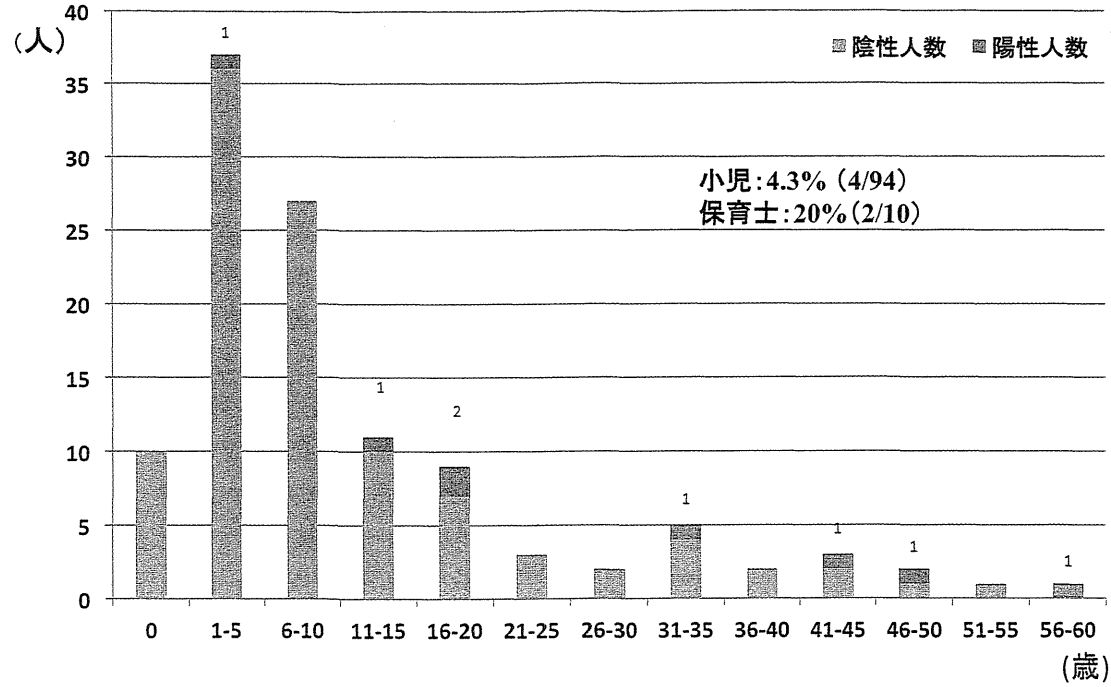

図1 北海道K町の年齢別*H. pylori*感染状況

それぞれPCRを行って増幅し、電気泳動で得られたバンドパターンを観察し3個以上のprimerでパターンが一致した場合に同一菌株由来と判定した。*H. pylori*陽性の母親から出生した44例の児のうちで感染が確認された5例すべてにおいて母と同じ菌株のDNA patternを確認し、母子感染を証明した。感染時期は1歳が4名、4歳が1名であった。これまでの報告[8]と同様、2歳未満での感染が多かった。

2) 家族検索での母子感染の優位性

2008年に報告した母子感染に関する研究[9]の概要を述べる。我々は吐血、下血、反復性腹痛や原因不明の鉄欠乏性貧血など有症状の小児42例に上部消化管内視鏡検査を施行し、*H. pylori*感染陽性を確認した。*H. pylori*胃炎の患児の42例中12例が十二指腸潰瘍を、2例が胃潰瘍を合併していた。家族の*H. pylori*感染率は、父が82%(32/39)、母が86%(36/42)、同胞が47%(18/38)であり、同世代の一般集団に比較して非常に高い陽性率であった。父母のうち希望者には内視鏡検査を施行し、胃粘膜生検も行った。それ以外の父母や同胞からは胃液を採取し、菌の分離培養を行った。

感染経路の解明のために患児42例とその家族の菌株を用いてRAPD法を施行し、fingerprinting patternの比較により菌株の異同を判別した。その結果、42 Familyのなかで、家族内に患児と同じfingerprinting patternが確認されたのは32 Family(76%)であり、32 Familyのうち29 Family(91%)で母子感染が確認された。つまり、42例の患児の中29例(69%)が母子感染であることが示され、感染ルートとして最も優位であることが明らかになった。

表1 わが国の小児の最近の感染率
(日本ヘリコバクター学会学術集会抄録集より引用)

District	Age	Prevalence	Diagnosis	Presenter	Year
篠山市	＜10Y	13/689 (1.9%)	*Hp*SA	奥田	2011
篠山市	中学生	19/337 (5.6%)	Urine *Hp*IgG Ab	上田	2013
長野&北海道	中学生	8/189 (4.2%)	Urine *Hp*IgG Ab	林	2013
長野	高校生	99/2113 (4.7%)	Urine *Hp*IgG Ab	赤松	2013
北海道K町	＜18Y	4/93 (4.3%)	*Hp*SA	今野	2013

3) 他の家族からの感染、家族外感染の検討

我々は2013年3月までに55例の患児とその家族の菌のRAPD検索を行ってきたが、その結果を2013年の本学会学術集会workshopで報告した[10]。母子感染以外で家族内感染が確定されたのは4 Familyのみで、父子感染の1 Familyと同胞感染の2 Family、そして同居の祖母から患児への感染の1 Familyであった。

前述したK町の便中抗原検査で判明した家族全員が*H. pylori*陽性であったFamilyを示す（**図2**）。同胞1例（C1）は胃潰瘍の既往あり、同胞2例（C2、C3）と母は当院で内視鏡検査を施行した。母は結節性胃炎、同胞（C2、C3）も結節性胃炎であり、そのうち1例（C3）は鉄欠乏性貧血を合併していた（**図2**）。父も突然のハプニングであったが、母子らの検査の2か月後に出血性胃潰瘍のため当院に緊急入院し内視鏡検査を施行した。保育士2名（N1、N2）は除菌目的で当院にて内視鏡検査を施行した。感染経路の検索のために家族ならびに保育士の菌株RAPD fingerprintingを施行したところ（**図3**）、同胞3例（C1、C2、C3）は同一のpatternであった。しかし父（F）、母（M）とはそれぞれ異なっており、保育士（N1、N2）とも異なるpatternであった。結局3人の同胞間感染は判明したが、父、母、保育士からの感染は否定され、その感染源を確定することはできなかった。最近Raymondら[11]は3家族のMacro-arraysによる検討で同胞間感染を示唆する結果を報告している。

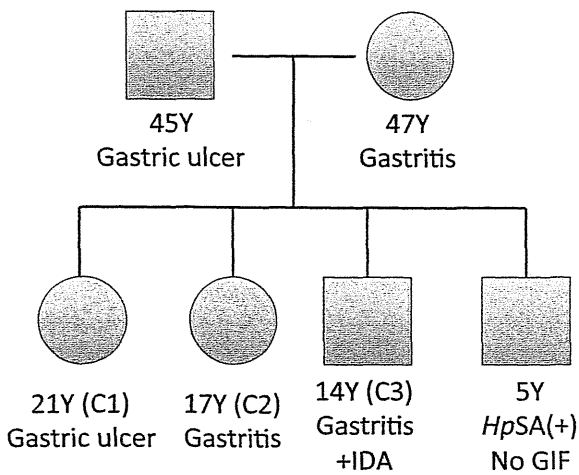

図2 K町の家族全員が*H. pylori*陽性のFamily

4）夫婦間感染

55 Familyのなかで患児の父母の*H. pylori*感染検査が行われ、かつ陽性例においてRAPD検索をなし得た父母は32 coupleであった。32 couple中、父母のRAPD patternが一致したのはFamily 1からFamily 9までの9 couple（28%）であった（**表2**）[10]。4 Familyでは患児の同胞の菌株も一致した。

家族全員の菌株DNA patternが一致したFamily 8のRAPD patternを**図4**に示す。患児と父、母、同胞において3種のprimerによるRAPD patternが一致した。

今回RAPD fingerprintingの他にさらにmulti locus sequence typing（MLST）解析によりこの家族の菌株の遺伝子型を検討した[12]。MLSTは数種類の遺伝子の部分配列を解析し、菌株どうしの遺伝子型を比較する方法であり、*H. pylori*の場合には7個のハウスキーピング遺伝子（*atpA*、*efp*、*mutY*、*trpC*、*ureI*、*ppa*、*yphC*）について、**表3**のprimerを用いてPCRで増幅し、ダイレクトシークエンス法で増幅産物の塩基配列を決定する。その後MLSTデータベース（http://pubmlst.org/helicobacter）から、それぞ

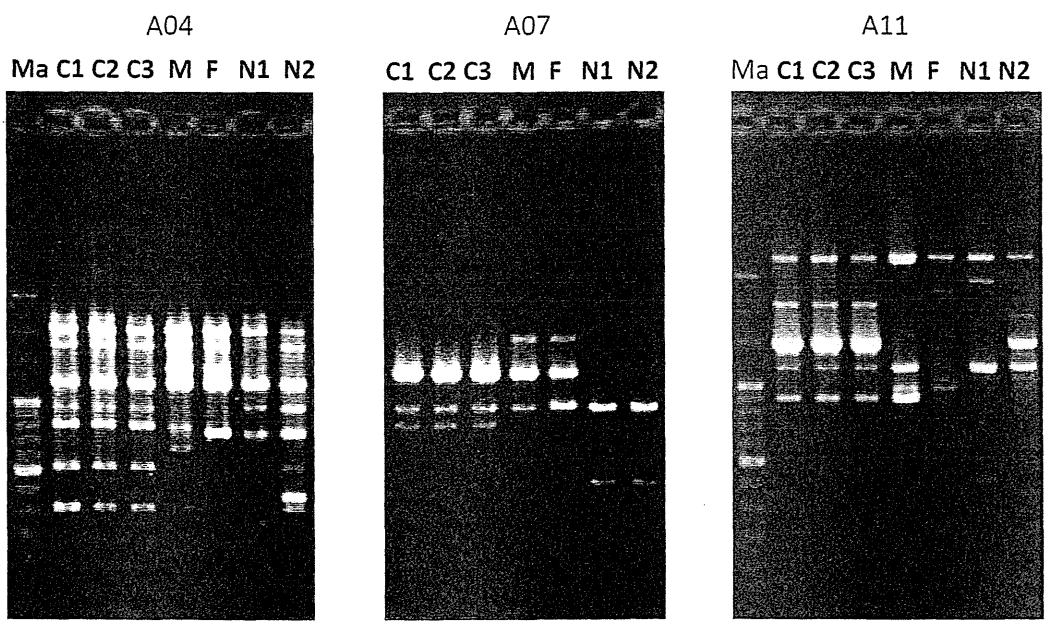

図3 子供と父、母、保育士の菌株 RAPD fingerprinting patterns
子供（C1, C2, C3）、母（M）、父（F）、保育士（N1, N2）。Ma：marker

れの遺伝子の多型（allele）を決定し、さらに各遺伝子多型の組み合わせにより菌株DNAの遺伝子配列型を決定した。RAPD法で全員のfingerprintingが一致したFamily 8はMLST解析においても、その家族全員において7遺伝子すべての配列型が一致し（**表4**）、同一の菌株に感染していることが示された。したがって、この家族ではRAPD法とMLST法の両方で親子感染のほかに夫婦間感染もあることが証明された。

これまで夫婦間感染に関する論文はあまり多くないが4篇の報告[13～16]があり、**表5**に頻度の高い順から示した。ギリシャのGeorgopoulosら[13]は十二指腸潰瘍の患者とそのパートナーの菌株rRNA遺伝子のribopatternを解析し、18組中8組（44%）の夫婦が同一菌株を示したことを報告した。次に我々の今回報告した25%（8/32）が続く[10]。SwedenのKiviら[14]は学校の調査で判明した小児（10～12歳）の*H. pylori*感染者を家族とともに受診させ内視鏡検査と生検を施行し、培養した菌株をRAPD法とRFLP法で解析した結果、22%（5/23）が夫婦で同一菌株であったとの成績を示した。

夫婦間感染が低頻度であったとの報告もある。日本のSuzukiら[15]はrestriction fragment length polymorphism（RFLP）解析によりdigestion patternが一

表2 患児と家族の*H. pylori*菌株PARD finger printing patternの異同

Family No	Mother	Father	First-born	Second-born	Third-born
1	○	○	patient	○	○
2	○	○	○	patient	
3	○	○	patient		
4	○	○	Hp(+), ND	patient	
5	○	○	patient	○	
6	○	○	patient	Hp(+)	
7	○	○	patient		
8	○	○	patient	○	
9	○	○	patient		
10	○	●	patient	Hp(−)	Hp(−)
11	○	●	patient	■	
12	○	●	patient	Hp(−)	Hp(−)
13	○	●	patient		
14	○	●	patient	Hp(−)	
15	○	●	patient	Hp(+)	Hp(−)
16	○	●	○	patient	
17	○	●	patient		
18	○	Hp(−)	patient		
19	○	Hp(−)	patient		
20	○	Hp(−)	patient		
21	○	Hp(−)	Hp(−)	patient	Hp(−)
22	○	Hp(−)	patient	Hp(−)	Hp(−)
23	○	Hp(−)	patient		
24	○	Hp(−)	patient	Hp(−)	
25	●	○	Hp(+), ND	Hp(−)	patient
26	●	■	patient	○	○
27	●	Hp(−)	patient		
28	●	Hp(−)	patient		
29	Hp(−)	●	patient	○	
30	Hp(−)	●	Hp(+), ND	patient	
31	Hp(−)	●	patient		
32	Hp(−)	●	patient		

Hp：*H. pylori*、patient：index patient、○：identical to index patient、
●：distinct from index patient、■：distinct from other family members、
Hp(+), ND：*H. pylori*(+)but culture not done

致したのは夫婦の5%（1/21）のみであり、また台湾のKuoら[16]はRAPD法とRFLP法の両方で解析し、一致したのは夫婦の4%（1/25）と低頻度であった。

このように、夫婦間感染の報告は限られており、今後症例数を増やして検討すべき課題と考えられる。内視鏡機器の洗浄が不十分であった20数年前には、内視鏡検査を介しての*H. pylori*感染が問題になったことがあったが、そのことは大量の菌が入れば成人でも感染することがあり得ることを示している。日本では最近若い世代での感染率が低下しており、結婚後に未感染の配偶者が*H. pylori*陽性のパートナーから感染した可能性が十分考えられる。家族間で

表3 Primers used for PCR

Target gene	PCR Primers		Product size
efp	efp-for1	GGCAATTTGGATGAGCGAGCTC	558
	efp-rev1	CTTCACCTTTTCAAGATACTC	
trpC	trpC_for8	AGCATCGCCCTCTAAAGGTT	618
	trpC_rev 6	AAGCCCGCACACTTTATTTTC	
ppa	ppafor1-1	GAARTKAGCCATGACGCTRA	698
	ppa-rev4	GGGTTAARATCGTTAAATTGTAG	
mutY	mutY-for4	TTATGAAGTCTCTATATCAGCGAAGT	529
	mutY-rev4	TACCTAAACAATAAGGATTGAAAGG	
atp	atpA_for2	GGACTAGCGTTAAACGCACG	840
	atpA_rev2	CTTGAAACCGACAAGCCCAC	
yphC	yphC_rev3	CATTYACCCTCCCAATGATGC	721
	yphC_for2	CACGCCTATTTTTTTGACTAAAAAC	
ureI	ureIfor	AGGTTATTCGTAAGGTGCG	721
	ureI-rev2	GTTTAAATCCCTTAGATTGCC	

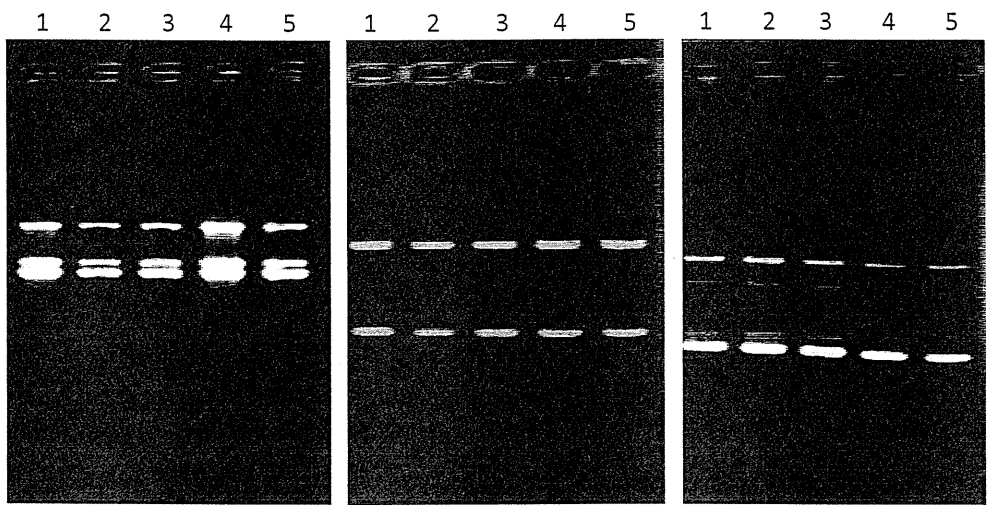

図4 全員の*H. pylori*菌株RAPD fingerprintingが一致したFamily 8
1. 患児（胃粘膜）、2. 母（胃液）、3. 母（胃粘膜）、4. 妹（胃液）、5. 父（胃液）

表4 MLST of *H. pylori* DNA isolated from family members

	atpA	*efp*	*mutY*	*ppa*	*trpC*	*ureI*	*yphC*
父	866	910	937	945	954	36	957
母	866	910	937	945	954	36	957
患児（15Y, F）	866	910	937	945	954	36	957
妹（14Y, F）	866	910	937	945	954	36	957

は小児も含めて日常的に濃厚な接触が繰り返され、Personnetら[17]が示したように、胃腸炎に罹患した際などに嘔吐物や下痢便からの大量の菌に汚染されることによる感染の機会もあるであろう。最近わが国では逆流性食道炎患者が増加しているが、胃液の口腔内への逆流による胃液－口感染が夫婦間でおこる可能性も考えられる。一方、**表2**に示すように夫婦間感染の9 Familyすべてにおいて、子供からも同一菌株が確認されており、子供から父親への感染の可能性も完全には否定できない。

Kiviら[14]は夫婦間感染についての考察のなかで、小児期に感染していたパートナーがその後、他のパートナーの異なる菌株に感染し、菌が置き換わったのではないかと述べている。しかし、最近我々が患者31例の菌株のRAPD fingerprinting法による検討[18]を行ったところ、同一患者においては幽門前庭部と体部の生検粘膜から分離培養した菌株も、胃液からの培養菌株も、それらのRAPD patternはすべて一致していた。また、初回から5～9年後に採取した同一患者の菌株RAPD patternも初回菌株のそれと一致していることが確認された。他の先進国からの報告[19]と同様、日本でもsingle hostにおいては単一クローンの*H. pylori*による感染が主体で、それが年余にわたって続くことが確認された。

表2に示すように我々の解析では異なる菌株の夫婦が11 coupleあったが、他のパートナーが陰性の夫婦も13 coupleあった。今後これらパートナーの感染の有無のフォローアップも含めて検討していく必要があろう。

おわりに

我が国の*H. pylori*の感染経路は乳幼児期における母子感染が主要なルートであることは明らかになったが、どのようにして感染するのかはまだ解明されてはいない。また、夫婦間感染も我々の成績では25%ほどあるということも判明し、今後さらなる検討が必要である。ただ我が国では*H. pylori*感染率が年々低下するなかで感染経路の詳細な解明は今後ますます難しくなるかもしれない。

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表5　*H. pylori*の夫婦間感染の報告

Country	Rate	Method Molecular typing	Author	Year Journal
Greece	High 44% (8/18)	Ribotyping (16S rRNA)	Georgopoulos	1996 Gut[13]
Japan	28% (9/32)	RAPD	Konno	2013 日本ヘリコバクター学会抄録[10]
Sweden	22% (5/23)	RAPD＋RFLP	Kivi	2003 J Clin Microbiol[14]
Japan	5% (1/21)	RFLP (ure B and ure C)	Suzuki	1999 J Clin Microbiol[15]
Taiwan	Low 4% (1/25)	RAPD＋RFLP	Kuo	1999 J Infect Dis[16]

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The stomach cancer pooling (StoP) project: study design and presentation

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Gastric cancer affects about one million people per year worldwide, being the second leading cause of cancer mortality. The study of its etiology remains therefore a global issue as it may allow the identification of major targets, besides eradication of *Helicobacter pylori* infection, for primary prevention. It has however received little attention, given its comparatively low incidence in most high-income countries. We introduce a consortium of epidemiological investigations named the 'Stomach cancer Pooling (StoP) Project'. Twenty-two studies agreed to participate, for a total of over 9000 cases and 23 000 controls. Twenty studies have already shared the original data set. Of the patients, 40% are from Asia, 43% from Europe, and 17% from North America; 34% are women and 66% men; the median age is 61 years; 56% are from population-based case–control studies, 41% from hospital-based ones, and 3% from nested case–control studies derived from cohort investigations. Biological samples are available from 12 studies. The aim of the StoP Project is to analyze the role of lifestyle and genetic determinants in the etiology of gastric cancer through pooled analyses of individual-level data. The uniquely large data set will allow us to define and quantify the main effects of each risk factor of interest, including a number of infrequent habits, and to adequately address associations in subgroups of the population, as well as interaction within and between environmental and genetic factors. Further, we will carry out separate analyses according to different histotypes and subsites of gastric cancer, to identify potential different risk patterns and etiological characteristics. *European Journal of Cancer Prevention* 00:000–000 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

The incidence and mortality of gastric cancer have been falling at least since the middle of the previous century in most high-income countries (Shibata and Parsonnet, 2006; Malvezzi *et al.*, 2010). This is largely explained by downward trends in the prevalence of *Helicobacter pylori* infection, and by improvements in diet and food conservation (Peleteiro *et al.*, 2012; Bosetti *et al.*, 2013a).

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The major reductions in the burden of gastric cancer were however achieved without specific interventions, before the identification of the major etiologic determinants (Howson *et al.*, 1986), which led to neglect of this neoplasm in terms of research and development efforts (Lunet *et al.*, 2004). Still, gastric cancer affects about one million people per year worldwide, being the second leading cause of cancer mortality (Bertuccio *et al.*, 2009).

Gastric carcinogenesis is a multistep and multifactorial process, involving both genetic and environmental factors (Boccia and La Vecchia, 2013). *H. pylori* infection is a major recognized risk factor for gastric cancer (International Agency for Research on Cancer, 1994) and appears to be a necessary condition for the disease (Peleteiro *et al.*, 2012). This notwithstanding, not all areas with high prevalence of *H. pylori* infection show a high gastric cancer incidence (Lunet and Barros, 2003). Gastric cancer is a tobacco-related cancer, and the decreased prevalence of smoking among men may explain a part of the recent decline in gastric cancer among men (Ladeiras-Lopes *et al.*, 2008). Diet and related nutrient intake also play an important role in the causation and prevention of gastric cancer [World Cancer Research Fund and American Institute for Cancer Research (WCRF/AICR), 2007]. A joint report from the WCRF/AICR (2007) concluded that there is 'probable evidence' that high consumption of nonstarchy vegetables and fruits decreases the risk of gastric cancer, whereas salt and salted foods are likely to increase the risk. 'Limited evidence' for a role of several other foods was also reported. Apart from salt, no specific constituent of diet has yet been identified to explain these associations (Pelucchi *et al.*, 2009). With reference to other factors, results have often been inconsistent (Shibata and Parsonnet, 2006). Similarly, a few hereditary syndromes and susceptibility genes of gastric cancer have been identified (Boccia *et al.*, 2008; Hartgrink *et al.*, 2009), but their role is far from being adequately understood and quantified.

The study of the etiology of gastric cancer remains therefore a global priority, as it may allow the identification of major targets, besides *H. pylori* infection, for the prevention and control of this major cancer worldwide. This should include, among others, understanding the almost two-fold higher risk for (intestinal-type) gastric cancer among men, despite the small sex differences in the prevalence of *H. pylori* infection (De Martel and Parsonnet, 2006; Derakhshan *et al.*, 2009), the interactions between complex dietary exposures, and the etiological differences between heterogeneous nosological entities jointly referred to as gastric cancer (i.e. cardia vs. noncardia subsite; intestinal vs. diffuse histological type).

Various epidemiological consortia have been established during the last two decades, to pool and analyze data on risk factors for breast, ovarian, head and neck, pancreatic, thyroid, and other neoplasms (Burgio *et al.*, 2013). These allowed us to identify and better quantify the role of risk factors in various cancers (Collaborative Group on Hormonal Factors in Breast Cancer, 1997; Franceschi *et al.*, 1999; La Vecchia *et al.*, 1999; Hashibe *et al.*, 2007; Galeone *et al.*, 2010; Bertuccio *et al.*, 2011). However, no consortial effort has yet been established for gastric cancer epidemiology. A large number of methodologically sound case–control studies on this neoplasm have been conducted over the years, and a concerted strategy for the joint analysis of these investigations may allow new insights into the etiology of gastric cancer. Therefore, our aim is to join several investigators together to set up a consortium of epidemiological investigations on risk factors for gastric cancer, named the 'Stomach cancer Pooling (StoP) Project'.

Our final aim is to examine the role of several lifestyle and genetic determinants in the etiology of gastric cancer through pooled analyses of individual-level data, after central collection and validation of the original data sets.

Methods

Participant studies, data collection, and harmonization

The StoP Project is a consortium of epidemiological studies on gastric cancer. Inclusion criteria for study participation are: case–control study design, including nested case–control within cohort studies, and inclusion of at least 80 cases of incident, histologically confirmed gastric cancer (including both cardia and noncardia locations).

Participating studies were recruited through personal contacts of participating investigators. Studies were identified through searches in electronic databases, including Medline and Embase, backward citation tracking, and contact with experts. Principal investigators of these studies were contacted and invited to participate in the consortium.

Tables 1 and 2 show the studies included in the StoP Project, the country of data collection, the number of cases and controls, and the information available on each study. In brief, to date (i.e. October 2013), 22 studies from 11 countries have agreed to participate, including a total of over 9000 patients and 23 000 controls. Study participation was confirmed through a signed data transfer agreement (DTA) form for 21 of 22 studies, and 20 studies have already provided the original data set and questionnaire. Subsequently, we plan to define the final core group of studies to participate in the first subproject analyses, while additional studies will be invited to join the consortium. Table 3 shows the frequency distribution of patients and controls enrolled in the studies that have already provided data to the StoP Project, according to sex, age, and other selected characteristics. Of the patients, 40% are from Asia, 43% from Europe, and 17% from North America; 34% are women and 66% men; the median age is 61 years; 56% are from population-based case–control studies, 41% from hospital-based ones, and 3% from nested case–control studies from cohort investigations.

Table 1 List of studies that have agreed to participate in the StoP Project

Study ID	Study area(s)	Period	Study type	Investigator(s)	References	Provided data
1	Milan, Italy	1985–1997	CC, hospital-based	Carlo La Vecchia	La Vecchia *et al.* (1995)	X
2	Harbin, China	1987–1989	CC, hospital-based	Jinfu Hu	Deandrea *et al.* (2010)	X
3	Milan, Italy	1997–2007	CC, hospital-based	Eva Negri, Carlo La Vecchia	Lucenteforte *et al.* (2008)	X
4	Rome, Italy	2006, ongoing	CC, hospital-based	Stefania Boccia	De Feo *et al.* (2012)	X
5	Four areas, Italy	1985–1987	CC, population-based	Monica Ferraroni, Domenico Palli	Buiatti *et al.* (1989)	X
6	Athens, Greece	1981–1984	CC, hospital-based	Dimitrios Trichopoulos, Pagona Lagiou	Lagiou *et al.* (2004)	X
7	Eight provinces, Canada	1994–1997	CC, population-based	Kenneth C. Johnson	Mao *et al.* (2002)	X
8	Taixing, Jiangsu, China	2000	CC, population-based	Lina Mu, Zuo-Feng Zhang	Mu *et al.* (2005)	X
9	Moscow, Russia	1996–1997	CC, hospital-based	David Zaridze, Dmitry Maximovich	Zaridze *et al.* (1999)	X
10	Ardabil, Iran	2004–2005	CC, population-based	Reza Malekzadeh, Farhad Pourfarzi	Pourfarzi *et al.* (2009)	X
11	Ardabil, Iran	2005–2007	CC, population-based	Reza Malekzadeh, Mohammadreza Pakseresht	Pakseresht *et al.* (2011)	X
12	Shanghai, Qingdao, China	1991–1993	CC, population-based	Guo-Pei Yu, Zuo-Feng Zhang	Setiawan *et al.* (2005)	X
13	Yangzhong, China	1995	CC, population-based	Guo-Pei Yu, Zuo-Feng Zhang	Setiawan *et al.* (2001)	X
14	New York, USA	1992–1994	CC, hospital-based	Zuo-Feng Zhang, Robert C. Kurtz	Zhang *et al.* (1999)	X
15	Aichi, Japan	2001–2005	CC, hospital-based	Keitaro Matsuo, Hidemi Ito	Matsuo *et al.* (2013)	X
16	New York, USA	1980–1990	CC, hospital-based	Joshua Muscat	NA	X
17	Porto, Portugal	1999–2006	CC, population-based	Nuno Lunet, Bárbara Peleteiro	Lunet *et al.* (2007)	X
18	Two counties, Sweden	1998–2010	Cohort, nested CC (SMC study)	Alicja Wolk, Nicola Orsini, Andrea Bellavia	Harris *et al.* (2013)	X
19	Ardabil, Iran	2001–2004	CC, hospital-based	Reza Malekzadeh, Mohammad Derakhshan	Derakhshan *et al.* (2008)	X
20	Two counties, Sweden	1998–2010	Cohort, nested CC (COSM study)	Alicja Wolk, Nicola Orsini, Andrea Bellavia	Harris *et al.* (2013)	X
21	Ten provinces, Spain	2008–2012	CC, population-based	Nuria Aragonés, Vicente Martín, Gemma Castano-Vinyals	NA	
22	Five counties, Sweden	1989–1995	CC, population-based	Weimin Ye	Ye *et al.* (1999)	

CC, case–control; COSM, cohort of Swedish men; NA, not available; SMAC, Swedish mammography cohort; X, the study has already provided the data set.

For each study, we asked for the completion of a study description form, providing information on the study characteristics, as well as the principal investigator and contact person. Investigators who agreed to participate provided a signed DTA and, thereafter, the complete original data set of the study. Investigators not wishing to share the complete data set were invited to provide a set of core variables including, among others, age, sex, education/social class, smoking habits, family history of gastric cancer, selected dietary variables, and – if available – markers of *H. pylori* infection. In addition to the data sets, we also collected the original questionnaires and any useful information to help with data handling (e.g. codebooks, labels, etc.) from the participating studies, to optimize data harmonization.

Data harmonization is a complex task. An identification number is assigned to each study, and then a unique identification number is defined for each participant, by combining the study number, the case–control status, and the original identification number of the participant. We split the whole body of information into several sections (e.g. sociodemographic characteristics, smoking habits, lifetime alcohol use, physical activity, etc.), and created a project codebook for each topic, reporting which variables are present in each study, their names and codes. We then standardized data for the core variables of the project (reported above), as well as progressively for selected topics of interest for analyses, as the project evolved by defining a uniform rule to recode each variable.

Completeness and consistency of the core variables of all the studies included in the project are centrally controlled, using ad-hoc developed programs. This will facilitate the identification of implausible values and outliers, inconsistencies between related variables (e.g. nonsmokers reporting number of cigarettes/day), and random and nonrandom missing values. It will also assist in checking the overall consistency of the data through frequency tables, median and mean values, and verification of extreme values. For each study, we prepared a set of tabulations, identified problems, and asked the contact person to check the data.

Policies

The consortium adopted a few forms to define the terms for sharing and use of research data, as well as the authorship policies.

The DTA form sets the terms and conditions of the agreement between parties, such as the ownership of original data and of their modifications, methods of data protection and storage, and date of termination of the agreement. The original investigators will maintain ownership of the data shared with the consortium.

Secretariat functions, that is, data collection, harmonization, and checking, are centralized at a single institution (IRCCS – Istituto di Ricerche Farmacologiche 'Mario Negri'). Within the consortium, working groups are formed to promote collaborative projects in specific areas of gastric cancer epidemiological research. Each group contributing data is invited to actively participate by developing its own specific subprojects – that is, proposing itself for the investigation of specific risk

Table 2 Main characteristics of the 22 studies involved in the StoP Project[a]

				Information collected in the study														
Study ID	Country	Cases	Controls	Sociodemographic	Smoking history	Alcohol drinking	Anthropometry	Physical activity	Coffee/tea consumption	Diet	Medical history	Occupational exposures	Menstrual and reproductive	Exogenous hormones	Drugs	Family history	*Helicobacter pylori* infection	Biological samples
1	Italy	769	2081	X	X	X	X		X	X	X		X	X	X	X		
2	China	266	533	X	X	X	X		X	X	X					X		
3	Italy	230	547	X	X	X	X	X	X	X	X		X	X	X	X		
4	Italy	164	444	X	X	X	X	X	X	X	X	X		X	X	X	X	X
5	Italy	1016	1159	X	X	X	X	X	X	X	X	X	X			X		
6	Greece	110	100	X	X	X	X	X	X	X	X		X		X	X		
7	Canada	1182	5039	X	X	X	X	X	X	X		X	X					
8	China	206	415	X	X	X	X	X	X	X	X	X				X	X	X
9	Russia	448	610	X	X	X	X	X	X	X	X	X	X		X	X	X	
10	Iran	217	394	X	X	X	X		X	X	X					X	X	
11	Iran	286	304	X	X	X	X			X	X					X	X	X
12	China	951	951	X	X	X	X			X	X					X		
13	China	133	433	X	X	X	X		X	X	X	X				X	X	X
14	USA	134	132	X	X	X	X	X		X	X	X			X	X	X	X
15	Japan	1250	3911		X	X	X	X	X	X	X	X	X	X		X	X	X
16	USA	87	261	X	X	X	X				X							
17	Portugal	692	1667	X	X	X	X		X	X	X				X	X	X	X
18	Sweden	88	352	X	X	X	X	X	X	X	X		X	X				X
19	Iran	119	119		X												X	X
20	Sweden	161	644	X	X	X	X	X	X	X	X							X
21	Spain	400[b]	1800[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
22	Sweden	514	1164	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Total	9423	23 060															

X, available information.
[a]On the basis of the study description form or reference papers provided by the study investigators.
[b]Approximate numbers. The study data set is under definition.

Table 3 Distribution of 8509 patients and 20 096 controls from 20 studies providing data to the StoP Project, according to selected characteristics[a]

Characteristic	Patients (%)	Controls (%)
Sex		
Females	2920 (34.3)	8139 (40.5)
Males	5589 (65.7)	11957 (59.5)
Age (years)[b]		
<40	318 (3.8)	1824 (9.2)
40–44	302 (3.6)	1267 (6.4)
45–49	509 (6.2)	1612 (8.1)
50–54	817 (9.9)	2150 (10.8)
55–59	1117 (13.5)	2550 (12.8)
60–64	1341 (16.2)	3022 (15.2)
65–69	1513 (18.3)	3171 (16.0)
70–74	1523 (18.4)	2754 (13.9)
≥ 75	835 (10.1)	1501 (7.6)
Study type		
Population-based case–control	4683 (55.8)	10362 (51.9)
Hospital-based case–control	3458 (41.2)	8619 (43.1)
Nested case–control (from a cohort)	249 (3.0)	996 (5.0)
Geographic area		
Asia	3428 (40.3)	7060 (35.1)
Europe	3678 (43.2)	7604 (37.8)
North America	1403 (16.5)	5432 (27.0)

[a]StoP database v.0. Studies no. 21 and 22 were not included as their data sets are not yet available.
[b]The full data set of study 12 is still under retrieval. Thus, the sums of the subgroups of age do not add up to the totals.

factors. Three-monthly conference calls of the Steering Committee and annual meetings are held to advance collaboration between groups, discuss results, share expertise and ideas, propose new research topics, and discuss further development of the project.

Participation in the StoP Project requires the willingness to share raw data in collaborative subprojects. However, for each subproject a specific data-use approval will be asked from each participating study. This will allow the investigators to decide each time whether to withdraw from a specific subproject (e.g. because they want to publish their results on that issue separately). Authorship policies have also been defined.

The consortium is regulated by a Steering Committee. This includes one member from the secretariat and six other members from different geographic areas, elected from among the principal investigators of the participating studies or because of their epidemiological expertise. The Steering Committee prepares the consortium guidelines, assesses and approves working group proposals, decides on acceptance of new members, and in general redeems every potential controversy within the consortium.

Statistical methods

We quantify the independent association between several potential risk factors and gastric cancer using adjusted odds ratios and the corresponding 95% confidence intervals, using a two-stage procedure. First, we compute study-specific estimates through logistic regression models (conditional or unconditional, according to the design of each study), including terms for relevant covariates for each individual subproject. Second, to calculate summary estimates, the study-specific estimates are included in a fixed-effect or random-effect model, as appropriate (DerSimonian and Laird, 1986).

We assess heterogeneity between studies using a likelihood ratio test. Further, heterogeneity between subgroups is explored through stratified analyses, in each subproject. Thus, we compare results across subgroups of age, sex, education level/social class, type of controls (i.e. population vs. hospital-based), geographic area (i.e. Europe, Asia, North America, others), and other strata. Interest is focused on histological types and subsites of gastric cancer (i.e. intestinal vs. diffuse histology; cardia vs. noncardia site).

Missing data are handled by applying multiple imputation procedures. They are estimated through logistic regression models, separately for each geographic region and including in the models terms for case–control status, age, sex, and study center.

We also perform at least two types of sensitivity analysis: (i) by excluding one study at a time to evaluate its influence on the overall results, to ensure that the statistical significance and magnitude of the summary estimate are not heavily dependent on a single study; (ii) by restricting the analyses to studies with information on *H. pylori* infection of controls, and comparing *H. pylori*-seropositive controls with all gastric cancer cases, regardless of their *H. pylori* serostatus, under the assumption that *H. pylori* is a necessary cause for gastric cancer (Peleteiro *et al.*, 2012; Bonequi *et al.*, 2013).

Forest plots presenting results for individual studies are provided, to guarantee transparency.

Pooled analyses on dietary and other lifestyle factors

We will analyze the role of those foods (or food groups) and nutrients with indications in the literature of a potential protective role against gastric cancer (e.g. fruit and vegetables, vitamin C, carotenoids, etc.), as well as those that are likely to increase the risk for this neoplasm (e.g. starchy and salty foods, red and processed meat, carbohydrates, etc.; Shibata and Parsonnet, 2006).

Selected dietary factors, including folate intake (Boccia *et al.*, 2008), will also be investigated together with their interaction with genetic susceptibility and family history, tobacco smoking, and other identified risk factors.

Other relevant factors considered include lifetime tobacco smoking and alcohol consumption; overweight and obesity at different ages; physical activity; socioeconomic status; history of gastric or duodenal ulcers and of gastroesophageal acid reflux disease; use of histamine-2-receptor antagonists, proton-pump inhibitors, and aspirin; and occupational exposures. For women, we also

investigate menstrual and reproductive factors and exogenous hormones.

Biological materials and methods

Among the 22 studies included, 12 reported collection of biological samples. Among them, three had frozen blood samples from patients and controls, five had stored DNA from entire blood samples/lymphocytes, and 10 had frozen serum and/or plasma samples. In addition, paraffin-embedded tissue samples of gastric cancer mucosa are, in principle, available from all the participating studies for the surgical cases, whereas freshly frozen tissues and/or frozen tissue preserved in dedicated preserving reagents are available only from two studies.

As shipment policies for biological samples outside the country of each participating center vary widely, the StoP Project in principle does not aim to pool individual samples centrally unless specifically requested from dedicated projects. However, the consortium will set up a data set including all the following information from each center:

(1) Reference single nucleotide polymorphisms (SNP) number(s) for the SNP investigated within candidate gene studies or genome-wide association studies (GWAs), along with the distribution of allelic variants among the compared groups.
(2) Gene expression profile(s) available, along with the expression levels among the compared groups.
(3) microRNA, along with their expression levels among the compared groups.
(4) *H. pylori* infection status and genotyping results (e.g. CagA positive/negative, VacA m1/m2, etc.) from gastric tissue samples.
(5) Any biochemical biomarkers investigated (e.g. folic acid levels, pepsinogen).

Discussion

This project takes advantage of the experience gained by several principal investigators participating in the International Head and Neck Cancer Epidemiology (INHANCE) consortium (Hashibe *et al.*, 2007; Conway *et al.*, 2009), and other similar ones (Kamper-Jorgensen *et al.*, 2013; Bosetti *et al.*, 2013b).

The StoP Project examines several dietary, nondietary, and genetic risk factors for gastric cancer, taking advantage of a large data set with original information from various geographic areas. The role of environmental and lifestyle risk factors in gastric carcinogenesis is in fact not well quantified (Shibata and Parsonnet, 2006). Further, the associations may vary according to the gastric subtype. For example, heavy alcohol drinking might be positively associated with gastric noncardia, but not gastric cardia, adenocarcinoma (Tramacere *et al.*, 2012), although not all studies are consistent with this finding (Zaridze *et al.*, 2000). In contrast, overweight and obesity might increase gastric cardia cancer risk only (Yang *et al.*, 2009).

We will also be in a position to investigate the role of a number of infrequent habits that cannot be examined in the single studies because of the relatively low number of exposed subjects. For example, we plan to consider the roles of pipe and cigar smoking, as well as of time since smoking and alcohol drinking cessation.

This study has the potential to improve the knowledge on the etiopathogenesis of stomach cancer through the identification of genetic variants associated with stomach cancer risk. In particular, the StoP Project aims to: (i) carry out pooled analysis on SNPs that have already been genotyped in studies of the consortium, starting from those significant from GWAs (replication study); (ii) perform investigations on the effect of gene–environment interactions on gastric cancer risk by selecting the available SNPs in association with potentially modifiable environmental risk factors; (iii) apply for funding within GWAs (replication study) on gastric cancer by pooling individual DNA/frozen blood samples; (iv) apply for funding to address the role of microRNA as a diagnostic tool in gastric cancer by pooling individual serum samples.

The major strength of the project is its collaborative framework, and thus the large number of cases available for analysis. This will allow us to examine separately, with adequate statistical power, the role of the above-reported risk factors in gastric cancer overall, as well as in different gastric cancer histological types (mainly intestinal vs. diffuse type) and subsites. Besides gastric cardia, we also plan to investigate differences between cancers of the fundus/body and pylorus.

The assessment of *H. pylori* infection in case–control studies is problematic. Blood samples obtained at stomach cancer diagnosis are of limited value, as the infection tends to decrease with the progression of the neoplastic disease (Shibata and Parsonnet, 2006; Peleteiro *et al.*, 2012). In addition, serological methods (mostly ELISA) used in old studies did not cover all antigenic parts of *H. pylori*, yielding inaccurate results. Another possible reason for lower *H. pylori* infection rates in surgical specimens from patients who have undergone gastrectomy is prophylactic antibacterial therapy, which may cause *H. pylori* infection rates to fall in the specimens. Thus, whereas almost all gastric cancer cases (at least noncardia ones) are attributable to chronic *H. pylori* infection, some cases show seronegative results on *H. pylori* testing. Therefore, in studies in which information on *H. pylori* infection is available, when assessing the effects of other risk factors we will conduct sensitivity analyses assuming that all patients with gastric cancer have been infected, comparing them only with seropositive controls, assuming that only these patients

would be at risk for developing gastric cancer if *H. pylori* infection is a necessary condition for it.

Information and selection bias are other potential limitations related to the case–control design of the studies included in the project (excluding nested case–control studies from cohort investigations), and confounding also needs to be addressed. To overcome these problems, multivariate analyses will be carried out, adjusting for a set of core variables known or suspected to influence gastric cancer risk, besides specific factors for each investigation. Stratified analyses will also be carried out according to covariates, such as study design (i.e. original or nested case–control study) and source of controls (i.e. hospital-based, population-based) and other relevant factors, including geographic area.

Pooled analyses of individual-level data have several advantages over systematic reviews (Blettner *et al.*, 1999). In particular, the individual-level data approach allows harmonization of information and analyses, consistency of adjustment terms and multivariate models, and efficient investigation of heterogeneity and interaction between covariates (Ioannidis *et al.*, 2013).

Conclusion

The uniquely large data set available will allow us not only to define and quantify more precisely than previously possible the main effects of each risk factor of interest, but also to adequately address interactions within and between environmental and genetic factors.

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Conflicts of interest

There are no conflicts of interest.

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The aldehyde dehydrogenase 2 (*ALDH2*) Glu504Lys polymorphism interacts with alcohol drinking in the risk of stomach cancer

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The impact of alcohol on the risk of stomach cancer is controversial. Although aldehyde dehydrogenase 2 (*ALDH2*) Glu504Lys (rs671) polymorphism has a strong effect on acetaldehyde metabolism, little is known about its impact on stomach cancer risk when combined with alcohol drinking. This case-control study included a total of 697 incident stomach cancer case subjects and 1372 non-cancer control subjects who visited Aichi Cancer Center between 2001 and 2005. We estimated odds ratios (OR) and 95% confidence intervals (CI) for ALDH2 genotypes and alcohol consumption using logistic regression models after adjustment for potential confounders, including *Helicobacter pylori* infection. The ALDH2 504Lys allele was associated with the risk of stomach cancer, with adjusted ORs of 1.40 (95% CI, 1.11–1.76) for Glu/Lys and 1.73 (1.12–2.68) for Lys/Lys compared with Glu/Glu. Heavy drinking was associated with risk (OR 1.72, 1.17–2.52) after adjustment for *ALDH2* genotype and other confounders. Moreover, ORs for heavy drinking were 1.28 (0.77–2.12) for those with *ALDH2* Glu/Glu and 3.93 (1.99–5.79) for those with the *ALDH2* Lys allele relative to non-drinkers with the Glu/Glu genotype (*P* for interaction = 0.0054). In conclusion, *ALDH2* and alcohol drinking showed interaction for risk factors of stomach cancer, indicating that acetaldehyde plays a role in stomach carcinogenesis.

Introduction

Alcohol consumption is an established risk factor for cancers of the upper aero-digestive tract (UADT) (1–3), majority of them are squamous cell carcinoma. One major hypothesized mechanism behind alcohol-related carcinogenesis in the UADT is the involvement of acetaldehyde, a metabolite of ethanol. Aldehyde dehydrogenase 2 (ALDH2) is a key enzyme in acetaldehyde metabolism, and molecular epidemiologic studies in East Asia (4–11), where the functional *ALDH2* Glu504Lys (rs671) polymorphism is prevalent, have contributed to the conclusion that acetaldehyde has a substantial impact on carcinogenesis in humans as a result of its strong interaction with alcohol drinking (3).

To date, the association between alcohol consumption and gastric cancer, of which majority are adenocarcinoma, has been controversial. A recent meta-analysis showed no appreciable association of moderate alcohol drinking with stomach cancer, but it did find a suggestive association between heavy drinking and non-cardia adenocarcinoma (12). Although it has been hypothesized that acetaldehyde contributes to gastric carcinogenesis, as it does for UADT cancer (13,14), evidence for this association to date has been limited (15–18). Taken evidences of no association between esophageal adenocarcinoma risk and alcohol in mind (19,20), there may not be neither association nor interaction. Anyhow, it is worth to be evaluated in the population in which functionally validated *ALDH2* polymorphism is prevalent.

In this study, we investigated the association between *ALDH2* Glu504Lys (rs671) polymorphism and alcohol consumption and risk of stomach cancer in Japanese population.

Abbreviations: AG, atrophic gastritis; ALDH2, aldehyde dehydrogenase 2; OR, odds ratios; CI, confidence intervals; PG, pepsinogen; PY, pack years; UADT, upper aero-digestive tract.

Materials and methods

Study population

The case participants were 697 patients with no history of cancer who were histologically diagnosed with stomach cancer between January 2001 and December 2005 at Aichi Cancer Center Hospital in Japan. All participants were recruited under written informed consent within the framework of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (21–23), and all provided blood samples. Among the 697 subjects, 684 (98.1%) were histologically confirmed as adenocarcinoma. Among 684 cases, 379 were diffuse type and 305 were intestinal type.

The control subjects were 1372 first-visit outpatients during the same period who were confirmed to have no cancer and no history of neoplasms. Non-cancer status was confirmed by medical examinations, including radiographic examinations, with participants suspected of having stomach cancer first examined by physical or endoscopic inspection, and subsequently radiographically when indicated. Controls were selected randomly and were individually matched by age (±5 years) and sex (male and female) with a case-control ratio of 1:1–2. A total of 2069 participants (697 cases and 1372 controls) were included in this study. Response rate was over 95% for both case and control subjects. The study was approved by the institutional ethical committee of Aichi Cancer Center.

Information on alcohol consumption

Information on alcohol consumption was collected from first-visit outpatients aged 20–79 years using a self-administered questionnaire. Each participant was asked at the time of first visit to our hospital about their alcohol consumption before the development of the current symptoms, which made them visit our hospital. For the present analyses, lifetime alcohol consumption of various common beverages (Japanese sake, beer, shochu, whiskey and wine) was determined in terms of the average number of drinks per day, which was then converted into a Japanese sake (rice wine) equivalent measure of 180 ml; termed a *go*, this is standard measure in Japan and contains 23 g of ethanol. Drinking status was classified into the four categories of never drinker, light drinker (fewer than 5 days per week, fewer than 2 go per day), moderate drinker (5 or more days per week, fewer than 2 go per day) and heavy drinker (5 or more days per week, 2 or more go per day).

Evaluation of other lifestyle factors

Information on smoking status was obtained in the three categories of non-smoker, former smoker and current smoker, with former smokers defined as those who had quit smoking at least 1 year before study enrolment. Cumulative exposure to smoking was categorized into five groups by pack years (PY), the product of the number of packs of cigarettes smoked per day and the number of years of smoking, namely as never, PY < 20, PY < 40, PY < 60 and PY 60 or more. Consumption of fruits and vegetables was determined using a food frequency questionnaire, which included 43 single food items in eight frequency categories (24). The food frequency questionnaire was validated using a 3 day weighed dietary record as standard, which showed that reproducibility and validity were satisfactory (25,26). Participants were divided into three groups based on the distribution of fruit and vegetable consumption among controls (tertiles).

Assessment of Helicobacter pylori infection and atrophic gastritis

All cases were examined for plasma IgG levels for *Helicobacter pylori* (*H.pylori*) using a commercially available direct enzyme-linked immunosorbent assay

kit ('E Plate "Eiken" *H.pylori* Antibody'; Eiken Kagaku, Tokyo, Japan). This enzyme-linked immunosorbent assay kit was developed in Japan using an antigen extracted from the domestic strain in Japan and is commonly used in medical studies in this country (27,28). A positive status for *H.pylori* infection was defined as an *H.pylori* IgG antibody level >10 U/ml in serum (27,28). Serum pepsinogens (PGs) were measured by chemiluminescence enzyme immunoassay, and gastric mucosal atrophy was defined by a PG I value ≤70 ng/ml and PG I/PG II ≤ 3 ng/ml (29–31).

Examination of ALDH2 Glu504Lys (rs671) polymorphism

DNA of each subject was extracted from the buffy coat fraction using a DNA blood mini kit (Qiagen). Genotyping for the *ALDH2* Glu504Lys polymorphism (rs671) was based on TaqMan Assays by Applied Biosystems (Foster City, CA). In our laboratory, the quality of genotyping is routinely assessed statistically using the Hardy–Weinberg test and by retyping of a random sampling of 5% of subjects.

Data analyses

To assess the association between ALDH2 polymorphism and alcohol consumption in the risk of stomach cancer, we estimated the odds ratios (OR) and corresponding 95% confidence intervals (CI) using multiple logistic regression models. First, we evaluated the impact of ALDH2 polymorphism and alcohol drinking separately using all subjects. For this analysis, conditional logistic regression models included terms for cumulative exposure to smoking, fruit/vegetable intake and *H.pylori* infection. We examined a model that separately evaluated ALDH2 genotype and alcohol drinking and a second model that included both. Further, we evaluated possible effect modification by ALDH2 polymorphism on the impact of alcohol consumption; for this analysis, we used unconditional logistic regression models adjusted for the same covariates as for the overall analysis. Effect modification was assessed by the likelihood ratio test between the models with and without interaction terms between the *ALDH2* polymorphism and alcohol consumption. We defined interaction term as a product of ALDH2 polymorphism (Lys allele carrier = 1 and wild-type homozygote = 0) and alcohol consumption as a continuous variable (never = 0, low = 1, moderate = 2 and heavy = 3); therefore, degree of freedom in the tests was 1. Consistency of the interaction between *ALDH2* polymorphism and alcohol consumption was assessed by stratified analysis according to the strata of the particular covariate considered with the model including three-way interaction term among ALDH2 polymorphism, alcohol consumption and stratifying factor. Association between the combination of *ALDH2* polymorphism and alcohol consumption and atrophic gastritis (AG) was evaluated in a multivariate unconditional logistic model among control subjects. Covariates considered in the model were the same as that for stomach cancer risk, except with regard to the status of AG. Missing values for covariates were treated as dummy variables in the models. All analyses were performed using Stata SE version 11.2 (STATA Corp, College Station, TX).

Results

Demographic characteristics and selected lifestyle habits of participants are shown in Table I. Age and sex were appropriately matched. The proportion of smokers was higher in cases than in controls. Cases were exposed to a higher smoking dose than controls. Prevalence of *H.pylori* infection was 82.2% in cases and 54.2% in controls. Fruit/vegetable intake between the two groups showed no apparent marked difference (27,28).

Table II presents the association between alcohol drinking and ALDH2 rs671 polymorphism and stomach cancer. We explored three models: model 1, a crude model; model 2, a confounder-adjusted model that evaluated alcohol drinking and *ALDH2* rs671 polymorphism separately and model 3, a complete model that included alcohol drinking and *ALDH2* polymorphism together. In model 3, ORs for drinking relative to non-drinking were 1.04 (0.77–1.40) for light, 1.15 (0.82–1.61) for moderate and 1.72 (1.17–2.52) for heavy drinking, indicating a dose-dependent positive association. This association remained significant after the exclusion of former drinkers from analysis (data not shown). The association between ALDH2 rs671 polymorphism was significant in model 3, with ORs relative to Glu/Glu, the normal enzyme activity genotype, of 1.40 (1.11–1.76) for Glu/Lys, 1.73 (1.12–2.68) for Lys/Lys and 1.42 (1.13–1.79) for the Lys allele carrier after adjustment for alcohol drinking. Although smoking and *H.pylori* status are potential sources of confounding for the effect of alcohol drinking, we did not observe clear evidence of confounding between these factors and ALDH2 rs671 polymorphism.

Table I. Subject characteristics

Overall	Cases		Controls	
	No. 697	%	No. 1372	%
Sex				
Male	521	74.7	1028	74.9
Female	176	25.3	344	25.1
Age (years)				
<40	34	4.9	146	10.6
40–49	72	10.3	154	11.2
50–59	245	35.2	429	31.3
60–69	210	30.1	435	31.7
>70	136	19.5	208	15.2
Smoking status				
Never	222	31.9	538	39.2
Former	181	26	403	29.4
Current	294	42.2	430	31.3
Unknown	0	0	1	0.1
PY				
Never	222	31.9	539	39.3
<20	99	14.2	286	20.9
<40	160	23.0	272	19.8
<60	117	16.8	153	11.2
60 or more	92	13.2	113	8.2
Unknown	7	1.0	9	0.7
Alcohol consumption				
Never	228	32.7	452	32.9
Light	167	24.0	412	30.0
Moderate	159	22.8	316	23.0
Heavy	132	18.9	177	12.9
Unknown	11	1.6	15	1.1
Fruit/vegetable intake				
Lowest tertile (<114.0 g/day)	263	37.7	446	32.5
Middle tertile (<199.96 g/day)	208	29.8	445	32.4
Highest tertile (≥199.96 g/day)	209	30	445	32.4
Unknown	17	2.4	36	2.6
Family history of gastric cancer				
Yes	153	22	239	17.4
No	544	78	1133	82.6
H.pylori IgG test				
Positive	124	17.8	628	45.8
Negative	573	82.2	744	54.2
AG defined by PG testing				
Negative	262	37.6	893	128.1
Positive	434	62.3	479	68.7
Unknown	1	0.1	0	0
Histologic classification				
Diffuse	379	54.4	—	
Intestinal	305	43.8	—	
Unknown	13	1.9	—	

Table III shows results for the interaction of ALDH2 rs671 polymorphism with alcohol consumption on the risk of stomach cancer. Among ALDH2 Glu/Glu, there was no statistically significant association. In contrast, heavy drinking among ALDH2 Lys allele carriers showed a statistically significant association, with ORs among *ALDH2* Lys+ subjects of 0.79 (0.55–1.11) for light, 1.18 (0.80–1.75) for moderate and 2.37 (1.37–4.12) for heavy drinking relative to non-drinking with *ALDH2* Glu/Glu. A significant interaction between drinking and *ALDH2 Lys* allele was seen (*P*-interaction = 0.0054). We further evaluated the consistency of the gene–environment interaction between the *ALDH2* Lys allele and alcohol drinking across strata of confounders. As shown in Table IV, interaction between the two factors was consistently observed, with some exception like fruit and vegetable consumption and *H.pylori* status.

Table II. Association between ALDH2 genotype and drinking and stomach cancer risk

	Case	Control	Model 1[a]	Model 2[b]	Model 3[c]
			OR (95% CI)[b]	OR (95% CI)[b]	OR (95% CI)[b]
Level of drinking					
Non-drinker	228	452	Reference	Reference	Reference
Ever drinker					
Light	167	412	0.81 (0.63–1.04)	0.89 (0.67–1.17)	1.04 (0.77–1.40)
Moderate	159	316	1.03 (0.79–1.34)	0.92 (0.68–1.24)	1.15 (0.82–1.61)
Heavy	132	177	1.52 (1.14–2.04)	1.29 (0.92–1.80)	1.72 (1.17–2.52)
Unknown subjects	11	15			
ALDH2 genotype[d]					
Glu/Glu	310	683	Reference	Reference	Reference
Lys+	386	689	1.24 (1.03–1.49)	1.27 (1.04–1.56)	1.42 (1.13–1.79)
Glu/Lys	323	580	1.23 (1.02–1.49)	1.25 (1.01–1.54)	1.40 (1.11–1.76)
Lys/Lys	63	109	1.27 (0.91–1.78)	1.42 (0.98–2.08)	1.73 (1.12–2.68)

[a]Crude ORs by the conditional logistic regression model.
[b]ORs were calculated by a conditional logistic regression model adjusted for PY of smoking, fruit/vegetable intake, family history of gastric cancer, gastric atrophy defined by serological PG testing and *H.pylori* status.
[c]ORs were calculated by unconditional logistic regression model adjusted for age, sex, PY of smoking, fruit/vegetable intake, family history of gastric cancer, gastric atrophy defined by serological PG testing, *H.pylori* status, levels of drinking and ALDH2 genotypes.
[d]One case was excluded because ALDH2 genotype was not defined.

Table III. Association between ALDH2 genotype and drinking and stomach cancer risk[a]

Level of drinking	ALDH2 Glu/Glu			ALDH2 Lys+			*P*-interaction
	Case	Control	OR (95% CI)[b]	Case	Control	OR (95% CI)[b]	
Non-drinker	49	112	Reference	179	340	1.24 (0.82–1.90)	0.0054
Ever drinker							
Light	87	208	1.07 (0.67–1.70)	80	204	1.03 (0.63–1.67)	
Moderate	79	208	0.89 (0.54–1.44)	80	108	1.57 (0.94–2.64)	
Heavy	87	145	1.28 (0.77–2.12)	44	32	3.03 (1.59–5.79)	
Unknown subjects	8	10		3	5		

[a]One case was excluded because ALDH2 genotype was not defined.
[b]ORs were calculated by an unconditional logistic regression model adjusted for age, sex, PY of smoking, fruit/vegetable intake, family history of gastric cancer, gastric atropy defined by serological PG testing and *H.pylori* status.

Table V explores the interaction between *ALDH2* genotype and alcohol drinking with regard to the prevalence of AG among non-cancer controls. Association with alcohol drinking was not significant. In analysis of the combination of *ALDH2* and alcohol drinking, heavy drinking with *ALDH2* Lys+ showed an OR of 4.50 (1.51–13.43, $P = 0.007$) relative to non-drinkers with *ALDH2* Glu/Glu, whereas that of heavy drinking with *ALDH2* Glu/Glu was 1.48 (0.74–2.98). The sources of confounding were age, sex, smoking status and *H.pylori* status.

Discussion

In this large case-control study, we found a significant interaction between the *ALDH2 Lys* allele and alcohol consumption after adjustment for *H.pylori* infection, cumulative exposure to smoking, and fruit/vegetable intake. Subjects with the *ALDH2* Lys allele who drank heavily showed a >2-fold higher risk than those with *ALDH2* Glu/Glu genotype who did not drink. A similar phenomenon was observed with regard to the prevalence of AG among non-cancer controls.

ALDH2 is a key enzyme that catalyzes acetaldehyde into acetate. The polymorphism Glu504Lys (rs671) has sufficient functional strength to influence many alcohol-related conditions (4,18,32). We first described a strong gene–environment interaction between alcohol drinking and the *ALDH2* Glu504Lys polymorphism in esophageal cancer risk (4), and subsequent studies, including our own, confirmed the same phenomenon in UADT cancers (5–11). This line of epidemiological evidence for an interaction between these two factors finally lead to the conclusion that 'acetaldehyde associated with alcoholic beverages' was Group 1 by the International Agency for Research on Cancer (3). Although the effect size of *ALDH2* or alcohol drinking was smaller than those for UADT cancers, our results are consistent with the phenomenon seen in UADT cancers, indicating the substantial attribution of acetaldehyde to stomach carcinogenesis, as previously hypothesized (13,14).

To date, several studies have evaluated the association between *ALDH2* rs671 polymorphism and risk of stomach cancer (15–18,33,34). However, these studies did not examine the interaction with detailed information on alcohol consumption. A recent study from Korea reported a similar phenomenon among 454 cases and 370 controls (17). Interestingly, a very recent study from Europe reported that a polymorphism in *ALDH2*, rs16941667, showed an allelic OR of 1.34 in a European population. But the interaction between rs16941667 and alcohol consumption is not remarkable, possibly because rs16941667 has less functional impact than rs671. In any case, their finding might indicate a substantial contribution of ALDH2 to stomach carcinogenesis across ethnicities. Clarification of the role of alcohol in gastric carcinogenesis awaits further studies of possible gene–gene interactions between the *ALDH2* and alcohol dehydrogenases genes.

In this study, we also explored the potential contribution of *ALDH2*–alcohol interaction in AG, which has been established as a pre-cancerous stage of stomach cancer (28,35,36). We defined AG status by PG I and II levels, which reflect the secretary function of gastric glands. We observed that the impact of heavy drinking was stronger in those with *ALDH2* Lys+ compared with *ALDH2* Glu/Glu, albeit that the statistical interaction was not significant. This finding might suggest that acetaldehyde plays a role in gastric carcinogenesis from the AG stage *via* induction of mutagenic adducts as reported (14) in the gastric mucosa. Against this, however, contradicting results have been reported from

Table IV. OR for heavy drinking compared with non-drinking stratified by potential confounders

Stratified by	*Glu/Glu*				*Lys+*				*P*-heterogeneity
	Non-drinker	Light	Moderate	Heavy	Non-drinker	Light	Moderate	Heavy	
	OR (95% CI)[a]	OR (95% CI)[a]	OR (95% CI)[a]	OR (95% CI)[a]	OR (95% CI)[a]	OR (95% CI)[a]	OR (95% CI)[a]	OR (95% CI)[a]	
Overall	Reference	1.07 (0.67–1.70)	0.89 (0.54–1.44)	1.28 (0.77–2.12)	1.24 (0.82–1.90)	1.03 (0.63–1.67)	1.57 (0.94–2.64)	3.03 (1.59 (5.79)	
Sex									
Male	Reference	1.10 (0.45–2.69)	1.04 (0.43–2.52)	1.43 (0.59–3.47)	1.42 (0.59–3.38)	1.16 (0.48–2.82)	1.85 (0.76–4.52)	3.47 (0.76–4.52)	0.823
Female	Reference	1.38 (0.72–2.67)	0.69 (0.26–1.80)	3.72 (0.52–26.7)	1.29 (0.75–2.20)	1.02 (0.43–2.41)	1.13 (0.16–7.90)	2.63 (0.16–7.90)	
Age category									
<60	Reference	0.67 (0.34–1.34)	0.68 (0.34–1.38)	1.33 (0.64–2.76)	1.29 (0.70–2.39)	1.20 (0.60–2.40)	1.32 (0.61–2.87)	1.71 (0.67–4.37)	0.751
60 or more	Reference	1.64 (0.85–3.19)	1.07 (0.54–2.14)	1.18 (0.57–2.44)	1.17 (0.65–2.12)	0.81 (0.40–1.65)	1.82 (0.89–3.70)	4.99 (1.94–12.8)	
Smoking status									
Never	Reference	1.15 (0.65–2.06)	1.05 (0.53–2.07)	1.08 (0.42–2.77)	1.16 (0.71–1.89)	1.22 (0.61–2.43)	1.66 (0.65–4.25)	2.50 (0.69–9.06)	0.187
Ever	Reference	1.10 (0.39–3.09)	0.93 (0.33–2.61)	1.63 (0.58–4.56)	1.60 (0.58–4.39)	1.12 (0.40–3.13)	1.84 (0.65–5.18)	3.89 (1.27–11.9)	
Fruit/vegetable intake									
Lowest tertile	Reference	0.45 (0.19–1.04)	0.47 (0.19–1.20)	0.51 (0.21–1.21)	0.84 (0.38–1.86)	0.48 (0.20–1.16)	0.79 (0.31–1.99)	0.95 (0.31–2.84)	0.023
Middle tertile	Reference	1.69 (0.67–4.25)	1.59 (0.63–4.06)	2.42 (0.93–6.27)	1.42 (0.61–3.27)	1.99 (0.76–5.03)	1.67 (0.62–4.51)	4.94 (1.60–15.3)	
Highest tertile	Reference	1.36 (0.62–2.95)	0.89 (0.39–2.06)	1.64 (0.60–4.51)	1.58 (0.80–3.14)	1.27 (0.56–2.91)	2.78 (1.12–6.87)	9.89 (2.16–45.3)	
H.pylori									
Positive	Reference	1.21 (0.71–2.08)	1.14 (0.65–1.98)	1.49 (0.83–2.64)	1.60 (0.8–2.61)	1.12 (0.64–1.97)	2.44 (1.35–4.42)	3.87 (1.82–8.24)	0.097
Negative	Reference	0.79 (0.30–2.10)	0.52 (0.18–1.53)	0.87 (0.29–2.57)	0.57 (0.24–1.40)	0.79 (0.29–2.14)	0.43 (0.12–1.51)	1.89 (0.50–7.11)	
AG defined by PG test									
Positive	Reference	1.00 (0.53–1.89)	1.11 (0.58–2.13)	1.18 (0.59–2.35)	1.26 (0.71–2.23)	0.92 (0.47–1.82)	1.75 (0.87–3.54)	2.35 (0.99–5.58)	0.808
Negative	Reference	1.38 (0.67–2.83)	0.73 (0.33–1.63)	1.56 (0.72–3.37)	1.46 (0.76–2.82)	1.27 (0.61–2.66)	1.84 (0.82–4.15)	5.95 (2.17–16.3)	
Family history of gastric cancer									
Yes	Reference	0.58 (0.19–1.76)	0.47 (0.14–1.54)	1.40 (0.42–4.62)	0.64 (0.23–1.73)	0.45 (0.14–1.41)	1.30 (0.40–4.25)	3.42 (0.82–14.2)	0.483
No	Reference	1.24 (0.74–2.09)	1.01 (0.59–1.73)	1.22 (0.69–2.14)	1.44 (0.90–2.31)	1.23 (0.72–2.12)	1.58 (0.88–2.84)	2.86 (1.37–5.94)	
Histology[b]									
Diffuse	Reference	1.11 (0.64–1.95)	0.97 (0.54–1.76)	1.68 (0.92–3.08)	1.50 (0.92–2.46)	1.19 (0.66–2.13)	2.00 (1.07–3.74)	3.76 (1.74–8.14)	NE[c]
Intestinal	Reference	0.89 (0.44–1.79)	0.66 (0.32–1.35)	0.82 (0.39–1.73)	0.82 (0.43–1.58)	0.66 (0.31–1.37)	1.04 (0.49–2.20)	1.96 (0.81–4.71)	
Location of stomach cancer									
Upper[d]	Reference	0.48 (0.03–8.53)	0.89 (0.07–11.7)	2.25 (0.20–25.9)	1.47 (0.15–14.3)	3.57 (0.36–35.8)	1.45 (0.10–20.3)	4.32 (0.29–64.6)	NE[c]
Others	Reference	1.09 (0.68–1.75)	0.89 (0.55–1.46)	1.26 (0.76–2.10)	1.24 (0.81–1.90)	0.98 (0.60–1.60)	1.58 (0.94–2.67)	2.89 (1.51–5.56)	

[a]ORs were calculated by an unconditional logistic regression model adjusted for age, sex, PY of smoking, fruit/vegetable intake, family history of gastric cancer, gastric atrophy defined by serological PG testing and *H.pylori* status.
[b]One case was excluded from analysis because of undefined histology.
[c]NE indicates not evaluable.
[d]Upper stomach cancer includes ICD O3T C16.0 (cardia, NOS, $n = 21$) and C16.1 (fundus of stomach, $n = 3$).

Table V. Associations between ALDH2 genotype and drinking and AG prevalence among controls

Level of drinking	Overall			Combined with ALDH2 genotype					
				ALDH2 Glu/Glu			ALDH2 Lys+		
	AG	Non-AG	OR (95% CI)[b]	AG	Non-AG	OR (95% CI)[b]	AG	Non-AG	OR (95% CI)[b]
Non-drinker	163	289	Reference	39	73	Reference	124	216	1.65 (0.92–2.93)
Ever drinker									
Light	128	284	0.99 (0.68–1.44)	68	140	1.71 (0.90–3.25)	60	144	1.27 (0.66–2.44)
Moderate	119	197	1.20 (0.81–1.79)	76	132	1.67 (0.88–3.17)	43	65	2.10 (1.00–4.41)
Heavy	66	111	1.19 (0.73–1.92)	51	94	1.48 (0.74–2.98)	15	17	4.50 (1.51–13.43)
Unknown subjects	3	12		1	9		2	3	

[a]One case was excluded because ALDH2 genotype was not defined.
[b]ORs were calculated by an unconditional logistic regression model adjusted for age, sex, PY of smoking, fruit/vegetable intake, family history of gastric cancer and *H.pylori* status.

Germany (37). In their population-based study in 9444 older adults, Gao *et al.* (37) found that alcohol drinking was associated with a reduced risk of AG, which they explained as due to the potentially bactericidal effect of alcohol. The attribution of *ALDH2* or alcohol consumption to gastric carcinogenesis thus remains to be elucidated.

This study had several methodological strengths. First, potential confounding by age, sex, smoking, fruit/vegetable intake, *H.pylori* infection and gastric atrophy status was considered by individual matching and statistical adjustment in the analyses. In particular, the consideration of *H.pylori* infection warrants the robustness of our observation. Second, as the *ALDH2* genotype does not change throughout life, we can assume that the impact of *ALDH2* polymorphism is subject to Mendelian randomization. Third, the size of the study was large, and the food frequency questionnaire was satisfactorily valid and reproducible (17,18). Potential limitations of this study also warrant mention. First, measurement of alcohol drinking might have been affected by the status of cases at recruitment. To avoid this, we asked about drinking behavior when the participants were healthy or before the current symptoms developed. Second, the control participants were selected from among non-cancer patients at our hospital. Because cases and controls were selected from the same hospital and almost all patients lived in the Tokai area of central Japan, the internal validity of this case-control study is likely acceptable (21). In addition, to dilute any bias that might have resulted from the inclusion of a specific diagnostic group that is related to the exposure, we did not set eligibility criteria for control diseases. Finally, it is difficult to completely rule out misclassification of *H.pylori* infection status or AG status by plasma measurement, or lifestyle factors considered as potential confounders based on self-reporting. If present, however, the effect of such misclassification in relation to possible under-adjustment would be limited, particularly considering the consistency of results across stratified analyses by several potential confounders.

In conclusion, we found that ALDH2 and alcohol drinking interact with each other in the risk of stomach cancer. This finding indicates a substantial role of acetaldehyde in carcinogenesis in the stomach, as has already been shown for cancers of the UADT.

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