6-24-2004

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Accuracy of MSI testing in predicting germline mutations of *MSH2* and *MLH1*: a case study in Bayesian meta-analysis of diagnostic tests without a gold standard

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June 24, 2004

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Abstract

Microsatellite instability (MSI) testing is a common screening procedure used to identify families that may harbor mutations of a mismatch repair gene and therefore may be at high risk for hereditary colorectal cancer. A reliable estimate of sensitivity and specificity of MSI for detecting germline mutations of mismatch repair genes is critical in genetic counseling and colorectal cancer prevention. Several studies published results of both MSI and mutation analysis on the same subjects. In this article we perform a meta-analysis of these studies and obtain estimates that can be directly used in counseling and screening. In particular we estimate the sensitivity of MSI for detecting mutations of *MSH2* and *MLH1* to be 0.78 (0.69–0.86).

Statistically, challenges arise from the following: a) traditional mutation analysis methods used in these studies cannot be considered a gold standard for the identification of mutations; b) studies are heterogeneous in both the design and the populations considered; and c) studies may include different patterns of missing data resulting from partial testing of the populations sampled. We addressed these challenges in the context of a Bayesian meta-analytic implementation of the Hui-Walter design, designed to account for various forms of incomplete data. Posterior inference are handled via a Gibbs sampler.

Keywords: Sensitivity; Specificity; Diagnostic test; Microsatellite instability (MSI); hereditary nonpolyposis colorectal cancer (HNPCC)

1 Introduction

Hereditary nonpolyposis colorectal cancer (HNPCC) (Lynch and de la Chapelle 1999) is the most common hereditary cancer syndrome. It can be caused by a deleterious germline mutation of one of the mismatch repair (MMR) genes, mainly *MSH2* and *MLH1*. These mutations confer substantially increased life-time risk of cancer of the colorectum and other sites (Vasen et al. 2001, Lin et al. 1998). Microsatellite instability (Thibodeau et al. 1993), or MSI, is the DNA amplification or deletion within microsatellites (tandemly repeated sequences that appear throughout the genome) caused by a failure of the DNA mismatch repair system, and is a key characteristic of the HNPCC syndrome. The detailed definition of MSI and the criteria used to determine the related tumor phenotype have been discussed and established on "The International Workshop on Microsatellite Instability and RER Phenotypes in Cancer Detection and Familial Predisposition" (Boland et al. 1998). MSI is found in more than 70% of HNPCC-related cancers (Liu et al. 2000). Meanwhile, up to 10–20% of sporadic colorectal cancers also exhibit MSI (Salovaara et al. 2000, Lothe et al. 1993). MSI testing has become widely used as a pre-screening procedure for possible HNPCC germline mutations thanks to the simpler and less expensive technique compared to most mutation analyses.

A reliable estimate of sensitivity and specificity of MSI for detecting germline mutations of mismatch repair genes is critical in genetic counseling and colorectal cancer prevention. In particular, knowing one's probability of carrying an inherited deleterious mutation on the *MSH2* and *MLH1* genes prior to genetic testing by mutation analysis is important for decision making about genetic testing, disease prophylaxis, family planning and more. As many colorectal cancer patients undergo MSI testing before mutation analysis, pre-test carrier probabilities need to incorporate information about MSI testing, as done in commonly used prediction software such as BayesMendel (Chen et al. 2004). This requires estimates of the sensitivity of MSI, defined as the probability of a subject's tumor sample being microsatellite unstable (MSI = 1) given he/she is carrying a deleterious germline mutation of *MSH2* or *MLH1* ($G = 1$), and of the specificity of MSI, defined as the probability of the subject's tumor sample being microsatellites stable (MSI= 0) given he/she is not carrying a mutation ($G = 0$). Also, accurate estimates of the test properties along with related costs are determinants in designing screening and surveillance programs for colorectal cancer (Ramsey et al. 2001, Reyes et al. 2002).

Several investigations reported testing a set of tumors both for germline mutations and for MSI, and then comparing the results. Among these, studies using tumors from high-risk colorectal cancer cases have relatively small sample sizes. Yet accurate risk prediction and genetic counseling require a reliable estimate of MSI sensitivity and specificity. In this article we perform a meta-analysis of these studies and obtain estimates of sensitivity and specificity as defined above. Statistically, challenges arise from the following: a) traditional mutation analysis methods used in published studies cannot be considered a gold standard for the identification of mutations (Yan et al. 2000); b) studies are heterogeneous in both the design

and the populations considered; and c) studies may include different patterns of missing data resulting from partial testing of the populations sampled. We addressed these challenges using a Bayesian meta-analysis motivated by the Hui and Walter approach (Hui and Walter 1980). This approach exploits the availability of multiple studies that sample populations with different mutation prevalences, to obtain estimates of sensitivity and specificity of two tests without requiring either one to be a gold standard. We developed specific adaptation designed to accommodate incomplete data and heterogeneity in the experimental designs of the available studies. Posterior inference are handled via a Gibbs sampler.

2 Data and Methods

For our meta-analysis, we consider published studies that involved testing colorectal cancer tumor tissues for MSI and/or germline mutations. We initially identified a number of such studies by interviewing experts, searching on PUBMED, searching reference sets of previously identified papers and searching citation indices. Not all studies with such results can be included in the analysis. The reason for exclusion and a more detailed description of each study are provided in the Appendix. We then selected studies with clearly reported test results. Examining the results of the literature search, the following issues arise.

The first issue is the absence of a gold standard. The tumor samples are tested with a variety of mutation analysis techniques including: direct sequencing, DGGE (denaturing gradient gel electrophoresis), PCR (polymerase chain reaction), RT–PCR (reverse transcriptase-polymerase chain reaction), PTT (protein truncation test), SSCA (single strand conformation analysis), and others. We refer to these tests collectively as "mutation analysis". However these conventional mutation analysis methods may miss a significant fraction of all HNPCC mutations. For example, studies have demonstrated that direct sequencing is not sensitive to certain complex mutations (Wang et al. 2001, Sophie et al. 2001), to large genomic deletions and rearrangements, or to mutations in only one of the two DNA copies (Yan et al. 2000, Payne et al. 2000, Nakagawa et al. 2002, Wang et al. 2003, Renkonen et al. 2003), while other techniques are considered less reliable than sequencing. The mutations that are likely to be missed by conventional methods make up a significant fraction of all mutations related to colorectal cancers. Because of these limitations, the true genotype is not always observed in the published studies, but rather a fraction of the subjects tested negative may be mutation carriers. Using germline testing results as the gold standard can lead to biased estimates of MSI's sensitivity and specificity. While we can safely assume that these mutation analysis methods are highly specific, we will also consider relaxation of this assumption in Section 3.

The second issue is population heterogeneity both across and within studies. For example, Lamberti et al. (1999) tested four groups of subjects: families fulfilling the stringent Amsterdam Criteria (Vasen et al. 1991), families who met a looser version of the Amsterdam Criteria, families that exhibit some familial clustering of colorectal cancer, and finally sub-

jects with sporadic colorectal cancer. It is likely that the prevalence of deleterious mutation differs significantly from group to group. While such a study design is typical in the relevant literature, other studies may focus on a single more homogeneous population. However the populations may be different across studies. This scenario is a challenge but also a strength because the existence of such strata allows to account for the lack of a gold standard, as we will discuss later.

The third issue is incompleteness in the data. An ideal study is one where the same set of subjects are tested *both* for MSI and germline mutations. However, not all studies have complete data in this sense. In Terdiman et al. (2002), Wang et al. (2003), Loukola, de la Chapelle and Aaltonen (1999) and Salovaara et al. (2000), since it was believed that the yield of mutations would be low among MSI negative tumors, only those samples that tested positive for MSI were then genotyped. In Cederquist et al. (2001), only 23 out of the 35 MSI positives were genotyped. Ponz de Leon et al. (2004) only gave mutation analysis results, whereas Percesepe et al. (2001), Salahshor et al. (1999) and Dietmaier et al. (1997) only gave MSI results. While the data in these studies are incomplete, they may provide important information on MSI accuracy. Therefore the inclusion of such data is worth the additional modeling effort required.

In our analysis, we stratify the study populations into a high-risk group and a low-risk group. The high-risk group includes families that fulfilled the Amsterdam Criteria, or met any modified clinical guidelines for the purpose of identifying HNPCC families. In addition, we have included in the high-risk group all families and individual cases that can be classified as being at significantly elevated risk based on descriptors such as "with age of onset before 35", "with two or more close relatives with colorectal cancer" or "exhibiting familial clustering of colorectal cancer (strong and positive family history)". The low-risk group includes cases from population-based registries. The data, stratified into two groups, are shown in Table 1.

If all missing counts in Table 1 were observed, then the stratified data would fit into the framework of a Hui and Walter design (Hui and Walter 1980). In this design, the sensitivity and specificity of MSI testing can be estimated without ascertaining the true genetic status of the subjects, by exploiting the existence of strata that are known to have different prevalences. Data consist of a three-way tabulation of the results of two tests and the stratum indicator. The Hui and Walter approach relies on two assumptions. The first is that the prevalence of mutations is higher in one of the groups (Parmigiani 2002, Johnson et al. 2001). This is guaranteed in our analysis by our stratification into a high-risk group and a low-risk group. The second assumption is that the results of MSI testing and mutation analysis are independent given the true genotype. In other words, if a specific mutation is difficult to detect by mutation analysis, this does not imply that the same mutation is also difficult to detect by MSI testing. So far, there is no biological evidence suggesting dependence between MSI test errors (with respect to true genotype) and mutation analysis errors on *MSH2* or *MLH1*.

Study	high-risk	low-risk	MSI=1 Mut=1	MSI=1 Mut=0	MSI=0 Mut=1	MSI=0 Mut=0
Bapat et al. (1999)	•		16	1	2	20
Calistri et al. (2000)	•		8	8	0	9
Debniak et al. (2000)	•		5	4	1	15
Dieumegard et al. (2000)	•		7	7	0	2
Lamberti et al. (1999)	•		13	22	2	10
Liu et al. (2000)	•		16	6	1	36
Scartozzi et al. (2002)	•		0	1	4	22
Cederquist et al. (2001)	•		$8 + x_1$	$15 + (12 - x_1)$	x_2	$43 - x_2$
Ponz de Leon et al. (2004)	•		x_3	x_4	$89 - x_3$	$75 - x_4$
Terdiman et al. (2002)	•		21	11	x_5	$63 - x_5$
Wang et al. (2003)	•		92	88	x_6	$188 - x_6$
Debniak et al. (2000)		•	0	5	0	38
Dieumegard et al. (2000)		•	0	0	0	7
Loukola et al. (1999)		•	10	53	y_1	$446 - y_1$
Percesepe et al. (2001)		•	y_2	$28 - y_2$	y_3	$308 - y_3$
Salahshor et al. (1999)		•	y_4	$22 - y_4$	y_5	$159 - y_5$
Dietmaier et al. (1997)		•	y_6	$23 - y_6$	y_7	$125 - y_7$
Salovaara et al. (2000)		•	18	48	y_8	$469 - y_8$

Table 1: A list of the studies included. Data are stratified into a high-risk group in the top portion and a low-risk group in the bottom portion. In each row, data are summarized in four columns by MSI test results (MSI=0 or 1) and mutation analysis results (Mut=0 or 1) and mutation analysis results. Missing counts due to samples without one of the two tests are represented by $x_.$ and $y_.$. For example, x_3 stands for the potential number of mutation analysis positive subjects in Ponz de Leon et al. (2004) who would have been tested positive for MSI. Among the MSI positive group of 35 in Cederquist et al. (2001), mutation analysis was performed on 23 samples among which 8 were found to harbor germline mutations. No mutation analysis were performed on the rest 12 samples. x_1 stands for the potential number of mutation positives among these 12 untested subjects.

To write the likelihood function we define the multinomial probabilities of an individual falling into the four cells MSI=1 & Mut=1, MSI=1 & Mut=0, MSI=0 & Mut=1, MSI=0 & Mut=0 as a, b, c and d respectively. Subscripts h and l will denote the high-risk and low-risk group, while subscript $+$ and $-$ will denote true carrier status. For example a_{h+} are MSI positive, mutation analysis positive, true carriers in the high risk group. We denote the sensitivity and specificity of MSI testing by β_M and α_M, those of the mutation analysis by β_T and α_T, and the mutation prevalences in the high-risk and low-risk group by π_h and π_l. Then, using the independence assumption for the two testing modalities, we can write, for the high risk group,

$$\begin{aligned}
a_h \equiv \quad & a_{h+} + a_{h-} = \pi_h \beta_M \beta_T + (1 - \pi_h)(1 - \alpha_M)(1 - \alpha_T) \\
b_h \equiv \quad & b_{h+} + b_{h-} = \pi_h \beta_M (1 - \beta_T) + (1 - \pi_h)(1 - \alpha_M)\alpha_T \\
c_h \equiv \quad & c_{h+} + c_{h-} = \pi_h (1 - \beta_M)\beta_T + (1 - \pi_h)\alpha_M (1 - \alpha_T) \\
d_h \equiv \quad & d_{h+} + d_{h-} = \pi_h (1 - \beta_M)(1 - \beta_T) + (1 - \pi_h)\alpha_M \alpha_T
\end{aligned}$$

Similar relationships apply to the low-risk group. We also use θ to denote the parameter

vector $(\alpha_T, \alpha_M, \beta_T, \beta_M, \pi_h, \pi_l)$ and O to denote the totality of observed variables in Table 1.

Using this notation, we can write the contribution of each study to the likelihood function, depending on the stratum and missing data pattern. For example, the contribution from Bapat et al. (1999) is

$$a_h^{16} b_h^1 c_h^2 d_h^{20},$$

that from Terdiman et al. (2002) is

$$a_h^{21} b_h^{11} (c_h + d_h)^{63},$$

and that from Percesepe et al. (2001) is

$$(a_l + b_l)^{28} (c_l + d_l)^{308}.$$

Assuming independence of study results conditional on θ, the likelihood is the product of the contribution of each study, that is:

$$\begin{aligned} L(\theta|O) &= \{a_h^{65} b_h^{49} c_h^{10} d_h^{114}\}\{a_h^8 b_h^{15} (a_h + b_h)^{12} (c_h + d_h)^{45}\}\{(a_h + c_h)^{89} (b_h + d_h)^{75}\} \\ &\quad \{a_h^{21} b_h^{11} (c_h + d_h)^{63}\}\{a_h^{92} b_h^{88} (c_h + d_h)^{188}\} \\ &\quad \{b_l^5 d_l^{38}\} d_l^7 \{a_l^{10} b_l^{53} (c_l + d_l)^{446}\}\{(a_l + b_l)^{28} (c_l + d_l)^{308}\}\{(a_l + b_l)^{22} (c_l + d_l)^{159}\} \\ &\quad \{(a_l + b_l)^{23} (c_l + d_l)^{125}\}\{a_l^{18} b_l^{48} (c_l + d_l)^{469}\} \\ &= a_h^{186} b_h^{163} c_h^{10} d_h^{114} (a_h + b_h)^{12} (c_h + d_h)^{294} (a_h + c_h)^{89} (b_h + d_h)^{75} \\ &\quad a_l^{28} b_l^{106} d_l^{45} (a_l + b_l)^{73} (c_l + d_l)^{1507} \end{aligned} \tag{1}$$

where each bracketed expression corresponds to a study. Under our modeling assumptions, all six parameters $\beta_M, \alpha_M, \beta_T, \alpha_T, \pi_h, \pi_l$ are identified, as long as the constraint $\pi_h > \pi_l$ holds.

We draw inferences about θ via a Bayesian analysis. Advantages include the possibility to derive exact probability intervals without relying on asymptotic approximations, and ease in handling the missing data patterns and the parametric constraint required for identifiability. Within this constraint, we adopt a uniform prior on all components of θ, with the exception of the specificity α_T of mutation analysis, which we assume to be 100%. This assumption is highly plausible biologically. However, as a sensitivity analysis, we also report results using a uniform prior on α_T. By Bayes theorem, the posterior is proportional to Expression (1) wherever the prior is non-zero.

Computing can be simplified by introducing two sets of auxiliary variables: the counts of test results for subjects who have not been tested by both tests, and the counts of true genotypes for all subjects. Denoting auxiliary variables by A, we implement a Markov chain Monte Carlo algorithm to draw from the distribution of (A, θ) given O (Tanner and Wong 1987).

Our algorithm requires an expression for the so-called complete data likelihood, that is $p(A, O|\theta)$. This is given by

$$\begin{aligned} &a_{h+}^{n_{a,h+}} \cdot a_{h-}^{n_{a,h-}} \cdot b_{h+}^{n_{b,h+}} \cdot b_{h-}^{n_{b,h-}} \cdot c_{h+}^{n_{c,h+}} \cdot c_{h-}^{n_{c,h-}} \cdot d_{h+}^{n_{d,h+}} \cdot d_{h-}^{n_{d,h-}} \cdot \\ &a_{l+}^{n_{a,l+}} \cdot a_{l-}^{n_{a,l-}} \cdot b_{l+}^{n_{b,l+}} \cdot b_{l-}^{n_{b,l-}} \cdot c_{l+}^{n_{c,l+}} \cdot c_{l-}^{n_{c,l-}} \cdot d_{l+}^{n_{d,l+}} \cdot d_{l-}^{n_{d,l-}} \end{aligned} \tag{2}$$

Each exponent in Expression (2) arises as the sum of contributions from studies with different missing data structures. Those need to be simulated separately, requiring additional auxiliary variables defined in Table 2. To explain the significance of the auxiliary variables, for example, we observe in the high-risk group that 186 subjects are MSI positive and mutation analysis positive (MSI+ & Mut+) and 163 are MSI+ & Mut−. This corresponds to the first two factors in Expression (1), $a_h^{186}b_h^{163}$. After we assume the carrier status known, among the 186 MSI+ & Mut+ subjects, $n^{(1)}_{a,h+}$ would be the true carriers and $n^{(1)}_{a,h-} = 186 - n^{(1)}_{a,h+}$ the true noncarriers. Similarly, among the 163 MSI+ & Mut− subjects, $n^{(1)}_{b,h+}$ would be true carriers and $n^{(1)}_{b,h-}$ noncarriers. Thus the corresponding factors in the complete data likelihood will be written as $a_{h+}^{n^{(1)}_{a,h+}} a_{h-}^{n^{(1)}_{a,h-}} b_{h+}^{n^{(1)}_{b,h+}} b_{h-}^{n^{(1)}_{b,h-}}$ with the constraints $n^{(1)}_{a,h+} + n^{(1)}_{a,h-} = 186$ and $n^{(1)}_{b,h+} + n^{(1)}_{b,h-} = 163$. In other words, $a_h^{186}b_h^{163}$ is the result of integrating out the auxiliary variables $n^{(1)}_{a,h+}$, $n^{(1)}_{a,h-}$, $n^{(1)}_{b,h+}$, $n^{(1)}_{b,h-}$ from $a_{h+}^{n^{(1)}_{a,h+}} a_{h-}^{n^{(1)}_{a,h-}} b_{h+}^{n^{(1)}_{b,h+}} b_{h-}^{n^{(1)}_{b,h-}}$ within the constraints.

	high-risk	
	Mut=1	Mut= 0
MSI=1	$n_{a,h+} = n^{(1)}_{a,h+} + n^{(2)}_{a,h+} + n^{(3)}_{a,h+}$	$n_{b,h+} = n^{(1)}_{b,h+} + n^{(2)}_{b,h+} + n^{(3)}_{b,h+}$
	$n_{a,h-} = n^{(1)}_{a,h-} + n^{(2)}_{a,h-} + n^{(3)}_{a,h-}$	$n_{b,h-} = n^{(1)}_{b,h-} + n^{(2)}_{d,h-} + n^{(3)}_{b,h-}$
MSI= 0	$n_{c,h+} = n^{(1)}_{c,h+} + n^{(2)}_{c,h+} + n^{(3)}_{c,h+}$	$n_{d,h+} = n^{(1)}_{d,h+} + n^{(2)}_{d,h+} + n^{(3)}_{d,h+}$
	$n_{c,h-} = n^{(1)}_{c,h-} + n^{(2)}_{c,h-} + n^{(3)}_{d,h-}$	$n_{d,h-} = n^{(1)}_{d,h-} + n^{(2)}_{d,h-} + n^{(3)}_{d,h-}$
	low-risk	
	Mut=1	Mut= 0
MSI=1	$n_{a,l+} = n^{(1)}_{a,l+} + n^{(2)}_{a,l+}$	$n_{b,l+} = n^{(1)}_{b,l+} + n^{(2)}_{b,l+}$
	$n_{a,l-} = n^{(1)}_{a,l-} + n^{(2)}_{a,l-}$	$n_{b,l-} = n^{(1)}_{b,l-} + n^{(2)}_{d,l-}$
MSI= 0	$n_{c,l+} = n_{c,l+}$	$n_{d,l+} = n^{(1)}_{d,l+} + n^{(2)}_{d,l+}$
	$n_{c,l-} = n_{c,l-}$	$n_{d,l-} = n^{(1)}_{d,l-} + n^{(2)}_{d,l-}$

Table 2: Notation: the number in each cell can be broken-down into those with a true germline mutation (subscript "+") and those without (subscript "−"). High-risk and low-risk are indicated by the letter "h" or "l" in the subscript. The superscripts in parentheses signify contributions from studies with different missing data structures.

The Gibbs sampler and the various constraints placed on the components are described in the Appendix.

3 Results

Posterior means and 95% credible intervals are shown in Table 3. The estimated specificity of MSI testing is 92% (95% c.i. 90–93%), indicating that MSI is a more specific test than previously suggested (Salovaara et al. 2000, Lothe et al. 1993). Combined with an estimated sensitivity of 78%, our estimates translate into a positive predictive value (PPV) of 0.93 (95% c.i. 0.90 – 0.95) among high risk families. However, the PPV of MSI is drastically lower

		$\alpha_T = 1$		$\alpha_T \sim \mathcal{U}(0,1)$	
		Mean	95% P.I.	Mean	95% P.I.
MSI specificity	α_M	0.92	(0.90, 0.93)	0.92	(0.90, 0.93)
MSI sensitivity	β_M	0.78	(0.69, 0.86)	0.79	(0.69, 0.89)
Mutation Analysis specificity	α_T	1		0.98	(0.93, 1.0)
Mutation Analysis sensitivity	β_T	0.62	(0.56, 0.68)	0.62	(0.56, 0.68)
Prevalence in high-risk group	π_h	0.57	(0.51, 0.65)	0.56	(0.49, 0.65)
Prevalence in low-risk group	π_l	0.052	(0.034, 0.075)	0.049	(0.029, 0.073)

Table 3: Summary of inferences on parameters. The left part of the table is obtained by setting α_T to 1. The right part is obtained by placing a uniform prior on α_T.

$\alpha_T = 1$	**high-risk**		**low-risk**	
	Mut=1	Mut=0	Mut=1	Mut=0
MSI=1	262+0	158+34	43+0	27+137
MSI=0	75+0	47+367	12+0	8+1531
$\alpha_T < 1$	**high-risk**		**low-risk**	
	Mut=1	Mut=0	Mut=1	Mut=0
MSI=1	260+1	158+34	41+3	26+138
MSI=0	70+8	44+367	11+32	7+1502

Table 4: The data table broken down by imputed genotype. The top table is imputated under the constraint that the specificity of mutation analysis is 1, the bottom without the constraint. In each cell, the first number is the expected number of true mutation carriers of all subjects in that corresponding column in Table 1, the second number is the number of noncarriers.

among unselected colorectal cancer cases: we estimate to be 0.34 (95% c.i. of 0.23 – 0.46). Both PPV estimates are consistent with practical clinical experiences in MSI screening. Our prevalence estimate of true carriers among unselected colorectal cancer, 3–7%, coincides with previous estimates (Aaltonen et al. 1994, Aaltonen et al. 1998). Importantly, we have also estimated the sensitivity of mutation analysis at a modest 62%.

At each iteration of the Gibbs sampler, we generate a draw of all entries in Table 2. These draws can be used to make inferences on the missing counts in Table 1. For example, in the Ponz de Leon et al. (2004) study, x_3 is imputed as 69 on average by the Gibbs sampler, which means that 69 out of the 89 mutation positive subjects in the study would have been tested positive for MSI. In addition, x_1 is on average 7 and x_4 is on average 24. The other missing counts can be estimated up to a sum. The quantities $x_2 + x_5 + x_6$, $y_2 + y_4 + y_6$ and $y_1 + y_3 + y_5 + y_7 + y_8$ have posterior means around 45, 15 and 12, respectively.

With the Gibbs sampler, in addition to inferences on missing portions of the original studies, we also obtain inferences on the true genotypes for all subjects. Computing the total of each of the rightmost four columns of Table 1, and then breaking them down using imputed carrier status results, we get Table 4. From Table 4 we can see that although MSI is quite specific, because of the low prevalence of mutations in the low-risk population, there will still be a significant portion of false positives (an estimated 137 out of 207).

As a sensitivity analysis we relaxed the assumption that the mutation analysis specificity

is 100%, that is that $\alpha_T \equiv 1$, in favor of a uniform prior. Results are also shown in Table 3 and Table 4. The posterior mean of the mutation analysis specificity is 0.98 (95% c.i. of 0.93–1.0) suggesting that the data are consistent with the assumption. Comparing these estimates with those obtained under $\alpha_T \equiv 1$, we can see that relaxing this assumption has a very small impact on the posterior inferences. The credible intervals are slightly wider, as the result of increased uncertainty. Also, since some of the true positives (in terms of mutation analysis) under the specificity assumption are now imputed as false positives, the posterior distributions of the proportion of carriers in the two groups shifted slightly to the left. The MSI sensitivity estimate increased slightly from 0.78 (0.69–0.86) to 0.79 (0.70–0.89). Comparing the upper portion of Table 4 to the lower portion, in all the mutation positives cells, a few are imputed as false positives in the lower portion. However, such imputation occurred more often among MSI negative subjects than among MSI positive ones, causing a small increase in the overall MSI sensitivity. The only sensitive figure is the posterior mean of $y_1 + y_3 + y_5 + y_7 + y_8$. When α_T is uniform that is 43 instead of 12.

4 Discussion

In this paper we presented a meta-analysis of the accuracy of MSI testing in detecting *MSH2* and *MLH1* mutations. Our meta-analysis overcomes the difficulty posed by the fact that no gold standard is available in the published literature about this issue, and provides estimates that can be directly used in genetic counseling and colorectal cancer screening. Our methodology is a Bayesian meta-analytic implementation of the Hui-Walter approach, adapted to account for various forms of incomplete data.

We found MSI to be a very sensitive and specific indicator of germline mutations. The estimates of sensitivity and specificity of MSI in detecting *MSH2* and *MLH1* mutations will be incorporated in the latest version of the carrier probability package BayesMendel (Chen et al. 2004) and the risk prediction model CRCAPRO. The false positive fraction associated with MSI testing can be large among unselected colorectal cancers. We have also estimated the sensitivity of mutation analysis at a modest 62%. This result suggests to use caution when interpreting negative genetic testing results for potential mutation carriers.

One of the limitations of our study is that we only considered the *MSH2* and *MLH1* genes. Other, less prevalent, HNPCC genes have been found. For example *MSH6*, has a mutation prevalence of about 1.7% among unselected colorectal or endometrial cancer cases (Goodfellow et al. 2003). At the present time, it would be difficult to consider other genes, because data from published studies involving such genes are scarce.

Another issue is that published studies used a variety of mutation analysis techniques. We pooled all these as though they had the same sensitivity. While adopting a more general statistical model allowing for different sensitivities would have been feasible, there were many practical reason to make this unlikely to be successful: the sample sizes for each testing modality were generally small; some studies used more than two methods in conjunction

without stating further details; the same type of test may have different sensitivities if performed in different laboratories. To investigate this issue further, we designed some simple simulation experiments that indicate that as long as the distributions of subjects undergone each test with a particular sensitivity are consistent across the groups, estimates of MSI specificity and sensitivity remain unbiased.

Our analysis relies on two modeling assumptions: independence of the testing modalities and different prevalence among the high and low risk groups. While we are aware of no evidence of a dependence between type of *MSH2* and *MLH1* mutation and MSI, some studies have pointed out that carriers of *MSH6* mutations are less likely to show MSI (Wu et al. 1999, de Leeuw et al. 2000, Lucci-Cordisco and *et al.* 2001). Therefore, further validation of the independence assumption would be helpful.

That the prevalence of mutations in the two groups should be different is not a restrictive assumption, given the way the groups were defined. However, groups have been separated based on a sensible but subjective choice and it is conceivable that the results may be sensitive to this choice. To address this issue we performed simulation experiments that indicate that different grouping of the data according to risk does not change the unbiasedness of the estimates of MSI's sensitivity and specificity, as long as the risk in one group is higher than the other.

Acknowledgments

Work supported by grants NCI-P50CA62924 and NCI-P30CA06973. The authors thank Hormuzd Kakti, Kenneth Kinzler and Jiang-wen Zhang for helpful comments.

Appendix

Summary of studies

List of studies identified during the search with reason for inclusion or exclusion.

While we included as many studies as possible, we had to exclude studies where the subjects are a mixture of high-risk and low-risk cases with no breakdown given, such as Ruszkiewicz et al. (2002) and Banno et al. (2003). We have also disregarded all IHC (immunohistochemistry) results if they are reported. Because literature suggests a very high correlation between MSI and IHC (Lindor et al. 2002, Debniak et al. 2000, Marcus et al. 1999), which may violate the conditional independence assumption. A few studies used novel methods to search for germline mutations that are hard to detect by conventional methods (Renkonen et al. 2003, Nakagawa et al. 2003, Wang et al. 2003). Unfortunately, since these technologies are much different from conventional methods, pooling them with the conventional methods may introduce bias, we have decided to exclude such studies or to exclude mutations found by using such technologies. However such data can serve as independent validation of the estimated low sensitivity for conventional methods.

- Banno et al. (2003) Thirty-eight endometrial tumor samples in Japan were tested for MSI and for germline mutation on *MSH2*. Some cases fulfilled Amsterdam criteria while others did not, no breakdown was given. Since the tumors are not colorectal tumors, this study was excluded.
- Bapat et al. (1999) Thirty-nine samples from 33 high risk families satisfied the Mount Sinai criteria, of which 14 met Amsterdam Criteria (AC). Germline mutations were identified by RT-PCR and PTT(protein truncation test), and confirmed by sequencing.
- Calistri et al. (2000) Thirteen samples are from families that met AC, seven samples from families that met modified guidelines, four selected on the basis of family history.
- Cederquist et al. (2001) Population-based double-primary CRC samples were searched for mutations on the *MSH2*, *MLH1* and *MSH6* genes using DHPLC confirmed by direct sequencing. However *MSH6* mutations are not included because of reason stated in the text.
- Debniak et al. (2000) Sequencing, IHC and MSI analysis were conducted on two groups: group A, late-onset sporadic CRC cases, which we include in the low-risk group; group B, definitive and suspected HNPCC cases, included in the high-risk group. IHC results are discarded.
- Debniak et al. (2001) Eleven patients diagnosed definitively or with high probability for HNPCC. Fifteen patients have sporadic late-onset colorectal cancer. No mutation analysis was performed and expression of *MSH2* and *MLH1* was examined by IHC, thus the study is excluded.

- Dieumegard et al. (2000) Two groups, one satisfied the International Collaborative Group criteria, one with strong family histories, are included with the high-risk group; one group of sporadic CRC at age before 50, is included in the low-risk group. All samples were analyzed for MSI, mutation (by SSCP) and IHC. IHC results are discarded.

- Dietmaier et al. (1997) MSI was tested on 148 CRC adenocarcinomas. No mutation analysis was performed. Eighteen was MSI high, five MSI low, and rest stable. This result is included in the low-risk group.

- Lamberti et al. (1999) This study consists of groups of families from different populations. Fourty-seven families fulfilled AC. Five families met looser AC. Methods used for mutation analyses include PCR, SSCA, HA (heteroduplex analysis), *etc* (included).

  The rest consists of families that exhibit familial clustering of colorectal cancer and patients with sporadic colorectal cancer, respectively (not included because of inadequate presentation of the data).

- Liu et al. (2000) Include 59 families that are "highly suspicious for HNPCC", including some families that fulfilled AC, some with two close relatives with colorectal cancer, some with early onset colorectal cancer before age 35, although the exact breakdown was not given. Mutation analysis involved DGGE, RT-PCR and PTT.

- Loukola et al.(1999) Population-based colorectal adenoma patients were screened for MSI. We did not include this study because we were afraid that the proportion of MSIs among colorectal ademonas may be different from that in all CRC patients.

- Loukola, de la Chapelle and Aaltonen (1999) This is a population-based study where 509 patients were drawn from a Finnish colorectal cancer registry and tested for MSI. Mutation analysis is conducted only on the samples that were MSI+.

- Nakagawa et al. (2003) The multiplex ligation-dependent probe amplification method was used to detect large genomic rearrangements that have been missed by conventional methods. Five were detected among 70 MSI positive but sequencing negative samples. This study cannot be included because of the use of unconventional technology.

- Percesepe et al. (2001) Families from an Italian local colorectal cancer registry were tested for MSI. Twenty-eight out of the 336 were h-MSI. No genotype information is available.

- Ponz de Leon et al. (2004) Members from 32 high-risk kindreds were tested for MSI and IHC expression. Mutation was searched by SSCP or direct sequencing. One hundred and sixty-four subjects underwent genetic testing, however their MSI status was not available as the results were pooled with those who did not undergo genetic testing. IHC results were ignored.

- Renkonen et al. (2003) Twenty-six population based Finnish families that had screened negative by conventional techniques were tested for RNA expression of *MSH2*, *MLH1* and *MSH6* by single nucleotide primer extension (SNuPE). This study cannot be included because of use of unconventional techniques.
- Salahshor et al. (1999) Twenty-two out of 181 unselected colorectal cancer cases were tested MSI positive.
- Salovaara et al. (2000) Another Finnish population based study following (Loukola, de la Chapelle and Aaltonen 1999) have reported 66 MSI+ among 535 colorectal cancer cases, among which 18 was detected with germline mutations.
- Terdiman et al. (2002) Among 114 high risk families, 23% met AC, 70% met the Bethesda Guidelines. MSI was detected in 47 out of 109 families, of the 47 MSI+'s mutation was detected in 16 families. The rest of the families were not screened for mutation.
- Wahlberg et al. (2002) Fourth-eight HNPCC families are tested for MSI and for expression of *MSH2* and *MLH1* by IHC. No mutation analysis was performed (not included).
- Wang et al. (2003) For 368 unrelated patients meeting the Bethesda criteria and were tested for MSI, genetic testing was performed on the 180 MSI positives first with conventional methods(SSCP, HA or DHPLC followed by sequencing) to find 68 disease-causing mutations and 24 rare missense and other variants, and then screened with a semiquantitative multiplex PCR to detect large deletions. The 19 mutations found by the latter method are not considered.

Computing

The Gibbs sampler constructed based on Expression 2 can be written as follows: start with an arbitrary value for each parameter and arbitrary values for the auxiliary variables; then, in iteration i

1. impute the auxiliary variables based on parameter values:

$$
\begin{aligned}
n^{(1)}_{a,h+} &\sim Bin(178, \frac{a_{h+}}{a_{h+}+a_{h-}}), \qquad n^{(1)}_{a,h-} = 178 - n^{(1)}_{a,h+} \\
n^{(1)}_{b,h+} &\sim Bin(148, \frac{b_{h+}}{b_{h+}+b_{h-}}), \qquad n^{(1)}_{b,h-} = 148 - n^{(1)}_{b,h+} \\
n^{(1)}_{c,h+} &\sim Bin(10, \frac{c_{h+}}{c_{h+}+c_{h-}}), \qquad n^{(1)}_{c,h-} = 10 - n^{(1)}_{c,h+} \\
n^{(1)}_{d,h+} &\sim Bin(114, \frac{d_{h+}}{d_{h+}+d_{h-}}), \qquad n^{(1)}_{d,h-} = 114 - n^{(1)}_{d,h+} \\
(n^{(2)}_{a,h+}, n^{(2)}_{a,h-}, n^{(2)}_{b,h+}, n^{(2)}_{b,h-}) &\sim MultNom(35, p = \frac{1}{a_{h+}+a_{h-}+b_{h+}+b_{h-}}(a_{h+}, a_{h-}, b_{h+}, b_{h-})) \\
(n^{(2)}_{c,h+}, n^{(2)}_{c,h-}, n^{(2)}_{d,h+}, n^{(2)}_{d,h-}) &\sim MultNom(294, p = \frac{1}{c_{h+}+c_{h-}+d_{h+}+d_{h-}}(c_{h+}, c_{h-}, d_{h+}, d_{h-})) \\
(n^{(3)}_{a,h+}, n^{(3)}_{a,h-}, n^{(3)}_{c,h+}, n^{(3)}_{c,h-}) &\sim MultNom(89, p = \frac{1}{a_{h+}+a_{h-}+c_{h+}+c_{h-}}(a_{h+}, a_{h-}, c_{h+}, c_{h-})) \\
(n^{(3)}_{b,h+}, n^{(3)}_{b,h-}, n^{(3)}_{d,h+}, n^{(3)}_{d,h-}) &\sim MultNom(75, p = \frac{1}{b_{h+}+b_{h-}+d_{h+}+d_{h-}}(b_{h+}, b_{h-}, d_{h+}, d_{h-})) \\
n^{(1)}_{a,l+} &\sim Bin(28, \frac{a_{l+}}{a_{l+}+a_{l-}}), \qquad n^{(1)}_{a,l-} = 28 - n^{(1)}_{a,l+} \\
n^{(1)}_{b,l+} &\sim Bin(106, \frac{b_{l+}}{b_{l+}+b_{l-}}), \qquad n^{(1)}_{b,l-} = 106 - n^{(1)}_{b,l+} \\
n^{(1)}_{d,l+} &\sim Bin(45, \frac{d_{l+}}{d_{l+}+d_{l-}}), \qquad n^{(1)}_{d,l-} = 45 - n^{(1)}_{d,l+} \\
(n^{(2)}_{a,l+}, n^{(2)}_{a,l-}, n^{(2)}_{b,l+}, n^{(2)}_{b,l-}) &\sim MultNom(73, p = \frac{1}{a_{l+}+a_{l-}+b_{l+}+b_{l-}}(a_{l+}, a_{l-}, b_{l+}, b_{l-})) \\
(n^{(2)}_{c,l+}, n^{(2)}_{c,l-}, n^{(2)}_{d,l+}, n^{(2)}_{d,l-}) &\sim MultNom(1507, p = \frac{1}{c_{l+}+c_{l-}+d_{l+}+d_{l-}}(c_{l+}, c_{l-}, d_{l+}, d_{l-}))
\end{aligned}
$$

2. sum up according to Table 2 to get total imputed genotypes in each cell.

3. update parameters based on imputed genotypes

$$
\begin{aligned}
\alpha_M &\sim Beta(n_{c,h-}+n_{d,h-}+n_{c,l-}+n_{d,l-}+1, n_{a,h-}+n_{b,h-}+n_{a,l-}+n_{b,l-}+1) \\
\alpha_T &\sim Beta(n_{b,h-}+n_{d,h-}+n_{b,l-}+n_{d,l-}+1, n_{a,h-}+n_{c,h-}+n_{a,l-}+n_{c,l-}+1) \\
\beta_M &\sim Beta(n_{a,h+}+n_{b,h+}+n_{a,l+}+n_{b,l+}+1, n_{c,h+}+n_{d,h+}+n_{c,l+}+n_{d,l+}+1) \\
\beta_T &\sim Beta(n_{a,h+}+n_{c,h+}+n_{a,l+}+n_{c,l+}+1, n_{b,h+}+n_{d,h+}+n_{b,l+}+n_{d,l+}+1) \\
\pi_h &\sim Beta(n_{a,h+}+n_{b,h+}+n_{c,h+}+n_{d,h+}+1, n_{a,h-}+n_{b,h-}+n_{c,h-}+n_{d,h-}+1) \\
\pi_l &\sim Beta(n_{a,l+}+n_{b,l+}+n_{c,l+}+n_{d,l+}+1, n_{a,l-}+n_{b,l-}+n_{c,l-}+n_{d,l-}+1)
\end{aligned}
$$

The Gibbs sampler quickly converged. The posterior estimates are stable regardless of the starting value of the sampler.

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