Clonal Hematopoiesis of Indeterminate Potential: An Emerging Risk Factor for Type 2 Diabetes and Related Complications

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Abstract

The accumulation of acquired somatic mutations is a natural consequence of aging, but the pathophysiological implications of these mutations beyond cancer are only beginning to be understood. Most somatic mutations are functionally neutral, but a few may confer a competitive advantage to a stem cell, driving its clonal expansion. When such a mutation arises in hematopoietic stem cells, it leads to clonal hematopoiesis, where a significant proportion of blood cells originate from the mutant stem cell and share the same mutation. Clonal hematopoiesis of indeterminate potential (CHIP), a specific subset of clonal hematopoiesis driven by myeloid leukemia-related somatic mutations, has been linked to a higher risk of various age-related conditions, particularly cardiovascular disease, by exacerbating inflammatory responses. Emerging evidence suggests that CHIP may also contribute to the pathogenesis of type 2 diabetes and some of its complications. This review synthesizes current knowledge on CHIP and its potential as a novel risk factor for type 2 diabetes, highlighting the need for further research to clarify this relationship and to explore its potential value in developing personalized preventive care strategies for type 2 diabetes and related conditions.

Keywords: CHIP, cardiovascular disease, DNMT3A, diabetes, inflammation, insulin resistance, kidney disease, somatic mutation, TET2.

Abbreviations: CH (clonal hematopoiesis), CHIP (clonal hematopoiesis of indeterminate potential), mCA (mosaic chromosomal alteration), SNP (single nucleotide polymorphism), SNV (single-nucleotide variant), VAF (variant allele fraction), WES (whole exome sequencing, WGS (whole genome sequencing).

Introduction

Genetic variation is fundamental to the etiology and progression of numerous human diseases, including metabolic disorders such as diabetes and its complications. In this context, genetic variants, or mutations, can be categorized into two types: those arising in germ cells and inherited by offspring (inherited germline variants), and those acquired during an individual's lifetime in non-germline cells (acquired somatic variants). Historically, human genetic studies have demonstrated that many chronic disorders, including type 2 diabetes, often result from the interplay between inherited genetic variants and environmental exposures throughout life [1, 2]. Recently, acquired somatic mutations, previously linked primarily to cancer, have also been recognized as contributors to non-cancer conditions [3]. Of particular relevance in this context, somatic mutations in the hematopoietic system that lead to clonal hematopoiesis (CH) are gaining clinical relevance across multiple domains [4, 5]. This review provides an overview of our current understanding of CH and explores the growing evidence linking some CH-related mutations to type 2 diabetes and its complications (**Figure 1**).

Clonal hematopoiesis: a primer

CH occurs when a substantial fraction of an individual’s blood cells is derived from a single hematopoietic stem cell (HSC) clone that has gained a competitive advantage. While non-mutational mechanisms may also contribute to CH, somatic mutations are the most frequent drivers. Over time, HSCs accumulate random somatic mutations, and estimates of mutation rates and HSC numbers in humans suggest that middle-aged individuals may harbor around one million mutations in their HSC pool [6-8]. This accumulation of mutations fosters competition among

different mutant clones. While most mutations are neutral or detrimental to HSC function, some confer a selective advantage by promoting the self-renewal, proliferation, or survival of the mutant HSC, leading to the progressive expansion of the mutant clone and the development of CH. The mutations driving CH are primarily single-nucleotide variants (SNVs) or small insertions or deletions (indels), though larger chromosomal changes, known as mosaic chromosomal alterations (mCAs), can also be involved. As further discussed below, these acquired mutations are now known to be present in many apparently healthy individuals, but most of them were previously identified as drivers of hematological cancer, and, accordingly, are considered precursors for hematological malignancies [4, 5].

CH is most frequently identified through next-generation sequencing of blood samples (**Box**), which allows for the detection of clonally expanded somatic mutations by calculating the variant allele fraction (VAF) —the proportion of sequencing reads supporting the mutant allele. While inherited variants usually have a VAF close to 50%, reflecting their presence in all cells as they are inherited from one parent, somatic mutations typically exhibit lower VAFs, indicating they are present in as subset of blood cells. VAFs also serve as estimates of mutant clone size; a VAF of 10%, for example, suggests that 20% of nucleated cells carry the mutation, as it typically affects only one allele.

Although a formal consensus classification is still lacking, different types of CH have been defined based on mutations characteristics, the extent of clonal expansion, and their association with hematological disease (**Figure 1**). One specific type of CH that has gained particular attention is clonal hematopoiesis of indeterminate potential (CHIP), defined as the presence of any expanded SNV or indel in a known hematological malignancy-related gene with a VAF of at least 2% in the

absence of a hematological malignancy diagnosis [9]. While not explicitly stated in the original definition, CHIP is increasingly being used to refer specifically to mutations in genes linked to myeloid leukemia, distinguishing it from CH associated with mutations in lymphoid leukemia-related genes, termed lymphoid-CH or lymphoid-CHIP [4, 10]. At a gene-specific level, CHIP is most commonly associated with mutations in genes related to epigenetic regulation (e.g., *DNMT3A, TET2, ASXL1*), although it is also driven by mutations affecting genes involved in the DNA damage response (e.g., *TP53*, *PPM1D*), splicing regulation (e.g., *SF3B1*, *SRSF2*, *U2AF1*) or intracellular signaling (e.g., *JAK2*), among other functions.

CHIP: epidemiology and role in human disease

CHIP mutations are acquired randomly during the lifespan. Accordingly, although they can be acquired at any point in life, even during embryonic development [11], the chances of having acquired one of these mutations evidently increase with age. In addition, once acquired, the expansion of the mutant clone may take years or decades to reach detectable levels. As a result, CHIP is strongly associated with aging. Analyses of large whole-exome sequencing (WES) and whole-genome sequencing (WGS) datasets indicate that CHIP is present in 2-3% of middle-aged individuals and over 10% of those older than 70 [12-14]. However, these estimates likely underreport the prevalence of CHIP due to the limited sensitivity of these sequencing approaches for detecting CHIP with VAF<10% [13, 15]. More sensitive, albeit smaller, sequencing studies suggest that the true prevalence of CHIP may range from 5 to 10% in middle-aged individuals and exceed 25% in the elderly [15, 16]. However, larger high-sensitivity sequencing studies are needed to determine CHIP prevalence across the lifespan accurately.

As discussed above, CHIP mutations are linked to an elevated risk of developing myeloid leukemia and are often viewed as a precursor state for this and other hematological malignancies [4, 5, 9]. However, despite the markedly increased relative risk of hematologic disease among individuals with CHIP, the absolute risk for this condition remains low, as malignant transformation typically requires the accumulation of multiple oncogenic mutations. Most CHIP carriers harbor only one or, less frequently, two mutations, exhibit normal blood cell counts and do not progress to blood cancer. Nevertheless, CHIP is associated with increased all-cause mortality, primarily due to its strong link to various age-related non-hematological conditions, particularly cardiovascular disease [4, 5]. Mechanistic studies in mice and observations in humans indicate that these associations are largely driven by exacerbated inflammatory responses from mutant immune cells, underscoring CHIP as a non-traditional risk factor for numerous age-related, inflammation-driven conditions.

CHIP as a risk factor for type 2 diabetes

Insights from human studies

The role of CH or CHIP as risk factors for disease is typically examined through DNA analyses in human populations, an approach that has also been applied to type 2 diabetes. Initial evidence suggesting CH as a risk factor for diabetes emerged from a SNP array analysis of 7,659 individuals, which revealed a significant cross-sectional association between autosomal mCAs and higher type 2 diabetes risk [17]. However, a recent study based on WGS data from 482,396 UK Biobank participants found no statistically significant association between autosomal mCAs and type 2

diabetes incidence [18], raising questions about this connection. In contrast, evidence linking CHIP to type 2 diabetes appears stronger, despite some conflicting findings. A 2014 cross-sectional study utilizing whole-exome sequencing data from approximately 17,000 individuals reported a 30% higher type 2 diabetes risk among CHIP mutation carriers, even after adjusting for confounding variables [12]. However, a similar cross-sectional analysis of over 200,000 individuals from the UK Biobank found no association between CHIP and prevalent type 2 diabetes [19]. Caution must be exercised when interpreting these conflicting findings on associations with prevalent disease due to the unknown timing of the onset of diabetes and CH. In this context, recent longitudinal evidence provides stronger support for a link between CHIP and incident diabetes. A study involving 17,637 participants from the TOPMed initiative with a mean follow-up of 9.8 years identified CHIP through WGS analysis and found that carriers with a variant allele fraction (VAF) ≥10% had a 23% increased risk of developing type 2 diabetes relative to non-carriers [20]. The size of the study population allowed some reasonably powered gene-specific analyses, finding that carriers of mutations in *TET2* and *ASXL1* show significantly elevated type 2 diabetes risks (48% and 76%, respectively). Similar trends were observed for *JAK2* and *TP53* mutations, although these did not reach statistical significance, likely due to limited statistical power. Although *DNMT3A* mutation carriers also showed a nonsignificant trend to higher type 2 diabetes risk, the effect size was considerably weaker than that of other CHIP genes. While this study provides compelling evidence, it is limited by the low sensitivity of CHIP ascertainment based on WGS data, which have modest sequencing depth. This approach misses many mutant clones with VAFs between 2% and 10% (or 4-20% mutant cells in blood) [13, 15], which have been shown to be pathophysiologically relevant in other contexts [15, 21-23]. Additionally, minor differences in variant interpretation and filtering can significantly influence results when using WGS or WES

data to study CHIP, as evidenced by recent partly conflicting findings regarding the association between CHIP and cardiovascular disease, even when using the same dataset [14, 24]. In this context, it should be noted that a study using higher-sensitivity targeted gene sequencing found that CHIP with VAF≥1.5% was associated with elevated type 2 diabetes risk in an East Asian cohort, although this association became a non-significant trend after adjusting for confounders [25]. Overall, while current data support a link between CHIP and type 2 diabetes, further studies employing high-sensitivity DNA sequencing and detailed information on diabetes status and outcomes are needed to clarify this relationship and establish clinically relevant VAF thresholds.

Insights from experimental studies in mouse models

The emerging genetic association between CHIP and type 2 diabetes raises the possibility of novel strategies for managing diabetes risk. However, the descriptive nature of these studies requires cautious interpretation, as they do not allow to determine whether CHIP and type 2 diabetes are causally linked or whether CHIP is simply a marker of aging or other confounding conditions. Nonetheless, given that CHIP mutations frequently affect genes with broad roles in immune regulation—a relevant factor in diabetes pathophysiology, particularly in obesity-related insulin resistance—it is plausible that CH contributes causally to type 2 diabetes in some individuals. In this context, experimental studies have started to shed light onto causality in the relationship between CHIP and the development of different age-related disorders, primarily using bone marrow transplantation strategies to introduce CHIP mutations in mouse models (**Figure 2**). In the context of type 2 diabetes and related alterations in glucose homeostasis, experimental studies have focused on the effect of *TET2* mutations on insulin resistance. However, the emerging genetic

evidence linking other CHIP genes to type 2 diabetes in humans is likely to inspire new research efforts in this area.

Studies in mouse models of CHIP driven by *TET2* loss-of-function mutations demonstrate that the expansion of TET2-deficient cells exacerbates insulin resistance in conditions of aging and diet-induced obesity [26]. Mechanistically, this was linked to interleukin-1β (IL-1β) overproduction by TET2-deficient macrophages, resulting in defective insulin signaling in adipocytes. In cultured adipocytes, exposure to conditioned media from TET2-deficient macrophages reduced IRS1 (insulin receptor substrate 1) expression and glucose uptake, both of which could be prevented with IL-1β-neutralizing antibodies. Furthermore, pharmacological inhibition of the NLRP3 inflammasome—the main proteolytic complex mediating IL-1β maturation and secretion—mitigated insulin resistance in mice exhibiting *TET2*-mutant CHIP. Further supporting a connection between *TET2*-mutant CHIP and IL-1β-driven inflammation, circulating IL-1β levels have been reported to be elevated in carriers of *TET2* mutations [13]. Considering the central role of IL-1β in pancreatic β-cell dysfunction and turnover in various settings [27-29], it is plausible that this same mechanism could also impair pancreatic function and accelerate transition from insulin resistance to type 2 diabetes. However, this possibility was not evaluated in the aforementioned study and remains to be investigated. Similarly, whether *TET2*-mutant macrophages impair insulin signaling in other organs key to glucose homeostasis besides the adipose tissue, such as the liver or the skeletal muscle, remains unexplored.

CHIP as a risk factor for frequent complications of type 2 diabetes

Beyond type 2 diabetes itself, CHIP is also associated with several common comorbidities in diabetic patients. While most evidence available to date does not come from populations of diabetic patients, it provides a solid basis for future studies. The most robust link is with macrovascular disease, as CHIP has emerged as a potent risk factor for subclinical atherosclerosis and overt atherosclerotic disease, including coronary artery disease and peripheral artery disease, independently of traditional cardiovascular risk factors [12, 15, 23, 30, 31]. CHIP also appears associated with ischemic stroke and cerebrovascular disease, although the effect is milder and dependent on the type of stroke [32]. Furthermore, longitudinal imaging and sequencing studies have shown that CHIP confers an increased risk of developing asymptomatic, subclinical atherosclerosis, whereas atherosclerosis itself does not influence mutant hematopoietic cell expansion [15]. Gene-specific analyses generally support a connection between CHIP mutations in *TET2*, *ASXL1*, *JAK2*, DNA damage response genes and splicing factors, and a higher risk of atherosclerotic vascular disease [23, 30, 31]. Mixed findings have been obtained regarding the effect of *DNTM3A* mutations, the most frequent in CHIP, with high-sensitivity sequencing studies supporting a moderate, but significant effect on atherosclerotic disease [15, 23], whereas larger, albeit less sensitive WES studies typically find a negligible effect [30, 31]. Beyond atherosclerotic disease, CHIP is also strongly associated with adverse clinical outcomes in heart failure [21, 22, 33], and has a more modest, but still significant effect on the development of new-onset heart failure [34, 35] . Experimental studies in mice support a causal role of several CHIP mutations in atherosclerosis and cardiac dysfunction through various gene-specific pro-inflammatory mechanisms (reviewed in depth elsewhere [36]). Consistent with its aforementioned effects in mouse models of insulin resistance, *TET2*-mutant CHIP accelerates atherosclerosis and cardiac

dysfunction in various experimental settings, primarily through overactivation of NLRP3/IL-1β-driven inflammation [37-39]. Other mutated genes also accelerate atherosclerosis in mice by activating distinct central drivers of innate immune signaling, such as IRAK1 in mouse models of *ASXL1*-mutant CHIP [40] or the AIM2 inflammasome in mouse models of *JAK2*-mutant CHIP [41]. *DNMT3A* loss-of-function mutations have been suggested to promote atherosclerosis through similar, albeit milder, effects on the expression of several pro-inflammatory cytokines and chemokines in murine macrophages [42]. However, the specific molecular pathways underlying the pro-inflammatory effects of *DNMT3A* mutations remain to be elucidated. Inactivating *TP53* mutations have been shown to accelerate atherosclerosis by increasing macrophage burden in the atherosclerotic wall, driven by heightened mutant macrophage proliferation [31].

Emerging evidence from multiple cohorts suggests that CHIP also has a role in kidney disease, including associations with predisposition to acute kidney injury, impaired recovery from kidney injury, and kidney function decline both in the general population and among chronic kidney disease (CKD) patients [43-47]. Additionally, experimental studies in mouse models support a direct contribution of *TET2*-mutant CHIP to kidney dysfunction via inflammation-driven renal fibrosis [48]. However, in the specific setting of diabetes, a nested case-control study of 294 type 2 diabetes patients found no evidence of an association between CHIP and kidney function decline [49]. Larger studies are needed to conclusively assess the impact of CHIP on kidney function in diabetic patients, with a particular focus on the effects of specific mutated genes.

Beyond cardiovascular and kidney disease, CHIP has also been associated with an increased risk of other chronic conditions that are relatively common in diabetic patients, including non-alcoholic steatohepatitis [50], among others [4, 5]. Experimental studies in these various settings highlight

the central role of inflammation in mediating the link between CHIP and organ damage at multiple levels, which may be exacerbated by metabolic dysregulation in type 2 diabetes.

Metabolic alterations as potential modulators of the dynamics of CHIP

Some recent findings suggest that metabolic alterations common in type 2 diabetes, particularly in obese individuals, may accelerate mutant clone expansion, establishing a feedback loop that may enhance the effects of CHIP on insulin resistance, diabetes or related conditions. A longitudinal assessment of CHIP in 40 obese individuals found that clonal expansion rates correlated positively with insulin levels and insulin resistance, measured by HOMA-IR, and inversely with HDL-cholesterol levels [51]. Additionally, in the UK Biobank, CHIP, particularly *TET2*-mutant CHIP, is more frequent in individuals with high waist-to-hip ratios, an indicator of abdominal obesity [52]. Consistent with this, the expansion of TET2-deficient cells has been reported to be accelerated in a mouse model of genetically induced obesity [52]. In contrast, serial WES analyses from the ARIC study showed no significant association between body mass index or type 2 diabetes and incident CHIP or pre-existing clone expansion [53]. Similarly, longitudinal high-sensitivity analyses of CHIP dynamics in healthy middle-aged participants in the PESA study showed no effect of obesity or diabetes on the expansion rate of mutant hematopoietic clones [15]. Given these mixed findings and the limited longitudinal data currently available, additional studies are needed to clarify, particularly at the gene-specific level, whether obesity or related metabolic abnormalities can influence the expansion of mutant clones in CHIP.

Implications of CHIP for the personalized prevention of type 2 diabetes or its complications

In the era of precision medicine, the prospect of using CHIP to improve disease prevention is of great interest. While much work lies ahead, specialized CHIP clinics have already been established in several hospitals in the US and Europe to guide the management of individuals who have been found incidentally to exhibit CHIP [54]. However, although it can be expected that CHIP mutation carriers will benefit from closer monitoring, general CHIP screening is not yet recommended, given the lack of evidence-based interventions to mitigate the heightened risk of disease associated with these mutations. Nevertheless, ongoing research in both humans and mouse models is revealing potential avenues for personalized preventive care interventions that target specific CHIP-related pro-inflammatory pathways.

Current knowledge points to *TET2*-mutant CHIP and its connection with the IL-1β/NLRP3 proinflammatory pathway as a promising target. Several strategies targeting this pathway have been tested pre-clinically or clinically in the context of insulin resistance, type 2 diabetes and its vascular complications, with mixed results [26, 55-61]. While these mixed findings raise questions about the overall value of targeting this pathway for the prevention of diabetes, they do not preclude its potential as a targeted intervention tailored to *TET2* mutation carriers. Indeed, preclinical and clinical data strongly suggest that the beneficial effects of such anti-inflammatory approaches may be substantially greater in individuals with *TET2* mutations. Although still untested for diabetes-related outcomes, the protective effects of canakinumab, an IL-1β neutralizing antibody, for ischemic CVD were approximately 9-fold greater in *TET2* mutation carriers that in those lacking CHIP mutations in the CANTOS trial. Additionally, colchicine, a broad-spectrum anti-inflammatory agent that also inhibits IL-1β-driven inflammation, has been

suggested as a potential preventive agent for CVD in *TET2* mutation carriers [62], and may hold promise for type 2 diabetes prevention. While the effects of colchicine on the risk of diabetes in CHIP mutation carriers remain untested, retrospective studies suggest a reduced incidence of type 2 diabetes in gout patients treated with colchicine [63, 64], as well as a numerically lower, though non-significant, rate of new-onset type 2 diabetes in the LoDoCo2 clinical trial [65]. Beyond *TET2* mutations, specific potential therapeutic targets have also been identified for other CHIP-associated genes, such as the AIM2 inflammasome for *JAK2*-mutant CHIP [41] or IRAK1 for *ASXL1*-mutant CHIP [40]. As our understanding of the regulation and effects of CHIP mutations deepens, translating these findings into clinical practice will require rigorous prospective clinical trials.

Concluding remarks and future directions

The identification of CH, particularly CHIP, as a potential contributor to age-related diseases signals a promising yet still emerging area in type 2 diabetes research. Current evidence positions CHIP as a possible risk factor for type 2 diabetes and its complications, with certain CHIP mutations potentially contributing to type 2 diabetes pathogenesis in a causal manner through proinflammatory mechanisms. However, the still-limited body of genetic and experimental evidence highlights the need for further research. Looking ahead, continued investigation into the role of CHIP in type 2 diabetes and other age-related diseases could ultimately integrate CHIP into personalized prevention strategies, potentially transforming how we approach type 2 diabetes and its complications.

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Authors' relationships and activities

The authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

Contribution statement

Both authors were responsible for drafting the article and reviewing it critically for important intellectual content. Both authors approved the version to be published.

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Figures and legends

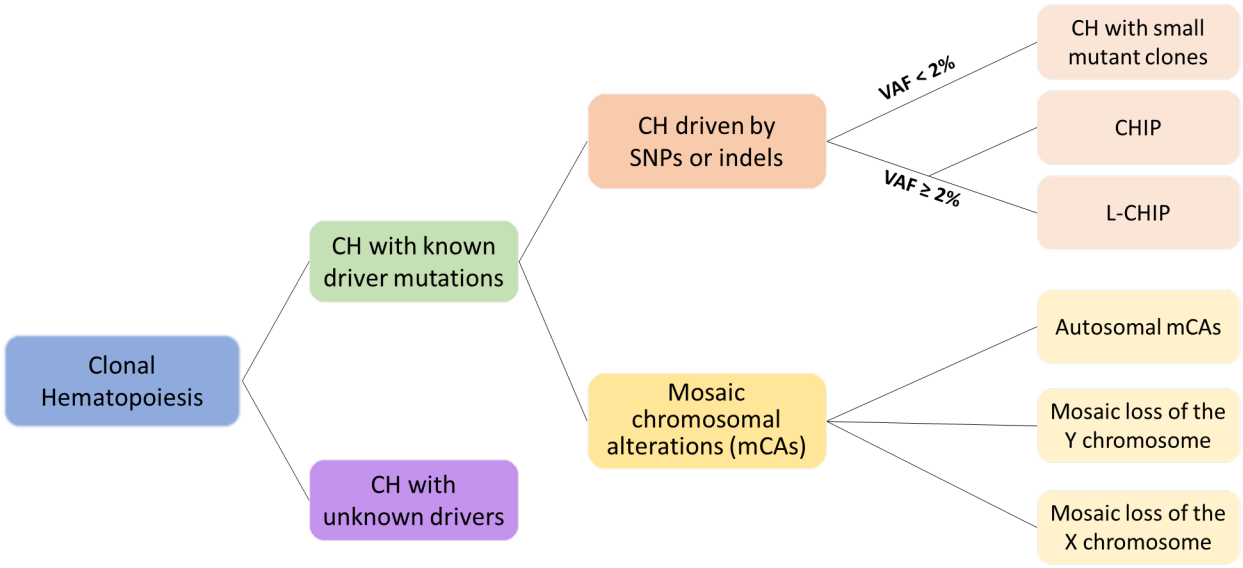
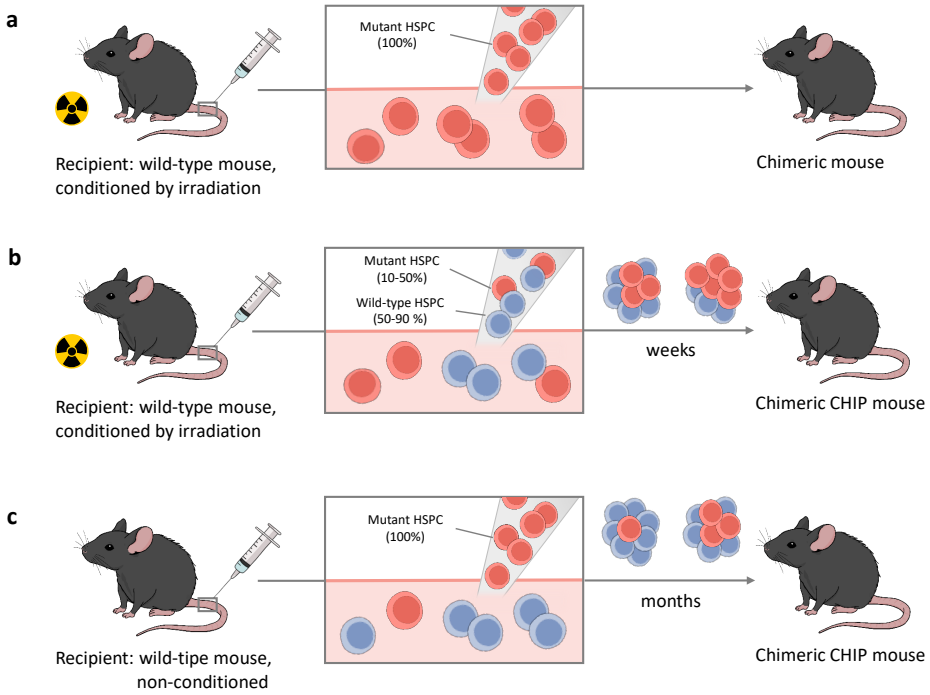


Figure 1. Spectrum and classification of clonal hematopoiesis. CH encompasses a broad range of conditions, frequently categorized based on the existence or absence of a known driver mutation and the characteristics of such driver mutation. CH with unknown drivers may arise from non-mutational mechanisms or by undetermined driver mutations. CH with known drivers can be subclassified further based on the type of driver mutation: small genetic alterations (i.e., single-nucleotide variants [SNVs] or indels) or variants affecting larger chromosomal regions (i.e., mosaic chromosomal alterations [mCAs]), which can affect either autosomes or sex chromosomes. CH driven by SNVs/indels is classified as clonal hematopoiesis of indeterminate potential (CHIP) when it is associated with mutations linked to myeloid hematological malignancies, the driver mutation has a variant allele frequency (VAF) ≥2% in bone marrow or blood, and the carrier has no major hematological abnormalities. Lymphoid CHIP (L-CHIP) is sometimes used to refer to CH driven by mutations linked to lymphoid malignancies with VAF ≥2%. CH can also occur with smaller mutant clones, but the clinical significance of these small clones remains to be fully elucidated.



525

526 **Figure 2. Approaches to model CHIP in mice.** CHIP is frequently modeled in mice using bone
527 marrow transplantation strategies with donor cells carrying murine surrogates of known CHIP
528 mutations in humans, either in heterozygosis or in homozygosis. Recipient mice may harbor
529 germline mutations and/or be exposed to specific dietary or environmental conditions to confer
530 susceptibility to particular disorders. Within this framework, several approaches can be used. **a.**
531 Conventional bone marrow transplantation. Donor bone marrow cells from mutant or wild-type
532 mice are transplanted into recipient mice following irradiation to ablate their native bone marrow.
533 This approach is useful to detect subtle effects of CHIP mutations, but does not replicate the
534 competition between mutant and non-mutant cells that is characteristic of CHIP. **b.** Competitive
535 bone marrow transplantation. A fraction of donor cells with a CHIP mutation is combined with
536 non-mutant cells and transplanted into irradiated recipient mice. This model mimics better the
537 human scenario of CHIP, enabling the investigation of whether mutant cells have specific
538 advantages in infiltrating or expanding within the bone marrow or other tissues.
539 **c.** Non-conditioned bone marrow transplantation or adoptive transfer. Relatively high numbers of
540 mutant bone marrow cells are injected into recipient mice without prior irradiation or conditioning.
541 This approach avoids the side effects of conditioning, but typically results in low baseline
542 chimerism (1–5% of white blood cells) and slow mutant cell expansion. It is preferred for long-
543 term studies or investigations focused on the dynamics of mutant cell expansion. HSPC:
544 hematopoietic stem and progenitor cells.

Box. Main strategies to detect clonal hematopoiesis-related mutations

- **Single nucleotide polymorphism (SNP) arrays** enable the detection of mosaic chromosomal alterations [17], but not clonal hematopoiesis driven by single nucleotide variants (SNVs) or small insertions/deletions (indels), such as clonal hematopoiesis of indeterminate potential (CHIP) mutations**.**
- **Whole genome sequencing (WGS)** enables the detection of both mosaic chromosomal alterations [18] and clonal hematopoiesis driven by SNVs or small indels, including CHIP mutations [13]. WGS at 30X sequencing depth can reliably detect most mutations with variant allele fractions (VAFs) ≥10% [13, 15]. Additionally, WGS may be used to estimate the expansion rate of mutant clones using data from a single time point [66].
- **Whole exome sequencing (WES)** enables the detection of clonal hematopoiesis driven by SNVs or small indels, including CHIP mutations. WES at 100X sequencing depth can reliably identify most mutations with VAF≥5% [13, 15].
- **Targeted gene sequencing** focuses on a predefined set of candidate genes, enabling detection of clonal hematopoiesis driven by SNVs or small insertions/deletions in these genes, including CHIP mutations. The typically higher sequencing depths in targeted sequencing allow reliable detection of mutations with VAF≥2%, or even lower when very deep sequencing and error correction techniques are employed [15, 22].