

# **Community Health Screening Report**

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COMMUNITY HEALTH SCREENING FOR JAK2 (V617F) MUTATION  
LUZERNE, SCHUYLKILL, and CARBON COUNTIES, PENNSYLVANIA

COST RECOVERY NUMBER: A08X

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Agency for Toxic Substances and Disease Registry  
Division of Health Assessment and Consultation  
Atlanta, Georgia 30333

COMMUNITY HEALTH SCREENING REPORT

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Prepared By:

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## **Executive Summary**

Polycythemia vera (PV) is a rare blood disease in which the bone marrow makes too many red blood cells. The extra red blood cells make the blood thicker than normal. As a result, blood clots can form more easily and block blood flow through arteries and veins. This can lead to heart attacks and strokes. Other symptoms of PV include headaches, dizziness, itching, and vision problems. The major goal of treatment is to prevent thrombotic events. This is accomplished by removal of blood through periodic phlebotomy and drug treatment to suppress red blood cell production by the bone marrow.

In 2009, ATSDR identified a cluster of polycythemia vera cases near the nexus of Luzerne, Schuylkill, and Carbon Counties and a second cluster in Schuylkill County, Pennsylvania. In response to these findings, ATSDR conducted community health screening. The purpose of the screening was to offer testing of blood specimens from residents of the tri-county area for the JAK2(V617F) genetic mutation. This mutation is found in approximately 95% of patients with PV, as well as in some patients with other kinds of myeloproliferative neoplasms (MPN).

ATSDR, in cooperation with the Pennsylvania Department of Health, collected blood samples from 1,170 self-selected residents, and tested them for the JAK2 mutation. About 1.2 % of participants in this screening, who had not been previously diagnosed with MPN or had symptoms of MPN, tested positive for the JAK2 genetic mutation. Available data are not adequate to conclude whether this represents an increased prevalence of the JAK2 mutation in the population tested.

A person with a positive JAK2 mutation is at increased risk of developing PV, but it is not known if everyone with this mutation eventually develops PV. It is possible that other mutations or predisposing factors are necessary for disease progression. All participants in this community health screening who had a positive test result were offered a referral for a free medical evaluation at Geisinger Health System. JAK2 positive individuals should have periodic evaluations to monitor for possible disease onset or progression.

## Introduction

In 2005, the Pennsylvania Department of Health (PA DOH) released a report in which they identified a statistically significant higher incidence of polycythemia vera (PV) in Luzerne and Schuylkill Counties as compared to the rest of the state. This finding was based on reports of PV to the state cancer registry since 2001. In response to this finding, PADOH invited the Agency for Toxic Substances and Disease Registry (ATSDR) to conduct an investigation in which they attempted to (1) locate all cases of PV in Luzerne and Schuylkill Counties and in adjacent Carbon County, (2) confirm the diagnosis of PV among the registry and non-registry cases using a test for the JAK2 (V617F) genetic mutation, and (3) describe the characteristics of these individuals (Seaman et al. 2009). An analysis of these data identified clustering of PV cases near the nexus of Luzerne, Schuylkill, and Carbon Counties and in a second area of Schuylkill County (Seaman et al. 2009). The cause of the cluster of PV cases is not known.

Polycythemia vera is a myeloproliferative neoplasm characterized by increased production of red blood cells and often other blood cell lines. In the past, PV was diagnosed by clinical symptoms and traditional laboratory hematological tests. In 2005, researchers discovered a mutation in the Janus Tyrosine Kinase 2 gene (JAK2 (V617F)), which plays a pivotal role in the regulation of blood cell production (Levine et al. 2005, Kralovics et al. 2005). Approximately 95 percent of PV patients carry this acquired mutation (Baxter et al. 2005). In addition, about half of patients with the closely related blood diseases, essential thrombocythemia (ET) and primary myelofibrosis (PMF), also carry the JAK2<sup>1</sup> mutation (Baxter et al. 2005). In this report, these three disorders will be collectively referred to as myeloproliferative neoplasms (MPN)<sup>2</sup>. Since the discovery of the JAK2 mutation, the presence of the mutation has become an important diagnostic criterion for identifying patients with PV and for reducing the potential for misdiagnosis of persons with elevated red blood cell counts.

Polycythemia vera is not considered to be a hereditary disease, although familial clustering of cases has been reported (Kralovics et al. 2003). In a recent prospective study of 1,638 patients, PV was diagnosed at a median age of 62 years, and only 4 percent of cases were below the age of 40 years (Finazzi et al. 2005). In this study, males accounted for slightly more than half (58%) of the cases. The cause (or causes) of PV is (are) not known. Risk factors, including familial predisposition, occupational history, and exposure to radiation and toxic chemicals, have been evaluated, but no clear evidence of causality has been established (Najean et al. 1998).

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<sup>1</sup> In this report, the “JAK2” mutation refers to the “JAK2 (V617F)” mutation, unless otherwise specified.

<sup>2</sup> Other MPNs, such as chronic eosinophilic leukemia and mastocytosis, that have not been associated with the JAK2(V617F) mutation, will not be considered in this report.

## **Purpose**

The purpose of this community health screening was to offer JAK2 testing to residents of the tri-county area of Luzerne, Schuylkill, and Carbon Counties in northeastern Pennsylvania. ATSDR conducted this Community Health Screening in cooperation with the PA DOH.

The intent of this screening was to address individual health concerns regarding the high prevalence of PV identified in this area. As a service oriented project, this screening was not conducted in such a way as to produce information that is generalizable to the entire population within the PV cluster area. Results are only applicable to the individual participants and cannot be used to assess the prevalence of this mutation in the general population, since those electing to be tested were self-identified.

## **Methods**

### **A. Criteria for participation**

Any current, full-time resident of Luzerne, Schuylkill, or Carbon County who had lived in the tri-county area for 1-year or longer was eligible to participate in this screening. Because of the concerns of some community members, ATSDR also accepted a few participants who were not currently residing in the area, but who had previously been long-term residents of the area.

### **B. Recruiting participants**

ATSDR focused its recruitment on residents who were living in the area of the previously-identified PV cluster near the confluence of the three counties. Because PV occurs primarily in people above the age of 40, recruitment was focused on this segment of the population. However, since this was a community health service, there was no minimum age requirement for eligibility.

Recruitment efforts included the following:

- (1) Representatives of ATSDR attended a public meeting on July 9, 2009 in Tamaqua. At this meeting, we described the Community Health Screening to the community and solicited participants.
- (2) We met with community leaders and asked them to encourage their friends and neighbors to participate.
- (3) We provided informational flyers to community leaders, who distributed them throughout the target area.

(4) We talked with the local news media, which ran newspaper and television stories on the community health screening being offered.

### **C. Sample collection**

Participants in the screening were instructed to call a toll-free telephone number to make an appointment at one of the three health screening centers (Attachment A). At the screening center, the participant was required to sign an informed consent form prior to testing. Children and their parents were required to complete an applicable consent or assent form. After consent was obtained, a phlebotomist collected, by veinipuncture, a 6-ml blood sample from each participant in a heparinized tube.

ATSDR conducted the community health screening over two periods of time. The first round of screening was held on August 3-6 and August 10-14, 2009. During this time period, blood samples were obtained from 356 participants.

A second round of screening was conducted on October 19-22, October 26-29, and November 2, 2009. During these time periods, four evening sessions were held to accommodate people who could not attend a daytime session. During the second round of screening, blood samples were collected from 814 participants. Therefore, we collected a total of 1,170 blood samples during rounds 1 and 2.

To protect privacy, ATSDR labeled the tubes with a coded identification number prior to shipping them to the laboratory for analysis.

### **D. Sample handling and shipping**

At the end of each day, ATSDR packaged the blood samples in protective Styrofoam containers and shipped them at room temperature by overnight delivery to the Mount Sinai Medical Center laboratory in New York City for analysis. Blood samples collected during the evening sessions were kept at refrigerator temperature (35-38 °F) prior to and during shipping the next day.

### **E. Laboratory analysis**

Blood samples were analyzed at the Molecular Pathology Laboratory at the Mount Sinai School of Medicine using published methodologies (Ishii et al. 2006). The laboratory is regulated under the Clinical Laboratory Improvement Amendments (CLIA) of 1988 as qualified to perform high complexity clinical testing. Pursuant to the requirements of CLIA of 1988, the laboratory has established the test's accuracy and precision.

All blood samples were tested as follows: DNA was extracted from granulocytes and amplified by multiplex Polymerase Chain Reaction (ARMS-PCR) enabling simultaneous detection of mutant and normal alleles specific for a sequence of the JAK2 gene (V617F). PCR products were separated by agarose gel electrophoresis and detected by fluorescence. The detection limit for this assay is 0.05% of JAK2 V617F allele in a

background of wild type allele.

Blood samples that tested positive for the JAK2 (V617F) mutation were further tested by a confirmatory, quantitative real-time PCR test as follows: Total DNA was extracted, amplified in two separate real-time PCR steps using primer sets specific for either JAK2 (V617F) or wild type JAK2. Using established standard curves, absolute quantitation of JAK2 (V617F) allele and wild type allele could be made. The JAK2 (V617F) allele burden was computed as the ratio of JAK (V617F) alleles to the total alleles. The limit of detection is 0.05% JAK2 (V617F) allele in a wild-type background.

#### **F. Notifying participants of test results**

All participants received written notification of their test results and a copy of the laboratory report. Upon request, ATSDR also mailed the test results and laboratory report to a health care provider designated by the participant. ATSDR telephoned participants with positive test results to personally discuss the findings and answer any questions.

#### **Results**

A total of 1,170 blood samples from rounds 1 and 2 of the community health screening were tested for the JAK2 mutation. Of the 1,170 samples collected, 19 (1.6%) tested positive for the JAK2 mutation. Five of the participants with positive test results had previously been diagnosed with or had clinical symptoms of PV (2) or ET (3). The other 14 JAK2 mutation-positive individuals, representing 1.2% of the participants, gave no history of an MPN and reported no signs or symptoms of illness.

Figure 1 depicts the age distribution of the test participants. The mean age of all of the participants was 54 years old, and the mean age of the 19 participants with the JAK2 mutation was 63 years old. A recent study reported that the median age of patients with PV was about 62 years old (Finazzi et al. 2005). The ages of the participants in this screening with a positive test result ranged from 23 to 88 years old. Ten of the positive test results were from men, and nine were from women.

Figure 1. Number of participants vs. age of participants in JAK2 community health screening. Total number of participants = 1,170.

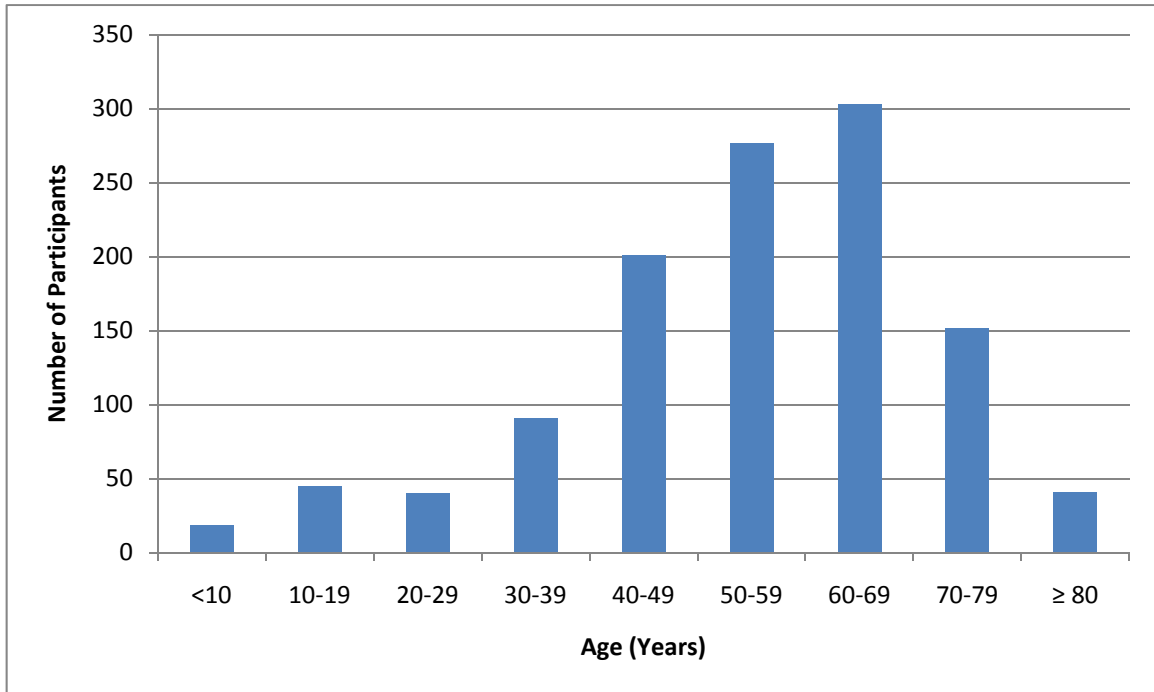


Table 1 provides summary statistics on the ages of the participants in the screening and how long they have lived at their current address.

Table 1. Ages of participants and length of residence.

	<b>Number</b>	<b>Age (range)</b>	<b>Age (mean)</b>	<b>Length of residence (mean)</b>
All participants	1,170	2-92	54	24
JAK2+ with disease <sup>(1)</sup>	5	50-77	64	20
JAK2+ without disease	14	23-88	63	28

(1) Diagnosed with or had clinical symptoms of an MPN



## Discussion

The health significance of the presence of the JAK2 (V617F) genetic mutation is not known. About 95 percent of PV patients test positive for the JAK2 mutation, and about half of patients with ET and PMF test positive for the mutation (Baxter et al. 2005). Therefore, it is likely that a person who tests positive for the JAK2 mutation is at increased risk for one of these three myeloproliferative neoplasms.

Although the JAK2 (V617F) mutation is present in most PV patients, other JAK2 mutations have also been detected in PV patients. Researchers have identified at least five other mutations in the JAK2 gene in the small subset (5%) of PV patients who lack the JAK2 (V617F) mutation (Scott et al. 2007; Li et al. 2008).

Although about 95 percent of PV patients have the JAK2 mutation, it is not known whether everyone with the JAK2 mutation will eventually develop the disease, or the time frame between acquisition of the mutation and disease onset. It has been suggested that other mutations or predisposing factors are also involved in the development and progression of PV (Nussenzveig et al. 2007; Dupont et al. 2007).

In this screening, the JAK2 positive individuals were confirmed by a second analytical test that measured the percent of the DNA from blood granulocytes that carried the JAK2 mutation. This is referred to as the JAK2 allele burden. In a published study, patients with clinically diagnosed PV had JAK2 allele burdens ranging from 1 to 100 percent with an average of 52 percent (Antoniolli et al. 2008). Longitudinal studies of PV patients have shown that the percent of mutated JAK2 DNA increases over time (Rumi et al. 2006). Furthermore, studies have shown that clinical symptoms (splenomegaly, pruritus, thrombosis) and laboratory parameters (increased hematocrit) are more likely to occur in patients with a high JAK2 allelic burden (Vannucchi et al. 2007).

In the JAK2 positive cases from this screening, the JAK2 allele burden ranged from 0.1 % to 26.8 % (detection limit of 0.05 %). The allele burden was higher in cases with previously diagnosed MPN or symptoms (8.4 %) than in cases without previously diagnosed disease (2.1 %). In 12 of 14 cases without a MPN diagnosis, the allele burden was 1.2 % or less.

Table 2. JAK2 (V617F) allele burden in positive cases

	<b>Number</b>	<b>Allele Burden % (range)</b>	<b>Allele Burden % (average)</b>
JAK2+ with disease <sup>(1)</sup>	5	2.2 – 26.8	8.4
JAK2+ without disease	14	0.1-22.5	2.1

(1) Diagnosed with or had clinical symptoms of MPN

The meaning and prognosis of a low allele burden is unknown. One possibility is that people with low allele burdens of the JAK2 mutation have only recently acquired the mutation. Disease may not occur until clonal expansion of the mutated cell line results in a higher proportion of JAK2 mutated cells. It is possible that other mutations or predisposing host factors are necessary for progression to clinically detectable disease. Alternatively, such persons may not progress to develop illness. Regardless, these individuals should be closely monitored for signs and symptoms of MPNs.

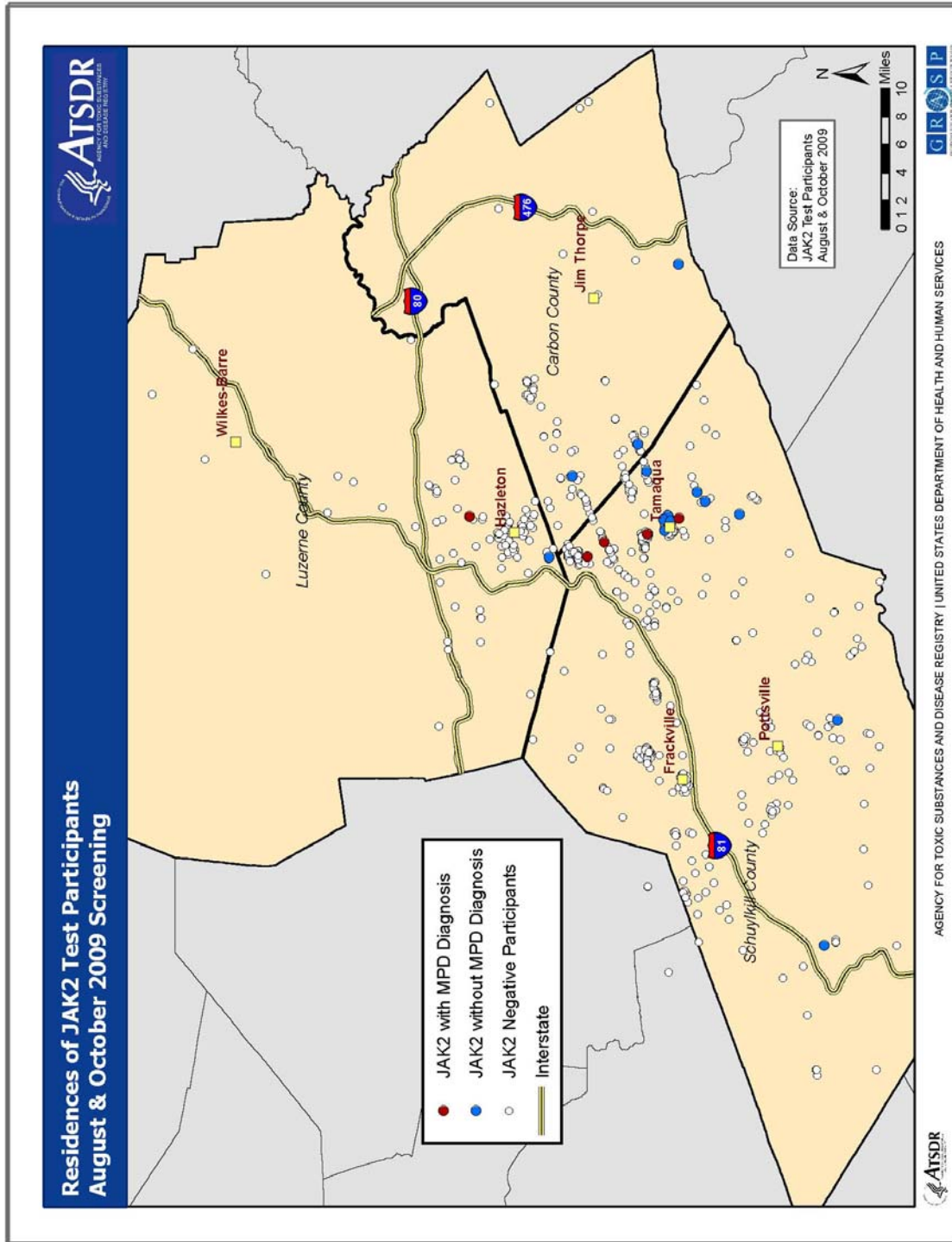
In this screening, 14 of 19 participants who tested positive for the JAK2 mutation did not self-report a diagnosis or clinical symptoms of PV or other MPN. It may be that because we used a very sensitive JAK2 screening test, we detected people who had only recently acquired the mutation. In PV patients, the initial symptoms are mild and progress as the disease develops over a period of years.

### **Geospatial analysis**

The geographical distribution by residence of the people tested in this screening is depicted in Figure 2. Many of the participants with a positive JAK2 test lived in the previously identified PV cluster area. This area was also the focus of our recruitment efforts.

This screening was offered as a public health service, and the participants were self-selected, rather than being randomly chosen. For this reason, it is not valid to compare the prevalence rates of JAK2 positive tests across the tri-county area. Although the prevalence rate of JAK2 positive mutations appears to be higher in the cluster area, self-selection of the participants may have introduced a bias for some unknown risk factor. This is known as participation bias. As an example of this type of bias, some of the positive individuals were known to have an MPN or to have symptoms.

Figure 2. JAK2 test results



## Prevalence of JAK2 positive cases in the general population

In this community health screening, 1.2 percent of the participants with no previous diagnosis or clinical symptoms of PV or other MPN tested positive for the JAK2 mutation. It is possible that the prevalence rate of JAK2 positive tests in our screening was influenced by the age distribution of the participants (Figure 1). The median age of all participants in this screening was 57 years old, which is considerably older than the national median age of 37 years old. MPNs are known to be more frequent in older persons. The median age at diagnosis of PV is 62 years old (Finazzi et al. 2005), and the median age of the JAK2 positive individuals in our screening was 68 years old.

Several published studies have examined the prevalence of the JAK2 mutation in convenience samples from various populations (Table 3). In these studies, the percent of people who tested positive for the JAK2 mutation ranged from 0.9 to 10 percent.

Table 3: Prevalence of JAK2 (V617F) positive test results in published studies.

Author	Number tested	Percent positive (%)	Analytic Sensitivity (%)	Location
Sideon 2006	57	10 (5/57)	0.01	Belgium
Sutton 2007	57	1.8 (1/57)	0.1	OK, TN
Xu 2008	3,935	0.9 (37/3935)	0.25	China
Rapado 2008	149	2.0 (3/149)	0.01-1	Spain
This report	1,165	1.2 (14/1165)	0.05	PA

The screening test results in Pennsylvania should not be directly compared to these other published test results. These other studies used different analytical methodologies and instruments, and the methodologies had different analytical sensitivities. Also, selection criteria for the persons being tested varied between studies. Except for the study in China, the number of persons tested was small, leading to imprecise estimates of prevalence.

The studies in Table 3 indicate that a small percent of the population without clinical symptoms of myeloproliferative neoplasms have the JAK2 mutation in their circulating white blood cells. However, because of the limitations and variability in these studies, we are not able to conclude whether the prevalence of the JAK2 mutation in the ATSDR screening population is elevated. To address this question, ATSDR is currently funding a study to determine the JAK2 prevalence in the general population. This study, being conducted by Geisinger Health System, is looking at the background prevalence of the JAK2 mutation from a region in Pennsylvania outside of Luzerne, Schuylkill, and Carbon Counties.

### Follow-up of Positive Cases

It is prudent public health policy to consider JAK2 positive individuals to be at increased risk for developing PV or other myeloproliferative diseases. Therefore, we recommend

that JAK2 positive individuals undergo periodic medical evaluations to monitor for possible disease onset or progression. Early diagnosis and treatment of PV may improve the patient's quality of life and reduce the risk of life-threatening complications.

All participants in this EI with a positive JAK2 test were offered a referral for a follow-up medical evaluation. In addition, longitudinal evaluation of these individuals is indicated to: (1) help us to learn more about the progression of the disease and (2) help determine if everyone with the mutation goes on to develop overt disease. Therefore, ATSDR encouraged all JAK2 positive individuals to participate in an ongoing study at Geisenger medical facilities that will follow these individuals over time.

### **Child Health Considerations**

Children are a low risk population for PV. The JAK2 mutation is an acquired mutation rather than an inherited one. Therefore, the prevalence of the JAK2 mutation is expected to increase with age, as does the prevalence of PV. The prevalence of PV in the general population for adults 65-74 years old is 99.5 cases per 100,000, whereas the prevalence of PV in patients 0-34 years old is 0.31 cases per 100,000 (Ma et al. 2008).

In the ATSDR screening, the JAK2 mutation was not detected in any children. The youngest participant who tested positive for the JAK2 mutation was 23 years old; the next youngest positive participant was 43 years old.

### **Conclusions**

(1) ATSDR conducted community health screening for the JAK2 (V617F) mutation in self-selected residents of Luzerne, Schuylkill, and Carbon Counties. A total of 1.2 % of participants in this screening, who had not been previously diagnosed with polycythemia vera or other myeloproliferative neoplasms or had symptoms of these diseases, tested positive for the JAK2 (V617F) genetic mutation. Available studies are not adequate to conclude whether this represents an increased prevalence of the JAK2 mutation in those tested.

(2) A person with a positive JAK2 mutation may be at increased risk of developing PV or other MPN, but it is not known if everyone with this mutation will develop an MPN or the time frame for this to occur.

(3) Additional studies are needed to better define the background prevalence of the JAK2 mutation in the tri-county area, other areas in Pennsylvania, and nation-wide.

### **Recommendations**

(1) Participants with a positive test result should have periodic medical evaluations to monitor for the development of PV and other myeloproliferative neoplasms and to assure necessary treatment if indicated.

(2) Residents who are concerned about their risk of developing PV or other myeloproliferative neoplasms should consult with their personal physician who can arrange for JAK2 genetic testing through a private laboratory.

### **Public Health Action Plan**

ATSDR is funding studies at the Pennsylvania Department of Health, Drexel University, University of Pittsburgh, Mount Sinai Medical Center, Geisinger Health System, and ATSDR/CDC to investigate PV and other MPN in the tri-county area.

In addition, ATSDR is funding the following studies that will provide additional information on the prevalence rate of JAK2 mutations.

1. Geisinger Health Systems will screen a random sample of 2,500 people from the PV cluster area for the JAK2 mutation.
2. Geisinger Health Systems will screen 6,000 participants from outside the tri-county area to determine the “background” prevalence of the JAK2 mutation in Pennsylvania.
3. ATSDR has submitted a proposal to determine the prevalence of the JAK2 mutation in the 1999-2002 NHANES DNA sample set (7,900 samples). This will reflect the national “background” prevalence.

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**Attachment A: Blood Collection Centers**

Schuylkill Mall  
Community Meeting Room  
Route 61 and I-81  
Frackville, PA 17931

Saint Jerome's Catholic Church  
School Gymnasium and Alumni Room  
250 West Broad Street  
Tamaqua, PA 18252

Hazleton General Hospital  
700 East Broad Street  
O & E Building – 1st floor  
Hazleton, PA 18201