IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence

AUTHER- BALKRUSHNA PRAKASH JANGAM, ASST. PROF. DR. RAJANDRA.M. KAWADE.
NANDKUMAR SHIND COLLEGE OF PHARMACY

➢ Abstract

Multiple acquired mutations, most likely over many years, are the cause of cancers. Years before tumors manifest clinically, they may be in the early stages of development. Cancer cells from primary tumors are often carried through the bloodstream to other parts of the body, where they may form metastases. However, many of these cells are destroyed by the blood or tissues, preventing further spread. Advanced cancer cases frequently show metastases, with tumor cells found in blood vessels or invading their walls. Schmidt's 1903 study of lung tissues in advanced cancer patients found tumor emboli in the pulmonary arteries in 15 out of 41 cases. Similarly, Quensel observed a tumor embolus in the heart's right auricle in a case of stomach cancer. Blood vessel invasion is a diagnostic criterion for certain cancers, such as thyroid carcinoma.

Key words:- Multiple acquired mutations, BLOODSTREAM, frequently,pulmonary

Data from whole-exome sequencing of DNA in peripheral blood cells from 12,380 individuals who were not selected for hematologic characteristics or malignancy were examined. Unusual allelic fractions were used to identify somatic mutations. We tracked health outcomes for two to seven years following DNA sample using information from Swedish national patient records

➢ RESULTS

Ten percent of people over 65 had clonal hematopoiesis with somatic mutations, but just one percent of people under 50 had this condition. Somatic mutations in three genes (DNMT3A, ASXL1, and TET2) that have been linked to hematologic malignancies in the past were the most common cause of detectable clonal expansions. A significant risk factor for later hematologic malignancy was clonal hematopoiesis (hazard ratio: 12.9; 95% CI: 5.8–28.7). among this cohort, about 42 percent of hematologic malignancies developed among individuals who were clonal at the time of DNA sample, which was more than six months prior to a cancer diagnosis. Bone marrow biopsy samples from two patients who were diagnosed with acute myeloid leukemia were analyzed, and the results showed that their tumors developed from the clones that came before.

➢ CONCLUSIONS

DNA sequencing may easily identify clonal hematopoiesis with somatic mutations, which is becoming more prevalent as people age and linked to a higher risk of hematopoietic malignancy and death. These mutations may be typical early events in the development of hematopoietic cancers. A subset of the genes that are mutated in individuals with myeloid tumors are often mutated in people who appear to be in good health. The National Human Genome Research Institute and other donors provided funding. Dynamic mechanisms that start years or decades before the clinical onset are frequently involved in the genesis of disease. However, the pathogenesis process is frequently not identified until the patient exhibits symptoms and a clinically evident illness. Multiple somatic mutations, which are likely to have been acquired at separate times, work together to cause cancer.One Many years before a disease manifests, early mutations may exist. Early mutations cause stem cells or other progenitor cells to expand clonally in certain cancer formation models.2. The probability that later, collaborating mutations will occur in cells that already carry the earlier, initiating mutations is significantly increased by such clonal expansions. In order to comprehend how proliferative illnesses are caused, it's critical tounderstand how much clonal expansions occur and how they come before cancer. Many hematologic malignancies, such as myeloproliferative neoplasms, myelodysplastic syndromes, acute myeloid leukemia (AML), chronic lymphocytic leukemia, and others, may be preceded by hematopoietic stem-cell population dynamics.7. For instance, in certain individuals, stem cells that possess a subset of the mutations found in the cancer cells can withstand chemotherapy; these cells then develop new mutations, which leads to a relapse.8–10 This implies that there might have been a clonally enlarged stem-cell population prior to the development of the malignancy. About 2% of older people appear to have clonal mosaicism, which is characterized by substantial chromosomal anomalies that represent the proliferation of a particular cellular clone. This condition is associated with a higher risk of later hematopoietic cancers.11–15 In theory, a process known as “clonal hematopoiesis,” or clonal proliferation among hematopoietic stem cells, may be far more frequent16 and only infrequently accompanied with chromosomal abnormalities. Nowadays, thousands of people’s blood-derived DNA is sequenced in numerous studies to find inherited risk factors for prevalent diseases. Such information, we reasoned, provided a chance to verify the notion that somatic mutation-driven clonal expansions are frequent and frequently precede blood malignancies, as well as to pinpoint the genes where mutations cause clonal expansions.

➢ Methods

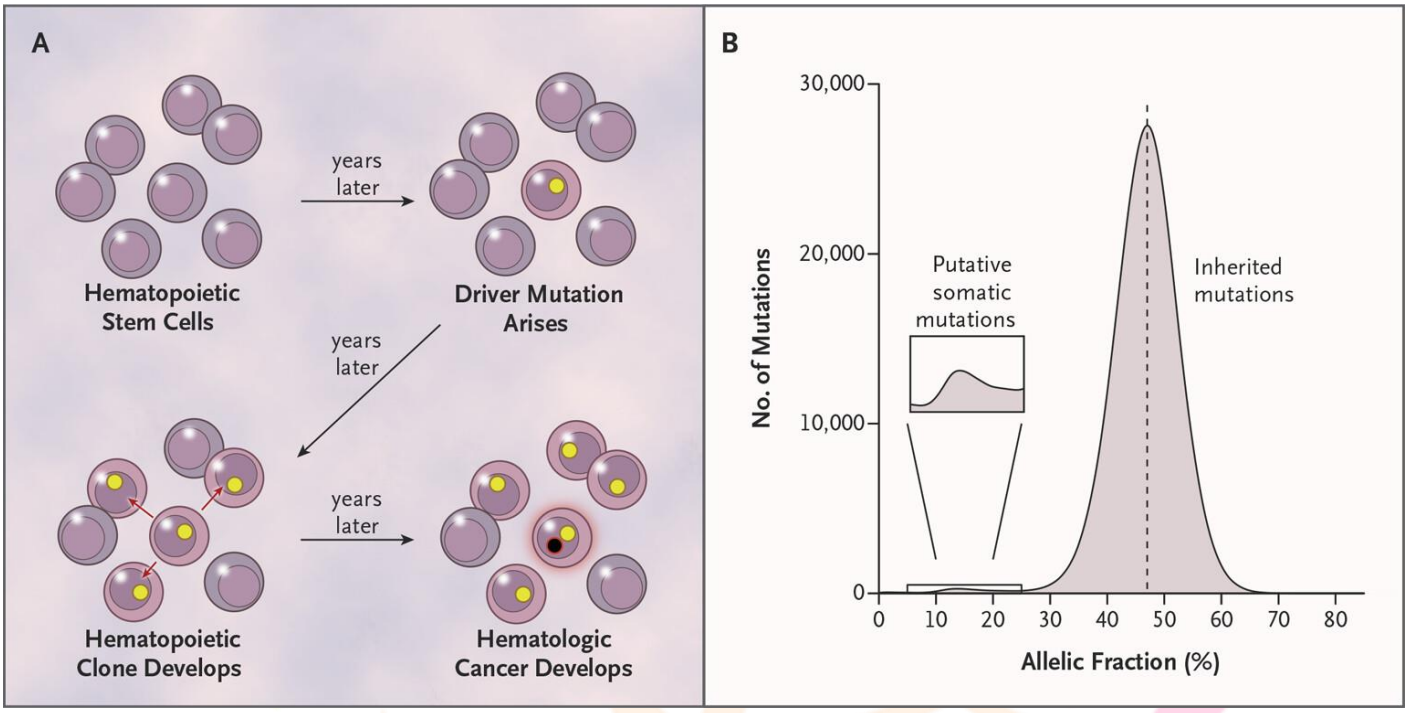
Participants in the Study and Whole-Exome Sequencing of DNA Derived from Blood

12,380 Swedish individuals (mean age at sample collection: 55 years [range: 19 to 93]) provided blood samples for DNA sequencing (refer to Fig. S1 in the Supplementary Appendix, which is accessible at NEJM.org along with the article's complete text). According to Table S1 in the Supplementary Appendix, this cohort comprised 6245 controls, 4970 individuals with schizophrenia, 17, 18, and 1165 individuals with bipolar illness. Since none of the mutational variables examined had any discernible correlation with psychiatric diagnosis once we adjusted for other variables like age and smoking status, we considered case patients and controls as a single cohort for all analyses shown below. Direct extraction of DNA was performed from peripheral venous blood samples. The appropriate ethics committees gave their approval to every procedure, and the appropriate ethical committees, and each participant provided written informed consent. The Supplementary Appendix provides a detailed description of DNA sequencing and analysis techniques.

Finding Somatic Mutations

Based on allelic fractions, we devised a method for detecting somatic mutations. We projected that the mutant allele would be present in less than 50% of the sequencing reads originating from that genomic region,

assuming that a somatic mutation would only be found in a portionof the cells from which we extracted DNA for study (Figure 1A and 1B). In the Supplementary Appendix, we provide a detailed description of this analytical methodology.



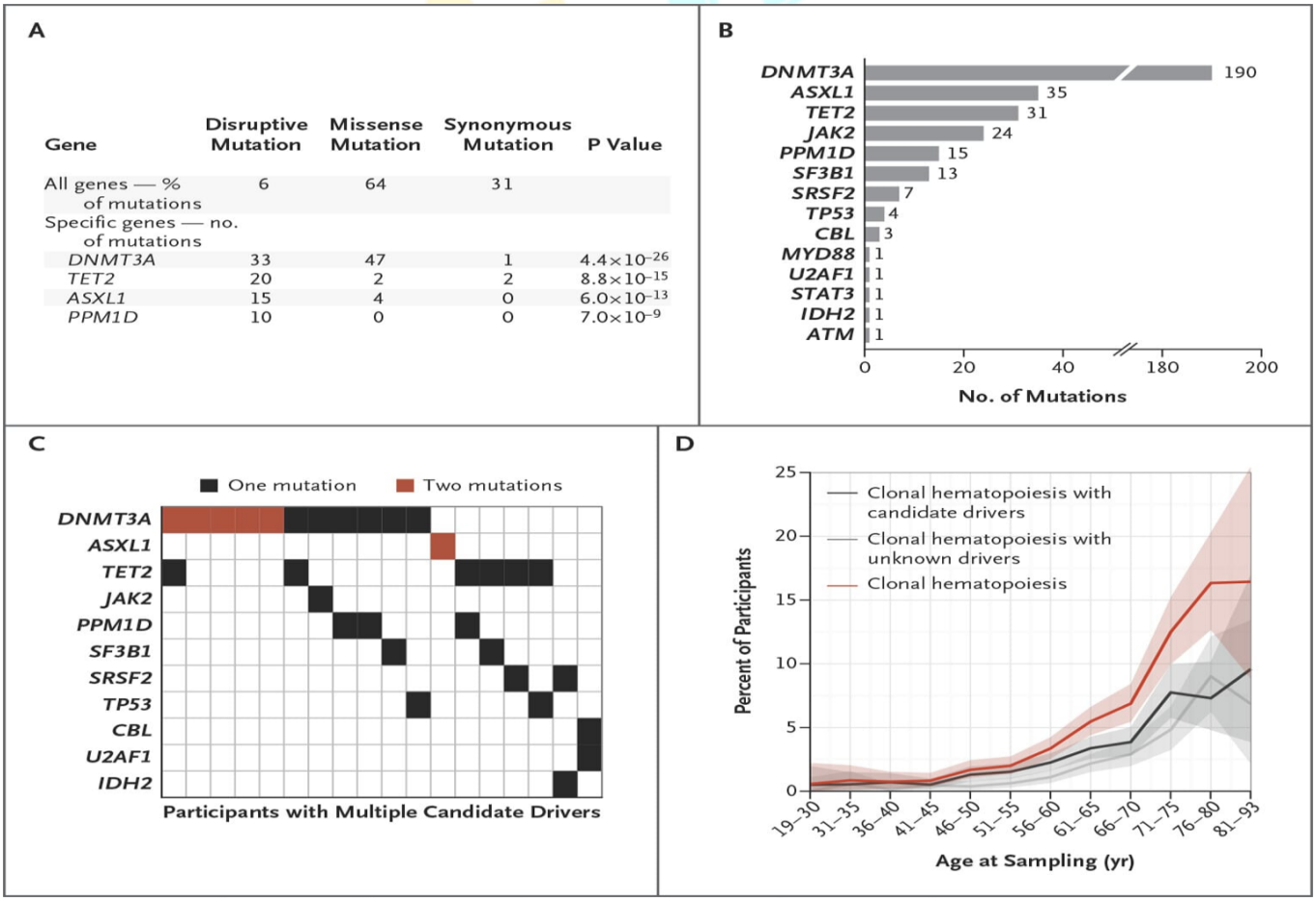
Examinations of Cancers and Later Medical Results

From 1965 to 2012, 11,164 participants' medical histories were taken from the Swedish national cause-of-death, inpatient, and outpatient registers. For two to seven years following DNA sample, we used these data to track health outcomes. Bone marrow biopsy specimens (obtained at the time of diagnosis) were subjected to whole-exome and whole-genome sequencing for two participants who were diagnosed with cancer two and thirty-four months after the initial DNA sample.

➢ Results

Clonal Hematopoiesis: Typical Mutations and Potential Drivers

Based on the occurrence of anomalous allelic fractions, we investigated whole-exome sequencing data from 12,380 individuals and found 3111 putative somatic mutations, indicating a frequency of roughly one putative somatic mutation every four participants. Molecular validation verified that the mutant allele was present at a low allelic fraction (<50%) for all 65 mutations evaluated, indicating that it could not have been inherited (Fig. S7 in the Supplementary Appendix). Most of the mutations were scattered throughout the genome. However, a disproportionately high number of somatic mutations were found in four genes: DNMT3A, TET2, ASXL1, and PPM1D. The somatic mutations found in DNMT3A, TET2, ASXL1, and PPM1D had a strong propensity to disrupt gene protein-coding sequences by introducing a frame-shift, nonsense, or splice-site disruption, even though over 94% of the mutations found throughout the genome were missense and synonymous changes (Figure 2A). These mutations are also frequently found in hematologic cancers19–21, and three of these four genes—DNMT3A, TET2, and ASXL1—are thought to act as epigenetic regulators.22



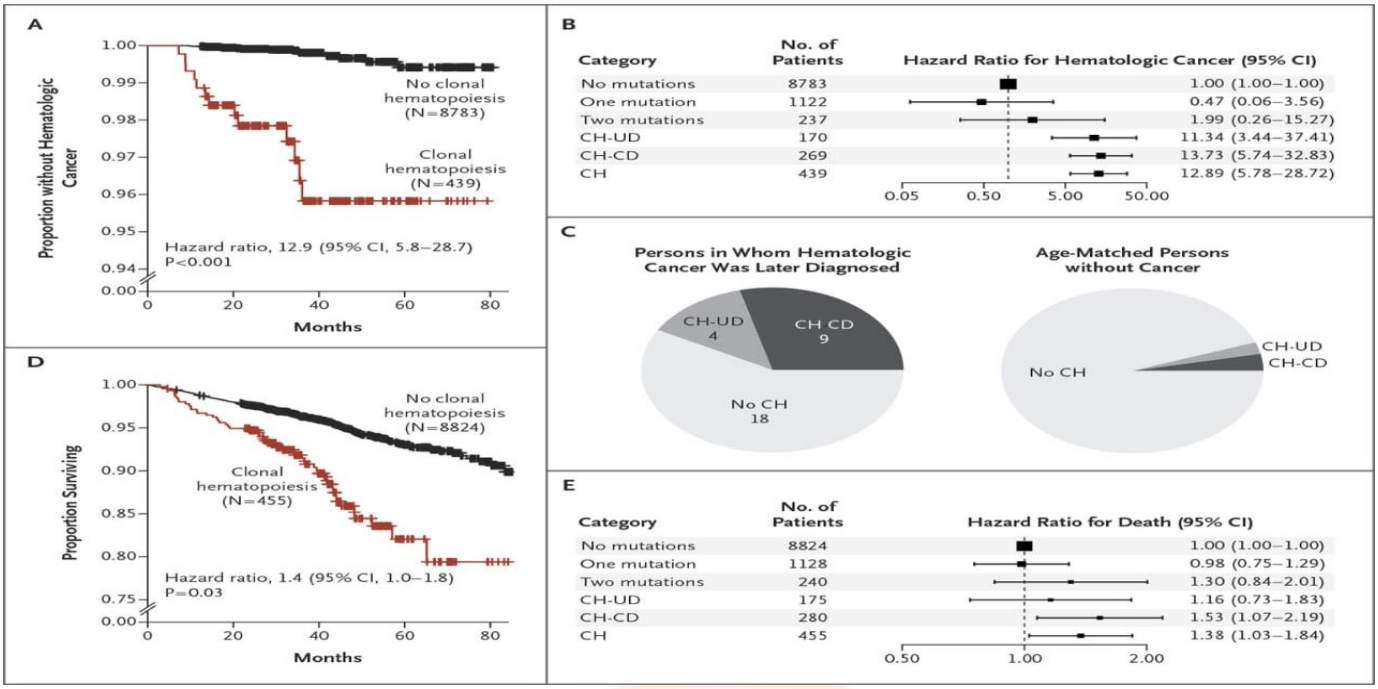
In nonhematologic malignancies, mutations in PPM1D, a protein that regulates the tumor-suppressor protein p53,23 have been reported more frequently. Twelve of the fifteen protein-truncating mutations found in PPM1D were found in the final exon, which is also where protein-truncating mutations found in cancer patients are found.24–27 It has been observed that PPM1D activation and p53 repression, which affects the p53-dependent G1 checkpoint and promotes proliferation, occur when the C-terminal localization domain of PPM1D is lost.26 Additionally, somatic mutations in DNMT3A were enriched for cysteine-forming changes (Fig. S9 in the Supplementary Appendix) and exhibited a large excess of missense mutations (P<0.001) (Figure 2A), all of which were located in exons 7 to 23. The tetrameric DNMT3A protein complex may be affected by such mutations in a dominant-negative manner (for more information, see the Supplementary Appendix). We reasoned that other recurrent cancer mutations would similarly encourage clonal hematopoiesis because DNMT3A, TET2, and ASXL1 are commonly mutated in hematologic cancers28,29. Therefore, we took into

account 208 distinct variations that were known to be recurring (discovered in ≥7 patients) in lymphoid and hematological malignancies, as documented in the Catalogue of Somatic Mutations in Cancer30 (for more information, see the Supplementary Appendix). Among the 98 cases of these recurrent mutations, we discovered that 24 patients had the JAK2 mutation p.V617F31, 15 participants had the DNMT3A mutation p.R882H32, and 9 participants had the SF3B1 mutation p.K700E33. A set of 327 candidate driver somatic mutations for clonal hematopoiesis in 308 participants (Figure 2B, and Table S3 in the Supplementary Appendix) were caused by disruptive mutations in DNMT3A, TET2, ASXL1, and PPM1D, missense mutations in DNMT3A, and recurrent cancer-associated mutations. Of these, 18 participants carried multiple candidate drivers (Figure 2C). The most mutations were found in DNMT3A (190), ASXL1 (35) and TET2 (31). For technical purposes, mutations in ASXL1 and TET2 were most likely undercounted. explanations (see Supplementary Appendix Figs. S6B and S6C).

Hematopoiesis Clonal with Unidentified Drivers

The presence of a clone may be indicated by somatic mutations that are either drivers, which aid in clonal expansion, or passengers, which do not. Individuals with candidate drivers in their clonal hematopoiesis tended to have more putative somatic mutations than those without (mean number of extra mutations, 1.5 vs. 0.2; $P<0.001$ after age correction) (Fig. S11A in the Supplementary Appendix). Multiple putative somatic mutations were found in 459 subjects, none of whom had any of the above-mentioned probable drivers. Pairs of somatic mutations seen in the same individual had estimates of the allelic fraction that were more comparable than pairs of somatic mutations seen in different people ($P<0.001$ by the Mann-Whitney test), which is consistent with the probability that the mutations were present. within the identical clone. We aimed to establish a highly specific criterion for clonal hematopoiesis that was based solely on the quantity of mutations rather than the type of mutations in order to account for situations of clonal hematopoiesis without evident driver alterations. 9927 persons had no putative somatic mutations, 1333 had one mutation, 313 had two, and 272 had three to eighteen mutations out of 11,845 participants with sequencing data of high enough quality to detect somatic mutations. This distribution suggested that the unexpectedly large proportion of subjects having 3–18 identifiable mutations could not be explained by a random (Poisson) process (with a constant mean). We identified 195 patients as having clonal hematopoiesis with unknown causes in the analyses that follow. In certain clonal hematopoiesis situations with unclear causes) was more pronouncedly age-dependent, occurring in 4.6% of individuals over 65 but only 0.3% of those under 50 (Figure 2D). With potential drivers, this age trajectory is comparable to that of clonal hematopoiesis. Overall, 10.4% of persons over 65 had clonal hematopoiesis with candidate or unknown causes, compared to 0.9% of participants under 50 (Figure 2D). The average number of putative somatic mutations found in persons with clonal hematopoiesis similarly rose with age ($P<0.001$ by linear regression) (Fig. S14 in the Supplementary Appendix).

Clonal Hematopoiesis and Subsequent Hematologic Cancer and Death Compared to participants without detectable putative somatic mutations, those with clonal hematopoiesis had a significantly higher chance of receiving a first diagnosis of hematologic cancer six months or more after DNA sampling (Table S6 in the Supplementary Appendix) (hazard ratio, 12.9; 95% CI], 5.8 to 28.7; P<0.001 by Cox proportional-hazards analysis of time to a diagnosis of hematologic cancer, with adjustment for age and sex) (Figure 3A). Risks were similarly higher for participants with unknown drivers and those with potential drivers (Figure 3B). Out of the 31 individuals who were diagnosed with hematologic cancer, more Thirteen subjects (42%) had clonal hematopoiesis in their original DNA sample more than six months following DNA sampling (Figure 3C). Although it did not influence the preceding conclusion, participants with clonal hematopoiesis were also more likely to have a history of hematologic malignancy than those without (Table S7 in the Supplementary Appendix).

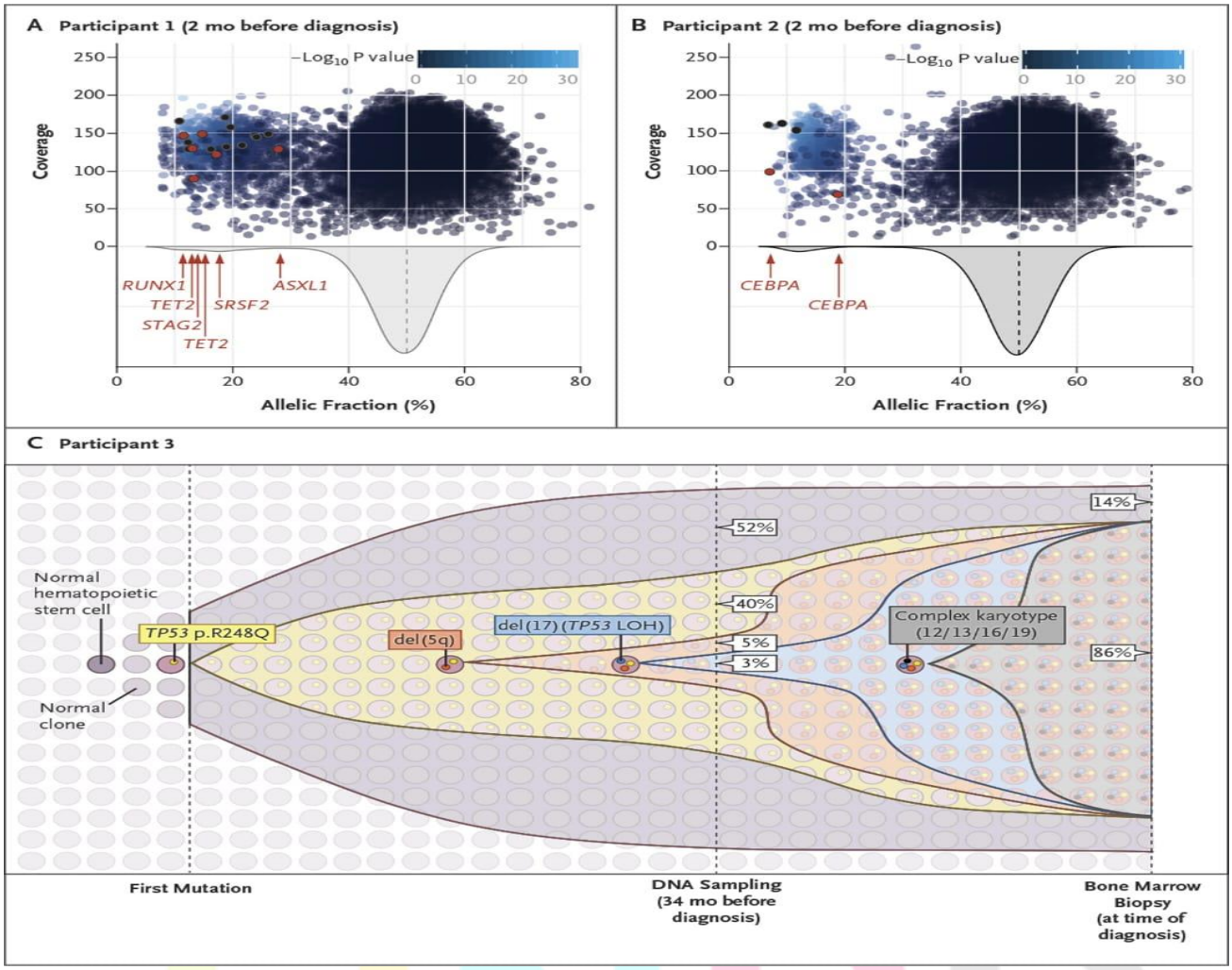


MALIGNANT CLONES IN DNA SAMPLES

Two months after DNA sample, two clonal hematopoiesis participants were diagnosed with myeloid malignancy. We postulated that the clone identified by whole-exome sequencing analysis may have been the preclinical malignant clone (for more information, see the Supplementary Appendix). The preclinical blood DNA sample was subjected to whole-genome sequencing analysis, which showed 660 putative somatic mutations in Participant 2 and 1153 putative somatic mutations at a characteristic frequency in Participant 1 (Figure 4A and 4B). This was consistent with the number of mutations found in AML genomes and provided evidence that a clone had amplified from a single cell.29 Numerous known harmful mutations were found at the clone's distinctive allele frequency, according to the whole-genome sequencing data. (Figures 4A and 4B). Participant 1 had mutations in RUNX1, STAG2, SRSF2, and TET2 at the allelic fractions typical of the clone

two months prior to being diagnosed with myelodysplastic syndrome. Additionally, Participant 1 had an ASXL1 mutation at a slightly higher allelic fraction, which may have been consistent with a founder mutation. In myelodysplastic syndromes, mutations in ASXL1, RUNX1, and STAG2 frequently co-occur.35 Two months prior to being diagnosed with AML, Participant 2 had two mutations in CEBPA: a frame-shift N-terminal deletion and an in-frame 33-bp insertion. In AML, these two kinds of CEBPA mutations commonly co-occur.36

Genetic Relationship of Leukemia to Earlier Clones



[11/26, 12:25 PM] Balkrushna Jangam: Two participants had bone marrow-biopsy specimens taken at the time of diagnosis: Participant 2, who had the two CEBPA mutations, and Participant 3, who was diagnosed with AML 34 months after DNA sampling (for more information, see the Supplementary Appendix). The presence of the mutations found in the previous DNA sample, including the two CEBPA mutations and the three passenger mutations now seen at higher allelic fractions (20.5% vs. 15.5% two months earlier; estimate based on the three single-nucleotide substitutions), was confirmed by analysis of the specimen taken from Participant 2. The prognosis for cancers with pairs of CEBPA mutations is generally favorable37, and this patient's cancer did not recur after chemotherapy and was completely remitted. [11/26, 12:27 PM] Balkrushna Jangam: It seemed that Participant 3's hematologic malignancy was a subclone of the original clone that had undergone further clonal evolution during the previous 34 months. The loss of the other copy of chromosome 17 and the bone marrow–biopsy specimen's 86% blast count were both compatible with a TP53 p.R248Q mutation that had grown from its original allelic fraction of 24% to 86%. DNA examination of the biopsy samples revealed a complicated pattern of gains and losses on chromosomes 12, 13, 16, and 19, as well as losses on chromosomes 17 and 5q (results that were in line with the karyotype findings) (Fig. S15 in the Supplementary Appendix).[11/26, 12:28 PM] Balkrushna Jangam: People with TP53 mutations frequently have losses of 17

and 5q.38 We calculated that there were already losses of 5q and 17 at low allelic fractions (8% and 3% of cells, respectively) in the original DNA sample, but no losses on chromosomes 12, 13, 16, and 19, or losses at an undetectable frequency at that time (Fig. S16 in the Supplementary Appendix), using these segmental losses to differentiate between alleles on the lost segments and alleles on the retained segments. We deduced that these mutations originated in a sequence of subclones since the biopsy samples displayed all of these events at high allelic fractions (Figure 4C). Two months after the diagnosis, the patient passed away.

- **Discussion**..

[11/26, 12:30 PM] Balkrushna Jangam: Ten percent of the elderly study participants had clonal hematopoiesis with somatic mutations, and the incidence increased with age (Figure 2D). Driver genes and mutations that are also driver mutations in hematologic malignancies seemed to be involved in the majority of these clonal expansions (Figure 2A and 2B). We discovered that having such clones increased the chance of death (hazard ratio, 1.4; 95% CI, 1.0 to 1.8) and subsequent hematologic malignancies (hazard ratio, 12.9; 95% CI, 5.8 to 28.7) (Figure 3A and 3B) (Figure 3D and 3E).[11/26, 12:32 PM] Balkrushna Jangam: Mutations in a particular group of the genes known to be responsible for blood cancers22, including DNMT3A, ASXL1, and TET2, seemed to be involved in the majority of clonal hematopoiesis cases (Figure 2A). These subclinical clonal expansions did not show activating mutations in FLT3 or NPM1 29, two other prominent mutational drivers of such malignancies. These data imply that mutations in FLT3 and NPM1 may typically be later, cooperative occurrences, whereas mutations in DNMT3A, ASXL1, and TET2 are frequentl initiating mutations that persist in subclinical states for extended periods of time. Data from studies involving cancer patients and mice models would support such an inference.12–14[11/26, 12:34 PM] Balkrushna Jangam: According to functional experiments, loss of TET2 causes hematopoietic stem cells to self-renew more frequently and gain a competitive growth advantage, while loss of DNMT3A hinders the differentiation of hematopoietic stem cells, increasing their number in the bone marrow39.40 42% of the patients who were diagnosed with cancer more than six months later had clonal hematopoiesis, which we found to be a significant risk factor for these tumors (Figure 3C). Our findings prompt the question of whether routine blood sample DNA sequencing could facilitate the early identification of blood malignancies. We think that such an approach would be premature based on the available facts. Somatic mutations in clonal hematopoiesis were found.[11/26, 12:34 PM] Balkrushna Jangam: Furthermore, there aren't any therapies on the market right now that look appropriate for sizable patient cohorts with a low risk of developing cancer. Although our data indicate that such findings may be prevalent, they do not support a diagnosis of hematologic cancer; rather, they indicate an enhanced risk. Therefore, caution is also necessary when cancer-associated mutations are discovered as an incidental finding in other studies or diagnostic procedures.[11/26, 12:36 PM] Balkrushna Jangam: However, in the future, we might be able to improve our DNA analysis to provide methods for hematopoietic cancer prevention and early diagnosis. The ability to identify high-risk states, track the development or remission of these states, and identify follow-on, transformative mutations prior to clinically evident illness are three significant capacities that DNA analysis will provide. A number of significant research avenues may advance the clinical utility of DNA sequencing for clonal hematopoiesis. First, larger studies could find somatic mutations that are likely to be linked to a heightened chance of developing cancer later on. Second, single-cell analysis could reveal combinations of mutations that are high-risk for[11/26, 12:37 PM] Balkrushna Jangam: identical cells. Third, mutation–cell-type combos with higher predictive value may be found through the sequencing of particular cell types. Fourth, clonal hematopoiesis may warrant routine screening for cooperative mutations at low allele frequencies that may be predictive of malignancy (Figure 4C). To collect the data required to assess these possibilities, larger research will be required. Furthermore, as described here, the use of DNA sequencing to track clonal expansions and identify at-risk groups may make it easier to conduct clinical trials of methods to lower the risk of cancer development. In other medical specialties, such In the development of preventive medicines for cardiovascular disease, biomarkers (such as low-density lipoprotein cholesterol) that identify high-risk patient groups and provide quick information on the effectiveness of interventions have proven essential. Understanding the degree to which clonal hematopoiesis serves as a marker for the deteriorating

health of hematopoietic stem-cell populations—possibly due to aging, attrition, and a decreased capacity to contain emerging neoplasms—will also be crucial.These results demonstrate how DNA sequencing provides insights into dynamic processes that evolve throughout an individual's life and are indicative of both clinical illness and mortality. We hypothesize that a continuum of ascertainable genomic states linked to increased risks of future illness may eventually replace the conventional dichotomy between illness and health in asymptomatic individuals with an improved capacity to forecast future harm.

➢ REFERENCES

1. Nowell, PC. The clonal evolution of tumor cell populations. Science 1976;194:23-282.

2. Reya, T, Morrison, SJ, Clarke, MF, Weissman, IL. Stem cells, cancer, and cancer stem cells. Nature 2001;414:105-1113.

3. Jamieson, CHM, Gotlib, J, Durocher, JA, et al. The JAK2 V617F mutation occurs in hematopoietic stem cells in polycythemia vera and predisposes toward erythroid differentiation. Proc Natl Acad Sci U S A 2006;103:6224-6229

4. Jaiswal, S, Ebert, BL. MDS is a stem cell disorder after all. Cancer Cell 2014;25:713-714

5. Potter, NE, Greaves, M. Cancer: persistence of leukaemic ancestors. Nature 2014;506:300-301

6. Vasanthakumar, A, Godley, LA. On the origin of leukemic species. Cell Stem Cell 2014;14:421-422

7. Damm, F, Mylonas, E, Cosson, A, et al. Acquired initiating mutations in early hematopoietic cells of CLL patients. Cancer Discov 2014;9:1088-1101

8. Ding, L, Ley, TJ, Larson, DE, et al. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. Nature 2012;481:506-510

9. Shlush, LI, Zandi, S, Mitchell, A, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature 2014;506:328-333

10. Corces-Zimmerman, MR, Hong, W-J, Weissman, IL, Medeiros, BC, Majeti, R. Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. Proc Natl Acad Sci U S A 2014;111:2548-2553
11. Forsberg, LA, Rasi, C, Razzaghian, HR, et al. Age-related somatic structural changes in the nuclear genome of human blood cells. Am J Hum Genet 2012;90:217-228

12. Laurie, CC, Laurie, CA, Rice, K, et al. Detectable clonal mosaicism from birth to old age and its relationship to cancer. Nat Genet 2012;44:642-650

13. Jacobs, KB, Yeager, M, Zhou, W, et al. Detectable clonal mosaicism and its relationship to aging and cancer. Nat Genet 2012;44:651-658

14. Schick, UM, McDavid, A, Crane, PK, et al. Confirmation of the reported association of clonal chromosomal mosaicism with an increased risk of incident hematologic cancer. PLoS One 2013;8:e59823-e59823

15. Forsberg, LA, Rasi, C, Malmqvist, N, et al. Mosaic loss of chromosome Y in peripheral blood is associated with shorter survival and higher risk of cancer. Nat Genet 2014;46:624-628

16. Busque, L, Mio, R, Mattioli, J, et al. Nonrandom X-inactivation patterns in normal females: lyonization ratios vary with age. Blood 1996;88:59-65

17. Ripke, S, O'Dushlaine, C, Chambert, K, et al. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 2013;45:1150-1159

18. Purcell, SM, Moran, JL, Fromer, M, et al. A polygenic burden of rare disruptive mutations in schizophrenia. Nature 2014;506:185-190

19. Ley, TJ, Ding, L, Walter, MJ, et al. DNMT3A mutations in acute myeloid leukemia. N Engl J Med 2010;363:2424-2433

20. Delhommeau, F, Dupont, S, Della Valle, V, et al. Mutation in TET2 in myeloid cancers. N Engl J Med 2009;360:2289-2301

21. Russler-Germain, DA, Spencer, DH, Young, MA, et al. The R882H DNMT3A mutation associated with AML dominantly inhibits wild-type DNMT3A by blocking its ability to form active tetramers. Cancer Cell 2014;25:442-454Gelsi-Boyer, V, Trouplin, V, Adelaide, J, et al. Mutations of polycomb-associated gene ASXL1 in myelodysplastic syndromes and chronic myelomonocytic leukaemia. Br J Haematol 2009;145:788-800

22. Shih, AH, Abdel-Wahab, O, Patel, JP, Levine, RL. The role of mutations in epigenetic regulators in myeloid malignancies. *Nat Rev Cancer* 2012;12:599-612

23. Chuman, Y, Kurihashi, W, Mizukami, Y, Nashimoto, T, Yagi, H, Sakaguchi, K. PPM1D430, a novel alternative splicing variant of the human PPM1D, can dephosphorylate p53 and exhibits specific tissue expression. *J Biochem (Tokyo)* 2009;145:1-12

24. Ruark, E, Snape, K, Humburg, P, et al. Mosaic PPM1D mutations are associated with predisposition to breast and ovarian cancer. Nature 2013;493:406-410

25. Kleiblova, P, Shaltiel, IA, Benada, J, et al. Gain-of-function mutations of PPM1D/Wip1 impair the p53-dependent G1 checkpoint. J Cell Biol 2013;201:511-521

26. Akbari, MR, Lepage, P, Rosen, B, et al. PPM1D mutations in circulating white blood cells and the risk for ovarian cancer. J Natl Cancer Inst 2014;106:djt323-djt323

27. Zhang, L, Chen, LH, Wan, H, et al. Exome sequencing identifies somatic gain-of-function PPM1D mutations in brainstem gliomas. Nat Genet 2014;46:726-73

28. The Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med 2013;368:2059-2074[Erratum, N Engl J Med 2013;369:98.]

29. Lawrence, MS, Stojanov, P, Mermel, CH, et al. Discovery and saturation analysis of cancer genes across 21 tumour types. Nature 2014;505:495-501

30. Forbes, SA, Bindal, N, Bamford, S, et al. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Res 2011;39:D945-D950

31. Kralovics, R, Passamonti, F, Buser, AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 2005;352:1779-1790

32. Russler-Germain, DA, Spencer, DH, Young, MA, et al. The R882H DNMT3A mutation associated with AML dominantly inhibits wild-type DNMT3A by blocking its ability to form active tetramers. *Cancer Cell* 2014;25:442-454

33. Papaemmanuil, E, Cazzola, M, Boultwood, J, et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. *N Engl J Med* 2011;365:1384-1395

34. Campbell, IM, Yuan, B, Robberecht, C, et al. Parental somatic mosaicism is underrecognized and influences recurrence risk of genomic disorders. Am J Hum Genet 2014;95:173-182

35. Chen, T-C, Hou, HA, Chou, WC, et al. Dynamics of ASXL1 mutation and other associated genetic alterations during disease progression in patients with primary myelodysplastic syndrome. Blood Cancer J 2014;4:e177-e177

36. Fasan, A, Haferlach, C, Alpermann, T, et al. The role of different genetic subtypes of CEBPA mutated AML. Leukemia 2014;28:794-803

37. Martelli, MP, Sportoletti, P, Tiacci, E, Martelli, MF, Falini, B. Mutational landscape of AML with normal cytogenetics: biological and clinical implications. Blood Rev 2013;27:13-22

38. Kulasekararaj, AG, Smith, AE, Mian, SA, et al. TP53 mutations in myelodysplastic syndrome are strongly correlated with aberrations of chromosome 5, and correlate with adverse prognosis. Br J Haematol 2013;160:660-672

39. Challen, GA, Sun, D, Jeong, M, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. Nat Genet 2012;44:23-31

40. Busque, L, Patel, JP, Figueroa, ME, et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. Nat Genet 2012;44:1179-1181