の 50.1%，女性患者の 9.6%を占めていた．重症急性膵炎例においてもアルコール性が 35.4%（男性 51.7%，女性 7.0%）と最多の成因であった．1998 年の全国調査[6]と比べると，1 年間の推定受療患者数は 19,500 人から大きく増加，アルコール性の成因別頻度も 30.1%から増加していた．飲酒による急性膵炎発症のリスクを定量化した研究は少ないが，玉腰ら[7]は症例対照研究により急性膵炎発症と関連するライフスタイルを検討している．その結果，発症前 24 時間以内にエタノール換算で 100 g 以上飲酒した場合のリスクは，飲酒しなかった場合と比べてオッズ比が 4.4（95%信頼区間：1.3～15.5）と高かった．また，1988～1995 年にドイツで行われたコホート研究[8]によると，60 g/day 以上のアルコール消費をする高リスク群が 25 年間にアルコール性急性膵炎を発症するのはわずか 2～3%であった．アルコール性膵炎は圧倒的に男性に多かったが，高リスク群における急性膵炎発症率は男女でほぼ同等であったという．

一方，同じく厚生労働省難治性膵疾患に関する調査研究班が行った全国調査[9]では，2002 年 1 年間における慢性膵炎の受療患者は 45,200 人（95%信頼区間 35,600～54,700 人），男女比は 2.8：1 と推定されている．臨床診断基準における確診および準確診例と診断された慢性膵炎の成因は，アルコール性 67.7%，特発性 20.5%，胆石性 3.0%であった．とくに男性患者の 76.6%がアルコール性であった．2002 年の全国調査と 1999 年のそれ[10]を比較すると，アルコール性の割合が 54.0%から 67.7%へ増加し，特発性が 30.0%から 20.5%に減少していた．なお現在では一般に，純エタノール換算で 80 g/day 以上を 10 年以上あるいはそれに相当する量を飲酒し，他の成因を除外した場合にアルコール性慢性膵炎と診断することが多い．

丸山らは，大量飲酒が明らかであるアルコール依存症患者における膵炎の発症頻度について実態調査を行った[11]．それによると，アルコール性膵炎症例は 40 歳代の前半に発症する症例が多く，飲酒開始年齢が比較的若年（19 歳）で，1 日の飲酒量が比較的多かったという（日本酒換算 1 日平均 7.3 合）．また，アルコール性膵炎例では“つまみ”の量が少ない傾向があり，その内容は膵炎既往がない症例に比べて魚介類が多く，野菜や乾きものが少なかったと報告している．

膵におけるアルコール代謝

体内においてエタノールはおもに肝における酸化的代謝を受ける．約 80%がアルコール脱水素酵素（ADH）によりアセトアルデヒドに，残りの約 20%は，肝ミクロソームの酸化酵素系，とくにチトクローム P4502E1（CYP2E1）によって酸化される．アセトアルデヒドはアルデヒド脱水素酵素（ALDH2）によって酢酸に代謝される．膵にも肝の 1/4 程度の ADH と 1/3 程度の ALDH2 が存在する[12]．一方，carboxyl ester lipase（CEL）などの作用によりエタノールをエステル化する非酸化的（嫌気性）代謝経路も膵には存在し，細胞傷害性の強い脂肪酸エチルエステル（fatty acid ethyl ester：FAEE）が産生される．肝では酸化的代謝経路が主であるが，膵では酸化的・非酸化的両方の代謝経路が働く．そのため膵におけるアルコール代謝の特徴として非酸化的代謝経路が注目されている．1986 年に急性アルコール中毒で死亡した剖検例で FAEE が臓器に沈着しており，とくに脂肪組織と膵にもっとも高濃度であったという[13]．また，FAEE を血中投与するとラットに膵炎様病態を惹起する[14]が，アルコールそのものの急性投与は組織学的な膵炎病変を惹起できないことから，FAEE が膵障害を生じる主役とも考えられている．

アルコールによる膵傷害機序

アルコールによる膵傷害機序についてはいくつかの説が提唱されてきたが，確立されていない．以下に代表的な仮説を列記する[12,15,16]．

① **アルコール毒性説**（toxic metabolic theory）……エタノールおよびその代謝産物であるアセトアルデヒドなどが直接膵腺房細胞に作用して，細胞内シグナル伝達や細胞内代謝，細胞膜，小器官の恒常性などを障害する．また，膵液に移行し直接作用により膵実質の破壊・線維化を引き起こす可能性も指摘されている．

② **逆流説**（reflux theory）……慢性飲酒によって Oddi 括約筋が収縮して胆道と膵管の間に共通

管(common channel)が形成され，胆汁が膵管内に逆流して膵管壁を傷害し，膵酵素が活性化されて膵炎が発症すると考える胆汁膵管内逆流説，逆に慢性飲酒によって Oddi 括約筋の収縮不全が起こり，エンテロキナーゼを含む十二指腸液が膵管内に逆流して膵液を活性化し，膵炎を発症させるという十二指腸液膵管内逆流説もある．

③ **閉塞過分泌説(obstruction hypersecretion theory)**……アルコールは胃酸分泌を刺激し，胃酸が十二指腸内へ流入すると CCK やセクレチンなどの膵外分泌刺激ホルモンが分泌される．一方，十二指腸内に流入したアルコールは Oddi 括約筋の収縮や Vater 乳頭の浮腫を引き起こし，胆汁・膵液の十二指腸内への流入を障害するのみならず，十二指腸内の pH を低下させ，セクレチン分泌を刺激する．また，十二指腸内への胆汁流入が減少することにより CCK 分泌も増加する．このような膵に対する分泌刺激の増加と胆汁・膵液の流出障害により膵胆管内圧が上昇して膵液が膵実質内に逸脱し，膵炎が発症する．

④ **蛋白塞栓説(ductal plug theory)**……慢性飲酒が膵液中のラクトフェリンなどの蛋白を増加させ，膵液の粘調度が高くなった結果，小膵管内に蛋白栓(protein plug)が形成される．さらに，アルコールにより膵液中のカルシウム濃度が上昇し，逆にクエン酸分泌を抑制して蛋白塞栓へのカルシウム沈着を促進．蛋白栓や膵石によって膵液の流出障害，うっ滞と上流膵管内圧の上昇をもたらし，炎症と膵実質の破壊が生じて膵炎に至る．

⑤ **活性酸素・過酸化脂質産生説(free radical theory)**……飲酒により発生したフリーラジカルが膵腺房細胞膜の脂質過酸化を起こし，リソソームやミトコンドリアの膜変性や血管透過性亢進により膵組織を破壊し，膵炎に至る．

これらの説のほか，常習的飲酒により膵腺房細胞内において不活性化型で貯蔵されている膵酵素が活性化されることにより膵炎が起こるという細胞内膵酵素活性化説や，飲酒によって生じる高脂血症が膵リパーゼを活性化し，その結果，遊離脂肪酸が産生されて膵内の毛細血管や腺房細胞を障害して膵炎を惹起するという脂質代謝障害説などがある．いずれの説もアルコールによる膵炎発症のある面を説明するが，決定的証拠を提供するまでには至っていない．当然，これらの因子が複合的に作用している可能性もある．

アルコール性膵炎を起こしやすい先天的素因とは?

さて上述したようにアルコールは急性ならびに慢性膵炎の主要な原因ではあるが，大酒家でも膵炎を発症しない人も多い．Haber ら[17]は，1 日当りエタノール 80 g および 10 年以上の飲酒歴を有する大量飲酒者のうち，慢性膵炎を発症する割合は 5%以下と報告している．1 日当りエタノール 80 g 以上を 6〜12 年以上継続した大量飲酒者の病理解剖にて慢性膵炎の所見が認められたのは 10%にすぎなかったという報告[18]もある．さらに，わが国では大酒家のうち膵炎を発症するのは 1%に満たないという試算[19]もある．すなわち，膵炎の病態が成立するためには，個体側の要因，換言すればアルコール膵炎を起こしやすい先天的素因があることが想定される．そのような遺伝的背景に関する検討が最近精力的に行われている．以下にアルコール膵炎との関連の可能性が指摘されている代表的な遺伝子について概説する．

1. 膵分泌性トリプシンインヒビター(SPINK1)遺伝子

膵分泌性トリプシンインヒビター(PSTI，UniGene 名：serine protease inhibitor, Kazal type 1：SPINK1)は膵腺房細胞で合成，膵液中に分泌される．SPINK1 はトリプシンと結合し，異所性に活性化されたトリプシンの活性部位を阻害する．膵の総トリプシン活性の 20%を阻害しうるため，自己消化から膵を守る第 1 の防御機構として働く．Witt ら[20]は，若年の慢性膵炎患者における SPINK1 遺伝子変異を検索し，96 例中 22 例(23%)に遺伝子変異があることを見出した．22 例の遺伝子変異陽性患者のうち 18 例は N34S 変異(ホモ接合 6 例，ヘテロ接合 12 例)で，他に M1T 変異，L14P 変異，IVS3+2T>C 変異および−53C>T 変異を認めた．一方，Chen ら[21]は遺伝性膵炎患者と散発性慢性膵炎患者において N34S 変異と P55S 変異を見出した．しかし，これらの変異は健常人 400 人中，それぞれ 3 および 2 例にも認められたた

表 1　日本人慢性膵炎患者における*SPINK1*遺伝子変異の頻度

成因	*n*	N34S 変異						IVS3+2T>C 変異					
		全体	ht	hm	頻度*	アレル頻度	*p* 値	全体	ht	hm	頻度*	アレル頻度	*p* 値
アルコール性	76	0	0	0	0%	0%	—	3	2	1		2.6%	0.0096
非アルコール性	77	11	10	1	14.3%	7.8%	<0.0001	7	5	2	9.10%	5.8%	<0.0001
遺伝性	8	0	0	0	0%	0%	—	0	0	0	0%	0%	—
家族性	12	5	4	1	41.7%	25%	<0.0001	1	1	0	8.3%	4.2%	0.0678
特発性	47	5	5	0	10.6%	5.3%	0.0024	6	4	2	12.8%	8.5%	<0.0001
自己免疫性	9	1	1	0	11.1%	5.6%	0.1009	0	0	0	0%	0%	—
膵管非癒合	1	0	0	0	0%	0%	—	0	0	0	0%	0%	—
健常対照者	165	1	1	0	0.6%	0.3%	—	0	0	0	0%	0%	—

*：N34S 変異と IVS3+2T>C 変異の頻度は各成因の慢性膵炎患者における頻度．
n：患者数，ht：ヘテロ接合体，hm：ホモ接合体．

め，これら変異の慢性膵炎における意義は低いと推察した．その後，慢性膵炎患者における SPINK1 遺伝子変異の検討が各国よりなされたが，N34S 変異は特発性膵炎の 6.4〜25%に認められるとされている．頻度が報告により大きく異なるのは，地域差のみならず診断基準の違いなども影響しているのかもしれない．一方，アルコール性慢性膵炎における SPINK1 遺伝子変異の頻度は特発性膵炎に比べて低く，5.8%に認められたという報告がある[22]．

わが国における慢性膵炎患者の SPINK1 遺伝子変異は最近のデータ[23]（**表 1**）によると，非アルコール性慢性膵炎 77 例中 11 例（14.3%）に N34S 変異が認められ，とくに家族性膵炎（41.7%）や 30 歳未満で発症した特発性慢性膵炎（25.0%）で高頻度であった．一方，76 例のアルコール性慢性膵炎では N34S 変異は 1 例も認められなかった．IVS3+2T>C 変異は 77 例の非アルコール性慢性膵炎の 7 例，9.1%に観察されたが，N34S 変異と異なり家族性膵炎での頻度は高くなく，特発性膵炎でも 30 歳未満発症の若年例と 30 歳以上の発症例で頻度に差は認められなかった．76 例のアルコール性慢性膵炎の 3.9%に IVS3+2T>C 変異が観察され，アルコール性慢性膵炎と IVS3+2T>C 変異の間に有意な関連が認められた．なお IVS3+2T>C 変異は Kaneko ら[24]が報告したプロモーター領域の −215G>A 変異と完全連鎖不均衡にあり，IVS3+2T>C 変異を有する患者はすべて −215G>A 変異をも有していた．

2. ADH遺伝子

ADH はおもに，α，β，γ の 3 種類のサブユニットの組合せによって代謝活性の異なるアイソザイムを構成する．α，β，γ はそれぞれ独立した遺伝子 ADH1A（旧名 ADH1），ADH1B（旧名 ADH2），ADH1C（旧名 ADH3）の産物である．ADH1B には，ADH1B*1，ADH1B*2 および ADH1B*3 の 3 種類の allele が存在し，それぞれエタノール代謝に重要な β1，β2，β3 のサブユニット蛋白を産生し，この組合せによって個体のエタノール代謝能に差が生じる[25]．ADH1B*1/ADH1B*1 遺伝子がコードする β1/β1 の ADH は至適 pH が 10.8 で typical ADH ともよばれる[25]．一方，ADH1B*1/ADH1B*2 および ADH1B*2/ADH1B*2 がコードする β1/β2 および β2/β2 は至適 pH が 8.8 であり，atypical ADH ともよばれている．Atypical ADH は typical ADH に比べてエタノール酸化能の Vmax が約 40 倍高く，約 100 倍のエタノール酸化能をもつとされ[25]，摂取されたエタノールが速やかにアセトアルデヒドに分解される．

Yamauchi ら[26]は，日本人のアルコール依存症患者 96 例について検討し，慢性膵炎を合併している 29 例では ADH1B*2 頻度が 67.2%と，非合併 69 例の 48.5%に比べて有意に高いことを報告している．Matsumoto ら[27]もアルコール依存症患者 296 例を対象として膵石，膵管拡張などの慢性膵炎所見や膵障害の既往を検討し，膵障害を認めた 52 例において ADH1B*2 頻度は 60.6%であり，対照とした膵障害を認めないアルコール依存症患者 244 例の 48.0%に比べ有意に高率であったとして

いる．一方，Maruyama ら[28]は，アルコール性慢性膵炎確診群 54 例と，非アルコール性慢性膵炎患者 30 例における ADH1B 遺伝子型を膵機能正常のアルコール依存症患者と比較した．アルコール性慢性膵炎患者の ADH1B*2 頻度は 76.9%であり，膵機能正常のアルコール依存症患者における 50%に比べ高頻度であった．また著者らは，ADH1B*2/*2 の慢性膵炎例は ADH1B*1/*1 や ADH1B*1/*2 の慢性膵炎患者に比べて膵仮性嚢胞を形成しやすく，手術の頻度も高い傾向を見出している[26]．

このようにエタノールからアセトアルデヒドを，速やかに生成する ADH1B*2/*2 が膵傷害や線維化と関連しており，日本人のアルコール性慢性膵炎発症の遺伝的背景因子として重要な役割をもつと考えられた．

3. CEL遺伝子

CEL 遺伝子の exon 11 には，塩基の単純反復配列の繰返し回数の多型性である VNTR(variable number of tandem repeat)が存在する[29]．その生物学的意義は明らかではないが，蛋白質の安定性や酵素の分泌に関与している可能性がある[30]．Miyasaka ら[31]は，アルコール性膵炎患者 100 人(うち男性 98 人)，膵炎のないアルコール依存患者 52 人(すべて男性)，非アルコール性膵炎患者 53 人(うち男性 16 人)，健常人 435 人(うち男性 328 人)において，VNTR 数を検討し，男性アルコール性膵炎群において繰り返しの多いタイプが高頻度であることを報告した．VNTR が多いことが膵炎発症とどのようにかかわるかは明らかではない．CEL の機能亢進の結果，FAEE 沈着を増加させ膵炎発症につながるのかもしれない．

4. その他の遺伝子

このほか，嚢胞性線維症の原因遺伝子である cystic fibrosis transmembrane conductance regulator(CFTR)遺伝子とアルコール性慢性膵炎の関連が報告されている[32]．

おわりに

大酒家のうち膵炎を発症するのは少なく，酒を飲みすぎてもかならずしも膵炎にはならない．その背景としてアルコール代謝速度を規定する代謝酵素などの遺伝子多型の関与が明らかとなってきている．しかし，単一の遺伝子多型では説明できないことから，種々の因子が複合的に関与しているのであろう．一方，現在の慢性膵炎診断基準により診断されるアルコール性慢性膵炎の大半はすでにかなりのアルコール摂取がされており，病状が終末像として完成しているという問題がある．このため，慢性膵炎確診，準確診に至らない軽微な変化を表す臨床的カテゴリーとして“アルコール性膵症(alcoholic pancreatopathy)”という概念が提唱されている[33](「サイドメモ」参照)．今後，アルコール性膵炎の病態解明とともに，早期診断法の開発が強く望まれる．

サイドメモ

アルコール性膵症

現在の診断基準を補い，アルコール性膵傷害の早期病態を拾い上げる目的で，“アルコール性膵症”の概念が 2005 年に提唱され，その診断基準案が作成されている[33]．この診断基準案はアルコールを原因として惹起される膵の異常をすべて包括する概念として“アルコール性膵傷害”を定義している．このうち，アルコール性急性膵炎とアルコール性慢性膵炎の 2 つの疾患群に含まれず，現行の慢性膵炎準確診症例を含むアルコール多飲による膵異常を“アルコール性膵症”としている．診断基準として継続的に飲酒している飲酒家または大酒家で，①急性膵炎の既往歴のあるもの，②反復する腹痛発作のあるもの，③血中 P 型アミラーゼあるいは血清リパーゼ値が高値を示すもの，④画像診断，膵組織像においてアルコール多飲に起因すると考えられる軽微な変化が観察されるもの，のいずれかを示し，他疾患が否定されるものを“アルコール性膵症”としている．

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Health Risk Appraisal Models for Mass Screening of Esophageal Cancer in Japanese Men

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Abstract

Background: **Because early squamous cell carcinoma (SCC) of the esophagus is detectable by endoscopic esophageal iodine staining with high accuracy and is easily treated by endoscopic mucosectomy, it is important to develop efficient methods for screening candidates for the endoscopic examination. Inactive aldehyde dehydrogenase-2 (ALDH2) is a very strong risk factor for esophageal SCC in alcohol drinkers and thus may be suitable as a screening tool.**

Purpose: **To assess the performance of health risk appraisal (HRA) models in screening for esophageal SCC in the Japanese male population.**

Methods: **Two types of HRA models were developed based on our previous case-control study, which included assessment of ALDH2 activity and selected risk factors (HRA-G and HRA-F: activities of ALDH2 assessed by genotype and questionnaire for alcohol flushing, respectively). Each individual's risk of esophageal SCC was calculated quantitatively as a risk score. The sensitivity and specificity of the HRA models at various cutoff values of risk score was estimated by a leave-one-out cross-validation. The positive predictive value was estimated assuming the prevalence of esophageal SCC in the whole population to be 0.17% or 0.39% according to literatures.**

Results: **When individuals ranked in the top 10% of the HRA-F risk score was screened, the sensitivity was 57.9% and positive predictive value was 0.93% or 2.12% according to the above assumptions, respectively. The sensitivity was slightly better by the HRA-G model than by the HRA-F model.**

Conclusion: **The HRA models may provide an important approach to early intervention strategies to control esophageal SCC in Japanese men.** (Cancer Epidemiol Biomarkers Prev 2008;17(10):2846–54)

Introduction

Because early squamous cell carcinoma (SCC) of the esophagus and oropharyngolarynx can be treated by endoscopic mucosectomy (1, 2) or endoscope-guided mucosectomy (3), it is important to develop methods to identify individuals at increased risk of cancer of the upper aerodigestive tract to provide detailed examinations by the upper aerodigestive tract endoscopy combined with esophageal iodine staining. Without using the esophageal iodine staining, more than half of intraepithelial or mucosal esophageal SCC would be missed (2, 4). A possible approach to mass screening of high-risk individuals is to classify them according to exposure to risk factors such as heavy alcohol drinking and smoking. However, the prevalence of drinkers and smokers in Japanese men is so high (e.g., 35.7% of men drink every day and 43.3% are current smokers in 2004; ref. 5) that it is not practical to conduct detailed endoscopic examinations on all of them; therefore, a more effective screening method is required.

A mutant allele encoding an inactive subunit of aldehyde dehydrogenase-2 (*ALDH2**2) is prevalent (42%) in the Japanese population (6), and the ALDH2 genotype determines an individual's blood acetaldehyde concentration (7). Acetaldehyde has been established as a carcinogen in experimental animals (8) and is suspected of playing a critical role in cancer development in humans (9). Case-control studies in Japanese (10-13) and Taiwanese (13-16) individuals and prospective studies in which esophageal iodine staining has been used in Japanese alcoholics (17-19) have consistently shown a very strong link between the risk of esophageal SCC and alcohol drinking in people possessing the *ALDH2**1/*2 genotype. Alcohol drinking together with the *ALDH2**1/*2 genotype has been reported to be a risk factor for multiple cancerization in the upper aerodigestive tract (13, 17, 19-21) and for oropharyngolaryngeal SCC (13, 18, 20, 22, 23). The IARC has recently concluded that substantial mechanistic evidence in humans with inactive ALDH2 indicates that acetaldehyde derived

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from the metabolism of ethanol in alcoholic beverages contributes to esophageal cancer (9). Because drinking a small amount of alcohol results in acetaldehydemia and unpleasant alcohol flushing responses in persons with inactive ALDH2, the activity of ALDH2 can be assessed by a simple questionnaire that asks about both current and past facial flushing (24, 25). This simple questionnaire about flushing as a marker of inactive ALDH2 is a highly reliable means of detecting inactive ALDH2 and predicting the risk of SCC in the upper aerodigestive tract (11, 18, 22, 25-27).

Increased mean corpuscular volume (MCV) is associated with alcohol drinking, especially drinking by inactive ALDH2 heterozygotes, and with smoking, low body mass index, and poor nutrition, all of which increase the risk of SCC in the upper aerodigestive tract (26, 27). We showed recently that MCV is a marker for drinkers who are at high risk of SCC in the upper aerodigestive tract (18, 26-28).

Based on our previous case-control study of esophageal SCC in Japanese men (12, 25), we developed simple health risk appraisal (HRA) models that predicted an individual's risk of developing esophageal cancer based on logistic regression analyses. In addition to drinking habits, smoking habits, and diet, the HRA models included either ALDH2 genotype or the results of a simple questionnaire about alcohol flushing (26). Each individual was ranked according to his risk of esophageal SCC. Persons in the top 10% risk category for esophageal SCC, as estimated by the HRA model that included ALDH2 genotype, were selected with high sensitivity by two simple criteria, that is, the combination of moderate/heavy drinking plus "heavy smoking or alcohol flushing" and the combination of moderate/heavy drinking plus "heavy smoking or MCV ≥ 99 fl" (26). If these models are valid for the screening of high-risk individuals for esophageal SCC, a detailed examination by endoscopy of such high-risk individuals may provide an efficient method for detecting early esophageal SCC. In the present study, we assessed the performance of screening methods with our HRA models in terms of sensitivity, specificity, and positive predictive value (PPV) to identify individuals with and without esophageal SCC.

Materials and Methods

Data of Case-Control Study

Study Subjects. We previously conducted a case-control study of 234 male cases with esophageal SCC and 634 male cancer-free controls and reported the results (12). The case participants were male Japanese patients with primary esophageal SCC undergoing treatment at the National Cancer Center Hospital, National Cancer Center Hospital East, Kawasaki Municipal Hospital, or National Osaka Hospital. The cancer-free controls were men who came to two Tokyo clinics for annual health checkups, and most of them were ordinary residents or workers living in Tokyo or surrounding areas. The age-adjusted prevalences of current smokers and habitual drinkers in the controls were very similar to those in the Tokyo metropolis assessed by the National Nutrition Survey in Japan, a nationwide population-based survey using representative samples (29). Thus, the controls represented the general population of Tokyo well, at least with regard to drinking and smoking habits (12). The ethics committee of each collaborating institute reviewed and approved the proposal for this study, and each of the participants gave his informed consent.

Measurement of Risk Factors. Each participant independently completed a structured questionnaire concerning his drinking, smoking, and dietary habits; those with cancer were instructed to report on their habits before they got sick. The contents of the questionnaire and the method of calculating alcohol consumption (1 unit = 22 g, the ethanol content of one serving of *sake*) were described previously (12). The subjects were classified as never/rare drinkers, ex-drinkers, or current drinkers who consumed 1 to 8.9 units/wk (light drinkers), 9 to 17.9 units/wk (moderate drinkers), or ≥18 units/wk (heavy drinkers). MCV was measured during the health checkups in the

Table 1. Risks of esophageal SCC according to selected risk factors including ALDH2 genotype or alcohol flushing

Model		Estimated multivariate risks of esophageal SCC, OR* (95% confidence interval)
Risk factors		
Multivariate model including *ALDH2* genotype		
ALDH2 genotype	Alcohol drinking	
2*1/*1	Never/rare	0 (not calculable)
	Light	1 (reference)
	Moderate	5.58 (1.54-20.25)
	Heavy	10.38 (2.85-37.84)
	Ex-drinker	8.81 (1.53-50.76)
2*1/*2	Never/rare	0.75 (0.14-4.11)
	Light	5.82 (1.59-21.38)
	Moderate	55.84 (15.40-202.51)
	Heavy	88.88 (23.97-329.57)
	Ex-drinker	50.50 (9.18-277.95)
2*2/*2	Never/rare	1.44 (0.22-9.54)
	Light	0 (not calculable)
Strong alcoholic beverages†		4.58 (2.10-9.99)
Smoking‡		2.36 (1.52-3.65)
Green-yellow vegetables§		1.63 (1.00-2.66)
Fruit§		1.73 (1.01-2.94)
Multivariate model including alcohol flushing		
Flushing	Alcohol drinking	
Any	Never/rare	1 (reference)
Never	Light	1.27 (0.27-5.88)
	Moderate	10.12 (3.45-29.69)
	Heavy	15.61 (5.19-46.91)
	Ex-drinker	27.31 (5.24-142.46)
Current/former	Light	6.69 (2.21-20.20)
	Moderate	42.66 (14.17-128.42)
	Heavy	72.86 (23.75-223.57)
	Ex-drinker	37.00 (7.66-178.76)
Strong alcoholic beverages†		3.59 (1.63-7.87)
Smoking‡		2.62 (1.71-4.00)
Green-yellow vegetables§		1.65 (1.03-2.64)
Fruit§		1.57 (0.94-2.62)

* Simultaneously adjusted for all the variables (including age; not shown) in each multiple logistic regression model. These ORs were estimated by our previously reported case-control study. See refs. 12 and 25 for details.
† Frequent versus never/sometimes (reference).
‡ ≥30 versus <30 pack-years (reference).
§ Not every day versus almost every day (reference).

Risk factors		Score (select one each for A-E)	
ALDH2 genotype and alcohol drinking			
*ALDH2*1/*1*			
Never/rare	(<1 unit/w)	-12.94	A
Light	(1-8.9 units/w)	0.00	
Moderate	(9-17.9 units/w)	1.72	
Heavy	(18+ units/w)	2.34	
Ex-drinker		2.18	
*ALDH2*1/*2*			
Never/rare	(<1 unit/w)	-0.29	
Light	(1-8.9 units/w)	1.76	
Moderate	(9-17.9 units/w)	4.02	
Heavy	(18+ units/w)	4.49	
Ex-drinker		3.92	
*ALDH2*2/*2*			
Never/rare	(<1 unit/w)	0.37	
Light	(1-8.9 units/w)	0.00	
Drinks strong alcoholic beverages frequently			
	Yes	1.52	B
	No	0.00	
Smoked 30 pack-years or more			
	Yes	0.86	C
	No	0.00	
Eats green-yellow vegetable almost every day			
	Yes	0.00	D
	No	0.49	
Eats fruit almost every day			
	Yes	0.00	E
	No	0.54	

Total score = A + B + C + D + E

Predicted risk	Total score
Bottom 25%	≤1.02
25-49%	1.03-2.33
50-74%	2.34-3.60
75-89%	3.61-4.56
Top 10%	4.57+

Figure 1. HRA model for esophageal cancer that includes ALDH2 genotype. The risk score is calculated as the sum of scores A to E. The higher the score, the higher the risk. For example, if an individual's risk is ≥4.57, his risk of esophageal SCC is in the top 10% in this study population.

controls and at the time of diagnosis of esophageal SCC in the cases. The activity of ALDH2 was assessed by ALDH2 genotype and a facial flushing response to alcohol drinking. The PCR-restriction fragment length polymorphism method had been done on lymphocyte DNA samples to determine the ALDH2 genotype (12). The flushing response was assessed by two questions (25): (*a*) Do you have a tendency to flush in the face immediately after drinking a glass of beer (yes, no, or unknown)? (*b*) Did you have a tendency to flush in the face immediately after drinking a glass of beer during the first to second year after you started drinking (yes, no, or unknown)? The designation "current flushing" was applied to individuals who answered "yes" to question (*a*) and "former flushing" to those who answered "no" or "unknown" to question (*a*) and "yes" to question (*b*). The remaining subjects were classified as "never flushing." Current or former flushing individuals were assumed to have inactive ALDH2.

This approach identified inactive ALDH2 genotype with 90% sensitivity and 88% specificity in the cancer-free controls (25). Data on ALDH2 genotype were available for 234 cases and 634 controls (12); data on alcohol flushing were available for 233 cases and 610 controls (25). The distribution of ALDH2 genotypes in the control subjects was similar to that reported in other Japanese studies (6, 10, 23).

HRA Models

Scoring Each Individual's Risk of Esophageal SCC. Table 1 summarizes the estimated odds ratios (OR) of selected risk factors for esophageal SCC in our previously reported case-control study (12, 25). We calculated the risk of esophageal SCC for each cancer-free control subject by the previously reported method (26) based on alcohol drinking, ALDH2 genotype (or alcohol flushing), smoking, and intake of vegetables and fruit, using the formula: $RR_i = \exp[z_i - z_0]'\hat{\beta}$, where RR_i is the ith individual's OR [vs. the reference individual, who is a light drinker with the *ALDH2*1/*1* genotype (or a never/rare drinker with any category of alcohol flushing), does not drink strong alcohol beverages frequently, smoked less than 30 pack-years, and eats vegetables and fruit every day]; z_i is an observed vector of the ith individual's risk factors; z_0 is a vector of the reference individual's risk factors; and $\hat{\beta}$ is the vector of logistic regression coefficients estimated in our previously reported case-control study (12, 25). In other words, the ith individual is RR_i times more likely to develop esophageal SCC than the reference individual. In actual practice, the above formula is equivalent to simply calculating the product of RRs that correspond to the individual's risk factors. For example, computation with the estimation model that includes the ALDH2 genotype is illustrated below. When the ith individual is a moderate drinker with

*ALDH2*1/*2* [log OR = 4.02 (OR = 55.84); see Table 1 and Fig. 1], does not drink strong alcohol beverages frequently (log OR = 0), smoked ≥30 pack-years (log OR = 0.86), and does not eat vegetables and fruit every day (log OR = 0.49 and 0.54, respectively), his risk is calculated as the sum of these log ORs (log RR_i = 4.02 + 0 + 0.86 + 0.49 + 0.54 = 5.91). Hereafter, each subject's RR_i (for the *i*th individual versus the reference subject) is designated as RR_{ind} to express his risk of esophageal SCC. The subjects were classified into five risk categories based on the percentiles of the log RR_{ind} value among all controls in our previous case-control study (12, 25): bottom 25%, 25% to 49%, 50% to 74%, 75% to 89%, and top 10%.

The procedures used to make these calculations are summarized in Figs. 1 and 2. Although the calculations are relatively simple, because an electronic calculator may be required to sum the scores with two decimal places and it may be inconvenient in clinical situations or for mass-screening purposes, we further simplified the HRA model that included alcohol flushing by converting the scores to small integers ("integer score" in Fig. 2). The integer score was obtained by multiplying the original score by a constant, 2.265, and then rounding to the nearest integer, with the constant having to meet the following three conditions: (*a*) each integer score must be between 0 and 10, so that most people are able to sum them in their head; (*b*) the Spearman rank correlation coefficient between totals of the original scores and totals of the integer scores must be close to 1.0; and (*c*) a cutoff value that divides the subjects into the approximately top 10% and bottom 90% should be established because we intend to identify the top 10% risk individuals from the population and conduct a detailed examination of them by endoscopy with esophageal iodine staining. When 2.265 was used as the constant, it yielded a Spearman rank correlation coefficient of 0.997 and a cutoff value of 11, which selected 11.1% of the subjects.

Cross-Validation Study. The performance of the HRA models was assessed in terms of sensitivity (the percentage of subjects predicted to have a cancer among the cancer cases), specificity (the percentage of subjects predicted to be cancer-free among the cancer-free controls), and PPV (the percentage of patients with cancer among the selected high-risk individuals). The maximum likelihood estimates of the logistic regression model most effectively predict the data that generated them but do not perform so well when used for predictions on new data (30). Therefore, the sensitivity and specificity were estimated by the cross-validation method, which is a data-oriented method to more correctly assess the performance of a statistical model for predicting new data (30). We used the leave-one-out cross-validation method as follows:

1. Let Q be the percentage of subjects ($0 < Q < 100\%$) to be selected as candidates for detailed examinations by endoscopy.
2. Remove one subject from the case-control data (n subjects in total) and generate a HRA model, as described above, using the remaining cases and controls (n - 1 subjects in total). Calculate RR_{ind} for each of the remaining controls and the left-out subject using this HRA model.
3. If the RR_{ind} for the left-out subject is above or equals to the $(1 - Q) \times 100$th percentile of the RR_{ind} for the remaining controls, the left-out subject is predicted to have a cancer; otherwise to be cancer-free.
4. Repeat nos. 2 and 3 by removing each of the cases and controls n times in total.

Risk factors		Score (select one each for A-E) Original score	*(Integer score)*	
Alcohol flushing and drinking				
Any flushing				
Never/rare	(<1 unit/w)	0.00	*(0)*	
Never flushing				
Light	(1-8.9 units/w)	0.24	*(1)*	
Moderate	(9-17.9 units/w)	2.31	*(5)*	
Heavy	(18+ units/w)	2.75	*(6)*	
Ex-drinker		3.31	*(7)*	
Current/former flushing				
Light	(1-8.9 units/w)	1.90	*(4)*	
Moderate	(9-17.9 units/w)	3.75	*(9)*	
Heavy	(18+ units/w)	4.29	*(10)*	A
Ex-drinker		3.61	*(8)*	
Drinks strong alcoholic beverages frequently				
	Yes	1.28	*(3)*	B
	No	0.00	*(0)*	
Smoked 30 pack-years or more				
	Yes	0.96	*(2)*	C
	No	0.00	*(0)*	
Eats green-yellow vegetable almost every day				
	Yes	0.00	*(0)*	D
	No	0.50	*(1)*	
Eats fruit almost every day				
	Yes	0.00	*(0)*	E
	No	0.45	*(1)*	

Total score = A + B + C + D + E

Predicted risk	Total score Original	*Integer*
Bottom 25%	≤1.18	*0-2*
25-49%	1.19-2.78	*3-5*
50-74%	2.79-3.80	*6-8*
75-89%	3.81-4.70	*9-10*
Top 10%	4.71+	*11+*

Figure 2. HRA model for esophageal cancer that includes alcohol flushing. The risk score is calculated as the sum of scores A to E. The higher the score, the higher the risk. The integer score is prepared for a self-administered questionnaire in mass screening, where each participant calculates his risk score in his head.

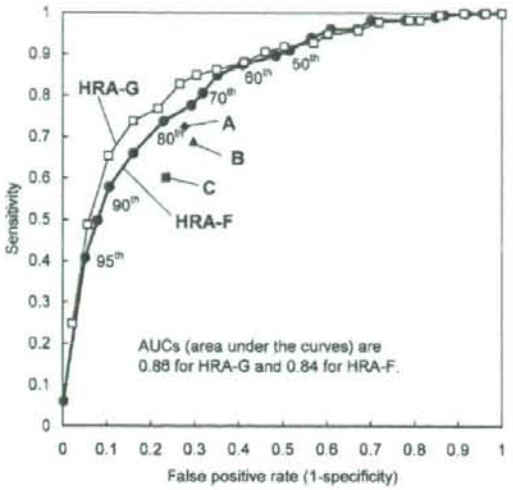

Figure 3. Sensitivity and specificity for screening esophageal SCC by two HRA models (HRA-G and HRA-F) and three other simple criteria (A-C). The ROC curves are for HRA models, where the sensitivities and specificities are calculated by the leave-one-out cross-validation method; the cutoff values for screening (denoted as 50th, 60th, etc.) are percentiles of the HRA risk score in the cancer-free control subjects. HRA-G: activity of ALDH2 assessed by genotype. HRA-F: activity of ALDH2 assessed by a questionnaire for alcohol flushing. **A.** Moderate-to-heavy drinking plus either smoking ≥30 pack-years or flushing. **B.** Moderate-to-heavy drinking plus either smoking ≥30 pack-years or MCV ≥ 99 fl. **C.** Moderate-to-heavy drinking and smoking ≥30 pack-years.

The percentage of models that correctly predict the left-out cases to have a cancer is the cross-validated sensitivity; the percentage that correctly predict the left-out controls to be cancer-free is the cross-validated specificity. We computed the receiver operating characteristic (ROC) curve for the HRA models by changing Q from 0 to 100% for calculation of cross-validated sensitivity and specificity. The area under the curve was computed via numerical integration of the ROC curve.

If PPV is extremely low, the esophagoscopy with iodine staining would not be practical in terms of cost efficiency. Therefore, we estimate PPV, which is a function of sensitivity, specificity, and prevalence of disease in a whole population (p) according to the following formula (31):

$$\mathrm{PPV} = \frac{p \times \text{sensitivity}}{p \times \text{sensitivity} + (1-p)(1-\text{specificity})}$$

The data on sensitivity and specificity are available as explained above. However, data on prevalence of the esophageal cancer in the whole population are very limited, and mass screening for targeting esophageal cancer by the esophageal iodine staining technique has not been conducted for the whole population. The detection rate of esophageal cancer by endoscopy in men ages ≥40 years was 0.39% in the Research Center for Cancer Prevention and Screening Program (32), where esophageal iodine staining was applied when the mucosal surface appeared abnormal. The detection rates of stomach cancer by mass screening using endoscopy were 0.87% among 11,679 people ages ≥40 years in Niigata city in 2004 (33). Because the incidence of esophageal cancer was only 19.3% of that of stomach cancer in men ages ≥40 years and the incidence of stomach cancer was higher in men than in women in Japan in 2001 (34), the detection rate of esophageal cancer would be higher than 0.17% (0.87% × 0.193) according to these data. Thus, we assumed two different detection rates of 0.17% and 0.39% (lower and higher assumptions, respectively) for the detection rate of esophageal cancer ($\approx p$ by esophageal endoscopy with iodine staining) in the whole population.

We also calculated the sensitivity and specificity of three simple combinations of criteria for estimating the risk of cancer: moderate-to-heavy drinking plus smoking ≥30 pack-years; moderate-to-heavy drinking plus "smoking ≥30 pack-years or alcohol flushing"; and moderate/heavy drinking plus "smoking ≥30 pack-years or MCV ≥ 99 fl" (26). The sensitivity is the percentage of cases who met the criterion; the specificity is that of controls who did not meet it.

All statistical analyses were done with the SAS statistical package version 9.1 (SAS Institute).

Results

Figure 3 shows the cross-validated ROC curves of the two HRA models (HRA-G: activity of ALDH2 assessed by the genotype and HRA-F: activity of ALDH2 assessed by the flushing questionnaire) for predicting esophageal cancer. The ROC curve of HRA-F model showed that when people in the top 10% of risk scores were selected for detailed endoscopic examinations, 57.9% (sensitivity at cutoff value of 90th percentile) of cancer cases in the whole population were expected to be included in them. The HRA-G model selected cancer cases with a higher sensitivity (65.4%) at this cutoff value. When the 80th percentile was used as the cutoff value, 73.8% (HRA-F model) or 76.9% (HRA-G model) of all cancer cases were selected as candidates for detailed endoscopic examinations, mildly improving the sensitivity at the cost of a doubled false-positive rate. It should be noted that the specificity is approximately equal to the cutoff value (denoted as the percentile) by definition but could be slightly different because of ties (equal ranking) of scores (e.g., 23% of controls are above or equal to the 80th percentile by the HRA-F model). The area under the curve was slightly higher for the HRA-G model than HRA-F model (0.86 and 0.84, respectively).

The sensitivity and specificity for the simple combinations of criteria are also shown in Fig. 3: (*A*) moderate-to-heavy drinking plus "smoking ≥30 pack-years or alcohol flushing," (*B*) moderate/heavy drinking plus "smoking ≥30 pack-years or MCV ≥ 99 fl," and (*C*) moderate-to-heavy drinking plus smoking ≥30 pack-years. Criterion (*C*) showed relatively low sensitivity (60.1%) and false-positive rate (23.3%); criterion (*A*) showed relatively high sensitivity (72.5%) and false-positive rate (27.7%). All

criteria were below the ROC curves of HRA models, indicating that these combinations of risk factors were not as useful as the HRA models.

Figure 4 shows the expected PPV among individuals selected by the HRA-F model at different cutoff values. When people in the top 10% of risk scores were selected for detailed endoscopic examinations (cutoff value = 90th percentile), the expected PPV were 0.93% and 2.12% according to the lower and higher assumptions for the prevalence in the whole population, respectively.

In the HRA-F model, the top 10% of individuals were selected by the original score of 4.71 or above, whereas 96% of them were selected by the integer scores of ≥11 (data not shown), showing that the integer score can be used instead of the original score for the mass-screening purpose.

Discussion

This study assessed the performance of screening methods for detecting individuals who have a high risk of esophageal cancer using HRA models that were developed based on our previous case-control study (12, 25). Because each individual's risk is calculated as a quantitative score and the risks differ extraordinarily among the individuals (e.g., RR_{ind} ranked at the bottom 25th and top 10th percentiles differ 83-fold by the HRA-G model), we can give a higher priority for detailed examination to those who have the higher scores. The sensitivity and specificity of the HRA-G model are slightly superior to those of the HRA-F model, but the low-cost is a significant advantage of the latter. When we select individuals with the top 10% risk scores of HRA-F, the sensitivity is 57.9%. This means that approximately 60% of esophageal cancer in the whole population can be detected by examining only 10% of them. Furthermore, the expected detection rate of esophageal cancer among the screened high-risk men (PPV) is 0.93% based on a lower assumption and may be more than 2% through the use of advanced diagnostic technology including esophageal endoscopy combined with iodine staining (higher assumption). If these indexes of screening are true, the application of our HRA models for the mass screening of esophageal cancer would be highly cost-efficient.

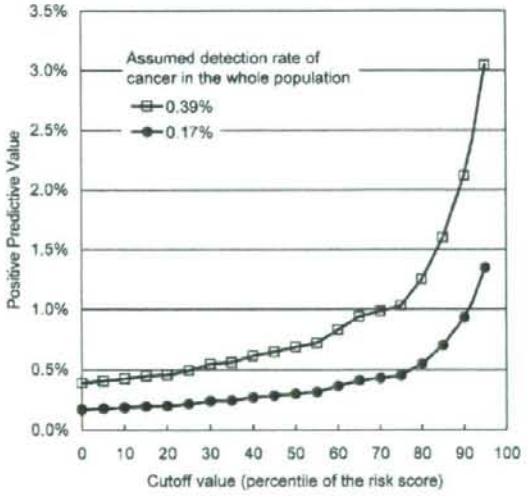

Figure 4. Expected PPV among individuals selected by the HRA-F model at different cutoff values. Because the overall detection rate of esophageal cancer in the whole population by endoscopy is unknown, two rates were assumed according to the literatures (0.17% and 0.39%). For example, if the overall detection rate in the whole population is 0.17% and the 90th percentile of risk score (that is, upper 10%) is used as the cutoff value, the expected PPV is 0.93%.

The generalizability of our estimates of sensitivity, specificity, and PPV depends on the difference in distributions of risk factors between the background population of our case-control study and the target population to which the HRA models are applied. Especially, the prevalence of inactive ALDH2 and alcohol drinking would strongly affect the performance of screening using the HRA models. The magnitude of the ALDH2-associated risk depends on the extent of the association between the evaluated cancer and alcohol consumption and the proportion of alcohol drinkers with inactive ALDH2. Case-control studies in high-risk rural regions in China, where alcohol drinking plays a less important role in esophageal carcinogenesis than in Japan and Taiwan, showed moderate-to-modest positive or no associations (35-37) between inactive heterozygous ALDH2 and esophageal cancer risk. A case-control study in a Thai population, where only 18% of the controls have inactive ALDH2, also showed a marginally significant modest positive association (38). The inhibitory effect of inactive heterozygous ALDH2 on alcohol drinking is influenced by sociocultural factors; thus, only 3% of Japanese alcoholics had the inactive heterozygous ALDH2 in 1979 as opposed to 8% in 1986 and 13% in 1992 (39).

This phenomenon inversely suggests the possibility, using the HRA models, that alcohol consumption by inactive ALDH2 heterozygotes is decreased by social intervention such as public education. Thus, the HRA models that include inactive ALDH2 or alcohol flushing should be changed according to different regions and different eras in Asia. ALDH2-related susceptibility to esophageal SCC has been shown in Japanese female heavy drinkers (11). However, a much smaller proportion of women with heterozygous ALDH2 are drinkers in comparison with men, resulting in a smaller population attributable risk of esophageal SCC for alcohol drinking plus heterozygous ALDH2 in women than in men (11, 12). Therefore, we intended to apply our HRA models for screening esophageal cancer in Japanese male populations. The proportion of people selected by our cutoff value for the top 10% risk may be smaller than 10% in a population where the prevalence of drinkers is small or vice versa. However, the importance of screening for esophageal cancer in such a low-risk population would be relatively small. The age-adjusted death rates from esophageal cancer differ considerably among the 47 prefectures in Japan (40) and are strongly correlated with annual per capita consumption of alcoholic beverages in each prefecture (ref. 41; Spearman's rank correlation coefficient = 0.68; $P < 0.0001$). Further studies are needed to clarify the geographical difference

in the distribution of HRA risk score among Japanese male populations.

From a cost-efficiency point of view, the HRA-F to detect esophageal cancer in a mass screening has the advantage that it is available almost at no cost and deliverable not only at the clinic but also via mass media, Internet, and other sources. For the screened top 10% high-risk individuals, the cost of endoscopic esophageal iodine staining is 12,000 Japanese yen (JPY; 1 US$ ≈ 105-108 JPY in June 2008) per person. Thus, the cost to identify one case of esophageal cancer is roughly estimated, by dividing 12,000 JPY by the PPV, to be 570,000 to 1,290,000 JPY (higher and lower assumptions, respectively), which is less expensive than that of gastric cancer by endoscopic screening (1,608,000 JPY) and photofluorography (3,290,000 JPY) in a Japanese population (33). As for the HRA-G, the expected PPV (2.40% and 1.06% for higher and lower assumptions, respectively, with the cutoff value of the top 10%) is slightly better; thus, the estimated cost of endoscopic examination to identify one case of esophageal cancer is somewhat smaller (500,000-1,130,000 JPY, respectively) than that of HRA-F. However, a large *initial* cost would be required for genotyping of ALDH2 for all individuals in the target population. The additional cost to identify one case of esophageal cancer is estimated as the unit cost of genotyping divided by the detection rate of cancer among all subjects, and this may far exceed the cost of endoscopic examination. However, it should be emphasized that genotyping is needed only once in a lifetime and the data are available repeatedly; and the unit cost would be largely discounted when a huge number of samples is analyzed.

On the other hand, we have no data to analyze the *effectiveness* of mass screening to decrease mortality from esophageal cancer because there has been no large-scale screening of esophageal cancer conducted in Japan. When an asymptomatic high-risk population was screened by a combination of endoscopy and esophageal iodine staining, 76% of the esophageal cancer detected did not invade the submucosa (4). Such early esophageal cancer can be safely treated by endoscopic mucosal resection, and the patients are generally hospitalized for only a week or less (1) with relatively low medical costs. Notably, the cause-specific survival rates 5 years after endoscopic mucosal resection for esophageal cancer not invading the submucosa have been reported to be 95% to 98% (1, 42), whereas the 5 year-survival rate of esophageal cancer based on data from a population-based cancer registry was reported to be 25% among seven prefectures of Japan (43). Therefore, it is expected that implementation of screening for esophageal cancer could improve the prognosis and decrease the mortality substantially. However, the actual effectiveness, considering the costs, must be analyzed based on the results of mass screening in a large population in the future.

A parallel study on oral and pharyngeal SCC conducted in men who came to the same clinics and hospitals showed that hypopharyngeal SCC and esophageal SCC shared several common risk factors including ALDH2 genotype, alcohol flushing, drinking habits, smoking habits, and diet (12, 22, 25). Figure 5 shows the ROC curves for detecting hypopharyngeal SCC by the HRA models for esophageal SCC. The sensitivity was 62.7% when the top 10% was screened by the HRA-F

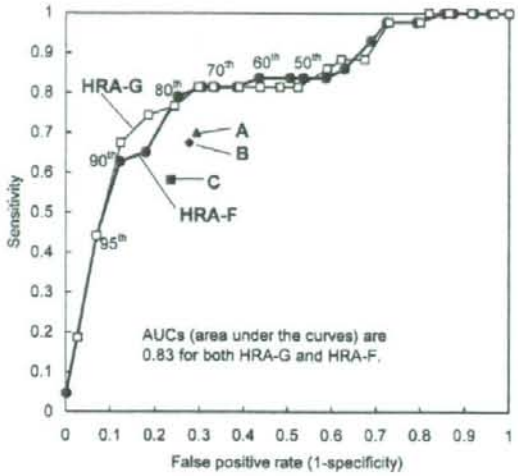

Figure 5. Sensitivity and specificity for screening hypopharyngeal cancer by two HRA models (HRA-G and HRA-F) and three other simple criteria (A-C). HRA-G: activity of ALDH2 assessed by genotype. HRA-F: activity of ALDH2 assessed by a questionnaire for alcohol flushing. **A.** Moderate-to-heavy drinking plus either smoking ≥30 pack-years or flushing. **B.** Moderate-to-heavy drinking plus either smoking ≥30 pack-years or MCV ≥ 99 fl. **C.** Moderate-to-heavy drinking and smoking ≥30 pack-years.

model and the areas under the curve are very similar to those of esophageal SCC. Although the HRA models were designed based on the results of a case-control study of esophageal SCC, they can be applied to hypopharyngeal SCC as well.

The combination of alcohol flushing and MCV with the traditional risk factors of drinking and smoking improved the predictive power of esophageal and pharyngeal SCC in comparison with the combination of drinking and smoking alone, which was in good agreement with our previous study (26). However, a substantially greater proportion (28.2% and 30.6%) of subjects met the two criteria, which is a disadvantage when using these criteria to select candidates for cancer screening.

One of the limitations of our study may be that the HRA models are not based on a prospective cohort study, but a case-control study that could have some biases in principle. However, it is unlikely that the potential biases affect the results notably because the estimated RRs are exceptionally strong. Another limitation is that we could not assess the risk of age. Because the incidence rate of esophageal cancer increases above the age of 50 years (34) and the majority of esophageal cancer patients in our case-control data is older than 50 years (12, 25), it may be appropriate that our HRA models are used for mass screening in those age groups. The age distributions of cases and controls did not match well in our case-control study, especially at ages 70 to 79 years; hence, there was concern that statistical

adjustment for age might have unexpected effects. To address this issue, we repeated the cross-validation study for men ages 70 to 79 years and confirmed that, at the cutoff value of top the 10%, the sensitivity was similar to (60.0% and 57.5% for HRA-G and HRA-F, respectively) and the specificity was somewhat better than (93.3% and 96.7%, respectively) those of other age groups, showing a good performance of our HRA models even in this old age group. However, the sample sizes in these groups are so small (40 cases and 30 controls) that further investigations are needed for the elderly ages ≥70 years.

Although alcohol flushing is a marker of inactive ALDH2, the sensitivity and specificity of the flushing questionnaire for identifying inactive ALDH2 in a Japanese male population was 90% and 88%, respectively (25). The advantage of the model that included alcohol flushing over the model that included ALDH2 genotype is that it facilitates risk estimation and can easily be applied to both public education and screening. Our HRA functions, which are very easily calculated on a simple sheet of paper (Figs. 1 and 2), may not only provide a means of screening the high-risk individuals but also help persuade those people to change their drinking habits, smoking habits, and diet. Cessation of drinking and smoking has been shown to reduce the risk of cancer of the upper aerodigestive tract (44, 45). Early cancer of the esophagus (1, 2) and pharynx (3) can be easily treated by endoscopic mucosectomy or endoscope-guided mucosectomy, and the HRA models we used may provide an important approach to early intervention strategies to control these high-mortality cancers in Japanese men. Further study is needed to confirm the effectiveness of this new approach in large Japanese populations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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Short Communication

Health Risk Appraisal Models for Mass Screening for Esophageal and Pharyngeal Cancer: An Endoscopic Follow-up Study of Cancer-Free Japanese Men

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Abstract

Purpose: **To assess the performance of our health risk appraisal (HRA) models for screening individuals at high risk of esophageal/pharyngeal squamous cell carcinoma (EPSCC).**

Methods: **Based on the results of our previous case-control study, we invented HRA models that enable screening for EPSCC cases in Japanese men with high sensitivity and specificity based on either their aldehyde dehydrogenase-2 genotype (HRA-G model) or alcohol flushing (HRA-F model) and drinking, smoking, and dietary habits. Follow-up endoscopy combined with esophageal iodine staining (median follow-up period: 5.0 years) was done on 404 Japanese men (50-78 years) who were registered as cancer-free controls in the previous study.**

Results: **The follow-up endoscopy resulted in a diagnosis of 6 esophageal SCC (T_{is} in 5 and T_1 in 1), 1 hypopharyngeal SCC (T_2), and 1 oropharyngeal SCC (T_2). Seven and 6 of the 8 EPSCC cases were in the top 10% risk group at baseline according to the HRA-G and HRA-F models, respectively. The EPSCC detection rates per 100 person-years in the top 10% risk groups by the HRA-G and HRA-F models were 4.38 (95% confidence interval, 1.76-9.01) and 3.48 (95% confidence interval, 1.28-7.58), respectively. Their age-adjusted relative risk was 95.1- and 26.3-fold, respectively ($P < 0.0001$), higher than in the bottom 90% risk groups.**

Conclusions: **The high detection rates for EPSCC in the top 10% risk group of this preliminary follow-up study were in good agreement with those predicted by the HRA models and thus encouraged the screening based on our HRA models in larger populations of Japanese men.** (Cancer Epidemiol Biomarkers Prev 2009;18(2):651–5)

Introduction

Recent technical improvements in endoscopes and growing understanding of the endoscopic findings of early squamous cell carcinoma (SCC) in the esophagus (1, 2) and pharynx (3) have made it possible to detect esophageal/pharyngeal SCC (EPSCC) early. Treatment of early esophageal SCC by endoscopic mucosectomy has become a widespread practice in Japan and has succeeded in improving the prognosis of this high-mortality cancer (2, 4, 5), and early pharyngeal SCC can also be treated by endoscope-guided mucosectomy (6). Therefore, it is important to identify individuals at increased risk of EPSCC and offer them the opportunity to undergo detailed examination by upper aerodigestive tract endoscopy combined with esophageal iodine staining. Without using the esophageal iodine staining, more than half of intraepithelial or mucosal esophageal SCC would be missed (1, 7).

A mutant allele encoding an inactive subunit of aldehyde dehydrogenase-2 (*ALDH2*2*) is prevalent in East Asian populations (e.g., prevalence of the *ALDH2*2* allele is 24% in a Japanese population; ref. 8) and drinking a small amount of alcohol results in severe acetaldehydemia and unpleasant alcohol flushing responses in individuals with inactive ALDH2 (9). Acetaldehyde is an established carcinogen in experimental animals (10) and is suspected to play a critical role in cancer development in humans (11). Case-control studies among Japanese (12-16) and Taiwanese (13, 17, 18)

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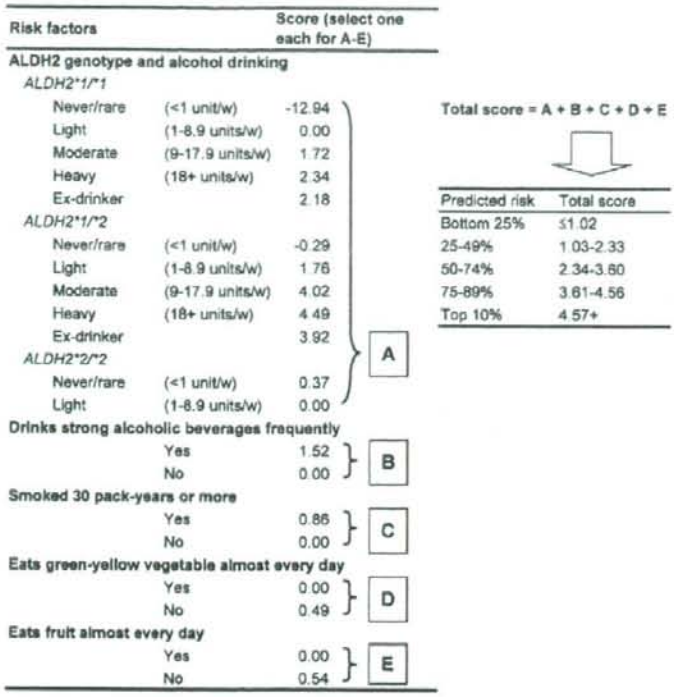

Risk factors		Score (select one each for A-E)	
ALDH2 genotype and alcohol drinking			
*ALDH2*1/*1*			
Never/rare	(<1 unit/w)	-12.94	A
Light	(1-8.9 units/w)	0.00	
Moderate	(9-17.9 units/w)	1.72	
Heavy	(18+ units/w)	2.34	
Ex-drinker		2.18	
*ALDH2*1/*2*			
Never/rare	(<1 unit/w)	-0.29	
Light	(1-8.9 units/w)	1.76	
Moderate	(9-17.9 units/w)	4.02	
Heavy	(18+ units/w)	4.49	
Ex-drinker		3.92	
*ALDH2*2/*2*			
Never/rare	(<1 unit/w)	0.37	
Light	(1-8.9 units/w)	0.00	
Drinks strong alcoholic beverages frequently			
	Yes	1.52	B
	No	0.00	
Smoked 30 pack-years or more			
	Yes	0.86	C
	No	0.00	
Eats green-yellow vegetable almost every day			
	Yes	0.00	D
	No	0.49	
Eats fruit almost every day			
	Yes	0.00	E
	No	0.54	

Total score = A + B + C + D + E

Predicted risk	Total score
Bottom 25%	≤1.02
25-49%	1.03-2.33
50-74%	2.34-3.60
75-89%	3.61-4.56
Top 10%	4.57+

Figure 1. HRA model for esophageal cancer that includes ALDH2 genotype. 1 unit = 22 g ethanol.

and prospective studies among Japanese alcoholics (19, 20) have consistently shown a markedly increased risk of EPSCC in drinkers possessing *ALDH2*1/*2*. Our previous case-control studies confirmed that alcohol drinking especially in individuals with inactive ALDH2, tobacco smoking, a preference for drinking concentrated alcoholic beverages straight, and less intake of green and yellow vegetables increased the risk of EPSCC in Japanese men (12, 16, 21). Based on the data we obtained in that study, simple health risk appraisal (HRA) models were developed to be able to quantitatively assess individual risk of developing EPSCC in the form of a risk score (22). A cross-validation study, which used a simulation-based approach to assess the performance of a statistical model, predicted that ~60% of the EPSCC in the entire population could be detected by examining only people with the top 10% risk scores of the HRA models (sensitivity ≈ 60% and specificity ≈ 90%; ref. 22). Furthermore, the detection rate of esophageal SCC in people with the top 10% risk score (positive predictive value) was expected to be >2%. If it is possible to achieve these performances levels in an actual mass screening, a very efficient approach to early detection of EPSCC in Japanese men will have been achieved.

The present study was a 7-year follow-up study of cancer-free men who were the controls in our previous case-control study and was conducted to confirm that the good performance of HRA models predicted by the cross-validation study was also achieved in an actual follow-up study, where the subjects were examined repeatedly by using a combination of upper aerodigestive tract endoscopy and esophageal iodine staining.

Materials and Methods

Study Population. We previously conducted a case-control study of 234 male cases with esophageal SCC and 634 male cancer-free controls and reported the results (12). The cancer-free controls were men who came to two Tokyo clinics for annual health checkups between September 2000 and December 2001, and most of them were ordinary residents or workers living in Tokyo or surrounding areas. The cancer-free controls who had been recruited in one of the two clinics and diagnosed as cancer-free by upper gastrointestinal endoscopy when they registered to participate in the previous study were sent annual letters of invitation to be screened by endoscopy. As of April 2007, 404 (81.3%) of the 497 eligible men ages 50 to 78 years had undergone a combination of follow-up endoscopic screening and esophageal iodine staining at least once, and they were enrolled in the present study. The Ethics Committee of the Kurihama Alcoholism Center reviewed and approved the proposal for this study, and each of the participants gave his informed consent.

Endoscopic Screening. Endoscopy was done with an Olympus Q240 or Q240Z panendoscope (Olympus Optical) by one of the authors (Y.K.), who is an expert in the field of upper gastrointestinal endoscopy. The endoscope was inserted into the pharynx, and it was carefully examined while removing secretions by

suction. After advancing the endoscope beyond the upper esophageal sphincter, the esophageal mucosa was flushed with 40 mL water through the biopsy port. Conventional endoscopic inspection was done from the esophagus down to the duodenum, and the esophagus was stained with then 10 mL of 1.5% iodine solution and inspected again. Mucosal biopsy specimens were collected from lesions that remain distinctly unstained by iodine, if their greatest diameter was ≥5 mm. At the end of the screening procedure, the esophageal mucosa was rinsed with 20 mL of 2.5% sodium thiosulfate solution, and the gastric contents were removed by suction.

Measurement of Risk Factors. At the time of baseline registration for the previous study, each participant was asked to fill out a simple questionnaire that asked the questions concerning alcohol flushing responses, drinking habits, smoking habits, and diet (12). Alcohol flushing is a surrogate marker of inactive ALDH2 and the sensitivity and specificity of the flushing questionnaire for identifying inactive ALDH2 in a Japanese male population were 90% and 88%, respectively (21). The PCR-RFLP method has been done on lymphocyte DNA samples to determine their ALDH2 genotype (refSNP ID: rs671) of all participants in the previous case-control study (12).

We calculated the HRA score to assess the risk of esophageal SCC in each subject at the time of registration based on alcohol drinking, either ALDH2 genotype (HRA-G model) or alcohol flushing (HRA-F model), smoking, and intake of vegetables and fruit according to the previously reported method (22). The subjects were classified into five risk categories according to their HRA scores: bottom 25%, 25% to 49%, 50% to 74%, 75% to 89%, and top 10%. The procedures used to make these calculations are summarized in Fig. 1 (HRA-G) and Fig. 2 (HRA-F). The HRA score was calculated as the sum of the scores (A-E), which were logarithms of the multivariate odds ratio of each factor estimated in the previous case-control study (12, 21). We further simplified the HRA-F model by converting the scores to small integers ("integer score" in Fig. 2), so that the categorization of the risk group was approximately the same as the categorization based on the original scores (22).

Statistical Analyses. The cancer detection rates during the follow-up period were calculated by the person-year method, with "person-year" defined as time from the baseline examination to either cancer detection or the most recent follow-up examination, whichever came first. The 95% confidence interval (95% CI) of the detection rate was estimated based on a Poisson distribution. The relationships between the HRA score at baseline and results of subsequent endoscopic screening are expressed as relative risk of cancer detection rate adjusted for decade of age by the Mantel-Haenszel method. All statistical analyses were done with the SAS statistical package (version 9.1; SAS Institute).

Results

The mean follow-up period was 4.4 years [median (25th and 75th percentiles), 5.0 (3.3, 5.6) years; range, 0.1- 6.7 years]. There were no significant differences between the distribution of the HRA scores of the subjects who underwent the follow-up screening and those who did not ($P > 0.4$, Fisher's exact test; data not shown). Follow-up endoscopy resulted in a diagnosis of primary esophageal SCC in 6 subjects, SCC in 5 (T_{is}), and SCC

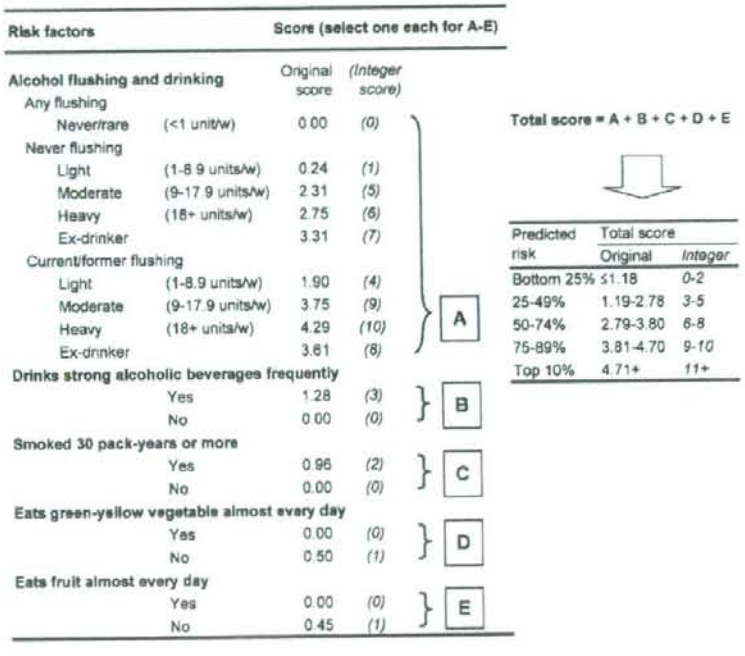

Figure 2. HRA model for esophageal cancer that includes alcohol flushing.

Table 1. Detection rate and relative risk of cancer during the follow-up period according to risk category based on the HRA models

	No. cohort members	Person-years of follow-up	SCC of esophagus			SCC of esophagus/pharynx		
			n	Per 100 person-years (95% CI)	RR* (95% CI)	*n*	Per 100 person-years (95% CI)	RR* (95% CI)
HRA models								
HRA-G (n = 404)								
Risk category								
Lower 25%	104	483.8	0			0		
25-49%	96	430.3	0			0		
50-74%	118	519.5	0	0.06 (0.00-0.34)	1.0 (reference)	0	0.06 (0.00-0.34)	1.0 (reference)
75-89%	44	189.0	1			1		
Top 10%	42	160.0	5	3.13 (1.01-7.29)	67.8 (6.44-714)	7	4.38 (1.76-9.01)	95.1 (10.4-1,048)
HRA-F, original score (n = 393)								
Risk category								
Lower 25%	101	463.5	0			0		
25-49%	98	446.7	0			0		
50-74%	88	394.8	2	0.13 (0.02-0.46)	1.0 (reference)	2	0.13 (0.02-0.46)	1.0 (reference)
75-89%	61	253.9	0			0		
Top 10%	45	172.3	4	2.32 (0.63-5.94)	17.5 (3.12-98.3)	6	3.48 (1.28-7.58)	26.3 (5.15-134)

NOTE: HRA-G: ALDH2 activity was assessed by genotyping; HRA-F: ALDH2 activity was assessed based on the results of a questionnaire regarding alcohol-related flushing (see also Figs. 1 and 2).

*Relative risk of person-year detection rate adjusted for age by the Mantel-Haenszel method. All $P < 0.0001$ for comparisons between the top 10% and the bottom 90% categories.

plus basaloid carcinoma in 1 (T_1) and primary SCC of the hypopharynx (T_2) and oropharynx (T_2) in 1 subject each. At baseline, all 8 subjects who were diagnosed with EPSCC at follow-up were moderate/heavy drinkers (>200 g ethanol/wk) and heterozygotes for inactive ALDH2, and 7 of them reported current/former alcohol flushing. Seven of the subjects with EPSCC had smoked ≥30 pack-years, 5 did not eat green-yellow vegetables almost every day, and 7 did not eat fruit almost every day.

Table 1 shows the detection rate of cancer during the follow-up period according to risk category based on the HRA-G and HRA-F models. Five of the 6 esophageal SCC patients and 7 of the 8 EPSCC patients had been classified in the top 10% risk category based on the HRA-G model, and 4 of the esophageal SCC patients and 6 of the EPSCC patients had been classified in the top 10% risk category based on the HRA-F model. The detection rate of esophageal SCC per 100 person-years (95% CI) was 3.13 (1.01-7.29) and 2.32 (0.63-5.94) in the top 10% risk group based on HRA-G and HRA-F models, respectively, and that for EPSCC was 4.38 (1.76-9.01) and 3.48 (1.28-7.58), respectively. The age-adjusted relative risk in the top 10% risk group was much larger than in the bottom 90% risk group and the difference was highly significant ($P < 0.0001$) in both models. The results based on the HRA-F model obtained with integer scores were very similar to the results obtained with the original scores (data not shown).

Discussion

We invented HRA models that allow prediction of ~60% of patients with EPSCC while referring only the top 10% of risk category of Japanese high-risk men for endoscopic screening (22). The present study investigated whether the HRA models would perform well in terms of actual endoscopic screening for cancer during a 7-year follow-up of Japanese men. The results showed that 7 (88%) of the 8 EPSCCs developed in individuals ranked in the top 10% risk category according to the HRA-G model and 6 (75%) developed in individuals ranked in the top 10% risk category according to the HRA-F model, showing better performance in comparison with the proportions (= sensitivity) predicted by the cross-validation method (65.4% and 57.9%, respectively; ref. 22). It was noteworthy that the esophageal cancers detected were in the very early stage (T_{is} cancer in 5 and T_1 cancer in 1). An esophageal cancer detection rate by endoscopy in men ages ≥40 years was reported to be 0.39% in the Research Center for Cancer Prevention and Screening of the National Cancer Center (23), where esophageal iodine staining was applied when the mucosal surface did not appear normal. We estimated the esophageal cancer detection rates in the top 10% category according to the HRA-G model and HRA-F model to be 2.40% and 2.12%, respectively, based on the overall detection rate (0.39%) in the Research Center for Cancer Prevention and Screening (22), and those rates were in good agreement with the results of the present study (3.13 and 2.32, respectively, per 100 person-years). The high skill level of the endoscopists and the use of esophageal iodine staining probably contributed to the high rates of esophageal cancer detection in the Research Center for Cancer Prevention and Screening and the present study, because more than half of the cases of intraepithelial or mucosal SCC in the esophagus would have been missed without esophageal iodine staining (1, 7). The precise incidence of esophageal T_{is} SCC in the Japanese general population and its natural course are unknown, and they will be topics of future research.

Our follow-up study had several potential limitations. The intervals between the follow-up screening and the baseline screening that confirmed freedom from cancer

were short, and the very small number of cancer cases may have limited the assessment of the relationship between the HRA scores and actual rate of cancer development. Although the follow-up was incomplete (81.3%), there were no significant differential follow-up biases. The performance of HRA models could depend on the difference in distributions of risk factors between the background population of the present study and the target population to which the HRA models are applied (22). Further investigation of the relationship in a large, long-term prospective study in different populations with a high follow-up rate is clearly warranted.

Our HRA-F model enables many people to identify their own risk of EPSCC very easily, and public awareness campaigns using the HRA-F model will help persuade high-risk persons to undergo endoscopic screening and enable detection of EPSCC early or enable them to change their lifestyle to prevent ESCC. Although the number of cancers detected was small, the very good performance of the HRA models in this preliminary follow-up study provided evidence supporting the validity of the HRA risk scores for selecting individuals at high-risk of EPSCC and encouraged the use of these new models for screening in larger populations of Japanese men. Further study is needed to confirm the effectiveness of this approach in large Japanese populations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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わが国のアルコール関連問題の現状

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