A protective role for early oral exposures in the etiology of young adult Hodgkin lymphoma

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The pattern of adolescent/young adult Hodgkin lymphoma (YAHL) suggests causation by a relatively late infection with a common childhood virus, but no causal virus has been found. Susceptibility is heritable and linked to lower interleukin 12 (IL12) levels, which can also result from fewer fecal-oral microbial exposures early in life. We studied twin pairs discordant for YAHL to examine exposures capable of altering the IL12 response and T-helper type 1 (Th1)–Th2 balance. One hundred eighty-eight YAHL-discordant twin pairs from the International Twin Study returned questionnaires (70% response). Exposure history of YAHL case-twins was compared with that of their unaffected control-twins using conditional logistic regression for matched pairs to calculate odds ratios (ORs). Behaviors likely to produce oral exposure to microbes conveyed decreases in risk (univariable OR range = 0.2-0.5, *P* = .003-.11). Significant adjusted ORs were seen for appendectomy (OR = 4.3, *P* = .001), eczema (OR = 4.2, *P* = .025), smoking (OR = 2.2, *P* = .054), and relatively more frequent behaviors associated with oral exposures (OR = 0.1; *P* = .004). Kappa statistics for intrapair agreement were higher than 0.8 for each significant finding. Our observations support a protective role for increased early oral exposure to the microbiome, suggesting that factors associated with increased Th2 and decreased Th1 cytokines are etiologically relevant to YAHL. (Blood. 2009;114:4014-4020)

Introduction

In the United States, Hodgkin lymphoma (HL) is the most common cancer diagnosed among females and the second most common among males from 15 to 30 years of age.[1] Young adult Hodgkin lymphoma (YAHL) occurs more commonly under conditions of economic development,[2] has a different incidence pattern than HL at younger and older ages,[3] consists largely of Epstein-Barr virus (EBV)–negative nodular sclerosis,[4] peaks in the third decade, and is now considered a distinct etiologic entity.[3,4] Multiple siblings, higher birth order, crowded living conditions, lower socioeconomic status, and exposure to day care/kindergarten have all been associated with decreased risk for this neoplasm.[5-10] This evidence led to the hypothesis that risk is increased after relatively late exposure to a ubiquitous infectious agent, analogous to paralytic polio before polio vaccine.[8] However, extensive searches have yet to identify a viral agent that could account for the majority of YAHL.[11-16] Epstein-Barr virus is etiologically linked only to that small proportion of YAHL with EBV evident in the malignant cells.[4,10]

An alternative hypothesis was first offered to explain the recent increase in the prevalence of atopic disease. A lower level of fecal-oral exposure to bacteria early in life might produce a decrease in interleukin 12 (IL12) secretion and thus persistence of the T-helper type 2 (Th2) immature immune response phenotype.[17,18] Clinically, YAHL is associated with a Th2-skewed immune response.[19,20] It is a heritable cancer,[21] and susceptibility has been linked to genetically determined higher IL6 (a Th2 cytokine) and lower IL12 (a Th1 cytokine) responses.[22,23] Because microbiologic exposures early in life influence the Th1/Th2 cytokine "balance,"[17,18] and could therefore also alter susceptibility to YAHL, we sought to compare cases with healthy persons with respect to various indicators of childhood infection. We elected to make these comparisons within twin pairs discordant for YAHL, who, although well matched on both genome and childhood socioeconomic status, still have had independent interactions with the biologic environment. Having the advantage of a lifetime of mutual comparison, we predicted that twins could independently recall otherwise obscure differences in agreement, as they have been able to do with respect to sun exposure[24] and markers of puberty.[25]

Methods

The study was approved by the Institutional Review Board of the Keck School of Medicine of the University of Southern California and all subjects provided signed written informed consent in accordance with the Declaration of Helsinki. In response to advertisements attracting twins with cancer placed in print media across North America from 1980 through 1992,[26] at least 1 member from each of 292 twin pairs reported a YAHL diagnosis made at an eligible age (< 51 years), and provided dates of birth and diagnosis, perceived zygosity, sex, address, and telephone number. Each living member of an affected pair was sent a 35-page questionnaire

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and completed questionnaires were received from both twins (108 double-respondent pairs) or single twins (95 single-respondent pairs, of which 85% were the unaffected sole survivor). Thus information from 203 (70%) of the 292 pairs was available. Excluding the 15 pairs that had become concordant for YAHL, 93 double-respondent and 95 single-respondent YAHL-discordant pairs were available for study.

Pathology reports and diagnostic histopathologic slides were requested and received for 85% of the YAHL cases and were reviewed by a single hematopathologist (B.N.N.). Hodgkin lymphoma was confirmed in all cases, although there was disagreement between the single expert reviewer and original pathologist regarding the subclassification in 4 cases. All 4 of these were reclassified from mixed cellularity subtype (from the original pathology report) to nodular sclerosis cellular phase, based on B.N.N.'s review. The diagnosis usually occurred decades before ascertainment, and thus tissue from only 19 of the cases was available for Epstein-Barr virus (EBV) screening (L.M.W.).[27] Of these, 15 were EBV-tumor negative and the remaining 4 were EBV-tumor positive.

Exposure history

The questionnaire covered a wide range of exposures including weight and height at different ages, early childhood exposures, infections, surgical, medical and pharmacologic history, occupational history, reproductive history, timing of puberty, exposure to electromagnetic fields, and alcohol and tobacco use, as well as demographic information such as ethnic background and education. Twins were instructed to answer all questions about exposure history as of at least 5 years before diagnosis in the case-twin and were also told to answer questions separately without consultation. We purposely set the reference date as 5 years before diagnosis to be certain that the exposures of interest occurred before development of disease. Recently, Hjalgrim et al showed that the risk of Epstein-Barr tumor–positive Hodgkin lymphoma was very high within a 5-year period after the exposure,[28] validating the use of this reference period.

Questions were framed as both absolute and relative (ranking comparisons of the experience between members of the pair). For relative questions on comparisons of behavior, such as "which twin sucked their thumb, fingers, or pacifier more?," each twin choose from the following responses: "me, much more; me, more; both the same; my twin, more; my twin, much more; don't know" (for analysis, "me, much more" was combined with "me, more" and "my twin, much more" was combined with "my twin, more"). For absolute differences between twins' exposures, each twin was queried on his/her own exposure history (eg, have you ever smoked at least 100 cigarettes? yes/no) and on his/her twin's exposure (has your twin ever smoked at least 100 cigarettes? yes/no). We were interested in exposure to infectious agents by the oral route and emphasized differences in early behavior that would facilitate fecal-oral or oral-oral transmission of infection (oral behaviors).

Statistical analysis

To assess the level of agreement beyond chance between paired twin responses about mutual exposures, we used the kappa statistic (kappa, k) with 95% confidence intervals (CIs). A value higher than 0.7 was considered good agreement between paired responses (both twins in the pair returned a completed questionnaire: double respondents), and indicated the reliability of using the proxy response when only 1 member of a pair responded (single respondents). Analyses were restricted to exposures for which there were at least 18 exposure-discordant pairs to reduce instability of the risk estimates.

To obtain a risk estimate for each exposure the response of the YAHL-affected case-twin (case) was compared with that of the matched unaffected cotwin (control). Results were obtained for all pairs (including single-respondent pairs), and separately for the subset of double-respondent pairs. All analyses were also repeated after first excluding unlike-sex pairs, and then pairs in whom the case was diagnosed between 40 and 50 years of age (n = 19 pairs). Results from all sensitivity analyses were essentially identical and only results based on the double and total respondent sets are presented.

Each risk estimate was computed using conditional logistic regression for matched pairs with the SAS procedure PHREG (SAS Version 9.1), estimating odds ratios (ORs) and 95% confidence intervals (CIs), according to standard methods.[29] *P* values were obtained using the Wald test. For single-respondent pairs, a "dummy" twin was created using the available proxy responses for that variable. Adjustment for the known confounders of age, ethnicity, sibship size, and childhood socioeconomic status was unnecessary because twins are already matched on these factors.

For relative comparisons, if the case, by mutual agreement, engaged in a given behavior more often than his/her cotwin control, the OR would be greater than 1.0 and the interpretation would be consistent with an increased risk associated with that behavior. Conversely, if the control engaged in the behavior more often than the case, the OR would be less than 1.0 and the interpretation would be consistent with a protective effect on risk. Absolute (dichotomous) exposures are interpreted, as in any case-control study, with an increase in the exposure resulting in an OR greater than 1.0 interpreted as associated with an increased risk.

For results that were statistically significant or exposures considered important a priori, analyses were repeated after stratification by histology (nodular sclerosis vs all other histologic subtypes) and by zygosity (monozygotic [MZ] vs dizygotic [DZ]). Results after stratification by sex are not presented because substantive differences were not observed. Finally, multivariable logistic regression was performed to evaluate the effect on risk after adjusting for all other statistically significant or strong a priori exposures simultaneously and *P* values were obtained using the χ^2 test (SAS Version 9.1).

Results

Among the double-respondent pairs, there was a slight excess of MZ twins and females and a deficit of unlike sex pairs (among whom the female members responded disproportionately; Table 1). Nodular sclerosis (NS) was the most common histologic type.

The kappa statistic ranged from 0.8 to 1.0 and the lower 95% CI was consistently above 0.7 when twins were asked about each others' experience with respect to easily recalled discrete events and behaviors (yes/no dichotomous exposures) such as tonsillectomy, appendectomy, and smoking (ever/never; supplemental Table 1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article). Agreement on a history of eczema was equally high in comparisons of the control proxy report versus case self-report (k = 0.8, 95% CI = 0.5-1.0) and the case-proxy report versus control self-report (k = 0.8, 95% CI = 0.6-1.0). Agreement between the control proxy and the case self-report was borderline for infectious mononucleosis (k = 0.7, 95% CI = 0.4-0.9), but higher between the case proxy and control self-report (k = 0.8, 95% CI = 0.6-1.0). Kappa statistics were lower with respect to history of specific childhood exanthemata including mumps, measles, chicken pox, and rubella (kappas all below 0.70; data not shown); thus data on these infections are not presented.

Among double-respondent twin pairs, kappa was high (k > 0.70, supplemental Table 1) for relative differences in behaviors associated with oral-oral or fecal-oral exposures, especially those behaviors occurring early in childhood. The kappa statistic was 0.9 for questions about which twin sucked their thumb, fingers, or a pacifier more (95% confidence interval [CI] = 0.8-1.0) and which twin put things in their mouth more often as an infant, toddler, and young child (95% CI = 0.8-1.0), but slightly lower for exposures occurring later in childhood, (for all other relative exposures: k = 0.7, 95% CI = 0.6-0.8; supplemental Table 1).

Table 2 shows the risk of YAHL in relation to absolute (dichotomous, yes/no) exposures occurring at least 5 years before

Table 1. Demographic distribution of double-respondent (both members) and single-respondent (1 member) twin pairs discordant for young adult Hodgkin lymphoma who completed and returned questionnaires

	Double respondent		Single respondent		Total pairs	
Characteristic	**No.**	**%**	**No.**	**%**	**No.**	**%**
Total twin pairs	93	100	95	100	188	100
Sex						
Male-male	28	30	27	28	55	29
Female-female	51	55	38	40	89	47
Male-female/female-male	14	15	30	32	44	23
Zygosity*						
MZ	47	51	38	40	85	46
DZ	46	49	54	57	100	53
Unknown zygosity	0	0	3	3	3	2
Age at diagnosis, y						
Younger than 21	20	22	20	21	40	21
21-30	46	49	44	46	90	48
31-40	19	20	20	21	39	21
41-50	8	9	11	12	19	10
Histology†						
Nodular sclerosis	53	69	33	40	86	54
Mixed cellularity	13	17	14	17	27	17
Lymphocyte predominant	3	4	5	6	8	4
Not otherwise specified	8	10	30	36	38	24
Other	0	0	1	1	1	1

*MZ indicates monozygotic; and DZ, dizygotic.

†Histology data were unavailable for 16 double and 12 single respondents.

diagnosis in the case. Odds ratios for all exposures were similar regardless of whether single respondents were included. A history of appendectomy was significantly linked to a 3.0- to 3.6-fold increase in risk ($P = .002$ for total twin pairs; $P = .01$ for double-respondent twin pairs). Odds ratios for tonsillectomy and smoking were increased, but not significantly ($P = .11$ and $P = .17$, respectively). A history of eczema was associated with an almost 3-fold increased risk of borderline significance ($P = .048$). The number of double-respondent twin pairs discordant for tonsillectomy, smoking, and eczema was less than 18, thus data are shown only for total pairs. Prior infectious mononucleosis was not associated with risk (Table 2).

Table 3 shows the effect of behaviors likely to increase exposure to infectious agents in childhood, especially those transmitted through the oral-oral or fecal-oral route. Relatively more frequent oral behaviors (sucking fingers and/or placing things in the mouth as an infant or preschooler) were linked to a 40% to 80% reduced risk (*P* values .11-.003; Table 3). The ORs were similar whether or not single respondents were included. Behaviors in older childhood (5-10 years old) that permitted exposure to oral secretions were not

Table 2. Risk factors for young adult Hodgkin lymphoma diagnosed between 13 and 50 years of age in disease-discordant twin pairs

Exposure/twin set by response	Ratio of exposure, discordant pairs*	Odds ratio†	95% CI‡	*P*§
Appendectomy				
Total‖	33/11	3.0	1.5-5.9	.002
Double respondent¶	18/5	3.6	1.3-9.7	.01
Tonsillectomy#**				
Total‖	16/8	2.0	0.8-4.7	.11
Double respondent¶	10/3			
Smoked cigarettes, ever/never#**				
Total‖	21/13	1.6	0.8-3.2	.17
Double respondent¶	10/7			
Eczema#				
Total‖	14/5	2.8	1.0-7.8	.048
Double respondent¶	8/2			
Infectious mononucleosis				
Total‖	22/19	1.2	0.6-2.1	.64
Double respondent¶	10/9	1.1	0.4-2.7	.82

*Total number of twin pairs in which case-twin was exposed and the unaffected cotwin was unexposed/total number of twin pairs in which unaffected cotwin was exposed and the case-twin was unexposed.

†Odds ratio estimated using conditional logistic regression using SAS Version 9.1.

‡Confidence interval estimated using conditional logistic regression.

§*P* value estimated from the Wald test.

‖One hundred eighty-eight total pairs = double- and single-respondent twin pairs. Single-respondent pairs are those in whom only 1 member of the pair sent a questionnaire but answered questions about their twin's exposures (eg, proxy report for the nonresponding twin).

¶Ninety-three double-respondent pairs. Double respondent pair = both members of the twin pair sent back a questionnaire; answers are based on self-report.

#Results are not presented for double-responding twin pairs separately since the number of exposure-discordant pairs was less than 18.

**Lower 95% confidence interval for kappa 0.70 or higher.

Table 3. Relative differences in childhood behaviors associated with transmission of infection and the risk of young adult Hodgkin lymphoma diagnosed between 13 and 50 years of age in disease-discordant twin pairs

Relative differences in childhood behaviors: which twin . . . ?	Ratio of exposure, discordant pairs*	Odds ratio†	95% CI‡	*P*§
Sucked pacifier/thumb/fingers more as an infant/young child?‖				
Total¶	18/34	0.5	0.3-0.9	.03
Double respondent#	11/20	0.6	0.3-1.1	.11
Put more things in mouth as an infant/young child?‖				
Total¶	10/30	0.3	0.2-0.7	.003
Double respondent#	6/17	0.4	0.1-0.9	.03
Sucked more and put more things in mouth as infant/child?				
Total¶	4/20	0.2	0.1-0.6	.003
Double respondent#	4/13	0.3	0.1-0.9	.04
Bit their nails more?				
Total¶	33/42	0.8	0.5-1.2	.30
Double respondent#	16/21	0.8	0.4-1.5	.41
Kissed people more as children?				
Total¶	22/32	0.7	0.4-1.2	.18
Double respondent#	11/13	0.8	0.4-1.9	.68
Had more contact with family pets?				
Total¶	25/22	1.1	0.6-2.0	.66
Double respondent#	13/8	1.6	0.7-3.9	.28

*Total number of twin pairs in which case-twin was exposed and the unaffected cotwin was unexposed/total number of twin pairs in which unaffected cotwin was exposed and the case-twin was unexposed.

†Odds ratio estimated using conditional logistic regression using SAS Version 9.1.

‡Confidence interval estimated using conditional logistic regression.

§*P* value estimated from the Wald test.

‖Lower 95% confidence interval for kappa 0.70 or higher.

¶Eighty-eight total respondent pairs = double- and single-respondent twin pairs. Single-respondent pairs are those in whom only 1 member of the pair sent a questionnaire but answered questions about their twin's exposures (eg, proxy report for the nonresponding twin).

#Ninety-three double-respondent pairs. Double-respondent pair = both members of the twin pair sent back a questionnaire; answers are based on self-report.

significantly associated with YAHL risk, although most of the ORs were less than 1, suggesting protection.

Relatively more oral behaviors appeared to be similarly protective for both nodular sclerosis and other histologic subtypes (data not shown). The effect of appendectomy was stronger for the nodular sclerosis subtype compared with all other YAHL subtypes (OR for nodular sclerosis = 5.0, $P = .01$; OR for all other histologic subtypes = 2.2, $P = .056$, based on 18 and 29 exposure-discordant pairs, respectively). Infectious mononucleosis was not associated with either type, however the OR for the nodular sclerosis subtype was less than 1.0 (OR = 0.6, $P = .26$), but elevated for other (mostly mixed cellularity) subtypes (OR = 2.1, $P = .10$).

A multivariable regression model that included all exposures significantly linked to YAHL showed positive associations with appendectomy ($P = .001$), eczema ($P = .025$), and smoking ($P = .054$) and an inverse association with relatively more early childhood behaviors that increase oral exposures ($P = .004$; Table 4).

Table 4. Results of multivariable analysis of significant exposures for risk of adolescent/young adult Hodgkin lymphoma in disease-discordant twin pairs diagnosed at 50 years or younger

Exposure	OR*	95% CI†	*P*‡
Appendectomy	4.3	1.9-10.0	.001
Eczema	4.2	1.2-14.8	.025
Smoked cigarettes, ever/never	2.2	1.0-5.0	.054
Relatively more behaviors resulting in early oral exposures§	0.1	0.0-0.5	.004

*Odds ratio estimated using multivariable conditional logistic regression adjusting for all variables above in the model simultaneously.

†Confidence interval estimated using conditional logistic regression.

‡*P* value estimated from the Mantel-Haenszel χ^2 test.

§Sucked thumb, finger, or pacifier more and put more things in mouth.

Discussion

YAHL tumors are characterized by exaggerated lymphoid hyperplasia, strong host Th2 cytokine responses, and scattered pathognomonic malignant giant cells[30] (Hodgkin-Reed-Sternberg cells), a pattern unique among cancers and one that led early investigators to suspect an infectious etiology.[31] Extensive studies based on serology or viral load have not yet identified an association between any specific known infectious agent and EBV-negative YAHL.[11-16] Although our study could not rule out a protective advantage after specific childhood exanthemata or other infections, risk was strongly reduced in the member of the twin pair who had more behaviors associated with oral-oral or fecal-oral exposure. Behaviors that increase oral exposures are risk factors for all infections transmitted by the fecal-oral or oral-oral route including but not limited to hepatitis A, *Helicobacter pylori, Salmonella,* and herpes simplex type 1.[32] These oral behaviors could also increase acquisition of nonpathogenic organisms to colonize the gut or increase exposure to components of organisms

Hrncir et al recently examined immune function in germ-free mice fed a sterile diet compared with wild-type mice fed a normal diet, and concluded that the gut microbiota and/or lipopolysaccharide were necessary for production of IL12 required to mount a Th1 immune response to antigenic stimuli.[33] A deficit of early oral exposures in humans has also been shown to alter immune response development and Th1/Th2 cytokine balance in favor of a Th2-skewed response with a corresponding reduced IL12 production and deficient Th1 response.[17,18]

We have previously reported that heritable lower IL12 (Th1) and higher IL6 (Th2) responses are associated with increased susceptibility to YAHL,[22,23] and here we observed that more frequent early oral exposure results in a substantially decreased

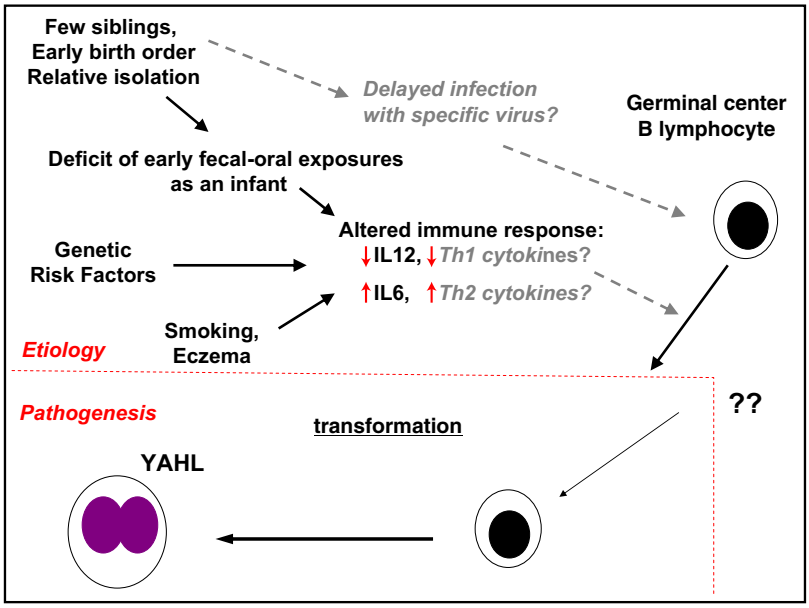

Figure 1. Etiologic model for adolescent/young adult Hodgkin lymphoma consistent with the hygiene hypothesis mechanism. IL12 indicates interleukin 12; IL6, interleukin 6; Th2, T-helper type 2; Th1, T-helper type 1; and YAHL, adolescent/young adult Hodgkin lymphoma.

risk. Together these results are quite consistent with the exposure model known as the "hygiene hypothesis" (Figure 1).

We found prior (at least 5 years before diagnosis in the case) appendectomy and to a lesser degree, tonsillectomy, to be associated with an increased YAHL risk. It has been suggested that similar previous observations were the result of confounding by socioeconomic status.[34] Because twins are matched on parental socioeconomic status, the high risk we observed cannot be so explained. The indications for either surgery may simply represent an early premalignant lymphoid hyperplasia, although the surgery occurred 5 or more years before diagnosis. Appendicitis can result from enteric infections by *Yersinia* or *Clostridium* species, both more commonly found in feces of children who have grown up in "clean"[35,36] environments. Alternatively, appendicitis may result from an abnormal microbial response after a deficit of early fecal-oral exposures.[37] Because both tonsillectomy and appendectomy are performed because of a suspected inflammatory response in lymphoid tissue, it is also possible that the association with YAHL reflects a tendency toward a reactive and inflammatory lymphoid hyperplasia in response to an endogenous or exogenous antigen.

We also found a positive link between YAHL and eczema and smoking, both exposures known to be associated with an increase in Th-2 cytokines.[38,39] Previous reports of an association between HL and smoking have usually been strongest in EBV-positive HL[40-43]; we did not have enough information on EBV tumor status to evaluate this relationship. Although adult-onset eczema is a common clinical manifestation of HL,[44] the majority of twins with a history of eczema reported an onset in early childhood, and the remainder at least 5 years before diagnosis, so reverse causality is not a likely explanation of the association.

Our findings are consistent with the possibility that susceptibility to YAHL is increased by an aberration in the cytokine response pattern produced by a reduction in the cumulative microbial exposures of childhood. Whatever the mechanism, our findings are unlikely to represent artifact. Other study designs would not provide the mutually validated comparison data of early life and childhood exposure information collected here, such as thumb sucking, which would not be captured by medical record. Although medical records were not available to document differences in clinically reported events such as tonsillectomy and appendectomy, twins are accustomed to being compared throughout their lives and are repeatedly reminded (by each other, their siblings, and their parents) of their differences, especially those in childhood.

Agreement between answers given by the members of each pair was high, even for those behaviors that were not discrete and eventful, and random misclassification is unlikely (and if present, would lead to a dampening of the risk estimate). We previously tested the recollections of healthy twins against their mothers' recollections of selected childhood conditions or events, including tonsillectomy, appendectomy, and infectious mononucleosis, and have found strong and significant correlations between twin subject and maternal responses (W.C., A. Hwang, A.S.H., and T.M.M., unpublished data, July 2007). Other studies have reported strong agreement between self-report of appendectomy and tonsillectomy and medical record documentation.[45] Furthermore, the kappa for relative differences in early oral behavior was 90%, supporting the reliability of the comparisons.

Although we made many comparisons, the exposures of interest were chosen because of a priori hypotheses, and several were logically related to a single hypothetical pathway. Some of the exposures were significantly correlated in control twins (tonsillectomy and appendectomy, $r^2 = 0.20$, $P = .006$; and the 2 early oral behaviors, $r^2 = 0.45$, $P \leq .001$), making adjustment for multiple comparisons inappropriate.

Recall bias is often a problem in case-control studies; cases may be more likely than controls to report a positive history of exposures known to be linked to risk of their disease. In this study, however, the exposures of interest are not generally known to be associated with YAHL, and if they were, the default assumption would be that they would be directly, not inversely, related to disease. Moreover, in response to an open-ended question about the cause of the YAHL, no causes related to the exposures of interest were mentioned by either case or control twins.

Another potential problem with self-reported information from case-control studies is that memory of past events, especially those occurring in childhood, may be poor. A unique advantage of twin studies is that they permit joint assessment of recall. When the kappa scores are high for relative comparisons (as they are here), the implication is that the twins within a pair generally agreed about the direction of the exposure difference (case more or

unaffected twin more), and that they more easily recalled strong differences than absent or subtle differences. Thus, the relative differences in oral behaviors reported by the twins most likely represent mutually agreed upon noticeable differences. Because these oral behaviors (eg, thumb sucking) are surrogates for exposure to infectious agents transmitted by the oral-oral or fecal-oral route, there is no meaningful way to quantify the absolute magnitude of the difference necessary to prevent disease.

The lack of EBV tumor status for the majority of subjects is a limitation. We were able to obtain tissue for only 19 case-twins because tumor tissue was not available from patients who were diagnosed, on average, 25 years before the study. Two of the 14 NS and 2 of the remaining 5 cases were positive for EBV, thus histologic subtype was highly correlated with EBV tumor status.[4] Therefore we examined associations within strata of histologic subtype of the case-twin and found that histology-specific results were generally similar to those previously reported according to EBV status.[10,28]

The inverse association between risk and early oral exposure and the positive associations with smoking and eczema suggest that alterations in the Th1/Th2 cytokine balance (in favor of Th2) may play a role in the etiology of YAHL. The association with appendectomy warrants further examination. Future studies will resolve the question of whether a specific infectious agent is a necessary risk factor for EBV-negative YAHL, and also how the observed risk factors interact with EBV to cause EBV-positive YAHL.

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Authorship

Contribution: W.C. designed the questionnaires and the research study and wrote the paper; A.S.H. developed and maintained the databases and twin registry and conducted statistical analyses; P.Z. and M.T.S. conducted statistical analyses; D.M.D. developed and maintained the databases and twin registry; B.N.N. reviewed histopathology slides; L.M.W. conducted analysis for EBV typing of tumors; and T.M.M. developed and maintained the twin registry and designed the questionnaires and the research study.

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