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Noise in multiple sclerosis: unwanted and necessary

Isabella Bordi[1], Vito A. G. Ricigliano[2,3], Renato Umeton[2], Giovanni Ristori[2], Francesca Grassi[4], Andrea Crisanti[1], Alfonso Sutera[1] & Marco Salvetti[2]

[1]Department of Physics, Sapienza University of Rome, Rome, Italy
[2]Neurology and Department of Neurosciences, Mental Health and Sensory Organs (NESMOS), Centre for Experimental Neurological Therapies (CENTERS), Sapienza University of Rome, Rome, Italy
[3]Neuroimmunology Unit, Fondazione Santa Lucia, (I.R.C.C.S.), Rome, Italy
[4]Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy

Correspondence
Marco Salvetti, Neurology and Department of Neurosciences, Mental Health and Sensory Organs (NESMOS), Centre for Experimental Neurological Therapies (CENTERS), Sapienza University of Rome, Rome, Italy.
Tel: +39 0633775994;
Fax: +39 0633775900;
E-mail: marco.salvetti@uniroma1.it

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Abstract

As our knowledge about the etiology of multiple sclerosis (MS) increases, deterministic paradigms appear insufficient to describe the pathogenesis of the disease, and the impression is that stochastic phenomena (i.e. random events not necessarily resulting in disease in all individuals) may contribute to the development of MS. However, sources and mechanisms of stochastic behavior have not been investigated and there is no proposed framework to incorporate nondeterministic processes into disease biology. In this report, we will first describe analogies between physics of nonlinear systems and cell biology, showing how small-scale random perturbations can impact on large-scale phenomena, including cell function. We will then review growing and solid evidence showing that stochastic gene expression (or gene expression "noise") can be a driver of phenotypic variation. Moreover, we will describe new methods that open unprecedented opportunities for the study of such phenomena in patients and the impact of this information on our understanding of MS course and therapy.

Introduction

> *This place is a mystery Daniel, a sanctuary. Each book, each one that you see, has a soul. The soul of who has written it and the soul of those that have read it, experienced it, and dreamed with it. Each time that a book changes hands, each time that someone takes a look at its pages, his spirit grows and becomes stronger.*
>
> La sombra del viento
> Carlos Ruiz Zafón

Broadly speaking, infectious and mendelian diseases originate from a perturbation which is traceable in most cases. In other terms, the outcome (disease) is a function of the initial condition (pathogen invasion or gene mutation).

This knowledge stems from decades of research, full of old and recent successes, that have to some extent framed our mind to think that, for each disease, initiating events or a confluence of initiating events should be identifiable. It is therefore not surprising that a similar attitude has influenced also etiological research in multifactorial diseases, including multiple sclerosis (MS). After decades of studies on the heritable and nonheritable causes of MS, it seems that something is perpetually missing.[1] Large genome-wide association studies (GWAS) and prospective surveys on environmental risk factors have unequivocally identified elements associated with the disease. Nonetheless, these associations are neither sufficient nor necessary for the development of the disease and for its prediction[2] (including familial cases[3–5]). The prevailing opinion is that there is a big gap of knowledge that someday will be filled, thanks to the identification of many common (or few rare) variants of small (or large) effect, gene–gene, or gene–environment interactions among others.[6–10] This is a strictly deterministic view: given a cause, the same effect (MS, for instance) invariably results. Moreover, a given effect always stems from the same set of causes. Thus, in a largely reductionistic frame, the medical/scientific prob-

lem becomes finding the function that connects the right set of initial conditions (yet unidentified, in some cases) to the pathology.

However, the available information is already enormous and offers various interpretative opportunities.[11–15] Insofar as it is possible that eventually the key heritable or nonheritable factor(s) will show up, it is somehow peculiar that, in many years, we have been able to identify many subtle variations while keeping on missing the rafters we might have had in our eyes. Recently, similar considerations prompted some reflection about the inadequacy of transferring, as such, the deterministic approach that applies for infectious and genetic diseases to complex traits.[2,15] So far, it is unclear which processes may substitute for these paradigms, or flank them in shaping a complete picture of disease etiology.

Here, in an attempt to take a further step, and suggest a new framework to interpret events that may lead to the disease, we will first discuss the limits of a strictly deterministic view of the etiology of multifactorial diseases, recalling that the behavior of complex physical systems (including biological ones) can be significantly modified by small random perturbations (or "noise"). We will then review evidence suggesting that a major nondeterministic component of phenotypic variation (which can include the occurrence of diseases) is stochastic gene expression (or gene expression "noise"), which, along with time, may be a necessary component for the development of MS.

Finally, we will discuss how this information may impact the studies on disease etiology, proposing a mechanistic model in which noise, superimposed onto a system moving on an energy landscape with many minima (multi-minima landscape), well describes erratic oscillations between relapses and remissions clinically observed in several individual patients in the initial phases of MS. In particular, the model integrates "noise" and time with the genetic and environmental risk, in a process that can induce phenotypic changes, including disease, also in the presence of heritable and nonheritable factors that mistakenly appear too weak.

Instability of Complex Systems

In this study, we refer to nonlinear systems (where the ratio between the output and the input is not constant) that have multiple steady states, associated with energy minima (multi-minima landscape). Energy barriers, corresponding to energy maxima, separate the minima, so that, in the absence of external energy inputs, the system settles down in a minimum. Extremely different steady states can be reached starting from relatively similar initial conditions: two water drops falling few millimeters apart at a watershed could end their course into different Oceans (Fig. 1). In the absence of external energy inputs, this disproportion in size between "causes" (small distance between drops) and "effects" (different terminal Oceans) may be fully predictable, although impressive. If energy to overcome the barriers is available, transitions between states are possible. To stay with our example, water can be pumped upwards and allowed to flow on the other side of the mountain. For physical systems, it is known that small random forces (noise) can occasionally sum up and provide this energy. In a system with more than one steady state, noise can induce transitions among different steady states (see Appendix). The theory of Earth's climate is an example: climate changes that induce glaciation cycles are negligible if compared to the temperature variations that accompany the shift from a temperate climate to an ice-covered Earth. It is the cooperative effect of small-scale stochastic perturbations and periodic forcing that amplifies the climate response.[16–19]

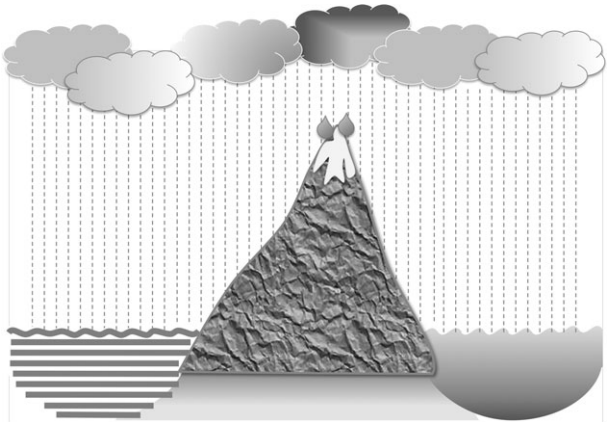

Figure 1. A nonlinear system with two energy minima (striped and gray Oceans) separated by a barrier (energy maximum, mountain peak). Minimal differences in the initial conditions (where the water drops fall) will determine completely different outcomes (the Ocean into which a water drop ends).

Obviously, the fact that the fate of nonlinear systems can be influenced by small random perturbations results into nondeterministic behavior: the state finally attained depends on noise, in addition to the initial conditions.

In medical conditions, behaviors similar to those observed for stochastic nonlinear systems manifest already in diseases whose pathogenesis may be less "complex" compared to MS. For instance, embolism that can be a casual response to normally distributed liability traits such as dyslipidemia or hypertension. Similarly, nonlinear feed-forward processes are involved in distinguishing between lethality and mild disease in influenza infection.[20] If stochastic and/or nonlinear pathogenetic events play a role in diseases with a relatively "simple" pathogenesis, even more so they should be considered as inescapable components of the mechanisms that lead to the

development of conditions such as MS. The attention to such processes should increase as the evidence grows that these diseases are indeed "complex": their heritability appears to be attributable to many common variants loci of seemingly weak effect,[10] with many additional susceptibility alleles that remain to be identified in MS[21] and non-heritable risk factors that also appear to be common and able to perturb the host with pleiotropic mechanisms.[22,23]

Gene Expression "Noise" is a Nondeterministic Component of Phenotypic Variation

If we accept that processes leading to multifactorial diseases can have a stochastic component, the next question to be asked is where does the noise of the system stem from. Keeping in mind that what we perceive as noise is the results of processes that act on a time scale much shorter than the ones we are considering, noise permeates biological phenomena. In processes such as multifactorial diseases that involve genes, environment and their interactions the most likely candidate source of noise resides in gene expression.

Gene expression is the outcome of intrinsically stochastic processes, for instance it depends on a Brownian random walk of transcription factors through the cellular volume before they find their promoters. As witnessed by paradigmatic experiments, allowing enough time, even genetically identical cells and organisms, grown in the same environment, can become phenotypically different.[24,25] This happens because the expression of individual genes takes place through discrete and random fluctuations (gene expression "noise") of the production of mRNAs and proteins (Fig. 2).

Interestingly, noise also enforces the coordinated expression of genes across large regulons,[28] all this implying that any noise change may have profound consequences. In fact, bursts in the expression of single genes, due to intrinsic noise, can propagate to the expression of downstream genes generating extensive and correlated fluctuations (extrinsic noise).[25] In mammalian cells, this may lead to long-lasting and concerted noise in protein levels that may, for example, cause the appearance of "outlier cells"[29] that react differently to environmental signals or drugs with respect to the bulk of the population.

The fact that the most basilar events that characterize life (information storage in genes and retrieval through gene expression) are intrinsically stochastic means that living organisms can adapt to changing conditions much better than any rigidly predetermined system could do. Thus, noise plays an essential role in key cellular activities and, at longer timescales, it may do so also at the evolutionary level. Furthermore, noise may be viewed as a buffer[25] between the conflicting functions of genome (that defines and constrains the system) and genes (that modify and diversify it).[30]

Although these observations are not new,[31] it took some time before they ignited interest in stochastic gene expression,[32] perhaps because noise in gene expression is instinctively perceived as a nuisance for a process that ought to be tightly regulated. In MS, it took even longer before the possibility of random events[15] and finally translational/transcriptional stochasticity[33] were proposed as a component of the etiology of disease, in spite of epidemiological evidence suggesting that unique, nonshared environmental factors cannot be invoked to explain disease discordance in monozygotic twins.

If we acknowledge that gene expression noise can be a source of clinically relevant phenotypic variation, we should also start looking at the heritable risk in a more dynamic way, that is, as a component that can fluctuate over time (Fig. 3).

Time is the Other Ingredient

In experimental models of autoimmunity, there is evidence of a multistep process where subtle alterations resulting from quantitative trait loci variations

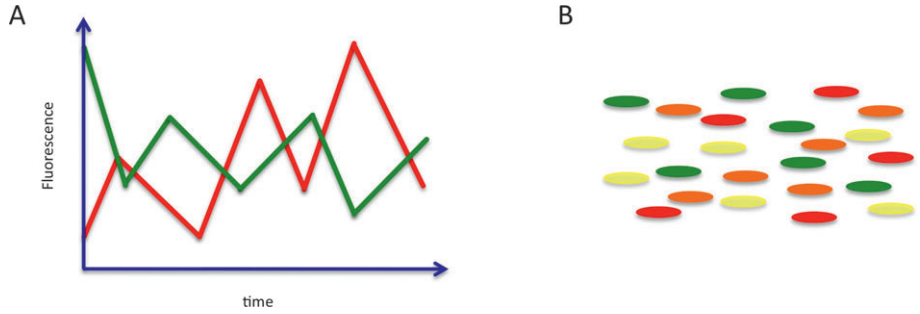

Figure 2. Effects of noise on gene expression and cell-to-cell variability. Individual E. coli expressing two identical promoters that control the expression of two different fluorescent proteins, red and green. Scheme of the temporal variations of gene expression (noise) (A) and different levels of the ratio of red to green intensity in the cell population at the end of the observation period (B). Adapted from refs. (26 and 27).

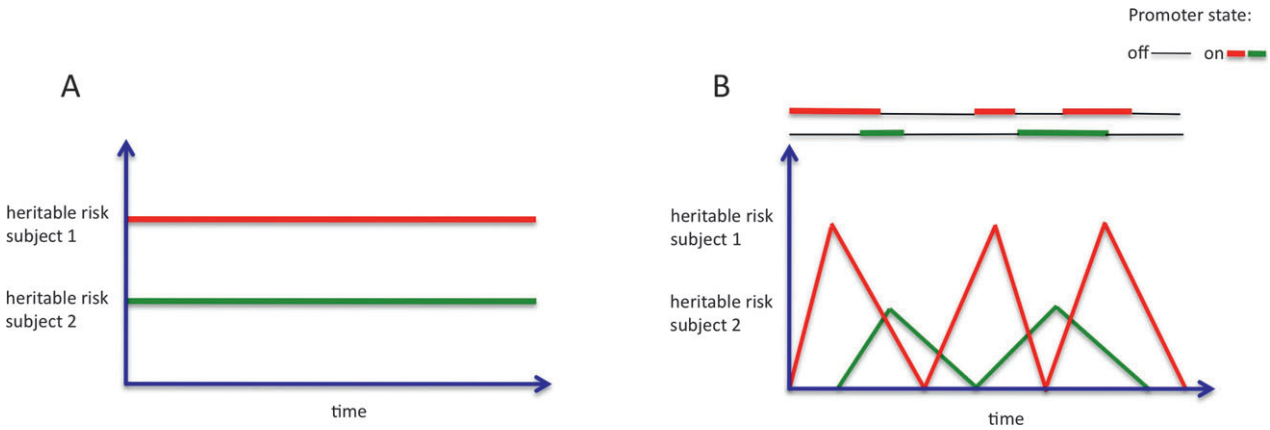

Figure 3. Dynamic effects of gene expression noise on the heritable risk. Let us consider two individuals, 1 and 2, with different heritable risk for multiple sclerosis (MS). According to the canonical view of the pathogenesis of multifactorial diseases, given an identical environmental input, the individual 1 will be at higher heritable risk than individual 2 (A). However, if we incorporate gene expression noise (bursty gene expression) in this model, heritable risk cannot be viewed any more as a monolithic entity. Rather, it will fluctuate over time with gene expression bursts. At given time points this may even revert the individual risk (B).

accumulate with time, resulting in large changes in the susceptibility to autoimmunity when a threshold for the occurrence of the disease is passed.[34] In human autoimmune conditions, various studies indirectly suggest that relatively long time intervals are a necessary factor in the development of complex diseases. Immune response defects, such as those linked to the *PTPN22* variant (which associates with many autoimmune diseases), precede the clinical onset of the disease itself (see ref. 35 for a review on the evidence of such mechanisms in human autoimmune conditions).

As far as MS is concerned, persons with "radiologically isolated syndromes" (i.e., incidental magnetic resonance imaging findings suggestive of MS) develop the first symptoms of the disease after 1–5 years in a 33% of the cases,[36] with a substantial proportion of subjects who may experience the first symptoms much later or never in a lifetime, reinforcing the idea that MS is a process starting a long time before its clinical "onset." And even after a first clinical episode has occurred, a new relapse or a radiological conversion into MS does not occur in a relatively large proportion of patients even after a 20-year follow-up.[37] The relapsing/remitting course that patients experience during the first years of the disease, suggests that they actually spend time wandering between active disease and a relatively healthy remission state.

But even more than clinical observations, epidemiological data support the importance of time, suggesting that it starts exerting its influence well before the subclinical or clinical onset of the disease.[38] After some debate, the correlation between risk of the disease and place of residence in childhood seems to be confirmed,[39] while the role of longer "exposures," perinatal, prenatal and ancestral, needs further scrutiny.[40] Finally, findings from twin studies strongly suggest that a proportion of heritable and nonheritable factors in MS are interactive rather than independent, indirectly supporting the importance of time to allow (presumably numerous and iterative) interactions to take place.[40]

Noise and Time Can Amplify the Effects of Weak Genetic and Environmental Factors

So far, we have seen that gene expression noise can be a source of phenotypic variation at the cellular level. Its pervasive presence in biological processes, essential role in regulation and phenotypic definition suggest that it may have "macrosopic" effects, including the development of pathologic states. Likewise, time is required for the unfolding of pathogenetic events that lead to a disease, and MS is no exception. The next step is to understand how noise and time may combine and contribute to the development of the disease.

Mechanistically, gene expression noise may contribute to reaching a disease threshold through the gradual accumulation of defects, by providing small random perturbations that amplify the effects of heritable and nonheritable predisposition or both. The multistep accretion of subtle alterations can be intuitively appreciated and is to some extent supported by data in animal models.[34] However, the erratic manifestations of a disease such as MS appear difficult to reconcile with this idea of progressive accumulation of pathogenetic events.

A model that incorporates small random perturbations, provided by gene expression noise, appears more

compatible with the relapsing-remitting course of the disease and with the pervasive and regulatory role of noise. Supporting this hypothesis in MS is the observation that the occurrence of relapses follows an exponentially decaying distribution, implying that disease activity manifests randomly in time. Hence, similar to models used for instance for the Earth's climate theory,[17–19] MS can be modeled as a nonlinear system with two steady states, diseased and healthy (Appendix and Fig. 4).[41] The system will remain in either steady state even if exposed to small random perturbations. However, these small stochastic events can eventually force the system out of its steady state and into the other one. Time is necessary to allow the occurrence of these low-probability events.

Thus, a mechanistic model with random forcing describes in a satisfactory manner, the disease course and how small stochastic perturbations induced by (gene expression) noise may interact with etiologic factors of small effect-size (genetic and environmental), occasionally reaching the disease threshold provided that enough observation time is given (Fig. 4).

Consequences for Studies on Disease Etiology

To verify this model, and to assess the proportion of phenotypic variance that can be attributed to gene expression noise, the usual attempts to correlate average measurements with the disease status should be complemented by single-cell measurements and stochastic analyses, particularly for genes with low transcription and high translation rates (which may bring about large protein fluctuations).[42] This approach is not frequent, and existing studies on cellular heterogeneity (because of methodological limitations) have quantified either few RNAs or proteins in relatively large samples of cells or more gene products but on much smaller numbers of cells. Furthermore, with few exceptions,[43–49] data refer to model microbial systems.

The recent advent of single-cell genomics may now open new opportunities to extend this kind of studies also to patient populations and has already shown unexpected levels of heterogeneity between cells also for genes that are very highly expressed at the population average.[48]

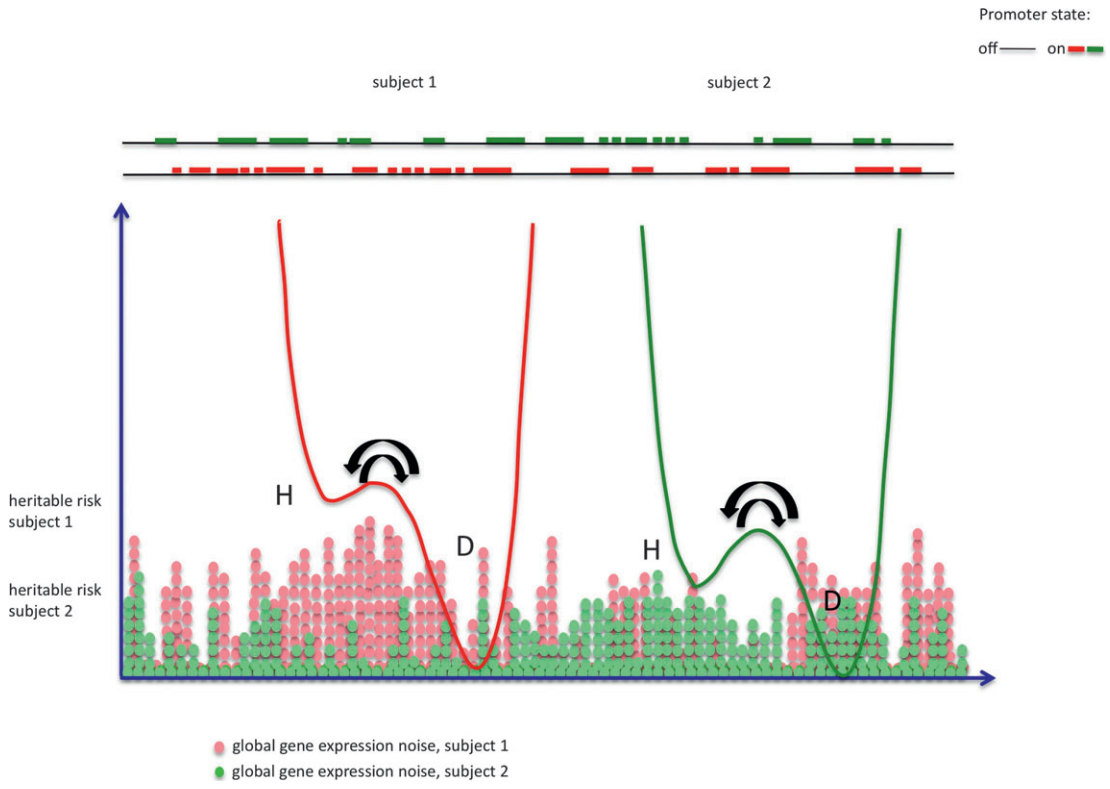

Figure 4. Schematic picture of a mechanistic stochastic model that describes disease risk and course. Health and disease are modeled as a nonlinear system with steady states, health (H) and disease (D). The disease risk and course for the same subjects as in Figure 2 are considered. A transition from health state (well H) to disease state (well D) is easier for subject 1 (green) than for subject 2 (red) as a result of his higher heritable risk. For the same reason, reverting from the disease to the health state is more difficult for subject 1. However, the transitions remain random as the underlying gene expression noise provides the required energy for switching from one well to the other.

Here, bimodality among highly expressed immune response genes was detected, suggesting the suitability of this kind of studies to reveal distinct cellular subtypes or stochastic differences in the activation of regulatory circuits. Other very recent and potentially useful technological approaches to go beyond average measurements and explore noise and cell-to-cell variations include super-resolution microscopy methods for live cell imaging, which promise to display single molecule dynamics with second- or even sub-second-scale time resolution.[50] Besides these new developments, also the complementary combination of more established methods such as dynamic array chips and single-molecule mRNA fluorescent in situ hybridization has yielded important information, for example, about variations in stochasticity in different phases of cellular reprogramming.[51] The same may be true for the use of new approaches for the direct measurement of ongoing transcription of nascent RNAs and other techniques.[52,53]

Other approaches may be less demanding in terms of methodological innovation. This is the case of the large and increasing number of studies on microRNAs, on epigenetic chromatin modifications and on more specific mechanisms of regulation such as those linked to the molecular chaperone Heat shock protein 90[54] or to ultrasensitive response motifs.[55] Any imperfection in these mechanisms of global regulation, but also mutations in genes with more specific functions[52] may have consequences on the robustness of transcriptional regulation, affecting the buffering effect of microRNAs on fluctuations in gene expression or through changes in the stability and transition rates of promoter-activity states brought about by chromatin modifications. The latter may also lead to variations in the expression noise of neighboring genes as "opening" of the chromatin surrounding one gene is likely to affect other genes nearby, leading to correlations in their expression.[24,25,56] New techniques to perturb histone modification patterns in a locus-specific manner through the induction of site-specific heterochromatin domains in vivo promise to bring new information about the connection between chromatin state and gene transcription.[57]

As far as MS is concerned, the need for technological advancements and data repositories (such as the recently established genome-wide chromatin modifications maps[58]) that may clear studies on gene expression noise in patients appears to be even stronger in light of results showing that disease- and trait-associated variants are concentrated in regulatory DNA.[59–61] In this context, it is interesting that transcription factors involved in chromatin remodeling such as SP1 may be central to explain gender-based variability between persons with MS and controls.[62] In addition, the presumptive role of vitamin D in the pathogenesis of MS and various other diseases may be ascribed to the vitamin D receptor preferential binding to intronic and intergenic regions with regulatory role and enhancer functions,[63] again supporting possible consequences on gene expression noise. Finally, an area of interest for MS pathogenesis, the interplay between virus and host, conceptually lends itself very well to the study of gene expression noise. Viral reactivation from a "latent" state can be stochastic, as recently shown for the reactivation of HIV reservoirs.[64] Also for viruses such as Epstein-Barr virus (EBV), which may contribute to MS etiology, the study of gene expression noise and cell-to-cell variability may be informative for the identification of pathogenetically relevant variations. Noncytopathic viruses have established coregulated genomes and gene expression programs with their hosts.[65–67] These include the regulation of higher order chromatin structure, "super-enhancers" or accelerators of transcription, all factors of potential impact on gene expression noise. An example of potential interest in MS is BRD4, a transcriptional coactivator that co-occupies thousands of enhancers and a small set of "super-enhancers"[68]; this factor is an interactor of the EBNA1 protein of EBV[69] and co-regulates *MYC* which, in turn, can accumulate in the promoter regions of active genes causing transcriptional amplification.[70,71] Furthermore, a low rate of transcription is a hallmark of virus ability to establish a latency state. The reactivation rate of latent to lytic cycle is also low, and intrinsically subjected to stochastic bursts such as those driven by antigen stimulation or receipt of a plasma cell differentiation signal, once more suggesting that disease-associated differences may be revealed by single-cell measurements and stochastic analyses rather than bulk assays.

Other studies that encourage analyses at the single-cell level in an immune-mediated disease such as MS are those that describe how stochastic processes regulate the physiology of the cytotoxic T cell (CTL) response to antigens[72] or the differentiation of T-cell subsets.[73] As for CTLs, time series of lymphocytes selectively activated to cytotoxicity (that lacks a mature immunological synapse) show that these cells have signaling patterns with lower average calcium mobilization compared to fully activated CTLs. However, when the time series are analyzed at the single-cell level, the less mature elements have higher spikes of calcium mobilization that comprise deterministic (e.g., oscillations) and stochastic aspects. In the differentiation of key T-cell subpopulations such as TH1 and TH2 T cells, single cell variability is relevant in physiology and may be altered in an autoimmune disease state: in the early phase of T helper cell differentiation a "noisy" cytokine expression is key in triggering a fully differentiated state in the subpopulations but it is buffered by the ubiquitous, high-level co-expression of antagonistic transcription factors in individual cells. Finally, as far as

the T and B cell responses to antigens are concerned, stochastic events such as the encounter of T and B cell epitopes contribute to the regulation or breakdown of immunological tolerance.[74,75]

Conclusion

A stochastic (i.e., nondeterministic) component has seldom been taken into account as a relevant actor in the pathogenesis of a multifactorial disease like MS. For some reason, our logical explorations tend to feel more comfortable in the presence of identifiable causes and effects. In the case of a disease, this attitude is perhaps reinforced by the fact that acknowledging a stochastic component in the pathogenesis of a disease may be viewed as an obstacle or, at best, useless in therapeutic terms.

On the contrary, particularly in the early phases of the disease, noise itself may represent a target for etiologic therapies, and might ultimately turn out to be less complex to attack than genes or viruses. Encouraging preclinical evidence – but also clinical data – about the possibility of affecting the early course of MS with "mild" interventions such as increase in vitamin D or other fat-soluble vitamin levels[22] or Bacille Calmette-Guerin (BCG) vaccination,[76,77] dietary sodium,[78,79] and modulation of the endocannabinoid system,[80] among others, may reflect the possibility of stabilizing noise and "deamplify" its effects thanks to the pleiotropic, although "soft," actions of these interventions. If and when the integration of genetic data with endophenotypes, magnetic resonance imaging or other biomarkers will become useful for prediction in a clinical circumstance,[81] the importance of such "soft" approaches will become even more evident.

We hope to have provided some conceptual and methodological information that will encourage and help those who will engage this new and promising field of research, in MS and in other multifactorial diseases.

Acknowledgments

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Conflict of Interest

None declared.

Appendix:

In the last years, there has been a growing interest in understanding the role and impact of noise on many aspects of biology and medicine[82]. With "noise" one generically means any small random perturbation acting on a system. Due to its intrinsic "smallness," it appears obvious to expect that noise effect is "small" and, in the presence of noise, the system behaves much as it would do in its absence. In such cases, noise represents a nuisance, which usually one tries to filter out.

However, despite its smallness, noise may have dramatic effects, which can drive the systems far away from its noiseless behavior. These situations are not so uncommon. An example is climate changes. Here, the cooperative effect of noise, arising from the internal dynamics of the atmosphere and ocean, and the small periodic variation in solar energy over a period of about 100,000 years, induced by the variation in the Earth's eccentricity, provides an amplification of the climate response that leads to the transition from a temperate climate to an ice-covered Earth state and vice versa.[17,18]

The phenotypic differences between individual organisms can often be ascribed to the underlying genetic and environmental variations. However, it is known that genetically identical organisms evolving in a homogeneous environment may present phenotypic differences, indicating that the small perturbation of developmental processes that stems from the gene expression noise may generate macroscopic diversity. At a larger scale level, the random fluctuations in the expression of individual genes may then contribute to, or be responsible for, the transition between health and disease by exposing hidden genetic and environmental risk. Following this idea, recently Bordi et al.[41] have shown that the transitions between remissions and relapses in MS can be well described by a simple stochastic model. The model can be visualized by thinking of the motion of a particle in an asymmetric double-well energy potential schematically illustrated in figure. The position of the particle represents the patient state, while the two minima account for the health (lower global minimum) and disease (higher local minimum) states. In the absence of noise, for (almost) all initial positions the particle will move down to one of the two minima (uniquely determined by the initial conditions), and there it will remain, with no transitions between states (remissions and relapses).

If noise is added, the particle will still move down to one of the two minima and remain close to it for some time. However, the stochastic perturbation due to noise may eventually drive the particle over the hill separating the minima and into the other minimum. In just the same manner, the fate of a patient is no more uniquely determined by the initial state and transitions between remissions and relapses are now possible. It is intuitively clear that as the transitions are driven by noise, they occur randomly in time. It is also evident that for an

asymmetric double-well the time the particle spends close to one minimum, i.e., the health/disease periods, is different for the two minima. Such a stochastic model gives a good description of the occurrence of remissions and relapses in single patients affected by Multiple Sclerosis.

Glossary of terms used

Deterministic system: a system whose evolution is (at least in principle) fully predictable from the knowledge of its condition (state) at any given time.

Noise: random perturbation acting on a system.

Nonlinear systems: systems in which changes in the output (or response) are not linearly related (i.e., directly proportional) to changes in the input (or stimulus).

Stochastic: Random.

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