Chapter 7
Linking Gulf War Illness to Genome Instability, Somatic Evolution, and Complex Adaptive Systems

Henry H. Heng, Guo Liu, Sarah Regan, and Christine J. Ye

7.1 Introduction

Gulf War illness (GWI) impacts nearly one-third of Gulf War veterans in the United States [1]. The complex etiology and diverse symptoms of GWI lead to difficulties both for diagnosis and treatment, which also slows down the acceptance of this clinical condition within the medical community [2]. Recently, an increasing number of symptoms, exposures, and molecular defects have been identified for GWI [3–7]. However, the general mechanism of GWI remains elusive, which prevents further developments of common biomarkers and treatment options. By considering GWI as a common and complex illness, we have searched for the somatic evolutionary mechanism of GWI. Because many different initial trigger factors of GWI occurred over 26 years ago, it is logical to study GWI in the context of complex adaptive systems that follows the principles of somatic evolution. In particular, based on the recently introduced genome theory [8–10], which suggests that the karyotype or chromosome sets (the physical relationship of genes) encode the blueprint of the bio-system by defining system inheritance, and genome-level

H. H. Heng (✉)
Center for Molecular Medicine and Genetics, and Department of Pathology, Wayne State University School of Medicine, 3226 Scott Hall, 540 E. Canfield, Detroit, MI 48201, USA
e-mail: hheng@med.wayne.edu

G. Liu · S. Regan
Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, 3226 Scott Hall, 540 E. Canfield, Detroit, MI 48201, USA
e-mail: gliu@med.wayne.edu; saregan@bu.edu

C. J. Ye
The Division of Hematology/Oncology, Department of Internal Medicine, Comprehensive Cancer Center, University of Michigan, 1500 East Medical Drive, Ann Arbor, MI 48109, USA
e-mail: jchrisy@med.umich.edu

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variations play an important role in both the initiation and progression of various diseases/illnesses, we have used our own finding that elevated genome instability is commonly observed from GWI patients to unify the diverse etiologies and symptoms of this illness. In this presentation, we share our journey of searching for the common mechanism of GWI by applying principles of complex adaptive systems into our synthesis. Furthermore, we introduce the framework of genome alteration-mediated somatic evolution to understand common and complex diseases and illnesses including GWI.

7.2 The Unexpected Journey of Studying GWI

Our journey to study GWI was unexpectedly initiated by a documentary production project of Discovery Channel back in 2007 [11]. At that time, we had just published a number of research articles that linked genome instability to cancer evolution, following years of reviewing by a number of journals [12–14]. One of the technologies we used to score stochastic chromosomal changes was spectral karyotyping or SKY, which can reliably measure cellular genome instability. SKY "paints" each pair of human chromosomes with a unique color, and any major chromosomal aberrations can be easily identified following SKY detection [15–17]. The TV show was interested in applying SKY to study GWI patients. The initial goal was to identify specific chromosomal changes associated with radiation (to establish a potential link of Gulf War syndrome to depleted uranium (DU)). Following further discussion about our novel concept of linking seemly random chromosomal aberrations to overall genome instability, they were highly interested in using our approach to examine five Gulf War veterans who displayed the mysterious illness. Back then, Gulf War syndrome was a rather sensitive and a highly controversial subject, as both the medical community and government agents were refusing to accept it as a legitimate illness due to how difficult it was to understand. In fact, many thought that GWI was imagined by patients, as there are no acceptable medical explanations. Knowing the sensitive nature of this subject, some of our colleagues have advised us not to be involved with this politically sensitive research, as the least thing a scientist wants is for their own research to be disrupted by politics. This was especially relevant for us given the fact that our genome-mediated cancer evolutionary research was already sharply in contrast to the main gene mutation centric cancer concept.

After some quick research, we were puzzled by how complicated GWI was: the duration of the Gulf War was obviously short, yet there is a large impacted patient population; there are some identifiable war factors that can be linked to GWI, but no uniformed exposure to all patients; there are diverse symptoms, and even the basic diagnosis is hard. Considering this, it becomes obvious that GWI likely represents a complex adaptive system based on many seemingly confusing factors within this medical mystery. We thought, "Why not check it from the genome perspective? We already know from our cancer research that the diverse

molecular mechanisms of cancer can be unified by elevated genome instability, and many illnesses/conditions should be understood using the principles of evolution and complex adaptive systems." Of course, multiple levels of consultations within the school were necessary due to the politically sensitive nature of this project at that time, but finally, after given the green light, we started to test samples from GWI patients. The plan was straightforward: we would culture the lymphocyte cells from patients and examine the cells' chromosomes. Following SKY detection, all karyotype changes would be recorded to see if there were radiation exposure-related chromosomal aberrations (such as dicentric chromosomes). At that time, one of the dominating viewpoints was the potential linkage between DU exposure and GWI. We anticipated that we would finish this project in 2–3 weeks.

To our surprise, after examining the chromosomal aberrations for these GWI patients, we observed that the majority of them did not display dicentric chromosomes. However, all of them displayed highly elevated levels of diverse chromosomal aberrations, including many novel types, some of which had never been reported in the literature. Based on our experience in cancer research, a high level of non-clonal chromosome aberrations (NCCAs) often indicates increased genome instability, and various stress conditions are linked to elevated NCCAs [9, 12–14, 18–22]. As it turns out, these five GWI patients represented different Gulf War exposure types, and not all of them had been exposed to DU. (To avoid any bias in our analyses, we only accessed patient profiles after we had presented our findings.) This made sense of our finding that most patients did not display radiation-specific chromosomal aberrations. In addition to elevated chromosomal aberrations across all patients, the most surprising discovery was the level of NCCAs. Many of them displayed a higher rate than many cancer patients!

Despite the fact that the exact mechanisms were unclear to us, we were convinced that the linkage between the unstable genome and GWI was real and could serve as a common feature among all tested patients. This observation supports the view that GWI has a physical or pathological basis, as patients' imaginations should not change the status of their genome instability. Therefore, this discovery could play an important role in understanding GWI and legitimizing GWI research and care.

Following the airing of the Discovery program in 2007, we received a large number of phone calls and emails from the veteran community. Many personal stories from GWI patients deeply touched us. We decided to make more efforts to study GWI based on our newly established genome theory, as the evolutionary framework of genome theory suits studies of complex adaptive systems. Since then, teamed up with Dr. Chowdhury from the Detroit VA hospital, we have applied for a research grant from DOD to link genome instability to GWI, the first key characterizations of the complexity of GWI.

7.3 The Complex Features of Chromosomal Aberrations in GWI Patients

Prior to our studies, there were limited chromosomal analyses examining the relationship between chromosomal abnormalities and exposure to DU in Gulf War veterans. A significant increase in the frequency of dicentric chromosomes (dic) and centric ring chromosomes (cR) was observed from 16 individuals, reflecting a possible previous exposure to DU [23]. However, our initial studies pointed in a different direction, as the majority of observed chromosomal aberrations differed from dicentric chromosomes and ring chromosomes. Another study of 35 DU-exposed veterans failed to exhibit a significant relationship between any cytogenetic endpoint and log [urine uranium] levels, smoking, or log [lifetime X-rays] [24], which further complicated the case. On the surface, this study even seemed to disagree with our observations that there is a significant elevation in overall chromosomal instability in GWI. But a brief analysis can easily reconcile the key differences. Despite using a similar cytogenetic-based platform, our chromosomal analyses have examined different aspects of GWI with advanced tools [7, 15–17, 25]. First, our correlation study has focused on GWI patients rather than types of exposure, such as DU exposure. Not all individuals with exposure will develop GWI, and, importantly, medical examination after longer periods of time (over 26 years) might not capture the initial response of the system. In contrast, as explained by our model, when somatic evolution is involved, the general feature of genome instability should be detected regardless of the different types of initial factors. Second, we have performed more systematic analysis by scoring different types of chromosomal and nuclear abnormalities rather than just chromosomal translocations. Using all different NCCAs to monitor genome instability has proven to be the most effective strategy compared to using translocations alone [26]. Some GWI patients displayed a high frequency of other types of chromosome aberrations, including DMFs and sticky chromosomes, without exhibiting a high level of translocations. These aberrations represent examples that likely would have been missed by previous studies [7]. Even for the detection of chromosomal translocations, the 24-color SKY method is much more effective than 3-color FISH used by previous reports due to its coverage and sensitivity. Third, the detection of multiple chromosome aberrations in a single cell is of special importance, as these “outliers” strongly suggest genome instability. In fact, it is rare to detect them in normal individuals. Interestingly, Bakhmutsky et al. have also observed such outliers even using interphase detection with limited probes (unfortunately, they did not pay enough attention to these important outliers) [27]. If SKY is performed to examine these individuals, a high level of genome instability will likely be observed. Finally, prior to the establishment of the genome theory, the meaning of many seemingly stochastic chromosomal aberrations was not clear, and many researchers have considered them as genetic “noise” [9, 22, 28]. Our synthesis, that different karyotypes represent different systems, has called to change the practice of ignoring them. As illustrated by our expression studies, these long-ignored NCCAs

represent evolutionary potential and have defined the transcriptome dynamics of cancer cells [29–31]. Increased attention is now being paid to multiple chromosomal translocations [27, 32]. Further analyses have unexpectedly revealed many novel types of chromosomal/nuclear abnormalities including sticky chromosomes and defective mitotic figures (DMFs) [7, 25, 32], suggesting that diverse molecular mechanisms can be linked to GWI, as stochastic chromosomal alteration has been linked to various molecular pathways in our cancer research [29, 30]. Furthermore, cellular stress has also been linked to GWI, explaining the relationship among stress, elevated genome instability, and the diverse phenotypes observed in GWI patients.

Obviously, even for studying chromosomal aberrations in GWI, a new approach is needed to understand seemly stochastic genome alterations. The fact that a high level of genome instability is observed in the cells of GWI patients also reflects the reality that GWI represents a complex clinical condition, which requires the action of treating it as a complex adaptive system.

7.4 GWI: A Case Study of Somatic Evolution and Complex Adaptive Systems

In our previous cancer research, elevated genome instability has been identified as a common key driver for cancer evolution [12, 21]. Of equal importance, we have proposed using the evolutionary mechanism of cancer to unify cancer's diverse molecular pathways [9, 18, 19]. The recent large-scale cancer genome sequencing data forcefully confirmed our genome theory of cancer evolution by revealing both punctuated cancer evolution and unmanageably high levels of genetic heterogeneity. Cancer is no longer a gene disease but a complex adaptive system featuring genome-mediated macro-cellular evolution. This realization calls for a new approach of using complex system-based thinking to study other disease conditions.

As most common and complex diseases/illnesses involve the cellular evolution process and constant multiple genotype-environment interactions, it is logical to consider them as various complex and adaptive systems [33–37]. Under such a framework, the multiple genetic variants and cellular/tissue/organ structural components of patients represent various "agents"; the initial Gulf War-specific environments and the patients' involved cellular environments following the Gulf War's original impact function as environments, and the diverse symptoms represent "emergent features." Due to the high level of genome instability present in GWI patients, all of these genetic alterations are different, leading to increased unpredictability. Furthermore, the mind/body/treatment interactions within an individual, and individual-society interactions, as well as over 26 years of cellular evolution, make GWI a highly complex illness featuring the clear involvement of somatic evolution.

Defining GWI as a complex adaptive system is thus of particular importance, since a portion of the current medical community still doubts the legitimacy of considering GWI as an illness condition, due to the very nature of complex adaptive systems. For example, different from many infectious diseases and single-gene Mendelian diseases, there seems to be no fixed dominant causative relationship between specific environmental or genetic factors and the diverse symptoms of GWI. This fact represents a major rationale for some to question if GWI is real. By accepting GWI as a complex adaptive system, most of the confusion should be resolved. Clearly, it is not suitable to use a reductionist approach to define GWI, as there is no dominant linear causation for either the etiology of the illness or its symptoms. In contrast, the observation of the generally unstable genome in GWI patients fits well with key features of somatic evolution and complex adaptive systems.

Interestingly, using evolution and complex adaptive systems to study GWI can also generate useful information to illustrate the relationship between bio-evolution and how complex adaptive systems work. For example, in the field of complex adaptive systems, evolution was thought by some to be a result of an adaptive biological system, while others use evolution and complex adaptive systems in an overlapping description. Thinking of GWI, we realized that evolutionary and genomic mechanisms, such as fuzzy inheritance [9, 20], selection acts on the genome [8], and genome alteration-mediated macro-cellular evolution, serve as effective means for the bio-system to adapt [12, 19], supporting the notion that bio-evolution also serves as a strategy for bio-complex adaptive systems. Furthermore, the details of how genome level change has an impact on the macroevolution of cancer (while gene mutation impacts microevolution) provide new insight for differentiating stepwise system adaptation and the emergent punctuated system of genome reorganization [20]. Genome reorganization can significantly alter the status of agents, which raises the issue of the emergence of new systems based on altered agents, further complicating the systems under investigation. The increased heterogeneity among agents will further complicate a system, which makes some complex adaptive systems more complicated than others. While the somatic cell evolution pattern of GWI is less clear compared to the two phases of cancer evolution, GWI can nevertheless be considered as stress-induced, genome instability-mediated evolution, which can be used to define some key characteristics of complex adaptive systems.

For example, unlike many cancer cases and some genetic diseases, in which clonal chromosomal aberrations can be observed, GWI patients often display high levels of non-clonal chromosome aberrations or NCCAs. The linkage between GWI and NCCAs is rather interesting when considering how the heterogeneity of lower level agents can have an impact on emergent properties. To explain how elevated NCCAs can lead to GWI, we hypothesize that NCCAs contribute to the degree of heterogeneity among individual cells, which alters emergent properties above the level of individual cells, such as immune response, tissue/organ function, or overall health status of individuals. Due to the different degree and types of altered genomes, the emergent properties (symptoms) could be highly diverse due

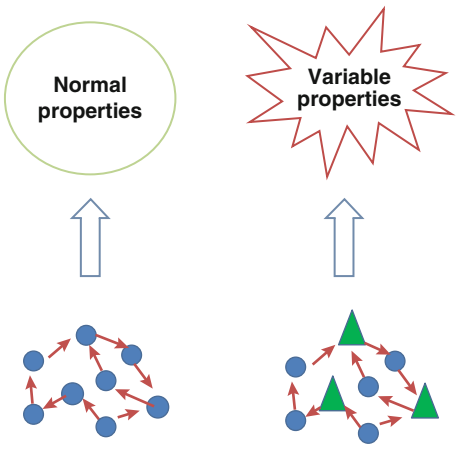

Fig. 7.1 The illustration of how the heterogeneity of agents (the cell population of tissues) impacts the emergent properties of tissue/organ functions. Circles represent cells with normal karyotypes, and triangles represent cells with altered genomes. These variable properties are the potential basis for diseases/illnesses

to cellular-environmental interaction. Our hypothesis is illustrated in Fig. 7.1, where the degree of genome heterogeneity itself leads to emergent variable properties at tissue or organ level, which can be considered as potential for abnormal system response. According to our definition that diseases are genotype/environment-induced variants that are not compatible with a current environment, the relationship between genetic heterogeneity and illness becomes obvious [2]. Note that the recently introduced concept of "fuzzy inheritance" likely plays an important role in these emergent properties based on the heterogeneity of agents [9], as how these agents pass the genetic information among somatic cells can influence the emergent properties as well. Clearly, further studies are needed to address this issue.

7.5 Search for the General Model for Common and Complex Diseases/Illnesses

By linking cellular stress, genome instability, and diverse molecular mechanisms to GWI in the context of complex adaptive systems [2, 35], we might have solved some key mysterious features of GWI. Stress-induced genome alterations, and their facilitative role in cellular evolution, have provided the mechanistic basis for understanding GWI, which also suggests that GWI is real and that a patient's imagination cannot significantly alter the genome (Fig. 7.2). Moreover, both GWI diagnosis and treatment could benefit from this finding. For example, treatment strategies should not further destabilize the genome of patients. Of equal importance, our research on GWI has further suggested that stress-induced, genome alteration-mediated cellular evolution might be used to explain other common and complex diseases as well.

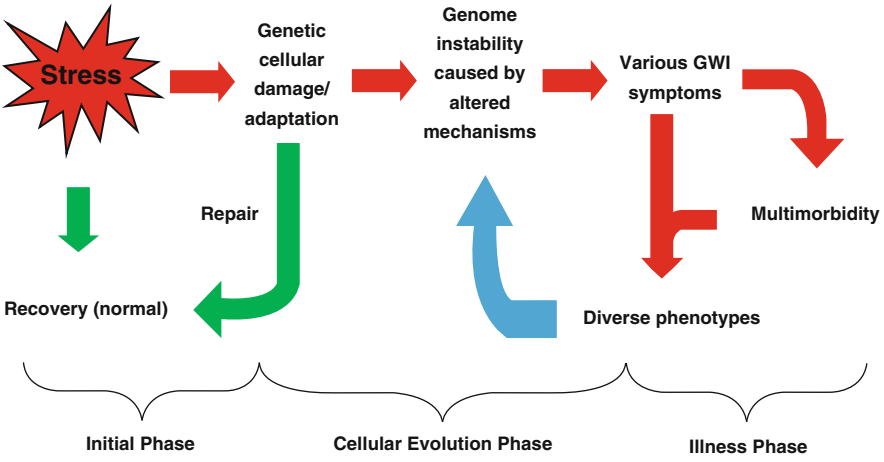

Fig. 7.2 The model of the stress-induced genome instability and GWI phenotypes. During the initial phase, Gulf War-specific stress can generate genetic or cellular damages. The unrepaired bio-damage can trigger cellular evolution, which is driven by genome instability. Importantly, genome alterations can stochastically activate different molecular pathways. Together, this somatic evolution will lead to diverse symptoms. Note that the three phases interact closely with each other through different feedback loops. This model is adapted from [2].

The linkage between elevated genome instability and somatic evolution was initially observed in our cancer research. In particular, we have established a strong link between stochastic genome alteration and large numbers of individual molecular mechanisms. We later proposed the evolutionary mechanism of cancer, which can be explained by three key components (system stress, increased frequencies of non-clonal chromosome aberrations, and genome-mediated macro-cellular evolution). Paradoxically, such a simple evolutionary principle can lead to a large number of individual molecular mechanisms and their combinations. Our prediction has recently been confirmed by the cancer genome sequencing project, as most of the cancer cases do not share the same gene mutations, and cancer heterogeneity is overwhelming.

Since cancer belongs to the category of common and complex diseases, we have proposed that genome instability should also be shared by other common and complex diseases [35, 37, 38]. However, most researchers consider cancer as a special case due to its invasiveness, and it is hard for them to accept our viewpoint. Our arguments are the following: Despite that all diseases have unique phenotypic characteristics, most of them involve somatic evolution and system adaptation, and the invasiveness of cancer, even though it is unique, still represents an abnormal feature, emergent from a cellular population with unstable genomes. In fact, out-of-control growth and invasiveness also mean that the constraint of normal tissue function is lost. Fortunately, increased studies have revealed that increased genome instability can be observed from many disease types as well as normal tissues [39–43].

Following GWI data analysis, we proposed a general model for common and complex diseases to unify stress, genome instability, diverse molecular pathways, and diverse symptoms. This model states:

1. Stress and/or the requirement of cellular adaptation can trigger genome alterations, which initiate genome alteration-mediated somatic evolution [44]. NCCAs are eliminated as the dead end of the evolution. With extremely low likelihood, some CCAs (clonal chromosome aberrations) will form. Usually, a number of NCCA/CCA cycles are needed to bring about disease conditions. It is also possible that a high level of NCCAs alone (without dominant CCAs) can lead to phenotype abnormalities.
2. Some rare outliers become successful to form the dominant population. The altered genome can stochastically be linked to different molecular pathways [29, 30], or different emergent properties, which reduces the normal function or response of specific organs or tissues. Unlike cancer, although there likely is no homogenously altered genome, significant genome heterogeneity will change the emergent function of the cellular population, leading to different symptoms.
3. Moreover, some responses can come from different levels of the system [8]. For example, the heterogeneous cell population can simply reduce normal function, or produce cellular stress, leading to altered functions at high levels of system organization. Combining this process with infection, immune reaction, the aging process, and medical intervention, the emergent properties can further change, leading to more complications or even multimorbidity [45], in a stochastic fashion.

7.6 Conclusions

Our journey of studying GWI has been both exciting and rewarding, especially since we adapted the concept of somatic evolution and complex adaptive systems. It is worth noting that the concepts of somatic evolution and complex adaptive systems have been largely ignored by the medical research community, despite the fact that complexity sciences have been around for decades and there has recently been a new push to bring them into mainstream medical care [46]. For example, the cancer research field has started to pay more attention to evolutionary analyses, based on the increased realization that the somatic cell evolutionary mechanism of cancer can unify large numbers of devised molecular mechanisms, but such a realization has yet to become popular in the field of medicine. It is interesting to point out that evolutionary medicine has also been around for decades [47]. However, possibly influenced by the popularity of molecular medicine, the bio-determinist has promised predictive power based on advanced molecular medical research. Now, there is an increased call to question the reductionist's approach of current medicine, as not appreciating the uncertainty of bio-systems during evolution leaves us with increased confusion [48–50]. To change the status quo, which not only wastes

research resources but also delays the search for new conceptual frameworks and compromises the needed care for patients, a new attitude and strategy is needed to consider many common and complex diseases/illnesses as complex adaptive systems, wherein somatic cell evolution plays a key role [8, 9]. Our GWI research likely will serve as such an example.

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The Journey

Our appreciation of the concept of complexity was paradoxically enhanced by the progress of our knowledge of the gene's function. As a graduate student (at the golden age of molecular cloning, and mentored by a leading gene hunter, Lap Chee Tsui, who cloned the cystic fibrosis gene), the power of the individual gene seemed obvious. However, increased data has clearly illustrated the nonlinear correlation between genes and their phenotypes. Some common explanations were: "Well, there is more than one gene responsible for a given phenotype; there exist modifiers and even modifiers of the modifiers ...." Clearly, life was complicated. Nevertheless, it was thought the reductionist approach should be able to solve this issue (if we just worked harder).

The overwhelming genomic heterogeneity in cancer has forced us to question our knowledge, from the gene mutation theory of cancer to how to define genetic information. In the past 20 years, we have reevaluated many important questions through the lens of complexity, including the function of sex (to ensure the genome system identity rather than simply increasing genetic diversity); the pattern of evolution ("punctuated" often in macroevolution and "stepwise" frequently in microevolution); the chromosomal coding which defines the "system inheritance" or blueprint (while a gene encodes only "parts inheritance"); and the concept that genetic information is rather fuzzy, representing a spectrum of potential phenotype variants (and should not be simply explained as either dominant or recessive without the consideration of continuous environmental-influenced variants). These realizations have provided new frameworks for us to understand many common and complex diseases.

We specifically benefit from learning and interacting with scholars in the community of complexity. Despite the relatively small number of members, we see the hope that comes from this collaboration, as we believe in the power of outliers during emergence. The new phase of our journey is to use unique features of biological systems (heterogeneity-mediated bio-evolution) to understand the principles of complexity.

Take-Home Message

- GWI (Gulf War illness) is a real illness condition which can be classified as an environmental illness
- The common mechanism of GWI is high stress-induced, genome instability-mediated somatic evolution. Cellular heterogeneity is likely responsible for the diverse abnormal properties observed
- Many common and complex diseases/illnesses should be considered as common adaptive systems

References

1. Research Advisory Committee. Gulf War illness and the health of Gulf War veterans: scientific findings and recommendations. Washington, DC: U.S. Government Printing Office; 2008.
2. Heng HH. Challenges and new strategies for Gulf War illness research. Environ Dis. 2016;1:118–25.
3. Koslik HJ, Hamilton G, Golomb BA. Mitochondrial dysfunction in Gulf War illness revealed by 31Phosphorus Magnetic Resonance Spectroscopy: a case-control study. PloS One 2014;9:e92887.
4. Craddock TJ, Harvey JM, Nathanson L, Barnes ZM, Klimas NG, Fletcher MA, Broderick G. Using gene expression signatures to identify novel treatment strategies in Gulf War illness. BMC Med Genomics 2015;8:36.
5. Parihar VK, Hattiangady B, Shuai B, Shetty AK. Mood and memory deficits in a model of Gulf War illness are linked with reduced neurogenesis, partial neuron loss, and mild inflammation in the hippocampus. Neuropsychopharmacology 2013;38(12):2348–62.
6. White RF, Steele L, O'Callaghan JP, Sullivan K, Binns JH, Golomb BA, Bloom FE, Bunker JA, Crawford F, Graves JC, Hardie A, Klimas N, Knox M, Meggs WJ, Melling J, Philbert MA, Grashow R. Recent research on Gulf War illness and other health problems in veterans of the 1991 Gulf War: effects of toxicant exposures during deployment. Cortex 2016;74:449–75.
7. Liu G, Ye CJ, Chowdhury SK, Abdallah BY, Horne SD, Nichols D, Heng HH. Detecting chromosome condensation defects in Gulf War illness patients. Cur Genomics 2018 (in press).
8. Heng HH. The genome-centric concept: resynthesis of evolutionary theory. Bioessays 2009;31(5):512–25.
9. Heng HH. Debating cancer: the paradox in cancer research. Hackensack, NJ: World Scientific; 2015.
10. Heng HH, Liu G, Stevens JB, Bremer SW, Ye KJ, Abdallah BY, Horne SD, Ye CJ. Decoding the genome beyond sequencing: the new phase of genomic research. Genomics 2011;98:242–52.
11. Discovery Channel. Discovery channel in 2007: Gulf War illness-conspiracy test. https://www.youtube.com/watch?v=jhvdkdMFVJQ.
12. Heng HH, Stevens JB, Liu G, Bremer SW, Ye KJ, Reddy PV, Wu GS, Wang YA, Tainsky MA, Ye CJ. Stochastic cancer progression driven by non-clonal chromosome aberrations. J Cell Physiol. 2006;208(2):461–72.
13. Heng HH, Bremer SW, Stevens J, Ye KJ, Miller F, Liu G, Ye CJ. Cancer progression by non-clonal chromosome aberrations. J Cell Biochem. 2006;98(6):1424–35.
14. Heng HH, Liu G, Bremer S, Ye KJ, Stevens J, Ye CJ. Clonal and non-clonal chromosome aberrations and genome variation and aberration. Genome 2006;49(3):195–204.

15. Ye CJ, Lu W, Liu G, Bremer SW, Wang YA, Moens P, Hughes M, Krawetz SA, Heng HH. The combination of SKY and specific loci detection with FISH or immunostaining. Cytogenet Cell Genet. 2001;93(3–4):195–202.
16. Heng HH, Ye CJ, Yang F, Ebrahim S, Liu G, Bremer SW, Thomas CM, Ye J, Chen TJ, Tuck-Miller C, Yu JW, Krawetz SA, Johnson A. Analysis of marker or complex chromosomal rearrangements present in pre- and post-natal karyotypes utilizing a combination of G-banding, spectral karyotyping and fluorescence in situ hybridization. Clin Genet. 2003;63(5):358–67.
17. Ye CJ, Stevens JB, Liu G, Ye KJ, Yang F, Bremer SW, Heng HH. Combined multicolor-FISH and immunostaining. Cytogenet Genome Res. 2006;114(3–4):227–34.
18. Ye CJ, Stevens JB, Liu G, Bremer SW, Jaiswal AS, Ye KJ, Lin MF, Lawrenson L, Lancaster WD, Kurkinen M, Liao JD, Gairola CG, Shekhar MP, Narayan S, Miller FR, Heng HH. Genome based cell population heterogeneity promotes tumorigenicity: the evolutionary mechanism of cancer. J Cell Physiol. 2009;219(2):288–300.
19. Horne SD, Pollick SA, Heng HH. Evolutionary mechanism unifies the hallmarks of cancer. Int J Cancer 2015;136(9):2012–21.
20. Horne SD, Ye CJ, Heng HH. Chromosomal instability (CIN) in cancer. eLS 2015;1–9.
21. Heng HH, Bremer SW, Stevens JB, Horne SD, Liu G, Abdallah BY, Ye KJ, Ye CJ. Chromosomal Instability (CIN): what it is and why it is crucial to cancer evolution. Cancer Met Rev. 2013;32:325–40.
22. Heng HH, Regan SM, Liu G, Ye CJ. Why it is crucial to analyze non clonal chromosome aberrations or NCCAs? Mol Cytogenet. 2016;9:15.
23. Schröder H, Heimers A, Frentzel-Beyme R, Schott A, Hoffmann W. Chromosome aberration analysis in peripheral lymphocytes of Gulf War and Balkans War veterans. Radiat Prot Dosimetry 2003;103(3):211–9.
24. Bakhmutsky MV, Squibb K, McDiarmid M, Oliver M, Tucker JD. Long-term exposure to depleted uranium in Gulf-War veterans does not induce chromosome aberrations in peripheral blood lymphocytes. Mutat Res. 2013;757(2):132–9.
25. Heng HH, Stevens JB, Liu G, Bremer SW, Ye CJ. Imaging genome abnormalities in cancer research. Cell Chromosome 2004;3:1.
26. Heng HH, Stevens JB, Bremer SW, Liu G, Abdallah BY, Ye CJ. Evolutionary mechanisms and diversity in cancer. Adv Cancer Res. 2011;112:217–53.
27. Poot M, Haaf T. Mechanisms of origin, phenotypic effects and diagnostic implications of complex chromosome rearrangements. Mol Syndromol. 2015;6(3):110–34.
28. Abdallah BY, Horne SD, Stevens JB, Liu G, Ying AY, Vanderhyden B, Krawetz SA, Gorelick R, Heng HH. Single cell heterogeneity: why unstable genomes are incompatible with average profiles. Cell Cycle 2013;12(23):3640–49.
29. Stevens JB, Horne SD, Abdallah BY, Ye CJ, Heng HH. Chromosomal instability and transcriptome dynamics in cancer. Cancer Metastasis Rev. 2013;32(3–4):391–402.
30. Stevens JB, Liu G, Abdallah BY, Horne SD, Ye KJ, Bremer SW, Ye CJ, Krawetz SA, Heng HH. Unstable genomes elevate transcriptome dynamics. Int J Cancer 2014;134(9):2074–87.
31. Liu G, Stevens JB, Horne SD, Abdallah BY, Ye KJ, Bremer SW, Ye CJ, Chen DJ, Heng HH. Genome chaos: survival strategy during crisis. Cell Cycle 2014;13(4):528–37.
32. Heng HH, Liu G, Stevens JB, Abdallah BY, Horne SD, Ye KJ, Bremer SW, Chowdhury SK, Ye CJ. Karyotype heterogeneity and unclassified chromosomal abnormalities. Cytogen Genome Res. 2013;139:144–57.
33. Heng HH. The conflict between complex systems and reductionism. JAMA 2008;300(13):1580–1.
34. Heng HH. Bio-complexity: challenging reductionism. In: Sturmberg JP, Martin CM, editors. Handbook on systems and complexity in health. New York: Springer; 2013. p. 193–208.
35. Heng HH, Horne SD, Stevens JB, Abdallah BY, Liu G, Chowdhury SK, Bremer SW, Zhang K, Ye CJ. Heterogeneity mediated system complexity: the ultimate challenge for studying common and complex diseases. In: Sturmberg JP, editor. The value of systems and complexity sciences for healthcare. Cham: Springer; 2016. p. 107–20.

36. Heng HH, Regan S, Ye CJ. Genotype, environment, and evolutionary mechanism of diseases. Environ Dis. 2016;1:14–23.
37. Heng HH. Heterogeneity-mediated cellular adaptation and its trade-off: searching for the general principles of diseases. J Eval Clin Pract. 2017;23(1):233–237.
38. Heng HH. Missing heritability and stochastic genome alterations. Nat Rev Genet. 2010;11(11):813.
39. Hultén MA, Jonasson J, Iwarsson E, Uppal P, Vorsanova SG, Yurov YB, Iourov IY. Trisomy 21 mosaicism: we may all have a touch of Down syndrome. Cytogenet Genome Res. 2013;139(3):189–92.
40. Iourov IY, Vorsanova SG, Yurov YB. Chromsomal mosaicism goes global. Mol Cytogenet. 2008;1:26.
41. Yurov YB, Vorsanova SG, Liehr T, Kolotii AD, Iourov IY. X chromosome aneuploidy in the Alzheimer's disease brain. Mol Cytogenet. 2014;7(1):20.
42. Biesterfeld S, Gerres K, Fischer-Wein G, Böcking A. Polyploidy in non-neoplastic tissues. J Clin Pathol. 1994;47(1):38–42.
43. Duncan AW, Taylor MH, Hickey RD, Hanlon Newell AE, Lenzi ML, Olson SB, Olson SB, Finegold MJ, Grompe M. The ploidy conveyor of mature hepatocytes as a source of genetic variation. Nature 2010;467:707–10.
44. Horne SD, Chowdhury SK, Heng HH. Stress, genomic adaptation, and the evolutionary trade-off. Front Genet. 2014;5:92.
45. Sturmberg JP, Bennett JM, Martin CM, Picard M. "Multimorbidity" as the manifestation of network disturbances. J Eval Clin Pract. 2017;23(1):199–208.
46. Sturmberg JP, Martin CP. Complexity in health: an introduction. In: Sturmberg JP, Martin CM, editors. Handbook on systems and complexity in health. New York: Springer; 2013. p. 1–17.
47. Nesse RM. Evolution: medicine's most basic science. Lancet 2008;372(S1):S21–7.
48. Evans JP, Meslin EM, Marteau TM, Caulfield T. Genomics. Deflating the genomic bubble. Science 2011;331:861–2.
49. Heng HH, Regan S. A systems biology perspective on molecular cytogenetics. Curr Bioinfom. 2016;12(1):4–10.
50. Noble D. Physiology in rocking the foundation of evolutionary biology. Exp Physiol. 2013;98:1235–43.