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Novel insight in the association between salmonellosis or campylobacteriosis and chronic illness, and the role of host genetics in susceptibility to these diseases

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SUMMARY

We studied the role of host genetics in the susceptibility to severe *Salmonella* and *Campylobacter* infections and chronic sequelae of these infections. Participants of a previous case-control study were sent a buccal swab kit and a questionnaire about occurrence of chronic sequelae. Single nucleotide polymorphisms (SNPs) in the *TLR4* (rs4986790), *IFNG* (rs2430561 and rs1861493), *STAT1* (rs1914408), *IL1B* (rs16944), *NRAMP* (*SLC11A1* rs2276631), *JUN* (rs11688) and *VDR* (rs10735810) genes were determined. In total, 687 controls, 457 *Campylobacter* cases and 193 *Salmonella* cases participated. None of the SNPs were associated with *Campylobacter* or *Salmonella* infections. None of the participants developed Guillain–Barré, Miller–Fisher or Reiter's syndrome. Reactive arthritis occurred in 5% and 2% of cases and controls, respectively. *Campylobacter* cases more frequently experienced gastroenteritis episodes than controls. *Campylobacter* or *Salmonella* infection in women, use of proton pump inhibitors and an SNP in the *IFNG* gene were independent risk factors for reactive arthritis. Another SNP in the *IFNG* gene and use of proton pump inhibitors were risk factors for recurrent episodes of gastroenteritis. In conclusion, reactive arthritis and recurrent gastroenteritis episodes are common after infection and host genetic factors play a role in susceptibility to these long-term health effects.

INTRODUCTION

Campylobacter and *Salmonella* are the most common causes of bacterial gastroenteritis in The Netherlands with around 100 000 and 50 000 cases per year, respectively [1]. Some of these cases consult a general practitioner and from these cases samples are collected for diagnostics. In 2005, about 6200 *Campylobacter* cases and 2100 *Salmonella* cases were laboratory-confirmed [2], which represent the more severe cases of gastroenteritis caused by these agents.

Campylobacter and *Salmonella* infections occasionally lead to chronic sequelae of infection. The association between *Campylobacter jejuni* infection and the Guillain–Barré and Miller–Fisher syndromes has been well-documented [3, 4]. Other well-known sequelae of both *Campylobacter* and *Salmonella* infections are reactive arthritis and Reiter's syndrome [5, 6]. Other studies suggested that *Campylobacter* and *Salmonella* infections cause chronic gastrointestinal diseases, such as irritable bowel syndrome (IBS),

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Table 1. *Immunomodulatory genes involved in the host's immune response to* Salmonella *that are of interest in the present study*

Gene	Protein	Function
TLR4	Toll-like receptor 4	Part of the host cell receptor complex involved in recognition of bacterial lipopolysaccharides [35]
NRAMP	Natural resistance-associated macrophage protein	Modifies the intraphagosomal milieu in such a way that it inhibits the replication of *Salmonella* in the macrophage [36, 37]
IFNG	Interferon-γ	Cytokine that activates macrophages to eliminate intracellular *Salmonella* [18, 19]
STAT1	Signal transducer and activator of transcription factor 1	IFN-γ associated signal transducer
JUN	c-JUN	Part of Activator Protein-1 (AP-1) which is important for the expression of pro-inflammatory cytokines [38]
VDR	Vitamin D receptor	Vitamin D stimulates the innate immune response, but suppresses the adaptive immune response and is important in immunity to tuberculosis [39]
IL1B	Interleukin 1β	Inhibitor of gastric acid secretion [40]

dyspepsia and inflammatory bowel disease (IBD) [7–9].

Case-control studies on risk factors for campylobacteriosis and salmonellosis have shown that multiple sources, transmission routes and risk factors are associated with illness [10–13]. For instance, our previous Dutch case-control study on campylobacteriosis (Y. Doorduyn, W. E. van den Brandhoff, Y. T. H. P. van Duynhoven, J. A. Wagenaar & W. van Pelt, unpublished data) and salmonellosis [14] revealed that besides exposure to the pathogen, factors that influence the host's gastrointestinal environment such as use of gastric acid inhibitors and antibiotics were associated with the development of both diseases. However, the risk and the severity of *Salmonella* and *Campylobacter* infections may also depend on host susceptibility. In addition, it is still unknown if host susceptibility plays a role in the development of chronic sequelae after a *Campylobacter* or *Salmonella* infection.

To study whether genetic factors influence host susceptibility to *Campylobacter* and *Salmonella* infections and chronic sequelae of these infections, we selected seven candidate genes involved in innate and adaptive immunity to infection. Since the host's defence against *Campylobacter* is still poorly understood, we focused on genes involved in the response to *Salmonella*.

The innate immune response to *Salmonella* is mainly triggered by the recognition of lipopolysaccharide on the outer membrane of the bacteria by the host cell receptor complex [15]. This is followed by an influx of phagocytes that engulf the pathogen and eliminate it. However, *Salmonella* is a facultative intracellular pathogen and is able to defend itself against intracellular killing [16, 17]. In a later stage, the adaptive immune response is triggered, and Th1 cytokine interferon-γ (IFN-γ) is one of the key cytokines involved in elimination of intracellular *Salmonella* [18, 19].

The importance of Th1 immunity in the host's defence against *Salmonella* is highlighted by the fact that deleterious mutations in genes involved in the Th1 pathway are linked to severe human infections due to intracellular pathogens, including *Salmonella*, that are normally only weakly pathogenic [20]. These mutations are rare and therefore unlikely to contribute much to the incidence of *Salmonella* infections in the general population. Therefore, the present study focused on more frequently occurring subtle genetic variations, single nucleotide polymorphisms (SNPs).

Participants of our previous case-control study on risk factors for campylobacteriosis and salmonellosis [14], the CaSa study, were re-contacted to ascertain whether SNPs in several immunomodulatory genes (shown in Table 1) influence the host's susceptibility to *Salmonella* and *Campylobacter* infection. In addition, we evaluated the occurrence of chronic sequelae in this study population. We also studied the influence of the determined SNPs (and previously registered risk factors) on the development of these chronic sequelae.

METHODS

Study design

Subjects were selected from participants of a previous Dutch case-control study [14], the CaSa study. In the

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CaSa study, questionnaires about risk factors were obtained from 1446 laboratory-confirmed cases with campylobacteriosis and 573 laboratory-confirmed cases with salmonellosis for the period April 2002 to April 2003. In addition, 3409 frequency-matched community controls (according to age, sex, degree of urbanization and season) completed the questionnaire. Participants in the CaSa study who did not object to being contacted for future studies, who were born in The Netherlands and whose parents were born in The Netherlands were selected for inclusion in the present study, to avoid population admixture. A total of 3706 participants were selected for the present study: 2114 former controls, 1143 former *Campylobacter* cases and 449 former *Salmonella* cases. In November 2005, these subjects were sent a self-administrable buccal swab kit, a questionnaire about chronic sequelae and an accompanying letter with instructions about the swab kit and information about the study. Informed consent was obtained from each subject in accordance with the guidelines of the medical ethics committee of the University Medical Centre, Utrecht.

SNP selection

To study the role of host-genetic factors in *Salmonella* and *Campylobacter* infection, SNPs in seven candidate genes were studied: *IFNG*, *STAT1*, *IL1B*, *TLR4*, *NRAMP*, *JUN* and *VDR* (see Table 1). SNPs in these genes were selected based on previously published associations with various diseases [21–27], thus increasing the chance of selecting SNPs with functional consequences.

DNA isolation and genotyping

DNA was isolated from buccal swabs using the QIAamp DNA Blood Mini kit (Qiagen NV, Venlo, The Netherlands). Polymorphisms in *IL1B* and *SLC11A1* (the gene encoding NRAMP) were genotyped using pre-designed TaqMan SNP genotyping assays (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). For each sample 2·5 μl TaqMan universal PCR master mix (Applied Biosystems) and 10 ng genomic DNA were used in a total volume of 5 μl. The reaction was run according to the following protocol: 10 min at 95 °C, 40× (15 s, 92 °C; 1 min, 60 °C), 4 °C. Polymorphisms in *IFNG*, *JUN*, *STAT1*, *TLR4* and *VDR* were genotyped using pre-designed or custom TaqMan SNP genotyping assays (Applied Biosystems). For each sample 2·5 μl TaqMan fast universal PCR master mix (Applied Biosystems) and 10 ng genomic DNA were used in a total volume of 5 μl. The reaction was run according to the following protocol: 20 s at 95 °C, 40× (3 s, 95 °C; 3 s, 60 °C), 4 °C. Primer and probe sequences and assay numbers where appropriate are given in Table 2. All genotyping assays were performed on a 7500 Fast Real Time PCR system (Applied Biosystems).

Questionnaire design

To study the occurrence of chronic sequelae in the 3 years after infection, a questionnaire was used. The questionnaire included questions regarding occurrence of gastroenteritis episodes, development of joint or back problems, reactive arthritis, Guillain–Barré, Miller–Fisher or Reiter's syndrome since the *Campylobacter* or *Salmonella* infection in 2002–2003 (for cases), or participation in the CaSa study in 2002–2003 (for controls). Cases and controls were also asked about visits to the general practitioner, hospital admission and use of medication for the above-mentioned conditions and about the presence of other chronic illnesses. We also asked them the native country of their grandparents. Parents were asked to complete questionnaires for their children.

Statistical analysis

In the analysis, only subjects whose grandparents were also born in The Netherlands were included. Genotype distributions of each determined SNP among *Campylobacter* cases, *Salmonella* cases and controls were studied using cross-tabulations, χ^2 tests and 'single' variable logistic regression models (which also included age, sex and degree of urbanization) for significance testing. In the same way, the occurrence of chronic sequelae (Guillain–Barré syndrome, Miller–Fisher syndrome, reactive arthritis or Reiter's syndrome) and other health outcomes after follow-up (occurrence of gastroenteritis episodes, development of joint or back problems, presence of other chronic illnesses) were compared between *Campylobacter* cases, *Salmonella* cases, and controls. To study the influence of the determined SNPs on the development of chronic sequelae, the genotype distributions of each SNP in subjects who developed chronic sequelae were compared with the genotype distributions in subjects who did not develop chronic sequelae using cross-tabulations, χ^2 tests and single variable logistic

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Table 2. *PCR primer and probe sequences, or assay numbers used to determine polymorphisms in* IL1B, SLC11A1 (NRAMP), TLR4, IFNG, JUN STAT1 *and* VDR *genes*

Gene	rs number	SNP name	Assay number	Primers	Probes	Reaction mix and method
IL1B	rs16944	c.-598G>A	C___1839943_10			Universal
SLC11A1 (NRAMP)	rs2276631	c.198G>A	C___1659795_20			Universal
TLR4	rs4986790	Asp299Gly		Fwd-TGACCATTGAAGAATTCCGATTAGCA	VIC-TACCTCGATGATATTATT	Fast
				Rev-ACACTCACCAGGGAAAATGAAGAA	FAM-CCTCGATGGTATTATT	
IFNG	rs2430561	c.115-483A>T		Fwd-ACTGTGCCTTCCTGTAGGGTATT	VIC-CACAAAATCAAATCTCACACAC	Fast
IFNG	rs1861493	c.366+497A>G	C__2683476_10	Rev-GCTGTCATAATAATATTCAGACAT-TCACAATTGAT	FAM-ACAAAATCAAATCACACACAC	Fast
JUN	rs11688	c.750G>A		Fwd-GGTTCCTCATGCGCTTCCT	VIC-TCCGCTCCTGGGACT	Fast
				Rev-CCCCTGTCCCCCATCGA	FAM-ATCCGCTCTTGGGACT	
STAT1	rs1914408	c.2136-318G>A		Fwd-CGGTGCACTACCCTGAGATG	VIC-CCACGAGGCATTG	Fast
				Rev-GGTGCTTTTCTGTCCAGGGTAAG	FAM-CCACAAGGCATTG	
VDR	rs10735810	Thr1Met		Fwd-GGGTCAGGCAGGGAAGTG	VIC-ATTGCCTCCATCCCTGT	Fast
				Rev-TGGCCTGCTTGCTGTTCTT	FAM-TGCCTCCGTCCCTGT	

regression models. If the occurrence of chronic sequelae differed between the type of participant (*Campylobacter* case, *Salmonella* case or control), the type of participant was added to the logistic regression model. In the same way, the effect of factors previously registered in the CaSa study on the occurrence of chronic sequelae were studied. Finally, a multivariate logistic regression model was obtained by forward selection of SNPs and risk factors that showed a P value of $\leqslant 0{\cdot}10$ in the single variable analysis. Factors that multivariably showed a P value of $\leqslant 0{\cdot}05$ were retained in the model. All analyses were performed using SAS statistical software version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Questionnaires and buccal swabs were received from 457 native Dutch *Campylobacter* cases (response 40%), 193 *Salmonella* cases (43%) and 687 controls (32%). The response for both cases and controls was lower among subjects in the 18–29 years age group (response respectively 29%, 27%, and 20%) and higher among subjects who used proton pump inhibitors in the CaSa study (respectively 48%, 57%, and 51%). Questionnaires were completed at a median of 3·2 years (range 2·4–3·8 years) after the CaSa study.

Role of host genetics in *Salmonella* or *Campylobacter* infection

We studied four SNPs in four genes involved in innate immunity (*TLR4*, *NRAMP*, *VDR*, *JUN*), and three SNPs in two genes involved in Th1 immunity (*IFNG*, *STAT1*) to *Salmonella* infection. In addition, we selected a SNP in *IL1B* as a candidate for susceptibility to both *Campylobacter* and *Salmonella* infection, because of its modifying effect on gastric acid production. No association was found between the determined SNPs and *Campylobacter* or *Salmonella* infection, although for the selected SNP in the *STAT1* gene a marginally significant difference in genotype distribution was found between *Salmonella* cases and controls (Table 3).

Chronic sequelae after a *Campylobacter* or *Salmonella* infection

The occurrence of chronic sequelae and other health outcomes after follow-up among cases and controls

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Table 3. *Genotype distribution of SNPs in* IFNG, STAT1, IL1B, NRAMP, TLR4, JUN *and* VDR *genes among* Campylobacter *cases,* Salmonella *cases and controls*

	Controls		*Campylobacter* cases				*Salmonella* cases			
Genotype	($n=687$)	%	($n=457$)	%	P*	OR† (95% CI)	($n=193$)	%	P*	OR† (95% CI)
IFNG (rs2430561)					0·76				0·29	
AA	193/679	28	134/450	30		1·0	45/191	24		1·0
TA	332/679	49	210/450	47		1·0 (0·7–1·3)	105/191	55		1·4 (0·9–2·0)
TT	154/679	23	106/450	24		1·0 (0·7–1·4)	41/191	21		1·1 (0·7–1·9)
IFNG (rs1861493)					0·59				0·43	
AA	337/676	50	226/448	50		1·0	97/190	51		1·0
GA	293/676	43	185/448	41		1·0 (0·8–1·3)	85/190	45		1·0 (0·7–1·4)
AA	46/676	7	37/448	8		1·2 (0·7–1·9)	8/190	4		0·6 (0·3–1·4)
STAT1					0·51				0·16	
GG	405/678	60	257/455	56		1·0	102/192	53		1·0
GA	228/678	34	168/455	37		1·1 (0·9–1·5)	79/192	41		1·4 (1·0–2·0)‡
AA	45/678	7	30/455	7		1·1 (0·7–1·9)	11/192	6		1·0 (0·5–2·1)
IL1B					0·97				0·62	
GG	298/685	44	200/455	44		1·0	80/193	41		1·0
GA	312/685	46	204/455	45		1·0 (0·8–1·3)	95/193	49		1·1 (0·8–1·6)
AA	75/685	11	51/455	11		1·1 (0·7–1·6)	18/193	9		1·0 (0·5–1·7)
NRAMP					0·75				0·55	
CC	355/683	52	236/454	52		1·0	96/193	50		1·0
CT	283/683	41	183/454	40		1·0 (0·8–1·3)	80/193	41		1·0 (0·7–1·4)
TT	45/683	7	35/454	8		1·2 (0·7–1·9)	17/193	9		1·4 (0·7–2·5)
TLR4					0·82				0·65	
AA	608/683	89	405/455	89		1·0	173/193	90		1·0
AG	72/683	11	49/455	11		1·0 (0·7–1·5)	20/193	10		1·0 (0·6–1·7)
GG	3/683	0	1/455	0		0·6 (0·1–5·6)	0/193	0		—
JUN					0·43				0·57	
CC	617/686	90	400/457	88		1·0	171/192	89		1·0
CT	66/686	10	55/457	12		1·3 (0·9–2·0)	21/192	11		1·2 (0·7–1·9)
TT	3/686	0	2/457	0		0·9 (0·1–5·5)	0	0		—
VDR					0·48				0·44	
GG	271/685	39	185/457	40		1·0	77/193	40		1·0
GA	308/685	45	213/457	47		1·0 (0·8–1·3)	93/193	48		1·0 (0·7–1·5)
AA	106/685	15	59/457	13		0·8 (0·6–1·2)	23/193	12		0·8 (0·5–1·3)

OR, Odds ratio; CI, confidence interval.
* χ^2 test.
† Adjusted for age, sex and degree of urbanization.
‡ $P=0·06$.

are shown in Table 4. None of the study subjects developed Guillain–Barré syndrome, Miller–Fisher syndrome or Reiter's syndrome since the CaSa study. Joint or back problems occurred in respectively 22% and 10% of the study subjects and these were as common among cases as among controls. Since the CaSa study, more (former) *Campylobacter* cases experienced gastroenteritis episodes than controls (Table 4).

Reactive arthritis occurred more frequently among *Campylobacter* and *Salmonella* cases than among controls (OR 1·9 and 2·3, respectively). Women especially have an elevated risk of developing reactive arthritis after a *Campylobacter* or *Salmonella* infection, compared to controls (OR 6·8 and 6·1, respectively, Table 4). Reactive arthritis occurred only in adults aged $\geqslant$23 years. The median age of reactive arthritis cases was 60 years.

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Table 4. *Occurrence of illnesses about 3 years after* Campylobacter *cases,* Salmonella *cases and controls participated in a case-control study in The Netherlands in 2002–2003*

	Controls		*Campylobacter* cases			*Salmonella* cases		
	(*n* = 687)	%	(*n* = 457)	%	OR* (95% CI)	(*n* = 193)	%	OR (95% CI)
Gastroenteritis episodes	74/643	12	68/435	16	1·5 (1·1–2·1)	23/180	13	1·0 (0·6–1·7)
Gastroenteritis episodes								
Once	44/637	7	31/430	7	1·2 (0·8–1·9)	11/179	6	0·8 (0·4–1·6)
Twice	16/637	3	7/430	2	0·8 (0·3–1·8)	5/179	3	1·0 (0·4–2·9)
More than twice	8/637	1	25/430	6	4·2 (2·0–8·6)	6/179	3	2·5 (0·9–7·1)
Joint problems	141/664	21	87/408	21	0·8 (0·6–1·1)	43/180	24	1·1 (0·8–1·7)
Back problems	70/671	10	36/415	9	0·7 (0·5–1·0)	16/182	9	0·8 (0·5–1·4)
Reactive arthritis	12/663	2	20/434	5	1·9 (0·9–3·9)	8/181	4	2·3 (0·9–5·9)
Men	8/12	67	2/20	10	0·4 (0·1–1·6)	1/8	13	0·8 (0·1–6·7)
Women	4/12	33	18/20	90	6·8 (2·2–20·7)	7/8	88	6·1 (1·7–22·7)
Presence of chronic illness†	105/664	16	113/415	27	1·8 (1·4–2·4)	41/169	24	1·9 (1·3–2·8)
Presence of chronic illness†								
Asthma	40/662	6	48/414	12	2·1 (1·4–3·1)	17/166	10	1·9 (1·1–3·3)
Psoriasis	21/662	3	5/414	1	0·4 (0·2–1·0)	1/166	1	0·2 (0·0–1·4)
Rheumatism	24/662	4	30/414	7	1·5 (0·9–2·5)	5/166	3	0·9 (0·4–2·3)
Chronic intestinal disease	6/662	1	12/414	3	2·4 (1·0–5·8)	12/166	7	8·2 (3·2–20·9)

OR, Odds ratio; CI, confidence interval.

* Adjusted for age, sex, degree of urbanization and level of education.

† It is unknown whether these illnesses developed before or after participation in the CaSa study.

Chronic disease and susceptibility to *Salmonella* or *Campylobacter* infection

In the present study, *Campylobacter* and *Salmonella* cases were more likely to have chronic diseases than controls, especially chronic intestinal diseases (IBS, IBD, coeliac disease), asthma and rheumatism (Table 4). The association between infection with *Salmonella* or *Campylobacter* and the occurrence of these chronic diseases may suggest that there is a causal link between the two. However, from the follow-up questionnaire it was unclear whether these chronic diseases developed before or after the infection and enrolment in the CaSa study.

To further investigate this link, data from the CaSa study were analysed in which participants were asked if, at that time, they had chronic intestinal illness, asthma, food allergy or allergic rhinitis. When adjusted for use of medication, these analyses showed that having a chronic intestinal illness was significantly associated with both *Campylobacter* and *Salmonella* infections. In addition, having asthma was associated with *Campylobacter* infections (Table 5). These data suggest that patients with these chronic diseases are more susceptible to *Campylobacter* or *Salmonella* infections.

Role of host genetics in chronic sequelae of infection

Genetic factors and registered risk factors from the CaSa study that were associated with reactive arthritis and recurrent gastroenteritis episodes are summarized in Table 6. For reactive arthritis, having had a *Campylobacter* or *Salmonella* infection was only a risk factor in women. In addition, use of proton pump inhibitors and a SNP in the *IFNG* gene (rs2430561) were independent risk factors for reactive arthritis. In a multivariate model including the use of proton pump inhibitors, *Campylobacter* infection was no longer associated with gastroenteritis episodes. Of the determined SNPs, a SNP in the *IFNG* gene (rs1861493) was associated with gastroenteritis episodes (Table 6).

DISCUSSION

Participants of a previous case-control study on *Campylobacter* and *Salmonella* infections 3 years ago, the CaSa study, were re-contacted. SNPs in genes involved in the host's immune response to infection were determined in DNA obtained from buccal swabs of these cases and controls to study the influence of these genes in the susceptibility to *Campylobacter* and

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Table 5. *Association between chronic illness and use of medication with campylobacteriosis and salmonellosis based on data from the CaSa study in The Netherlands, 2002–2003*

	Controls		*Campylobacter* cases				*Salmonella* cases			
	($n=3409$)	%	($n=1446$)	%	Single variable OR* (95% CI)	Multivariate OR* (95% CI)	($n=573$)	%	Single variable OR* (95% CI)	Multivariate OR* (95% CI)
Chronic illness										
Chronic intestinal illness	46/3347	1	60/1403	4	3·4 (2·3–5·1)	3·1 (2·1–4·7)	18/553	3	3·8 (2·1–6·8)	3·6 (2·0–6·5)
Food allergy	160/3346	5	71/1396	5	1·2 (0·9–1·6)		39/556	7	1·6 (1·1–2·3)	
Asthma	301/3344	9	167/1388	12	1·4 (1·2–1·7)	1·3 (1·1–1·5)	65/548	12	1·5 (1·1–2·0)	
Allergic rhinitis	502/3340	15	239/1398	17	1·2 (1·0–1·4)		82/552	15	1·4 (1·0–1·8)	
Recent use of medication†										
Antibiotics	143/3385	4	48/1423	3	0·8 (0·6–1·1)	0·6 (0·4–1·0)	42/557	7	1·7 (1·2–2·4)	1·7 (1·2–2·6)
Proton pump inhibitors	75/3409	2	150/1446	10	4·6 (3·4–6·2)	4·5 (3·3–6·1)	50/573	9	4·6 (3·1–6·8)	4·3 (2·9–6·5)
H2 receptor antagonists	23/3409	1	25/1446	2	2·6 (1·5–4·6)	2·8 (1·6–5·1)	11/573	2	3·2 (1·5–6·9)	3·5 (1·6–7·6)
Other medication prescribed by a physician	963/3375	29	483/1418	34	1·2 (1·0–1·4)		169/557	30	1·2 (1·0–1·5)	

OR, Odds ratio; CI, confidence interval.
* Adjusted for age, sex, degree of urbanization and level of education.
† Use of medication in the 4 weeks prior to infection (cases) or in the previous 4 weeks (controls).

Salmonella infections. In addition, participants were asked to complete a questionnaire in order to study whether they developed chronic sequelae after infection. We also studied the influence of the determined SNPs on the development of chronic sequelae after infection.

None of the determined SNPs were associated with *Campylobacter* and *Salmonella* infections. However, a marginal effect was observed between a candidate, *Salmonella* susceptibility SNP in *STAT1* and *Salmonella* infection. *STAT1* is involved in IFN-γ mediated signal transduction. The genotype distribution of this polymorphism was different for *Salmonella* cases compared to controls, but due to the relatively low number of *Salmonella* cases, the present study lacked the power to statistically demonstrate this difference. The present study would have the power to indicate an OR of at least 1·6, but the power was too limited to indicate an OR of 1·4, as was found for the association between a SNP in *STAT1* and *Salmonella* infection. Therefore, the possible association between this SNP in *STAT1* and *Salmonella* infection should in future be studied using larger numbers of *Salmonella* cases.

None of the study subjects developed Guillain–Barré, Miller–Fisher or Reiter's syndrome, which confirms the low incidences of these illnesses after a *Campylobacter* or *Salmonella* infection [4, 28]. This study showed a high association between *Campylobacter* and *Salmonella* infections and reactive arthritis. Interestingly, we found that women especially were at increased risk of developing reactive arthritis after a *Campylobacter* or *Salmonella* infection. Of the *Campylobacter* and *Salmonella* cases in our study, about 5% developed reactive arthritis. This is relatively low compared to outbreaks where proportions from 3% to 18% have been reported [6, 29, 30]. We observed that reactive arthritis only occurred in adults and the median age of these reactive arthritis cases was high. This may explain (part of) the high incidence of reactive arthritis found in outbreak settings where most of the cases were adults. In two other population-based studies, 7% of *Campylobacter* cases and 6% of *Salmonella* cases developed reactive arthritis [5, 31], which is close to our estimate. Among *Campylobacter* cases, reactive arthritis occurred mainly in women, just as we observed in our study [5].

Both *Campylobacter* and *Salmonella* cases were more likely to have asthma and chronic intestinal illnesses (IBD, IBS, coeliac disease). From data of the

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Table 6. *Risk factors for reactive arthritis and gastroenteritis episodes developed within 3 years after controls,* Campylobacter *cases and* Salmonella *cases participated in the CaSa study in The Netherlands, 2002–2003*

	Reactive arthritis			Gastroenteritis episodes		
	No ($N=912$†) *n* (%)	Yes ($N=40$) *n* (%)	Multivariate OR* (95% CI)	No ($N=1093$) *n* (%)	Yes ($N=165$) *n* (%)	Multivariate OR* (95% CI)
Type and gender of participant						
Male participants	422 (46)	11 (28)	1·0			
Female controls	263 (29)	4 (10)	0·9 (0·3–2·9)			
Female *Campylobacter* cases	173 (19)	18 (45)	5·1 (2·3–11·5)			
Female *Salmonella* cases	54 (6)	7 (18)	8·0 (2·8–23·0)			
Use of proton pump inhibitors in the CaSa study	90 (10)	14 (35)	2·9 (1·4–6·1)			
IFNG (rs1861493)						
AA				551 (51)	68 (42)	1·0
GA				442 (41)	87 (54)	1·7 (1·2–2·4)
GG				81 (8)	7 (4)	0·7 (0·3–1·6)
IFNG (rs2430561)						
AA	254 (28)	19 (48)	1·0			
TA	435 (48)	15 (38)	0·4 (0·2–0·9)			
TT	212 (24)	6 (15)	0·3 (0·1–0·8)			

OR, odds ratio; CI, confidence interval.

* Adjusted for age, sex, degree of urbanization.

† Only adults were included, because reactive arthritis was only observed among subjects aged $\geqslant 23$ years.

CaSa study it appeared that these chronic illnesses were already present before infection, which suggests that patients with these chronic diseases are more susceptible to infection. This contradicts the suggestion of others that gastrointestinal infections may be a cause of chronic intestinal illnesses [7–9]. Patients with IBD or IBS have a disturbed intestinal function and it is conceivable that this may facilitate enteric pathogens to cause infection. It has been shown that patients with asthma may have oesophageal dysmotility or gastroesophageal reflux [32]. However, even when adjusted for gastric acid inhibitors, the association between asthma and *Campylobacter* infection remained significant.

Recurrent episodes of gastroenteritis were common among *Campylobacter* cases, but this was mainly due to the use of proton pump inhibitors by these cases. It is well known that use of these gastric acid inhibitors increases the susceptibility to gastrointestinal infections [33, 34]. A SNP in the *IFNG* (rs1861493) gene was also independently associated with gastroenteritis episodes. As IFN-γ is a crucial cytokine in the immune response against enteric infections, it is conceivable that changes in the expression of this protein would increase the susceptibility to gastroenteritis. However, it is unknown if this SNP results in such a functional alteration.

Use of proton pump inhibitors, *Campylobacter* and *Salmonella* infections and a SNP in the *IFNG* (rs24305621) gene were independently associated with reactive arthritis. So far, except for HLA-B27 no other genetic factors have previously been described that may increase the susceptibility to reactive arthritis. We hypothesize that the effect of both proton pump inhibitors and SNPs in the *IFNG* gene on reactive arthritis susceptibility is mediated by prolonged or more frequently occurring gastrointestinal infections, because of the gastric acid inhibitory effect of proton pump inhibitors and the crucial role of IFN-γ in the defence against gastrointestinal pathogens.

Limitations of the present study are the retrospective design and the reliance on subjective information about chronic sequelae of infection. In the questionnaire, we asked the participants if a physician ever diagnosed reactive arthritis. Participants reporting reactive arthritis or joint symptoms were not examined by a rheumatologist to confirm the diagnosis. This may have resulted in misclassification of reactive arthritis cases. However, the occurrence of reactive arthritis among *Campylobacter* and *Salmonella* cases

in our study was comparable to the incidence found in two other population-based studies where participants with joint symptoms were examined by a rheumatologist [5, 31].

Furthermore, we conducted our study in a selected group of *Campylobacter* and *Salmonella* cases and controls. In the CaSa study, 33% of the approached controls and 47% of all laboratory-confirmed *Campylobacter* and *Salmonella* cases in 2002–2003 were enrolled. In the present study, another selection of these cases and controls participated. Therefore, the study population in the present study may not have been fully representative of all initially approached cases and controls.

In conclusion, our study revealed a clear association between *Campylobacter* and *Salmonella* infections and reactive arthritis. Women are especially at increased risk of developing reactive arthritis after infection. However, such an association was not found for chronic intestinal diseases like IBD and IBS. In fact, our study showed that people who have chronic intestinal diseases and also people with asthma are at increased risk of acquiring *Campylobacter* or *Salmonella* infections. We did not find an association between the determined SNPs and *Campylobacter* or *Salmonella* infections. The results of our study suggest that polymorphisms in *IFNG* genes are involved in long-term health effects of *Salmonella* or *Campylobacter* infections, such as reactive arthritis and recurrent episodes of gastroenteritis. It is not yet clear whether these gene variants are indeed the causal variants in the development of human disease. Future research is needed to confirm these associations as well as to elucidate the functionality of these SNPs.

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DECLARATION OF INTEREST

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