Effects of idiopathic erythrocytosis on the left ventricular diastolic functions and the spectrum of genetic mutations
A case control study

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Abstract

Background: We have aimed at exposing left ventricular diastolic functions and the presence of known genetic mutations for familial erythrocytosis, in patients who exhibit idiopathic erythrocytosis.

Methods: Sixty-four patients with idiopathic erythrocytosis (mean age, 46.4 ± 2.7 years) and 30 age-matched healthy subjects were prospectively evaluated. The regions of interest of the erythropoietin receptor, hemoglobin beta-globin, von Hippel-Lindau, hypoxia-inducible factor 2 alpha, and Egl-9 family hypoxia-inducible factor genes were amplified by PCR. Left ventricular (LV) mass was measured by M-mode and 2-dimensional echocardiography. LV diastolic functions were assessed by conventional echocardiography and tissue Doppler imaging.

Results: As a result of genetic analyses, genetic mutations for familial erythrocytosis were detected in 5 patients. It has been observed in our study that the risk of cardiovascular disorders is higher in patients. Interventricular septum thickness, left atrial diameter, and some diastolic function parameters such as deceleration time and isovolumetric relaxation time have been found to be significantly higher in idiopathic erythrocytosis group than in the controls.

Conclusion: This study has shown that LV diastolic functions were impaired in patients with idiopathic erythrocytosis. In this patient group with increased risk of cardiovascular disorders, the frequent genetic mutations have been detected in 5 patients only. Therefore, further clinical investigations are needed as novel genetic mutations may be discovered in patients with idiopathic erythrocytosis because of cardiovascular risk.

Abbreviations: a = diastolic late wave, A = late phase peak flow velocities, DT = deceleration time, e = diastolic early wave, E = early phase peak flow velocities, E/A = early phase peak flow velocity/late phase peak flow velocity, EGLN1 = Egl-9 family hypoxia-inducible factor, EPO = erythropoietin, EPOR = erythropoietin receptor, FE = familial erythrocytosis, HBB = hemoglobin beta-globin, HGVS = Human Genome Variation Society, HIF2a = hypoxia-inducible factor 2 alpha, IE = idiopathic erythrocytosis, IVCT = isovolumetric contraction time, IVRT = isovolumetric relaxation time, IVST = interventricular septum thickness, LAD = left atrial diameter, LVDD = left ventricular diastolic diameter, LVM = left ventricular mass, LVSD = left ventricular systolic diameter, MPI = myocardial performance index, PDH2 = prolyl hydroxylase domain protein 2, PFCP = familial congenital polycythemia, PV = polycythemia vera, PWT = posterior wall thickness, s = systolic wave, T = ejection time, VHL = von Hippel-Lindau.

Keywords: cardiovascular diseases, mutation, polycythemia, ventricular function

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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### 1. Introduction

Erythrocytosis or polycythemia as commonly known refers to an increase in erythrocyte mass. Both acquired and familial erythrocytosis (FE), except relative erythrocytosis, are examined by subgrouping as primary and secondary.[12] Primary-acquired erythrocytosis involves polycythemia vera (PV), which is characterized by the presence of JAK2 mutations, although these markers are not found in about 1% of patients with PV.[2–4] Secondary-acquired erythrocytosis, however, occurs when erythropoietin (EPO) drives the production of red cells. This situation can develop in various cardiac, pulmonary, and renal diseases or from external hypoxic conditions.[1] For familial polycythemias, a third subgroup defined as a mixed polycythemia has been identified other than its primary and secondary subgroups; this group includes erythrocytosis associated with the Chuvash polycythemia and other von Hippel-Lindau (VHL) gene mutations.[6] Genes carrying mutations associated with familial polycythemias have been identified as erythropoietin receptor (EPOR), VHL, prolyl hydroxylase domain protein 2, and hypoxia-inducible factors 2 alpha (HIF2a) genes in the online Mendelian inheritance in man classification.[7,8] Other polycythemias, defined by molecular lesions, are congenital methemoglobinemia, polycythemias caused by high oxygen and 2,3-bisphosphoglycerate deficiency.[9,10] Once all the known causes of erythrocytosis have been ruled out, there remains a group of patients classified as having idiopathic erythrocytosis (IE). IE is characterized by an increase in red blood cell mass of unknown etiology.[11] The frequency of IE is considered to be 1.1 for 1000 subjects. Diagnosing IE requires the exclusion of PV, secondary-acquired polycythemia, and various congenital primary and secondary polycythemias. It has also been reported that the risk of thrombosis is low in IE, and there may be spontaneous progress to acute leukemia or myelofibrosis.[12]

In patients with PV, it has been shown that blood rheology is associated with cardiovascular risk, and the mechanism of this is associated with impaired left ventricular (LV) function.[13] Increased left ventricular mass (LVM) and deterioration in diastolic function parameters are risk factors for future heart failure development. In people with risk factors for the development of heart failure, asymptomatic LV dysfunction progresses to symptomatic heart failure over time.[14] Approximately half of the cases of left heart failure are in the form of diastolic LV dysfunction.[15,16] Heart failure that results from impairment of myocardial relaxation and compliance is called diastolic heart failure.[17] LV diastolic dysfunction refers to increased cardiac filling pressures as a result of decreased LV relaxation and increased stiffness.[18] It is important to monitor LV diastolic function parameters before diastolic heart failure develops. Measurement of transmitial blood flow with pulsed-wave Doppler and measurement of myocardial velocities from mitral annulus, septal, and lateral regions by tissue Doppler have become the preferred tool for noninvasively evaluating diastolic functions.[19] Although there are studies investigating the relationship between LV diastolic functions in anemia and polycythemia vera, no similar studies have been found in IE.[20,21]

In our study, we have aimed at investigating and understanding whether isolated erythrocytosis would present as a risk factor for cardiovascular diseases. For this purpose, first, the primary- and secondary-acquired erythrocytoses were excluded, in which factors other than erythrocytosis could increase the risk of cardiovascular disease. Mutations in genes associated with FE have been investigated in all the cases. We have attempted to determine the frameshift mutations in proposed regions and tried to reveal new mutations related to erythrocytosis where possible. In addition, we have tried to determine the differences between the control group and the risk group with reference to the LV diastolic functions.

### 2. Materials and Methods

#### 2.1. Patients

The study was conducted in the hematology and cardiology clinics of Tekirdağ Namık Kemal University between January 2014 and January 2017. The inclusion criteria were as follows: isolated erythrocytosis (hemoglobin [Hb] > 18.5 g/dL in males or >16.5 g/dL in females), absence of JAK2 V617F and exon 12 mutations, and absence of any defined causes of acquired secondary erythrocytosis except smoking. Exclusion criteria were as follows: the patients under 18 years of age and patients with known coronary artery disease, cerebrovascular disease, or peripheral arterial disease. Furthermore, patients with chronic obstructive pulmonary disease, congestive heart failure, congenital heart disease, history of cardiac operation, atrial fibrillation, atrial flutter, and left bundle branch block were excluded from the study.

Sixty-four patients evaluated to have IE, and 30 healthy subjects that made up the control group were included in the study. Comprehensive clinical evaluations of the patients and controls who agreed to participate in the study were performed, and the patients and their families were questioned for erythrocytosis and related features. Moreover, routine laboratory findings (including blood count and lipid parameters) were recorded. Cardiovascular risk factors (diabetes, smoking, hypertension, and lipid) were designated/assessed in the patients and controls. EPO levels were measured in all patients. Written informed consent was obtained from all subjects. This study was approved by the Institutional Review Board of Tekirdağ Namık Kemal University.

#### 2.2. Genetic analysis

All of the cases included in the study group were assessed for FE-related genomic alterations by studying the mostly reported genes and mutations in the literature. Exon 8 of the EPOR gene (MIM number; 133171), entire coding and regulatory sequences of the hemoglobin beta-globin (HBB) gene (MIM number; 141900), the genomic region encompassing the Arg200Trp mutation that constitutes more than 90% of mutations of the VHL gene (MIM number; 608537), the genomic region including the most frequent mutations Ile533Val and Phe540Leu of the HIF2a gene (MIM number; 603349), and the genomic sequences containing the Cys127Ser and the Gln157His mutations in the first exon of the Egl-9 family hypoxia-inducible factor (EGLN1) gene (MIM number; 606225) were screened by direct sequencing. For this purpose 5-cc peripheral blood samples were collected from the patients, and the samples were taken into K3-EDTA tubes, and genomic DNA isolations were carried out through a commercial kit (Roche, Germany) according to the manufacturer’s directives. The amount and purity of the isolated genomic DNA samples were determined. The regions of interest of the EPOR, HBB, VHL, HIF2a, and EGLN1 genes were amplified by PCR. The primer sequences used in PCR are given in Figure 1.

After the PCR, the products were checked by agarose gel electrophoresis. Then, PCR products were purified, and DNA sequence analysis reactions were performed. Repurified reaction products were subjected to capillary electrophoresis in an automated DNA sequencing device (GeFF; BeckmanCoulter) to determine the sequence of DNA fragments. Tekirdağ Namık Kemal University, the Scientific Technological Research and Application Center, performed the processes starting from DNA isolation to obtaining the sequence results. The results of the DNA sequence analyses were evaluated by comparing with the reference sequences, and genonomic changes that could be related to FE were determined.

#### 2.3. Echocardiography

Echocardiographic evaluation was performed using high-resolution B-mode ultrasound with a 2.5-MHz transducer by

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2 experienced cardiologists who did not know the diagnosis of patients (GE Vivid S5: General ElectricVingMedSystems, Horten, Norway). While taking echocardiographic recordings, attention was paid to the appropriate room temperature and dim lighting. During the procedure, patients were asked to breathe normally. The interobserver variability for all measurements was found to be between 8.5% and 9.8%. Measurements of 20 patients were repeated 2 days later to evaluate intraobserver variability, and intraobserver variability for all measurements was between 6.8% and 7.8%.

2.4. Conventional echocardiography

Two-dimensional, M-mode, pulse-wave, and tissue Doppler echocardiography was performed for the measurement of left heart cavity dimensions, wall thicknesses, LVM, systolic, and diastolic function parameters. Left atrium diameters, LV systolic diameters, left ventricular diastolic diameters (LVDD), interventricular septum thicknesses (IVST), and posterior wall thicknesses (PWT) were measured according to the recommendations of the American Society of Echocardiography by 2-dimensional and M-mode echocardiography.[22] The modified biplane Simpson method was used for LV ejection fraction measurements. The LVM was calculated with the Devereux formula as follows: $0.8 \times \left[\frac{[(1.04 \times (LVDD + IVST + PWT) - LVDD)]}{3}\right] + 0.6$.

2.5. Measurement of diastolic functions

In order to evaluate the conventional diastolic functions, diastolic transmitral flow was measured by pulsed-wave Doppler between the mitral leaflet tips at a level of 1 cm below the plane of the mitral annulus, in the apical 4-chamber view. The early (E) and late (A) phase peak flow velocities and their ratio (E/A), E wave deceleration time (DT), isovolumetric relaxation time (IVRT), isovolumetric contraction time (IVCT), and ejection time were measured.[18]

2.6. Tissue Doppler echocardiography

Tissue Doppler measurements were made using a 2.5-MHz variable frequency phased array transducer. To obtain LV tissue Doppler recordings, Doppler sample volume was placed in the septal and lateral corners of the mitral annulus from the apical 4-chamber view. The Nyquist limit was set to a velocity range from -20 to 20 cm/s. The monitor weep speed was set at 100 mm/s. Diastolic early (e), late (a), and systolic (s) waves were measured from septal and lateral regions of the mitral annulus, sepal e/a, lateral e/a, E/septal e, and E/ lateral e ratios.

2.7. Statistical evaluation

Statistical analysis of the data was carried out by using SPSS for Windows 18.0 software. The Shapiro-Wilk test was used to examine whether the data were normally distributed. All variables were found to be normally distributed. Continuous variables were expressed as mean ± standard deviation, and categorical variables as numbers and percentages. In statistical analysis, continuous variables between control and patient groups were compared with Student t test. Chi-square tests were used for comparison of categorical variables. Pearson correlation analysis was performed to investigate the relationship between
Hb values and diastolic echocardiographic parameters. A P value less than .05 was accepted as genetic statistically significant.

3. Results

3.1. The results of the genetic analysis

As a result of genetic analyses, genetic mutations related to FE were detected in 5 patients (Fig. 1). EPO serum levels were heterogeneous among the patients; only 2 patients with Hgb San Diego had high EPO levels. We detected the EPOR gene 8 exon Arg437His change (EPOR: c.1310G>A [p. Arg 437His]) in heterozygous form in a male patient (Fig. 2). His highest hematocrit value was 53%. We have looked for the same genetic change in his relatives and detected the same change in 3 of his relatives. This change has been previously reported to be associated with erythrocytosis. A C>G change as heterozygous form in the promoter region of the beta-globin gene was detected in a male patient (Fig. 2). The patient was a 58-year-old man who had experienced syncope 2 years ago. His highest hematocrit value was 52%. This genetic change had not been reported in the medical literature previously and had not been present in any relevant databases. This genomic change was designated as HBB: c.-169 C>G according to the nomenclature reported by Human Genome Variation Society. A T>C change at the +96 3’UTR of the beta-globin gene HBB: c. +96 T>C (rs34029390) was detected in heterozygous form in a male patient (Fig. 2). He was a 25-year-old patient who had been admitted with complaints of headache and had a hematocrit value of 52%. He did not have any history of arterial or venous thrombosis.

HBB: c.328 G>A (Hb San Diego) in heterozygous form was detected in a patient and his mother (Fig. 2). She was a 64-year-old woman who had undergone phlebotomy due to erythrocytosis for 20 years. A change in G>C at position 380 of EGLN1 gene (EGLN1: c.380G> C [p. Cys127Ser]) was detected in heterozygous form in a woman (Fig. 2). She was 45 years old, and her highest hematocrit value was 52%. Such a change had already been associated with erythrocytosis. There were no significant differences between the 2 groups in terms of age, gender, hypertension, diabetes, smoking, and lipid profile (P > .05 for all). Hb (18.1 ± 1.2 versus 14.5 ± 0.8 g/dL; P < .001) and hematocrit (58.2% ± 2.7% versus 43.9% ± 1.8%; P > .001) values were significantly higher in the IE group compared with the control group (Table 1).

3.2. Cardiovascular findings

There was no difference between the patients and control group members in terms of LV diameters, PWT, ejection fraction, mitral A wave, IVCT, ejection time, septal a, septal s, lateral a, lateral s, E/septal c, and E/lateral c and LVM (P > .05 for all). IVST (11.08 ± 0.87 vs 10.56 ± 1.13; P = .019), left atrial diameter (34.5 ± 2.8 vs 33.2 ± 2.6; P = .039), DT (128.84 ± 16 vs 121 ± 10.3; P = .019), IVRT (84.86 ± 20.46 vs 73.93 ± 6; P = .001), and LV myocardial performance index (MPI; 0.52 ± 0.14 vs 0.44 ± 0.03; P = .006) values were significantly higher in the patient group compared with the control group. Mitral E (62.80 ± 16.16 vs 74.48 ± 10.9; P = .001), E/A (0.93 ± 0.29 vs 1.14 ± 0.2; P = .001), septal e (8.75 ± 2.71 vs 10.52 ± 1.66; P = 0.001), septal e/a (0.89 ± 0.33 vs 1.1 ± 0.3; P = 0.006), lateral e (9.2 ± 1.7

Figure 2. Genetic mutations for familial erythrocytosis in 5 patients. EGLN1 = Egl-9 family hypoxia inducible factor, EPOR = erythropoietin receptor, HBB = hemoglobin beta-globin.
Table 1

| Variables | Patients (n = 64) | Controls (n = 30) | P |
|-----------|------------------|------------------|---|
| Age (mean ± SD) | 46.33 ± 2.7 | 46.72 ± 3.1 | .879 |
| Gender (F/M), n (%) | 3/61 (4.7/95.3) | 1/29 (3.3/96.7) | .540 |
| Hypertension, n (%) | 26 (40.6) | 11 (36.7) | .320 |
| DM, n (%) | 6 (9.37) | 2 (6.66) | .120 |
| Smoking, n (%) | 27 (42.2) | 10 (33.3) | .680 |
| TC (mg/dL) | 193.0 ± 40.3 | 199.6 ± 47.4 | .504 |
| HDL-C (mg/dL) | 42.1 ± 11.1 | 41.56 ± 10.2 | .825 |
| LDL-C (mg/dL) | 118.4 ± 31.4 | 125.15 ± 40.8 | .408 |
| Triglyceride (mg/dL) | 176.8 ± 88 | 176.3 ± 103.8 | .981 |
| Hemoglobin (g/dL) | 18.1 ± 1.2 | 14.5 ± 0.8 | .001 |
| Hematocrit (%) | 58.2 ± 2.7 | 43.9 ± 1.8 | .001 |

DM = diabetes mellitus, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, SD = standard deviation, TC = total cholesterol.

A positive correlation was found between Hb values and IVST (r = 0.267; P = 0.012), there was a negative correlation between Hb values and E (r = −0.319; P = .002), E/A (r = −0.410; P = .001), septal e (r = −0.301; P = .004), septal e/a (r = −0.337; P = .001), and lateral e/a (r = −0.321; P = .002) (Table 3). Related parameters comparing 2 groups are shown in Table 2.

4. Discussion

Our study was one of the very few genetic research studies conducted on familial erythrocytosis in our country. In addition to this fact, a rare cardiovascular risk assessment dimension was incorporated into the study. When one probes for similar research on a global scale, it becomes obvious that combined genetic and cardiovascular risk assessment studies have been limited both in number and in the number of patients included in such studies. In our study, we began by collecting data on mutations that have been known to have caused familial erythrocytosis. The second phase of our study consisted of how patients with IE presented themselves clinically. We assessed whether erythrocytosis caused any increase in cardiovascular risk. In connection with this, echocardiography results of patients both in the FE and in the control group were studied. Based on the echocardiography data available, it has been understood that cardiovascular risk increased in FE patients independent of the underlying genetic mutations.

In our study, 2 new genomic changes that had not been associated with FE and 3 known mutations associated with familial polycythemia have been detected. One of the 2 new genomic changes, HBB: c. + + 96 T>C (rs34029390) (HBB: c. + + 96 T>C, rs34029390) was detected in heterozygous form in 1 individual. It has been reported that HBB: c. + + 96 T>C is a silent mutation that does not cause any change in the Hb level in neither heterozygous nor homozygous states, but its compound heterozygosity with beta-thalassemia mutations increases the severity of beta-thalassemia.[24] The possible relationship of this mutation with erythrocytosis needs further investigation. In another case, a C>G change in heterozygous form in the promoter region of the beta-globin gene was detected. This change had not been previously reported in the medical literature and has not been existent in the relevant databases. Our medical literature search has revealed that HBB: c.-169 C>G change was involved in the binding site of the erythroid specific transcription factor, which has an effect on the expression of the beta-globin gene.[25] Further studies are needed to show whether this mutation has an effect on the oxygen affinity of Hb.[24]

Table 2

| Variables | Patients (n = 64) | Controls (n = 30) | P |
|-----------|------------------|------------------|---|
| LVDD (mm) | 46.58 ± 0.04 | 46.14 ± 2.17 | .429 |
| LVSD (mm) | 32.25 ± 0.26 | 31.21 ± 1.78 | .058 |
| NIWT (ms) | 11.08 ± 0.87 | 10.56 ± 1.13 | .019 |
| RWT (ms) | 10 ± 1.1 | 9.6 ± 0.9 | .077 |
| LAD (mm) | 34.5 ± 2.8 | 33.2 ± 2.6 | .039 |
| EF (%) | 64.2 ± 3.6 | 65.2 ± 2.1 | .20 |
| E wave (cm/s) | 62.80 ± 16.0 | 74.48 ± 10.9 | .001 |
| A wave (cm/s) | 69.15 ± 14.44 | 65.4 ± 9.3 | .197 |
| E/A ratio | 0.93 ± 0.29 | 1.14 ± 0.2 | .006 |
| DT (ms) | 128.84 ± 16.0 | 121 ± 10.3 | .019 |
| IVRT (ms) | 84.86 ± 20.46 | 73.93 ± 6.0 | .001 |
| VCT (ms) | 51.64 ± 19.6 | 46.3 ± 5.5 | .146 |
| ET (ms) | 264.6 ± 25.4 | 272.6 ± 14.2 | .113 |
| LV MPI | 0.52 ± 0.14 | 0.44 ± 0.03 | .006 |
| Septal e (cm/s) | 8.75 ± 2.71 | 10.52 ± 1.66 | .001 |
| Septal a (cm/s) | 10.12 ± 2.26 | 9.8 ± 1.9 | .568 |
| Septal s (cm/s) | 8.06 ± 1.94 | 7.45 ± 1.32 | .08 |
| Septal e/septal a | 0.89 ± 0.33 | 1.1 ± 0.3 | .006 |
| Lateral e (cm/s) | 9.2 ± 1.7 | 10.5 ± 3.1 | .001 |
| Lateral a (cm/s) | 10.5 ± 2.8 | 9.6 ± 2.3 | .125 |
| Lateral s (cm/s) | 9.00 ± 2.92 | 8.91 ± 2.17 | .839 |
| Lateral e/lateral a | 0.93 ± 0.3 | 1.2 ± 0.26 | .003 |
| E/septal e | 7.54 ± 1.95 | 7.24 ± 1.38 | .464 |
| E/lateral e | 6.6 ± 1.9 | 7.12 ± 2.2 | .257 |
| LVM (g) | 178 ± 29.4 | 166 ± 29 | .071 |

A = late phase peak flow velocities, DT = deceleration time, E = early phase peak flow velocities, E/A = early phase peak flow velocity/late phase peak flow velocity, EF = ejection fraction, ET = ejection time, VCT = isovolumetric contraction time, IVRT = isovolumetric relaxation time, LAD = left atrial diameter, LV MPI = left ventricular myocardial performance index, LVDD = left ventricular diastolic diameter, LVSD = left ventricular systolic diameter, PWT = posterior wall thickness, SD = standard deviation.

Table 3

| Variables | Patients (n = 64) | Controls (n = 30) | P |
|-----------|------------------|------------------|---|
| Hb | 0.245 | .021 |
| LAD | 0.194 | .068 |
| E | -0.319 | .002 |
| E/A | -0.410 | .001 |
| DT | 0.267 | .012 |
| IVRT | 0.205 | .054 |
| Septal e | -0.301 | .004 |
| Septal e/septal a | -0.337 | .001 |
| Lateral e | -0.199 | .061 |
| Lateral e/lateral a | -0.321 | .002 |
| LV MPI | 0.142 | .133 |

DT = deceleration time, E = early phase peak flow velocities, E/A = early phase peak flow velocity/late phase peak flow velocity, IVRT = isovolumetric relaxation time, LAD = left atrial diameter, LV MPI = left ventricular myocardial performance index.

Except for those newly defined HBB mutations, Hb San Diego was also detected in 1 case and her son. Hb San Diego has been described in subjects of various origins, and only 1 case had been previously reported from Turkey so far.[21] We detected EPOR exon 8 Arg437 His mutation (c.1310G>A) in 1 case and 3 family members. Pathological mutations in EPOR exon 8 lead to familial congenital polycythemia (FPCP). The diagnosis of FPCP has been made in a patient with IE in the presence of low or normal EPO serum level and a family history compatible with autosomal transition.[24] The diagnosis of FPCP can be confirmed by detection of heterozygote EPOR mutation in
only 12%–15% of cases. To date, mostly, mutations of EPOR, which cause PFCP, within exon 8 have been described.[49] Until
now, 2 cases with c.1310G>A variant have been described in the literature. One of them was a male 24 years of age who
required regular phlebotomy. The other one was a 52 years old patient with a clinical history of recurrent venous thrombosis
with normal Hb and hematocrit levels and no familial history of hematologic disorder.[50] Our case represents the third subject in
literature detected with this variant.

We detected EGLN1:c.380G>C (p.Cys127Ser) missense mutation in 1 case, which had been first detected by Lorenzo
et al.[31] The EGLN1 mutations cause secondary FE of the familial erythrocytosis type 3 online Mendelian inheritance in
man classification. Already, EGLN1 encodes PHD2, which is the principal negative regulator of (HIFs) and EPAS1 encoding
HIF-2a.[32] It has been reported that EGLN1 c.380G>C together with EGLN1 c.12C>G as a haplotype can be a component of a
complex array of genetic adaptations involving both PHD2 and HIF-2a that results in lower Hb in Tibetan highlanders.[33]

In PV and other Ph-negative myeloproliferative neoplasms, there is a high incidence of thrombosis due to an acquired
thrombophilic state.[34] The mechanism of the acquired thrombophilic state in these diseases is multifaceted. Prothrombotic
features of myeloproliferative neoplasm clone–derived blood cells, not only erythrocytes but also platelets and leukocytes and
procoagulant changes of normal vascular cells, in response to inflammatory stimuli, can be considered as mechanisms of this
thrombophilic state. In fact, hyperviscosity due to erythrocytosis does not seem as an important factor for thrombosis in PV.[35]
Chuvash polycythemia causes thrombotic and hemorrhagic vas-
cular complications that lead to early mortality usually before
the age of 40 years.[16–38] Apart from Chuvash polycythemia, the
association of FE and IE with thrombosis and cardiovascular
complications are not clear.[39]

In previous publications, it had been demonstrated that PV and some types of anemia had impaired LV diastolic func-
tions.[21] Aging, coronary artery disease, diabetes, hypertension, and atrial fibrillation are important factors contributing to dia-
stolic dysfunction.[40] To clearly assess the contribution of erythro-
ocytosis in diastolic dysfunction, we have excluded individuals
with coronary artery disease and atrial fibrillation. Also, there
was no difference between the groups in terms of age, gender,
hypertension, diabetes, and smoking habits. In this study, we
found some parameters such as decreased mitral E wave, trans-
mitral E/A ratio, septal e, septal e/a, lateral e, and lateral e/a ratio
and increased DT and IVRT values, as evidences of decreased
LV relaxation. In the presence of mild diastolic dysfunction,
relaxation of the left ventricle is impaired; early filling of the
left ventricle in diastole becomes difficult; accordingly, mitral
E wave, and septal e and lateral e wave amplitudes decrease,
and E wave DT and IVRT get prolonged. These findings show
that stage 1 diastolic dysfunction exists in patients with IE. In
addition, the atrial contribution increases exaggeratedly due to
late filling of the left ventricle in diastole, thereby increasing the
mitral A wave amplitude. As a result, mitral E/A, septal e/a, and
lateral e/a ratios decrease.[41]

We have not found any difference between the cases with erythrocytosis and healthy controls for cardiovascular risk
factors such as hypertension, lipid parameters, diabetes, and
smoking. Furthermore, we assessed cardiovascular status of all subjects with transthoracic echocardiography. It has been
suggested that transthoracic echocardiography can be used a
detection method for LV hypertrophy, which is considered as
a cardiovascular risk factor in asymptomatic adults who have
hypertension.[42] LV hypertrophy is an important risk factor for
diastolic dysfunction. Therefore, we have performed M-mode
and Doppler echocardiography for our patients and healthy
controls. M-mode echocardiography showed a higher thick-
ness of interventricular septum in our cases than the control

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