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Review

Trypanosoma cruzi Genetic Diversity Challenges Eco-Epidemiological Associations and Requires Integrated Biological-Social Approaches for Chagas Disease Management

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Abstract: *Trypanosoma cruzi*, the etiological agent of Chagas disease, exhibits extensive genetic diversity with important implications for parasite biology, disease manifestation, and control strategies. This review synthesizes current knowledge on *T. cruzi* genetic diversity. It emphasizes how these findings inform both biological and community-based interventions. The parasite employs a dual reproductive strategy combining clonal propagation and sexual reproduction, with evidence of meiotic sex, hybridization, and genetic recombination in natural populations. Genomic plasticity is characterized by chromosomal aneuploidies, gene amplification, and extensive copy number variations, predominantly in gene family-rich regions including mucins, trans-sialidases, and mucin-associated surface proteins. *T. cruzi* is classified into seven discrete typing units: TcI–TcVI and TcBat. Although DTUs have been historically associated with specific epidemiological and ecological scenarios, recent comprehensive analyses demonstrated extensive sympatry among all clades across the Americas with no significant niche differences. This challenges assumptions about strong associations between parasite diversity and ecology which requires adaptive surveillance strategies. Different DTUs elicit distinct innate and adaptive immune responses, with variations in cytokine production, cell invasion rates, and surface antigen expression affecting disease outcomes and vaccine development approaches. Drug resistance patterns are influenced by both inter-DTUs and intra-DTU genetic variability, with no exclusive association between natural resistance and particular DTUs, highlighting the need for community-based treatment monitoring. Understanding these complex interactions is essential for developing effective therapeutic strategies, vaccines, and diagnostic tools. Future research should integrate social sciences approaches including health education programs, community surveillance, and sustainable control interventions tailored to local parasite populations.

Keywords: *Trypanosoma cruzi*; Chagas disease; DTU; immune response; drug resistance

1. Introduction

Trypanosoma cruzi, the etiological agent of Chagas disease, exhibits remarkable genetic diversity that has been considered to shape the epidemiology and clinical outcomes of this neglected tropical disease affecting millions across the Americas. The complex genetics of the parasite arises from multiple mechanisms, including clonal propagation, sexual recombination, hybridization, and extensive genomic plasticity, resulting in seven discrete typing units (DTUs: TcI–TcVI and TcBat) with distinct biological characteristics. Understanding *T. cruzi* genetic diversity is essential for addressing the multifaceted challenges in Chagas disease control. This variation directly influences three critical aspects of disease biology: first, it affects the ecological dynamics of transmission cycles across diverse environments and host species; second, it influences the heterogeneity of host immune responses that shape disease progression from acute infection to chronic manifestations; and third, it influences the differential susceptibility to trypanocidal drugs that complicates therapeutic management. The interplay between parasite genetics and these biological processes has profound implications for diagnosis, treatment efficacy, and control strategies.

This review is part of a special section on Chagas disease examining the integration of biological and social sciences for effective disease control. Within this framework, we synthesize current knowledge on *T. cruzi* genetic diversity with particular emphasis on three areas: transmission dynamics, host-pathogen immunological interactions, and patterns of drug resistance. These areas were selected because understanding parasite genetic diversity in these contexts is essential for developing comprehensive control strategies that bridge biological research and social science interventions.

2. Reproductive Biology and Mechanisms of Genetic Diversity

The reproductive biology of *T. cruzi* is more complex than initially understood. The parasite uses a dual reproductive strategy that combines both clonal propagation and sexual reproduction, contributing significantly to its genetic diversity and adaptability. Clonal propagation remains a significant mode of reproduction, with studies documenting clonal diversity in various hosts and vectors [1–3]. However, high diversity detected within single mammalian reservoir hosts, with complex patterns across host populations challenged traditional view of clonal reproduction [4,5]. Laboratory studies have shown non-Mendelian genetic exchange, while field studies provide evidence of natural hybridization and genetic recombination [3,5–7]. The incongruence between nuclear and mitochondrial markers observed in natural populations further suggests widespread genetic exchange [3]. Moreover, genomic evidence of meiotic sex in *T. cruzi* has been demonstrated through the identification of patterns of genetic diversity consistent with sexual reproduction [7]. *T. cruzi* populations exhibit remarkable reproductive plasticity, with some groups maintaining highly clonal structures while others show evidence of sexual recombination. The parasite's ability to switch between reproductive modes appears to be influenced by environmental factors and host interactions [7,8]. This dual reproductive strategy has important implications for the epidemiology and control of Chagas disease. While clonal propagation enables expansion and persistence of successful genotypes, sexual reproduction and genetic recombination generate novel genotypic combinations that may enhance parasite adaptability [7,8]. This reproductive plasticity contributes to the genetic diversity of the parasite with potential implications for immune evasion and drug resistance development. However, the specific mechanisms by which these reproductive modes influence transmission dynamics, treatment response, and control interventions require further research.

Hybridization in *T. cruzi* occurs through a distinctive process where diploid parents fuse, followed by genome erosion, leading to tetraploid hybrids that eventually stabilize as diploid or triploid organisms [9,10]. This process differs from classical sexual reproduction observed in many eukaryotes and introduces novel mutations while increasing genetic diversity. The hybrid strains TcV and TcVI exemplify this phenomenon, showing products of genetic recombination between different parental strains [11]. Experimental studies have shown that hybrid formation can occur in the mammalian host, the insect vector, or potentially in culture [5,12]. Growing evidence of genetic exchange among field populations challenges earlier views that *T. cruzi* reproduced exclusively clonally with natural hybrid strains isolated from various geographic regions, demonstrating that genetic exchange occurs in wild populations [3–5,11]. These natural hybrids typically show heterozygosity at many loci, with alleles derived from different parental DTUs [13].

Homologous recombination, facilitated by proteins such as Rad51, plays a key role in genetic exchange and hybrid formation, as demonstrated by knockdown experiments showing that Rad51 depletion significantly reduces hybrid frequency in *T. cruzi*, confirming its essential role in genetic exchange processes [14]. Additionally, both nuclear and mitochondrial genetic material can be exchanged independently, increasing genetic through distinct mechanisms [10,15,16]. Mitochondrial introgression, where the mitochondrial genome from one strain is found in

the nuclear genetic background of another, has been documented in natural *T. cruzi* populations [17,18], adding an additional layer of complexity to the parasite's genetic diversity and evolutionary history [19].

T. cruzi demonstrates remarkable genomic plasticity, characterized by chromosomal aneuploidies, gene amplification, and extensive copy number variations [20–23]. These variations occur predominantly in gene family-rich regions, including mucins, trans-sialidases, and mucin-associated surface proteins (MASPs) [24,25], which are critical for parasite survival and host interaction [26].

Chromosomal aneuploidies are particularly prevalent, with strains exhibiting variable chromosome copy numbers that appear tolerable and potentially beneficial [7,21,23]. Moreover, the polycistronic transcription mechanism may facilitate tolerance to aneuploidy by buffering gene dosage variations maintaining functional gene expression that contribute to the adaptability and survival in different environments [27,28].

T. cruzi genome comprises core conserved regions and disruptive variable segments. The latter showed extraordinary structural variation, including extensive copy number variations in multi-gene families and complex patterns of heterozygosity [22,29]. Whole-genomes analysis has demonstrated extreme genome flexibility [30] which is crucial for parasite adaptation, pathogenicity, and the development of new drugs and diagnostic tools for Chagas Disease.

3. DTUs Characteristics and Distribution

The genetic diversity of *T. cruzi* has been a subject of intensive research, with significant advancements in molecular techniques enabling increasingly sophisticated analyses. From early isoenzyme studies to contemporary whole-genome sequencing approaches, our understanding of the parasite's genetic complexity has evolved substantially. This has transformed our perspective on Chagas disease from a single-pathogen infection to a complex disease with heterogeneous manifestations influenced by parasite genetic factors. *T. cruzi* is currently classified into seven discrete typing units (DTUs): TcI–TcVI and TcBat [13,31]. This classification system, formalized by an international consensus in 2009 [32] and refined in subsequent years [33], represents a significant advancement in our understanding of *T. cruzi* population structure.

The distribution of *T. cruzi* DTUs results from complex interactions between historical biogeographic factors and contemporary ecological conditions [34]. Associations between different DTUs with specific mammalian reservoirs and vectors had been reported to influence transmission dynamics across the Americas [8,13]. However, recent comprehensive analysis demonstrated extensive widespread and sympatry among all clades across the continent, with high beta-diversity indicating diverse host assemblages across regions [34,35]. In humans, the detected DTUs typically reflect the principal DTU circulating in domestic transmission cycles of a particular region, while orally transmitted outbreaks may involve sylvatic strains [13,35]. Mixed infections involving multiple DTUs have been reported in vectors, mammalian hosts and humans, particularly in regions with diverse ecological profiles [36–39].

TcI represents the most widespread and genetically diverse DTU, found throughout the Americas in both sylvatic and domestic transmission cycles [13], representing approximately 60% of genotyped strains and circulating in both sylvatic and domestic cycles [31]. Geographic distribution varies considerably across the Americas. TcI predominates in Central America, where it represents 94% of infections in *Triatoma dimidiata* across its range from Mexico to Colombia [40]. In Venezuela, TcI accounts for 94.1% of genotyped isolates and 79% of human disease cases [41]. In Ecuador, TcI showed remarkable genetic diversity in triatomines and sylvatic animals, with active dispersal across localities [42], and has been associated with fatal acute disease [43]. In Bolivia and Chile, TcI predominates in sylvatic triatomine populations [44,45], as well as in naturally infected rodents and dogs [46,47].

TcI showed high genetic variability and structural plasticity, including chromosomal aneuploidies and rearrangements [23]. Studies using polymorphic microsatellite loci have revealed high genetic diversity within sylvatic TcI populations, with spatial structuring at a continental scale [48], and significant gene flow between biomes providing evidence of genetic exchange [49]. This complexity was supported by findings of up to 49 distinct multilocus genotypes across just eight mammals and as many as 10 genotypes from a single host [4]. In Ecuador and Colombia, TcI has been subdivided into domestic and sylvatic genotypes reflecting adaptation to different transmission cycles [6,50,51]. In Colombia, domestic TcI isolates showed reduced genetic diversity compared to sylvatic populations, suggesting a genetic bottleneck during adaptation to domestic environments [51]; however, this substructure is not conserved across all the ecogeographic distribution of TcI [49,51].

TcII is widely distributed across the Americas, mainly associated with domestic transmission but also reported in sylvatic cycles. This DTU has been reported across a broad geographic range, including the southeastern United States, Mexico, Brazil and Chile [46,52–57]. Although less frequent and more restricted than

TcI, TcII has been detected in multiple triatomine vector across this range, including *T. dimidiata* and *Panstrongylus rufotuberculatus* in Mexico, *Rhodnius pictipes* in the Amazon Basin, *Triatoma sanguisuga* in the southeastern United States, *Triatoma infestans* in Paraguay and Bolivia, and *Mepraia* species in Chile [45,52,55,56,58,59] TcII also infects various mammalian hosts, including dogs in the Amazon Basin, rodents in Mexico and the United States, and small mammals in Chile [46,52,56]. Human infections with TcII have been associated with cardiac manifestations [53,54].

TcIII and TcIV, though rarely sampled, display an important genetic diversity and are predominantly found in sylvatic transmission cycles [31]. Both DTUs have been widely reported across the Americas. TcIII has been reported from southern countries such as Chile and Argentina [47,60], through Brazil, Colombia and Venezuela [38,61,62] to Mexico [63]. TcIV has been detected from Colombia through Central America and Mexico, reaching far north as Illinois in the United States [40,64,65]. Within Brazilian biomes, TcIV is more frequently detected than TcIII [38]. TcIII showed different ecological associations across the region. In Bolivia, TcIII has been detected in *Panstrongylus geniculatus* invading houses [66], and in wild *T. infestans*, although at low frequency [44]. In Paraguay, TcIII is strongly associated with armadillo species, particularly *Dasypus novemcinctus*, *Euphractys sexcinctus*, and *Chaetophractus* spp. [59]. Additionally, TcIII has been reported in dogs in northern Chile [47]. Both DTUs infect a wide range of mammalian hosts, particularly species from the Didelphimorphia, Rodentia, Artiodactyla and Carnivora orders [38,47,65] and have been transmitted by vectors from the *Triatoma* and *Rhodnius* genera [40,60,63,64]. A distinct TcIV-USA variant documented in *Dipetalogaster maxima* vectors represents regional adaptation and potential historical bottlenecks [67]. Although these DTUs are predominantly associated with sylvatic cycles, human infections have been reported and associated with cardiac disease [31,61].

TcV and TcVI, are hybrid lineages associated with domestic transmission cycles that circulate mainly in the Gran Chaco region [31]. These hybrid DTUs originated from genetic recombination between TcII and TcIII parental strains, as demonstrated through analysis of 32 loci across *T. cruzi* lineages [11]. Phylogenetic analyses showed clear mosaicism, with different genetic markers clustering alternatively with either TcII or TcIII, providing compelling evidence for historical hybridization events [11,68]. These lineages are predominantly distributed in the Southern Cone countries of Latin America where TcV and, to a lesser extent, TcVI have been reported in *T. infestans* from Paraguay [59] and Bolivia [69]. And in *Mepraia* vectors from Chile [45], as well as in human cases in Bolivia [70] and Chile [71]. However, molecular typing methods vary in their capacity to discriminate between TcII, TcV and TcVI DTUs, which should be considered when analyzing epidemiological patterns. Both DTUs are also present in Argentina, Peru and Paraguay [72–74] with scarce reports in other regions [34]. Although these lineages have been more frequently found in human infections associated with chronic pathologies, TcV was also reported in asymptomatic individuals [72].

TcBat has been identified in sylvatic cycles associated with bats. Its presence has been recorded in South America (Brazil, Ecuador and Colombia) and Central America (Panama) [13,75,76]. This DTU is closely related to TcI but displays distinctive genetic and biological characteristics [77,78]. TcBat was initially described in Phyllostomidae bat species from Panama and extended to bats of the Vespertiliondae family [76,77]. The identification of this bat-associated lineage has expanded our understanding of the evolutionary history and ecological versatility of the parasite, emphasizing the importance of considering non-human reservoirs in disease ecology studies [76]. Human infection with TcBat has been documented in Colombia, though it remains rare and appears to be non-pathogenic [75]. Also, TcBat was detected in mummies from Chile [79].

The ecological dimensions of *T. cruzi* genetic diversity reveal a complex scenario that integrates both historical associations and contemporary mixing patterns. The associations between specific DTUs and geographic regions, transmission cycles and hosts described above represent important epidemiological patterns that inform our understanding of disease ecology and control strategies. However, recent comprehensive analyses have demonstrated that these associations, while epidemiologically meaningful, should not be interpreted as absolute [34,35]. Extensive sympatry among all clades has been observed across the continent, with statistical analysis showing no significant ecological differences among DTUs when examined at a continental scale [34]. These findings challenge earlier assumptions that different DTUs occupy distinct ecological niches with separate geographical and host distributions [34,38]. While regional predominance patterns and host preferences do exist, they do not represent significant biological or ecological barriers to the transmission and establishment of DTUs across different biomes [34,38]. The persistence of genetic diversity between lineages despite extensive sympatry suggests that factors beyond spatial separation, such as reproductive biology, vector competence, and host immune interactions, contribute to maintaining DTU differentiation [34,35]. Understanding the geographic distribution of DTUs is essential for designing targeted surveillance and control programs, as different regions may require different approaches based on the local parasite genetic landscape [34,35]. These findings align with recent comprehensive epidemiological analyses that found no definite evidence for clear-cut associations between *T. cruzi* DTU and

distinct transmission cycles, transmission routes, or disease severity [80]. The recognition that multiple DTUs can circulate within the same geographic area requires community-based surveillance approaches that can detect and respond to local transmission patterns rather than relying on assumptions about fixed parasite-ecology associations.

4. Host Immune Response and Antigenic Diversity

At the innate immunity level, distinct patterns emerge from *in vitro* and *ex vivo* studies, with different DTUs showing contrasting immune profiles across multiple cell types. In human monocytes, TcI strains (Col cl1.7) tend to induce anti-inflammatory IL-10 expression, while TcII strains (Y strain) promote inflammatory TNF expression [81]. TcIV (AM-64) and TcV (3253) strains produce opposing profiles (anti-inflammatory and highly inflammatory responses, respectively) [82]. In human neutrophils, both TcI (Col cl1.7) and TcII (Y) strains triggered activation followed by apoptosis, a response not observed in monocytes infected with these same strains [83]. Dendritic cells, critical components of the innate immunity, also showed DTU-specific response. TcII strains (1849 and 2369) presented higher invasion rates of bone marrow-derived dendritic cells than TcI strains (AQ1.7 and MUTUM), with differential production of cytokines (IL-10, TLR-2, TNF-α, IL-12, IL-6, and CCL2) and maturation markers (CD40, CD80, MHC-II, CCR5, and CCR7), indicating the evolution of specific evasion strategies [84]. More recently, transcriptomic analysis of peripheral blood mononuclear (PBMCs) cells from chronically infected macaques has revealed important insights [85]. Parasite strain diversity is a key determinant of the initial innate immune response. This results in distinct profiles of trained immunity that modulate subsequent adaptive responses and determine whether infection is controlled or persists during the chronic phase [85]. Additionally, moderately effective immune control has been reported in *T. cruzi* infections. CD8+ T cells, critical for controlling *T. cruzi* infection and usually produce a robust immune response in infected host cells [86]. However, they are only partially effective. Most hosts maintain low level infection throughout life due to several factors: slow CD8+ T cell responses, expression of highly variant CD8+ T cell epitopes and pathogen immune evasion mechanisms [86].

The transition from asymptomatic infection to clinical disease appears to involve shifts in cytokine balance. Cardiac patients showed elevated pro-inflammatory cytokines (IL-2, IL-6, IL-9, IL-12) compared to asymptomatic patients, who show higher levels of anti-inflammatory cytokines (IL-5, IL-10, IL-13) [87]. This switch in cytokine profiles suggests that dysregulation of immune modulation may play an important role in the development of Chagas cardiomyopathy [87]. However, when comparing chronic cardiac patients infected with different DTUs (TcI, TcII, or mixed TcI/TcII), all groups displayed pro-inflammatory profiles with no clear cytokine pattern distinguishing the DTUs, although specific cytokines showed DTU-associated differences [87]. These findings indicated that while parasite genetic diversity can influence specific cytokine responses, all DTUs can promote a pro-inflammatory environment in chronic cardiomyopathy [87]. Moreover, differential immune responses have been reported in experimental models. A study showed some differences concerning parasite load, parasite tropism and cytokines production between two mice lineages infected with the same DTUs (TcI and TcIV) [88]. These studies suggested that disease outcome may depend more on immune modulation and host background rather than the infecting strain. Recent analysis emphasizes that both parasite and human genetics play crucial roles in defining Chagas disease pathogenesis [89,90], with the immunological condition of the patient potentially being as important as the parasite strain itself [90]. Understanding these complex host-parasite immune interactions is crucial for patient education programs, as it explains the variability in disease outcomes and informs realistic expectations regarding vaccine development and therapeutic interventions.

The antigenic diversity between DTUs directly influences how the immune system responds. For example, tGPI-mucins, that play an immunomodulatory role during the course of Chagas Disease, induced variations in nitric oxide and IL-12 expression profiles in peritoneal macrophages, as well as a differential expression of O-linked α-galactosyl residues among different DTUs [91]. Additionally, the trypomastigote small surface antigen (TSSA) mucin, used as a serological marker that differentiates TcII-VI from TcI infection, displayed DTU-specific variants [92]. TcI, TcIII, and TcIV shared key features in a lineage-specific TSSA epitope region, while TcII, TcV, and TcVI shared a common epitope [92]. Furthermore, TcII strains expressed and released significantly higher amounts of the virulence factor trans-sialidase than TcI lineage strains, which affects immune evasion capabilities [93]. These molecular differences in surface antigens demonstrate how genetic diversity can translate into differential immune recognition and evasion strategies.

5. DTU-Specific Drug Susceptibility Patterns

Studies comparing drug susceptibility across DTUs reveal marked differences in treatment responses. A systematic review and meta-analysis of *in vitro* susceptibility to benznidazole demonstrated that TcI trypomastigotes exhibit lower sensitivity to this drug compared to TcII strains at 24 h of drug incubation, although

considerable heterogeneity exists within each DTU [94]. Research on ergosterol biosynthesis inhibitors, including posaconazole and ravuconazole, demonstrated variable activity that was both compound- and strain-specific across different DTUs, with these inhibitors unable to eradicate intracellular infection even after prolonged exposure [95]. In contrast, studies of newly isolated TcV strains from Argentina found higher susceptibility to benznidazole compared to TcII strains, which are considered moderately resistant to this drug [96]. These contradictory findings within the same DTU underscore the complexity of genetic variability and its impact on drug response.

An experimental study evaluating benznidazole and itraconazole treatment in mice infected with twenty laboratory-cloned stocks representing the total phylogenetic diversity of *T. cruzi* demonstrated important differences among genotypes [97]. Members of the *T. cruzi* I group were highly resistant to both drugs, whereas members of the *T. cruzi* II group were partially resistant to both drugs, despite susceptibility to itraconazole during the chronic phase. The correlation between treatment response and phylogenetic classification was clearer for itraconazole than benznidazole, indicating that lesser genetic subdivisions within DTUs show considerable heterogeneity for *in vivo* drug susceptibility [97]. Additional studies examining TcV isolates from Argentina confirmed marked phenotypic diversity within this DTU [98]. Epimastigote sensitivity assays demonstrated different responses to benznidazole, nifurtimox, pentamidine, and dihydroartemisinin *in vitro* among different TcV isolates by different expression patterns of antioxidant proteins [98]. Benznidazole-resistant isolate decreased expression of some enzymes (Old Yellow Enzyme and cytosolic superoxide dismutase) while overexpressing mitochondrial superoxide dismutase and tryparedoxin-1, compared to the benznidazole-susceptible isolate [98]. These results demonstrate high levels of intra-DTU diversity, which may represent an important obstacle for the testing of chemotherapeutic agents.

Regional variations in treatment efficacy further support the role of genetic diversity in drug resistance. Differences in benznidazole efficacy observed in children and adolescents from Honduras, Guatemala, and Bolivia have been potentially linked to the presence of different DTUs circulating in these regions [99]. However, comprehensive analyses indicate that natural resistance to benznidazole and nifurtimox is not exclusively associated with any particular DTU, suggesting that both inter- and intra-DTU genetic variability contribute to treatment outcomes [68]. An early study examining sixteen natural stocks representing the overall genetic diversity of *T. cruzi* found important variation of IC_{50} values (7.3–16.9 μM) among stocks belonging to different DTUs [100]. However, correlation analysis showed that natural susceptibility to benznidazole expressed as IC_{50} level was not related to the genetic structure represented by the different DTUs [100]. These results suggest that while DTU classification provides a framework for understanding parasite diversity, drug susceptibility is influenced by additional genetic factors beyond DTU membership. This complexity highlights the need for community-based treatment monitoring programs and health education that prepare patients and health care workers for variable treatment responses, emphasizing the importance of follow-up and adherence despite potential therapeutic challenges.

6. Genetic Polymorphisms and Resistance Mechanisms

The genetic basis of drug resistance in *T. cruzi* involves polymorphisms in genes encoding membrane transporters that can affect drug retention within parasites. Analysis of the ABCG-like transporter gene (TcABCG1) across fourteen *T. cruzi* strains from different DTUs revealed DTU-specific SNPs and amino acid changes [101]. Although these genetic variations distinguished parasite lineages, no direct correlation between specific SNPs and benznidazole resistance phenotypes was found. However, strains naturally resistant to benznidazole showed overexpression of TcABCG1, suggesting that the level of transporter expression, rather than sequence polymorphisms alone, may be the critical determinant of resistance across different genetic backgrounds [101]. This finding showed that genetic diversity influences drug resistance through differential gene expression patterns among DTUs.

Beyond transporter gene expression, genetic diversity within DTU populations can influence treatment outcomes through polymorphisms in drug-activating enzymes. A study of an oral Chagas disease outbreak in Venezuela investigated patients who experienced treatment failure with nitroheterocyclic drugs (benznidazole and nifurtimox) [102]. The study revealed significant intra-DTU variability within TcI populations by the analysis of minicircle signatures [102]. Additionally, a polyclonal source of infection was identified through SNPs from the nitroreductase gene (*TcNTR-1*) [102]. The poor drug response in these naturally occurring parasite populations was further evidenced by the wide range of half-maximal inhibitory concentration (IC_{50}) values. These ranged from 5.3 to 104.7 μM among isolates, suggesting that naturally resistant parasite clones were present within the polyclonal infections [102]. Moreover, in some post-treatment samples, SNPs that produced stop codons were found, potentially generating truncated proteins associated with resistance [102].

The genetic heterogeneity observed at the molecular level translates into variable treatment responses in different geographic regions. Studies of Mexican *T. cruzi* strains using microsatellite analysis identified three distinct genetic subgroups (genotypes 1 and 2 formed by human isolates of TcI, and genotype 3 from isolates of wild mammals of TcII-TcVI) with heterogeneous susceptibility to nifurtimox and benznidazole [103]. Microsatellite genotypes 2 and 3 were significantly more susceptible to benznidazole than microsatellite genotype 1 [103]. In Brazil, experimental treatment of mice infected with TcII strains isolated from children demonstrated the complexity of assessing treatment efficacy in resistant strains [104]. Most strains exhibited resistance to benznidazole, with 95-100% of treated animals showing positive parasitological and/or serological tests during both acute and chronic phases, indicating therapeutic failure. However, despite the lack of parasitological cure, benznidazole treatment provided significant benefits, including suppression of parasitemia in all strains during the acute phase and reduction or elimination of inflammation and fibrosis in two of the eight TcII strains tested [104]. These findings demonstrated that outcomes vary among strains within the same DTU.

The role of genome instability in generating genetic diversity that facilitates drug resistance adaptation is increasingly recognized. *T. cruzi* exhibits extensive genome flexibility through aneuploidy, copy number variations, and genetic rearrangements, which may be strategically exploited for host adaptation and drug resistance [105]. Chromosomal copy number variation has been linked to *in vitro* drug resistance in *T. cruzi* [105], and karyotypic heterogeneity is observed even within clonal populations, suggesting that aneuploidy represents a dynamic response with the potential to adapt to rapidly changing environmental challenges [105]. Other studies have demonstrated that *T. cruzi* has an intrinsic ability to develop drug resistance through independent sequential genetic changes even within a single population, with multiple mechanisms acting in concert to generate varying levels of resistance [106].

In conclusion, the genetic diversity of *T. cruzi* profoundly influences drug resistance patterns through DTU-specific susceptibilities, intra-DTU polymorphisms, and genome instability mechanisms. Understanding the complex interactions between parasite genetics and treatment response is essential for developing effective therapeutic strategies and predicting treatment outcomes in different geographic regions where distinct DTUs predominate [89].

7. Conclusions and Prospective Studies

The extensive genetic diversity of *T. cruzi* influences multiple dimensions of Chagas disease biology, presenting both scientific insights and practical challenges for disease control [29,89]. Phylogenetic analyses consistently support the classification of strains into seven discrete typing units (DTUs), with most DTUs exhibiting substantial intragroup genetic variation shaped by both sexual reproduction and broad clonal expansion. This genomic complexity, characterized by chromosomal aneuploidies, extensive copy number variations, and significant expansion of surface protein gene families, provides *T. cruzi* with substantial adaptive capacity across diverse mammalian hosts and insect vectors. Indeed, such genomic variability presents considerable challenges at different levels. Although DTUs have been historically associated with specific epidemiological and ecological scenarios, recent comprehensive analyses have demonstrated that these associations should not be considered absolute, aligning with recent epidemiological reviews that found no definitive evidence for strict ecological or geographic boundaries among DTUs [80,89]. Extensive sympatry has been observed among all clades across the Americas, with statistical analysis showing no significant ecological segregations among DTUs at continental scale [35]. This finding has important implications for disease surveillance and control programs, as strategies cannot rely on fixed geographic predictions of DTUs distribution and must instead account for the dynamic circulation of multiple genetic lineages within communities.

Nevertheless, host-pathogen interactions remain complex, with disease outcomes influenced by numerous factors beyond the infecting DTU, including host immune modulation and parasite evasion strategies [87,88]. The crucial roles of both parasite and human genetics in defining disease pathogenesis and clinical outcomes have been increasingly recognized [90]. The high polymorphisms in surface molecules may facilitate immune evasion mechanisms that challenge the design of serological markers [92] and potentially broadly protective vaccine [107]. On the other hand, the interplay between parasite genetic diversity and host immune responses proves crucial in determining disease evolution, with different DTUs eliciting distinct immunological profiles and varying propensities for specific pathologies [87,88].

This complex relationship between parasite genetics and drug susceptibility represents another layer of difficulty. Studies reveal variable and complex relationships between DTU classification and benznidazole or nifurtimox susceptibility [90,100,101] with therapeutic failures likely influenced by multiple factors including both inter-DTUs and intra-DTU genetic variability rather than DTU identity alone [90,100,101]. Addressing these

challenges requires that new vaccines, therapies, and diagnostic tools be screened against diverse strains from different DTUs and geographic regions, as intervention performance may vary substantially according to parasite genetic backgrounds. Understanding this genetic complexity is essential for designing effective health education programs that communicate realistic treatment expectations to affected communities and inform community-based surveillance of treatment.

Furthermore, advances in next-generation sequencing technologies, particularly the integration of long and short-read sequencing methods, have significantly enhanced genome quality and revealed extensive genetic complexity, providing essential resources for studies on genomic plasticity crucial for understanding adaptation, pathogenicity, and developing novel drugs and diagnostics. Moving forward, comprehensive investigations that consider the full complexity of the transmission nature including parasites, hosts, vectors, and reservoirs, while accounting for both inter and intra-DTU variation will prove essential for advancing effective solutions to this disease. These biological insights into genetic diversity must inform social science approaches to ensure that control strategies are tailored to local parasite population and community contexts.

Recent reviews of Chagas disease control strategies emphasize the critical need for enhanced surveillance systems, integrated vector control programs, and sustained political commitment to public health initiatives [108]. The recognition that *T. cruzi* DTUs show broad geographic distributions without strict ecological boundaries [35,80] requires adaptive surveillance approaches that account for local parasite diversity while maintaining flexibility to respond to changing epidemiological patterns. Effective control efforts must address diverse transmission routes including both vectorial and non-vectorial mechanisms, with particular attention to the involvement of local governments, international organizations, and civil society in program implementation [108].

Future research should integrate social and ecological sciences through eco-bio-social approaches [109,110]. These approaches should examine how social institutions, economic factors, and historical-political contexts shape disease transmission, vector control sustainability, and healthcare access, while incorporating knowledge of local parasite genetic diversity to optimize intervention design. Such integration is particularly relevant for vaccines, early diagnosis and treatment, where understanding the circulating DTUs can inform both biological tool development and community engagement strategies [109,110]. Moreover, community-based interventions incorporating interdisciplinary and intersectoral participation [111], alongside community empowerment strategies, represent promising directions for sustainable disease control and improved quality of life for affected populations [112].

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