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Commentary

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New approaches to eliciting protective immunity through T cell repertoire manipulation: the concept of thymic vaccination

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Abstract

Conventional vaccines afford protection against infectious diseases by expanding existing pathogen-specific peripheral lymphocytes, both CD8 cytotoxic effector (CTL) and CD4 helper T cells. The latter induce B cell maturation and antibody production. As a consequence, lymphocytes within the memory pool are poised to rapidly proliferate at the time of a subsequent infection. The "thymic vaccination" concept offers a novel way to alter the primary T cell repertoire through exposure of thymocytes to altered peptide ligands (APL) with reduced T cell receptor (TCR) affinity relative to cognate antigens recognized by those same TCRs. Thymocyte maturation (i.e. positive selection) is enhanced by low affinity interaction between a TCR and an MHC-bound peptide in the thymus and subsequent emigration of mature cells into the peripheral T lymphocyte pool follows. In principal, such variants of antigens derived from infectious agents could be utilized for peptide-driven maturation of thymocytes bearing pathogen-specific TCRs. To test this idea, APLs of gp_{33-41}, a D^b-restricted peptide derived from the lymphocytic choriomeningitis virus (LCMV) glycoprotein, and of VSV8, a K^b-restricted peptide from the vesicular stomatitis virus (VSV) nucleoprotein, have been designed and their influence on thymic maturation of specific TCR-bearing transgenic thymocytes examined *in vivo* using irradiation chimeras. Injection of APL resulted in positive selection of CD8 T cells expressing the relevant viral specificity and in the export of those virus-specific CTL to lymph nodes without inducing T cell proliferation. Thus, exogenous APL administration offers the potential of expanding repertoires *in vivo* in a manner useful to the organism. To efficiently peripheralize antigen-specific T cells, concomitant enhancement of mechanisms promoting thymocyte migration appears to be required. This commentary describes the rationale for thymic vaccination and addresses the potential prophylactic and therapeutic applications of this approach for treatment of infectious diseases and cancer. Thymic vaccination-induced peptide-specific T cells might generate effective immune protection against disease-causing agents, including those for which no effective natural protection exists.

Introduction

Vaccination has improved healthcare by providing the most cost effective means to prevent disease on a global basis [1,2]. Since the first safe vaccine against smallpox infection was introduced by Sir Edward Jenner more than 200 years ago [3], a myriad of killed or live viral and bacterial vaccines as well as subunit (i.e. component) vaccines have been developed and proven to be highly

Table 2

Interacting proteins encoded at adjacent loci on the *P. horikoshii* OT3 genome

Protein 1		Protein 2	
ORF	Annotation	ORF	Annotation
PH1354	SNO like protein	PH1355	SNZ like protein
PH0487	Chemotaxis protein CheC	PH0490	Chemotaxis protein CheD
PH1978	AtpE, archaeal or vacuolar-type H^+-ATPase subunit E	PH1983	AtpH, archaeal or vacuolar-type H^+-ATPase subunit H
PH0073	Hypothetical protein (Paralog to PH0074)	PH0074	Hypothetical protein (Paralog to PH0073)
PH0812	Hypothetical protein (Paralog to PH0813)	PH0813	Hypothetical protein (Paralog to PH0812)
PH0126	3-isopropylmalate dehydratase	PH0127	Hypothetical protein
PH0353	Hypothetical protein	PH0354	Hypothetical protein
PH0402	Hypothetical protein	PH0403	Hypothetical protein
PH0468	Hypothetical protein	PH0469	Hypothetical protein
PH1025	Hypothetical protein	PH1024	Hypothetical protein
PHS014	Hypothetical protein	PHS013	Hypothetical protein

ORF, open reading frame.

[27-33]. Together with these results, it is reasonable to expect that many of the obtained interactions reflect functional, *in vivo* interactions.

Interacting proteins are likely to be encoded in the same operon [38]; of 49 independent hetero-interactions, we identified 11 hetero-interactions belonging to the same operons. Similar results have also been reported for the PPIs of *Helicobacter pylori*, in which the genomic localization of genes in interacting pairs was used to predict the functions of uncharacterized proteins [16]. Interestingly, we also found that protein pairs encoded in the same operon (marked by red frames in Figure 5) were much more frequent in the hetero-interactions between proteins of the same class than in the hetero-interactions between proteins of different classes (10 to 0, respectively, $P < 0.02$). This result suggests that interacting proteins in the same operon are more likely to evolve at similar rates.

Classifying the *Pyrococcus* proteins according to their homology data enabled us to better annotate them and characterize their interactions. We obtained many protein interactions between the archaea-specific proteins and between the archaea-specific proteins and other classes of proteins. It will be interesting to analyze the structures of such archaea-specific interacting proteins because they may possess novel protein interaction domains. Alternatively, although we did not observe any known domains in these proteins from their primary amino acid sequences, such proteins may possess novel domains that are structurally quite similar to known ones, as suggested by other reports [39,40]. We also found that the number of hetero-interactions between proteins of the same class was significantly more than the expected value. This observation may be explained by postulating that the protein interactions essential for many organisms are preferentially conserved beyond three kingdoms. Such interacting proteins may evolve at similar rates and show slower evolutionary changes than other proteins because substitutions in one protein would result in selection pressure for reciprocal changes in the interacting partners. This postulation has been generally confirmed [41].

Conclusions

We analyzed 960 soluble proteins of *P. horikoshii* OT3 using the mammalian two-hybrid system, and found 107 reliable PPIs. Furthermore, proteins in the identified interactions were classified by ortholog level, and we found a trend that proteins were more likely to interact with proteins within the same ortholog class than with proteins from different classes. Although we could not identify a large amount of protein interactions in our assay, the data are still valuable for several reasons.

We found thirteen unannotated proteins that interacted with previously annotated proteins. These interaction data are useful for predicting the functions of the unannotated proteins from the annotations of their interacting partners; a prediction that could not be achieved by the analysis of operons because most of the protein pairs (12 out of 13 interactions) are not in the same operon. This information is important because many proteins of *P. horikoshii* OT3 have no similar-

Figure 3
Shows how to prevent breast problems when breastfeeding.

patory qualitative research, this study aimed to adapt the international WHO/UNICEF guidelines on HIV and infant feeding and related generic counselling tools to the local social and cultural context of infant feeding and HIV in the Kilimanjaro Region of northern Tanzania. Because infant feeding practices are socially and culturally embedded, community norms and the cultural beliefs and practices of mothers and those who influence them must be taken into consideration in designing an intervention. Tailoring the present educational intervention to the specific needs and characteristics of the study participants helped to ensure that this intervention would be socially and culturally acceptable to the targeted study population, and underscores the importance of formative research in the intervention development process.

Although the utility of applying theoretical frameworks to the design and execution of interventions has been questioned [35], IM provided a useful reference to guide the development of the educational material (job aids) presented in this study, through a dual focus on health promotion theory and empirical evidence obtained through formative research,. Given restrictions on time and other resources, a modified version of IM was applied, which restricted the complexity of the change objectives. Nonetheless, IM was a valuable tool in the development of objectives, methods, strategies, materials and procedures. Through the definition of performance objectives, modifiable determinants and specified learning objectives as outlined in Tables 1 and 2, outcome indicators were identified at both the individual and environmental levels.

To ensure "ownership" of or "buy in" to the intervention by key stakeholders and to position this pilot intervention for subsequent scale up, the development process required the active and strategic participation of all relevant stakeholders, including participation in the initial review of the intervention strategy and technical reviews of the related products. Through the participatory approach prescribed, IM facilitated an active and systematic dialogue with all relevant actors.

Through the needs assessment, the intervention planning and the strategy and job aids development process, a number of questions related to potential impact and sustainability of the intervention emerged. As the IM framework underscores, change is very often the result of change in the behaviour of decision makers and key actors on multiple levels. For example, as documented in the current paper, there is no doubt that a woman's husband, her mother in law and her pMTCT counsellor are important actors in her infant feeding environment. pMTCT as a family issue remains tricky, in particular because of challenges related to the issues of confidentiality and disclo-

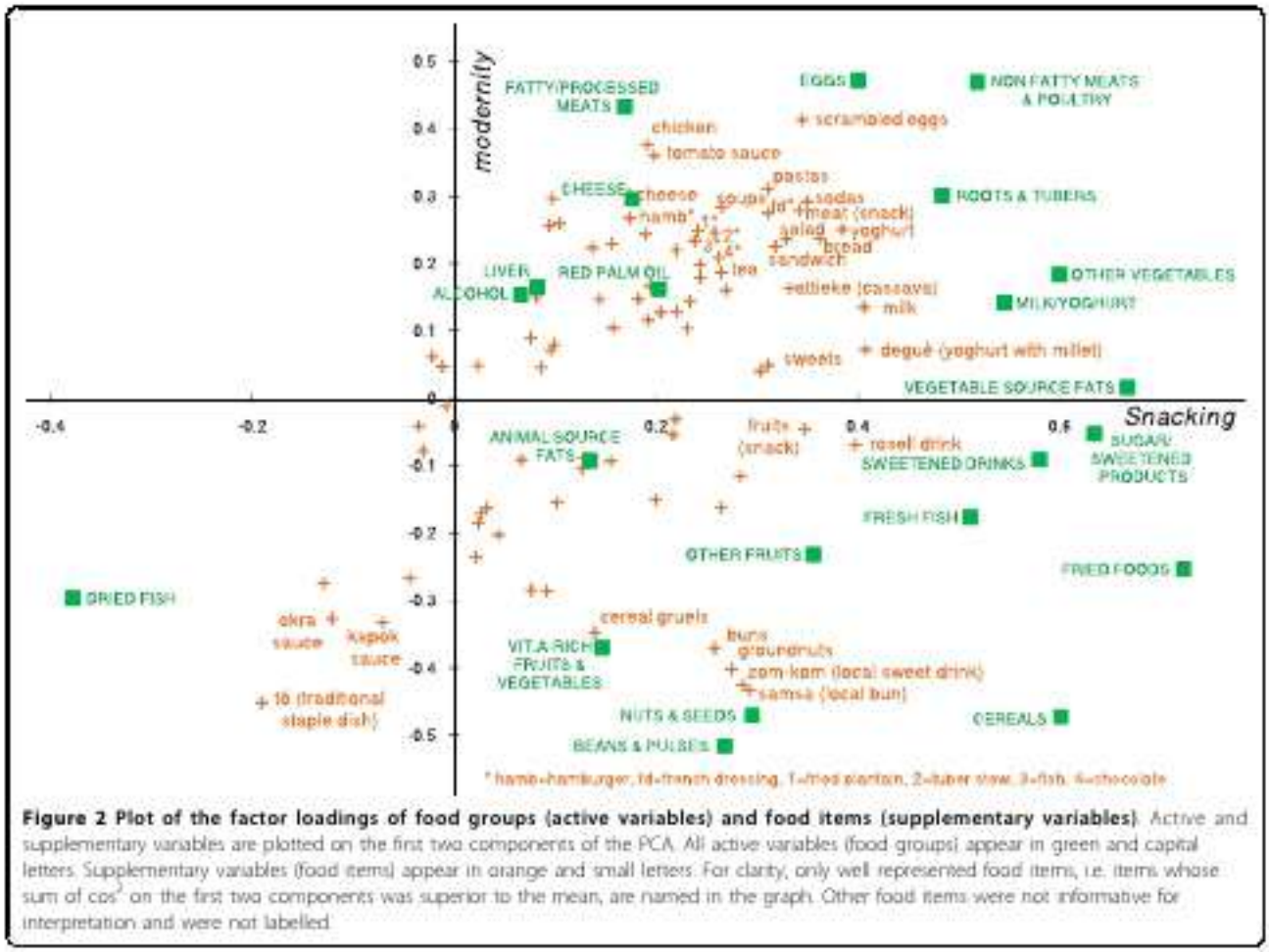


Figure 2 Plot of the factor loadings of food groups (active variables) and food items (supplementary variables). Active and supplementary variables are plotted on the first two components of the PCA. All active variables (food groups) appear in green and capital letters. Supplementary variables (food items) appear in orange and small letters. For clarity, only well represented food items, i.e. items whose sum of cos^2 on the first two components was superior to the mean, are named in the graph. Other food items were not informative for interpretation and were not labelled.

values obtained matched correctly, suggesting that our sample covered a large social and demographic diversity of individuals living in the city. Hence, we assume that our sample also caught a maximum of variety in diets and food patterns that should not be dramatically different from those observed in the whole population of Ouagadougou. Second, our q-FFQ was pre-tested in a population similar to our study sample but was not validated against another dietary method or biomarkers. However, the purpose of this study was to explore food consumption in Ouagadougou in order to draw a very first picture of diets to fill a gap of knowledge in such a setting. The pre-test revealed that our q-FFQ was acceptable and understandable by the subjects. In addition, the food grouping used had been proved to be associated with several socio-economic and demographic characteristics of women of the same sample [20]. It is also often argued that the lack of quantification of food intake is a major limit of the FFQ method. Nevertheless, FFQ has been identified a quick and valid tool to assess dietary consumption that, when combined with factor analysis, allows studying dietary patterns in relation to health outcomes, even without adjustment on energy intake or portion size [18,29]. Given the challenge of estimating portion sizes in such a setting and given the explorative purpose of the study, development of a semi-quantitative version of our questionnaire was not considered. In addition, our q-FFQ was designed to minimise some other sources of bias by means such as the accuracy of the list of foods, the aggregation of frequencies from single items or the frequency options which could be expressed weekly or monthly [30]. The semi-open interview method allowed staying close to what subjects really ate and allowed including all sort of foods even if very specific. Finally, in order to account for the two major co-factors of dietary intake, namely households' economic level and individuals' physical activity, we relied on proxy scores that were not validated as such. However, the economic score was constructed using a standard method recommended by several authors [31-33]. As far as the physical activity score is concerned, we ensured that our proxy was significantly and independently associated with overweight and overfatness, in the expected direction, in the models

TABLE 1. Baseline demographic and clinical characteristics (safety population[a])

	Placebo (n = 96)	Rotigotine (n = 191)
Age, mean (SD); range	64.4 (10.6); 37–86	64.8 (9.3); 37–85
Gender, n (%)		
Male	61 (64)	123 (64)
Female	35 (36)	68 (36)
BMI, mean (SD) kg/m^2; range	26.6 (4.5); 16–41	26.6 (4.1); 16–43
Race, n (%)		
White or Caucasian	85 (89)	177 (93)
Black	1 (1)	2 (1)
Asian	1 (1)	1 (<1)
Other	9 (9)	11 (6)
L-DOPA use, n (%)		
No	17 (18)	36 (19)
Yes	79 (82)	155 (81)
Time since first diagnosis, mean (SD) years; range	4.9 (4.6); 0–26	4.6 (4.2); 0–23
Disease severity at baseline, UPDRS Part III sum score categories, n (%)		
0–9	2 (2)	5 (3)
10–19	12 (13)	38 (20)
20–29	32 (33)	60 (31)
30–39	22 (23)	49 (26)
≥40	28 (29)	38 (20)
UPDRS Part III mean (SD) score[b]	32.0 (13.3)	29.6 (12.3)
PDSS-2 total mean (SD) score; range[b]	20.5 (10.4); 3–49	19.3 (9.3); 1–49

[a]1 subject randomized to placebo received one dose of rotigotine during dose de-escalation and is counted in the rotigotine group for the safety population.
[b]Rotigotine, n = 190.

experienced a sleep attack. One rotigotine-treated subject had positive findings of compulsive sexual behavior on the structured psychiatric interview, which was not reported as an impulse control disorder AE (this subject experienced one mild AE of appetite disorder/decreased appetite). A further 9 subjects (placebo, 2 [2%]; rotigotine, 7 [4%]) had a positive result on at least one mMIDI module.

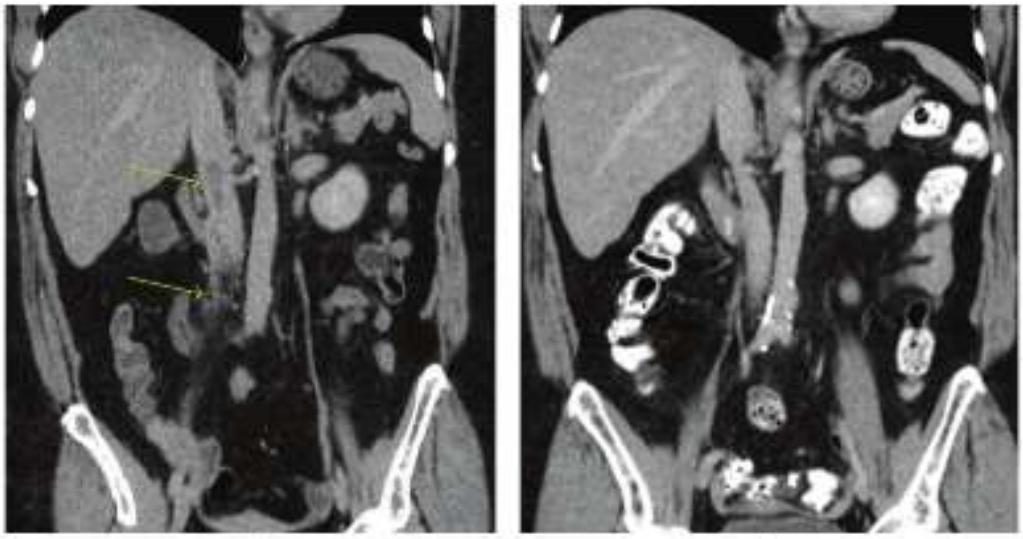
Most reported AEs were mild or moderate in intensity (placebo, 96%; rotigotine, 97%). In both treatment groups 6% of subjects discontinued due to any AE. Five (3%) rotigotine-treated subjects discontinued due to application/instillation site reactions, none of which was considered serious. There was no clear relationship between rotigotine dose and application site reaction rates. Serious AEs were reported by 5 (5%) placebo-treated subjects and 10 (5%) rotigotine-treated subjects; only visual hallucination in 1 rotigotine-treated subject led to study withdrawal. Two subjects, both in the placebo group, died during the study (one committed suicide and the other died of pneumonia aspiration). There were no clinically relevant changes in vital signs or ECG findings, and few laboratory test values of clinical relevance.

Discussion

RECOVER is the first large-scale, double-blind, randomized trial to investigate early morning motor function and sleep as coprimary outcome measures in PD. In this study, 24-hour transdermal rotigotine treatment was associated with significant benefits versus placebo in the management of early morning motor impairment and nocturnal sleep disturbances. Rotigotine was also associated with improvements in night-time disabilities (such as limb restlessness, immobility, pain, and cramps), and possibly dopaminergic nonmotor daytime symptoms (such as fatigue and mood) as well.

Other dopamine agonists and continuous L-dopa/DDCI infusion have been used successfully for treating early morning motor disabilities.[3,17,18] The treatment difference of 3.55 points on the UPDRS Part III seen in this 4-week study is consistent with treatment differences of 3.91 and 3.82 points on the UPDRS Part III at the end of 11 weeks of treatment with rotigotine at doses of 6 mg/24 hr and 8 mg/24 hr in an earlier dose-ranging study.[19] Likewise, as for rotigotine in the current study, improvements from baseline of approximately 7 points on the UPDRS Part III have been described over periods of 6–24 weeks for cabergoline,[3] L-dopa continuous infusion,[17] and ropinirole controlled release.[18]

The clinimetrics of the PDSS are well established[21] and validated in several studies across the world in independent populations.[22–25] In this study, a modified version of the PDSS—the PDSS-2[11]—was used; this modified scale was developed to better reflect treatment effects on nocturnal disabilities and has been



(a) (b)

Figure 1: (a) Septic deep vein thrombosis of the right ovarian vein with extension into the inferior vena cava. Dilation of the right ovarian vein, with a filling defect and air bubble, is seen in the proximal right ovarian vein, surrounded by fat stranding (lower arrow). This filling defect extends into the IVC at the level of the renal veins (upper arrow). These findings are consistent with septic deep thrombophlebitis of the proximal right ovarian vein with extension into the IVC. (b) 2.5 months later, there has been a complete resolution of septic thrombophlebitis of the right ovarian vein.

was discharged home in stable condition after a 5-day hospitalization; she was continued on oral antibiotics (amoxicillin and clindamycin) for 10 days, as well as oral anticoagulation (warfarin) for 3 months. The patient was followed up as an outpatient and was clinically doing well with no complaints and had remained persistently afebrile; her international normalized ratio (INR) remained therapeutic. A repeated CT two and a half months later showed a complete resolution of the septic thrombophlebitis of the right ovarian vein and IVC, Figure 1(b). Also there has been a complete resolution of the pulmonary nodule which may suggest septic emboli to the lung versus upper respiratory infection at presentation.

2. Background

Streptococcus constellatus is a species of group C nonhemolytic viridans streptococci. It is gram-positive, nonspore forming, nonmotile, catalase-negative cocci. *Streptococcus anginosus*, *Streptococcus intermedius*, and *Streptococcus constellatus* together make up the anginosus group (formerly milleri group). This group was first found in dental abscesses in 1956 [1]. It is usually considered part of the normal flora of the respiratory, gastrointestinal, and genitourinary tract. However, it can cause systemic purulent infections. Septic thrombophlebitis can be one of its rare clinical presentations, as was the case with our patient. Due to their nonspecific symptoms, including fever and lack of physical findings, early diagnosis of septic thrombophlebitis requires high clinical suspicion as well as the use of appropriate imaging techniques [2]. We present a rare case of septic pelvic thrombophlebitis caused by *Streptococcus constellatus* in an immunocompetent 60-year-old female.

3. Discussion

Septic pelvic thrombophlebitis is an uncommon entity, with approximately 4 cases occurring per year as seen in one study [13]. Furthermore, cases of septic pelvic thrombophlebitis caused by *S. constellatus* are extremely rare. When it is seen, *S. constellatus* is more common among intravenous drug abusers [5, 14]. Up to our knowledge, including our case, there have been eleven cases of septic thrombophlebitis reported in the literature where *S. constellatus* was involved either alone or among other bacteria; see Table 1. The majority of cases were located in the head and neck (7 out of 11); of those, cavernous sinus thrombophlebitis was the most commonly reported location. The remaining four cases were located in the intra-abdominal and pelvic area. Besides this case, three other cases were described: septic iliofemoral thrombophlebitis in an IV drug abuser [5], septic thrombosis of the portal vein and its branches (pylephlebitis) as a complication of diverticulitis [3], and a case of appendicitis that was complicated by portal pylephlebitis [4]. Predisposing factors for those eleven cases were variable.

Other bacteria, such as *Staphylococcus aureus*, are more commonly associated with septic thrombophlebitis. In a small study, *Staphylococcus aureus* was found in 4 out of 7 patients who had septic thrombophlebitis, while other bacteria isolated included coagulase-negative *Staphylococcus* species, *Proteus mirabilis*, and *Propionibacterium* species [14]. *Streptococcus constellatus* has been associated with other infectious presentations including septic shock after tooth extraction [15], mitral and aortic valve endocarditis [16], and subdural empyema [17].

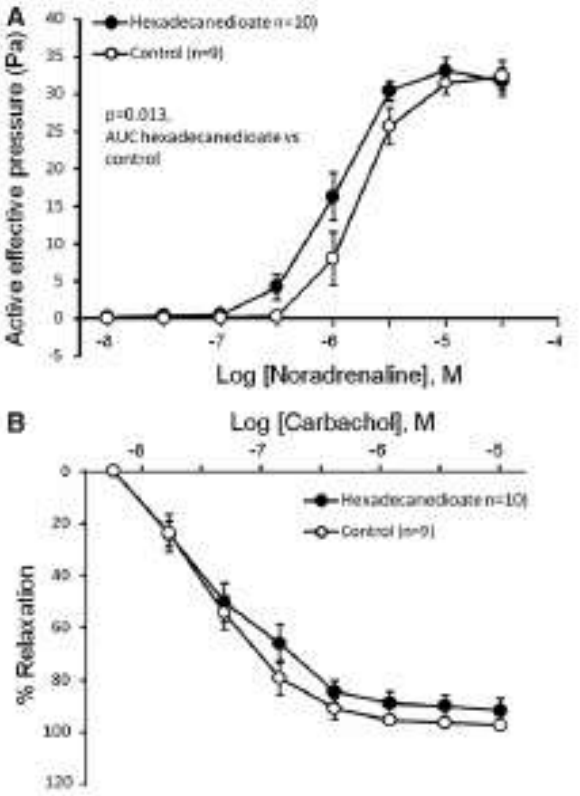


Figure 2. Mesenteric resistance artery contractile response to noradrenaline (**A**) and relaxation to carbachol (**B**) in control (n=9) and hexadecanedioate treated (n=10) WKY rats. AUC indicates area under the curve.

dihomo-linoleate (20:2n6) in larger cohorts with more events is warranted before further functional studies are conducted. The lack of a detrimental effect on survival for caffeine levels reflects the acute effect of caffeine on blood pressure, which varies with intake. We had a priori excluded individuals with renal impairment in our analysis and adjusting for estimated glomerular filtration rate did not alter the BP hexadecanedioate association. There is evidence that long-chain fatty acids, such as docosahexaenoic acid influence BP potentially through an effect on large-conductance Ca(2+)- and voltage-activated K+ (BK) channels,[21] but this was not evident in our study.

We performed 3 in vivo experiments to establish causality of hexadecanedioate on BP regulation. In the first, after oral intake of hexadecanedioate, normotensive WKY rats showed an increase in circulating hexadecanedioate levels along with an increase in BP, both of which were statistically significant. In the second experiment, we measured hexadecanedioate levels in the SHRSP rat before and after administration of salt, which resulted in a further increase in BP but no change in circulating hexadecanedioate levels. Interestingly hexadecanedioate levels in the SHRSP rats were higher than that of WKY rats at baseline, and levels were similar between SHRSP and posthexadecanedioate WKY rats. We used radio-telemetry to provide confirmation of our tail-cuff plethysmography data and to determine the time course of the hexadecanedioate-induced rise in blood pressure. From the radio-telemetry data, the increase in SBP and mean arterial pressure is not immediate after hexadecanedioate administration, with separation of SBP and mean arterial pressure between control and hexadecanedioate-treated animals occurring after day 15 of treatment. This suggests that the mechanism by which hexadecanedioate affects BP is probably through secondary downstream pathways activated by higher hexadecanedioate levels. The increased vascular reactivity to noradrenaline shown by mesenteric arteries of hexadecanedioate-treated rats point to a vascular mechanism underlying the association of hexadecanedioate and blood pressure. Although these are preliminary data, overall they provide strong corroborative evidence of a causal role for hexadecanedioate in modulating blood pressure and support further research in validating and elucidating the mechanisms.

Hexadecanedioic acid is a long-chain dicarboxylic acid, which is generated during fatty acid ω-oxidation and thence metabolized by β-oxidation in peroxisomes. ω-oxidation is a minor metabolic pathway that occurs in the endoplasmic reticulum and also contributes to 5% to 10% of total fatty acid metabolism in the liver. ω-Oxidation is increased in conditions that are characterized by increased levels of mono-carboxylic free fatty acid (obesity, starvation, diabetes mellitus, and chronic alcohol consumption), as well as disturbances in β-oxidation.[22,23] A lack of carnitine can lead to increased ω-oxidation.[24] In our data, we find that levels of hexadecanedioate are indeed negatively correlated with carnitine (β=−0.05; 95% confidence interval, [−0.08 to −0.01]; P=0.01).

Multiple strands of evidence from related pathways point to potential mechanisms that can inform future studies to dissect the hexadecanedioate BP association. Nonesterified fatty acids reduce Na^+K^+-ATPase activity in vascular smooth muscle cells through increase in intracellular Na^+ and decrease of passive Na^+/Ca^{2+} exchange or through partial depolarization of cell membrane and activation of voltage-dependent Ca^{2+} channels resulting in increased intracellular Ca^{2+} concentration and a relative elevation of vascular tone, thus promoting the development of hypertension.[25,26]

Interestingly, a recent report showed multiple dicarboxylic acids, including hexadecanedioate, to be significantly accumulated in pulmonary arterial hypertension tissues indicating a disruption of β-oxidation and an increase of ω-oxidation in this condition and pointing to a putative role in elevating pressure in both the systemic and the pulmonary circulations.[27] Indeed metabolic dysfunction and acquired mitochondrial abnormalities leading to abnormal glycolytic and fatty acid metabolism are now recognized as a potential biological mechanism leading to both pulmonary vascular remodeling with aberrant cellular proliferation and apoptosis, and in the development of right ventricular failure.[28,29] Fatty acid metabolism could offer a novel therapeutic pathway, which would potentially target both pulmonary vasculature and the right ventricle.

Some of the specific functional effects of hexadecanedioate have been studied using β,β′-tetramethylhexadecanedioic acid (MEDICA 16), which is not metabolized and hence the effects of downstream metabolic products that may mask the effects of hexadecanedioate are minimized. MEDICA 16 has been shown to be effective as a hypolipidemic and antiobesity/anti-insulin resistance agent in experimental models,[30,31] and has a liver-specific calorigenic-thyromimetic action characterized by a decrease in liver phosphate potential and liver redox potential with an increase in oxygen consumption, but

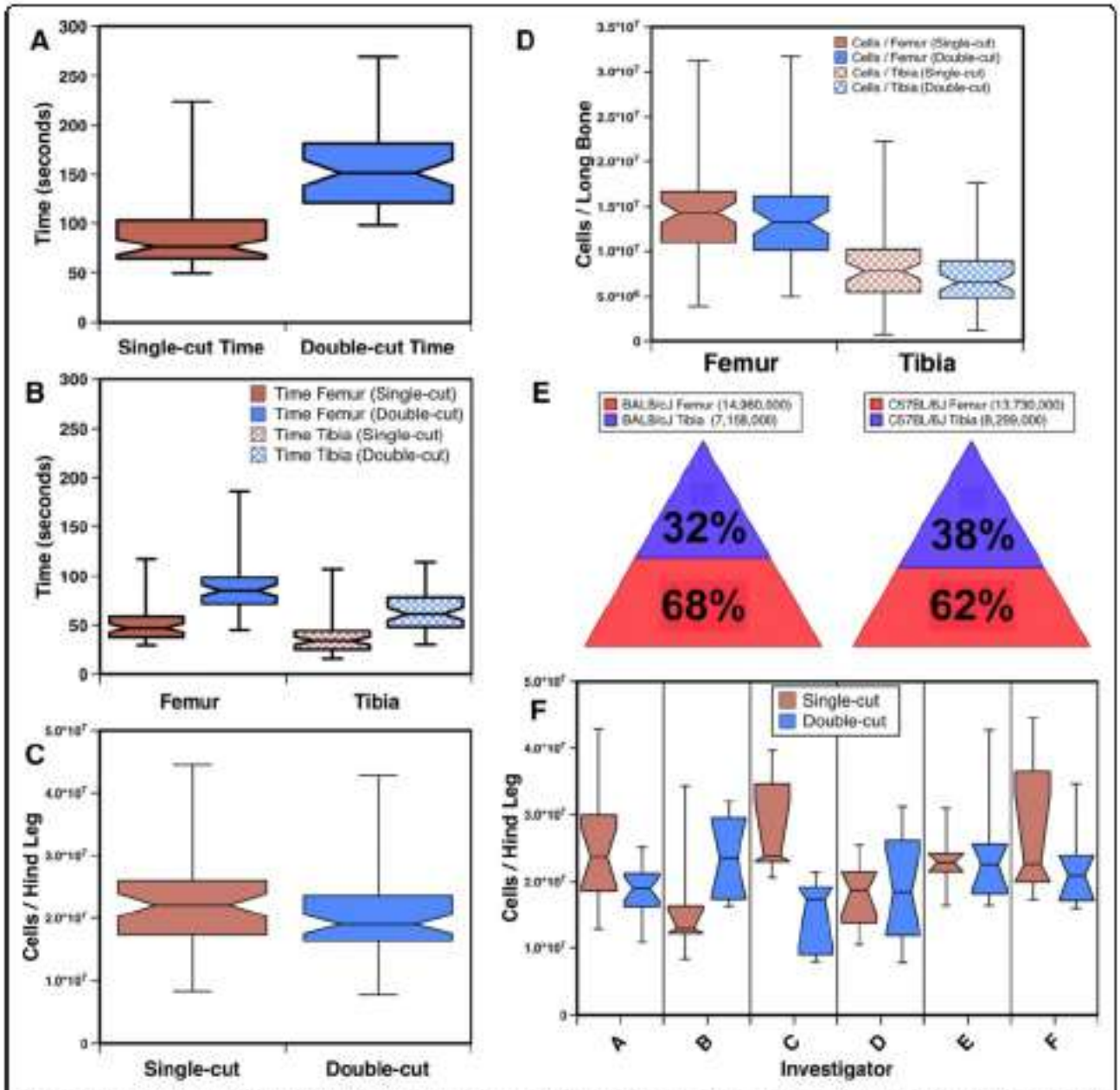


Fig. 3 Comparisons of mouse cBM isolations using the single-cut and double-cut methods. The time required to isolate cBM by the two methods is shown for both the femur and tibia (**a**) and for each of the long bones individually (**b**). The number of cells isolated by the two methods from both long-bones is shown in (**c**) and cell yields for the individual bones are shown in (**d**). The percentages of tibial and femoral BM cells recovered from BALB/cJ (n = 19) and C57BL/6J (n = 37) mice are shown (**e**). The median number of cells recovered from each bone using the single-cut method are indicated in the legends. Cell yields are shown by investigator in (**f**). Refer to Table 1 for the number of harvests performed and the level of experience of the investigator

within the marrow cavity affected the ability to flush the bones.

Comparison of hematopoietic precursors yields from fetal human cBM and eBM

The eBM compartment was also examined in human midgestation long bones. Four specimens of fetal human long-bones were used to isolate cBM and eBM and the recovered cells were analyzed by flow cytometry to determine the yield of HSCs as well as other BM cell populations. In 3 of 4 experiments, eBM cells represented a consistent 7 % of all BM cells recovered (Fig. 5). However, in one experiment, 57 % of cells were recovered from the eBM fraction. We attribute this outlier to the older age of the specimen and the fact that all the long bones found in the legs and arms were processed instead

Impact, scalability, and sustainability can be thought of as an equation where maximum impact, scale, and sustainability are achieved by having the maximum number of stakeholders adopt the maximum number of open practices.

Case Example 1: China

The global food system has changed dramatically as multinational supermarkets and their procurement channels have rapidly expanded into emerging markets. Consumers are demanding safe, high-quality food. In response, governments and industry are collaborating to assure quality and food safety consistently around the world. One area of focus has been the development of protocols and training for suppliers and people responsible for food safety compliance.

Starting in 2008, the Food Safety Knowledge Network (FSKN) a collaboration between Michigan State University (MSU), the Global Food Safety Initiative (GFSI) of the Consumer Goods Forum, and other food industry and public sector partners began strengthening the food industry's response to the complex food safety knowledge and training challenges that affect emerging markets by providing free access to high-quality, standardized OERs[4]. These OERs, for basic and intermediate levels of food manufacturing, are based on competencies developed by the Consumer Goods Forum. Their Global Markets Working Group has defined company characteristics of suppliers which have been used by MSU to create OER and proprietary pre- and post-tests. These are also based on the global and country-specific standards.

GFSP's 5-year work program of demand-driven food safety capacity building and advisory services for low and middle income countries was preceded by an initial programming and preparatory year (2012) that included implementation of a training program developed in partnership with the Asia Pacific Economic Cooperation (APEC) and other partners, on food safety prerequisites and Hazard Analysis & Critical Control Points (HACCP) delivered in Beijing in June, 2012. This program was comprised of 3–4 weeks of online learning followed by a 6 day intensive face-to-face session (with real-time live translation) focused on skills development. More recently in the summer of 2013 a similar program was conducted in Shanghai.

These programs are making use of existing OERs, building on those developed by FSKN/MSU, in a range of formats from PowerPoint presentations to more narrative and full curricula developed in partnership with APEC and the World Bank. Content for the Basic Global Markets Training Program (Archived at http://www.webcitation.org/6Y28HOsqN) is now up to version 2 and version 3 China specific translations.

The cumulative build-out of this work has established a global food safety knowledge base made up of knowledge assets and knowledge experts as depicted in Figure 2.

GFSP's current focus on China as a priority is building on this predecessor work. The main effort will be focused on generating economic growth by building out food safety knowledge and competencies of the estimated four hundred thousand food manufacturers and suppliers in China. The plan is to scale up use of existing open resources and roll out a program using a train-the-trainer approach in the fall of 2014.

The China work involves the formation of partnerships including:

- funders - such as United Nations Industrial Development Organization (UNIDO), Global Food Safety Partnership (GFSP), World Bank, International Finance Corporation and others
- nonprofits - U.S. Pharmacopeial Convention, Grocery Manufacturers Association Foundation
- universities - Shanghai Jiao Tong University

Additional China-based partners are still being established.

The business model for the China train-the-trainer program is to reuse existing open educational resources and make little to no upfront investment in training materials. Public investment is being sought to support the initial train-the-trainer delivery. Downstream delivery to food manufacturers and suppliers would entail participants paying a fee.

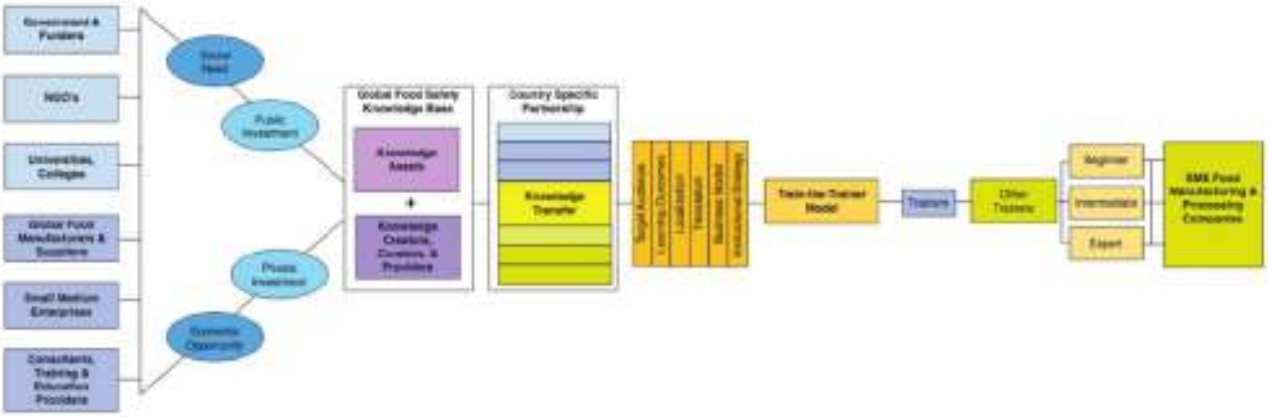


Figure 2. Case Eexample of China. This figure provides an overview of collaborators, resources and approaches being implemented in China.

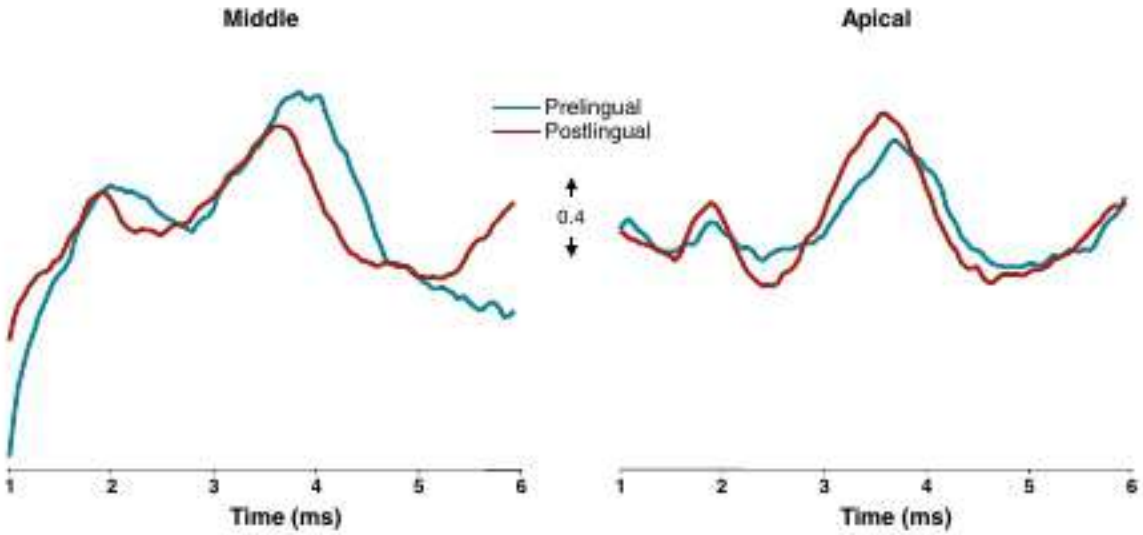


FIG. 3. Grand average eABR waveforms measured at Cz for all subjects in both groups, presented for the two stimulation electrode locations separately. The *blue and red traces* represent the waveforms of the prelingual and postlingual groups, respectively. Waveforms were first corrected for stimulus artifact by fitting a first-order polynomial and subtracting it from the signal and then they were normalized by dividing the signal by the difference in amplitude between the top of wave V and its preceding trough.

wave V latency (r=0.516, $F_{(1,18)}$=6.535, P=0.020). Implant experience was not associated with wave V latency on this electrode (P=0.846; Fig. 5B). Contrarily, implant experience was found to be the only significant predictor of wave V latency on the middle electrode (r=–0.665, $F_{(1,16)}$= 12.693, P=0.003; Fig. 5A). Group was not a significant predictor of wave V latency on this electrode (P=0.887). When examining both groups separately, wave V latency was significantly correlated to implant experience in the prelingual group (r=–0.706, P=0.034; Fig. 5A), but not in the postlingual group (r=–0.324, P=0.395). Previous studies on eABRs in children demonstrated that the wave V latency as function of CI experience could be best described by an exponential decay (Gordon et al. 2006; Thai-Van et al. 2007). If we describe the wave V latencies on the middle electrode in the prelingual subjects as an exponential, it would yield a time constant of about 4 years.

Wave V latency was not correlated to age at implantation on either tested electrode. Moreover, the other preoperative patient characteristics presented in Table 1 were not a factor determining wave V latency. Within the prelingual or postlingual group the wave V latency was not significantly correlated to age at onset of deafness.

The III–V interval was not associated with age at implantation, age at onset of deafness, or implant experience on either electrode location.

Relationship Between eABRs and Speech Perception

As demonstrated in Figure 2, typical eABRs exhibiting both waves III and V could be recorded in almost all subjects irrespective of their speech perception scores

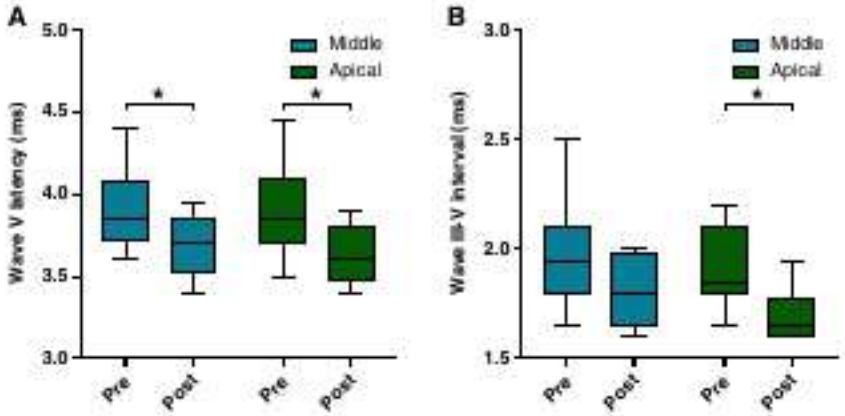


FIG. 4. Wave V latencies (*A*) and III–V interwave intervals (*B*) of the prelingual (pre) and postlingual (post) groups presented for the two stimulation electrode locations. The *box plots* represent the lower and upper quartile with the median. *Whiskers* indicate the 5–95 percentiles. *P<0.05.

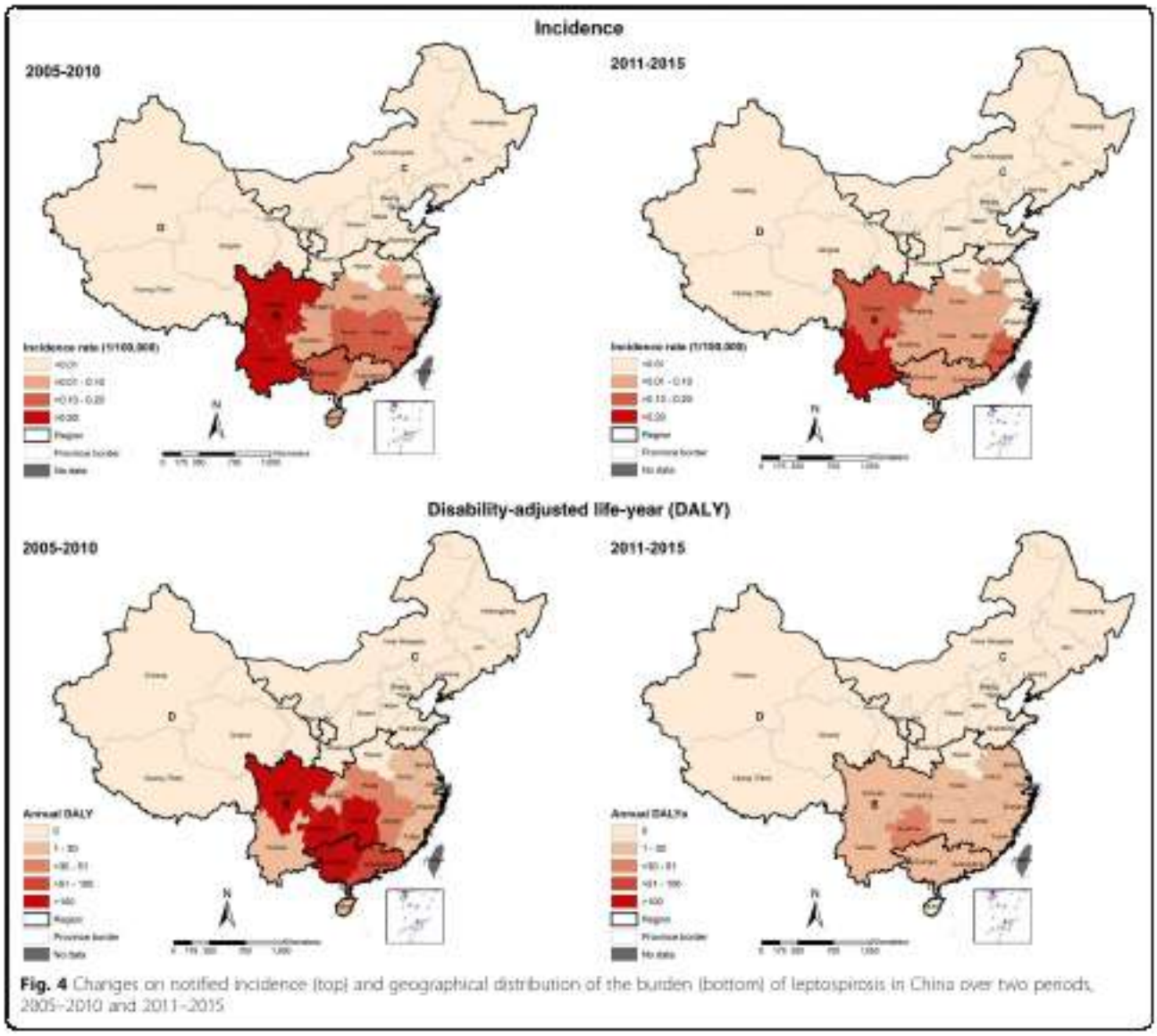


Fig. 4 Changes on notified incidence (top) and geographical distribution of the burden (bottom) of leptospirosis in China over two periods, 2005–2010 and 2011–2015

following outbreaks of the severe acute respiratory syndrome (SARS) in 2003 have helped efficiently improve the timeliness, completeness, and coverage of the data across China as well as facilitates early detection of diseases outbreaks [16]. However, it has been confirmed that during 2005–2015, there has been no significant change in the surveillance systems, specifically for leptospirosis. While, in terms of the vaccination programme, human leptospirosis vaccine has been developed since 1958 and until now it has been administered to high-risk populations in China during the epidemic seasons. A multivalent inactivated vaccine is currently the only available in China [29].

Changes in ecological and social conditions that have been underway in China in the past 20 years may also have played an essential role in leptospirosis epidemiology. Changes in the landscape, agricultural practices and livestock husbandry, for instance, restriction on livestock herding, farming commercialization, and pigs or livestock vaccination [10, 30] that happened in China could have impacted the transmission rate of leptospirosis in China. Industrialization, for example, has led to significant epidemiological shifts in rural areas through the introduction of agricultural technology and mechanization which might reduce the rate of human exposure to the *Leptospira*-contaminated environment. Also, we had noticed that there were significant anthropogenic ecological changes following the development of the Three Gorges Dam and the nationwide reforestation programme called "Grain for Green", which probably had an effect of leptospirosis transmission. Water impoundment in many endemic areas has been known to have an impact on rodents' habitat and population dynamics of the pathogen