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Short Communication

High Rate of Positive Fecal Occult Blood Test in Healthy Infants: A Nested Case-control Study

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Abstract

Background and objectives: Guaiac fecal occult blood test (gFOBT) is often used to evaluate evidence of food protein-induced allergic proctocolitis (FPIAP) in children in primary care and gastroenterology settings; however, it has not been validated for this diagnosis, and little is known about the positivity rates in early infancy. In this study, we used samples from healthy asymptomatic infants aged two weeks to two months to evaluate the gFOBT positivity rate compared to those diagnosed with FPIAP.

Methods: This was a nested case-control study. Frozen stool samples from infants aged two days to five months enrolled in the Gastrointestinal Microbiome and Allergic Proctocolitis study were evaluated using gFOBT (n = 123). The results were interpreted by three blinded staff members, including a trained clinical research coordinator, a pediatric gastroenterologist, and an experienced medical assistant. Additionally, the samples were analyzed using a quantitative fecal immunochemical test (FIT) for hemoglobin to compare with gFOBT results.

Results: Eight percent of samples from the 100 healthy asymptomatic infants were gFOBT positive (11% when including positive and equivocal results). Seventy-four percent of samples from infants diagnosed with FPIAP were gFOBT positive. The interrater reliability of gFOBT interpretation was 81%. Of the healthy samples that yielded a positive gFOBT result, 50% also yielded a positive FIT result. Of the 23 FPIAP samples that yielded a positive gFOBT result, 29% yielded a positive FIT result.

Conclusions: Healthy asymptomatic infants in early infancy were gFOBT positive up to 11% of the time. Caution should be used when interpreting gFOBT results in young infants in a diagnostic setting.

Introduction

The guaiac fecal occult blood test (gFOBT) is a non-invasive, qualitative method widely utilized in both adult and pediatric medicine to detect occult blood in stool, though its sensitivity and specificity in many clinical scenarios have not been investigated. In young infants, it is commonly used to aid diagnosis of food protein-induced allergic proctocolitis (FPIAP), also referred to as cow's milk protein allergy (CMPA) or milk soy protein intolerance,[1–4] but its validity for this indication or in this particularly young age group has not been well studied. FPIAP is a non-immunoglobulin E-mediated food allergy presenting in the first months of life (median age of diagnosis is 35 days),[1] with fussiness and mucus and/or blood in the stool in an otherwise healthy infant. It is often associated with nonspecific symptoms such as reflux or "colic", watery stools, or constipation.[2–4] Diagnosis is made clinically, with no reliable biomarkers, and while oral food challenges are the recommended confirmatory method, these are very rarely done in clinical practice.[4–8]

The gFOBT has been used very frequently as a supportive tool in diagnosis, but its sensitivity and specificity in this context are not well established, with false positives being reported.[3,9–11] In older studies, in many children thought to have FPIAP clinically based on

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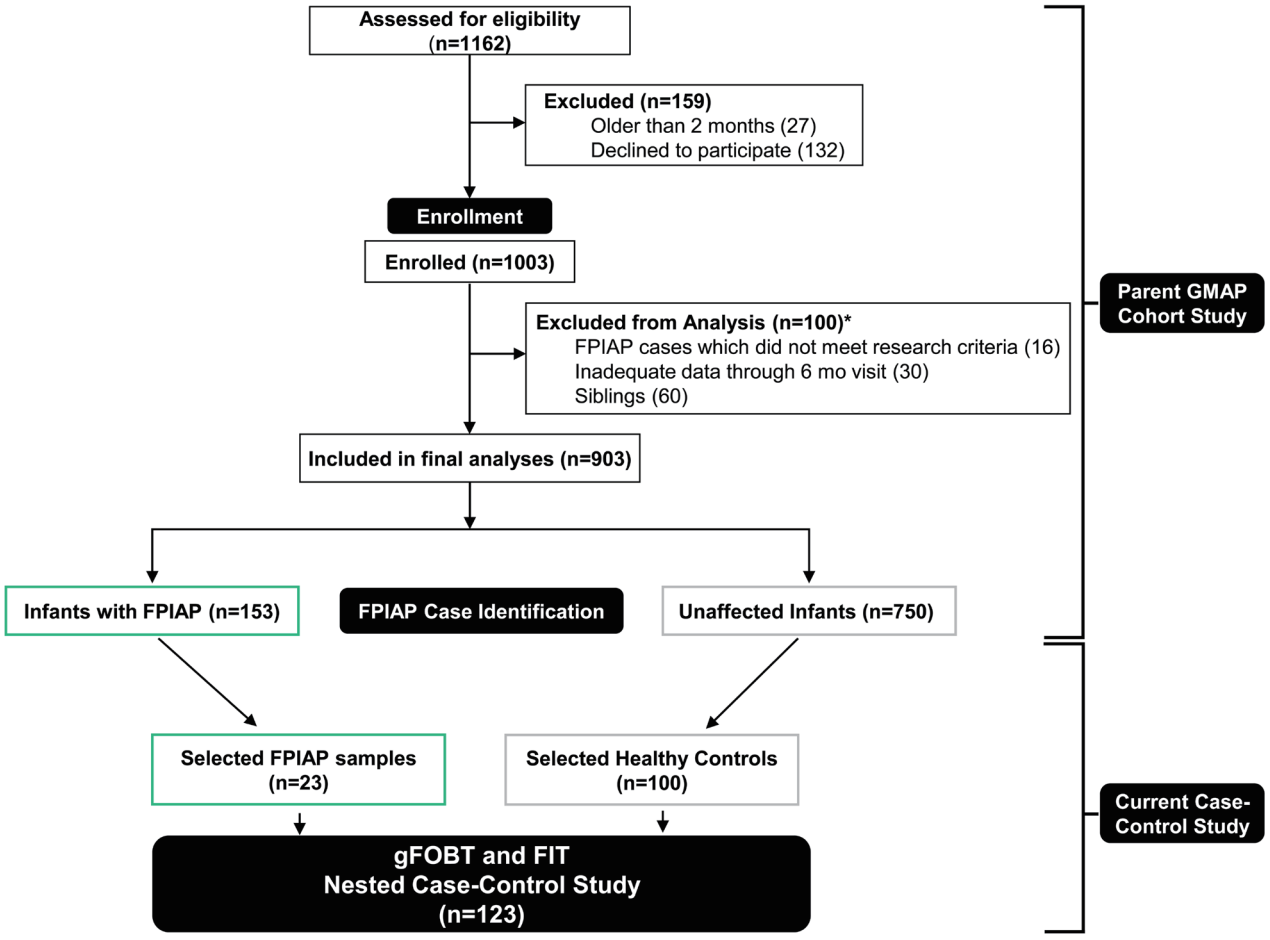

Fig. 1. Consort flow diagram of the parent GMAP Cohort Study and the nested case-control presented here. FIT, fecal immunochemical test; FPIAP, food protein-induced allergic proctocolitis; gFOBT, guaiac fecal occult blood test; GMAP, Gastrointestinal Microbiome and Allergic Proctocolitis.

rectal bleeding,[12] the diagnosis was not confirmed on biopsy or oral food challenges. As such, there is rising concern that FPIAP may be overdiagnosed based on the presence of rectal bleeding alone (gross or occult), particularly in otherwise healthy infants.[13] There is little to no evidence on the use of gFOBT to diagnose FPIAP or to assess response to treatment. There is significant risk associated with dietary elimination in this age group, making overdiagnosis and treatment of FPIAP a newly important area of study.

In this nested case-control study, we sought to evaluate the gFOBT positivity rate in healthy, asymptomatic infants from a large prospective healthy infant cohort. We focused on children aged two weeks to two months (when the majority of cases of FPIAP are identified). We also sought to evaluate the inter-rater reliability of interpretation of guaiac cards in the clinical setting. Finally, we evaluated the relative performance of newer quantitative fecal immunochemical test (FIT) for hemoglobin, now more commonly used for colorectal cancer screening, in both healthy infants and those diagnosed with FPIAP from the same cohort.

Materials and methods

Study design and sample collection

The Gastrointestinal Microbiome and Allergic Proctocolitis (GMAP) study is an ongoing prospective, observational healthy infant cohort study evaluating the early development of food allergies in infants, as previously published.[1,14,15] The GMAP study was approved by the Massachusetts General Hospital Institutional Review Board (IRB - #2013P002374), in accordance with the Declaration of Helsinki (as revised in 2024), and a parent of all enrolled infants gave written informed consent. Infants in GMAP were prospectively identified as having FPIAP based on prespecified diagnostic criteria (symptoms, pediatrician diagnosis, documented blood in the stool), as previously published. Stool samples were collected longitudinally from diapers at every infant well-child visit and were stored in cryovials at −80 degrees Celsius. We selected infants from the GMAP study who developed FPIAP and those who did not for this nested case-control study (n = 123) to evaluate testing for occult blood in the stool (Fig. 1). Inclusion in this nested case-control study was defined as not having diagnosed FPIAP (for the healthy, asymptomatic control group) or having diagnosed FPIAP (for the positive control group). Exclusion from this nested case-control study was defined as being out of the age range (older than six months) or having a documented reason for having blood in stool other than FPIAP. From the stored samples available, we selected "positive control" or FPIAP samples from infants diagnosed with FPIAP (as per clinical diagnosis from the treating physician and documented positive guaiac test or gross

Table 1. Demographics stratified by food protein-induced allergic proctocolitis (FPIAP) status of this subset of analyzed infants from this cohort

	No FPIAP	FPIAP
N	100	23
Median age (months)	1.04	0.92
Age range (months)	0.46–2.17	0.10–5.40
Sex (Female)	46 (46%)	7 (30.4%)
Vaginal Delivery	64 (64%)	16 (69.6%)
Initial diet		
Exclusively breastfed	50 (50%)	12 (52.2%)
Formula-fed	9 (9%)	3 (13%)
Partially breastfed	41 (41%)	8 (34.8%)
Perinatal antibiotic exposure	0 (0.0%)	10 (10.0%)
Eczema	12 (52.2%)	40 (40.8%)
Immunoglobulin E-mediated food allergy	2 (8.7%)	0 (0.0%)

blood in stool not attributable to another cause, as previously published),[1] who had stored samples collected on the date of known guaiac positivity in real time in the clinician's office. We first thawed and repeated gFOBT on these samples (stored at −80°C for several years). We then selected samples from healthy infants without FPIAP (our healthy controls), based on parent report, physician assessment, and chart review. These were age-matched to the range of ages represented by the positive controls (median age of 1.04 months, with a range of 0.46 to 2.17 months for the healthy infants and a median age of 0.92 month, with a range of 0.099 to 5.49 months for the positive controls) (Table 1). For cases and controls, we processed the first 23 and 100 samples, respectively, that had adequate volume of frozen stool and met the inclusion criteria above.

gFOBT interpretation

Samples were slowly thawed, and several distinct smears from the same tube were collected. The Beckman Coulter (Indianapolis, IN, USA) gFOBT kit was used following the manufacturer's instructions. The result of each card was read by blinded staff, including a trained clinical research coordinator performing the test, a clinical pediatric gastroenterologist on research study staff, and a trained blinded medical assistant who frequently reads these tests in the pediatric primary care setting. All three were instructed to record a binary "positive" or "negative" result. A "positive" result occurred when the smear on the card turned blue upon application of the developer solution, indicating the presence of blood. Additionally, the clinical research coordinator and gastroenterologist were instructed to record another answer, choosing from three choices: "weakly positive/borderline", "positive", or "negative" result. A "weakly positive" result was defined as a card that turned light blue or had small blue spots upon application of the developer solution. In cases where the three readers disagreed on a binary result, the majority ruled.

Chart review

Healthy infants without FPIAP whose gFOBT were positive were then chart reviewed for any evidence of possible contributors to false-positive results (comorbidities, medications, supplements, clinical symptoms, diet), and these were recorded.

FIT testing

A hemoglobin enzyme-linked immunosorbent assay from American Laboratory Products Company Diagnostics (Salem, NH, USA) was performed on the same sample set as above to quantify human hemoglobin present. A cut-off value to determine positivity according to the FIT was presented as one standard deviation above the median hemoglobin concentration of the healthy infant samples, as suggested by the manufacturer.

Statistical analysis

We used Fisher exact and chi-square testing to assess the association between breastfeeding and occult blood testing results. We used a point-biserial correlation to assess the correlation between gFOBT and FIT testing, with FIT results treated as a continuous variable and gFOBT treated as binary (positive/negative). Comparison of median FIT results between infants with and without FPIAP was also conducted through a Mann-Whitney U test.

Results

Healthy infants' gFOBT and FIT results

We analyzed samples from 100 healthy asymptomatic infants (median age 1.04 months [0.459, 2.17]) from the GMAP study,[1] 46% of whom were female, 50% of whom were exclusively breastfed, 41% were partially breastfed, and 9% were fed formula at their initial visit (Table 1).

Of the 100 healthy asymptomatic infant samples, eight (8%) yielded a positive gFOBT result and 92 (92%) yielded a negative result when using the binary "positive" or "negative" interpretations (Table 2). When using this binary interpretation, the interrater reliability between the three readers was 81%. When allowing for a sample from an infant without FPIAP to be interpreted as "weakly positive", three (3%) were determined to be "weakly positive", eight (8%) were positive, and 89 (89%) were negative (Table 2).

Of the eight positive result samples, four (50%) had a FIT hemoglobin concentration above the 0.603 µg/g cut-off value, and four (50%) had a hemoglobin concentration below the cut-off value (Fig. 2). Of the 92 negative result samples, three (3%) had a hemoglobin concentration above the cut-off value, and 89 (97%)

Table 2. Results of guaiac fecal occult blood test (gFOBT) compared to fecal immunochemical test (FIT) in healthy infants and infants with food protein-induced allergic proctocolitis (FPIAP)

	Positive FIT	Negative FIT	Total
Healthy infants			
Positive gFOBT	4 (50%)	4 (50%)	8
Negative gFOBT	3 (3%)	89 (97%)	92
Total	7 (7%)	93 (93%)	100
Infants with FPIAP			
Positive gFOBT	5 (29%)	12 (71%)	17
Negative gFOBT	1 (17%)	5 (83%)	6
Total	6 (26%)	17 (74%)	23

had a hemoglobin concentration below the cut-off value (Fig. 2). Of the three "weakly positive" samples, three (100%) had a hemoglobin concentration below the cut-off value (Fig. 2). If the "weakly positive" samples were considered positive, this would bring the overall positivity rate to 11%. Upon chart review of the 11 healthy infants whose samples yielded either a positive or "weakly positive" result, at the time their samples were collected, none were on iron supplementation, two were being treated for a diaper rash, two were reported as being colicky/fussy, one had mucousy stool, and one was noted to have reflux. Receiving breast milk was not associated with an increased rate of positive gFOBT ($p = 0.432$) or FIT ($p = 0.669$).

Infants with FPIAP gFOBT and FIT results

We also analyzed the samples from 23 infants diagnosed with FPIAP (median age 0.92 month [0.099, 5.49]) from the GMAP study,[1] 30.4% of whom were female, 52.2% of whom were exclusively breastfed, 34.8% were partially breastfed, and 13% were fed formula (Table 1).

Of the 23 positive control samples from infants with known FPIAP, 17 (73.9%) yielded a positive gFOBT result, and six (26.1%) yielded a negative gFOBT result (Table 2). The interrater reliability between the three readers was 91%. Of these 17 positive result samples, five (29%) had a hemoglobin concentration above the cut-off value used in the FIT, and 12 (71%) had a hemoglobin concentration below the cut-off value. Of the six negative result samples, one (17%) had a hemoglobin concentration above the cut-off value, and five (83%) had a concentration below the cut-off value (Fig. 3).

We found no significant difference in median FIT results between infants with and without FPIAP (0.095 µg/g and 0.178 µg/g, respectively, $p > 0.2$). Across all samples analyzed, there was a very weak correlation between gFOBT and FIT results, with a point-biserial coefficient of 0.23 ($p = 0.008$) when utilizing continuous FIT results, and a Spearman correlation of 0.39 when treated as binary ($p < 0.001$).

Discussion

In this study, we first sought to evaluate the gFOBT positivity rate in healthy young infants. We found that 8% (11% when including "weakly" positive results) of healthy asymptomatic infants aged zero to two months had a positive gFOBT. This adds to a few prior reports of high rates at this age.[9,16] This is a relatively high positivity rate in infants without symptoms, which clinicians should consider carefully when using gFOBT for diagnostic purposes, particularly in this age range. Concha *et al*.,[9] who confirmed FPIAP diagnoses through oral food challenges, found an FOBT sensitivity of 84% and a specificity of 66%, with 34% of healthy infants testing positive, concluding that FOBT is not specific enough to confirm FPIAP in infants with rectal bleeding, as a significant portion of healthy infants also had positive results.

Guaiac testing also has variability in interpretation, with discrepancies among providers in identifying what constitutes a "positive" or "weakly/borderline positive" result, leading to accuracy issues, particularly among noncertified providers.[17] We found the inter-rater reliability to be 81%. Due to many limitations of gFOBT, relatively newer immunochemical fecal occult blood tests (FIT) have emerged, with higher specificity in detecting human

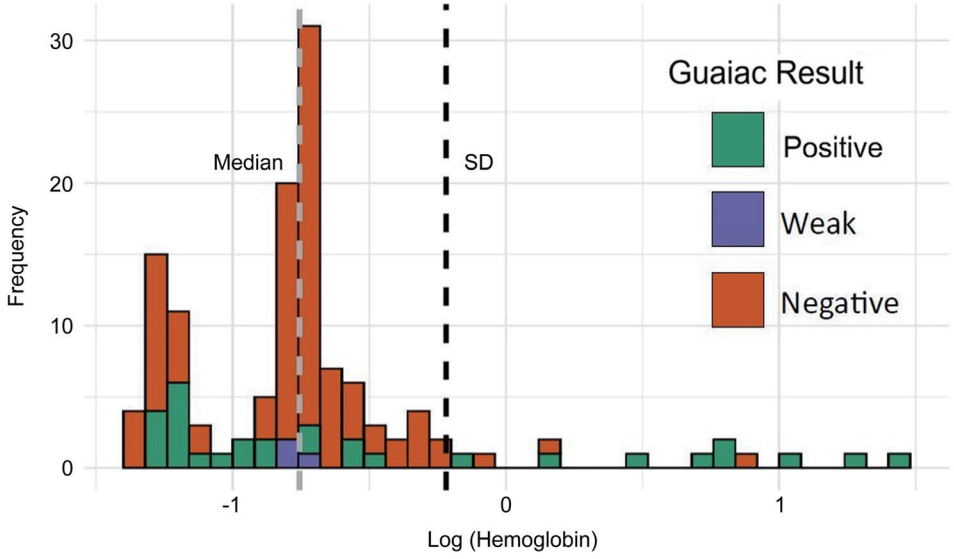

Fig. 2. Visualization of hemoglobin concentration in samples from infants without food protein-induced allergic proctocolitis (FPIAP) compared to guaiac fecal occult blood test (gFOBT) results of the same samples. SD, standard deviation.

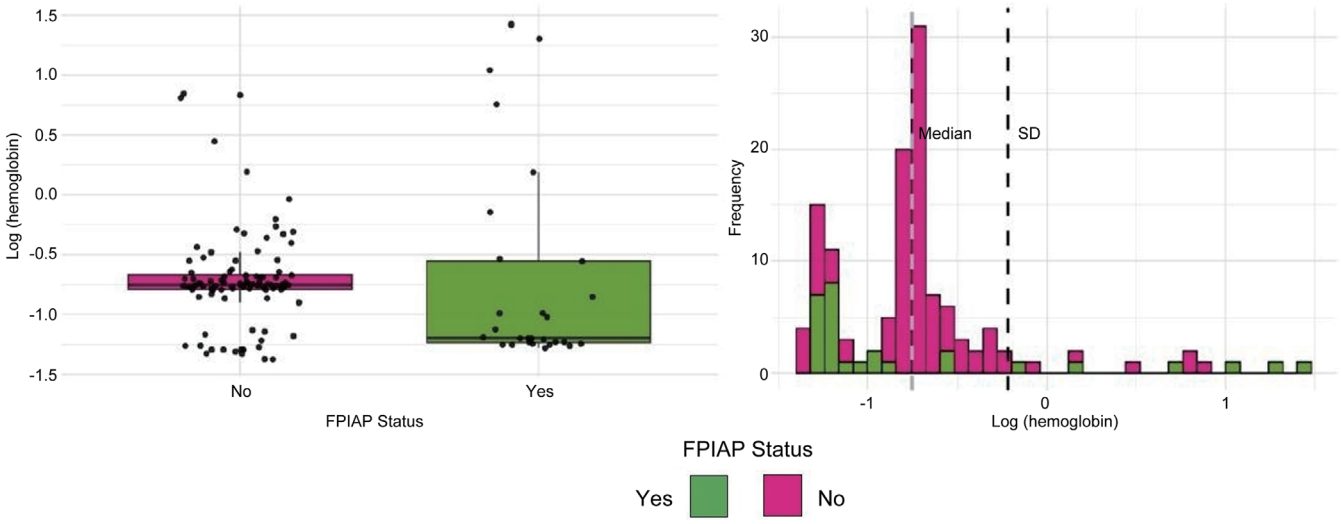

Fig. 3. Fecal immunochemical test (FIT) of hemoglobin in infants with and without food protein–induced allergic proctocolitis (FPIAP).

hemoglobin.[18] These tests detect antibody-human hemoglobin complexes, making them less susceptible to interference from non-human hemoglobin sources.[18,19] However, a meta-analysis of studies on detecting colorectal cancer in patients with iron deficiency anemia has shown no significant difference between gFOBT and FIT.[20] FIT application in diagnosing gastrointestinal disorders involving bleeding in infants and children remains underexplored. We evaluated the performance of a quantitative FIT assay in this infant population, both in healthy asymptomatic infants and in those diagnosed with FPIAP, and found poor correlation between the FIT and gFOBT results. The majority of the samples that were gFOBT positive were FIT negative, and yet (depending on cut-off values) there were more FIT-positive results than positive gFOBT results in the healthy control samples. The poor correlation between FIT and gFOBT could be explained by a number of factors that warrant further investigation: different methodologies to measure heme (FIT is human-specific, guaiac is not), resulting in different causes of false positives and false negatives for each test.

There are several possible explanations for positive FIT and/or positive gFOBT in healthy young infants, including increased permeability of the infant's GI tract leading to small amounts of heme, dysbiosis, maternal blood in breast milk,[21] and previously identified causes of false positives of the tests themselves. While we were not able to distinguish maternal from infant blood in these samples, we did show that there was no association between breastfeeding and positive gFOBT or FIT.

There are several limitations to this study. Samples were previously frozen at −80°C for several years, which likely lowered the sensitivity (however, the number of positive results is therefore likely under- rather than over-reported, making the findings, if anything, more striking).[22] The positive control samples were positive 74% of the time, which may represent a combination of freeze-thaw effects as well as the non-homogenization of stool samples. Because enrollment was closed for several years, we were not able to reproduce these findings in fresh samples, but this is an important area for future research.

Conclusions

In summary, caution should be used in interpreting gFOBT results in young infants, as we found that up to 11% were positive in healthy, asymptomatic infants. More prospective research is needed to understand the role (if any) of gFOBT or FIT testing in this age group, as well as work toward discovery of novel, better-performing noninvasive biomarkers. In the meantime, we advise against using gFOBT as a primary diagnostic tool for CMPA or FPIAP. Instead, we strongly advise following published clinical guidelines in the diagnosis and management of infants suspected to have CMPA or FPIAP, which require an open challenge of the offending food one month after symptoms resolve before confirming the diagnosis and continuing dietary antigen restriction.

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Conflict of interest

Drs. Virkud and Martin were supported by the NIH NIAID (K23AI130408 and K23AI151556, respectively) during this work. Dr. Shreffler reports personal consultant fees from ALK, Allerfund, Allergy Therapeutics, FARE, Milk Care Co., Mabylon, Novartis, Paraxel, Phylaxis, and Third Rock Ventures; and clinical trial agreements with Aravax, DBV, Genentech, Moderna, Novartis, and Regeneron. Dr. Martin is a paid consultant who serves on the Scientific Advisory Board for Milk Care Co. All other authors report no potential conflicts of interest.

Author contributions

Drafting of the initial manuscript (AK, FA), data collection (AK, AJ, TS, IOC), initial analyses (AK, AJ, TS), critically review and revision of the manuscript (AK, AJ, FA, TS, IOC, YV, WS, QY, VM), data visualization (AJ), conceptualization, study design, funding support, resources, and supervision of data collection (VM). All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Ethical statement

The GMAP study was approved by the Massachusetts General Hospital Institutional Review Board (IRB - #2013P002374), in accordance with the Declaration of Helsinki (as revised in 2024), and a parent of all enrolled infants gave written informed consent.

Data sharing statement

The original contributions presented in the study are included in the article and/or supplementary material. Further inquiries can be directed to the corresponding author.

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