferase, together with LOH at the *ABO* locus and hypermethylation of the *ABO* gene promoters. Loss of A or B antigen expression was found in 21 of 25 tumors (84%), while the expression of the glycosyltransferase was absent in all of tumors showing negative expression of A or B antigens. Loss of the A or B allele was found in 3/20 tumors (15%) heterozygous for the *ABO* locus and hypermethylation of the promoter region in 10 of 30 tumors (33.3%).[22] Furthermore, Habuchi *et al*[23] reported that the region 9q32–9q33, which is in the vicinity of the *ABO* gene locus at 9q34.1, is a frequent target of LOH and methylation in bladder cancer. These findings prompted us to hypothesize that deletion of blood-group A antigen expression in TCC of the bladder might be regulated by a combination of genetic and epigenetic mechanisms, that is, an LOH of the *ABO* gene locus and hypermethylation of the *ABO* gene promoter region. The purpose of this study was to elucidate the relevant mechanisms underlying the loss of blood group A antigen expression in TCC of the bladder and whether it could be used as a phenotypic marker to estimate any underlying genetic and epigenetic abnormalities in normal urothelium and concomitant bladder dysplasia in patients with bladder cancer.

Materials and methods

Samples and DNA Extraction

A total of 81 cases of TCC of the bladder were studied, of which 44 underwent TUR and 37 underwent radical cystectomy (Table 1). The histo-blood group for all cases was A (72 cases) or AB (nine cases) examined by routine hemagglutination tests at hospital. Tumors were graded and staged according to the WHO classification or the 1997 UICC TNM classification system. Based on patients'

Table 1 Patient background

	TUR-BT	*Radical cystectomy*	P-*value*
No. of cases examined	44	37	
Gender			NS
Male	37 (84.1%)	33 (89.2%)	
Female	7 (15.9%)	4 (10.8%)	
Age (median)	66 (45–79)	66 (39–89)	NS
Pathological stage			$P<0.01$
pTa	14 (31.8%)	0 (0%)	
pT1	25 (56.8%)	11 (29.8%)	
pT2	4 (9.1%)	8 (21.6%)	
pT3	0 (0%)	10 (27.0%)	
pT4	1 (2.3%)	8 (21.6%)	
Hitological grade			$P<0.01$
G1	7 (14.9%)	0 (0%)	
G2	21 (51.1%)	2 (5.4%)	
G3	16 (34.0%)	35 (94.6%)	
Blood group			
A	38	34	
AB	6	3	

history, the proportion of cases with advanced stage or high-grade tumors was significantly higher in those who underwent radical cystectomy than those who underwent TUR ($P<0.01$). In 44 patients who underwent TUR, DNA was extracted from fresh specimens and normal DNA was extracted from peripheral blood lymphocytes (PBL) by a standard procedure using proteinase K digestion followed by phenol–chloroform extraction. In 37 cases that underwent radical cystectomy, a total of 1130 paraffin-embedded specimens obtained from mapping study of the bladder were histologically confirmed by hematoxylin and eosin staining as being composed of tumor, dysplasia and normal tissues. DNA was extracted from manually dissected tumors and corresponding normal tissues using DEXPAT (TAKARASHUZO Co., Ltd, Shiga, Japan) according to the manufacturer's recommendation.

Expression of Blood-Group A Antigen by Immunohistochemical Staining

In all, 4-μm-thick sections from formalin-fixed, paraffin-embedded specimens of resected tissues that underwent TUR or radical cystectomy were used for immunohistochemical staining (IHC). A mapping study of the bladder specimens revealed concomitant dysplastic lesions in 23 cases that underwent radical cystectomy, and they were then subjected to IHC performed as described previously.[24] Mouse monoclonal antibody (mAb) directed against A antigen (clone 81FR2.2; DAKO, Carpinteria, CA, USA) was used as the primary antibody and the avidin–biotin-conjugated immunoperoxidase technique was performed with a DAKO LSAB2 Kit (DAKO, Carpinteria, CA, USA). Reportedly, the specificity of the mAb 81FR2.2 was characterized by transfection experiment of the A-glycosyl transferase gene to the HeLa cell (genotype OO).[25] Erythrocytes, normal epithelium and vascular endothelium were used as internal positive controls, while muscle and connective tissues served as negative controls. To determine the specificity of A antigen, IHC was performed for normal urothelium of blood group B and O donors. Immunohistochemistry for A antigen was classified as follows: 'negative' if the section had no positively (0%) stained tumor cells, 'positive' if staining was seen across the section (>70% positively stained tumor cells), and 'heterogenous' if <70% of tumor cells stained positively. As to the correlation with A allelic loss or methylation status, cases showing positive or heterogenous expression were compared with those showing negative expression.

Allelic Status on 9q Loci Defined by Blunt-End Single-Strand DNA Conformation Polymorphism Analysis

LOH of the *ABO* gene locus was examined by blunt-end Single-strand DNA conformation polymorphism (SSCP) analysis,[26] using genetic polymorphisms at nucleotide positions 261 and 297 in exon 6 of the *ABO* gene. Genotypes and their allelic frequencies in Japanese population were previously reported[15] and shown in Figure 1. Four groups of alleles, A (A101, A102, A103), B (B101, B102, B103, A104), O1 (O101, O102, O202, O203) and O2 (O103, O201) were identified by the analysis of two genetic polymorphisms (nucleotides 261, 297) in exon 6 of the *ABO* gene. The 5′-terminus of the reverse primer

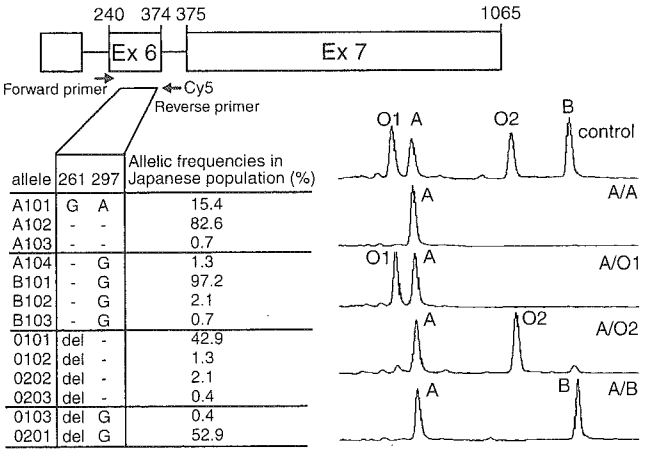

Figure 1 Schema of single nucleotide polymorphisms (SNPs) in exons 6 of the *ABO* gene and electropherogram of the blunt-end SSCP analysis showing examples of normal DNA from blood group A or A/B donors. SNPs in nucleotide positions 261 and 297 were used for analysis in this study. DNA variants and their allelic frequencies reported in the Japanese are indicated.[15] The blood group *O* gene has a single base deletion at position 261 resulting in a frame-shift mutation and causing protein termination at codon 117.

was labeled with Cy5 flourescent dye. The nucleotide sequences of the forward and reverse primers were 5′-TCTCCATGTGCAGTAGGAAGGATG-3′ and 5′Cy5-ATGGCAAACACAGTTAACCCAATG-3′, respectively. PCR conditions were as follows: 0.5–1.0 μg of genomic DNA as a template, 0.2 μmol/l of each primer, 0.125 mmol/l deoxynucleoside triphosohate (dNTP), 0.25 units of AmpliTaq Gold DNA polymerase (Perkin Elmer-Cetus, Norwalk, CT, USA) in a total reaction volume of 25 μl. After the first denaturation step at 95°C for 12 min, 40 cycles were performed for amplification consisting of 30 s at 95°C, annealing for 30 s at 57°C, and extension for 30 s at 72°C followed by a final extension at 72°C for 7 min. PCR products were then treated with Klenow fragment (TAKARA SHUZO Co., Ltd, Shiga, Japan) to generate DNA fragments with blunt ends. To 1 μl of each PCR product, 0.5 units of Klenow fragment was added, and the mixture was incubated at 37°C for 30 min. One microliter of this reaction mixture was diluted with 10 μl of loading solution (90% deionized formamide, 20 mM EDTA, 0.05% bromophenol blue) and heat denatured at 95°C for 5 min. An ALF red automated DNA sequencer™ (Pharmacia, Tokyo, Japan) was used for blunt-end SSCP analysis. One microliter of the diluted mixture was applied onto a 15% polyacrylamide gel (30:1, acrylamide:bisacrylamide ratio) containing Tris/glycine buffer (25 mM Tris, 192 mM glycine). Electrophoresis was performed at 30 W for 16 h using a continuous buffer system consisting of 25 mM Tris and 192 mM glycine. During electrophoresis, the gel was maintained at a constant temperature of 18°C by a circulating water bath. The data were analyzed using the ALF Win Fragment analyzer 1.02™ software package (Pharmacia, Tokyo, Japan). LOH was determined by measuring the signal ratio between the opposing alleles and defined as tumor cellularity according to the equation that we previously reported.[4,26,27] Supposing that the A1 allele is lost in a heterozygote carrying A1 and A2 alleles, T is the peak height of the signal from the tumor samples and N is the peak height of the signal from normal control. The tumor cellularity in the sample is thus given as follows:

$$\text{Tumor cellularity (\%)} = [(N_{A1}/N_{A2}) - (T_{A1}/T_{A2})] \times 100/(N_{A1}/N_{A2})$$

Genomic DNA from normal PBL was analyzed to set the cutoff values for tumor cellularity. As previously reported, the mean + 3s.d. values of the normal heterozygous DNA were used as a cutoff value for tumor cellularity, and tumor samples showing tumor cellularities above the cutoff level were considered to have LOHs.[4] A104 allele was indistinguishable from B allele in this analysis, while the observed frequency of the A104 allele in the Japanese is reported to be as low as 1.3%. In fact, in all samples tested, the genotypes coincided with the patient's ABO isotypes. In addition, two single base nucleotide polymorphism markers (*ALDOB*, 9q21.3 and *VAV2*, 9q34.1) were used to assess the allelic status on 9q according to the method that we previously reported;[4] the former is centromeric and the latter is telomeric to the *ABO* gene locus, respectively (Figure 4). Nucleotide sequences of the forward and reverse primers for *ALDOB* and *VAV2* were as follows: 5′Cy5-GGGCTTGACTTTC CAACACG-3′ and 5′-TCTAGCCTCAATCCTCATAC-3′ (*ALDOB*), 5′-GTGTCTGCACTGGCCACACT-3′ and 5′Cy5-TCCAAAGGACCTTCTCCAAA-3′ (*VAV2*).

Bisulfite PCR-SSCP Analysis and Methylation-Specific PCR

In cases that underwent TUR, methylation status in the promoter region of the *ABO* gene was analyzed by bisulfite PCR-SSCP (BiPS) and methylation specific PCR (MSP).[24,28,29] Seven primer sets were designed to amplify seven overlapping regions spanning the CpG island located from −765 to +21 relative to the translation start site (Figure 2). Primer sets *re 1* through *re 6* were designed for BiPS analysis and *RE7*.M and *RE7*.UM were for MSP. Bisulfite treatment was performed using the CpGenome DNA Modification Kit (Intergen Co., New York, NY, USA). In all, 1 μg of tumor-derived DNA was treated with Na-bisulfite according to the manufacturer's recommendations. PCRs were performed in 25 μl reaction volumes containing 10 × buffer, 1.0 μl bisulfite-modified DNA corresponding to 50 ng of genomic DNA as a template, 0.2 μmol/l of each primer, 0.125 mmol/l dNTP and 0.25 units of AmpliTaq Gold DNA polymerase. PCR conditions were 95°C for 9 min for heat denaturation, 40 cycles of 94°C for 1 min, 1 min at the different annealing temperatures for each primer set (Table 2), 72°C for 2 min for amplification, followed by a final extension at 72°C for 10 min. The BiPS procedure was performed as previously described.[28,29] Nondenaturing polyacrylamide gels of 8% for *re 2* and *re 6*, 10% for *re 1*, *re 4* and *re 5*, and 15% for *re 3* were used for the analysis. CpGenome™ Universal Methylated DNA (CHEMICON International, Temecula, CA, USA) was used as a positive control, and PBL obtained from healthy control donors were used as a negative control. When extra bands were observed, they were cut from the gels, reamplified and subjected to direct sequencing using ABI 3100 PRISM sequencer with a Big-Dye terminator sequencing kit (Perkin-Elmer). In analysis of cases that underwent radical cystectomy, BiPS analysis was not employed due to the technical difficulty for reliable amplification of relatively long sized DNA fragments from formalin-fixed paraffin-embedded sections. In cases that underwent radical cystectomy, methylation status was assessed by MSP of region 7, the most proximal to the translation start site. The size of the PCR product was as short as 96 bp and amplifiable from archival samples with

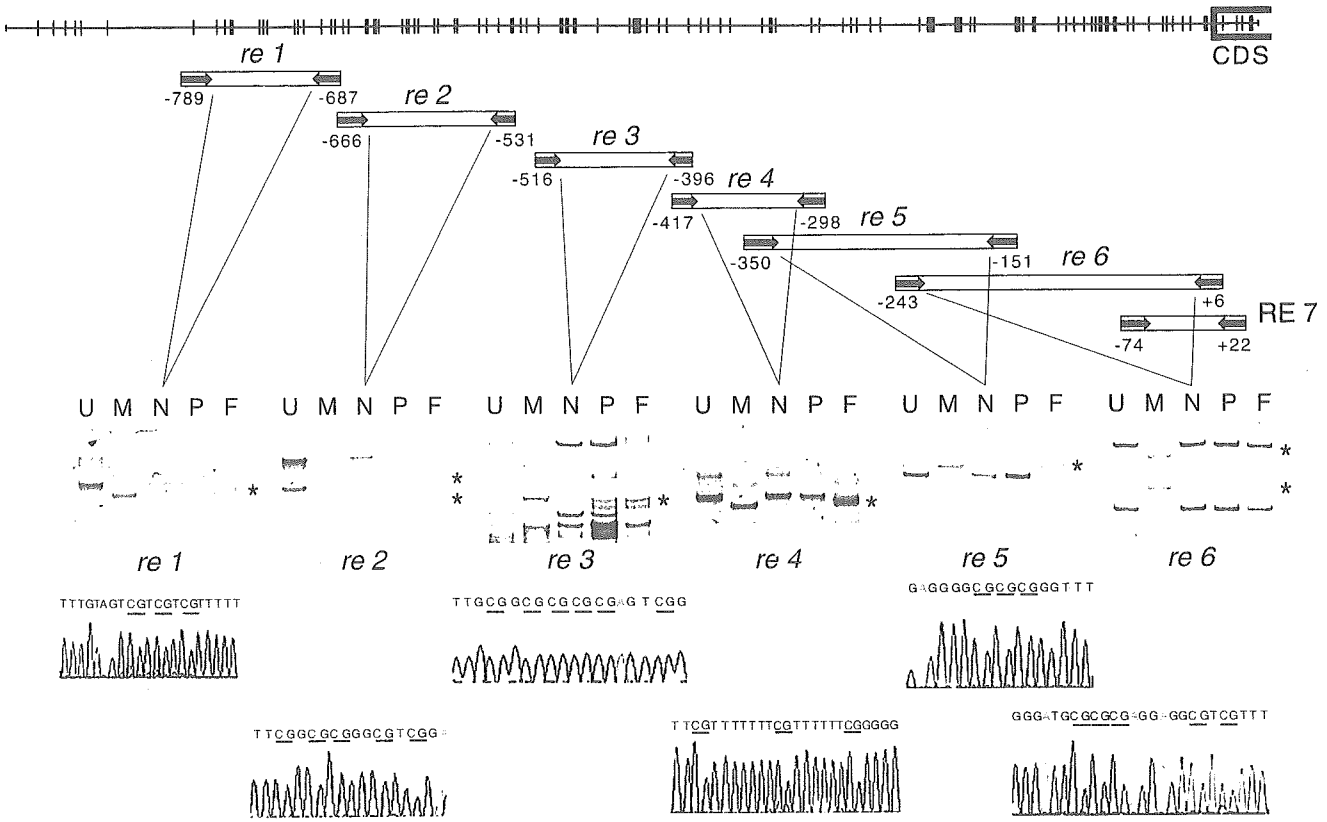

Figure 2 Map of the 5′ CpG island of the *ABO* gene and result of BiPS analysis. (Top) CpG sites in the promoter region of the *ABO* gene are indicated by vertical lines. (Middle) The amplified DNA fragments from regions 1 to 7 are indicated. PCR primer set of each region was indicated by arrows. (Bottom) BiPS analysis of the *ABO* gene. Extra bands are indicated by asterisks. After SSCP analysis, the extra bands were excised from gels, reamplified by PCR, and sequenced. Results of the direct sequencing of the case with full methylation were shown in the lower panel. U: unmethylated control, M: methylated control, N: no methylation, P: partial methylation, F: full methylation, *extra band showing mobility shift.

Table 2 Primer sequences for BiPS analysis and MSP

Primer name	*Forward primer sequence*	*Reverse primer sequence*	*Products length (bp)*	*No. of CpG sites*	*Annealing temperature (°C)*
re 1	5′-TTGGGATTTTTCGGGAGGTAATTT-3′	5′-CCCCGCTACGACCCCCGCCCTTAC-3′	103	11	54
re 2	5′-GGGCGGAGCGGGGTTTTGTTTACG-3′	5′-CGCGACCCCACGAAACTCTACGTC-3′	136	20	48
re 3	5′-AGCGATTTTGTTTAGGGGGA-3′	5′-ACTACGACCCCAAAACCCAC-3′	121	15	59
re 4	5′-TCGTGGGTTTTGGGGTCGTAGTTT-3′	5′-CCCCGTCCCCGAAAACCCCTTAAC-3′	120	11	54
re 5	5′-GGGGGTCGTTTTCGTTCGGGAGAT-3′	5′-CGAATCCCCAAAAACCCCTACTAA-3′	200	19	48
re 6	5′-TAAGGTATTAGGGTTACGAGG-3′	5′-GACCATAACTCCGCGTCTAAT-3′	248	33	49
RE 7.M	5′-GAGGGGGCGTTTCGGGTTTATTTC-3′	5′-ACGTCCGCAACACCTCGACCATAA-3′	96	16	70
RE 7.UM	5′-GGAGGGGGTGTTTTGGGTTTA-3′	5′-ATCCACAACACCTCAACCATAACT-3′	96	13	60

M, methylated; UM, unmethylated.

relative ease; however, five out of 37 cases that underwent radical cystectomy failed in PCR amplification. Methylation status of region 7 was used as the surrogate indicator for extensive methylation of the CpG sites or full methylation.

Statistical Analysis

Statistical analysis was performed using a likelihood χ^2 analysis or Fisher's exact test. Probability (P) values of <0.05 were considered to be significant.

Results

Expression of the A Antigen in TCC of the Bladder by IHC

Expression of the A antigen in tumor and normal urothelium was examined by IHC (Figure 3A). The corresponding staining of A antigen on the normal

urothelium from histo-blood-group B or O donors resulted in background levels only (data not shown). All of the normal urothelium from blood-group A individuals stained positively. The numbers of cases showing positive, heterogeneous and negative staining were 11 (25.0%), 11 (25.0%) and 22 (50.0%) in 44 tumor specimens that underwent TUR, while they were 14 (37.8%), 5 (13.5%) and 18 (48.6%) in 37 tumor specimens that underwent radical cystectomy. The overall frequencies of negative A antigen