Published in final edited form as:
Hum Immunol. 2010 July ; 71(7): 727–730. doi:10.1016/j.humimm.2010.04.004.

Polymorphisms in innate immunity genes and risk of childhood leukemia

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Abstract

Objectives—To evaluate whether candidate genes in innate immunity are associated with childhood leukemia, we conducted an association study with the 1,536 SNPs in 203 genes related to innate immunity.

Methods—Incident childhood leukemia cases (n=136) aged from 0 to 18 were recruited from three teaching hospitals in Seoul between 2003 and 2006. Non-cancer controls (n=140) were frequency-matched to cases by age and gender. The information on the characteristics of children and their parents were collected by trained interviewers using structured questionnaire. Candidate genes were selected based on SNP databases (CGAP and SNP500 database), and genotype assay was performed using GoldenGate (Illumina) oligonucleotide pool assay (OPA). False discovery rate (FDR), permutation test, and haplotype analyses were used to identify the SNP with significant association with childhood leukemia. Childhood leukemia risk was estimated as ORs and 95% CIs adjusted for age, gender and birth weight.

Results—Fourteen SNPs in 13 genes (*LMAN1, TLR4, STAT4, CCR9, MBP, ZP1, C8B, XDH, C7, C1QG, FGF2, LOC390183,* and *STAT6*) were significantly associated with childhood leukemia risk (FDR *p*-values <0.05). In particular, *LMAN1* rs1127220, *TLR4* rs11536897, *STAT4* rs13020076, *CCR9* rs1471962, and *MBP* rs10514234 were significant in 5,000 permutation tests (Permutation *p*-value <0.05). The most significant association with childhood leukemia risk was for the *LMAN1* rs1127220 that is in the protein-coding region, this finding was also supported by haplotype analysis.

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Conclusions—A number of innate immunity related genes are associated with childhood leukemia, suggesting possible links between the innate immunity system and development of the childhood leukemia.

Keywords

Childhood Leukemia; Innate Immunity; Single Nucleotide Polymorphism

Introduction

Childhood leukemia is the most common pediatric cancer. Despite great improvements in survival, childhood leukemia still remains a major cause of morbidity and mortality among children (1). The etiology of childhood leukemia is mostly unknown, although ionizing radiation, chemotherapy and chromosomal anomalies are the alleged risk factors (2).

The innate immune system, which includes leukocytes, epithelial barriers, circulating effector proteins (complement, collectins, pentraxins), and cytokines (e.g. TNF, IL-1, chemokines, IL-2, type I IFNs, and IFN-γ), plays a primary role in the defense mechanisms against infections and cancers (3). Little is known, however, regarding the role of genes involved in innate immunity such as mannose-binding lectin (MBL) (4), NOD2/CARD15 (5–7), and TLR4 (7) in the development of cancer or its response to treatment.

Leukemia is a heterogeneous disease characterized by the dysregulated proliferation of blood precursor cells with myeloid or lymphoid origin. Functional polymorphisms of selected SNPs in the *CYP*, *GST*, *NAT*, *MTHFR*, *NQO1*, *XRCC1*, *MDR1*, and *CCND1* have been suggested to be associated with the risk of developing leukemia (8,9). Sequence variants in the childhood leukemia development haven't been reported for a high-throughput genotyping of SNP.

Dysfunction of the innate immunity system could be related to childhood leukemogenesis (10). It has been hypothesized that innate immunity related genes may play an important role in the carcinogenesis of childhood leukemia. Thus, we hypothesized that genetic variants in genes related to innate immunity may be associate with childhood leukemia risk, and evaluated the association between 1,536 SNPs in 203 innate immunity genes and childhood leukemia risk in Korea through high-throughput genotyping with the GoldenGate assay.

Materials and Methods

Subjects

Eligible cases were histologically-confirmed incident childhood leukemias diagnosed at three teaching hospitals located in Seoul, Korea, between May 2003 and August 2006. More than 70% of childhood leukemia patients in Korea are diagnosed at one of the three hospitals participated in this study. Eligible non-cancer controls were patients without a medical history of childhood cancers recruited from the department of pediatrics, pediatric surgery, pediatric orthopedic surgery, pediatric urology, and pediatric otorhinolaryngology from the same hospitals, and frequency-matched by 5-year interval age (<1, 1–4, 5–9, 10–14, and ≥15) and gender. The clinical diagnoses of the 140 control patients included bone fracture (9.3%), hernia (8.6%), acute gastroenteritis (7.9%), LCP (Legg-Calve-Perthes disease) (7.1%), respiratory diseases such as bronchitis and pneumonia (5.7%), and others. Participation rate was approximately 80% for eligible cases and 70% for controls. Informed consent was obtained from all study subjects. The study was approved by the Institutional Review Board for human research of Seoul National University Hospital (IRB No. H-0407-128-001).

Information on the characteristics of the child (e.g., birth weight (<3.25 kg, 3.25–3.70, and >3.70), breast feeding (0 month, 0–6, 6–12, and >12 etc.)), parental characteristics including smoking habit (never, ex-smoker, and current smoker) and alcohol consumption, and maternal medication during pregnancy were collected by trained interviewers using structured questionnaire. The detailed information on subject selection and data collection was described elsewhere (11).

Gene and SNP selection and genotyping

Genomic DNA for genotyping was extracted from peripheral blood using the Gentra Puregene Blood Kit (gentra, USA).

Illumina customer oligonucleotide pool assay (OPA) chip composed of genes involved in innate immunity developed at the Core Genotyping Facility (CGF) of the Division of Cancer Epidemiology and Genetics, National Cancer Institute. Many cancer study group including our study group joined to analysis the innate immunity OPA chip.

Illumina GoldenGate™ OPA Panel was designed using 203 candidate genes for innate immunity according to some criterias: Initial candidate genes and SNPs were proposed by Stephen Chanock, Gilles Thomas in NCI/NIH.on the basis of relevant literature, SNP database (CGAP and SNP500 database) and were selected using dbSNP IDs (http://www.ncbi.nlm.nih.gov/SNP) to Illumina's Custom OPA Assay Design Tool (ADT) to obtain design scores for all SNPs in dbSNP in the region 20kb 5' of the start of transcription (exon 1) and 10kb 3' of the end of the last exon (N) of each candidate gene. In the cases where there were multiple transcripts available for genes, the primary transcript was submitted. The Tag SNPs were chosen from the designable set of SNPs that were genotyped as part of the international HapMap Project. Finally, 1536 SNPs with a minor allele frequency (MAF) > 0.05 in control, design score = 1.1, $r^2 > 0.8$ selected from the HapMap Caucasian (CEU). Among them, about half (55%) were located in intron, 22% in promoter (flanking region, utr), 15% in 3' of STP, and 9% in exon. For SNPs located in exon, 73 % were synonymous and 27 % were non-synonymous change. Genotyping was performed using GoldenGate™ assay (Illumina®, San Diego, CA) at the Core Genotyping Facility (CGF) of the Division of Cancer Epidemiology and Genetics, National Cancer Institute.

The 322 SNPs deemed unusable due to failure of genotyping or monomorphism (100 SNPs), low yield (19 SNPs were genotyped only for 11 subjects), HWE $p < 0.0001$ (9 SNPs), MAF < 0.05 (194 SNPs) were excluded from the analysis. And, 30 cases (22 %) and 17 controls (12 %) with no available DNA at the time of analyses or poor performance on the assay were additionally excluded. In total, 106 cases and 123 controls containing data for 1214 SNPs in 190 genes were used in the analyses. Genotype completion rates and concordance rates for all SNPs exceeded 93%. The median (range) minor allele frequency (MAF) among controls was 0.73 (0.4–0.95).

Quality control (QC) was performed using 82 duplicate SNPs identified CGF. The genotyping results of 82 SNPs for 272 subjects were 99% concordant (data not shown).

Statistical Analysis

Fisher's exact test for genotype distribution was conducted to evaluate deviation from the Hardy-Weinberg equilibrium in the control group complying R statistics package (genetics) and this result was reviewed using SAS software.

The association between individual SNPs and leukemia risk was estimated based on the global *p*-value computed using the likelihood ratio test (LRT) with 1 degree of freedom, comparing the (full) model with each SNP to the (null) model excluding the SNP. The 1 df-LRT assumes

a dose response effect with regard to the number of variant alleles, coding genotypes as 0, 1, and 2 according to the number of variant alleles.

Childhood leukemia risk was estimated as odds ratios (ORs) and 95% confidence intervals (CIs) using conditional logistic regression analysis accounting for the matching variables age (continuous) and sex, while controlling for birth weight (divided into two categories with > 3.70 kg and ≤ 3.70 kg). The homozygote of the most common allele in the subjects was used as the referent group. If any genotype frequency in either cases or controls was less than 5, exact conditional logistic regression analysis was performed to calculate the ORs and CIs (12).

The Benjamini-Hochberg false discovery rate (FDR) method (13) and permutation test with 5,000 times were conducted for multiple comparison correction to reduce spurious association by an uncorrected threshold.

Finally, haplotype analyses were assessed for the genes of FDR-adjusted global $p < 0.05$ using unconditional logistic regression with the program HaploStats (http://mayoresearch.mayo.edu/mayo/research/schaid_lab/software.cfm), which infers haplotypes based on Expectation-Maximization (EM) algorithm. Exact conditional logistic regression was also employed to estimate ORs and 95% CIs when the haplotype frequencies are low (<5).

All statistical procedures were conducted using “genetics”, “dgc.genetics” and “elrm” packages in the R project (http://www.r-project.org), the SAS® software version 9.1 (Cary, North Carolina), and Haploview version 3.11. (14).

Results

The distributions of the selected characteristics are summarized in Table 1. We did not find any significant difference between cases and control group except for birth weight: higher birth weight was associated with increased risk of childhood leukemia (p trend = 0.02).

Fourteen SNPs in 13 genes (*LMAN1, TLR4, STAT4, CCR9, MBP, ZP1, C8B, XDH, C7, C1QG, FGF2, LOC390183,* and *STAT6*) were significantly associated with childhood leukemia risk after correction for multiple comparisons (FDR p-value < 0.05, Table 2). There was no difference in the frequency of these 14 SNPs in three SNP databases (e.g. NCBI, SNP500, and KoreanHapMap: http://www.hkapmap.org/) (data not shown). The *LMAN1* rs1127220, *TLR4* rs11536897, *STAT4* rs13020076, *CCR9* rs1471962, and *MBP* rs10514234 were associated with childhood leukemia risk after 5,000 permutations (Permutation p-value < 0.05, Table 2).

Table 3 shows the result of haplotype association, presenting only the LD blocks with global p value (LRT) less than 0.01. We observed significant associations between haplotypes and childhood leukemia risk in the LD block including the SNPs (*LMAN1* rs1127220, *TLR4* rs11536897, *MBP* rs10514234 & rs3794845, *ZP1* rs530880, *XDH* rs207444, *C8B* rs1013579, *FGF2* rs308447, and *LOC390183* rs2511990) (Table 3).

When restricted to acute lymphoblastic leukemia (ALL) cases, 4 SNPs in 3 genes were significantly associated with ALL risk after correcting for multiple comparison (*MBP* rs3794845: FDR p-value = 0.024, *ZP1* rs530880: FDR p-value = 0.030, *Ly96* rs7838017: FDR p-value = 0.030, *MBP* rs10514234: FDR p-value = 0.036) (data not shown).

Discussion

To evaluate whether candidate genes in innate immunity are related to childhood leukemia, we conducted an association study with 1,536 SNPs in 203 genes related to innate immunity. Fourteen SNPs in 13 genes were significantly associated with childhood leukemia risk after correcting for multiple comparisons with FDR *p*-value less than 0.05 (*LMAN1, TLR4, STAT4, STAT6, CCR9, MBP, ZP1, C8B, XDH, C7, C1QG, FGF2 and LOC390183*) and after 5,000 permutations, 5 SNPs in 5 genes were significantly associated with childhood leukemia risk (*LMAN1* rs1127220, *TLR4* rs11536897, *STAT4* rs13020076, *CCR9* rs1471962, and *MBP* rs10514234).

There are several biological mechanisms to explain the association between the five genes associated with childhood leukemia suggested from our results.

The most significant association with childhood leukemia risk was identified by *LMAN1* rs1127220, located in the protein-coding region. The *LMAN1* (lectin, mannose-binding, 1, *MBL*) gene plays a critical role in the immune response. Zhang et al reported that mutations in LMAN1 result in combined deficiency of factor V and factor VIII an autosomal recessive bleeding disorder characterized by coordinate reduction of both clotting proteins (15). However, the association with cancer haven't been reported.

Toll like receptor (TLR) activation leads to the production of NF-κB, which is a transcription factor that mediates antiapoptotic signals in several cancer cell lines (16). Human TLR4 contains many rare missense mutations, and it is possible that these unusual mutations influence disease susceptibility. On the other hand, chronic lymphocytic leukemia (CLL) and some types of lymphoma cells strongly express TLR7 and TLR9 on their surface (17).

Constitutive activation of STATs has been found in a wide variety of human tumors (18). These proteins have been described as playing a role in hematologic cancers including leukemias, both acute and chronic forms (19).

The *CCR9* (chemokine (C-C motif) receptor 9) was frequently expressed on AML blasts (M4 and M5), and original expression of *CCR9* on the leukemic cells contributed to the relapse location in the gut of T-ALL patient (20).

Two out of 14 SNPs selected in our study were located on intron 5 of the *MBP* (myelin basic protein) gene. The *MBP* has an important role to play in apoptosis which has been critically reviewed as a process in cell loss from normal tissue and tumours for reasons; The p36 MBP kinase cascade might be a common cascade of the diverse signaling pathways leading to apoptosis (21). In addition, *MBP* was used as a substrate during cadmium induced apoptosis in human leukemia HL-60 cells (21). The *MBP* gene are strongly modulated by TNF-alpha through the activation of NF-kB pathway, and TNF-RI expression on AML blasts revealed to be a strong positive predictor for the percentage expression of CD40.

When data were analyzed among only ALL cases, we observed that rs7838017 polymorphism on intron 3 of *Ly96* gene activating the NF-κB which constitutively activated in some cancer and leukemia cells significantly decreased the risk of childhood ALL.

Although there are some limitations including small sample size, a lack of functional information and established epidemiological studies supporting the result for selected SNPs, this study identified innate immunity genes that may be associate with childhood leukemia. Further large study is needed to confirm our findings.

Acknowledgments

This study was funded by Korea Electrotechnology Research Institute, Ministry of Knowledge Economy (MKE) and by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, R.O.K. (AO30001: 03-PJ10-PG13-GD01-0002).

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Table 1

Selected characteristics of the childhood leukemia cases (n=136) and controls (n=140).

Variables	Cases (n=136)	Controls (n=140)	OR (95% CI)*/*P*
Sex			
Male	89 (65.4)	90 (64.3)	
Female	47 (34.6)	50 (35.7)	0.8
Age at diagnosis (years)			
< 1	0 (0.0)	2 (1.4)	
1–4	46 (33.8)	45 (32.1)	
5–9	52 (38.2)	50 (36.0)	
10–14	31 (22.8)	34 (24.3)	
≥ 15	7 (5.2)	9 (6.4)	
Mean± S.D.	7.00 ± 4.21	6.95 ± 4.47	0.9
Birth weight (kg)			
< 3.25	58 (42.6)	77 (55.4)	Ref.
3.25–3.70	45 (33.1)	40 (28.8)	1.5 (0.9–2.6)
> 3.70	33 (24.3)	22 (15.8)	2.0 (1.1–3.9)
P_{trend}			0.02
Mean ± S.D.	3.33 ± 0.52	3.18 ± 0.54	0.02
Breast feeding (≥ 1 month)			
No	56 (41.2)	50 (36.0)	Ref.
Yes	80 (58.8)	89 (64.0)	0.8 (0.5–1.3)
Education levels for both father and mother			
High school or less	49 (36.0)	41 (29.3)	Ref.
University graduate	35 (25.7)	38 (27.1)	0.7 (0.4–1.4)
Graduate school or more	52 (38.3)	61 (43.6)	0.7 (0.4–1.2)
P_{trend}			0.24
Family history of cancer			
No	68 (50.8)	79 (58.5)	Ref.
Yes	66 (49.2)	56 (41.5)	1.4 (0.8–2.2)

*Adjusted for age, sex and birth weight

Table 2

Polymorphisms associated with *P* values <0.001 in the Innate Immunity-Illumina OPA and risk of childhood leukemia among 106 leukemia cases and 123 controls in the Korean.

db SNP ID (allele)*	Gene	SNP region	Frequency		*P*†	*BH*-FDR *P*	*Permutated P*‡	OR (95% CI)§
			Cases (%)	Controls (%)				
rs1127220 (G)	LMAN1	Ex2-19A>G	0.00	0.07	0.000005	0.005	0.01	0.02 (0.0–0.2)‖
rs11536897 (A)	TLR4	3083bp 3' of STP A>G	0.00	0.07	0.000010	0.006	0.01	0.05 (0.0–0.3)‖
rs13020076 (C)	STAT4	7522bp 3' of STP T>C	0.00	0.08	0.000015	0.006	0.02	0.06 (0.0–0.3)‖
rs1471962 (C)	CCR9	5796bp 3' of STP C>G	0.00	0.07	0.000024	0.007	0.03	0.07 (0.0–0.3)‖
rs10514234 (G)	MBP	IVS5+6056A>G	0.01	0.10	0.000028	0.007	0.04	0.08 (0.0–0.4)‖
rs3794845 (G)	MBP	IVS5+7288 C>G	0.05	0.18	0.000068	0.010	0.08	0.25 (0.1–0.5)
rs530880 (T)	ZP1	−2893T>C	0.01	0.09	0.000052	0.010	0.06	0.12 (0.0–0.4)‖
rs1013579 (G)	C8B	Ex3-43 G>A	0.00	0.05	0.000066	0.010	0.08	0.07 (0.0–0.4)‖
rs207444 (T)	XDH	IVS33+398T>C	0.00	0.06	0.000078	0.011	0.09	0.05 (0.0–0.3)‖
rs7732104 (A)	C7	1760bp 3' of STP A>G	0.03	0.12	0.000096	0.012	0.11	0.20 (0.0–0.6)‖
rs17433222 (A)	C1QG	4369bp 3' of STP A>G	0.13	0.24	0.000155	0.017	0.18	0.33 (0.1–0.6)‖
rs308447 (T)	FGF2	−9384 T>C	0.06	0.16	0.000245	0.025	0.26	0.25 (0.1–0.6)‖
rs2511990 (T)	LOC390183	−18210 C>T	0.10	0.22	0.000289	0.027	0.30	0.34 (0.1–0.7)‖
rs703817 (A)	STAT6	Ex22-638 G>A	0.18	0.34	0.000371	0.032	0.35	0.43 (0.3–0.7)

Abbreviations: *LMAN1*=lectin, mannose-binding, 1; *TLR4*=toll-like receptor 4; *STAT4*=signal transducer and activator of transcription 4; *CCR9*=chemokine (C-C motif) receptor 9; *MBP*=myelin basic protein; *ZP1*=zona pellucida glycoprotein 1; *C8B*=complement component 8, beta polypeptide; *XDH*=xanthine dehydrogenase; *C7*=complement component 7; *C1QG*=complement component 1, q subcomponent, C chain; *FGF2*=fibroblast growth factor 2 (basic); *LOC390183*=similar to 40S ribosomal protein S4, X isoform; *STAT6*=signal transducer and activator of transcription 6, interleukin-4 induced

*Allele names are based on SNP500Cancer database, For additional information on the nomenclature for description of the sequence variants, please see the SNP500Cancer database.

†Global p-value for LRT with 1 df based on trend test for individual,

‡Permutated *p* values calculated from 5,000 permutations,

§Estimated by conditional logistic regression models accounting for matching variables of age and sex and controlling for birthweight,

‖Estimated by exact logistic regression models (3,000,000–5,000,000 iterations), where a cell count was <5

Table 3

Associations between risk of childhood leukemia and frequencies of inferred haplotypes on the basis of the observed genotypes in 106 leukemia cases and 123 controls in the Korean.

Gene	Hploatype block *	SNP	Haplotypes	Haplotype frequencies		OR (95% CI)	*P*	Global *P*
				Cases N (%)	Controls N(%)			
LMAN1	Block2	rs1127220	TC	110 (89.8)	103 (97.1)	ref.		0.000016
		rs2282583	TT	4 (3.0)	3 (2.9)	0.78 (0.30–2.04)	0.61	
			CT†	8 (6.8)	0 (0.0)	0.04 (0.00–0.26)	0.000017	
TLR4	Block1	rs10759930	TTGG	71 (58.6)	67 (64.6)	ref.		0.000044
		rs10759932	CCTG	29 (24.1)	27 (25.8)	0.90 (0.57–1.42)	0.65	
		rs2149356	CTTG	11 (9.0)	10 (9.5)	0.81 (0.41–1.59)	0.54	
		rs11536897	CCTA†	5 (4.1)	0 (0.0)	0.05 (0.00–0.32)	0.00015	
			TTGA†	2 (2.0)	0 (0.0)	0.29 (0.00–3.97)	0.17	
MBP	Block5	rs3794845	GAGT	81 (65.5)	77 (74.3)	ref.		0.000063
		rs470895	GATT	21 (16.7)	22 (20.8)	1.00 (0.58–1.72)	0.99	
		rs2051344	CGGC	11 (8.7)	2 (1.8)	0.12 (0.03–0.46)	0.0022	
		rs10514234	CAGT	8 (6.1)	3 (2.5)	0.35 (0.10–1.26)	0.11	
	Block7	rs921336	TTCG	50 (40.9)	31 (29.0)	ref.		0.000068
		rs8096433	GACA	24 (19.2)	27 (25.9)	1.60 (0.93–2.76)	0.09	
		rs17660901	GTGG	21 (17.3)	26 (24.3)	1.61 (0.92–2.81)	0.1	
		rs2282574	GTCG	10 (8.2)	8 (7.5)	1.10 (0.49–2.48)	0.81	
			GACG	8 (6.5)	13 (12.7)	2.92 (1.28–6.63)	0.011	
			GAGA†	7 (5.3)	0 (0.0)	0.10 (0.00–0.68)	0.011	
ZP1	Block 1	rs679682	TC	88 (72.2)	75 (73.8)	ref.		0.00019
		rs530880	CC	23 (19.0)	25 (25.2)	1.27 (0.76–2.12)	0.36	
			CT	11 (8.8)	1 (1.0)	0.08 (0.04–0.36)	0.0013	
XDH	Block1	rs10490361	CAG	58 (47.2)	54 (50.6)	ref.		0.00063
		rs207432	GCG	36 (29.5)	32 (30.3)	0.81 (0.50–1.29)	0.37	

Gene	Hploatype block *	SNP	Haplotypes	Haplotype frequencies		OR (95% CI)	*P*	Global *P*
				Cases N (%)	Controls N(%)			
		rs207444	GAG	20 (16.0)	20 (18.5)	0.95 (0.54–1.65)	0.84	
			GCA†	7 (5.7)	0 (0.0)	0.06 (0.00–0.37)	0.00045	
			CCG	2 (1.6)	1 (0.6)	0.35 (0.03–3.63)	0.37	
C8B	Block3	rs647571	CT	68 (55.4)	57 (54.3)	ref.		0.00079
		rs1013579	TT	48 (39.6)	48 (45.7)	1.18 (0.80–1.73)	0.41	
			TC†	4 (3.4)	0 (0.0)	0.10 (0.00–0.65)	0.0097	
			CC†	2 (1.6)	0 (0.0)	0.68 (0.00–26.45)	0.4	
FGF2	Block1	rs308447	CA	95 (82.3)	93 (91.6)	ref.		0.0012
		rs308428	TG	15 (13.0)	5 (4.9)	0.29 (0.13–0.64)	0.0025	
			TA	3 (2.9)	1 (0.5)	0.13 (0.01–1.15)	0.068	
			CG	2 (1.8)	3 (3.0)	1.58 (0.42–5.94)	0.5	
LOC390183	Block 1	rs2649663	AGG	79 (65.6)	79 (75.1)	ref.		0.0069
		rs2511990	AAA	18 (14.7)	7 (6.8)	0.39 (0.17–0.87)	0.022	
		rs3758919	GGG	15 (12.0)	15 (14.5)	1.08 (0.59–1.96)	0.81	
			GAA	9 (7.7)	4 (3.7)	0.35 (0.11–1.15)	0.085	

Estimated by conditional logistic regression models accounting for matching variables of age and sex and controlling for birthweight

*Haplotype blocks defined by solid spine of LD in our control population

†ORs, CIs and *p* values are estimated by exact conditional logistic regression