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PERSPECTIVE

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The triple code model for advancing research in rare and undiagnosed diseases beyond the base pairs

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ABSTRACT

Rare and undiagnosed diseases pose significant challenges for understanding their mechanisms, diagnosis, and treatment. The Triple Code Model, an integrative paradigm described here, considers the combined influence of the genetic code, epigenetic code, and nuclear structure (an emerging code), as fundamental biochemical mechanisms underlying many rare diseases. Studies demonstrate dysfunctional membrane and cytoplasmic signals instruct the epigenome to ultimately impact the 3D structure and dynamics of the nucleus, highlighting their close interrelationships. Consequently, this model offers a holistic perspective on rare and undiagnosed diseases by moving beyond a solely genetic view. We propose that this integrated framework will efficiently guide rare disease research by taking it 'Beyond the Base Pairs,' leading to improved diagnostics and personalized treatments.

PLAIN LANGUAGE SUMMARY

Rare and undiagnosed diseases are difficult to understand and treat because they are diverse and occur infrequently. To address these challenges, we need a new way to think about these diseases. The Triple Code Model, discussed in this article, looks at three important factors: the genetic code (our DNA), the epigenetic code (how our genes are turned on or off), and nuclear structure (how DNA is organized inside the cell). Research shows that problems with signals from the cell membrane and cytoplasm can change how genes are expressed and affect the 3D organization of the genome. This model highlights how these factors are interconnected and suggests that we should study rare diseases from a broader perspective, rather than focusing only on genetics. By using the Triple Code Model, we can better understand these diseases and work toward improved diagnostics and treatments.

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1. Navigating the multifaceted pathobiological landscape of rare and undiagnosed diseases

Notably, while rare diseases are defined in the USA as conditions affecting fewer than 1 in 200,000 individuals and in the European Union as less than 1 in 2,000 [1], they collectively represent a significant global health burden despite their prevalence. Current estimates indicate the existence of more than 6,000 distinct rare diseases, which translate to 260–440 million people affected, or up to 5.9% of the world's population [2]. However, these are diseases for which we know its cause. This consideration is important since a large number of individuals with conditions of unknown causes exist despite comprehensive evaluation, and they are categorized as undiagnosed diseases. It is believed that many in this category may represent previously undescribed rare disorders or rare variations of known diseases [3]. Unfortunately, patients with rare and undiagnosed diseases most often embark on a prolonged diagnostic odyssey, facing delays of 4–5 years or more, sometimes decades, before being diagnosed. Interestingly, several years of experimentation and data analyses from our laboratories and many others have accumulated evidence that the interplay among genetics, epigenetics, and nuclear dynamics, forming the basis of our Triple Code Model [4], may play a crucial role in their pathogenesis.

Here, we apply the Triple Code Model, which departs from the genetic-centric view that has dominated rare and undiagnosed diseases for several decades. A less developed version of this paradigm published by our laboratory for cancer applications has guided investigations into how a somatic mutation induces tumor development [4]. Following this model, we first discuss genetics, namely how mutations in distinct genes contribute to the pathobiology of rare diseases. Secondly, we address how changes in the epigenetic landscape of cells and tissues impact rare diseases, underscoring how epigenetic modifications, such as DNA methylation and histone modifications, modulate gene expression and influence disease phenotypes at the cell, tissue, and whole organism levels. Moreover, we consider the role of nuclear dynamics in the development and progression of rare diseases, including nuclear architecture, chromatin organization,

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Article highlights

Introduction:
- Over 6,000 distinct rare diseases affect 260–440 million people worldwide, or up to 5.9% of the global population.
- Patients often endure years without a diagnosis while navigating a diagnostic odyssey.
- The "Triple Code Model" aims to integrate and understand how genetic mutations, epigenetic changes, and nuclear structure interact with each other in disease development.

Genetics in Rare and Undiagnosed Diseases:
- Approximately 80% of rare diseases are caused by genetic factors, and genomic sequencing technologies like WES and WGS have transformed how these conditions are diagnosed.
- Understanding the genetic basis of rare diseases has opened new avenues for developing novel gene therapies and targeted treatments, driving pharmaceutical and research efforts to advance interventions in this field.

Epigenetics in Rare and Undiagnosed Diseases:
- Rare diseases are increasingly associated with disruptions in DNA methylation, histone modifications, and non-coding RNA activities, highlighting the complex mechanisms of regulating gene expression.
- Epigenetic changes play a key role in driving rare disease phenotypes, with examples including Prader-Willi syndrome (resulting from differential DNA methylation), Rubinstein-Taybi syndrome (caused by altered histone modifications), and spinal muscular atrophy (linked to non-coding RNAs).

Nuclear Structure and Dynamics in Rare and Undiagnosed Diseases:
- Laminopathies illustrate the essential role of nuclear envelope integrity and chromatin organization in cellular function, with disruptions leading to rare diseases.
- The study of nuclear dynamics reveals key insights into disease mechanisms and offers promising new approaches for treating rare diseases by targeting nuclear structure and function.

Integration of the Triple Code Model for Rare and Undiagnosed Diseases:
- The Triple Code Model provides a unified framework that integrates genetics, epigenetics, and nuclear architecture to enhance our understanding of disease mechanisms in rare and undiagnosed conditions.
- Research using this model has illuminated how disruptions in genetics, epigenetics, and nuclear structure contribute to disease, exemplified by findings in chromatinopathies, rasopathies, and rare cancers.
- Implementing this model into diagnostic workflows could revolutionize rare disease diagnosis and treatment by combining advanced sequencing, epigenomic profiling, and techniques for analyzing nuclear structure and dynamics.

Future Perspective:
- The Triple Code Model represents a significant advancement in rare and undiagnosed disease research, leveraging multi-omics technologies and computational modeling to generate new hypotheses and improve patient outcomes through personalized medicine approaches.
- Embracing a systems biology approach through the Triple Code Model promises to enhance our understanding of complex biological systems, drive innovative therapeutic strategies, and promote collaborative research to address the challenges of rare diseases more effectively.

and nucleolar function. The rationale underlying this model is that the genome acts as a self-protecting and self-regulating system that senses and responds to input signals. Thus, many genes, specifically those that encode signaling proteins and are altered by mutations (Genetics), change the types of signals they send to the nucleus. This alteration influences how the nucleus responds, modifying the structure, composition, and function of chromatin (Epigenetics) and leading to changes in nuclear compartments important for gene regulation (3D nuclear structure and dynamics) (Figure 1). Thus, we highlight the potential avenues of research and practice that are better leveraged by integrating these three regulatory layers of genomic control. We are optimistic that these insights may lead to the discovery of new therapeutic targets, biomarkers, and personalized treatment approaches, paving the way for improved diagnostic and therapeutic strategies.

2. The genetic code as the Rosetta Stone that revolutionized research and practice in rare and undiagnosed diseases

Advances in genetics and genomics have profoundly shaped the field of rare diseases to the point that approximately 80% of rare diseases known to date originate from genetic factors [5]. Without a doubt, genomics has heralded an unprecedented era for discovering causes and mechanisms underlying rare and undiagnosed diseases. Prominent examples of how genomic efforts impacted the field include the identification of mutations in the *CFTR* gene that cause cystic fibrosis, mutations in the *DMD* gene responsible for Duchenne muscular dystrophy, mutations in the *FBN1* gene causing Marfan syndrome, and mutations in the *LDLR* gene leading to familial hypercholesterolemia, among others [6]. Understanding the genetic basis of these conditions has been pivotal for advancing research on rare diseases. In particular, the emergence of powerful genomic sequencing technologies, like whole-exome sequencing (WES) and whole-genome sequencing (WGS), has revolutionized the diagnosis of these diseases by enabling the precise identification of causative genomic variants [7]. For instance, WES led to the identification of *MLL2* as a causative gene for Kabuki syndrome. WGS offers expanded diagnostic capabilities beyond WES by evaluating DNA structural changes, deep intronic variants, alterations in non-coding regions, and repeat expansions [8]. These advancements have begun to offer opportunities for more targeted and personalized treatment approaches.

Genetic mutation-based diagnoses provide insights into rare disease mechanisms and consequently build the trajectory for precision medicine approaches with targeted therapies tailored to the specific genetic profile of a patient. For example, developing ivacaftor, lumacaftor, tezacaftor, and elexacaftor as specific therapeutics for cystic fibrosis patients with distinct *CFTR* mutations illustrates the potential for personalized treatments based on genetic information [9]. However, when analyzing the genetic landscape of rare diseases, we find many disorders exhibiting genetic heterogeneity, where mutations in the same gene (e.g., *ABCA4* for various retinal dystrophies) produce different clinical phenotypes [10] or different genes (e.g., *FKRP, POMT1*, and *POMT2* for congenital muscular dystrophies) often lead to similar clinical phenotypes [11]. Some rare diseases also display patterns of inheritance that introduce additional complexities outside traditional Mendelian models, such as unstable trinucleotide repeat expansions in Huntington's disease, requiring more in-depth levels of analysis in this field [12]. As genetic testing becomes more common among rare disease patients, databases like ClinVar and HGMD, containing genetic variants and their clinical interpretations, have become invaluable resources [13]. However, we should remain cognizant of the bias present in each database that can affect the data mining approach, particularly for methods that use training sets. For

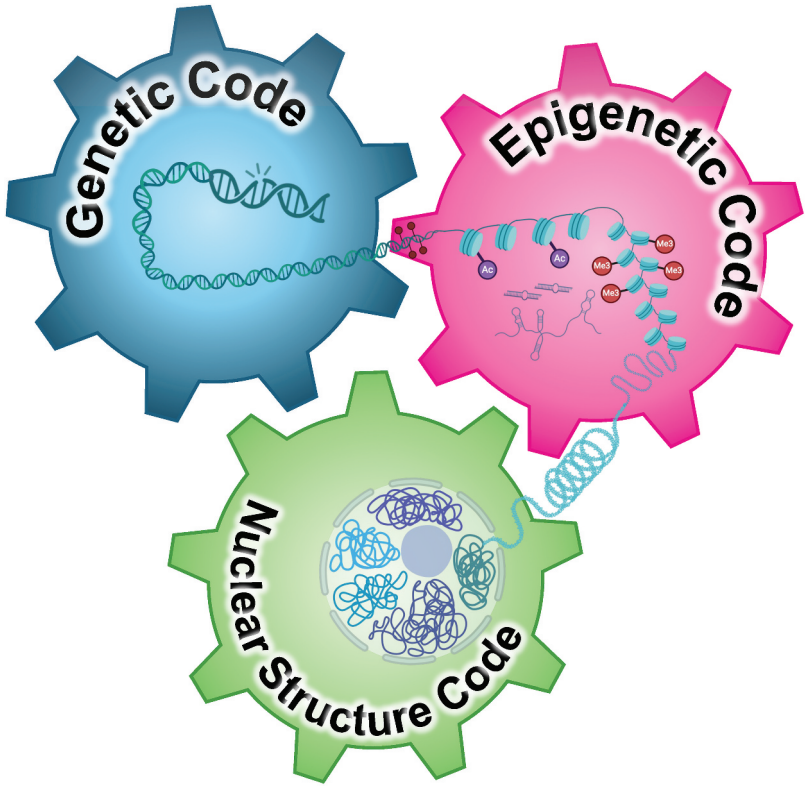

Figure 1. Unifying triple code model integrates genetics, epigenetics, and nuclear structure for better understanding the pathobiology of rare and undiagnosed diseases.

The Triple Code Model, depicted as interlocking gears, illustrates the interconnected dynamics of genetic, epigenetic, and nuclear structure codes, emphasizing their collective impact on rare and undiagnosed diseases. Advances in genomics have revolutionized our understanding of these diseases by uncovering the genetic mechanisms involved. Epigenetic processes, such as DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs, regulate gene expression without altering the underlying DNA sequence. The Nuclear Structure Code encompasses the nuclear matrix and chromatin organization in 3D. Genetic mutations can alter signaling inputs to the nucleus, affecting chromatin structure and gene regulation. The gear-like model underscores the complex network between genetics, epigenetics, and nuclear architecture, where perturbations in one layer can propagate to others, causing the diverse symptoms seen in rare and undiagnosed syndromes. Gears are depicted in Rare Disease Day colors to honor those affected by rare diseases. Portions of the figure were created with BioRender.com.

instance, many of the available databases display an underrepresentation of non-European ancestry groups, which can pose challenges for variant interpretation in diverse populations [14]. Beyond diagnoses, understanding the genetic basis of many rare diseases, such as spinal muscular atrophy, Duchenne muscular dystrophy, hemophilia, Leber congenital amaurosis, and certain lysosomal storage disorders, has presented exciting opportunities for developing novel gene therapies and other targeted molecular treatments [15,16]. Consequently, supported by many emerging sequencing efforts, numerous pharmaceutical companies and research institutions are using such data to pursue gene therapy programs to treat rare genetic disorders, making this field a fertile ground for creating advanced interventions.

3. Discovery of the epigenetics code unravels new mechanisms of rare and undiagnosed diseases

Extensive data, particularly gathered during the last three decades, reveal that an increasing number of rare and undiagnosed diseases are associated with dysregulated epigenetic mechanisms governing hierarchical cascades of gene expression that give rise to both normal and diseased phenotypes. Epigenetic mechanisms include DNA methylation, histone modifications, chromatin remodeling, and the actions of non-coding RNAs, which can either shut down or increase gene expression without altering the DNA sequence itself [4,17]. DNA methylation is the process of adding methyl groups to cytosines in the DNA. It is often associated with repression of transcription at specific gene promoters, the imprinting of genes at disease-causing loci, and differences in the levels of gene expression between alleles in various human tissues due to skewed methylation. Posttranslational modifications of histones, such as acetylation, methylation of lysine and arginine, phosphorylation of serine and threonine, ubiquitination, citrullination, and other emerging modifications, are generated by histone modifying complexes [4,18]. These modifications serve as binding platforms for recruiting other protein complexes, including nucleosome remodeling complexes, which work in a coordinated manner to condense or relax chromatin structure, thereby impacting gene accessibility and expression levels. Non-coding RNAs can serve as guides that direct epigenetic machinery to specific genomic locations, influencing gene expression through modifications of chromatin structure. In addition, promoters and enhancers, which are regions of DNA that initiate or enhance gene expression, can produce long non-coding RNAs (lncRNAs) that act as signals to influence gene activity in other regions of the genome, although the precise mechanisms remain poorly understood [19]. Congruently, dysregulation of these epigenetic processes can

disturb gene expression patterns crucial for the development and the homeostasis of cells, organs, tissues, and the individual. In this manner, epigenomic alterations contribute to disease pathogenesis. Consequently, epigenetics represents a transformational conceptual and methodological new frontier for revealing the complexities of rare and undiagnosed diseases, shedding light on their underlying molecular mechanisms.

Beginning with alterations in DNA modification, genomic imprinting disorders, such as Prader-Willi syndrome, Angelman syndrome, and Beckwith-Wiedemann syndrome, are caused by altered methylation patterns of genes expressed in a parent-of-origin-specific manner [20]. This dysregulation is caused by various molecular mechanisms, including genomic mutations or chromosomal abnormalities within imprinted genes, uniparental disomy resulting in inheritance of both copies from one parent, imprinting defects causing improper silencing or activation of allele-specific genes, and isolated or multi-locus epimutations affecting DNA methylation at imprinting control regions (ICRs) or differentially methylated regions (DMRs). Interestingly, individuals with imprinting disorders often harbor abnormal DNA methylation patterns at several imprinted loci, called multi-locus imprinting disturbance (MLID), resulting from cis-acting elements or trans-acting factors involved in establishing, maintaining, or erasing genomic imprints during gametogenesis or early embryogenesis [20]. Thus, imprinting alterations manifest as diverse clinical features due to aberrant expression of genes critical for development, growth, metabolism, and neurological function [21]. Furthermore, environmental factors in various assisted reproductive technologies (*e.g., in vitro* fertilization or IVF and intracytoplasmic sperm injection or ICSI) may also increase the risk of imprinting errors [22]. Noteworthy, however, alterations in DNA methylation can contribute to rare diseases through mechanisms other than imprinting errors. For example, a subgroup of disorders, named rare diseases of epigenetic origin (RDEOs), are diseases in which genetic defects occur in epigenetic regulators or chromatin remodelers themselves [23]. A well-known example of these disorders is mutations in genes that encode DNA methyltransferases or methyl-binding proteins like methyl-CpG binding protein 2 (MeCP2) [24], in which global methylation patterns become a defining molecular feature of the disease. Thus, distinct epigenetic landscapes can help undiagnosed patients, highlighting the potential significance of epigenetics as a diagnostic tool for rare diseases.

Beyond DNA methylation, RDEOs also encompass those caused by mutations in histone genes or the enzymes and complexes that modify them [23]. Rubinstein-Taybi syndrome is an example of a rare congenital disorder brought about by mutations in *CREBBP* or *EP300*, both of which encode histone acetyltransferases that regulate enhancers and super-enhancers, thus leading to dysregulated expression of defined gene networks [25]. Mutations in histones are also a well-known cause of rare diseases and rare cancers [23,26]. For example, Rahman syndrome results from mutations in the *HIST1H1E* gene for the linker histone H1.4, disrupting chromatin organization. Other histone mutations disrupt their post-translational modification, such as H3K27M mutations, which are found in up to 80% of pediatric diffuse midline gliomas and 60% of adult diffuse gliomas [27], and H3.3K36M mutations, identified in up to 95% of chondroblastomas, a rare type of noncancerous bone tumor [28]. Both of these mutations disrupt the methylation patterns at their respective residues on the remaining wild-type histones. Similarly, alterations are found in molecular machines that can move nucleosomes to form either heterochromatin or euchromatin in cooperation with histones and their modifying enzymes [23]. Within this group of disorders, a prominent example is Coffin-Siris syndrome (CSS), which arises from mutations in genes encoding various subunits of BAF nucleosome remodeling complexes, including *ARID1A*, *ARID1B*, *SMARCA4*, and *SMARCB1* [23]. On the other hand, mutations in another ATP-dependent chromatin remodeling factor encoding gene, *SMARCAL1*, cause the multisystem disorder Schimke immuno-osseous dysplasia (SIOD) [29]. In addition, NEDDFAC (neurodevelopmental disorder with dysmorphic facies and thin corpus callosum) is caused by mutations in the *SUPT16H* gene. The protein encoded by this gene is a subunit of the FACT histone chaperone complex, required for transcriptional elongation and involved in histone deposition, particularly during DNA replication, giving rise to epigenomic inheritance at the replication fork [30]. In summary, the provided examples of defects in histone genes, histone-modifying enzymes (e.g., acetyltransferases), and nucleosome remodeling machinery profoundly impact normal histone dynamics, chromatin structure, and gene regulation. These alterations ultimately contribute to the development of various disorders that, while rare, often require the expertise of genetic counselors and clinical geneticists.

Regarding the third epigenetic mechanism, it is relevant to note that non-coding RNAs, particularly microRNAs (miRNAs) and lncRNAs, have been implicated in the pathogenesis of several rare and undiagnosed diseases. In Duchenne muscular dystrophy, a neuromuscular disease, we find dysregulation of specific miRNAs, including miR-1, miR-133, and miR-206, contributing to muscle degeneration and fibrosis [31]. Moreover, circulating miRNAs, like the aforementioned plus miR-26a, miR-30c, and miR-181a among others, have been investigated as potential noninvasive biomarkers of this disease [32]. Similarly, altered expression of miRNAs such as miR-145, miR-223, and miR-494 is often observed in cystic fibrosis, a disorder affecting the lungs, sweat glands, and digestive system, particularly the pancreas, leading to organ insufficiency [33]. Regarding the role of lncRNAs, one of the first described lncRNAs is H19, which is used as a biomarker for diagnosis of Silver-Russell syndrome, the clinically opposite growth-affecting congenital imprinting disorder of Beckwith-Wiedemann syndrome [34]. For other examples, such as the upregulation of the lncRNA *NEAT1* in idiopathic pulmonary fibrosis (IPF), lncRNA silencing is being proposed as a potential therapeutic strategy [35]. In β-thalassemia, an inherited blood disorder, the lncRNA *RP11-196G18.23* regulates the expression of the *HBG1*/*HBG2* genes encoding fetal hemoglobin by recruiting DNMT3A to promote DNA hypermethylation-mediated downregulation of *ERF*, a transcription factor involved in γ-globin activation [36]. Furthermore, the dysregulation of two antisense lncRNAs SMN-AS1 and SMN-AS1* is associated with spinal muscular atrophy (SMA) pathogenesis

[37]. These lncRNAs inhibit *SMN* expression by recruiting the Polycomb repressive complex, and use of antisense oligonucleotides to target them increases SMN levels. Notably, there is evidence to support that targeting of these lncRNAs will enhance the efficacy of the FDA-approved splicing-correcting antisense oligonucleotide nusinersen to further increase SMN levels. These examples highlight the diverse roles of ncRNAs in the molecular pathways underlying various rare genetic diseases, offering potential avenues for developing novel diagnostic and therapeutic approaches.

Lastly, by considering the broader impact of epigenetic dysregulation across various rare diseases, we can shed light on the complexity of pathophysiological mechanisms already discovered. A notable example of this complexity is the Immunodeficiency Centromeric Instability Facial Syndrome (ICF), where disruptions in one of the three main epigenetic inheritance mechanisms, DNA methylation, can affect chromatin structure leading to chromosomal instability, altered gene expression, and complex clinical manifestations [38]. Another interesting mechanism is reflected in neurodevelopmental and mental disorders thought to be caused by epigenetic alterations triggered by environmental factors, stress, diet, lifestyle, or aging rather than solely genetic mutations affecting epigenetic machinery [39]. An example of this type of pathological regulation is adverse reactions to small molecules in the environment, such as cosmetics or foods, which act as synthetic endocrine disruptors, interfering with the action of endogenous hormones [40]. The lack of cofactors needed to close the neural tube, namely folate deficiency, alters the metabolic cycle of the amino acid methionine. This disruption affects the generation of S-adenosylmethionine (SAM), which is used in all DNA, histone, and RNA methylation pathways [41]. These examples highlight how epigenetic mechanisms can lead to rare disease phenotypes independent of underlying genetic defects. Despite the apparent complexity of epigenomics, identifying epigenetic molecules as biomarkers holds promise for improving the diagnosis, prognosis, and monitoring of rare diseases. Furthermore, ongoing research into epigenetic therapies, such as inhibitors of DNA methyltransferases, histone deacetylases, and histone methylases, offers treatment options, particularly in rare cancers and some diseases such as epilepsy [17,42,43]. With technological advancements in single-cell epigenome sequencing and data analysis tools, coupled with machine learning approaches, including several modalities of deep learning, we will continue gaining insights into the epigenetic landscapes that eventually give rise to the phenotypes of many rare and undiagnosed diseases.

4. Nuclear structure and dynamics emerges as another code for better understanding the pathobiology of rare and undiagnosed diseases

Historically, genetics was initiated with the analyses of traits and their association with genes, mostly using a reductionist approach. The completion of the human genome launched the era of big data analyses with not only genes but also the characterization of entire gene networks, which could be altered in disease states [44]. This development gave birth to systems biology, which also prompted looking at genome regulation in 3D. In other words, it was the beginning of studying the nucleus as a system rather than as isolated genes. Over more than two decades, the study of nuclear dynamics has begun to extend our understanding of the pathogenesis of various rare diseases. For this reason, modern practitioners in rare and undiagnosed diseases can greatly benefit from studying the cell biology of this organelle and considering its underlying framework. Briefly, the nuclear membrane connects the interior of the nucleus with the cytoplasm through the nuclear pores and its external membrane, which continues into the endoplasmic reticulum [45]. Similar to the cytoplasm, the nucleus has a cytoskeleton formed by intermediate filaments made of lamin proteins. Lamin proteins form a basket that contacts the nuclear membrane and extend invaginations to create domains for housing other membraneless organelles. This organization ensures the proper functioning of the nucleolus, the spliceosome, Topologically Associating Domains (TADs), and insulators, allowing genes to be expressed to fulfill the desired normal phenotype. Unfortunately, disruptions of the intricate interplay between the nuclear envelope, lamina, and chromatin organization can lead to significant consequences, ultimately leading to the onset of various rare diseases.

One prominent example illustrating the significance of nuclear dynamics and architecture in rare diseases is the group of disorders collectively known as laminopathies [46]. These conditions are caused by mutations in genes encoding nuclear lamins, such as Emery-Dreifuss muscular dystrophy (EDMD), familial partial lipodystrophy (FPLD), and the premature aging disorder Hutchinson-Gilford progeria syndrome (HGPS). Mechanistically, mutations in lamins can compromise the structural integrity of the nuclear envelope, changing the elasticity of the cell nucleus and eventually resulting in altered gene expression patterns, impaired mechano-signaling, disrupted nuclear-cytoskeletal interactions, and aberrant cell cycle regulation [47], which all contribute to the diverse clinical manifestations. Another class of rare diseases associated with nuclear dynamics is displaying defects in the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex, which physically links the nuclear lamina to the cytoskeleton via KASH and SUN domain proteins [48]. This physical connection from the plasma membrane through the cytoplasm to the nucleus is relevant to cell homeostasis by the fact that cells sense their environment through ECM receptors, which transduce the signal via the cytoskeleton and, ultimately, the nucleus via LINC to regulate gene expression responses needed to support migration [49]. Interestingly, mutations in LINC complex components are implicated in conditions like EDMD, cerebellar ataxia, and neuropathy, underscoring the crucial role of this complex in maintaining nuclear positioning, mechanotransduction, and gene expression regulation [48].

However, nuclear architecture and dynamics extend beyond the examples of laminopathies and LINC complex disorders since they have been implicated in other diseases. For instance, limb-girdle muscular dystrophy can arise from mutations in the gene encoding the nuclear envelope protein emerin, *EMD* [50], and a group of rare increased bone density disorders are caused by mutations in the *MAN1/LEMD3* gene,

another LEM domain-containing protein [51]. Moreover, nuclear architecture and dynamics disruptions are also a defining feature of certain rare cancers, potentially contributing to genomic instability and aberrant gene expression patterns [52,53]. In conclusion, the study of nuclear dynamics and architecture constitutes a new and exciting area of genomic research, which has shed light on the pathogenesis of several diseases. We predict that targeting the nuclear environment, such as modulating nuclear import/export pathways, restoring nuclear envelope integrity, or correcting aberrant chromatin organization, are being explored in the preclinical setting as potential treatment approaches for various nuclear architecture-related rare diseases.

5. The Triple Code Model: when genetics meets epigenetics and nuclear structure to give rise to phenotypes in rare and undiagnosed diseases

Integrating the dynamic interplay among the genetic, epigenetic, and nuclear architectural codes, the Triple Code Model serves as a conceptual and methodological framework that transcends the impact of each of these disciplines independently. This model acknowledges their contributions and underscores their interconnectedness in the processes that give rise to normal cellular phenotypes and disease states, offering additional etiopathological insight into rare and undiagnosed diseases. As advancements in artificial intelligence (AI) and deep learning further refine our capacity to interpret these interconnected codes, the Triple Code Model continues to evolve as a powerful tool for understanding complex pathologies. We envision that this integrated model, stemming from a guiding paradigm in our laboratory for understanding pancreatic cancer subtypes [4], has a valuable application in the context of germline variation in rare and undiagnosed diseases. The robust scientific evidence supporting this model highlights the significance of considering all three regulatory layers in deciphering pathophysiological mechanisms underlying rare and undiagnosed diseases.

Traditionally, and to a certain degree still, the genetic code within the DNA sequence was considered the primary determinant of a genotype. However, years of investigations have revealed a more intricate regulatory landscape that works beyond the base pairs. Epigenetic mechanisms, such as DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs, dynamically modulate the expression and regulation of genes. Thus, a phenotype should be considered the consequence of the collective interaction between genetics and epigenetics, which is faithful to the original coining of the latter term by Conrad Waddington in 1942 [54]. Accordingly, these epigenetic processes control chromatin structure and accessibility, influencing gene expression patterns independently of the underlying genes. Notably, the Triple Code Model introduces a third layer – the nuclear architectural code. This governs the 3D organization and dynamics of the genome and epigenome inside the nucleus and is influenced by interactions with the plasma membrane through the LINC complex [55]. The nuclear code encompasses the interplay between the nuclear envelope, lamina, and chromatin organization, including the positioning and movement of chromosomes and gene loci. Integrating these three regulatory codes, genetics, epigenetics, and nuclear structure, is essential for regulating the hierarchical cascade of gene expression networks that results in a particular normal or diseased phenotype. In this sense, logically, disruptions in any of these codes, or in their coordinated crosstalk, can lead to dysregulated gene expression and aberrant variant phenotypes, such as those in rare and undiagnosed diseases.

Throughout the years, our investigations into genetic mutations responsible for rare diseases, such as chromatinopathies, rasopathies, and rare cancer subtypes, have been fruitful after integrating these layers and linking them with pathophysiological outcomes. For example, at the genetic level, we described the $MRAS^{G23V}$ mutation as causing Noonan Syndrome due to constitutive activation of the MRAS protein, which triggers cellular responses of physiopathological relevance [56]. However, our subsequent studies on both diverse RAS mutations and rare pancreatic cancer subtypes highlight alterations in the epigenome, most conspicuously by inducing enhancer-promoter looping within the nucleus [17,57–60]. Extending these investigations on enhancer regulations and rare diseases, we have focused on mutations affecting proteins within the COMPASS complex, such as *KMT2C*, *KMT2D*, and *KDM6A* [61–63]. This protein complex is increasingly linked to a spectrum of congenital syndromes like Kabuki (by *KMT2D* or *KDM6A*) and Kleefstra Syndrome II (*KMT2C*) by disrupting the normal regulation of gene expression and nuclear architecture. In this regard, through our most recent endeavors in advanced computational modeling and its integration with existing experimental data, we are providing an even more comprehensive understanding of molecular mechanisms driving pathogenesis in these diseases. Another noteworthy example is the rare disease, Camurati-Engelmann, caused by *TGFB1* gene mutations that result in the production of an overly active TGFβ-1 protein [64]. This abnormal TGFβ-1 protein activity drives increased signaling, which phenotypically leads to accelerated bone turnover. Importantly, enhanced TGFβ-1 signaling leads to chromatin remodeling and nuclear shape abnormalities [65], highlighting the complex mechanisms by which *TGFB1* mutations can contribute to disease pathogenesis in the context of this Triple Code Model. Collectively, these examples underscore the intricate, interconnected network between genetics, epigenetics, and nuclear architecture, challenging the notion of the unidirectional influence of single genes and revealing a system where perturbations in one layer can propagate to others, causing variable signs and symptoms often found in rare and undiagnosed syndromes.

To fully harness the complexity of the Triple Code Model, advanced computational tools, including AI, are essential for synthesizing high-resolution data across genetic, epigenetic, and nuclear dimensions. Recent advances in systems biology have introduced numerous high-resolution multiomic techniques that leverage integrative data science modeling to examine complex systems through their emergent properties [66]. These approaches expand the potential of the Triple Code Model even to single-cell, molecular,

and atomic levels, advancing insights needed to understand rare and undiagnosed diseases. With the integration of enhanced computational modeling and machine learning, including deep learning and generative AI, we can now better define genomic variations and interpret the transcriptome, epigenome, and alterations in nuclear territories [67]. Additionally, advanced AI algorithms enhance the processing of high-resolution microscopy data [68], which can aid in detecting subtle atomic structure changes associated with specific genetic mutations. The ultimate clinical purpose of these tools is to integrate the vast array of information derived from such high-resolution analyses, enabling more precise diagnoses, disease risk evaluations, and therapeutic developments. As AI technology continues to evolve, the field moves ever closer to these goals, significantly enhancing the capabilities and impact of the Triple Code Model in rare disease research to support precise, personalized solutions.

To integrate the Triple Code Model into the diagnostic workflow of rare diseases and evaluate its potential impact, it is valuable to present thoughtful examples and consider well-defined methodologies for implementation. For instance, the role of BAF complex members in CSS is significant, as they are involved in transducing developmental signals such as Wnt and Notch to the nucleus [69–71]. This signaling affects gene expression networks involved in cellular migration, particularly in neuroblasts. It has been observed that during cell migration, fragility of the nucleus due to disruptions in nuclear architecture can result in cellular damage [72]. Currently, CSS diagnosis is accurately obtained using WGS, WES, or gene panels. However, there is potential to develop severity and progression scores that correlate with specific nuclear abnormalities identified through cytopathology, cytogenetics, immunohistochemistry, or perhaps in the future, more innovative techniques for assessing 3D genome organization. AI-driven analyses enhance our ability to interpret these large datasets, distilling complex biological interactions into actionable insights and allowing for more precise assessments of high-resolution genomic and nuclear features [73]. In addition, the rapid development of spatial genomics and the miniaturization of epigenomic methodologies at single-cell resolution are advancing this field and are likely to soon enter clinical practice [74,75]. A cutting-edge diagnostic workflow would seamlessly integrate these layers of biological information, leveraging advanced sequencing technologies, epigenomic profiling, and techniques for assessing 3D genome organization. However, the implementation of such a comprehensive workflow requires overcoming challenges in data integration, interpretation of complex biological interactions, and the need for specialized expertise. The ultimate goal is to translate advanced diagnostic capabilities into tangible benefits for patients, shortening the diagnostic odyssey and advancing the management of rare diseases with more targeted interventions.

Beyond diagnostics, harnessing the holistic approach of the Triple Code Model has opportunities for understanding disease mechanisms, drug discovery, and personalized medicine. This multi-layered view could lead to the identification of novel drug targets that were previously overlooked by traditional genomic approaches. Indeed, CSS is an example of a rare disease where repurposing cancer drugs could be beneficial [76]. Similar approaches would be applicable to other chromatinopathies, such as those involving the COMPASS complex, which also have potential therapeutic interventions [77]. Moreover, understanding how nuclear architecture influences gene expression could lead to innovative gene therapy strategies or epigenetic interventions [78]. In terms of precision medicine, more specific patient stratification could offer tailored treatment strategies that consider not just genetic variants, but also epigenetic profiles and chromatin states. Importantly, this approach could considerably improve treatment efficacy and reduce adverse effects, as well as significantly advance current initiatives like the Treatabolome database, expanding existing variant-specific treatments for rare diseases [79]. Furthermore, this integrated perspective could reveal unexpected connections between seemingly unrelated conditions, leading to new classification systems based on molecular pathways rather than clinical symptoms alone. Therefore, we foresee an expansion of this field into rare disease clinics, guided by this paradigm, which will inspire further discoveries.

6. Future perspective

6.1. Value of the triple code model in guiding rare disease research toward new horizons

The Triple Code Model represents a transformative paradigm in deciphering rare and undiagnosed diseases, integrating genetics, epigenomics, and nuclear structure dynamics into a unified framework (Figure 1). Because of their nature, approaches for analyzing these layers are also proxy for reading proteomic or metabolomics events, helping generate hypotheses and leverage advanced multi-omics technologies and computational modeling. These links enhance our understanding further while guided by these paradigms. Translating scientific discoveries into clinical applications promises to improve patient outcomes and quality of life. We predict that personalized medicine approaches, informed by the Triple Code Model, will enable tailored interventions addressing specific molecular drivers of rare diseases. Advancements in diagnostic techniques, employing new high-throughput sequencing and imaging technologies to study genetics, epigenetics, and nuclear structure, along with their impact on proteomics and metabolomics, can facilitate more rapid and accurate diagnosis, enabling timely intervention and improved prognosis.

Consequently, it is important to consider collaborative efforts to integrate multi-omics data and patient-derived models to prioritize future research endeavors that can significantly deepen our understanding of many aspects of rare diseases. Developing innovative therapeutic modalities, including several types of gene therapies and epigenetic modifiers, holds the potential for reversing underlying pathobiological mechanisms and improving outcomes. These considerations bring us to the proposal that embracing a multidisciplinary and collaborative approach grounded in systems biology can harness the power of

the Triple Code Model and drive transformative advancements in rare disease research and clinical care. This code can also help as a framework for comprehending the intricate operations of biological systems and conceptualizing them as cohesive networks of genes, proteins, metabolites, and other molecular components. In the spirit of systems biology, the Triple Code Model can discover emergent properties arising from complex system interactions, providing insights into their dynamics, regulation, and function. This conceptualization is paramount since traditional reductionist approaches often fail to capture the full complexity of biological phenomena. In contrast, to elucidate interconnectedness and emergent properties, systems biology integrates experimental data, computational models, quantitative analyses, and a large amount of data derived from large-scale experimental initiatives. Exploring genetics, epigenomics, and nuclear dynamics and incorporating them into a systems biology framework will lead to applying advanced analytical methodologies that make the biological system comprehensive in its multidimensionality. Therefore, we are optimistic that the Triple Code Model will advance our understanding of rare and undiagnosed diseases by guiding further investigations that explore the concepts described in this perspective article.

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Grammarly for Windows (Version 1.2.92.1464) was utilized during the production of this manuscript to correct grammatical errors, improve sentence structure, and enhance readability.

Author contributions

Both Gwen Lomberk and Raul Urrutia conceptualized, conducted the literature search, wrote and revised the manuscript.

Disclosure statement

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