In 2012, blood, skin and soft tissue infections caused by clindamycin resistant *Streptococcus pyogenes* (group A streptococcus; GAS) appeared to be increasing in the Helsinki metropolitan area. We compared monthly percentages of clindamycin resistant isolates in the area between 2012 and 2013, with those in 2010 and 2011. Resistance frequency in terms of patient age was also studied. We reviewed the medical records of bacteraemic cases in 2012 and 2013 and linked the data to *emm* types. To inform on the *emm* distribution among GAS isolated from skin and soft tissue infections during the epidemic, GAS isolates of one month (March 2013) were *emm* typed. For GAS blood, skin, and soft tissue isolates taken together, the proportions of clindamycin resistant isolates were significantly higher in 2012 and 2013 (23% and 17%, respectively) compared with the two previous years (3%, p<0.001). The erythromycin resistance percentages were almost equal to clindamycin (22% and 17%) in 2012 and 2013, respectively. Clindamycin resistance was most frequent in GAS isolates of 40 to 60 year-old patients (148/417; 36%). Among clindamycin resistant isolates, 12 of 14 blood isolates from 2012 to 2013, and 11 of 13 skin and soft tissue isolates from March 2013, were *emm33*. *Emm33* GAS bacteraemia was associated with clindamycin and erythromycin resistance (odds ratio (OR): 7.0; 95% confidence interval (CI): 1.9–25.3). Infection focus was mainly the skin; either cellulitis (7/12) or necrotising fasciitis (3/12). All *emm33* GAS isolates harboured the *ermTR* resistance gene with constitutive macrolides, lincosamides and streptogramines B (MLSβ) phenotype. *Emm33* GAS was responsible for the higher proportion of clindamycin resistance in skin, soft tissue, and blood isolates locally in 2012 and 2013.

**Introduction**

*Streptococcus pyogenes* (group A streptococcus; GAS) causes pharyngitis, skin and soft tissue infections, and invasive septic diseases [1]. Certain GAS *emm* types have been associated with tissue-specific infections [2], antibiotic resistance [3], and local epidemiics [3]. Erythromycin resistance has been linked to various *emm* types, such as 4, 6, 12, 75, and 77 [4-7]. Most of these *emm* types have been identified in throat isolates.

Depending on the erythromycin resistance mechanism, isolates may also be resistant to clindamycin, although relatively rarely [8].

There is limited information concerning clindamycin resistance in GAS isolates causing skin and soft tissue infections. In Finland, the annual percentages of erythromycin and clindamycin resistance was only 2 to 3% in 2012 when all GAS isolates (including throat isolates) were analysed together [9]. The figures for all GAS isolates have been the same also in Helsinki metropolitan area [10]. In February 2013, while making the annual local antibiotic resistance statistics of 2012, a high proportion of clindamycin resistance was noticed among blood, skin and soft tissue GAS isolates.

In this study, we investigated whether a specific *emm* type was behind this phenomenon, by examining the laboratory data of GAS isolates in 2012 and 2013 in the Helsinki metropolitan area. We used baseline data of years 2010 and 2011 for comparison. The invasive GAS cases of 2012 and 2013 were analysed in detail and linked to *emm* types (blood isolates) to characterise common denominators behind the increase in clindamycin resistance. To obtain more information on the *emm* distribution in GAS isolates from skin and soft tissue infections, *emm* typing was performed on a set of such GAS isolates obtained during March 2013 in the Helsinki metropolitan area.
Methods

Setting
In Finland, the Division of Clinical Microbiology at HUSLAB is a clinical diagnostic laboratory that serves the Helsinki metropolitan area of ca 1.5 million population. It receives from the local laboratories all blood cultures flagged positive for bacteria by the BacT/ALERT3D system (bioMérieux, Marcy l’Etoile, France) for bacterial identification and resistance analyses. The GAS blood isolates are routinely stored and sent to the national reference laboratory at the National Institute for Health and Welfare for emm typing. HUSLAB does the final identification and resistance analyses also for all other GAS isolates e.g. throat, skin, and soft tissue isolates of the Helsinki metropolitan area and keeps records of the resistance data for statistical analyses, however the bacterial isolates are not routinely stored. For this study, GAS isolates of skin and soft tissue infections of March 2013 were collected specifically, and stored at -70 °C. This was done to characterise the emm type distribution of both clindamycin susceptible and resistant GAS isolates from skin and soft tissue infections (while the epidemic was still going on), and to verify if the distribution was the same in these isolates compared with the routinely stored blood isolates. We were able to gather 78% (45/58) of the total skin and soft tissue GAS isolates of the month.

Microbiological methods
At HUSLAB GAS isolates are routinely identified by colony morphology with beta-haemolysis on sheep blood agar and Lancefield grouping with latex agglutination (Latex Reagent A, Oxoid Ltd, Basingstoke, Hants, England). The resistance for erythromycin and clindamycin is routinely determined using the double-disc diffusion method on Mueller-Hinton agar with 5% defibrinated horse blood with 20 mg/L beta-NAD (MH-F broth). In this study, additional minimum inhibitory concentrations (MICs) were determined for the clindamycin resistant skin and soft tissue GAS isolates of March 2013 and blood isolates of 2012 to 2013 after twice sub-culturing on horse blood agar. MICs were determined by Etests (bioMérieux SA, Marcy l’Etoile, France) for azithromycin, clindamycin, doxycycline, erythromycin, levofloxacin, moxifloxacin, tetracycline, and vancomycin, on Mueller-Hinton (MH-F) broth using 0.5 McFarland inoculum and incubated for 18±2h with 5% CO2 at 35±1°C. Telithromycin susceptibility was tested by disc-diffusion method in similar conditions as in MIC determinations. European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2013 breakpoints were used to determine whether the zone inhibitions or MICs were considered susceptible (S), intermediate (I) or resistant (R).

Emm typing and resistance gene analysis of the collected group A streptococcus isolates
At the National Institute for Health and Welfare, the 45 GAS isolates of March 2013 and 109 blood GAS isolates from 2012 to 2013 were emm typed according to the guidelines provided by Centres for Disease Control and Prevention (http://www2a.cdc.gov/ncidod/biotech/streplast.asp) as previously described [11].

DNA of the erythromycin and clindamycin resistant GAS isolates (blood isolates of 2012–2013; n = 14 and isolates of March 2013; n = 13) was extracted by suspending the colonies in 100 μl of TE buffer and boiling for 15 min, followed by centrifugation at 13,000 rpm for 2 min. The presence of erm, including ermB and ermTR, and mefA genes was detected by multiplex-polymerase chain reaction (PCR) including primers for amplification of mefA, ermB and ermTR genes, as described previously [12]. Positive controls were S. pyogenes A569 for mefA, Escherichia coli with plasmid pIR229 for ermB, and S. pyogenes A200 for ermTR [13].

Susceptibility data analysis
The susceptibility analyses of clinical isolates were made using WHONET 5.6 software. We analysed the resistance figures of GAS isolates of the Helsinki metropolitan area between January 2012 and December 2013, and compared the data to the baseline years, namely 2010 and 2011. Blood, skin, and soft tissue isolates were analysed separately from throat isolates. One isolate per patient, the most resistant one, was included in the analysis (WHONET definition). Data were expressed as percentage of isolates resistant or intermediate for erythromycin together and as percentage of isolates resistant for clindamycin, according to EUCAST 2013 standard (http://www.eucast.org). The reason for this was that the standard did not include a zone diameter breakpoint for intermediate
for clindamycin, but for erythromycin it did. Inducible clindamycin resistance was detected by antagonism of clindamycin activity by erythromycin (the D phenomemon) and if not present the isolate was reported susceptible. In HUSLAB the detected antagonism was reported resistant for clindamycin.

Analysis of clinical data and statistics
Electronic medical records of patients with a GAS positive blood culture between January 2012 and December 2013 in Helsinki metropolitan area were reviewed to identify underlying conditions and any common exposure between the patients. Age, sex, C-reactive protein (CRP) value, and leucocyte count at the time of diagnosis were registered. Diagnosed diabetes was recorded. Alcohol abuse was defined as a known social or medical problem caused by alcohol noted in the medical records. Intravenous drug abuse was registered similarly when mentioned in the records. Suspected focus of infection was registered. Presence of a cutaneous infection was described as either cellulitis (infections of the skin and underlying tissues such as erysipelas and deeper non-necrotising soft-tissue infection) or necrotising fasciitis (progressive, destructive, subcutaneous streptococcal infection with necrosis observed either directly or under surgery). Need for surgical procedures, complications, and stay at an intensive care unit due to GAS bacteraemia was recorded. Mortality within seven days after GAS positive blood culture was recorded. Data were analysed and compared using Fisher’s exact or Pearson chi-squared tests, or t-test, Mann–Whitney U-test or analysis of variance (ANOVA), when appropriate, using SPSS for Windows statistical package (SPSS Inc., Chicago, IL). Logistic regression analysis was used to identify the risk factors. A p-value < 0.05 was considered statistically significant.

Results

Resistance data of clinical group A streptococcus isolates
When the resistance data were analysed without throat isolates, the proportions of skin, soft tissue, and blood GAS isolates obtained from Helsinki metropolitan area that were clindamycin resistant in 2012 and 2013 were respectively 23% (199/866) and 17% (153/899). The baseline figures were 3% (22/745) in 2010 and 3% (24/734) in 2011 (p < 0.001; 2012 and 2013 figures compared with 2010 and 2011 figures). The proportions of erythromycin intermediate-resistant and resistant isolates were almost equal to those for clindamycin, namely 22% (191/866) and 17% (152/899) in 2012 and 2013, respectively. Baseline proportions of erythromycin intermediate-resistant and resistant isolates were 4% (26/745) and 5% (33/734) in 2010 and 2011 (p < 0.001; 2012 and 2013 figures compared with 2010 and 2011 figures), respectively.

The increase in proportion of isolates with clindamycin resistance began in the spring 2012 and was the highest at 49% (40/82) in October 2012 (Figure 1).

At the end of the study period, in December 2013, still 12% (10/81) of skin, soft tissue, and blood GAS isolates were clindamycin resistant. The proportion of clindamycin resistant isolates varied between age groups, and was highest in the 41 to 50 (35%; 84/238) and 51 to 60 year-olds (36%; 64/179), and lowest among those < 16 years of age (2%; n = 12/569; Figure 2).

The proportion of clindamycin resistant throat isolates remained at the baseline level being 3% (233/8,953), and 4% (354/9,083) in 2012 and 2013, respectively. The proportion of throat isolates which were intermediate-resistant or resistant to erythromycin were also
3% (278/8,953) and 4% (391/9,083) in 2012 and 2013, respectively.

**Emm types, clindamycin resistance, and clinical data of invasive group A streptococcus cases in 2012 and 2013**

A total of 109 GAS positive blood isolates were identified in the Helsinki metropolitan area between January 2012 and December 2013. Figure 3 shows the *emm* type distribution of these invasive isolates.

Of the 109 GAS positive blood isolates, 14 were clindamycin resistant and these included 12 *emm33*, one *emm28*, and one *emm89*. Figure 4 shows the time distribution of the invasive GAS isolates resistant or sensitive for clindamycin with respective resistance genes. None of the *emm33* isolates were susceptible for clindamycin. During the baseline years 2010 and 2011, when clindamycin resistance among isolates was at a low level, no invasive *emm33* GAS were isolated in the Helsinki metropolitan area.

Table 1 compares the clinical data of *emm33* cases to the cases with another *emm* type. In logistic regression analysis clindamycin and erythromycin resistance, alcoholism, and intravenous drug abuse (Table 1) associated with *emm33* GAS bacteraemia. Of the 12 *emm33* cases, 10 had infection focus on the skin or soft tissue. Three *emm33* cases underwent a surgical procedure due to complications of GAS infection. There were no re-infections or need for intensive care in *emm33* cases.

**Emm types and laboratory referral data of skin and soft tissue group A streptococcus isolates of March 2013**

A total of 45 GAS isolates from skin and soft tissue infections were gathered in March 2013 and *emm* typed. *Emm* typing revealed two isolates being *S. dysgalactiae* subsp. *equisimilis* and these were discarded from the analysis. The remaining 43 isolates represented *emm* types shown in Figure 5. Of these, 13 showed clindamycin resistance and these included 11, which were *emm33*. None of the *emm33* were susceptible for clindamycin. Ten *emm33* isolates were from a skin lesion or abscess as shown by the laboratory referral data in Table 2.

![Figure 4](https://www.eurosurveillance.org)

**Figure 4**

Time distribution of invasive group A streptococcus isolates, Helsinki metropolitan area, 2012–2013 (n = 109)

- *Emm33*: constitutive ermTR
- *Emm89*: constitutive ermB
- *Emm28*: inducible ermTR
- Clinda S

Clinda S: isolates susceptible to clindamycin.
Clindamycin resistant isolates with respective resistance genes are shown separately (all *emm33* (n = 12), one *emm89*, and one *emm28*).
Susceptibility and resistance genes of the collected \textit{emm}33 group A streptococcus isolates

All studied \textit{emm}33 GAS (skin and soft tissue isolates of March 2013 and blood isolates of 2012–2013, totally \(n = 23\)) showed the constitutive macrolides, lincosamides and streptogramines B (MLS\(B\)) phenotype with similar antibiotic resistance profiles and harboured the \textit{erm\(TR\)} resistance gene. The isolates were non-susceptible for azithromycin (MIC range: 12–\(> 256\) mg/L), clindamycin (all MICs \(> 256\) mg/L), and erythromycin (MIC range: 2–8 mg/L). They showed susceptibility for doxycycline (MIC range: 0.125–0.38 mg/L), levofloxacin (MIC range: 0.25–0.75 mg/L), moxifloxacin (MIC range: 0.064–0.19 mg/L), tetracycline (MIC range: 0.25–1.0 mg/L), and vancomycin (MIC range: 0.38–0.75 mg/L). All isolates were susceptible for telithromycin (diameter range: 27–38mm), which was tested by disc-diffusion method.

Discussion

During 2012 and 2013 \textit{emm}33 GAS caused a local epidemic of skin and soft tissue infections in the adult population in Helsinki metropolitan area, Finland. The outbreak was detected as a marked increase in the proportion of isolates resistant to erythromycin and clindamycin. In most cases the primary infection focus was the skin, but a few GAS \textit{emm}33 infections were invasive and caused necrotising fasciitis. All \textit{emm}33 isolates were resistant to both erythromycin and clindamycin.

There was an association of \textit{emm}33 with alcohol and intravenous drug abuse, however the number of patients was very low so these results have to be interpreted with caution. Alcohol abuse was marked positive if mentioned in the patient records. Since alcoholism is not always evident and not even always actively asked about by the doctor in the hospital, this information is most probably partly lacking from our data. The same counts for intravenous drug abuse. An association with alcohol abuse has nevertheless been reported for certain other \textit{emm} types, such as \textit{emm}59 and \textit{emm}1 [14,15].

\textit{Emm}33 belongs to the \textit{emm} superfamily group D, which includes \textit{emm} types causing skin infections, such as impetigo [2,16]. \textit{Emm}33 has further been characterised as a member of the D4 \textit{emm}-cluster, which is able to bind plasminogen [17]. Plasminogen binding may contribute to the skin tissue tropism by possible break down of tissue barriers facilitating dissemination and prolonged bacterial persistence in the skin [18].

There is limited data concerning infections caused by this \textit{emm} type. It caused some of the severe GAS infections of intravenous drug users reported in a study in the United Kingdom (UK) in 2003 and 2004, but was
Several mechanisms underlie the macrolide and lincosamide resistance. M phenotype isolates carry the mefA gene, which causes efflux of the antibiotic and confers resistance to many macrolides with preserved susceptibility to clindamycin and streptogramin B [22]. Emm4 GAS with mefA has previously caused high erythromycin resistance locally in Finland [23]. Interestingly, in our study none of the tested isolates carried the mefA resistance gene. The resistance data of the Helsinki metropolitan area showed also that most of the isolates with decreased susceptibility to erythromycin were also clindamycin resistant (either with inducible or constitutive phenotype) suggesting that the M phenotype was not generally present in the GAS isolates during the years 2012 to 2013.

Ribosomal methylation of the target of the antibiotics (ermA, ermB or ermTR) prevents binding of the antibiotics by causing a conformational change in the 23S ribosome [24]. The ermB isolates usually show constitutive MLS\textsubscript{a} (cMLS\textsubscript{a}) resistance to macrolides, clindamycin, and streptogramin B, while the ermA and ermTR isolates may show macrolide-induced resistance to clindamycin [13]. In our study all emm33 isolates shared the ermTR macrolide resistance gene with a cMLS\textsubscript{a} resistance phenotype. This resistance phenotype is more common among ermB but has in rare occasions also been shown by isolates carrying the ermTR gene [25].

All the emm33 isolates were also susceptible to telithromycin. Usually isolates with the cMLS\textsubscript{a} phenotype are resistant or intermediate to telithromycin, however, such phenotypes harbour typically the ermB gene [26]. Accordingly, two isolates in our study (a blood emm89 and a skin isolate of March 2013 emm28) with cMLS\textsubscript{a} phenotype with ermB gene showed intermediate susceptibility to telithromycin (data not shown). In contrast, an isolate with inducible MLS\textsubscript{a} phenotype with ermB (skin isolate of March 2013 emm33) was susceptible for telithromycin. The only isolate with inducible MLS\textsubscript{a} phenotype with ermTR gene (a blood isolate emm28) was susceptible for telithromycin, as were all the emm33 isolates. Similar results have shown Giovanetti et al. regarding inducible ermTR GAS isolates [27]. All the emm33 isolates in our study showed large disc-diameters growth suppression by telithromycin. Unfortunately at the time of investigation, telithromycin Etests were not available in our laboratory, so we were not able to determine MICs, which would have represented more precise data.

The emm33 isolates in our study were also tetracycline susceptible, while Kataja et al. showed that inducible ermTR GAS isolates in Finland in 1994 and 1995 were mostly tetracycline resistant [25]. To fully understand the resistance mechanism underlying emm33, the isolates should be examined in more detail. The fact that

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**Table 2**

Laboratory referral data of the group A streptococcus isolates from skin and soft tissue that were clindamycin resistant, Helsinki metropolitan area, Finland, March 2013 (n = 13)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age groups in years</th>
<th>Emm type</th>
<th>Resistance gene</th>
<th>Infection focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>81–90</td>
<td>33</td>
<td>ermTR\textsuperscript{a}</td>
<td>Decubital wound, sacrum</td>
</tr>
<tr>
<td>Male</td>
<td>31–40</td>
<td>33</td>
<td>ermTR\textsuperscript{a}</td>
<td>Auricular canal</td>
</tr>
<tr>
<td>Male</td>
<td>41–50</td>
<td>33</td>
<td>ermTR\textsuperscript{a}</td>
<td>Suppurative wounds, foot and elbow</td>
</tr>
<tr>
<td>Female</td>
<td>31–40</td>
<td>33</td>
<td>ermTR\textsuperscript{a}</td>
<td>Postoperative wound infection, gynaecological</td>
</tr>
<tr>
<td>Female</td>
<td>51–60</td>
<td>33</td>
<td>ermTR\textsuperscript{a}</td>
<td>Postoperative wound infection, ankle</td>
</tr>
<tr>
<td>Male</td>
<td>41–50</td>
<td>33</td>
<td>ermTR\textsuperscript{a}</td>
<td>Abscess, finger</td>
</tr>
<tr>
<td>Male</td>
<td>17–30</td>
<td>33</td>
<td>ermTR\textsuperscript{a}</td>
<td>Impetigo, perioral</td>
</tr>
<tr>
<td>Male</td>
<td>41–50</td>
<td>33</td>
<td>ermTR\textsuperscript{a}</td>
<td>Postoperative wound infection, finger</td>
</tr>
<tr>
<td>Female</td>
<td>17–30</td>
<td>33</td>
<td>ermTR\textsuperscript{a}</td>
<td>Abscess, leg</td>
</tr>
<tr>
<td>Female</td>
<td>31–40</td>
<td>33</td>
<td>ermTR\textsuperscript{a}</td>
<td>Nasal discharge</td>
</tr>
<tr>
<td>Male</td>
<td>17–30</td>
<td>33</td>
<td>ermTR\textsuperscript{a}</td>
<td>Impetigo, foot</td>
</tr>
<tr>
<td>Male</td>
<td>17–30</td>
<td>92</td>
<td>erm\textsuperscript{b}</td>
<td>Suppurative wound, heel</td>
</tr>
<tr>
<td>Male</td>
<td>61–70</td>
<td>11</td>
<td>erm\textsuperscript{b}</td>
<td>Penile sores</td>
</tr>
</tbody>
</table>

\begin{itemize}
  \item \textsuperscript{a} Constitutive ermTR.
  \item \textsuperscript{b} Inducible ermB.
  \item \textsuperscript{c} Constitutive ermB.
\end{itemize}

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not the most common emm type in that study [19]. Intravenous drug abuse has been shown to be a risk factor for severe disease caused by GAS [20].

GAS is able to cause very local and timely limited epidemics, as shown in intravenous drug users in the UK [19]. Interestingly, in 2012 in our neighbouring country, Sweden, there was an increase of invasive emm1 GAS diseases occurring mostly in patients over 80 years of age [21]. The invasive emm1 numbers have remained stable in Helsinki metropolitan area between 2010 and 2013 indicating that the Swedish epidemic is local or has not reached Finland yet.

The Finnish National Institute for Health and Welfare receives all invasive GAS isolates from Finland for genotyping and strain collection. Since 2007, the main genotyping method has been emm typing. In Finland, the first emm33 invasive GAS isolates were found only in 2012 and they all originated from the Helsinki metropolitan area. From June 2013 onwards sporadic emm33 cases have been found also in other hospital districts, however emm33 still remains an uncommon genotype in Finland (Pieter Smit, personal communication, April 2014).
emm33 isolates had the same resistance gene and similar antibiotic resistance patterns suggests the idea that they belong to the same clone (Pieter Smit, personal communication, April 2014).

Clindamycin is an important drug in the primary health-care, especially, as it is used for treating GAS infections in penicillin allergic patients. The skin and soft tissue infections of intravenous drug abusers and of diabetic patients are often polymicrobial with anaerobic bacteria and staphylococci present making clindamycin the drug of choice for empirical treatment. In invasive, septic GAS infections clindamycin is used in combination with penicillin for better outcome possibly diminishing the bacterial toxin production [28]. Spreading of a skin-tropic emm type with clindamycin resistance is of concern considering the empirical antibiotic treatment of the abovementioned patient groups. An announcement, aimed at the primary care and hospital doctors of the city of Helsinki, was released in spring 2013 by HUSLAB together with the infectious disease specialists of the Helsinki city hospitals concerning the proportion of clindamycin resistant GAS figures. It guided the empirical therapy of adult skin infections recommending that clindamycin should not have been used as monotherapy. The infectious disease specialists of the whole Helsinki metropolitan area were also informed, and additional antimicrobial susceptibility testing was conducted for the clindamycin resistant GAS isolates to find alternative drugs for penicillin-allergic patients. Surveillance of the situation is important because emm33 GAS may spread to children since it is a potential impetigo-causing emm type [2].

The study shows that for resistance statistics it is important to analyse skin and soft tissue GAS separately from the numerous throat GAS. Different emm types are typical for distinct anatomical locations and important resistance phenomena may be masked if isolates are analysed only together. Here we documented a single, local, epidemic of a previously rare emm33 GAS causing skin and soft tissue infections with also invasive cases. This emm type caused rapid changes in macrolide and clindamycin resistance locally in the adult population. These findings had an impact on the empirical treatment of skin and soft tissue infections of the area.

Acknowledgments

We thank the personnel of the Department of Bacteriology for gathering and analysing the GAS isolates. Tuula Randell is thanked for technical assistance with the erm PCR. Pieter Smit is thanked for his comments and revision of the article.

Conflict of interest

None declared.

Authors’ contributions

Anne-Katrine Pesola investigated the medical records of the bacteraemic patients of years 2012-2013 and was mainly responsible for the statistical analyses. Reetta Silvonen made the antimicrobial susceptibility testing (MIC determinations) for the resistant skin, soft tissue, and blood GAS isolates and determined the erm genes of the resistant isolates. Laura Lindholm was responsible for the erm typing of the GAS isolates. Anu Pätäri-Sampo made the original finding of the high resistance figures of GAS isolates in the Helsinki metropolitan area. Pätäri-Sampo designed the study, applied for the permission to run the study in HUSLAB, and was primarily responsible for the writing of the manuscript. All authors contributed substantially to the manuscript, and have seen and approved the final version.

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