The transition from a rural to an urban environment alters expression of the human Ebola virus receptor Neiman-Pick C1: implications for the current epidemic in West Africa

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The 2014 Ebola epidemic in West Africa has captured the world's attention and has been described as a humanitarian crisis and a threat to international security by Dr. Margaret Chan, director general of the World Health Organization (Chan 2014). As of October 25th of this year, a total of 10,141 confirmed, probable, and suspected cases of Ebola virus disease (EVD) have been reported primarily in five countries, including Guinea, Liberia, Nigeria, Senegal, and Sierra Leone, resulting in 4922 deaths thus far (WHO 2014a). The prediction is that without drastic control measures the number of cases and deaths could increase to thousands per week in the coming months (WHO Ebola Response Team 2014).

EVD is caused by a filamentous shaped, single-stranded negative-sense RNA virus, which was first described by Johnson et al. (1977) and Bowen et al. in 1977. EVD is a zoonotic infection with the animal reservoir being monkeys and fruit bats (Martines et al. 2014). The current epidemic

began in December 2013 in a village located in the rainforest of Guinea, most likely through exposure to bush meat or urinary discharges of fruit bats. The Ebola virus is spread through contact with body fluids of symptomatic patients. As such, transmission can be stopped by a combination of early diagnosis, contact tracing, patient isolation and care, infection control, and safe burial (WHO 2014b). Ebola virus is thought to be extremely infectious. Indeed, only one or two viral particles may be needed to infect a person, which is in contrast with the lower limit for influenza infection ranging in hundreds of particles per infection.

In humans, Ebola virus causes a fatal hemorrhagic fever. The disease manifests itself abruptly with non-specific flu-like symptoms, including chills, fever, myalgia, and general malaise. These symptoms are followed by nausea, vomiting, diarrhea, abdominal pain, coughing, headache, and even unexplained bleeding from mucosal surfaces. In the most severe cases, patients develop hypotensive shock and multiple organ failure with death occurring 6–16 days after the onset of symptoms (Martines et al. 2014). The case-fatality rate of the ongoing outbreak in Guinea, Sierra Leone, and Liberia has been estimated to be about 70 % with a range of 61–89 % (WHO Ebola Response Team 2014). In contrast, the case-fatality rate of the recent Nigerian outbreak was about 40 %, which is at the lower end of the mortality rate of 41–89 % recorded in previous outbreaks. This lower mortality rate in the Nigerian outbreak has been attributed to supportive treatment (Chowell and Nishiura 2014). Under-reporting of milder symptomatic cases and unknown final outcome are just two of the factors that could bias case-fatality estimates. One of the greatest concerns with the current EVD epidemic in West Africa is that the disease would spread to the large urban centers and could ultimately reach outside of Africa, due to the large number of international flights in and out of the region, typical of our highly dynamic and mobile modern society.

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Despite the rarity of this infectious disease, there is a surprisingly large amount of information regarding the mechanism of Ebola virus cellular infection. The process begins when the viral spike glycoprotein recognizing multiple cell surface molecules in target cells, particularly macrophages and dendritic cells. The virus is internalized by macropinocytosis and delivered into endosomes (Martines et al. 2014). Within late endosomes, cathepsin B cleaves the viral glycoprotein by a process requiring acidification (Martines et al. 2014; Ng et al. 2014). The cleaved glycoprotein binds to a receptor within the endosome and fuses with the compartment membrane to deliver the viral genetic material necessary for replication. At an astronomical replication rate, the emerging viruses infect other cell types, including hepatocytes and endothelial cells.

Perhaps the most important discovery during the past few years has been the identification of the Ebola virus receptor Niemann-Pick C1 (NPC1) protein. NPC1 is a transmembrane endosome protein responsible for the transport of internalized cholesterol into other intracellular compartments. The name NPC1 is derived from studies indicating that this gene is mutated in patients presenting with the rare neurovisceral pathology known as Niemann-Pick disease, type C, which results in accumulation of cholesterol and sphingolipids in late endosomes and lysosomes (Rosenbaum and Maxfield 2011). NPC1 was initially identified as the Ebola virus receptor in a genome-wide screening of human cells (Carette et al. 2011). This observation was confirmed by studies in which fibroblasts isolated from Niemann-Pick disease, type C1, patients were found resistant to Ebola virus infection. Moreover, Chinese hamster ovary cells in which *NPC1* was deleted were also resistant to viral infection and reverted by expression of human *NPC1* (Carette et al. 2011). Finally, two inhibitors of NPC1 activity, U1866A and the antidepressant imipramine, were effective in inhibiting infection by Ebola virus (Carette et al. 2011). A contemporary report by Coté et al. (2011) also showed that benzylpiperazine adamantane diamide derivatives that blocked Ebola virus infection were targeted to NPC1. These authors also demonstrated that genetic manipulation of *NPC1* altered the pattern of Ebola infection. More recent studies have confirmed the recognition of NPC1 by Ebola virus (Miller and Chandran 2012; Krishnan et al. 2012; Ng et al. 2014). Finally, cationic amphiphiles that target NPC1 were also found to inhibit Ebola virus cell entry and infection (Shoemaker et al. 2013).

Given the central role of the NPC1 in Ebola virus infection, an important emerging question is whether variation in *NPC1* expression could affect either the transmission or outcome of the disease. This information could be helpful in predicting the course of the epidemic and might also lead to novel prevention and treatment strategies for this deadly disease. As a first step in assessing this concept, we addressed whether *NPC1* gene expression might be different in rural and urban populations

living in Africa. The rationale for this inquiry is that different environments impact physiology and disease susceptibility (Bickler 2000, 2006; Bickler and De Maio 2008). This principle is perhaps best illustrated by a gene expression study performed using peripheral blood leukocytes in genetically similar nomadic, rural, and dense urban Moroccan Amazigh populations (Idaghdour et al. 2008). In this study, more than one third of the transcriptome was differently expressed in the three different regions, suggesting an effect of environmental geography on gene expression. Similar findings were reported from Fiji, where there were also major differences between the peripheral blood transcriptomes of rural villagers and residents of the capital city, Suva (Nath et al. 2012). In both studies, differences in genome-wide expression signature were attributed to a combination of lifestyle, geography, and biotic factors. To test the hypothesis that *NPC1* expression is different in rural and urban populations in Africa, we queried the gene array data used in the Moroccan study (GSE8847). Our analysis showed that *NPC1* is upregulated in rural villagers compared to inhabitants of an urban area in Morocco ($p=0.0001$, by ANOVA). Additionally, *NPC2* ($p=1.0\text{e}^{-10}$) and *LDLR* ($p=0.004$) expression were increased in the rural population as opposed to urban inhabitants. All of these genes encode proteins that play a role in cholesterol uptake. Our analysis has some limitations including the small sample size ($n=12$ for the rural sample), different geographic location within Africa of the current EVD epidemic, and the restricted gene expression analysis of peripheral blood leukocytes. Nevertheless, our findings illustrate that environmental factors alter expression of the *NPC1* gene.

The observation that *NPC1* expression is greater in rural as compared to urban areas of Africa raises several important questions. Perhaps the most important is whether variation in this receptor level may alter the infectivity or the fatality rate of the Ebola virus. For instance, could the difference in *NPC1* expression have a role in the lower case fatality rate observed in Nigeria compared to other areas of West Africa? Or, could it explain why there was not a more rapid spread of the disease to urban centers early on when limited control measures were in place? An appealing hypothesis is that the nutritional differences between rural and urban areas of Africa play a role in determining *NPC1* expression. Subclinical malnutrition is a common finding in rural areas of low-income countries and results from a complex interaction of diet and chronic and recurrent infections. Urbanization is known to have a major influence on nutrition, eating habits, and lifestyle. In the poor rural areas, perhaps reduced consumption of fat triggers a cellular response to increase the expression of proteins necessary for cholesterol internalization to maximize the utilization of this important lipid for cellular function. Thus, it is not surprising that *LDLR* expression is also increased in rural versus urban areas. In contrast, individuals living in urban areas where rates of infection are lower, food more available

and perhaps richer in fat, react with a reduction of the machinery for cholesterol uptake to avoid an excess of cellular sterol. Of note, lower serum lipids levels were inversely correlated with high infection rates and inflammatory markers in an indigenous population of Bolivia Amazon (Vasunilashorn et al. 2010). If compromised nutrition increases the risk for EVD, it illustrates the importance of maintaining food security in the hardest hit areas.

Although the interaction of Ebola virus with NPC1 does not apparently alter cholesterol transport per se (Carette et al. 2011), there is no direct evidence that Ebola infection does not affect cholesterol cellular homeostasis. The dramatic invasion and replication of Ebola virus into cells is likely to induce a major stress response that alters cellular function. Indeed, prior studies have shown cellular stress altered cholesterol metabolism, in particular the subcellular distribution of this sterol in macrophages. This process predisposes cells to a hyperinflammatory response when challenged with pro-inflammatory agents, such as LPS (Frey and De Maio 2007, 2009), and reduces their phagocytic capacity (Niño et al. 2014). Since Ebola infection triggers an overwhelming inflammatory condition, known as a cytokine storm, it could be speculated that an alteration of cellular cholesterol homeostasis during Ebola infection is part of the pathology of this horrible disease. Moreover, membrane cholesterol has been shown to be important in the release of viruses from target cells and propagation of infection (Mañes et al. 2003). In addition, statin therapy has been shown to improve the outcome from viral infections (Kohjima et al. 2013; Fedson 2014). Alterations in cellular cholesterol homeostasis is contemplated as a contributing factor for the development of other diseases that are products of an exaggerated and poorly controlled inflammatory responses, such as sepsis. There are some parallels between EVD and sepsis: both conditions produce a cytokine storm, coagulopathies, and the cause of death is multiple organ failure after cardiovascular collapse. In addition, the only effective treatment for both conditions is supportive therapy within intensive care units. Perhaps, the intersection of these two conditions may be related to the primary alteration of macrophage or dendritic cell function. Investigations on the molecular and cellular mechanisms that contribute to the pathology of both sepsis and EVD may provide insight into mutual therapeutic targets or interventions to ameliorate these conditions.

In summary, the observation that the Ebola virus receptor NPC1 has different levels of expression in rural and urban areas of Africa may add a new perspective in the understanding of the current Ebola epidemic and perhaps may shed light on possible interventions to reduce the spread and decrease the mortality from this condition. Further, it illustrates an important epidemiological principle that disease modeling should consider biological differences in the populations affected, especially when there are such profound biological differences

as those observed between rural and urban populations in Africa.

Finally, it is important to recognize that it was basic science research that has helped us understand some of the most important aspects of EVD. For example, the current knowledge about NPC1 was not the product of investigations directed at identifying the Ebola virus receptor, but rather derived from the basic interest in elucidating cellular cholesterol metabolism. In this process, knowledge generated from basic science research clearly played a crucial role in the understanding of a mechanism of infection for a virus that had not even been identified. Perhaps there is a cautionary lesson here that lack of support for the basic sciences could have a detrimental effect on managing public health problems in the future. The current EVD epidemic should alert legislators about the importance of basic research in our society.

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