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Helicobacter pylori multiplex serology and its dynamics within families during a 3-year prospective follow-up

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ABSTRACT

Objectives: Transmission routes of *Helicobacter pylori* (*Hp*) have been extensively studied, but many aspects remain unclear. This study explored the dynamics of multiplex *Hp* serology within regular families during a 36-month prospective follow-up.

Methods: Altogether, 329 families from the Finnish Family HPV study were subjected to sequential blood sampling and now tested also for six *Hp* proteins, HP0010, HP0073, HP0547, HP0875, HP0887, and HP1564, using multiplex serology assay.

Results: *Hp* seropositivity, defined as being seropositive to at least three of the six *Hp* proteins, was more common among the fathers (20%) than mothers (10%). After maternal antibody decay, only a few children tested *Hp*-seropositive at later follow-up visits, indicating that acquisition of *Hp* infection is practically non-existent (0.4-2.0%) at an early age. No evidence was found to support the person-to-person transmission of *Hp* in this cohort because there was no correlation in *Hp* seropositivity or antibody levels between the spouses and/or their offspring, and individuals who were *Hp*-seropositive did not seem to increase the risk of other family members to co-test *Hp*-seropositive.

Conclusions: Our results perfectly agree with a recently published register-linkage study from Finland, where *Hp* and *Hp*-related co-morbidity are predicted to disappear among the native Finns during the 21st century.

Introduction

Helicobacter pylori (*Hp*) infection is the single most important cause of chronic gastritis [1–3] and, if untreated, results in atrophic gastritis (AG), progressing to gastric cancer (GC). Because of the significant global GC burden ascribed to *Hp*, International Agency for Research on Cancer already declared *Hp* as a class I human carcinogen in 1994 [4]. Since the discovery of this bacterium in 1982 [1], a wide variety of diagnostic techniques have been developed for *Hp* testing of symptomatic (dyspeptic) patients and screening of asymptomatic populations [1,2]. The caveats of these different *Hp* tests are well-known [2,3,5]. In sizable sero-epidemiologic studies, the population prevalence of *Hp* seropositivity variety is wide, from a few percent up to >80% [1,2,6]. Apart from the geographic region, this variation is related to age, ethnicity, associated co-morbidity, socioeconomic status, and hygiene conditions [6–8].

The prevalence of *Hp* infection in Finland has declined rapidly, being currently approximately 5% [9], with seropositivity influenced by age and co-morbidities [9–13]. Thus, (i) *Hp* is rarer in children than in adults [9], (ii) *Hp* carriage increases with advanced age, up to 16% in those aged 80 years [10], and (iii) *Hp* is high (16%) among patients referred for gastroscopy [11], (iv) among those with dyspeptic symptoms (25%) [12], and (v) among the patients with autoimmune disorders (18%) [13]. A recent register-linkage study in Finland predicts Hp-associated morbidity would practically disappear among the native Finns by 2080 [14], paralleling global prevalence declines [15].

Transmission routes of *Hp* have been extensively studied, but many of the details remain unclear [6]. Once contracted with *Hp*

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infection, the pathogen usually persists for life, unless effectively eradicated [2,3,6]. Spontaneous clearance is rare but can occur, e.g. in patients with advanced AG [7–9]. Fecal-oral route appears to be the most common transmission route, although oral-oral and gastric-oral routes also play minor roles [16,17]. In addition, anal-oral and genital-oral routes remain only hypothetical [16], and divergent environmental and occupational factors limit animal-to-human transmission [16,17]. In settings with apparently high hygienic standards, *Hp* infection can be transmitted in childhood and adolescence through outdoor toilets, private well water, and farm animals [18].

Person-to-person transmission within families, especially from mothers and siblings with *Hp* infection, is common in low-resource countries [6,19]. This is evidenced by the high concordance (56%) in *Hp* strains between mother-offspring pairs [20]. Such a concordance in father-offspring pairs was practically lacking, whereas concordant strains among siblings were detected in 81% of the families [20]. According to the existing data [9], the role of *Hp* transmission between spouses remains controversial [21], but it is generally believed to be quite low [22].

The present study exploits the unique longitudinal setting of the prospective Finnish Family HPV (FFHPV) study, originally designed for monitoring the outcomes of human papillomavirus (HPV) infections within families [23,24]. Since the study's onset in 1998, the family members of the FFHPV cohort have been subjected to multiple blood samples for testing of other infectious agents as well, including *Hp*. The present analysis was undertaken to cast further light on the dynamics of *Hp serology* among the family members during a 36-month prospective follow-up.

Material and methods

Subjects

The subjects of the present analysis are participants in the prospective FFHPV cohort study jointly conducted since 1998 at the Department of Obstetrics and Gynecology, Turku University Hospital and the Institute of Dentistry, Faculty of Medicine, University of Turku, Finland [23,24]. The participants were enrolled between 1998 and 2001. The cohort included 329 women (mothers-to-be; mean age 25.5 years [SD 3.4 years]), 135 spouses (fathers-to-be; mean age 28.8 years [SD 4.9 years]), and 331 newborns who all were subsequently followed up for 3 years. All study participants were of Caucasian descent and shared the same ethnic background [23,24]. The participants were requested to complete a structured questionnaire at baseline with questions on demographics sexual behavior, smoking habits, history of sexually transmitted infections, and general health. The research ethics committee of Turku University Hospital has approved this study's design (#3/1998 and 2/2006, with amendments 45/1801/2018).

Serological assay for Helicobacter pylori

From both spouses, blood samples were collected at the baseline and subsequently at the 12-, 24- and 36-month follow-up visits. From the newborn, additional blood samples were collected at the 1- and 2-month visits [23,24]. The serological assays were performed in collaboration with the German Cancer Research Center, Heidelberg, Germany using a validated multiplex serology assay, as described earlier [25]. In the original study, complete open reading frames of 17 *Hp* proteins were amplified by polymerase chain reaction from genomic DNA of the strains 26695, G27, and 151, using strain 26695-derived primers elongated with BamHI and SalI cloning sites, respectively [25]. For the present analysis, six of original 17 Hp proteins were included (HP0010, HP0073, HP0547, HP0875, HP0887, and HP1564) based on previous studies [25–27], where they demonstrated a high sensitivity and specificity in detecting *Hp* seropositivity. HP0010 was chosen for its strong immunogenicity, whereas HP1564 is a potential virulence factor. HP0073 plays a role in bacterial stress response, HP0875 contributes to immune evasion, and HP0087 is involved in bacterial adhesion and colonization [26–28]. The key characteristics of these proteins are summarized in Supplementary Table 1.

The multiplex serology is based on a glutathione-S-transferase (GST) enzyme capture immunosorbent assay combined with fluorescent bead technology [25]. Bacterially expressed recombinant GST-*Hp* fusion proteins were used as antigens. A Luminex 200 analyzer identified the internal bead color and, thus, the antigen carried by the bead [25]. For each bead set, the quantity of antibodies bound to the respective antigen was determined as the median reported fluorescence intensity (MFI) of at least 100 beads per bead set per serum. The final antigen-specific MFI values were generated by subtraction of GST tag and individual bead background values. Antigen-specific cutoffs (Supplementary Table 1) have been determined using the cutoffs for seropositivity established through comparison with urea breath test, as described previously [26–28]. HP0010, HP0887, and HP1564 are very sensitive and specific in determining *Hp* infection and commonly associated with gastrointestinal (GI) cancer outcomes. HP0547, HP0875, and HP0073 also have functional importance as virulence factors and are important as associated markers with GI cancer risk [25,28]. *Hp* seropositivity was defined as positivity to at least three of six *Hp*-specific proteins, which has been shown to increase specificity compared with single-antigen serological tests. Furthermore, the multiplex serology we used has been extensively validated in previous studies [25,27], demonstrating high sensitivity and specificity for detecting *Hp* infection.

Statistical analysis

All statistical analyses were performed using SPSS 29.0.2.0 for Windows (IBM, NY, USA) and STATA/SE 18.0 software (STATA Corp., TX, USA). The descriptive statistics were calculated according to routine procedures. Frequency tables were analyzed using the χ^2 test, with the likelihood ratio or Fisher's exact test for categorical variables. For the examination of differences in means of continuous variables, a nonparametric test (Friedman test for K-related samples) was used. The risk estimates were calculated using conventional univariate regression models, expressed as odds ratio (ORs) and their 95% confidence interval (CI).

The data were arranged in the panel format, suitable for analyses with generalized linear models, e.g. panel Poisson regression. In this study, the co-variates of *Hp* point prevalence (*Hp* seropositivity at any follow-up visit) and the role of the six *Hp* proteins as an independent determinant of point *Hp* seropositivity were estimated using population-averaged Poisson regression modeling [19,23,24]. The results for all co-variates were expressed as the incidence rate ratio (IRR) with 95% CI. All co-variates recorded in the baseline questionnaire were first tested in the univariate model, followed by the multivariate model, adjusted for age and all significant co-variates of the univariate analysis. All tests were run two-sided and $P < 0.05$ were considered statistically significant.

Data availability

The data generated in this study are available upon request from the corresponding author (KL).

Table 1
MFI levels of the antibodies to six *Hp* proteins, HP0010, HP0073, HP0547, HP0875, HP0887, and HP1564, in the spouses and their offspring during the 3-year follow-up.

Hp protein	Follow-up visits of the parents					
	Baseline MFI (95% CI)[a]	1-month MFI (95% CI)[a]	2-month MFI (95% CI)[a]	12-month MFI (95% CI)[a]	24-month MFI (95% CI)[a]	36-month MFI (95% CI)[a]
Mother:	n = 318	[b]n = 0	[b]n = 0	n = 269	n = 256	n = 241
HP0010	365 (236–518)			348 (223–489)	351 (227–505)	383 (243–543)
HP0073	453 (366–557)			452 (357–551)	444 (339–564)	569 (453–700)
HP0547	2465 (2136–2778)			2605 (2287–2943)	2404 (2075–2759)	2451 (2138–2813)
HP0875	859 (703–1027)			727 (585–888)	708 (564–884)	657 (535–811)
HP0887	406 (320–503)			371 (302–451)	392 (305–493)	444 (357–550)
HP1564	690 (550–832)			711 (557–883)	663 (503–819)	735 (574–903)
Father:	n = 133	[b]n = 0	[b]n = 0	n = 111	n = 101	n = 97
HP0010	822 (507–1186)			681 (356–1025)	737 (380–1160)	686 (331–1065)
HP0073	868 (639–1144)			751 (564–977)	755 (524–1032)	789 (522–1128)
HP0547	3372 (2832–3967)			2963 (2487–3503)	2894 (2324–3497)	3252 (2528–4036
HP0875	1584 (1108–2168)			1384 (935–1924)	1197 (784–1706)	1357 (849–1967)
HP0887	672 (478–924)			582 (420–774)	571 (382–831)	577 (403–781)
HP1564	1254 (894–1664)			1051 (699–1472)	946 (617–1338)	1213 (812–1690)
	Follow-up visits of the children					
Child:	1-month n = 228	2-month n = 236	6-month n = 255	12-month n = 270	24-month n = 247	36-month n = 238
HP0010	371 (205–573)	377 (206–563)	297 (210–414)	119 (75–199)	93 (57–148)	65 (39–105)
HP0073	359 (242–516)	235 (155–342)	189 (118–281)	252 (156–364)	325 (245–421)	288 (211–380)
HP0547	1747 (1360–2133)	1394 (1052-1782)	762 (550–1002)	478 (383–592)	516 (432–604)	638 (511–792)
HP0875	682 (486–917)	599 (385–824)	372 (259–514)	250 (205–307)	403 (312–526)	360 (283–456)
HP0887	261 (191–355)	184 (125–265)	137 (118–160)	127 (115–139)	185 (157–218)	168 (140–201)
HP1564	726 (542–929)	528 (377–701)	1725 (1471-2004)	550 (465–641)	1183 (992–1399)	725 (587–888)

CI, confidence interval; Hp, *Helicobacter pylori*; MFI, Median reported fluorescence intensity.
[a] 95% CI calculated by non–parametric bootstrapping
[b] Not sampled at this visit; Mother: Friedman test (K–related samples) for titer comparisons across FU visits: HP0010, P <0.001; HP0073, P <0.001; HP0547, P <0.001; HP0875, P <0.001; HP0887, P <0.001; HP1564, P = 0.003: Father: Friedman test (K–related samples) for titer comparisons across FU visits: HP0010, P = 0.385; HP0073, P = 0.612; HP0547, P = 0.121; HP0875, P <0.001; HP0887, P = 0.049; HP1564, P = 0.053: Child: Friedman test (K–related samples) for titer comparisons across FU visits: HP0010, P <0.001; HP0073, P <0.001; HP0547, P <0.001; HP0875, P <0.001; HP0887, P <0.001; HP1564, P <0.001.

Results

Table 1 summarizes the mean MFI values of the multiplex serology for the six *Hp* proteins, stratified by follow-up visits of the mothers, fathers, and children. Among mothers, the antibody titers of all six *Hp* proteins were significantly different across the four follow-up visits, with P <0.001 for all, except HP1564 (P = 0.003). This is because of the skewed distribution of MFI values in the mothers' serology at those visits. Among the fathers, only HP0875 showed significant variation (P <0.001), and HP0887 and HP1564 were marginally different (P = 0.049 and P = 0.053) between the four samples. Among the children, the antibody titers of all six *Hp* proteins showed a significant difference (P <0.001) across the six follow-up visits. There was a declining trend in all antibodies from 1 month until 12 months, followed by an increase starting from 24 months for all, except HP0010.

Of all *Hp* proteins, HP0547 seropositivity was the most prevalent in all visits of both parents (from 27% up to 41%), the seropositivity of all other *Hp* proteins being substantially lower (from <10% up to 20%) in both parents (Figure 1). *Hp* seropositivity among the fathers at any time point was almost twice as high as among the mothers, varying between 17.1% and 21.8% and between 7.8% and 12.0%, respectively (Figure 2). Among the children, seropositivity to the six *Hp* proteins was more equally distributed. From 6 months onward, HP1564 was the protein with the highest seropositivity, reaching 44.3% (at 6 months) and 33.6% (at 24 months) (Figure 1c). *Hp* seropositivity of the children was peaking (7%) at 1 month, followed by a steady decline until the age of 12 months when only one of 270 (0.4%) children was classified as *Hp*-seropositive (Figure 2). At subsequent visits, five and three of the children were classified as *Hp*-seropositive at 24- and 36-months, respectively.

The impact of the six individual *Hp* proteins as independent determinants of global *Hp* seropositivity was tested using multivariate Poisson analysis (considering all the follow-up visits) (data not shown). The two most powerful independent determinants of *Hp* seropositivity in the offspring included HP0010 and HP1564, with IRRs of 10.7 (95% CI 3.2-36.3) and 4.99 (95%CI 2.1-11.8), respectively. *Hp* seropositivity among the mothers was most significantly determined by seroreactivity to HP0547 (IRR = 4.4; 95% CI 2.6-7.5) and HP0010 (IRR = 4.2; 95% CI 2.3-7.9). Among the fathers, the single most powerful component of *Hp* seropositivity was HP1564, with IRR = 4.4 (95% CI 1.8-10.5), followed by HP0010 (IRR 3.0; 95% CI 1.1-8.7). In the mothers (IRR = 0.162) and their offspring (IRR = 0.765), HP0073 was the only *Hp* protein that had no significant independent impact on *Hp* seropositivity, whereas, among fathers, there were two such proteins (HP0875 and HP0887), with IRR = 0.225 and IRR = 0.905, respectively.

The antibody levels to all six *Hp* proteins were not significantly correlated between the spouses at any time point (Supplementary Table 2). Only scattered (marginally) significant R-values (shown in bold) to individual protein antibodies were found, with no temporal patterns. The only exception was HP0010 antibodies, which were significantly (P = 0.005) correlated at the 36-month visit of the mothers and the fathers.

Maternal antibodies to all *Hp* proteins measured at the baseline visit (third trimester of pregnancy) showed a highly significant (P <0.001 for all) correlation with the respective antibodies of the newborn at 1 month (Table 2). This almost perfect correlation is also shown by scatter plots in Figure 3, where R^2 values for all bivariate correlations exceed 0.700 (mostly, close to 0.900), indicating a highly significant (P <0.001) correlation between the maternal and newborn antibodies to all six *Hp* proteins.

Figure 1. Seropositivity to individual *Hp* protein antigens (HP0010, HP0073, HP0547, HP0875, HP0887, and HP1564) among the mothers (a), fathers (b), and offsprings (c) during the 36–month follow-up period.
Hp, Helicobacter pylori.

Table 2
Bivariate correlations of the six *Helicobacter pylori* protein antibodies between the mothers and their offspring at each time point during the follow-up visits.

H. pylori protein/ Mother's follow-up visit	Follow-up visits											
	Child											
	1-month		2-month		6-month		12-month		24-month		36-month	
	[a]R	[b]*P*	[a]R	*P*	[a]R	*P*	[a]R	*P*	[a]R	*P*	[a]R	*P*
Baseline												
HP0010	0.623	**<0.001**	0.615	**<0.001**	0.182	**0.004**	0.113	0.067	−0.030	0.649	0.067	0.310
HP0073	0.820	**<0.001**	0.661	**<0.001**	0.352	**<0.001**	0.027	0.663	0.065	0.317	0.042	0.529
HP0547	0.886	**<0.001**	0.793	**<0.001**	0.469	**<0.001**	0.136	**0.027**	−0.153	**0.018**	−0.045	0.493
HP0875	0.794	**<0.001**	0.685	**<0.001**	0.251	**<0.001**	0.038	0.542	−0.022	0.740	−0.007	0.917
HP0887	0.724	**<0.001**	0.555	**<0.001**	0.259	**<0.001**	0.176	**0.004**	0.070	0.280	0.024	0.719
HP1564	0.896	**<0.001**	0.805	**<0.001**	0.135	**0.033**	−0.057	0.358	0.003	0.959	0.036	0.592
12-Month												
HP0010	0.602	**<0.001**	0.585	**<0.001**	0.187	**0.004**	0.128	**0.040**	−0.039	0.558	0.010	0.877
HP0073	0.805	**<0.001**	0.609	**<0.001**	0.312	**<0.001**	0.086	0.167	0.111	0.093	0.034	0.618
HP0547	0.868	**<0.001**	0.773	**<0.001**	0.473	**<0.001**	0.143	**0.022**	−0.141	**0.032**	−0.013	0.848
HP0875	0.786	**<0.001**	0.701	**<0.001**	0.219	**<0.001**	0.073	0.241	−0.109	0.098	0.004	0.957
HP0887	0.680	**<0.001**	0.563	**<0.001**	0.235	**<0.001**	0.149	**0.016**	0.062	0.349	0.036	0.598
HP1564	0.865	**<0.001**	0.789	**<0.001**	0.110	0.096	−0.078	0.213	0.030	0.648	0.081	0.230
24-Month												
HP0010	0.538	**<0.001**	0.587	**<0.001**	0.165	**0.014**	0.114	0.076	−0.076	0.247	0.054	0.420
HP0073	0.744	**<0.001**	0.626	**<0.001**	0.304	**<0.001**	0.012	0.851	0.095	0.145	0.045	0.498
HP0547	0.846	**<0.001**	0.782	**<0.001**	0.475	**<0.001**	0.153	**0.016**	−0.143	**0.029**	0.003	0.962
HP0875	0.746	**<0.001**	0.650	**<0.001**	0.228	**<0.001**	0.056	0.383	−0.097	0.139	−0.002	0.973
HP0887	0.569	**<0.001**	0.560	**<0.001**	0.189	**0.005**	0.091	0.159	−0.024	0.716	0.070	0.292
HP1564	0.821	**<0.001**	0.783	**<0.001**	0.155	**0.021**	−0.006	0.925	0.042	0.525	0.105	0.115
36- Month												
HP0010	0.510	**<0.001**	0.562	**<0.001**	0.244	**<0.001**	0.143	**0.030**	0.030	0.659	0.064	0.346
HP0073	0.754	**<0.001**	0.585	**<0.001**	0.369	**<0.001**	0.141	**0.032**	0.114	0.093	0.026	0.702
HP0547	0.848	**<0.001**	0.783	**<0.001**	0.450	**<0.001**	0.196	**0.003**	−0.134	**0.047**	−0.028	0.686
HP0875	0.683	**<0.001**	0.644	**<0.001**	0.214	**0.002**	0.041	0.539	−0.030	0.653	−0.016	0.812
HP0887	0.640	**<0.001**	0.585	**<0.001**	0.168	**0.015**	0.158	**0.016**	0.074	0.274	−0.002	0.980
HP1564	0.816	**<0.001**	0.746	**<0.001**	0.098	0.160	0.001	0.991	−0.003	0.969	0.033	0.628

[a] Spearman's rho (data violating normal distribution)
[b] *P*, significance level (2-2-tailed), significant *P*-values bolded.

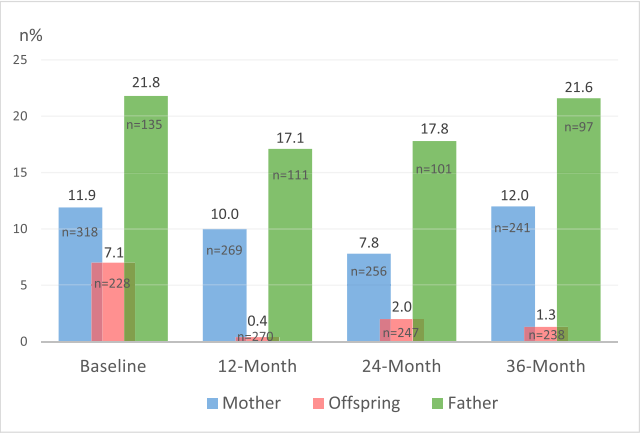

Figure 2. *Hp* seropositivity of the spouses and their offspring during the different follow-up visits over a 3-year period. Seropositivity was defined as positivity to at least three of six *Hp*-specific proteins (HP0010, HP0073, HP0547, HP0875, HP0887, and HP1564) by their median reported fluorescence intensity values.
Hp, Helicobacter pylori.

This significant correlation for all six *Hp* protein antibodies continued until the 6-month samples of the offspring. At 12 months, a considerable correlation persisted for HP0547 ($P = 0.027$) and HP0887 ($P = 0.004$) but, at 24-months, only for HP0547 antibodies ($P = 0.018$). At 36 months, the maternal and offspring antibody levels were completely unrelated. This pattern is consonant with the transplacental transfer of immunoglobulin G *Hp* antibodies to newborn babies and not an indication of mother-to-child transmission of *Hp*. There were no significant bivariate correlations in *Hp* protein antibodies between the fathers and their offspring simultaneously at the same time point (Supplementary Table 3).

The temporal relationships of *Hp* seropositivity in mother-child pairs demonstrated the OR (and κ agreement) for the children being seropositive when the mothers were seropositive at the same time points (baseline,12 months, 24 months, and 36 months) (Supplementary Table 4). Significant association (OR = 180; 95% CI 21-1517) was only established for the (mother) baseline and (child) 1-month visit; however, even then, the agreement by Cohen's κ was ranking only substantial (0.600; 95% CI 0.50-0.68). In the subsequent testing at 12, 24, and 36 months, there was no correlation between the mother-child pairs in their *Hp* seropositivity. Similar calculations for the father-child pairs co-tested at the same follow-up visits showed that the *Hp* seropositivity of the father-child pairs was completely unrelated at all time points. However, because of the limited numbers of *Hp*-seropositive pairs, the calculation of ORs was seriously compromised. Concordance in *Hp* seropositivity between the two spouses was extremely low, as measured by the κ test, and *Hp* seropositivity of one spouse did not increase the likelihood of *Hp* seropositivity in the other spouse (all ORs were non-significant).

Discussion

This study analyzed serum samples from the FFHPV cohort, comprising mothers, fathers, and their offspring, to evaluate antibody responses to six selected *Hp* proteins: HP0010, HP0073, HP0547, HP0875, HP0887, and HP1564. These proteins, previously validated for their sensitivity and specificity, were chosen for their relevance in detecting *Hp* infections and their association with GI cancer risk [26,27]. Although CagA (HP0547) is a well-known oncoprotein, our study aimed to include a broader range of serological markers to capture different aspects of *Hp* infection dynamics beyond the traditional focus on CagA/VacA.

Antibody levels showed distinct patterns across cohort groups. The fathers' levels remained quite stable (with HP0875 as the exception) during the 3 years, whereas the levels of antibodies to all *Hp* proteins in the mothers showed a significant ($P < 0.001$) change across the follow-up visits. Among the offsprings, maternal immunoglobulin G antibodies declined at 6-12 months, followed by a gradual increase (excluding HP0010) at 24-36 months. Compared with their parents, the children's antibody levels remained markedly lower, suggesting limited early life exposure to *Hp*.

In the original validation study of all 17 proteins [25], HP0010 demonstrated the highest seroprevalence (88%) (i) against a commercial enzyme-linked immunosorbent assay test, (ii) in multiplex serology (92%), in (iii) type classification of CagA- and/or VacA-positive sera (87%), (iv) CagA- and/or VacA-negative sera (89%), and (v) in multiplex assay for high CagA (89%) and low CagA (95%) antibody reactive sera [25]. Thus, the consistent decline of HP0010 antibodies in children from 297 MFI (at 6 months) down to 65 MFI (at 24 months) suggests, at least, indirectly, minimal Hp exposure during early infancy. HP0547 (CagA) seropositivity was >40% among parents throughout the follow-up. For children, seropositivity to CagA decreased sharply after the decay of maternally transferred antibodies, with ~3% remaining seropositive. CagA is the only bacterial oncoprotein linked to GC [29]. Still, its activity appears to be downregulated at a younger age because early infections in high-risk countries are common [6–8] but GC develops in individuals past the age of 50 years [29]. This seems to apply to the children of our cohort because CagA antibody levels decreased by >50% by 6 months and then remained low until 36 months at levels much lower than their parents. Many parents had likely been exposed to CagA-positive *Hp* strains, and chronic carriers may be at increased risk of GC later in life [1–3,7,29].

The fathers were classified as *Hp*-seropositive almost twice as often (~20%) as their spouses (~10%), with stable rates over 3 years. Among offsprings, *Hp* seropositivity rates decreased significantly, reaching only 1.3% at 36 months. Interestingly, only five families had both parents seropositive at any time, and no family had all members classified as *Hp*-seropositive, indicating that our study failed to show evidence for *Hp* infections clustering in families, a concept that has gained strong evidence in large cross-sectional epidemiological studies from China [30]. The reason for this could lie in different lifestyle factors and hygiene standards and in our relatively short follow-up period of 36 months. Our results could be indicative of *Hp* transmission being uncommon in early childhood in high-income settings. Larger studies with longer follow-up periods could give us insight on possible geographical differences in *Hp* infection clustering within families and among children beyond early childhood.

Considering the potential transmission routes (fecal-oral, oral-oral, gastric-oral, anal-oral, genital-oral [16]), these might remain relevant in low-income countries with a high risk of *Hp* but are likely less common in low-risk countries with high hygiene standards [8–10,14]. Our results implicate that these children born between 1998 and 2001 have a very low risk of contracting *Hp* infection during early infancy, and no mother-to-child transmission of *Hp* infection was detected. These findings are in perfect alignment with the recent register-linkage study from Finland [14], where the authors predicted that the *Hp*-related GC and AG will gradually disappear among the native Finns during the 21st century when the age groups currently carrying *Hp* [9,10,12] will have passed away by the year 2080 [14]. However, our limited follow-up data constrains conclusions on long-term infection dynamics.

Examining the person-to-person transmission of *Hp,* the antibody levels in the mothers and fathers showed no significant correlation, countering the hypothesis of spousal transmission. In agreement with the previously published data on father-offspring pairs, indicating a lack of concordance in *Hp* strains [20], the results in the FFHPV cohort confirm that the antibody levels of the fathers and their offspring were unrelated. However, although no

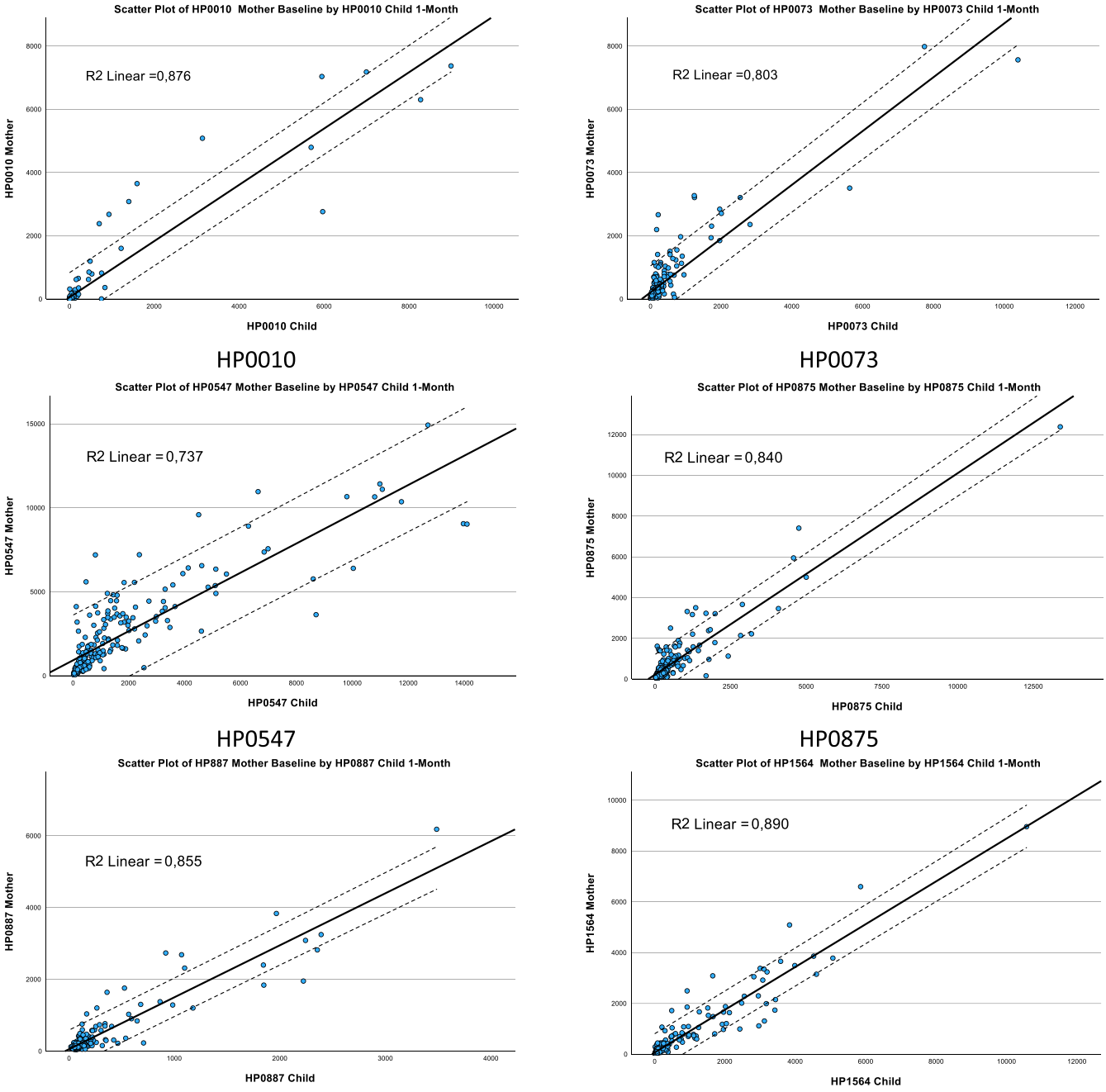

Figure 3. Mothers' baseline *Hp* antibody levels associations to their offsprings' antibody levels at 1-month-old using six different *Hp* proteins presented by scatter plot. Y-axis represents the mother's and X-axis represents the children's median reported fluorescence intensity antibody levels to each antigen.
Hp, Helicobacter pylori.

strong evidence of person-to-person transmission was observed, our findings do not entirely exclude other transmission routes. Our study focused on familial transmission, but we lack data on the participants' dietary habits, water sources, or other environmental exposures to *Hp*. Such factors may influence transmission dynamics, especially in high-prevalence regions with varying hygiene practices and sanitation infrastructure [6–8,18]. Finland's high hygiene standards, safe drinking water, improved food safety regulations, and reduced household crowding likely minimize early *Hp* exposure [9]. In addition, the widespread use of antibiotics for childhood infections may suppress *Hp* colonization, leading to lower infection rates in young children. This aligns with broader epidemiological trends in high-resource countries, where *Hp* prevalence has been declining. Host genetic factors, immune responses, and gut microbiota composition may also influence susceptibility to *Hp* infection.

Regarding the other potential *Hp* transmission routes discussed in the literature [16,17], we made some effort to explore the eventual sexual (genital-oral, anal-oral) transmission of *Hp*, albeit suggested to be only hypothetical [16,18]. The FFHPV cohort was originally designed to assess the transmission routes of HPV infections, in which sexual transmission plays a role [23,24]. We used the Poisson regression to disclose the potential co-variates of *Hp* seropositivity but found no significant associations. Consequently, we found no evidence supporting person-to-person *Hp* transmission via sexual routes among marital couples or to their offspring.

Finally, we estimated the odds of individuals who are *Hp*-seropositive increasing the risk of seropositivity in co-testing of mother-offspring, father-offspring, and mother-father. As expected, the risk of the newborn testing *Hp*-seropositive at 1 month was substantially (OR = 180) increased by the baseline *Hp* seropositivity of the mother. As to the κ value of 0.60, it only indicates a substantial agreement, being less than almost perfect (κ >0.8). Undoubtedly, the limited numbers of mothers and newborn babies who are *Hp*-seropositive play a role here. By 12 months, maternal antibodies had disappeared, rendering risk calculations non-significant. These results are exactly as expected given that the maternal *Hp* antibodies transferred to her newborn disappeared after 6 months of follow-up. Fathers being *Hp*-seropositive did not increase the risk of coexistent seropositivity of the offsprings at any of the visits when co-tested, and no association was found between spousal serostatus. Taken together, any of the family members testing *Hp*-seropositive did not increase the risk of the other family member to co-test *Hp*-seropositive at the same time point, irrespective of whether mother-offspring, father-offspring, or mother-father pairs are concerned.

The strengths of our study were our well-characterized longitudinal cohort with repeated serum sampling, allowing us to assess antibody dynamics over time. In addition, the inclusion of multiple *Hp*-specific proteins providing a broader perspective beyond CagA/VacA. However, the relatively short 36-month follow-up limits our conclusions on long-term transmission patterns. Serology alone cannot distinguish active from past infections; it only indicates whether a person has encountered *Hp* in their lifetime. The lack of other diagnostic tests, e.g. urea breath test or stool antigen testing, further limits our ability to differentiate between active and past infections. In addition, we did not have information on the participants' dietary habits, water sources, or other environmental factors. Future research with longer follow-up periods and expanded knowledge on environmental and lifestyle factors is needed to further clarify *Hp* transmission dynamics.

Conclusion

The FFHPV cohort was used to analyze *Helicobacter* serology in parents and their offspring over a 3-year follow-up. *Hp* seropositivity was twice as prevalent in fathers compared with mothers, whereas early seropositivity in children, attributed to maternal antibody transfer, declined after 6 months. Few children tested *Hp*-seropositive (0.4-2.0%) at later visits, indicating minimal early-age acquisition of infection. No evidence of person-to-person transmission was observed, supported by (i) stable antibody levels in adults, (ii) lack of correlation in antibody levels or seropositivity between family members, and (iii) no increased risk of seropositivity in co-testing of parent-child or spousal pairs. These longitudinal data perfectly agree with a recently published register-linkage study from Finland [14], where *Helicobacter* and *Hp*-related CG and AG were predicted to disappear among the native Finns during the 21st century.

Declarations of competing interest

TW serves on advisory boards for Merck (MSD) Sharp & Dohme. The other authors declare no conflicts of interest.

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Ethical approval

The research ethics committee of Turku University Hospital has approved this study's design (#3/1998 and 2/2006, with amendments 45/1801/2018).

Author contributions

KS, SG, TW, SS, and KL conceptualized and designed the study. KS, BM, JB, SG, TW, SS, and KL contributed to the methodology and data collection. KS, SR, NK, and KL conducted data analysis. KS, SR and NK prepared the original draft of the manuscript. KS, SR, NK, BM, JB, SG, TW, SS, and KL critically reviewed and revised the manuscript. SG, TW, SS, KL supervised the overall study implementation and ensured adherence to standards. SR, NK and KL contributed to journal formatting. All authors had access to the full dataset, verified the reported results, discussed the findings, and approved the final version of the manuscript for submission.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2025.107893.

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