Genetic Deletion of AEG-1 Prevents Hepatocarcinogenesis

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Abstract
Activation of the oncogene AEG-1 (MTDH, LYRIC) has been implicated recently in the development of hepatocellular carcinoma (HCC). In mice, HCC can be initiated by exposure to the carcinogen DEN, which has been shown to rely upon activation of NF-xB in liver macrophages. Because AEG-1 is an essential component of NF-xB activation, we interrogated the susceptibility of mice lacking the AEG-1 gene to DEN-induced hepatocarcinogenesis. AEG-1–deficient mice displayed resistance to DEN-induced HCC and lung metastasis. No difference was observed in the response to growth factor signaling or activation of AKT, ERK, and β-catenin, compared with wild-type control animals. However, AEG-1–deficient hepatocytes and macrophages exhibited a relative defect in NF-xB activation. Mechanistic investigations showed that IL6 production and STAT3 activation, two key mediators of HCC development, were also deficient along with other biologic and epigenetics findings in the tumor microenvironment, confirming that AEG-1 supports an NF-xB–mediated inflammatory state that drives HCC development. Overall, our findings offer in vivo proofs that AEG-1 is essential for NF-xB activation and hepatocarcinogenesis, and they reveal new roles for AEG-1 in shaping the tumor microenvironment for HCC development. Cancer Res; 74(21); 6184–93. ©2014 AACR.

Introduction
Hepatocellular carcinoma (HCC) is a highly fatal disease with mortality running parallel to its incidence (1). In the majority of cases, HCC arises in a setting of chronic inflammation, such as infection with HBV or HCV, alcoholism, and nonalcoholic fatty liver disease (1, 2). NF-xB is a key transcriptional regulator of the inflammatory response and plays an essential role in regulating inflammatory signaling in the liver (3, 4). NF-xB activation is a frequent and early event in human HCC of viral and non-viral etiologies and has been attributed to the acquisition of transformed phenotype during hepatocarcinogenesis (5–9). Both HBV X protein (HBX) and HCV core protein have been shown to activate NF-xB by multiple mechanisms (7, 10, 11). Many patients with advanced liver disease present with increased levels of LPS, resulting in activation of NF-xB in the liver (12).

Fatty acids may also activate NF-xB in patients with nonalcoholic fatty liver disease (13). The role of NF-xB in HCC development and progression has been interrogated in several mouse models. Mdr2−/− mice develop spontaneous cholestatic hepatitis and HCC (3). Overexpression of a nondegradable mutant IkBα that blocks NF-xB activation significantly inhibited HCC progression in this model (3). Hepatocyte-specific knockout of IKKβ abrogated HCC development in a transgenic mouse overexpressing lymphotoxin α and/or β (14). These findings indicate that NF-xB activation in hepatocytes is necessary in inflammation-induced HCC. In contrast, hepatocyte-specific knockout of IKKβ promoted HCC development in N-nitrosodimethylamine (DEN)-initiation model, and hepatocyte-specific knockout of IKKγ (NEMO) resulted in spontaneous development of HCC (15, 16). Reactive oxygen species-induced JNK and STAT3 activation have been suggested to promote HCC in IKKβ knockout model and IKKβ has been suggested to inhibit both hepatic injury and proliferation (17). However, deletion of IKKβ in macrophages significantly abrogated DEN-induced HCC (15). In addition, genetic deletion of IL6 or inhibition of inflammatory cytokines, such as TNFα, provided a significant reduction in tumor load (18). Injury to hepatocytes, such as those caused by DEN, leads to release of IL1α that activates NF-xB in liver macrophages (Kupffer cells) with subsequent release of cytokines, such as IL6 (18–20). IL6 promotes proliferation and survival of hepatocytes by activating STAT3 signaling (18). Thus, NF-xB activation in the tumor microenvironment plays a fundamental role in hepatocarcinogenesis. Astrocyte elevated gene-1 (AEG-1), also known as Metadherin (MTDH) and LYRIC, is an oncogene that is overexpressed...
in all cancers (21). AEG-1 overexpression is detected with the progression of cancer, especially in the aggressive metastatic stage, and negatively correlates with poor survival and overall adverse prognosis (21). In vitro studies and investigations using nude mice xenograft and metastatic models with diverse cancer cell lines documented that AEG-1 overexpression induces an aggressive, angiogenic, and metastatic phenotype, whereas knockdown of AEG-1 inhibits proliferation and invasion and markedly abrogates tumor growth and metastasis (22–25). AEG-1 plays an important role in regulating hepatocarcinogenesis. We documented that AEG-1 is overexpressed at both mRNA and protein levels in a high percentage (>90%) of patients with HCC and a significant percentage of patients harbored genomic amplification of the AEG-1 locus in chromosome 8q22 (22). AEG-1 is transcriptionally regulated by c-Myc (26), an oncogene frequently upregulated in HCC (27). The tumor suppressor miRNA miR-375, which is downregulated in patients with HCC, targets AEG-1 (28). Thus AEG-1 overexpression occurs by multiple mechanisms in patients with HCC. HCCs with more microvascular invasion or pathologic satellites, poorer differentiation, and tumor–node–metastasis stages II to III are prone to exhibit higher AEG-1 expression (29). Patients with HCC with high AEG-1 expression documented higher recurrence and poor overall survival (29, 30). Overexpression of AEG-1 in a poorly aggressive HCC cell line HepG3, which expresses low level of AEG-1, significantly increases in vitro proliferation, invasion and anchorage-independent growth and in vivo tumorigenesis, angiogenesis, and metastasis in nude mice (22). Conversely, knockdown of AEG-1 in highly aggressive QGY-7703 cells, expressing high levels of AEG-1, significantly abrogates in vivo tumorigenesis (22, 31). We have shown that transgenic mice with hepatocyte-specific overexpression of AEG-1 (Alb/AEG-1) do not show spontaneous HCC but develop highly aggressive angiogenic HCC with significantly accelerated kinetics upon treatment with DEN when compared with their wild-type (WT) counterparts (32). AEG-1 overexpression profoundly modulates expression of genes associated with proliferation, invasion, chemoresistance, angiogenesis, and metastasis in both human HCC cell lines and Alb/AEG-1 hepatocytes (22, 32).

Multiple prosurvival signaling pathways, such as NF-κB, PI3K/AKT, Wnt/β-catenin, and MEK/ERK, become activated upon overexpression of AEG-1 in human cancer cells and Alb/AEG-1 hepatocytes (22, 32). Pharmacologic and genetic inhibition studies have elucidated the importance of all these signaling pathways in mediating AEG-1–induced oncogenesis (22). However, apart from NF-κB, the molecular mechanism by which AEG-1 activates these signaling pathways is not known. More importantly, whether AEG-1 is required for activation of these pathways under physiologic conditions has not been investigated. We have documented that AEG-1 directly interacts with the p65 subunit of NF-κB and CBP thereby functioning as a bridging factor between NF-κB and basal transcriptional machinery promoting NF-κB–induced transcription (33, 34). A recent study has documented that AEG-1, anchored on the ER membrane, associates with upstream ubiquitinated activators of NF-κB, such as RIP1 and TRAF2, facilitating their accumulation and subsequent NF-κB activation (35).

In the present study, we analyzed the response of AEG-1 knockout (AEG-1KO) mouse to DEN-induced HCC development and progression. Our experiments unravel a fundamental role of AEG-1 in regulating NF-κB activation, especially in the tumor microenvironment, thereby rendering AEG-1KO mice to be significantly resistant to initiation and progression of HCC.

Materials and Methods

Mouse model

AEG-1KO mouse was generated in C57BL/6:129/Sv background and the procedure is described in detail in the Supplement. We have backcrossed the line to C57BL/6 for 10 generations and obtained similar results for both the WT and AEG-1KO mice on the C57BL/6:129/Sv background as on the C57BL/6:129/Sv background. AEG-1KO mice were viable and fertile, although litter sizes were very small (1–2 pups per litter). Furthermore, even litters generated by crossing AEG-1+/- breeding pairs were very small (2–3 pups per litter), which precluded generating large numbers of WT and AEG-1KO mice as littermates. Therefore, the majority of the experiments were carried out with age-matched mice generated by breeding WT and AEG-1KO mice separately. However, it should be noted that the same phenotypes were observed in AEG-1KO mice generated from AEG-1KO X AEG-1KO matings as from AEG-1KO X AEG-1KO matings. Thus, our findings are not restricted to strains or littermates. All animal studies were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University (Richmond, VA), and were conducted in accordance with the Animal Welfare Act, the PHS Policy on Humane Care and Use of Laboratory Animals, and the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training.

Cell culture

Primary mouse hepatocytes were isolated as described (32) and were cultured in Williams E Medium containing NaHCO3, L-glutamine, insulin (1.5 μmol/L), and dexamethasone (0.1 μmol/L) at 37°C and in 5% CO2. For isolating primary peritoneal macrophages, mice were injected intraperitoneally with 4% thioglycollate and 4 days later macrophages were harvested in PBS via intraperitoneal injection. Macrophages were cultured in DMEM with 10% FCS at 37°C and in 5% CO2.

Transient transfection and luciferase assay

For transfections, 1 × 10⁶ hepatocytes were plated in 24-well collagen-coated plates and the next day, transfected using Promofectin-Hepatocyte Transfection Reagent in 1:1 ratio of NF-κB luciferase reporter plasmid and Renilla luciferase reporter plasmid. After 48 hours, cells were treated with LPS (200 ng/ml) for 24 hours. Luciferase assays were measured using Dual Luciferase Reporter Assay Kit (Promega) following the manufacturer’s protocol, and firefly luciferase activity was normalized by Renilla luciferase activity. Each experiment was performed.
performed in triplicates and three times to calculate mean and SE.

**Total RNA extraction, cDNA preparation, and real-time PCR**

Total RNA was extracted from hepatocytes, macrophages, or mouse tissues using the QIAGEN miRNAeasy Mini Kit (QIAGEN). cDNA preparation was done using ABI cDNA Synthesis Kit (Applied Biosystems). Real-time PCR (RT-PCR) was performed using an ABI ViiA7 fast real-time PCR system and TaqMan gene expression assays according to the manufacturer’s protocol (Applied Biosystems).

**RNA sequencing**

Total RNA, extracted using Qiagen miRNAeasy mini kit (Qiagen) from livers of 3 adult mice per group, was used for RNA sequencing (RNA-Seq). RNA-Seq library was prepared using Illumina TruSeq RNA Sample Preparation Kit and sequenced on Illumina HiSeq2000 platform. RNA-Seq libraries were pooled together to aim about 25 to 40 million read passed filtered reads per sample. All sequencing reads were aligned with their reference genome (UCSC mouse genome build mm9) using TopHat2 and the Bam files from alignment were processed using HTSeq-count to obtain the counts per gene in all samples. The counts were read into R software using DESeq package and plot distributions were analyzed using Reads Per Kilobase Million (RPKM) values. Data were represented as the mean ± SEM and analyzed for statistical significance using ANOVA followed by Newman–Keuls test as a posthoc test.

**Results**

**Aged AEG-1KO mice do not develop spontaneous tumors**

We generated an AEG-1KO mouse in which the promoter region, exon 1 and part of intron 1 of the AEG-1 gene was deleted using a Cre-loxP system. The authenticity of AEG-1 knockout was confirmed by Southern blotting, genomic PCR, TaqMan qRT-PCR, Western blot analyses, and IHC (Supplementary Fig. S1). AEG-1KO mice were viable and fertile and AEG-1 in regulating tumor invasion (Fig. 1C). Marked infiltration of macrophages, evidenced by staining for macrophage marker F4/80, was observed in the aged (16-month-old) WT liver but not in AEG-KO liver (Fig. 1D), as well as in aged WT spleen but not in AEG-1KO spleen (Supplementary Fig. S3). No difference in infiltration of neutrophils, as evidenced by Ly6G staining, was observed between aged WT and AEG-1KO mice (data not shown), suggesting that aging-associated chronic inflammatory responses are blunted in AEG-1KO mice.

**AEG-1KO mice are resistant to experimental hepatocarcinogenesis**

WT and AEG-1KO mice were given a single intraperitoneal injection of DEN (30 μg/g) and tumorgenesis was monitored at 32 weeks. Compared with WT mice, AEG-1KO mice showed profound resistance to DEN-induced HCC. AEG-1KO mice either developed no tumor or the tumors were very small (<2 mm; Fig. 2A and Supplementary Table S3). WT livers presented with AFP-positive HCC with vascular invasion (arrow in Fig. 2B) and high AEG-1 expression, while the liver architecture was preserved in AEG-1KO mice (Fig. 2B). Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were significantly higher in WT mice than compared with AEG-1KO mice, indicating liver damage (Fig. 2C). One important aspect of AEG-1 is its ability to induce metastasis. We therefore tested a more aggressive experimental procedure where tumorgenesis was induced by injection of DEN (10 μg/g) and then it was promoted by providing phenobarbital (PB; 0.05%) daily in drinking water. Tumorgenesis was monitored at 28 weeks. WT mice exhibited an intensified hepatocarcinogenic response evidenced by large necrotic liver tumors with a 52% rate of lung metastasis (Table 1 and Fig. 2D). AEG-1KO mice remained remarkably resistant even to this combinatorial treatment with no distant metastasis. Histologic analysis of liver demonstrated HCC in WT mice (Fig. 2E). Although some level of dysplasia was observed in AEG-1KO mice, frank HCC was not detected. The metastatic nodules in the WT lung were positive for AFP, indicating their origin in the liver (Fig. 2F). Staining for macrophage marker F4/80 showed significant infiltration of macrophages both in DEN- and DEN/PB-treated WT tumors but not in AEG-1KO mice (Fig. 2G). Quantification of macrophage infiltration is shown in Supplementary Fig. S4. We stained DEN-treated WT and AEG-1KO liver for α-smooth muscle actin (α-SMA) as an indicator of activation of stellate cells and fibrogenic response. A substantial increase in α-SMA staining was observed in the tumor in WT mice when compared with nontumor region (Supplementary Fig. S5). α-SMA staining in AEG-1KO liver was similar to that in the nontumor region of WT liver.

A possible cause of the pronounced resistance of AEG-1KO mice to DEN-induced HCC might be improper metabolism of DEN in these mice so that DEN is not capable of adequately damaging hepatocytes. To rule out this possibility, we injected WT and AEG-1KO 2 weeks old pups with DEN and then...
measured serum liver enzymes 48 hours later. Both WT and AEG-1KO mice showed significant induction of liver enzymes, indicating that DEN could damage both hepatocytes in a similar manner (Supplementary Table S4).

**Growth factor signaling is not affected in AEG-1KO mice**

Activation of prosurvival signaling pathways, such as PI3K/AKT, MEK/ERK, and β-catenin, have been shown to play a role in mediating oncogenic effects of overexpressed AEG-1 in human HCC cells as well as in Alb/AEG-1 mice (22, 32). We treated WT and AEG-1KO hepatocytes with EGF (50 ng/mL) and analyzed temporal activation of EGFR, AKT, and ERK1/2. Both WT and AEG-1KO hepatocytes showed similar kinetics and magnitude of activation, suggesting that under physiologic condition, AEG-1 does not modulate growth factor signaling (Fig. 3A). No difference was observed in the activated (phosphorylated) forms of AKT, ERK1/2, and β-catenin in adult WT and AEG-1KO liver samples under basal condition (Fig. 3B). These results indicate that AEG-1 is not required for physiologic regulation of AKT, ERK1/2, and β-catenin. We next tested activation of these signaling pathways in DEN-treated WT and AEG-1KO liver samples (Fig. 3C). No significant difference was observed in the activation of AKT, ERK1/2, and β-catenin in the two groups. In naïve mice, inhibition in activated STAT3 and p65 NF-κB, known regulators of HCC, was observed in AEG-1KO livers versus WT (Fig. 3D). Upon DEN treatment, there was further induction of both p-p65 NF-κB and p-STAT3 in WT mice but not in AEG-1KO mice (Fig. 3D). These findings were confirmed by IHC in DEN-treated liver samples (Fig. 3E). Upon DEN-treatment, a significant increase in IL6 protein level was observed in liver homogenates of WT mice but not in AEG-1KO mice (Fig. 3F).

**NF-κB activation is abrogated in AEG-1KO mice**

We measured NF-κB luciferase reporter activity in primary hepatocytes isolated from WT and AEG-1KO mice. Both basal and LPS-induced luciferase activity was significantly blunted in AEG-1KO hepatocytes when compared with WT hepatocytes.
(Fig. 4A). As a corollary, LPS-induced phosphorylation (Fig. 4B) and nuclear translocation (Fig. 4C) of the p65 subunit of NF-κB and induction of NF-κB-target genes IL1β and IL6 (Fig. 4D) were significantly abrogated in AEG-1KO hepatocytes versus WT hepatocytes. Because NF-κB activation in macrophages is crucial for HCC, we next analyzed peritoneal macrophages isolated from WT and AEG-1KO mice. AEG-1 mRNA expression in macrophages was significantly higher compared with...
that in hepatocytes (Fig. 5A). In primary hepatocytes, AEG-1 is localized predominantly in the nucleus, whereas in macrophages, it is located both in the nucleus and in the cytoplasm (Fig. 5B). Upon LPS treatment nuclear translocation of p65, NF-κB was substantially abrogated in AEG-1KO macrophages compared with WT macrophages (Fig. 5C). LPS-mediated induction of IL6 and IL1β was also markedly blunted in AEG-1KO peritoneal macrophages versus WT (Fig. 5D and E). We collected conditioned media (CM) from LPS-treated WT and AEG-1KO hepatocytes and treated WT and AEG-1KO macrophages with the CM. CM from WT hepatocytes induced IL6 mRNA expression only in WT macrophages but not in AEG-1KO macrophages (Fig. 5F). In addition, CM from AEG-1KO hepatocytes failed to induce IL6 mRNA in either WT or AEG-1KO macrophages.

To extend these observations further, we performed RNA-sequencing analysis using liver samples from WT and AEG-1KO mice. Using cutoff of log2 fold change of 1.5 or 1/C0.15, 597 genes showed differential change, out of which, 247 genes were upregulated and 350 genes were downregulated in AEG-1KO liver versus WT liver (Supplementary Table S5). These differentially changed genes were analyzed using Ingenuity Pathway Analysis software. The data were analyzed to identify the upstream regulators the activation or inhibition of which might lead to alterations in downstream genes. An activation z-score >2 indicates activation and a score of <−2 indicates inhibition. The most significant inhibition (P < 0.02) was observed for genes downstream of IL6, IL1B, TNF family, IL17RA, and NF-κB complex (Supplementary Table S6). These upstream regulators were analyzed for regulator effects to predict functional endpoints. It was observed that collective inhibition of these genes leads to suppression of movement of myeloid cells and decreased activation of granulocytes (Fig. 6A). These analyses further support our hypothesis that inhibition of activation of myeloid cells is the major mechanism for resistance of AEG-1KO mice for developing HCC.

### Discussion

The observation that Alb/AEG-1 mice do not develop spontaneous HCC (32) prompted us to hypothesize that AEG-1 is not able to transform hepatocytes; hence, it is not required for initial development of HCC. In human HCC cells, overexpression of AEG-1 or knockdown of AEG-1 markedly affects invasion, angiogenesis, and metastasis, and in comparison, the effects on cell proliferation is significant but small (22). In breast cancer cells, modulation of AEG-1 does not affect proliferation at all, rather all phenotypes are reflected in more aggressive behavior, such as invasion and metastasis (23, 24). These findings lead to the conclusion that the primary role of AEG-1 is to promote aggressive behavior and owing to its profound effect on metastasis, it was named Metadherin (24). Our studies using AEG-1KO mice demonstrate for the first time that even after a mutagenic effect, such as by DEN, AEG-1 is required for initial development of the tumor, at least in the context of HCC. We document that abrogation of NF-κB signaling in hepatocytes and the tumor microenvironment cells, such as macrophages, might be the underlying mechanism that prevents paracrine

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signaling from macrophages to stimulate mutated hepatocytes to proliferate. Upon DEN treatment, a significant decrease in activated NF-κB and STAT3 and IL6 levels was observed in AEG-1KO livers versus WT providing supporting evidence for our hypothesis. In the short-term experiment, DEN was able to cause liver damage to both WT and AEG-1KO mice, indicating that the tumor-inhibitory effect observed in AEG-1KO mice is not because of ablation of DEN effect. Damaged hepatocytes release factors, such as IL1α, that activate NF-κB in macrophages, leading to secretion of IL6 that stimulates STAT3 activation in hepatocytes promoting their proliferation (19). In AEG-1KO mice, inhibition of NF-κB activation in macrophages stalls the subsequent processes, therefore, profoundly abrogating initial development of the tumor (Fig. 6B). This scenario might be applicable to other cancers as well because chronic inflammation is a core component of almost all cancers. Indeed, AEG-1KO mice show low basal level of inflammation, which might protect them from spontaneous tumorigenesis as observed in aged WT mice.

Multiple studies have documented the important roles of PI3K/AKT, MAPK, and Wnt/β-catenin signaling pathways in mediating oncogenic functions of overexpressed AEG-1 (22, 25, 36). However, AEG-1KO hepatocytes do not show abrogated response upon EGF stimulation when compared with WT, and no difference was observed in the activated status of AKT, ERK1/2, and β-catenin in the livers of WT and AEG-1KO mice under basal condition or upon DEN treatment. These observations might be explained by the localization of AEG-1 in normal hepatocytes versus HCC cells. In normal hepatocytes, AEG-1 is almost exclusively located in the nucleus (32). In cancer cells, overexpressed AEG-1 is monoubiquitinated, which facilitates its cytoplasmic accumulation (32, 37). In the cytoplasm, overexpressed AEG-1 might exert promiscuous interaction with other signaling molecules leading to their activation, a function that is attributed to oncogenic AEG-1 but not under physiologic condition.

The observation that AEG-1 is required for NF-κB activation in macrophages has profound implications in diverse physiologic and pathologic states. Activation of NF-κB pathways
dendritic cells is essential for their optimal functioning, including antigen processing and presentation (38). Using the human promonocytic cell line U937, it was documented that LPS induces AEG-1 and this induction is required for subsequent NF-κB activation (39). In addition, NF-κB activation is required for LPS-induced AEG-1 induction thus establishing a positive

Figure 4. NF-κB activation is inhibited in AEG-1KO hepatocytes. A, NF-κB luciferase reporter activity was measured in WT and AEG-1KO hepatocytes. Firefly luciferase activity was normalized by Renilla luciferase activity. The activity of empty pGL3-basic vector was considered as 1. RLU, relative luciferase units. Data represent mean ± SEM of three independent experiments. †, P < 0.01. B, WT and AEG-1KO hepatocytes were treated with LPS for the indicated time points and Western blotting was performed for the indicated proteins. C, immunofluorescence followed by confocal microscopy of WT and AEG-1KO hepatocytes after LPS treatment for 30 minutes showing p65 nuclear translocation. D, WT and AEG-1KO hepatocytes were treated with LPS for 4 hours and the mRNA level of Il1b and Il6 was measured by TaqMan Q-RT-PCR. Data represent mean ± SEM of three independent experiments. †, P < 0.01.

Resistance of AEG-1KO Mice to HCC

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feedback loop between AEG-1 and NF-κB. NF-κB activation is a key requirement for generating interferon-induced antiviral immunity. Thus, AEG-1 might be a key component regulating immune function. In addition, AEG-1 might be a key regulator of chronic inflammatory diseases.

In summary, our studies unravel a novel and important role of AEG-1 in regulating inflammation and activation of cells in the tumor microenvironment. AEG-1KO mice will be a valuable tool to interrogate in detail the role of AEG-1 in physiologic regulation of immunity and inflammation and diseases generated from deregulation of these systems.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: C.L. Robertson, D. Sarkar
Development of methodology: C.L. Robertson, S. Ghosh, D. Sarkar
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.L. Robertson, R. Gredler, L. Emdad, D. Rajasekaran, M. Akiel, C. Guo, S. Giashuddin, X.-Y. Wang, S. Ghosh, M.A. Subler, J.J. Windle, D. Sarkar
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.L. Robertson, J. Srivastava, L. Emdad, D. Rajasekaran, M. Akiel, S. Giashuddin, X.-Y. Wang, M.A. Subler, D. Sarkar
Writing, review, and/or revision of the manuscript: C.L. Robertson, S. Giashuddin, M.A. Subler, P.B. Fisher, D. Sarkar
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C.L. Robertson, J. Srivastava, A. Siddiq, R. Gredler, X.-N. Shen, D. Sarkar
Study supervision: C.L. Robertson, D. Sarkar

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