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Heterogeneity Across Biomedical Research: Variation and the Limits of Inference

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Introduction

Biomedical research often identifies differences, but those differences do not always have the same meaning. Within tumors, cancer stem cells may occupy different states and respond differently to treatment. At the molecular level, computational studies can compare predicted interactions across alternative conformations of a protein. In experimental models, outcomes measured in the same study may not change in parallel. Differences also emerge in psychosocial measures and population health estimates. Interpreting such findings requires attention not only to what varies, but also to how the variation is measured and what the study design allows researchers to conclude.

The contributions to this issue illustrate these challenges in different research settings. They include a review of cancer stem cells and treatment resistance, a computational study of the predicted interactions of selected compounds with different conformations of hemoglobin S, and two animal studies investigating plant-derived preparations in models of pancreatic ischemia/reperfusion injury and cutaneous wound healing. The issue also features a cross-sectional study of social capital, happiness, and perceived suffering among people living with HIV/AIDS and an ecological analysis of age-specific suicide mortality across 25 countries. Although these studies address different questions, they share an important concern: how can findings be interpreted without drawing conclusions that go beyond the available evidence?

The answer depends in part on recognizing the limits of each research approach. Computational docking can help identify hypotheses for further investigation, but its predictions do not establish binding or therapeutic efficacy. Findings from animal experiments need to be assessed in relation to the outcomes measured and the consistency of the results, with replication and further investigation needed before their clinical relevance can be determined. Cross-sectional studies identify

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associations but cannot establish temporal or causal direction, while ecological analyses describe population-level patterns that cannot be directly translated into individual-level risk. Similarly, finding a statistically significant association in one population but not another does not necessarily mean that the associations differ; a direct statistical comparison is needed to support that conclusion.

These considerations provide the focus for this editorial. Rather than treating different forms of variation as equivalent, we consider what the studies in this issue can establish, where uncertainty remains, and how their findings can inform further research. Careful interpretation depends on matching conclusions to the evidence, reporting methods and results transparently, and using replication and confirmatory research to determine which findings are robust.

Variation as an inferential problem

Variation is not a single scientific phenomenon. In tumors, it may describe differences among cell states; in hemoglobin S, the conformational alternatives evaluated computationally; in animal experiments, differences among measured endpoints; in psychosocial research, variation in reported well-being; and in epidemiology, differences across populations and age groups. Treating these phenomena as interchangeable would blur distinctions that matter for interpretation. Their common thread is methodological: any observed difference must be understood in relation to what was measured, at what level, and under which assumptions. Without this discipline, mechanistic plausibility can be mistaken for therapeutic benefit, discordant endpoints for a unified mechanism, and population-level patterns for individual risk. The contributions in this issue are valuable not because they reveal one shared biological process, but because they show why conclusions must remain proportionate to the evidence that supports them.

Biological states and molecular hypotheses

Mozaffari's review (1) considers cancer stem cells (CSCs), tumor initiation, invasion, metastasis, and resistance to therapy, emphasizing how the tumor microenvironment and signaling networks, including Wnt, bone morphogenetic protein (BMP), Notch, and Hedgehog pathways, may support CSC self-renewal and treatment evasion. Together with evidence that CSC states are plastic and context-dependent (2), this account challenges the assumption that tumors can always be treated as uniform cellular populations. A mechanistic review can clarify plausible routes to recurrence and resistance, but it does not by itself establish the effectiveness of a particular CSC-targeted intervention. The translational question is whether proposed targets remain relevant across tumor contexts and whether interventions against them improve outcomes in validated experimental and clinical settings.

The in silico study by Yousefi and colleagues (3) approaches variation at a different level by comparing predicted interactions of curcumin, rosmarinic acid, and related derivatives with tense and relaxed conformations of hemoglobin S (HbS), using voxelotor as a comparator. The reported docking scores favored the relaxed (R2) conformation over the tense (T) conformation. Such rankings can help prioritize hypotheses, but docking scores are model-dependent predictions rather than direct measurements of binding, inhibition of HbS polymerization, or clinical efficacy. Their interpretation depends on structural preparation, search-space definition, scoring assumptions, and validation. The next evidentiary steps include independent assessment of binding, testing effects on HbS polymerization, and determining whether predicted interactions are robust across relevant experimental conditions.

Voxelotor illustrates why the path from molecular rationale to clinical use cannot be assumed to be linear. The drug was designed

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to increase hemoglobin oxygen affinity and thereby reduce polymerization of deoxygenated HbS (4,5). The phase 3 HOPE trial demonstrated hematologic activity, including improved hemoglobin response and reduced markers of hemolysis (5); these findings, however, did not settle the question of overall long-term benefit and risk. On September 26, 2024, the US Food and Drug Administration reported the manufacturer's voluntary withdrawal of voxelotor after emerging safety data indicated that its benefits no longer outweighed its risks. The FDA described higher rates of vaso-occlusive crises in postmarketing clinical trials and two real-world registry studies, as well as more deaths in the treatment group than in the placebo group in postmarketing trials (6). These findings do not establish that the proposed molecular mechanism caused the safety outcomes, nor do they invalidate conformational targeting as a broader research strategy. They do show why predicted interactions and favorable intermediate outcomes must be evaluated alongside safety and clinically meaningful endpoints.

Experimental signals and translational boundaries

In the rat study by Astaneh and colleagues (7), oral pretreatment with Bunium persicum essential oil was reported to attenuate ischemia/reperfusion-induced pancreatic injury and significantly increase superoxide dismutase (SOD) activity, whereas changes in malondialdehyde (MDA) and Bcl-2 expression were not statistically significant. These findings support further investigation but do not, on their own, establish a specific antioxidant or anti-apoptotic mechanism. Interpreting the pattern requires attention to the measured endpoints, sampling times, assay properties, experimental precision, and the consistency of the findings across independent studies.

In a separate wound-healing experiment, Johari and colleagues (8) reported improved healing indices following topical application of Camellia sinensis, Daphne mucronata, and their combination relative to negative controls. The study reported 97.5% healing on day 21 in the C. sinensis group, together with lower MDA and tumor necrosis factor alpha (TNF-α) levels and higher SOD activity. A favorable result for a combined treatment does not establish pharmacological synergy. Synergy is an interaction claim and requires an appropriate design, dose-response evidence, and an analysis that tests whether the combined effect exceeds what would be expected from the component treatments.

Neither rat study is an example of biological heterogeneity in the strict sense. Their relevance to the present theme is more specific: outcomes measured in the same experiment need not move in parallel, and a favorable change in one endpoint does not automatically explain another. Such discordance may reflect biological processes, measurement choices, timing, or statistical uncertainty. It should prompt targeted investigation rather than a post hoc assumption of a single underlying mechanism. More broadly, plant-derived preparations remain potential sources of therapeutic leads (9), but translation requires reproducible formulation and chemical characterization, dose-response assessment, appropriate comparators, safety evaluation, and independent replication. Preclinical promise is a starting point for this process, not its conclusion.

Psychosocial and population patterns

The cross-sectional study by Bijani and colleagues (10) examined 120 people with HIV/AIDS attending a behavioral-diseases counseling center in Fars Province. Greater social capital, particularly trust and cohesion, was associated with greater happiness and lower perceived suffering. The regression analyses also identified economic condition and social capital as predictors of happiness and suffering (10). These results are compatible with broader evidence that social relationships are associated with health outcomes (11), but a cross-sectional

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association cannot determine temporal order or exclude reverse direction and residual confounding. People with stronger social ties may experience better well-being; people who feel better may also perceive or maintain social ties differently. Because the study was conducted at a single center, the transferability of its estimates to other populations and settings also requires caution. Longitudinal and intervention research would help establish whether strengthening social connectedness produces measurable improvements in well-being.

The ecological study by Yousefi and colleagues (12) compared suicide mortality in Iran and 24 neighboring or regional comparator countries in 2019 using WHO Global Health Estimates. It analyzed rates across 11 ordered age-group categories and reported the highest mean across these age-group-specific rates in Kazakhstan (32.12 ± 27.18 per 100,000) and the lowest in Syria (3.03 ± 1.10 per 100,000) (12). These values should be described as the study's reported means across age-group-specific rates, not casually relabeled as crude or age-standardized national rates. The analysis reported a statistically significant positive age-related gradient in the Russian Federation, but not in Iran, Oman, Saudi Arabia, Uzbekistan, or Yemen (12). A significant association in one country and a nonsignificant association in another do not, by themselves, demonstrate that the underlying gradients differ; that claim requires a direct comparison of slopes or another suitable test of between-country differences.

Geographic proximity does not guarantee epidemiological similarity, and country-level estimates cannot be translated directly into individual-level risk. The study's ecological design limits individual-level inference, while possible underreporting associated with stigma and differences in the completeness or uncertainty of secondary estimates may complicate cross-national comparisons (12). These concerns do not make comparative epidemiology uninformative. They mean that estimates should be interpreted with attention to how they were generated, their uncertainty, and the comparability of the data sources. Prevention strategies should therefore be informed by local evidence and strengthened surveillance rather than transferred between neighboring countries on the assumption that their risk profiles are interchangeable. Communication of suicide-related findings should also follow guidance for responsible media reporting (13).

The social-capital and suicide studies address different populations, outcomes, and analytical levels. Reading them together may motivate questions about social context and population health, but they do not establish a direct association between social capital and suicide mortality. Their connection is not a demonstrated causal pathway; it is a shared reminder that health patterns are shaped and measured within social contexts that must be described rather than assumed.

Three lessons for interpreting biomedical evidence

First, the kind of variation must be named precisely. Differences among tumor-cell states, predicted molecular conformations, biomarker responses, self-reported well-being, and country-level rates are not equivalent phenomena. Some concern heterogeneity within a biological system; others describe alternative structural states, variation among outcomes, associations between measured constructs, or population distributions. A single umbrella concept is useful only if these distinctions remain visible. Otherwise, the language of heterogeneity risks becoming so broad that it loses explanatory value.

Second, claims must be matched to the inferential capacity of the design. Docking generates hypotheses and can prioritize compounds; it does not establish therapeutic activity. Animal experiments test effects under defined conditions but do not by themselves

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demonstrate clinical benefit. Cross-sectional studies estimate associations at a particular time but cannot establish temporal direction. Ecological analyses identify population-level patterns but do not show how those patterns map onto individual risk. Evidence hierarchies can be useful for questions about causal effects, yet they do not fully capture the different purposes of exploratory, mechanistic, descriptive, and population-level methods. The relevant question is not simply which design ranks highest, but whether a design provides an appropriate and reproducible answer to the question being asked.

Third, uncertainty and reporting quality are part of the evidence, not administrative details to be addressed after interpretation. Computational reports should provide sufficient information on protein and ligand preparation, search spaces, scoring procedures, validation, simulation settings, and accessible data or code where feasible. ARRIVE 2.0 supports transparent reporting of animal research (14), and STROBE supports reporting of observational studies (15). GATHER can be relevant when health estimates are derived from multiple data sources, provided the study falls within its scope (16). These guidelines improve the completeness of reporting but do not guarantee sound design or valid conclusions. Replication, confirmatory analyses, transparent uncertainty reporting, and publication of informative negative results help distinguish robust findings from patterns that depend on analytical choices or sampling variability.

Conclusion

The unifying message of this issue is not that every form of variation reflects the same process. It is that biomedical inference becomes more reliable when researchers specify what varies, what has been measured, at which level, and under which assumptions. Cellular plasticity is not a population gradient; a changed biomarker is not, by itself, a mechanism; a docking score is not drug activity; a cross-sectional association is not a causal pathway; and statistical significance in one setting is not proof of a difference from another. Making these boundaries explicit does not weaken translational ambition. It provides the conditions under which findings can accumulate, be challenged, and ultimately inform practice. We thank the authors, reviewers, and editorial team for their contributions to this issue and welcome future submissions that pair innovative questions with rigorous methods and transparent reporting.

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