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Clonal Hematopoiesis as the Fundamental Mechanism to Launch Molecular-Biological Events Leading to Neurodegenerative Diseases of the Humans and as a Promising Target for their Therapy

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Abstract

The goal of this work is to detect clonal hematopoiesis in the onset of Neurodegenerative Diseases (NDD) of the humans and to test the hypothesis that the NDDs are initiated and supported by the genomic damage and pathology-specific clones of Hematopoietic Stem Cells (HSCs). The trial included 20 cases of various NDDs including Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis (ALS) and others. The DNA was isolated from the HSCs of bone marrow and lymphocytes of the peripheral blood and tested by the method of New Generation Sequencing (NGS). Targeted paired-end exome sequencing of 22,000 genes was conducted to search for genetic polymorphisms specific to NDD and to identify Additional Somatic Mutations (ASMs) in clonality genes and stemness genes. Germ cell polymorphism, indicating a hereditary genesis of the disease, was detected in only one patient with NDD; all other diseases had a sporadic genesis. In all cases of progressing NDD, various mutations of clonality genes were identified, which indicates Clonal Hematopoiesis (CH). 123 ASMs were identified, of which 114 mutations were nucleotide substitutions of clonality genes and 9 mutations of stemness genes. If the total number of ASMs clonality genes is taken as 100%, then in patients with NDD, 18 types of mutations of the AKT1 gene clonality gene (16.5%), 16 types of mutations of the ASXL1 gene (14.67%), 20 types of mutations of the CBL gene (18.3%), 17 types of JAK2 gene mutations (15.6%), 12 types of PTEN gene mutations (11%), 1 type of PPM1D gene mutation (0.9%), 10 types of TET2 gene mutations (9.2%), 12 types of TP53 gene mutations (11%), 2 types of DNMT3A gene mutations (1.8%), 3 types of DNMT3b gene mutations (2.75%) and 1 type of DNMT1 gene mutation (0.9%). The mutations of stemness genes were few: 6 types of mutations of the NANOG gene and 3 types of mutations of the MTOR gene. It has been shown that ASMs in NDD are structurally different from ASMs in aging, suggesting a molecular genetic difference between these age-related disorders. Clonal hematopoiesis, identified in all patients with NDD, explains the constant activity of blood ICCs, which support systemic neuroinflammation in the "clinical axis of neurodegeneration" in NDD. Blocking clonal hematopoiesis may be a new strategy for treating and stopping the progression of NDD.

Keywords: Neurodegenerative diseases; Alzheimer's disease; Parkinson's disease; Amyotrophic lateral sclerosis; Clonal hematopoiesis; Germ cell and somatic mutations; Hematopoietic stem sell

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Introduction

In the 21st century, Human Neurodegenerative Diseases (NDD) remain one of the most important unsolved problems of world health and the main reason for the constant increase in the cases of progressive dementia and incurable fatal degenerative-atrophic diseases of the Central Nervous System (CNS) [1-4]. Currently, a fairly large number of NDDS are known to affect the CNS, damage interneuronal connections, disrupt sensory, motor and cognitive processes, including vision, hearing, movement, speech, memory and other functions of higher nervous activity. Scientists explain this damage of neural connections by the deposits of neurospecific (beta-amyloid, tau proteins, Lewy bodies, a-synuclein, FUS proteins, SOD1 proteins, etc.) proteins inside the neuron and microenvironment, progressive degradation of synapses and axons, which ultimately leads

to the death of neurons [5,6]. Neurodegenerative diseases include Alzheimer's Disease (AD), Parkinson's Disease (PD), Amyotrophic Lateral Sclerosis (ALS), Huntington's Disease (HD), Multiple System Atrophy (MSA), Lewy body dementia, and more than 20 others. As the population ages, the incidence of neurodegeneration and dementia is projected to boost by 2050, posing a significant threat to global health systems in the world in general, and to healthcare in individual countries, in particular [2,4]. Although NDDs are very complex and may be etiologically distinct, identifying commonalities in disease mechanisms and pathologies may lead to a better understanding of triggering events in neurodegeneration and provide opportunities for new pan-neurodegenerative therapeutic avenues. When typing a query on the mechanisms of the etiopathogenesis of NDD in the Google search engine, more than 42,700 sites contain detailed specialized information on the known mechanisms of these diseases, which indicates the interest of researchers and scientists as well as multiple challenges in the area. The lack of systematic knowledge in this sphere of neurology is the inability of modern medicine to understand their cause, the inability to cure these diseases or even stop the progression of these deadly diseases [3]. The key motif of most publications is the absence of objective scientific evidence about the onset of these diseases and the causes of their progression. None of the known molecular biological markers of NDDs is capable of diagnosing these diseases at the preclinical stage. Most NDDs can be diagnosed only when more from 50% to 90% of neurons in the brain and/or spinal cord have died.

The scientific scandal that erupted in 2022 after the revelatory publication of the Journal Science [7], in which it was stated that the theory and pathogenesis of the development of Alzheimer's disease and other NDDs are based on false data presented by Professor Sylvain Lesne in 2006 in J. Nature [8], led to the discrediting of a large number of scientific studies of the mechanisms of NDDs. The cheat in the evidence of the most prestigious scientific journal in the world, that was detected discovery 20 years later, mislead an entire field of scientific research in the world. These data "were cited... thousands of times" in all world journals as the main cause of disease and led to the unjustified spending of billions of dollars on scientific research to confirm falsified results. As a result, for many years the existing scientific ideas that the onset of AD is conditioned by the beta-amyloid accumulation in neurons, as the fundamental root cause of this disease, have been severely criticized in J. Science (2022) due to gross manipulation and falsification of scientific facts and photographs [7]. Accordingly, the facts about the deposits of pathology-specific proteins in neurons and nervous tissue in other NDDs, as their key have become doubtful and ambiguous.

But not all scientists falsify and manipulate the results obtained. With all the variety of recent modern scientific literature on the pathogenetic mechanisms of neurodegeneration, in our opinion, one of the most fundamental and systematic research of recent years was performed by a scientific consortium consisting of 21 American researchers from various US universities [9]. The research analyzes the main basic mechanisms of neurodegeneration in humans in a multi-faceted fashion, as well as displays its unsolved problems and the main gaps in our knowledge in the area. The reliability of the results presented in this paper is beyond doubt. Unquestionably, the identification, systematization, and generalization of molecular features in common will improve the understanding of neurodegenerative events to be later used in the development of complex therapy for neurodegenerative mechanisms in various diseases. According to Wareham L.K., Liddelow S.A., Temple S. et al., there remain significant gaps in our knowledge that prevent us from the development of a broadly applicable and effective therapy for NDD. This unknown evidence can be summarized as follows: (i) common molecular events at the early stages of disease progression, those trigger events in the amplification cascade leading to neurodegeneration, (ii) events in progression that catalyze pre-existing neurodegenerative events, (iii) the involved cell types are still unknown, (iv) common pathological endpoints are not defined, i.e. we are unable to define these events to prevent or replace diseased tissue, and finally (v) there is no definition of the pro-degenerative events, as well as reparative or even pro-regenerative [9].

In our opinion these American researchers, have closely approached understanding the systemic fundamental mechanisms of neurodegeneration, but failed to notice the most important molecular biological event, which is both a “starter” and an “engine” of this negative process, which they defined as a “clinical axis of neurodegeneration”. This process constantly maintains a high activation of all short-lived Immunocompetent Cells (ICCs) in the case of NDD and a high activity of neuroinflammation in the nervous tissue and ensures the progression of neuronal degeneration and atrophy. This "engine" of the backbone pathological neurodegenerative process, in our opinion, is Clonal Hematopoiesis (CH) or pathological mono-/oligoclonal hematopoiesis. We believe that at the start neurodegenerative diseases are first a genomic-postgenomic disease of autologous Hematopoietic Stem Cell (HSC) and its descendants, that is, a blood disease, and it becomes a CNS disease only at the finish line.

The goal of this work was to test the hypothesis that the neurological manifestations of NDDs of the brain and spinal cord are not the cause of these diseases, but the consequence and outcome of immune-mediated blood diseases that are initiated and supported by genomic damage to HSCs of bone marrow and immunoaggressiveness of their descendants to the cells of the nervous tissue in the form of the formation of pathological clonality of hematopoiesis (hematopoiesis). The hypothesis was verified by whole exome sequencing of 22,000 genes, identification of Additional Somatic Mutations (ASM) of clonality genes in the DNA of autologous bone marrow HSCs and Peripheral Blood (PC) lymphocytes in NDD cases and definition of the role and place of clonal hematopoiesis in the onset and progression of these diseases as well as of its potential use as a therapeutic target for the promising treatment of these diseases.

Material and Methods

The study included 20 (twenty) patients with various neurodegenerative diseases being treated at the NeuroVita Clinical Hospital in 2022 and 2023: 10 patients with ALS, 3 patients with Alzheimer's disease, 4 patients with Parkinson's disease and 3 patients with systemic neurodegenerative disease. All patients underwent a bone marrow puncture from the posterior iliac crest (right or left) and isolation of 2 ml of bone marrow under aseptic conditions. Then autologous HSCs were sorted out on the CliniMacs cell sorter (Germany) using magnetic beads with antibodies to CD34. The HSCs were subsequently sent to the genetic laboratory of the Moscow Research Institute of Oncology named after P.A. Herzen, the Branch of the Federal State Budgetary Institution National Medical Research Center for Radiology of the Ministry of Health of Russia (Russia, Moscow) for genetic tests.

The DNA of the bone marrow HSCs and of peripheral blood lymphocytes from all 20 patients was tested by Next Generation Sequencing (NGS).

Targeted pair-end sequencing was performed on the MGISEQ-G400 apparatus using the MGIEasy Exome Capture V5 multiplex panel with a coverage rate of x=100 to search for Additional Somatic Mutations (ASM) in the genes of clonal hematopoiesis (AKT, ASXL1, CBL, mTOR DNMT1, DNMT 3a, DNMT 3b, JAK2, MYD88, Pi3-K, PPM1D, PTEN, SF3B1, TET, TP53) and in the stemness genes of HSCs and lymphocytes (nanog, Oct-4, SOx2). Bioinformatic data were processed using the software for demultiplexing, mapping (BOWTIE2 v 2.2.5), variant identification (GATK 3.8-0), and annotation (SNPEff 4.3T Annovar 2017J). Pathogenicity risk was assessed according to the ACMG (SF v 2.0)/MCSC recommendations, including the following criteria: Low population frequency (variant frequency 1000 genomes and/or EXAC<0.01), damaging effect (nonsense, frameshift), availability of information about the variant at the locus specific databases, etc.

Results

As a result of targeted double-end sequencing of DNA of bone marrow HSCs and PC lymphocytes, various mutations of clonality genes were detected in all examined 20 cases of NDDs, which confirmed clonal hematopoiesis in all these patients. At the same time, the comparison revealed almost complete identity of the detected ASMs of DNA nucleotide substitutions in the clonality genes in HSC of bone marrow and in lymphocytes of peripheral blood in all patients, which indicated the monoclonal nature of hematopoiesis (the dominance of descendant cells of one pathological clone of HSC) or the oligoclonality of hematopoiesis (the dominance of several HSC clones). The dominance of the pathological clone (clones) of HSC in NDD cases was observed both in peripheral blood lymphocytes and in bone marrow HSC. The fact of hereditary NDD was confirmed only for one case. To illustrate this fact, we present the genetic data of the identified mutations in the clonality genes of bone marrow HSC of the patient with F., 55 years old, with Parkinson's disease (Table 1). In this case, the neurodegenerative disease is hereditary: pathogenic polymorphism (NM_198578.4):c.6055G>A (p.Gly2019Ser) rs34637584 was detected in exon 41 of the LRRK2 gene. This variant is a pathogenic polymorphism and is associated with the hereditary development of Parkinson's disease.

The mutations of clonality genes in HSC of bone marrow of patient F. with Parkinson's disease, were identical to the mutations that had been detected in lymphocytes of peripheral blood, which indicates the monoclonal nature of the patient's hematopoiesis.

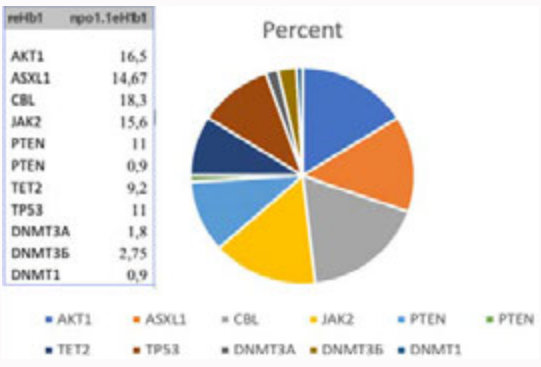

Figure 1: The share of frequent mutations of clonal hematopoiesis in 10 cases of amyotrophic lateral sclerosis, in which the driver genes could have been identified. These vary from study to study, but the general proportions are indicated.

This example is used to demonstrate the possibilities of whole genome sequencing of the human exome in identifying specific NDD polymorphisms and the phenomena of monoclonal hematopoiesis.

In Alzheimer's Disease (AD), we also diagnosed clonal hematopoiesis in all cases of the experimental group, although we had not detected hereditary disease. All cases of AD appeared to be sporadic. To illustrate, we present the genomic and proteomic arrays of the patient M., 72 years old, diagnosed with AD in Germany (Table 2, 3).

In this case, the patient M., 72, with Alzheimer's disease, has slight differences in the mutations of the clonality genes (TET2 and AKT1) in the DNA of the bone marrow HSC and the DNA of leukocytes, which indicates the oligoclonal nature of hematopoiesis. Mutations in the genes involved in the process of sporadic clonal hematopoiesis have been found in the studied sample. In a DNA sample of the bone marrow HSC, eleven mutations of the AKT1 gene, were detected. The gene is an intracellular enzyme, one of the three members of the protein kinase B family and oncogene in many malignant diseases. Nine mutations of the ASXL1 gene have been identified. Mutations in this gene impair the self-renewal ability of HSCs. One mutation was found in the CBL gene encoding the CBL protein, which is an E3 ubiquitin protein ligase involved in cell signaling and protein ubiquitination. Mutations in this gene are known to be involved in a number of human cancers, especially acute myeloid leukemia. The dynamic observation by an oncologist and a hematologist is recommended. Five mutations of the JAK2 gene have been found. Mutations in this gene are often found in myeloproliferative neoplasms, where they may be the only driver mutation. The mutations in the JAK2 gene are known to reduce the self-renewal of individual HSCs, but give an advantage to the descendants of these cells compared to other clones. Five mutations in the PTEN gene have been identified that may be associated with a disease called Cowden's syndrome. Cowden syndrome increases the risk of certain types of cancer (breast cancer; uterine (endometrial) cancer; thyroid cancer; colorectal cancer and colon polyps; kidney cancer). Mutations in the PTEN gene also increase the risk of developing other types of cancer. Two mutations were found in the TET2 gene. Clonal hematopoiesis associated with mutations in this gene dramatically increases the risk of cardiovascular disease. TET2 mutations in hematopoietic stem cells promote self-renewal of HSCs, giving them a competitive advantage in terms of expansion compared to other clones. Thus, mutations in the TET2 gene play an important role in the development of both myeloid and lymphoid malignancies. Four mutations were found in the TP53 gene encoding the p53 protein. p53 is a transcription factor that regulates a large number of genes in response to a variety of cellular damage, including oncogene activation, DNA damage, and inflammation. These factors activate p53 through post-translational modifications, which lead to upregulation of p53 protein and transactivation activity. Activated p53 induces growth arrest, apoptosis, DNA repair and differentiation in damaged cells to suppress cellular transformation. p53 is a critical regulator of the behavior of hematopoietic stem cells, maintains the resting state of HSCs and regulates their response to DNA damage. p53 activates transcription of target genes, mediating DNA damage repair, growth arrest, or apoptosis. TP53 is among the top five genes mutated in clonal hematopoiesis. The spread of clones

Table 1: The list of additional somatic mutations of clonality genes in hematopoietic stem cells of bone marrow of the patient F, 55 yr., Parkinson's disease.

Gene	Nucleotide substitution	rs (if available)	Pathogenicity criterion
ASXL1	ENST00000375687.4:c.*22A>G	NM_015338.6	Benign variant
ASXL1	ENST00000375687.4:c.*90T>C	NM_015338.6	Benign variant
ASXL1	ENST00000375687.4: p.Ser1253=	NM_015338.6	Benign variant
ASXL1	ENSP00000364839.4:p.Pro 815=	NM_015338.6	Benign variant
DNMT1	ENST00000676610.1_1:exon20:c.A1734G:p.Thr 594T	NM-001130823.3	Benign variant
DNMT1	ENST00000676610.1_1:exon20:c.A1734G:p.Pro463=	NM-001130823.3	Benign variant
DNMT3b	ENST00000456297.6_1:exon12:c.T1386C:p.Cys524=	NM_006892.4	Benign variant
DNMT3b	ENST00000624081.1_1:exon2:c.A139G:p.Tyr 558=	NM_006892.4	Benign variant
DNMT3A	ENST00000624081.1_1:exon2:c.A139G:p.Pro9=	NM_006893.4	Benign variant
DNMT3A	ENST00000624081.1_1:exon2:c.A139G:p.Leu422=	NM_006893.4	Benign variant
JAK2	ENSP00000371067.3:p.His163=	NM_004972.4	Benign variant
JAK2	ENSP00000371067.3:p.Leu830=	NM_004972.4	Benign variant
MTOR	ENST00000361445.9_1:exon33:c.G4731A:p.Asn999=	NM_004958.4	Benign variant
MTOR	ENST00000361445.9_1:exon19:c.C2997T:p.Asp479=	NM_004958.4	Benign variant
MTOR	ENST00000361445.9_1:exon10:c.T1437C:p.Leu2303=	NM_004958.4	Benign variant
MTOR	ENST00000361445.9_1:exon10:c.T1437C:p.Ser1891=	NM_004958.4	Benign variant
MTOR	ENST00000361445.9_1:exon10:c.T1437C:p.Ala1577=	NM_004958.4	Benign variant
PTEN	ENST00000371953.3:c.-653C>G	NM_000314.8	Benign variant
PTEN	ENST00000371953.3:c.-326 G >C	NM_000314.8	Benign variant
PTEN	ENST00000371953.3:c.*1516 T>C	NM_000314.8	Benign variant
PTEN	NM_001304717: 89623901: c.*2175 C>T	NM_000314.8	Benign variant
PTEN	ENST00000371953.3:c.-366 _363del	NM_000314.8	Benign variant
SF3B1	ENSP00000442788.1:p.Gly877=ENST00000540549.1:c.5284A>G	NM_12433.4	Null variant
SF3B1	ENST00000540549.1:p.Val 1219=	NM_12433.4	Benign variant
SF3B1	ENST00000540549.1:p.Lys141=	NM_12433.4	Benign variant
TP53	ENSP00000269305.4:p.Pro72Arg	NM_000546.6	Null variant
TP53	ENST00000269305.4:c.72+38C>G	NM_000546.6	Benign variant
TP53	ENST00000269305.4:c.376 -91G>A	NM_000546.6	Benign variant
TP53	ENST00000269305.4:c.*826 G>A	NM_000546.6	Benign variant

with TP53 mutations is known to be associated with the development of hematological neoplasms in the elderly.

We obtained similar results of gross molecular biological damage of the dominant HSC clone(s) in all 10 examined patients with ALS. Here is a typical example of clonality of hematopoiesis in ALS patient A., 35 years old (Table 4).

Since there was a statistically significant number of patients with ALS (10 patients), we analyzed all ASM variants that were observed in these patients. These data are summarized in Table 5.

Whole genome sequencing of DNA of HSCs of the NDD patients revealed 123 ASMs, of which 114 mutations were nucleotide substitutions of clonality genes and 9 mutations of stemness genes. If the total number of ASM clonality genes is taken as 100%, then the NDD patients showed 18 types of mutations of the AKT1 clonality gene (16.5%), 16 types of mutations of the ASXL1 clonality gene (14.67%), 20 types of mutations of the CBL clonality gene (18.3%), 17 types of mutations of the JAK2 clonality gene (15.6%), 12 types of mutations of the PTEN clonality gene (11%), 1 type of mutations of the PPM1D gene (0.9%), 10 types of mutations of the TET2 gene (9.2 %), 12 types of mutations of TP53 gene (11%), 2 types of DNMT3A gene mutations (1.8%), 3 types of DNMT3b gene mutations (2.75%), 1 type of DNMT1 gene mutations (0.9%). Besides, there were few mutations in stemness genes: 6 types of NANOG gene mutations and 3 types of MTOR gene mutations.

The analysis of the obtained data helped us detect the main clonality driver genes based on the frequency of occurrence of these mutations in blood of the patients with advanced NDDs.

We have found that mutations in the AKT1 clonality gene in the form of the ENST00000554581.1:c.46+42T>C nucleotide substitution in bone marrow DNA and peripheral blood lymphocytes occurred in 100% of cases, which requires additional verification of this mutation as a driver mutation in most NTDs.

Discussion

We have studied 20 cases of different NDDs, and have provisionally confirmed that the onset of pathological CH plays a key role in diseases pathogenesis. Each case has a certain set of ASMs and driver mutations in clonality genes, which, in our opinion, trigger and maintain the molecular biological pathogenesis. So far, we have

Table 2: The list of additional somatic mutations of clonality genes in hematopoietic stem cells of bone marrow of the patient M, 72 yr., Alzheimer's disease.

Gene	Nucleotide substitution	rs (if available)	Pathogenicity criterion
AKT1	ENST00000554581.1:c.1173-89C>T	rs149990267	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.46+42T>C	rs2494749	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.46+43G>A	rs2494748	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.287+30A>G	rs2494735	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.1260+106_1260+110del	rs55839843	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.1260+98G>A	rs2498800	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.1172+23A>G	rs2494732	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.726G>A	rs1130233	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.288-92A>G	rs2498797	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.1260+110G>C	rs12590657	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.1260+106_1260+107insC		Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.140+26A>G	rs2295454	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.471+89A>G	rs3818190	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.3759T>C	rs4911231	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.*22A>G	rs2295764	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.*90T>C	rs2295763	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.2444T>C:exon13:p.L815P	rs6058694	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.719-100C>T	rs2295765	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.*394A>G	rs2295762	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.*548G>C	rs41289852	Non-pathogenic mutation
CBL	ENST00000264033.4:c.*4931T>C	rs2510145	Non-pathogenic mutation
JAK2	ENST00000381652.3:c.1514-88G>A	rs7869668	Non-pathogenic mutation
JAK2	ENST00000381652.3:c.2886+71G>A	rs10974955	Non-pathogenic mutation
JAK2	ENST00000381652.3:c.2490G>A	rs2230724	Non-pathogenic mutation
JAK2	ENST00000381652.3:c.3060-72A>G	rs10815163	Non-pathogenic mutation
JAK2	ENST00000381652.3:c.489C>T	rs2230722	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.-326G>C	rs2943772	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.80-96A>G	rs1903858	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.-511G>A	rs12573787	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.1026+32T>G	rs555895	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.802-3_802-2insT	rs34003473	Non-pathogenic mutation
TET2	ENST00000540549.1:c.4538-113C>T	rs2647243	Non-pathogenic mutation
TET2	ENST00000540549.1:c.5284A>G:exon11:p.I1762V	rs2454206	Non-pathogenic mutation
TP53	ENST00000269305.4:c.672+62A>G	rs1625895	Non-pathogenic mutation
TP53	ENST00000269305.4:c.376-91G>A	rs2909430	Non-pathogenic mutation
TP53	ENST00000269305.4:c.215C>G:exon4:p.P72R	rs1042522	Non-pathogenic mutation
TP53	ENST00000269305.4:c.74+38C>G	rs1642785	Non-pathogenic mutation

not found the evidence in the published research that confirms clonal hematopoiesis in various human NDDs.

The CH is known to be caused by the accumulation of Additional Somatic Mutations (ASM) in long-lived cells of the body (nerve cells, tissue-specific stem cells, muscle cells, etc.) and, most importantly, in Hematopoietic Stem Cells (HSCs). GA Challen and MA Goodell (2020) state that the recent discovery of the prevalence of clonal hematopoiesis has changed the way hematologists, oncologists, and other clinicians think about HSCs [10]. Although fluctuations in the activity of Stem Cell (SC) clones have long been known, in general, the contribution of HSCs to blood production was considered to be fairly stable in the absence of overt diseases such as leukemia or BM deficiency. In fact, CH is the result of competition between long-lived hematopoietic SCs in BM [10].

The term clonal hematopoiesis refers to any state of clonal expansion in the hematopoietic system. Such blood cancer such as Chronic Myeloid Leukemia (CML) or Myelodysplastic Syndrome (MDS) is a typical example of clonal hematopoiesis. However, the mutations found in these cancers also occur in a large proportion of the healthy older population. To distinguish these mutations in non-

Table 3: The list of additional somatic mutations of clonality genes in lymphocytes of peripheral blood of the patient M, 72 yr., Alzheimer's disease.

Gene	Nucleotide substitution	rs (if avalable)	Pathogenicity criterion
AKT1	ENST00000554581.1:c.1173-89C>T	rs149990267	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.46+42T>C	rs2494749	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.46+43G>A	rs2494748	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.287+30A>G	rs2494735	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.1260+106_1260+110del	rs55839843	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.1260+98G>A	rs2498800	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.1172+23A>G	rs2494732	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.726G>A	rs1130233	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.288-92A>G	rs2498797	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.140+26A>G	rs2295454	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.471+89A>G	rs3818190	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.3759T>C	rs4911231	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.*22A>G	rs2295764	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.*90T>C	rs2295763	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.2444T>C:exon13:p.L815P	rs6058694	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.719-100C>T	rs2295765	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.*394A>G	rs2295762	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.*548G>C	rs41289852	Non-pathogenic mutation
CBL	ENST00000264033.4:c.-95_-94insGGC	rs57028199	Non-pathogenic mutation
CBL	ENST00000264033.4:c.*4931T>C	rs2510145	Non-pathogenic mutation
JAK2	ENST00000381652.3:c.1514-88G>A	rs7869668	Non-pathogenic mutation
JAK2	ENST00000381652.3:c.2886+71G>A	rs10974955	Non-pathogenic mutation
JAK2	ENST00000381652.3:c.2490G>A	rs2230724	Non-pathogenic mutation
JAK2	ENST00000381652.3:c.3060-72A>G	rs10815163	Non-pathogenic mutation
JAK2	ENST00000381652.3:c.489C>T	rs2230722	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.-326G>C	rs2943772	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.80-96A>G	rs1903858	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.-511G>A	rs12573787	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.1026+32T>G	rs555895	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.802-3_802-2insT	rs34003473	Non-pathogenic mutation
TET2	ENST00000540549.1:c.4538-113C>T	rs2647243	Non-pathogenic mutation
TET2	ENST00000540549.1:c.-46-39_-46-38insTAGA	rs58201766	Non-pathogenic mutation
TET2	ENST00000540549.1:c.4183-53_4183-52del	rs149641287	Non-pathogenic mutation
TET2	ENST00000540549.1:c.5284A>G:exon11:p.I1762V	rs2454206	Non-pathogenic mutation
TET2	ENST00000540549.1:c.86C>G:exon3:p.P29R	rs12498609	Non-pathogenic mutation
TP53	ENST00000269305.4:c.672+62A>G	rs1625895	Non-pathogenic mutation
TP53	ENST00000269305.4:c.376-91G>A	rs2909430	Non-pathogenic mutation
TP53	ENST00000269305.4:c.215C>G:exon4:p.P72R	rs1042522	Non-pathogenic mutation
TP53	ENST00000269305.4:c.74+38C>G	rs1642785	Non-pathogenic mutation

malignant conditions from malignant clonal hematopoiesis, the term Clonal Hematopoiesis of Uncertain Potential (CHIP) was introduced [11]. CHIP is defined by the occurrence of a cancer-associated somatic mutation in the blood or bone marrow in individuals without known hematologic cancers or other clonal conditions such as monoclonal gammopathy.

It is believed that CH develops with age over a long period of time, similar to a marathon race. GA Challen and MA Goodell, bring the same comparison: "In a marathon that would start with runners initially well matched, a small endurance advantage can pay off, while a runner that expends a large amount of energy in the early phase may not last. Similarly, runners that are injured will drop out along the way. Over a long race, many minor factors can come into play, including psychology, weather, and terrain. Chance always plays some role, and finally, the likelihood of winning also depends also on number of competing runners." [10].

Table 4: The list of additional somatic mutations of clonality genes in lymphocytes of peripheral blood of the patient A 35 yr., motor neuron disease.

Gene	Nucleotide substitution	rs (if available)	Pathogenicity criterion
AKT1	ENST00000554581.1:c.703-30del		Non-pathogenic mutation
AKT1	ENST00000554581.1:c.1173-31C>G	rs61761201	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.46+43G>A	rs2494748	Non-pathogenic mutation
AKT1	ENST00000554581.1:c.46+42T>C	rs2494749	Non-pathogenic mutation
ASXL1	ENST00000375687.4:c.2444T>C:exon13:p.L815P	rs6058694	Non-pathogenic mutation
CBL	ENST00000264033.4:c.-103_-95del	rs57028199	Non-pathogenic mutation
CBL	ENST00000264033.4:c.869+44_869+45insT	rs3842642	Non-pathogenic mutation
CBL	ENST00000264033.4:c.*366_*365insGG	rs397958007	Non-pathogenic mutation
CBL	ENST00000264033.4:c.*2698G>A	rs1207361567	Non-pathogenic mutation
CBL	ENST00000264033.4:c.195+84C>G	rs1592364794	Non-pathogenic mutation
CBL	ENST00000264033.4:c.747+74del	rs373788107	Non-pathogenic mutation
JAK2	ENST00000381652.3:c.-108-61A>G	rs2274471	Non-pathogenic mutation
JAK2	ENST00000381652.3:c.*1119G>T	rs12000101	Non-pathogenic mutation
PPM1D	ENST00000305921.3:c.*68T>C	rs370221881	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.802-3_802-2insT	rs34003473	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.-798A>G	rs577569375	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.-366del	rs71022512	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.253+109_253+110insTCTTA	rs1799734	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.*1457_*1459del	rs5786797	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.*1458_*1459del	rs5786797	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.-326G>C	rs2943772	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.80-96A>G	rs1903858	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.1026+32T>G	rs555895	Non-pathogenic mutation
PTEN	ENST00000371953.3:c.*1516T>C	rs701848	Non-pathogenic mutation
TET2	ENST00000540549.1:c.3035C>T:exon3:p.P1012L	rs587778707	Non-pathogenic mutation
TET2	ENST00000540549.1:c.86C>G:exon3:p.P29R	rs12498609	Non-pathogenic mutation
TET2	ENST00000540549.1:c.-46-39_-46-38insTAGATAGA	rs58201766	Non-pathogenic mutation
TET2	ENST00000540549.1:c.3954+26C>A	rs369415952	Non-pathogenic mutation
TET2	ENST00000540549.1:c.2771A>G:exon3:p.H924R	rs34485921	Non-pathogenic mutation
TET2	ENST00000540549.1:c.4538-113C>T	rs2647243	Non-pathogenic mutation
TP53	ENST00000269305.4:c.215C>G:exon4:p.P72R	rs1042522	Non-pathogenic mutation
TP53	ENST00000269305.4:c.672+62A>G	rs1625895	Non-pathogenic mutation
TP53	ENST00000269305.4:c.376-91G>A	rs2909430	Non-pathogenic mutation
TP53	ENST00000269305.4:c.74+38C>G	rs1642785	Non-pathogenic mutation
TP53	ENST00000269305.4:c.993+409_993+410insTT	rs34308401	Non-pathogenic mutation
TP53	ENST00000269305.4:c.673-71_673-70insA	rs752774596	Non-pathogenic mutation
TP53	ENST00000269305.4:c.96+41_97 -54del	rs59758982	Non-pathogenic mutation

However, this approach to CH does not explain the driver mutations in clonality genes in young people with autoimmune, neurodegenerative and oncological diseases of civilization, which we also found in our patients [1]. These findings prompt that the mechanism of accumulation of a critical amount of dramatic ASMs in HSCs is not necessarily determined by age. It is based on other mechanisms of CH formation. Grant A. Challen and Margaret A. Goodell provide a typical contribution of clonality genes in aging [10]. Comparing the distribution of mutations in clonality genes in the elderly and the distribution of mutations in the progressing neurodegenerative diseases, it becomes obvious that these are completely different diseases, although both depend on the age of people.

Various mutations of clonality genes dominate the onset and progression of these clinical conditions, and our study fully confirms this. The systemic aging process and the process of neurodegeneration at the genome level are completely different molecular biological processes, although bear outward similarity of clinical manifestations and age-association.

The dominant mutations of clonality genes in aging are DNMT3A (48.3%) and TET2 (15.5%), while for the neurodegenerative process,

Table 5: The list of additional somatic mutations of clonality genes in autologous HSCs of ALS cases.

Gene	Nucleotide substitution	rs (if available)	Pathogenicity criterion	Number of the patients with this mutation
AKT1	ENST00000554581.1:c.1251C>T:exon11:p.Y417Y	rs139297659	Benign variant	1
AKT1	ENST00000554581.1:c.1260+93C>T	rs150861537	Benign variant	1
AKT1	ENST00000554581.1:c.288-92A>G	rs2498797	Benign variant	2
AKT1	ENST00000554581.1:c.287+30A>G	rs2494735	Benign variant	4
AKT1	ENST00000554581.1:c.46+43G>A	rs2494748	Benign variant	4
AKT1	ENST00000554581.1:c.703-30del		Non-pathogenic mutation	1
AKT1	ENST00000554581.1:c.1260+106_1260+110del	rs55839843	Benign variant	3
AKT1	ENST00000554581.1:c.1260+98G>A	rs2498800	Benign variant	3
AKT1	ENST00000554581.1:c.288-92A>G	rs2498797	Benign variant	1
AKT1	ENST00000554581.1:c.1172+23A>G	rs2494732	Benign variant	2
AKT1	ENST00000554581.1:c.726G>A	rs1130233	Benign variant	2
AKT1	ENST00000554581.1:c.1172+69G>C	rs3803304	Benign variant	2
AKT1	ENST00000554581.1:c.46+42T>C	rs2494749	Benign variant	10
AKT1	ENST00000554581.1:c.1172+23A>G	rs2494732	Non-pathogenic mutation	2
AKT1	ENST00000554581.1:c.1173-31C>G	rs61761201	Non-pathogenic mutation	1
AKT1	ENST00000554581.1:c.1173-89C>T	rs149990267	Non-pathogenic mutation	1
AKT1	ENST00000554581.1:c.175+18C>T	rs3730358	Non-pathogenic mutation	1
AKT1	ENST00000554581.1:c.567+35G>A	rs3730346	Benign variant	1
ASXL1	ENST00000375687.4:c.-26A>G		Benign variant	3
ASXL1	ENST00000375687.4:c.57+41T>G	rs1569227911	Non-pathogenic mutation	2
ASXL1	ENST00000375687.4:c.57+36C>G		Non-pathogenic mutation	1
ASXL1	ENST00000375687.4:c.140+26A>G	rs2295454	Non-pathogenic mutation	1
ASXL1	ENST00000375687.4:c.2444T>C(p.Leu815Pro)	rs6058694	Benign variant	5
ASXL1	ENST00000375687.4:c.719-100C>T	rs2295765	Benign variant	2
ASXL1	ENST00000375687.4:c.471+89A>G	rs3818190	Non-pathogenic mutation	2
ASXL1	NST00000375687.4:c.1085+95A>G	rs56016114	Non-pathogenic mutation	1
ASXL1	ENST00000375687.4:c.159C>T	rs751085375	Non-pathogenic mutation	1
ASXL1	ENST00000375687.4:c.*90T>C	rs2295763	Non-pathogenic mutation	3
ASXL1	ENST00000375687.4:c.3759T>C	rs4911231	Non-pathogenic mutation	2
ASXL1	ENST00000375687.4:c.*22A>G	rs2295764	Non-pathogenic mutation	2
ASXL1	ENST00000375687.4:c.2444T>C:exon13:p.L815P	rs6058694	Non-pathogenic mutation	4
ASXL1	ENST00000375687.4:c.719-100C>T	rs2295765	Non-pathogenic mutation	2
ASXL1	ENST00000375687.4:c.*394A>G	rs2295762	Non-pathogenic mutation	2
ASXL1	ENST00000375687.4:c.*1239G>A	rs112187626	Non-pathogenic mutation	1
CBL	ENST00000264033.4:c.747+74del	rs373788107	Non-pathogenic mutation	2
CBL	ENST00000264033.4:c.-103_-95del	rs57028199	Non-pathogenic mutation	1
CBL	ENST00000264033.4:c.*2698G>A	rs1207361567	Non-pathogenic mutation	1
CBL	ENST00000264033.4:c.*7978A>G	rs2511836	Non-pathogenic mutation	1
CBL	ENST00000264033.4:c.747+74_747+75insT	rs373788107	Benign variant	1
CBL	ENST00000264033.4:c.869+44_869+45insT	rs3842642	Non-pathogenic mutation	2
CBL	ENST00000264033.4:c.748-67_748-66insT	rs367590251	Benign variant	2
CBL	ENST00000264033.4:c.*6610G>C		Benign variant	1
CBL	ENST00000264033.4:c.*7655A>C	rs1361522685	Benign variant	1
CBL	ENST00000264033.4:c.*366_*365insGG	rs397958007	Non-pathogenic mutation	3
CBL	ENST00000264033.4:c.*4864_*4863insT	rs572265582	Non-pathogenic mutation	2
CBL	ENST00000264033.4:c.*559_*560insCAA	rs3833768	Undefined value option	2

CBL	ENST00000264033.4:c.1432-61G>T	rs2298650	Benign variant	1
CBL	ENST00000264033.4:c.*7580_*7581del	rs376134331	Non-pathogenic mutation	1
CBL	ENST00000264033.4:c.2251+50G>C	rs2509660	Non-pathogenic mutation	1
CBL	ENST00000264033.4:c.2592C>T	rs1893177	Non-pathogenic mutation	1
CBL	ENST00000264033.4:c.*4931T>C	rs2510145	Non-pathogenic mutation	2
CBL	ENST00000264033.4:c.*5907T>C	rs2511844	Non-pathogenic mutation	1
CBL	ENST00000264033.4:c.*7559C>G	rs1052121	Non-pathogenic mutation	1
CBL	ENST00000264033.4:c.195+84C>G	rs1592364794	Non-pathogenic mutation	1
JAK2	ENST00000381652.3:c.-197T>C	rs2274472	Benign variant	1
JAK2	ENST00000381652.3:c.1514-88G>A	rs7869668	Non-pathogenic mutation	2
JAK2	ENST00000381652.3:c.*1329_*1330del	rs139964957	Benign variant	2
JAK2	ENST00000381652.3:c.3059+61_3059+63del	rs3831163	Non-pathogenic mutation	1
JAK2	ENST00000381652.3:c.1514-88G>A	rs7869668	Non-pathogenic mutation	2
JAK2	ENST00000381652.3:c.2886+71G>A	rs10974955	Non-pathogenic mutation	2
JAK2	ENST00000381652.3:c.2490G>A	rs2230724	Non-pathogenic mutation	4
JAK2	ENST00000381652.3:c.-108-61A>G	rs2274471	Non-pathogenic mutation	2
JAK2	ENST00000381652.3:c.3060-72A>G	rs10815163	Non-pathogenic mutation	3
JAK2	ENST00000381652.3:c.489C>T	rs2230722	Non-pathogenic mutation	4
JAK2	ENST00000381652.3:c.*1185A>C	rs567730823	Non-pathogenic mutation	1
JAK2	ENST00000381652.3:c.3059+23A>T	rs2274649	Non-pathogenic mutation	1
JAK2	ENST00000381652.3:c.-197T>C	rs2274472	Non-pathogenic mutation	1
JAK2	ENST00000381652.3:c.*1119G>T	rs12000101	Non-pathogenic mutation	2
JAK2	ENST00000381652.3:c.614+79C>T	rs7872649	Non-pathogenic mutation	1
JAK2	ENST00000381652.3:c.1929T>C	rs2230728	Non-pathogenic mutation	1
JAK2	ENST00000381652.3:c.3291+96T>G	rs12005968	Non-pathogenic mutation	1
PTEN	ENST00000371953.3:c.802-3_802-2insT	rs34003473	Undefined value option	4
PTEN	ENST00000371953.3:c.-457T>G	rs904599717	Benign variant	1
PTEN	ENST00000371953.3:c.*1516T>C	rs701848	Benign variant	4
PTEN	ENST00000371953.3:c.-366del	rs71022512	Benign variant	5
PTEN	ENST00000371953.3:c.253+109_253+110insTCTTA	rs1799734	Non-pathogenic mutation	2
PTEN	ENST00000371953.3:c.*1457_*1459del	rs5786797	Non-pathogenic mutation	4
PTEN	ENST00000371953.3:c.-326G>C	rs2943772	Benign variant	5
PTEN	ENST00000371953.3:c.-798A>G	rs577569375	Non-pathogenic mutation	1
PTEN	ENST00000371953.3:c.1026+32T>G	rs555895	Non-pathogenic mutation	3
PTEN	ENST00000371953.3:c.80-96A>G.	rs1903858	Non-pathogenic mutation	3
PTEN	ENST00000371953.3:c.-653C>G		Benign variant	1
PTEN	NM_001304717: 89623901: c.G194C p.C65S		Benign variant	1
PPM1D	ENST00000305921.3:c.*68T>C	rs370221881	Non-pathogenic mutation	1
TET2	ENST00000540549.1:c.3954+26C>A	rs369415952	Non-pathogenic mutation	1
TET2	ENST00000540549.1:c.5162T>G(p.Leu1721Trp)	rs34402524	Null variant	1
TET2	ENST00000540549.1:c.4538-113C>T	rs2647243	Benign variant	5
TET2	ENST00000540549.1:c.3035C>T:exon3:p.P1012L	rs587778707	Non-pathogenic mutation	1
TET2	ENST00000540549.1:c.-46-46_-46-39del	rs58201766	Non-pathogenic mutation	2
TET2	ENST00000540549.1:c.-46-39_-46-38insTAGATAGA	rs58201766	Non-pathogenic mutation	2
TET2	ENSP00000442788.1:p.Ile1762Val ENST00000540549.1:c.5284A>G	rs2454206	Null variant	1
TET2	ENST00000540549.1:c.86C>G:exon3:p.P29R	rs12498609	Non-pathogenic mutation	1
TET2	ENST00000540549.1:c.2771A>G:exon3:p.H924R	rs34485921	Non-pathogenic mutation	1
TET2	ENST00000540549.1:c.5284A>G:exon11:p.I1762V	rs2454206	Non-pathogenic mutation	1

TP53	ENST00000269305.4:c.993+409_993+410insTT	rs34308401	Benign variant	1
TP53	ENST00000269305.4:c.376-91G>A	rs2909430	Benign variant	3
TP53	ENST00000269305.4:c.96+41_97-54del	rs59758982	Benign variant	1
TP53	ENST00000269305.4:c.993+352C>T	rs77697176	Benign variant	1
TP53	ENST00000269305.4:c.672+62A>G	rs1625895	Benign variant	3
TP53	ENST00000269305.4:c.215C>G p.Pro72Arg	rs1042522	Benign variant	2
TP53	ENST00000269305.4:c.74+38C>G	rs1642785	Benign variant	3
TP53	ENST00000269305.4:c.673-71_673-70insA	rs752774596	Benign variant	2
TP53	ENST00000269305.4:c.215C>G:exon4:p.P72R	rs1042522	Null variant	3
TP53	ENST00000269305.4:c.993+409_993+410insTT	rs34308401	Benign variant	5
TP53	ENST00000269305.4:c.673-71del	rs752774596	Benign variant	2
TP53	ENST00000269305.4:c.96+41_97-54del	rs59758982	Non-pathogenic mutation	3
DNMT1	ENST00000676610.1_1:exon20:c.A1734G:p.T578T		Benign variant	1
DNMT3b	ENST00000456297.6_1:exon12:c.T1284C:p.C428C		Benign variant	1
DNMT3b	ENST00000456297.6_1:exon12:c.T1386C:p.Y462Y		Benign variant	1
DNMT3b	ENST00000624081.1_1:exon2:c.A139G:p.R47G		Benign variant	1
DNMT3A	ENST00000624081.1_1:exon2:c.A139G:p.Leu422=		Benign variant	1
DNMT3A	ENST00000624081.1_1:exon2:c.A139G:p.Pro9=		Benign variant	1
MTOR	ENST00000361445.9_1:exon33:c.G4731A:p.A1577A		Benign variant	1
MTOR	ENST00000361445.9_1:exon19:c.C2997T:p.N999N		Benign variant	1
MTOR	ENST00000361445.9_1:exon10:c.T1437C:p.D479D		Benign variant	1
NANOG	ENST00000229307.9_1:exon2:c.T165C:p.P55P		Benign variant	1
NANOG	ENST00000229307.9_1:exon2:c.G246T:p.K82N		Benign variant	1
NANOG	ENST00000229307.9_1:exon2:c.G276A:p.P92P		Benign variant	1
NANOG	ENST00000229307.9_1:exon2:c.C363T:p.S121S		Benign variant	1
NANOG	ENST00000229307.9_1:exon4:c.T531C:p.L177L		Benign variant	1
NANOG	ENST00000528386.4_1:exon1:c.A47C:p.E16A		Benign variant	1

Table 6: The highest occurrence (in percentage) of clonality gene mutations in 10 cases of neurodegenerative diseases.

Gene	Nucleotide substitution	rs (if available)	Pathogenicity criterion	Occurrence (%) of driver mutation
AKT1	ENST00000554581.1:c.46+42T>C	rs2494749	Pathology-specific variant	100
AKT1	ENST00000554581.1:c.287+30A>G	rs2494735	Benign variant	40
AKT1	ENST00000554581.1:c.46+43G>A	rs2494748	Benign variant	40
ASXL1	ENST00000375687.4:c.2444T>C(p.Leu815Pro)	rs6058694	Frequent variant	50
ASXL1	ENST00000375687.4:c.2444T>C:exon13:p.L815P	rs6058694	Non-pathogenic mutation	40
JAK2	ENST00000381652.3:c.2490G>A	rs2230724	Non-pathogenic mutation	40
JAK2	ENST00000381652.3:c.489C>T	rs2230722	Non-pathogenic mutation	40
PTEN	ENST00000371953.3:c.802-3_802-2insT	rs34003473	Null variant	40
PTEN	ENST00000371953.3:c.*1516T>C	rs701848	Benign variant	40
PTEN	ENST00000371953.3:c.-366del	rs71022512	Frequent variant	50
PTEN	ENST00000371953.3:c.*1457_*1459del	rs5786797	Non-pathogenic mutation	40
PTEN	ENST00000371953.3:c.-326G>C	rs2943772	Frequent variant	50
TET2	ENST00000540549.1:c.4538-113C>T	rs2647243	Frequent variant	50
TP53	ENST00000269305.4:c.993+409_993+410insTT	rs34308401	Frequent variant	50

the dominant mutations of clonality genes are mutations of the CBL genes (18.3%), mutations of the AKT1 gene (16.5%) and mutations in the ASXL1 gene (14.7%). But how does the CH that we have identified for all NDDs triggers the onset and progression of NDDs? The established facts of pathological clonality of hematopoiesis in NDDs logically fit into the "triggering molecular biological principle" of the "clinical axis of neurodegeneration" proposed by a consortium of American scientists [9]. The initiating and supporting events of neurodegeneration are the formation of pathological HSC clones, their consequent dominance in hematopoiesis, gradual expansion

in the hematopoietic system, inhibition of other 100,000-200,000 embryonic relatively healthy bone marrow HSC clones and constant targeted reproduction and migration of activated blood ICCs, which are aggressive to the nervous tissue of brain and spinal cord and infinitely maintain systemic neuroinflammation in the nervous tissue of the brain/spinal cord.

Apparently, it is CH that is the main initiating and system-forming mechanism of the clinical axis of neurodegeneration, described by American scientist [9]. A large scientific team of American researchers from different US Universities, Wareham, Liddelow, Temple et al. have identified several so-called "common mechanistic areas of focus that may provide potential pan-neurodegenerative therapeutic strategies. These included: Environmental factors, neuroinflammation, metabolic stress, neurovascular connectivity, and genetic contribution to disease development." Everything seems correct. But we are convinced that these coherent scientific constructions contain a systemic fundamental methodological error. Environmental factors, as the main initiating moment of the disease, are not a link in pathogenesis, it is a possible etiological factor, but not the molecular mechanism of the pathogenesis of NDD. As for the rest, the team of researchers are undoubtedly right in their conclusions. Also, the American researchers did not answer the most important questions, posed by themselves, about the molecular biological events that trigger, catalyze and support neurodegeneration. These are the most important aspects of the pathogenesis of these diseases, since various mechanical areas of focus that they have identified (genetic contribution, neuroinflammation, metabolic stress, pathological proteins, vascular damage, etc.) are natural consequence of the disease, and not its causes. Neurodegeneration includes complex interactions between neighboring cells and their axonal projections; neurons have both proximal and distal regions, which have a different cellular environment and, in turn, different mechanisms of pathology [5]. In addition, the CNS does not always act in isolation; the Peripheral Nervous System (PNS) and the peripheral immune system, as well as the vascular system, are also involved into CNS degeneration [12].

What is the root cause of systemic neurodegeneration? Wareham, Liddelow, Temple et al. clearly and strictly defined the clinical axis of neurodegeneration development: activated immune cells trigger the activation of glial cells, astrocytes and microglia, leading to chronic neuroinflammation, oxidative stress, metabolic and vascular damage, accumulation of pathological proteins inside the cell and in the intercellular space of the nervous tissue and as a consequence to the degeneration of neurons [9]. And what causes the activation of most ICCs? Why, with the lifespan of activated ICCs no more than 80 to 120 days, the process of neurodegeneration lasts for years and leads to the death of an individual? What is the "bonfire" that keeps "warming up" the high level of ICCs activation in each of their new generation? Why does the disease not end with the natural death of short-lived activated ICCs? The answer is trivial: The CH in all NDD cases, and we are the first to show this in this study.

The expert in Jaiswal [13] has shown in his publication on CHIP that somatic mutations accumulate in all cells of the body with the time, which has been confirmed by others [14-16]. These mutations are most often base substitutions (known as Single Nucleotide Variants [SNVs]), small insertions or deletions (indels), or copy number changes in large chromosomal regions (known as Structural Variants [SVs]). It is estimated that HSCs acquire approximately 20 somatic mutations per year throughout the genome [17,18] and approximately 0.1 mutation per year in protein coding exons [14], the majority of which are SNVs. In the bone marrow, only long-lived HSCs have the ability to self-renew throughout the life of the organism [19]. Therefore, in most cases, only mutations that arise in HSCs persist throughout a person's life. Given that there are from ~50,000 to ~200,000 HSCs per person, by the age of 70 people are expected to have between 350,000 and 1,400,000 coding mutations in the HSC pool. If at least one of these mutations is capable of providing a selective advantage, clonal expansion in the blood should be a common occurrence during aging [20] and the emergence of other diseases of civilization [1], including NDDs. Indeed, this phenomenon, called clonal hematopoiesis, is closely associated with aging and has been shown in several studies in individuals unselected for studies of hematological disorders [21-24].

In most research, the mutations that have been used to define CH are similar to those found in hematological cancer [21,24]. The most frequently mutated genes in CH include DNMT3A, TET2, ASXL1, JAK2, TP53 and SF3B1, which are also frequently mutated in Acute Myeloid Leukemia (AML) [25], Myelodysplastic Syndrome (MDS) [26], and Myeloproliferative Neoplasms (MPN) [27]. Therefore, it is not surprising that individuals with CH develop these types of cancer at a faster rate than people without mutations [21,28,29]. However, CH inducing mutations can also be found in such circulating immune cells as granulocytes, monocytes, and lymphocytes. This finding raises the possibility that CH may lead to altered immune responses that could potentially influence many of the diseases of civilization and aging. The central task of our research was to confirm CH for neurodegenerative diseases and to theoretically substantiate the fact of pathological CH as a fundamental event that triggers NDD and supports its progression.

It is not even theoretically possible to stop the progression of NDD and prevent its recurrence against the background of CH. Our data about CH in autoimmune, oncological and hereditary diseases described in our monograph [1], led us to the conclusion that the restorative mechanisms of sanogenesis in damaged organs and tissues are rigidly tied to hematopoiesis and immunity. It is the system-forming and regulatory, controlling role of HSC that underlays the sanogenetic mechanism of the restoration of damaged organs and tissues. In case of a polyclonal hematopoiesis in the eukaryotic organism (when all or most of the existing HSC clones' function), the sanogenetic mechanisms of the body work properly. Polyclonal hematopoiesis provides regular replacement or repair of affected specialized blood cells and restoration of specialized differentiated cells of organs and tissues [13]. The more HSC clones are involved in the process of forming a pool of all 36 billion blood cells circulating in the body, the less likely it is that NDD or sanogenesis disorders will occur. With a normally functioning immune system and polyclonal hematopoiesis, the accumulation of pathological proteins in tissues, including nervous tissue, is not possible.

For many years, there dominated a dogma in immunology, oncology and hematology that HSCs are just parent cells of all blood cells and immunity. The reproduction of new blood cells was considered their main function in the body. It was believed that they are able to restore the entire multi-billion cell blood pool from one cell, but they do not have any control and regulatory functions [30]. Scientific evidence that has been accumulated over the past decade demonstrate that HSCs are not only the progenitors of all blood cells, but they, are the "composers", "conductors" and "arrangers"

in the "orchestra of all existing immune reactions" occurring in mammalians [1]. All 230 known cell types of the human body [31] are dependent and controlled by them in cellular subsystems. HSCs are the main regulatory and system-forming structural elements of the body, forming the required number of regulatory, killer and cytotoxic blood cells under specific conditions in response to a pathogen (oncogene, virus, bacterium, protozoan, etc.) and provide for the immune memory of the body [32]. HSCs are the slowest cells in the human and animal body. The cell cycle of HSC is 360 days. Normally, there are no cells with a slower cell cycle in the human body [33]. As the founder of cybernetics and mathematical theory of systems, the great mathematician Norbert Wiener said: "The slowest phase is the control in any most complex system" [34]. This provision always "works" for all chemical, biochemical, physical and mathematical systems and today is not subject to discussion in the mathematical theory of systems [35]. Therefore, HSC occupies the highest dominant position in the hierarchy of all cells of the body, and all cells of the body are subordinate to it. The role and place of a queen bee in a bee hive or a queen in an anthill or termite mound is analogous to the role of HSC. If the queen bee decides to leave the hive, then the whole swarm will fly away with it.

HSCs are theoretically "immortal", or rather the longest-lived cells in the body, like neurons in the brain and spinal cord. Back in 2010, the calculations of the outstanding Russian mathematician Professor A.I. Galushkin (the data have not been published due to his death) using a neurocomputer network showed that of all cells in the body only neurons and HSCs are theoretically able to live up to 1000 years. This is fully consistent with their mission in the body: The number of neurons determines the structure of consciousness and personality, while the number of HSCs determines the entire hierarchy of cells in the body and the ability to form immune surveillance and to organize a systemic protective immune response [1].

Thus, our research allows us to fill the significant gaps in our knowledge about five mechanisms of NDDs, which have been stated by Wareham, Liddelow, Temple et al. [9]. We consider the formation of clonal hematopoiesis in NDD to be the main molecular biological event that initiates and supports the pathogenesis of NDDs, while their neurological manifestations are not the cause of the disease, but the consequence and outcome of the immune-mediated effect of activated ICCs of the blood on cells of nervous tissue of the brain, leading to the progression of the mechanisms of neuroinflammation and neurodegeneration.

Conclusion

For the first time, on a very small clinical material, we have demonstrated the fundamental scientific fact of the initiation and progression of NDD in the form of clonal hematopoiesis and substantiated the assumption that this phenomenon is a defining and system-forming molecular biological event that triggers neurodegeneration and ensures its progression. Undoubtedly, these studies require scaling up and a larger sample of NDD cases. During the life time or environmental stress, a large amount of ASMs accumulates in long-lived cellular systems and in all stem cells. Some of these ASMs in such long-lived cells as HSCs can theoretically become driver and dramatic and lead to the formation of CH. One (monoclonality) or several dozen (oligoclonality) dominant hematopoietic clones "win the competition" among other HSCs [13]. They gain priority in the reproduction of blood cells in the BM, regulatory and control functions, as their cell cycle becomes the largest and slowest among the other 100,000-200,000 HSC clones of BM. Gradually expanding in volume, the descendants of HSCs and hematopoietic precursors of the dominant clone (clones) of hematopoiesis displace ICCs of other clones in blood and tissues and form their pathological activity against glial cells, astrocytes and microglia in NDD. The number of ICCs of the dominant clone (clones) of HSC in the general circulation of blood cells reaches up to 70%, and sometimes up to 93% with different NDDs. They begin to support neuroinflammation in the nervous tissue of the brain and spinal cord and the pathogenesis of NDD. Therefore, we consider clonal hematopoiesis a genomic-postgenomic disease of HSCs, the key initiating process in the etiopathogenesis of most immune-associated NDDs and one of the central therapeutic molecular biological targets for innovative treatments of NDDs.

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