Supplemental Information

ROTAVIRUS VE, SAFETY, AND TOLERABILITY AMONG MEDICAL RISK INFANTS

Patient and Methods

Laboratory Analyses

Fecal samples were placed in biosecurity envelopes and mailed to the study laboratory for PCR testing. RNA and DNA were isolated from the samples by using the MagnaPura96 (Roche Diagnostics, Pleasanton, CA) and amplified by ABI75000 realtime PCR system (ThermoFisher Scientific, Foster City, CA). Before extraction, a nonhuman internal control was spiked into the lysis buffer of the samples to monitor for sample inhibition. Positive and negative controls for each pathogen were tested in every run. All samples with valid amplification curves are assessed as positive. In validation of the assay and yearly External Quality Assessment (Quality Control for Molecular Diagnostics program), there are no indications for aspecific results (false-positives). Fecal samples collected within 14 days of symptom onset were defined as AGE samples. Rotavirus-positive fecal samples were additionally genotyped, and RNA was extracted from fecal samples by using the MagnaPure96 nucleic acid extraction system. The purified RNA was subsequently subjected to PCR amplification and sequencing of the VP4 and VP7 genes according to Simmonds et al and Zeller et al, respectively.[37,38] The obtained sequences were used for rotavirus type determination in the Web-based typing tool RotaC.[39]

Three Sources of AGE Reporting

Episodes actively reported by parents with or without a fecal sample collected and including a daily symptom severity score and health care attendance. For these episodes we calculated the modified Vesikari score. A score of <8 was defined as mild, 9 to 10 was defined as moderate, and a score of 11 and more was defined as severe.[16,17]

Episodes reported on the monthly questionnaire for which the study team was not notified. For these episodes, information was available on duration of symptoms and health care attendance.

Episodes retrieved from medical chart review. These included hospitalized episodes only, with or without diagnostic fecal testing performed. Nosocomial AGE was defined as AGE occurring ≥48 hours of admission.

Sample size calculation was based on 8 participating hospitals, an assumed VE of at least 60% against severe rotavirus AGE, at a cumulative incidence of 4% until 18 months of age, and a between hospital intraclass correlation of 0.002, as documented in study protocol.[13]

Statistical Analyses

The proportional hazard assumption was tested by using Schoenfelds residuals. The primary analysis was adjusted for rotavirus seasonality (prespecified). Weighted rotavirus epidemic intensity was calculated by using the weekly data on rotavirus-positive tests from the national virological surveillance. This surveillance includes aggregated test results from 20 sentinel laboratories in the Netherlands serving primary and secondary care.[40] Weight per week was derived by Rpackage *timeseries* accounting for each years' rotavirus season and amount of reporting laboratories. We considered the following additional variables as covariates: breastfeeding, young siblings (5 years of age or younger) in the household, day care attendance, GA, household socioeconomic status (defined as the highest obtained parental education), and parental origin.[41] We used likelihood ratio test to select the final model with covariates and present 95% CI of the HR.

We reported this cohort study according to the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) checklist.[42]

Results

Secondary Outcomes

Analyses for complete series HRV resulted in a univariate HR of 0.68 (95% CI: 0.35 to 1.34) for rotavirus vaccinated versus unvaccinated infants in ATP cohorts; adjusted for attending day care and season, the HR was 0.62 (95% CI: 0.31 to 1.21). This was translated into a VE of 38% (95% CI: −21% to 69%) after completing the 2-dose HRV series against severe rotavirus AGE.

The incidence rate of rotavirus AGE of any severity in the cohort with at least 1 dose of HRV (ATP) cohort was 67.1 per 1000 person-years (95% CI: 51.3 to 86.1). In the willing-to-vaccinate (ATP) cohort, the incidence rate was 61.5 per 1000 person-years (95% CI: 44.3 to 83.1). There was no statistical significant difference, IRR 1.05 (95% CI: 0.72 to 1.55). The negative binomial model resulted in an adjusted IRR of 1.02 (95% CI: 0.69 to 1.50), corresponding with a VE of −2% (95% CI: −50% to 31%).

Post Hoc Analyses

The post hoc analyses on severe all-cause AGE resulted in a VE estimate of 19% (95% CI: −19% to 45%) for vaccinated versus unvaccinated infants in the ATP cohort. HRV impact on all-cause AGE

hospitalizations in the post- versus preimplementation cohort was −5%, with adjusted RR of 1.05 (95% CI: 0.68 to 1.65). Effectiveness against all-cause AGE of any severity among HRV-vaccinated infants revealed an adjusted IRR of 0.87 (95% CI: 0.76 to 1.01), translated into 13% VE. The incidence rate of all-cause AGE of any severity in the pre- and postimplementation ATP cohorts was 733.9 (95% CI: 670.7 to 800.7) and 660.3 (95% CI: 608.7 to 715.4) per 1000 person-years, respectively. The applied Bayesian analysis yielded a posterior probability of 0.049 for estimating a VE >60% (Supplemental Fig 4).

Safety

The vaccine-related SAEs included 2 cases of intussusceptions (1 ultrasound confirmed), 2 cases of necrotizing enterocolitis, and 1 case of clinical sepsis (no pathogen detected). Two infants developed AGE that required hospital admission (shortly after vaccination, no stool sample available) and 1 infant had lactose intolerance (occurrence of diarrhea and severe abdominal cramps after both vaccine doses, which resolved after lactose-free formula milk was introduced). Three infants developed sudden cardiorespiratory events. For detailed information see Supplemental Table 5. All infants recovered and there were no deaths. Three infants with hospitalized rotavirus AGE were classified as vaccine failures, and partially heterotypic genotypes were detected in sampled feces, in particular regarding the VP4 genotype, which in both cases did not match the genotype of the vaccine strain.

Subgroup Analyses

Overall, there was little difference in VE results per prespecified subgroup compared with the full cohorts, but CIs were wider because of smaller numbers. The estimated VE after at least 1 dose against severe rotavirus AGE varied between 3.3% for the subgroup of infants with a GA of 30 to 32 weeks and 49.0% for subgroup of term infants with congenital disorders. See Supplemental Table 7.

With all-cause severe AGE as outcome in subgroup analyses, premature infants (GA 32–37 weeks) and term infants with a congenital disorder had a higher estimated VE (44.6% and 68.0%, respectively). Also, preterm infants of lowest GA (<30 weeks) or infants with multiple conditions seemed to benefit less from HRV vaccination (VE −3.6% and 4.4%, respectively).

For the outcome of HRV tolerability, characteristics of infants with GA <27 weeks were relatively similar, with the exception of an older age at first vaccination for HRV-vaccinated infants (72 vs 63 days), shown Supplemental Table 8. Tolerability for infants with a GA <27 weeks is different from for those born past 27 weeks' gestation; fewer AEs were reported, but numbers are small (see Supplemental Tables 8 and 9).

Among 134 term infants with congenital disorders participating in the before-after cohort study, 79 infants were immunized with the NIP primary series vaccines and 55 infants were immunized with NIP and HRV vaccines; characteristics are shown in Supplemental Table 10. Out of all vaccine moments, 49.1% (79 of 161) experienced a solicited AE versus 59.1% (13 of 22), respectively (Supplemental Table 11).

SUPPLEMENTAL REFERENCES

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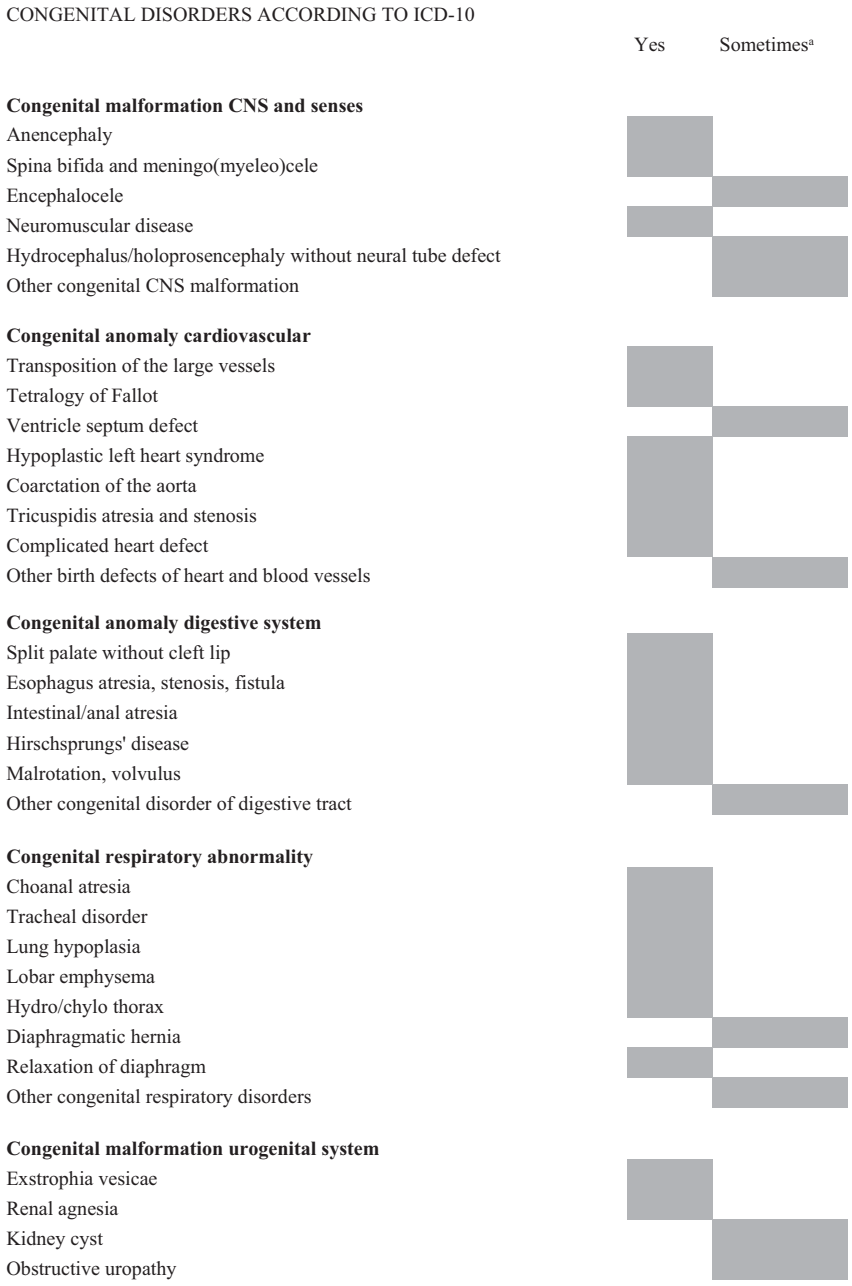
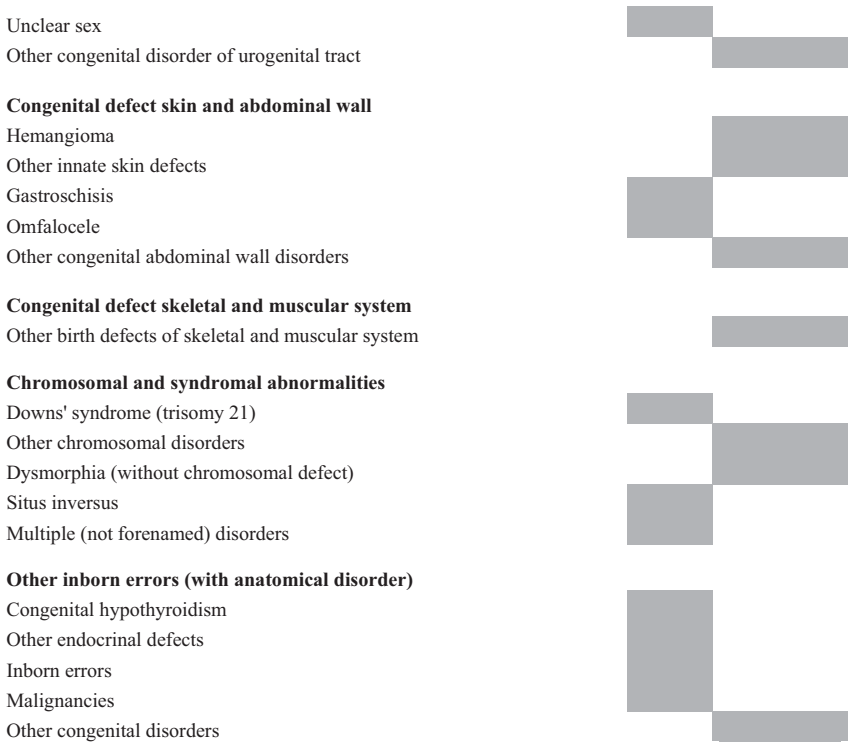


SUPPLEMENTAL FIGURE 3

List of eligible congenital disorders.[43] [a] Qualifying conditions are those that last longer than 12 months, involve multiple organ systems, and/or are expected to require pediatric specialty care. Disorders are defined into the following categories: (1) cardiovascular, (2) pulmonary, (3) neurodevelopmental, (4) chromosomal, (5) perinatal, and (6) other. CNS, central nervous system; ICD-10, International Code of Diseases 10th Edition.



SUPPLEMENTAL FIGURE 3
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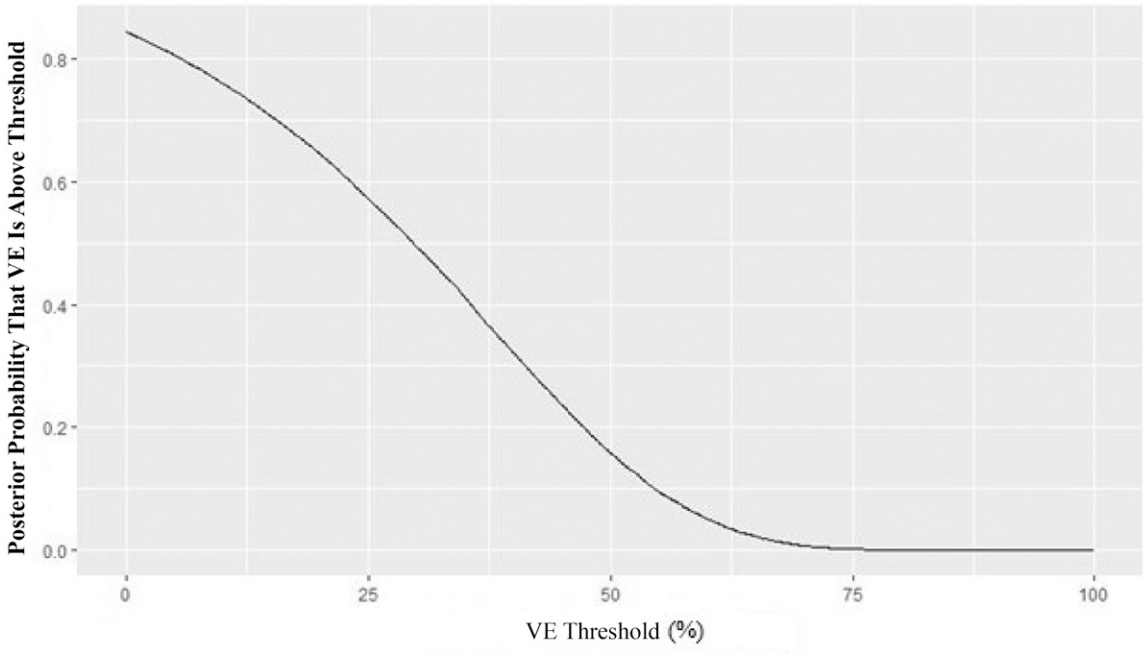

SUPPLEMENTAL FIGURE 4
Posterior probability per VE threshold.

SUPPLEMENTAL TABLE 5 Detailed Information on Vaccine-Related SAE and Hospitalized Vaccine Failure After First and Second HRV Administration

No.	Event	Sex	GA, wk + d	Birth Wt, g	Congenital Disorder	Age at HRV1, d	Age at HRV2, d	Age at SAE, d	Rotavirus-Positive Feces Sample
1	Severe AGE	Male	32 + 3	2000	No	52		55	—
2	Intussusception	Female	33 + 5	1500	No	49	120	124	—
3	Cardio-resp incidents	Female	30 + 3	1300	No	50	80	51	—
4	Sepsis	Female	28 + 4	800	No	56	91	92	—
5	NEC	Male	27 + 4	1000	No	58		62	No
6	Lactose intolerance	Male	32 + 0	1600	No	68	120	120	—
7	NEC	Male	33 + 1	2000	Yes	68	—	68	Yes
8	Severe AGE	Male	37 + 2	2000	Yes	47	—	55	—
9	Intussusception	Female	33 + 0	1500	No	95	126	127	—
10	Cardio-resp incidents	Female	28 + 3	700	No	58	108	109	—
11	Cardio-resp incidents	Male	27 + 3	2900	Yes	99	—	99	—
12	Vaccine failure	Female	38 + 0	3500	Yes	97		103	Yes (genotype G3P8)
13	Vaccine failure	Male	27 + 2	800	No	109	144	344	Yes (genotype G3P8)
14	Vaccine failure	Male	31 + 6	1800	No	51	125	384	Yes (genotype G9P8)

Cardio-resp, cardiorespiratory events; HRV1, first dose; HRV2, second dose; NEC, necrotizing enterocolitis; —, not available.

SUPPLEMENTAL TABLE 6 Characteristics of Infants With Severe Rotavirus AGE Among HRV-Vaccinated and Unvaccinated Infants

Case	Sex	Gestation, wk + d	Birth Wt, g	Congenital Disorder	SGA	HRV	Age Severe Rotavirus AGE, mo	Age HRV1, mo	Age HRV2, mo
1	Male	34 + 6	1800	No	Yes	Yes	10	2	—
2	Male	37 + 0	2100	No	Yes	Yes	14	1	3
3	Male	25 + 2	900	No	No	No	7	—	—
4	Male	36 + 1	2500	Yes	No	Yes	11	1	
5	Male	37 + 2	3500	Yes	No	Yes	3	2	4
6	Female	41 + 0	3500	Yes	No	Yes	15	2	3
7	Male	29 + 4	1300	No	No	No	8	—	—
8	Female	35 + 6	2500	No	No	No	16	—	—
9	Female	29 + 0	1200	No	No	Yes	10	1	3
10	Female	33 + 2	2000	No	No	No	4	—	—
11	Female	32 + 2	2200	No	No	Yes	2	1	—
12	Female	32 + 0	1800	No	No	Yes	14	1	4
13	Male	35 + 0	3000	No	No	Yes	5	1	4
14	Male	32 + 4	1800	No	No	No	12	—	—
15	Female	30 + 6	1600	No	No	No	9	—	—
16	Male	31 + 4	1300	No	Yes	Yes	13	1	—
17	Male	31 + 6	1800	No	No	Yes	12	1	4
18	Male	30 + 1	1600	No	No	No	12	—	—
19	Female	27 + 5	1000	No	No	Yes	17	1	3
20	Male	31 + 2	1700	No	No	No	9	—	—
21	Female	29 + 2	1300	No	No	No	15	—	—
22	Male	35 + 6	1900	No	Yes	No	13	—	—
23	Male	36 + 0	2200	No	Yes	Yes	10	2	3
24	Male	31 + 0	2000	No	No	Yes	9	1	4
25	Female	30 + 3	1600	No	No	Yes	13	1	2
26	Male	35 + 0	2100	No	Yes	No	9	—	—
27	Male	36 + 0	2200	No	Yes	No	15	—	—
28	Female	25 + 6	800	Yes	No	No	15	—	—
29	Female	25 + 6	700	Yes	Yes	No	15	—	—
30	Male	35 + 1	3700	No	No	No	10	—	—
31	Female	38 + 5	3200	Yes	No	No	5	—	—
32	Male	35 + 6	3100	No	No	No	13	—	—
33	Female	41 + 3	3000	Yes	Yes	Yes	5	3	4
34	Female	34 + 0	1700	Yes	Yes	Yes	15	2	—

HRV1, first dose of human rotavirus vaccination; HRV2, second dose of human rotavirus vaccination; SGA, small for gestational age; —, not available.

SUPPLEMENTAL TABLE 7 Coefficients and VE HRs for Subgroups

Subgroup	Coefficient[a]	95% CI	Multivariate HR[a]
GA 32–37 wk	−0.12	−2.08 to 0.37	0.89
GA 30–32 wk	−0.03	−1.45 to 2.80	0.97
GA <30 wk	−0.26	−1.74 to 1.53	0.77
Term and congenital disorder	−0.67	−3.30 to 5.50	0.51
Multiple risk conditions	0.25	−1.41 to 4.79	1.28

Rotavirus severe AGE as outcome comparing vaccinated versus unvaccinated infants in ATP cohorts using the main analysis Cox model with an interaction term for subgroup.
[a] Adjusted for day care attendance and rotavirus epidemic intensity.

SUPPLEMENTAL TABLE 8 Baseline Characteristics and Solicited AE for Receipt of Primary Series With or Without HRV per Infant, for Off-label Subgroup of High-risk Premature Infants (GA <27 Weeks)

Characteristic	NIP Vaccinated (n = 5)	NIP + HRV Vaccinated (n = 22)
GA, mean (SD), wk + d	26 + 1 (0 + 4)	26 + 0 (0 + 5)
Birth wt, median (IQR), g	925 (805–1045)	820 (523–1199)
Small for GA, yes (%)[a]	0	3 (13.6)
Sex (male, %)	2 (40.0)	12 (54.5)
Multiple birth, yes (%)	0	6 (27.3)
Congenital pathology, yes (%)	0	0
Age at first vaccination, median (IQR), d	63 (54–72)	72 (45–99)
Concomitant NIP + HRV administration	NA	10 (45.5)
Sibling, yes (%)	1 (20.0)	4 (18.2)
Parental education[b]		
High	3 (60.0)	15 (62.8)
Medium	2 (40.0)	6 (27.3)
Lower	0	1 (4.5)
Parental background[c]		
European	3 (60.0)	14 (63.6)
Non-European	—	4 (18.2)
Mixed	2 (40.0)	4 (18.2)
Average parental age, median (IQR), y	30.5 (17.7–43.3)	34.5 (27.5–41.5)
Parent reported solicited AE, n (%)		
Any solicited AE	4 (80)	15 (68)
Fever	3 (60)	8 (36)
Gastrointestinal AE	2 (40)	4 (18)
Any AE-related health care attendance	3 (60)	8 (36)

For the subgroup of infants with GA before 27 wk, we only took those receiving care in a hospital with a policy to vaccinate off-label as our study population. Percentages are derived excluding subjects with missing data on the variable.
[a] Based on 10th percentile perinatal growth curves.[44,45]
[b] Based on highest parental educational level.
[c] Based on parental background and categorized according to world population by country.

SUPPLEMENTAL TABLE 9 Solicited AE After Receipt of NIP Vaccines or Concomitant NIP + HRV Vaccination as Part of the Primary Series in Off-label Subgroup of High-risk Premature Infants (GA <27 Weeks)

Reported AEs After Primary Series Vaccine Administrations	Any NIP Vaccination (n = 14), n (%)	Concomitant NIP + HRV Vaccination (n = 17), n (%)
At least 1 solicited AE	9 (64.3)	7 (41.2)
Fever	4 (28.6)	4 (23.5)
Gastrointestinal AE	2 (14.3)	2 (11.8)
Any health care attended	3 (21.4)	3 (17.6)
Hospitalization	3 (21.4)	2 (11.8)

For the subgroup of infants with GA before 27 wk, we only took those receiving care in a hospital with a policy to vaccinate off-label as our study population.

SUPPLEMENTAL TABLE 10 Characteristics of Term Infants With Congenital Disorders and Solicited AE for Receipt of the Primary Series With or Without HRV per Infant

Characteristic	NIP Vaccinated (n = 79)	NIP + HRV Vaccinated (n = 55)	P
Mean GA (SD), wk + d	39 + 2 (1 + 2)	39 + 0 (1 + 3)	.22
Median birth wt (IQR), g	3340 (2685–3995)	3330 (2525–4135)	.96
Sex, male (%)	44 (55.7)	32 (58.2)	.76
No. congenital disorder, n (%)[d]			.25
1	55 (69.6)	37 (67.3)	
2	21 (26.6)	11 (20.0)	
>2	3 (3.8)	7 (12.7)	
Type of congenital disorder, n (%)			
Neurodevelopmental	4 (5.1)	2 (3.6)	.99
Cardiovascular	31 (3.2)	28 (50.9)	.18
Pulmonary	2 (2.5)	5 (9.1)	.12
Chromosomal	14 (17.7)	90 (18.2)	.95
Perinatal	1 (1.3)	1 (1.8)	.99
Other[a]	36 (45.6)	23 (41.8)	.67
Age at first vaccination, median (IQR)	62 (40–84)	61 (34–88)	.86
Concomitant NIP + HRV administration	NA	10	NA
Sibling, yes (%)	34 (49.3)	18 (34.6)	.11
Parental education[b,d]			.13
High	46 (65.7)	39 (75.0)	
Medium	16 (22.9)	12 (23.1)	
Lower	8 (11.4)	1 (1.9)	
Parental background[c,d]			.90
European	60 (85.7)	45 (86.5)	
Non-European	10 (14.3)	7 (13.5)	
Average parental age, median (IQR), y	33.5 (27.5–39.5)	34.3 (27.7–40.9)	.49
Parent reported solicited AE, n (%)			
Any solicited AE	57 (72.2)	46 (83.6)	.12
Fever	41 (51.9)	34 (61.8)	.26
Gastrointestinal AE	20 (30.8)	22 (41.5)	.23
Any AE-related health care attendance	8 (10.1)	4 (7.2)	.40

Percentages are derived excluding subjects with missing data on the variable.

[a] Infants can have multiple congenital disorders; in category Other there are duplicates.

[b] Based on highest parental educational level.

[c] Based on parental background and categorized according to world population by country.

SUPPLEMENTAL TABLE 11 Solicited AE After Receipt of NIP Vaccines or Concomitant NIP + HRV Vaccination as Part of the Primary Series in Term Infants With Congenital Disorders

Reported AE After Primary Series Vaccine Administrations	Any NIP Vaccination (n = 161), n (%)	Concomitant NIP + HRV Vaccination (n = 22), n (%)
At least 1 solicited AE	79 (49.1)	13 (59.1)
Fever	47 (29.2)	4 (18.2)
Gastrointestinal AE	11 (6.8)	5 (22.7)
Any health care attended for AE	8 (5.0)	1 (4.5)
Hospitalization	3 (1.9)	0