Thymic Stromal Lymphopoietin Variation, Filaggrin Loss of Function, and the Persistence of Atopic Dermatitis

David J. Margolis, MD, PhD; Brian Kim, MD; Andrea J. Apter, MD, MSc; Jayanta Gupta, MD, PhD; Ole Hoffstad, MA; Maryte Papadopoulos, MBE; Nandita Mitra, PhD

importance
Atopic dermatitis (AD) is a common chronic illness of childhood.

objective
To evaluate the association between thymic stromal lymphopoietin (TSLP) variation and the persistence of skin symptoms of AD.

Design, Setting, and Participants
A prospective cohort study was conducted in the general community. Participants included 796 children enrolled in the Pediatric Eczema Elective Registry.

exposure
Evaluation of TSLP variation.

main outcomes and measures
Self-reported outcome of whether a child's skin had no symptoms of AD and required no medications for 6 months at 6-month intervals.

results
We evaluated 14 variants of TSLP. The variant rs1898671 was significantly associated with the outcome in white children (P = .01). As measured by overlapping CIs, similar odds ratios (ORs) were noted among whites (OR, 1.72; 95% CI, 1.11-2.66) and African Americans (1.33; 0.52-3.45). Further within the subcohort of individuals with a filaggrin protein (FLG) loss-of-function mutation, those with TSLP variation were more likely to have less-persistent disease (OR, 4.92; 95% CI, 2.04-11.86).

conclusions and relevance
The TSLP variation is associated with less persistent AD. Therefore, TSLP may be a potential therapeutic target for the treatment of AD, especially in individuals with diminished barrier function due to FLG mutations. This is an attractive hypothesis that can be tested in clinical trials.

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Atopic dermatitis (AD) is a common chronic relapsing inflammatory disease of the skin that is seen most often in childhood.1 The development of AD has been associated with genetic polymorphisms, skin barrier dysfunction, environmental exposures, and host immune dysregulation.2,3 Conceptually, the pathophysiology of AD may be associated with a child’s sensitization to specific environmental or food allergens in association with skin barrier dysfunction, which is hypothesized to result from defects in the production of filaggrin (FLG) protein, a key constituent of the granular cell layer.3 In 2006, loss-of-function mutations in the FLG gene (OMIM 135940) were shown to be associated with AD.4-5 Ultimately, FLG proteolysis results in the creation of natural moisturizing factors that are part of the skin barrier. Animal models have shown that a defect in the production of FLG creates a more porous skin surface, resulting in eczematous sensitization to environmental allergens and activating the host immune system, thereby resulting in local inflammation, pruritus, and visible skin lesions.7

In 2010, Barnes8 and Gao et al9 reported an association between thymic stromal lymphopoietin (TSLP) and AD as well as a decreased susceptibility to an infectious inflammatory complication of AD called eczema herpeticum. Thymic stromal lymphopoietin promotes the differentiation of naïve T cells into type 2 helper T cells, a cell type associated with and thought to be pathogenic in atopic diseases.10,11 Increased expression of TSLP has been strongly associated with AD as well as other allergic diseases, including asthma, allergic rhinitis, and food allergy.12-14 Furthermore, a report by Beck et al15 revealed that individuals who developed eczema herpeticum had more severe AD as well as biomarkers consistent with increased allergic inflammation. This led to our hypothesis that genetic variations that result in increased or decreased TSLP activity could be associated with either more severe or milder skin disease.
More specifically, alterations in the activity of TSLP should directly influence the association between FLG and AD persistence. As noted, FLG mutations result in skin barrier dysfunction and are associated with an increased risk of developing AD and persistence of skin symptoms. Because TSLP acts to promote type 2 helper T-cell responses, it is conceivable that people with diminished TSLP expression, even in the setting of skin barrier dysfunction due to an FLG loss-of-function mutation, would be less likely to exhibit active symptoms of AD. The goal of this study was first to evaluate the association between TSLP (OMIM 607003) variation and the persistence of skin symptoms of AD and then to determine whether TSLP variation alters the known association between FLG loss-of-function mutations and the persistence of AD.

Methods

The Pediatric Eczema Elective Registry (PEER) (http://www.thepeerprogram.com) is an ongoing prospective 10-year observational registry that is part of a postmarketing commitment originally by Novartis, and now by Valeant, to the US Food and Drug Administration and the European Drug Agency. The goal of this longitudinal safety study is to determine whether FLG loss-of-function mutations and the persistence of AD. At the time of enrollment, 3.2% of the children had complete disease control, and 10.7% experienced limited disease control, or uncontrolled disease control. "To be symptom free the child had to have complete disease control for the preceding 6-month period. Our evaluation of symptoms was primarily based on pruritus and skin breakdown and may not include other symptoms or signs of AD, such aslichenification, pityriasis alba, xerosis, and keratosis pilaris. Because individuals in this study were monitored longitudinally and surveyed every 6 months, this outcome was reported on more than 1 occasion. We assessed the statistical association between the repeated measures of the binary outcome and each SNP, assuming an additive genetic model within a mixed-effects framework called a generalized linear latent and mixed model (GLLAMM). Because our outcome was binary, the logistic link function was used with a binomial family with an adaptive quadrature. The GLLAMM model has both random-effects and fixed-effects terms, allowing for subject-specific regression coefficients or subject-specific estimates of the association of a risk factor and the outcome. In other words, the GLLAMM models can be more useful if relationships between an outcome and an individual are more important than inferences about the population. The decision to correct or not correct for multiplicity is a complex and complicated issue. There is no universal solution because the decision to adjust a P value depends on the study design and hypothesis. The primary question in our study was the confirmation of previously described SNPs by Gao et al with respect to a new outcome (ie, persistence of AD) and a prespecified hypothesis; therefore, we did not correct for multiple testing. All SNP association tests were conducted separately within the 2 subgroups on the basis of race—white and African American. In addition, a pooled analysis that combined whites and African Americans (with adjustment for principal components derived from ancestry informative markers), as well as stratified analyses based on FLG and asthma status, were conducted. Finally, a meta-analysis was performed to combine SNP association results across races. The meta-analysis was implemented and heterogeneity was assessed using available software. All analyses were conducted using Stata, version 12.1 (StataCorp).

Results

Samples for DNA genotyping were available from as many as 796 children enrolled in PEER. A description of PEER participants who provided DNA and those who did not showed that no clinically important differences exist. With respect to our present study cohort, 51.9% were girls (n = 413), 46.1% were African American (n = 367), the mean (SD) age at PEER enrollment was 7.1 (3.7) years, and participants were monitored for 5.7 (1.4) years or approximately 4799 person-years. At the time of enrollment, 3.2% of the children had complete disease control, 43.9% noted good disease control, 42.2% reported limited disease control, and 10.7% experienced uncontrolled disease.

Table 1 presents the unadjusted association of TSLP tagging SNPs with the persistence of AD over time by race. One
SNP, rs10213865, was not evaluated further because the genotyping call rate was less than 90% and thus was considered inadequate. The rs1898671 SNP was significantly associated with the outcome in white children \( (P = .01) \). As measured by overlapping CIs, similar odds ratios (ORs) were noted among whites (OR, 1.72; 95% CI, 1.11-2.66) and African Americans (1.33; 0.52-3.45); rs764916 also attained statistical significance in whites \( (P = .031) \). However, the ORs were in opposite directions within the white and African American subgroups, and the SNP was not significant in the pooled analysis (white and African Americans analyzed as a single group) with adjustment for covariates.

The Figure shows the frequency of reporting that an individual’s AD was not persistent based on their age in categories at the time of reporting and stratified by the presence or absence of variation in rs1898671. As expected, persistence of AD symptoms decreased as the PEER population became older, but within each age category, those with the rs1898671 variation were less likely to have persistent AD. The results of fur-

**Table 1. Association of TSLP SNPs With Outcome**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Minor Allele</th>
<th>Major Allele</th>
<th>White, OR (95% CI)</th>
<th>No. b</th>
<th>MAF</th>
<th>African American, OR (95% CI)</th>
<th>No. b</th>
<th>MAF</th>
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</thead>
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<td>rs1872753</td>
<td>A</td>
<td>G</td>
<td>0.64 (0.40-1.01)</td>
<td>393</td>
<td>0.288</td>
<td>1.16 (0.58-2.32)</td>
<td>334</td>
<td>0.250</td>
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<tr>
<td>P value</td>
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<td>.67</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>rs1751570</td>
<td>A</td>
<td>G</td>
<td>0.70 (0.39-1.26)</td>
<td>393</td>
<td>0.147</td>
<td>2.15 (0.84-5.49)</td>
<td>334</td>
<td>0.097</td>
</tr>
<tr>
<td>P value</td>
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<td>.11</td>
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<td></td>
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<td>rs3806933</td>
<td>A</td>
<td>G</td>
<td>0.82 (0.51-1.26)</td>
<td>393</td>
<td>0.430</td>
<td>1.42 (0.74-2.71)</td>
<td>334</td>
<td>0.277</td>
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<td>.29</td>
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<td>rs2289276</td>
<td>A</td>
<td>G</td>
<td>0.96 (0.60-1.54)</td>
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<td>0.278</td>
<td>0.99 (0.46-2.10)</td>
<td>333</td>
<td>0.191</td>
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<td>P value</td>
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<td>.97</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>rs1898671</td>
<td>A</td>
<td>G</td>
<td>1.72 (1.11-2.66)</td>
<td>393</td>
<td>0.333</td>
<td>1.33 (0.52-3.45)</td>
<td>334</td>
<td>0.100</td>
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<tr>
<td>P value</td>
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<td>.55</td>
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<tr>
<td>rs10066929</td>
<td>A</td>
<td>C</td>
<td>0.65 (0.38-1.10)</td>
<td>386</td>
<td>0.165</td>
<td>1.90 (0.78-4.60)</td>
<td>330</td>
<td>0.097</td>
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<tr>
<td>P value</td>
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<td>.16</td>
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<tr>
<td>rs11466750</td>
<td>A</td>
<td>G</td>
<td>0.84 (0.50-1.43)</td>
<td>390</td>
<td>0.165</td>
<td>0.94 (0.46-1.92)</td>
<td>334</td>
<td>0.232</td>
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<tr>
<td>P value</td>
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<td>.87</td>
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<tr>
<td>rs2289277</td>
<td>C</td>
<td>G</td>
<td>0.84 (0.55-1.29)</td>
<td>393</td>
<td>0.440</td>
<td>1.26 (0.68-2.33)</td>
<td>335</td>
<td>0.412</td>
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<td>P value</td>
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<td>.46</td>
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<tr>
<td>rs10035870</td>
<td>G</td>
<td>A</td>
<td>0.54 (0.06-5.17)</td>
<td>391</td>
<td>0.009</td>
<td>0.51 (0.19-3.13)</td>
<td>335</td>
<td>0.119</td>
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<td>P value</td>
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<td>.17</td>
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<tr>
<td>rs11466749</td>
<td>G</td>
<td>A</td>
<td>0.96 (0.57-1.64)</td>
<td>393</td>
<td>0.174</td>
<td>2.02 (0.83-4.90)</td>
<td>334</td>
<td>0.112</td>
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<tr>
<td>P value</td>
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<td>.12</td>
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</tr>
<tr>
<td>rs2416259</td>
<td>G</td>
<td>A</td>
<td>0.88 (0.48-1.59)</td>
<td>393</td>
<td>0.144</td>
<td>1.35 (0.32-5.64)</td>
<td>335</td>
<td>0.048</td>
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<td>P value</td>
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<td>.69</td>
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<td>rs764916</td>
<td>C</td>
<td>G</td>
<td>0.44 (0.20-0.93)</td>
<td>387</td>
<td>0.074</td>
<td>1.02 (0.49-2.11)</td>
<td>332</td>
<td>0.202</td>
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<tr>
<td>P value</td>
<td>.03</td>
<td>.95</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>rs11466741</td>
<td>A</td>
<td>G</td>
<td>1.01 (0.64-1.60)</td>
<td>393</td>
<td>0.291</td>
<td>0.76 (0.40-1.44)</td>
<td>334</td>
<td>0.310</td>
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<tr>
<td>P value</td>
<td>.96</td>
<td>.40</td>
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<tr>
<td>rs10043985</td>
<td>C</td>
<td>A</td>
<td>1.49 (0.87-2.55)</td>
<td>392</td>
<td>0.073</td>
<td>1.09 (0.62-1.91)</td>
<td>335</td>
<td>0.197</td>
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<tr>
<td>P value</td>
<td>.15 c</td>
<td>.77</td>
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<td></td>
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</tr>
</tbody>
</table>

**Abbreviations:** MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism; TSLP, thymic stromal lymphopoietin.

* Symptom free for 6 months while not requiring topical medication.

**Figure. Age by Category and the Absence of Symptoms**

The child’s age at the time a survey was received is presented. The individuals were monitored over time and provided more than 1 survey. TSLP is thymic stromal lymphopoietin.
ther analysis of rs1898671 after adjustment for multiple co-

Table 2. Association of rs1898671 With Outcome*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Adjusted[^a]</th>
<th>FLG Wild Type</th>
<th>FLG Loss-of-Function Mutation</th>
<th>Asthma[^a]</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No. OR (95% CI)</td>
<td>No. OR (95% CI)</td>
<td>No. OR (95% CI)</td>
<td>No. OR (95% CI)</td>
</tr>
<tr>
<td>Combined white and African American</td>
<td>732 1.55 (1.01-2.30)</td>
<td>598 1.52 (1.01-2.29)</td>
<td>134 4.92 (2.04-11.86)</td>
<td>732 1.02 (0.81-1.30)</td>
</tr>
<tr>
<td>P value</td>
<td>.03</td>
<td>.046</td>
<td>&lt;.001</td>
<td>.84</td>
</tr>
<tr>
<td>White</td>
<td>398 1.72 (1.12-2.63)</td>
<td>284 1.18 (0.73-1.91)</td>
<td>114 5.68 (2.18-14.82)</td>
<td>398 1.11 (0.81-1.50)</td>
</tr>
<tr>
<td>P value</td>
<td>.01</td>
<td>.48</td>
<td>&lt;.001</td>
<td>.52</td>
</tr>
<tr>
<td>African American</td>
<td>334 1.32 (0.52-3.34)</td>
<td>314 1.53 (0.58-4.05)</td>
<td>20 Not estimated[^b]</td>
<td>334 1.07 (0.64-1.80)</td>
</tr>
<tr>
<td>P value</td>
<td>.56</td>
<td>.39</td>
<td>.78</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: FLG, filaggrin; OR, odds ratio.
*Symptom free for 6 months while not requiring topical medication.
[^a]Adjusted for age of onset of atopic dermatitis, presence of FLG mutation, sex, and ancestry.
[^b]Logistic regression model evaluating rs1898671 as a risk for asthma diagnosis.
[^c]Not estimated because only 5 individuals had FLG loss-of-function mutations and the thymic stromal lymphopoietin (TSLP) variant.

Discussion

Thymic stromal lymphopoietin has been shown[^11,14] to be a master initiator of allergic inflammation. Although FLG protein contributes to the skin barrier, TSLP expression occurs after antigen sensitization through a disrupted skin barrier and subsequently promotes the immune responses that result in diminished TSLP protein activity that is protective in terms of development of cutaneous inflammation and allergy[^11,28]. This hypothesis is further substantiated by the effect of rs1898671 on FLG loss-of-function mutations. Individuals who have the FLG loss-of-function mutation are more likely to have persistent AD[^16]; however, within the subcohort of individuals with that mutation, those who also had an rs1898671 variant were nearly 5 times less likely to have persistent AD compared with those without the variant. Furthermore, previous findings[^3] have shown that persons with increased TSLP activity are more prone to asthma.

The FLG loss-of-function mutations are most commonly found in individuals of European and Asian ancestry vs those of African ancestry[^4]. FLG mutations assayed in the present study are most commonly noted in people of European ancestry and are rarely seen in those of African ancestry[^4,16]. For example, in a previous study[^16] of this cohort, 27% of whites carried an FLG loss-of-function mutation (minor allele frequency, 0.16) compared with only 5.7% of African Americans (minor allele frequency, 0.03). As a result, many studies have focused solely on individuals of European ancestry to diminish concerns about population stratification, which is a form of bias that can result when genetic variation is associated with ancestry[^24]. In the present study, differing rates of carriage of rs1898671 occurred based on ancestry but not to the extreme noted for FLG. In addition, our ultimate question was based on the interaction between TSLP and FLG. We therefore have presented results from a pooled analysis as well as from analyses carried out separately in whites and African Americans.

As is true of all studies, there are limitations to the interpretation of our results. First, children enrolled in the PEER must have received at least 6 weeks (cumulative) of pimecrolimus, 1%, cream in the 6 months prior to entry into PEER. Most children, however, do not continue with this therapy while in PEER[^29]. Pimecrolimus, 1%, cream is approved for use in individuals with mild to moderate AD. It is therefore possible that the results of our study will not generalize to everyone with AD. However, rs1898671 was identified in a cohort of individuals with AD[^9]. The primary interest of that investigation was to study eczema herpeticum, so it is likely that those individu-
tions. These inflammation, diminishing the function of the TSLP protein
activity parallels the assumptions used by those who origi-
finally investigated
may have had more severe AD. However, animportant con-
stitution of our study is the potential confirmation of the in-
terplay between barrier dysfunction and immune activation,
and it is likely that this observation is generalizable. In addi-
tion, we tested the 4 most common European FLG muta-
tions. These FLG mutations have been found rarely in people of African ancestry. It is possible that mutations also exist in
other barrier proteins.30-32 Interactions between these yet-to-
be-identified mutations and the TSLP variation described in
our study could potentially influence the outcome in a differ-
ent manner than that described in the present study. This con-
cern, however, can be addressed only as additional muta-
tions are found. Because all children enrolled in PEER had to
have used pimecrolimus, 1%, cream before entry into the study,
it may be possible that the rs1898671 variation interacts with
pimecrolimus therapy, resulting in an improved outcome. How-
ever, by the third year of enrollment, more than 45% of PEER
participants were no longer using pimecrolimus, so we be-
lieve that this explanation is unlikely.29

We chose to not correct our P values for multiple compari-
sions. This was based on the notion that our study was guided
by a hypothesis established from previously published data.9
Our hypothesis was that diminished TSLP activity would lead
to less persistent AD even in the setting of an FLG loss-of-
function mutation. Our initial interest in diminished protein
activity parallels the assumptions used by those who origi-
nally investigated FLG.4 Because TSLP is a master inducer of
inflammation, diminishing the function of the TSLP protein
should diminish the inflammatory component of AD.10,11 This
hypothesis is consistent with the observation by Gao et al9 with
respect to eczema herpeticum and is also consistent with our
observations (eg, diminished inflammation resulting from di-
minished TSLP activity resulted in fewer cases of eczema her-
peticum and less persistent AD, respectively).10,11,15 A more per-
istent statistical observation would be the probability that 2
independent studies evaluating 14 SNPs achieved the same sta-
tistical results. The probability of finding this observation is
P = .009.

Conclusions
Because rs1898671 is a tag SNP of the TSLP gene, further inves-
tigations, including fine-mapping and functional investi-
gations of the TSLP protein, are needed to determine the causal variant with respect to the persistence of AD. We also did not evaluate whether rs1898671 is associated with the onset of AD. As with many illnesses, agents that cause a disease or affect its severity and persistence are often not the same. Further-
more, we did not study TSLP protein levels in our partici-
pants. It is, however, noteworthy that rs1898671 has now been
validated in at least 2 different cohorts by different investiga-
tors (Gao et al9 and us). Based on our evaluation of the persist-
ence of AD, inhibition or diminution of the effect of TSLP may
be a potential therapeutic target for the treatment of AD, es-
pecially in individuals with diminished barrier function due
to FLG mutations. This is an attractive hypothesis that could
be tested in clinical trials.

REFERENCES

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Study concept and design: Margolis, Apter, Mitra.
Acquisition of data: Margolis, Hoffstad, Papadopoulos.
Analysis and interpretation of data: Margolis, Kim, Apter, Gupta, Mitra.
Drafting of the manuscript: Margolis, Apter, Gupta, Mitra.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Margolis, Gupta, Hoffstad, Mitra.
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Study supervision: Margolis, Mitra.
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