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ENVIRONMENTAL MEDICINE GENOME BANK (EMGB): CURRENT COMPOSITION

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13. ABSTRACT *(Maximum 200 words)*
The Environmental Medicine Genome Bank (EMGB) project is an ongoing effort to identify and characterize genes relevant to environmental illnesses and to human physical performance. To accomplish this, the EMGB banks DNA samples from human volunteers who have participated in environmental and human performance studies or material obtained under approved Brigham and Women's Hospital protocols that would otherwise have been discarded. The EMGB maintains a registry of this phenotypic information. The EMGB can be used to identify polymorphisms in genes that are potentially of interest to environmental medicine and to obtain an estimate of the frequency of these polymorphisms in young, healthy U.S. adults because of the ethnically diverse and geographically dispersed backgrounds of the donors. Additionally, this resource also serves as a valuable source of control material for genetic studies of human diseases, such as asthma. The project is performed as part of a cooperative research and development agreement (CRDA) with the Division of Pulmonary and Critical Care Medicine at Brigham and Women's Hospital.

B-lymphocytes immortalized using the Epstein Barr Virus (EBV) have been added to the EMGB in attempts to maintain stocks of genetic material that are phenotypiclly characterized. These samples include cells and DNA from asthmatics as well as characterized non-asthmatics.

This report provides updated information about the samples currently stored in the EMGB. It is intended as a reference document for researchers who wish to make use of this resource, and fulfills the annual reporting requirement of CRDA number DAMD 17-00-0017.

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ENVIRONMENTAL MEDICINE GENOME BANK (EMGB): CURRENT COMPOSITION

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Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR70-25 and USAMRMC Regulation 70-25 on the Use of Volunteers in Research. For protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law CFR 46.

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EXECUTIVE SUMMARY

The Environmental Medicine Genome Bank (EMGB) project is an ongoing effort to identify and characterize genes relevant to environmental illnesses and to human physical performance. To accomplish this, the EMGB banks DNA samples from human volunteers who have participated in environmental and human performance studies or material obtained under approved Brigham and Women's Hospital protocols that would otherwise have been discarded. The EMGB maintains a registry of this phenotypic information. The EMGB can be used to identify polymorphisms in genes that are potentially of interest to environmental medicine and to obtain an estimate of the frequency of these polymorphisms in young, healthy U.S. adults because of the ethnically diverse and geographically dispersed backgrounds of the donors. Additionally, this resource also serves as a valuable source of control material for genetic studies of human diseases, such as asthma. The project is performed as part of a cooperative research and development agreement (CRDA) with the Division of Pulmonary and Critical Care Medicine at Brigham and Women's Hospital.

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INTRODUCTION

Based on recent reports, it seems likely that there is a significant genetic contribution to some aspects of human physical performance (1-5;7;9) and to the susceptibility to environmental illness and injury. However, very few candidate genes have been identified, in part because few laboratories have access to large populations of well-characterized subjects drawn from a wide variety of genetic backgrounds. The U.S. Army Research Institute of Environmental Medicine (USARIEM) is uniquely qualified to undertake a search for these genes, by virtue of its access to Army personnel and its ability to define precisely those phenotypes relevant to environmental illnesses and human performance.

Large numbers of samples are typically needed to identify genes that contribute to complex traits (2). Accordingly, the Environmental Medicine Genome Bank (EMGB) banks DNA samples obtained from donor white blood cells and catalogues phenotypic information obtained over the course of multiple approved studies. By pooling samples and data from several studies, it becomes possible to undertake genetic analyses that would otherwise not be feasible.

The EMGB serves as an Institute resource, and anonymous aliquots from the bank are available to individual investigators upon request. This document summarizes the current contents of the bank.

DNA samples of the EMGB entered previously were purified from terminally differentiated cell lines and were non-renewable sources of genetic material. Immortalized lymphocytes have been added to the EMGB so the cells can be grown at any time to extract the DNA. These cell lines represent a renewable source of genetic material. The samples of the EMGB that have not been immortalized will eventually be irreplaceably consumed.

MATERIALS AND METHODS

VOLUNTEERS

With the exception of study #1 (normal controls) and study #7, subjects were participants in other USARIEM studies of environmental medicine and physical performance (Table 1). All subjects gave consent in accordance with 45 CFR 46. In study #1, subjects were recruited directly for the purpose of creating a core cohort of DNA samples from anonymous, young, otherwise healthy volunteers. In study #7, the 56 immortalized samples were obtained through approved Brigham and Women's Hospital studies using material that otherwise would have been discarded. These samples are classified by asthmatic phenotypes such as FEV1, %FEV and skin testing for atopy.

From each volunteer 20 ml of blood were drawn into 10 ml, heparin-containing tubes. Samples drawn from locations remote from the analytical laboratory were shipped overnight (at room temperature) to the laboratory for processing.

DNA ISOLATION AND STORAGE

DNA is obtained from leukocyte nuclei after erythrocyte lysis, using the QIAamp Maxi Kit (Qiagen, Inc., Santa Clara, CA). The isolated DNA is stored in aqueous solution (in water), at a concentration of 35-150 ng / μl as determined by UV absorption at 260 nm.

Additional DNA samples have been added to the EMGB since the July 2000 Technical Note (14) and include immortalized B-lymphocytes. These samples were purified without nuclei isolation and therefore may include mitochondrial DNA. The DNA from these samples have been standardized to 50 ng / μl dilutions and tested using CLONTECH's β - Actin PCR primers (Cat# 5402), as outlined in the manufacture's instructions. This is performed to ensure constant quality of the template DNA during genetic studies. All future samples will meet this standard before aliquots are produced or dispensed.

ALIQUOTS

The EMGB was replenished by diluting the master DNA samples to 50 ng / uL, unless otherwise labeled, and dispensed in 25 uL aliquots. To minimize damage from repeated freeze-thaw cycles, each sample is divided into a master sample and several aliquots at the time of original isolation. At present, all samples are maintained at -80°C. Aliquots are used until exhausted. The master samples are thawed only when new aliquots are needed.

LYMPHOCYTE IMMORTALIZATION PROTOCOL

Immortalization Solution

The Esptein Barr Virus (EBV) Infection Solution is made from EBV-infected marmoset leukocyte cells. Marmoset cells produce viable virus, which is shed into the cell supernatant of the growth media (8). The supernatant is considered to be potent after the cells have been starved for approximately 1 week, when the phenol red in the media has turned yellow. The cell suspension is collected in a 50 ml centrifuge tube and centrifuged at 400 x g for 10 min. The supernatant is filtered with a 0.2 μm filter to ensure the removal of marmoset cells. This virus containing filtrate solution is the infection solution that can be stored at 4°C for up to 3 months, or can be frozen at -80°C for long-term storage.

PBMC Purification

Subject peripheral blood monocytes (PBMC) are purified for immortalization. Twenty (20) mL of room temperature, heparin treated blood is added to 20 ml of Hank's balanced salt solution (HBSS) in a 50 ml conical centrifuge tube. Contents are gently mixed to make a homogenous suspension. Fifteen (15) mL of Histopaque – 1077 (Sigma cat# 10771) is added to two separate 50 mL Falcon centrifuge tubes. Histopaque is a density gradient solution that separates blood into two cell types: red blood cells (RBC) and PBMC. The blood-HBSS solution is slowly layered on top of each Histopaque solution. A sharp interface should be seen between the two solutions. The tubes are centrifuged at 400 x g for 30 min with the brake left off. Centrifugation results in four distinct density layers: plasma; PBMC, Histopaque 1077, and RBC. The second layer, the PBMC, is the buffy coat layer that contains the cells required to produce the immortalized cell lines. This layer is extracted and pipetted into a separate 50 mL centrifuge tube and washed with HBSS. Cells are then centrifuged at 400 x g for 10 minutes, the supernatant decanted, and the washing step repeated. Recovery should be approximately $1x10^6$ cells per mL of blood drawn.

Immortilization of PBMC

The PBMC are suspended in 2 ml of EBV Infection Solution and the vented tube is placed in the incubator at 37°C for 1-2 hours. 2 ml of media (RPMI w/ L-Glutamine, Penn-Strep, and 20% heat-inatcivated FBS) is added to the cell suspension. 1 ml aliquots are added into four wells of a treated 24-well culture plate. Cell are placed at 37°C in 5% CO_2 for 4 days and transferred to T-25 or T-75 culture flasks at a concentration of $0.5x10^6$ cells per milliliter.

The immortalized cell lines are stored in liquid nitrogen in a step-wise fashion at concentrations of 5×10^6 cells per milliliter in DMSO Cell Freezing Media by Sigma (Catalog # C-6164). Cells are first stored at -20°C for 2 hours, then -80°C for 12 hours before finally transferring the cells to liquid nitrogen.

RESULTS

CONTRIBUTING STUDIES AND SAMPLE USE

Studies that have contributed samples to the EMGB and the current inventory of samples are listed in Table 1. This table also lists some of the phenotypic information available and summarizes some of the genotypic information that has been obtained on the samples to date. So far, 7 studies have contributed samples to the EMGB. DNA was obtained from most (but not all) donated samples, and some samples (especially those with low DNA yields) have been used in their entirety. At present, the bank contains DNA samples from 342 distinct donors.

Samples from the EMGB have been used in nine genetic studies through July 2001. This has diminished the total DNA aliquots available for future experiments. Eighteen master aliquots from the original 283 terminal samples have been consumed. The changes in the total number of available samples during the past year are presented in Figure 1. Fifteen samples have been consumed and are no longer available for study. Nineteen samples have 5 or less aliquot available for study. Sixty samples have been added to the EMGB that have their B-lymphocytes immortalized. These represent a renewable supply of DNA that will not be completely consumed as long as the cell lines are maintained.

Table 1. Summary of the Contents of the EMGB.

Study #	Study Designation	PI, Division	Study Location	Study Dates	Samples Submitted	Samples Currently Banked	Phenotypic Information	Genotypes Studied
1	Normal Controls	Sonna, TMD	USARIEM	Mar - Apr 1998	74	68	1,2	ACE, EOT,Gal-3, MCP-4, IL-18, CatS, NOS3
2	H98-07 Physical Fitness of Soldiers Entering and Completing Basic Combat Training and its Role in Injury Incidence	Sharp, MPD	Ft. Jackson, South Carolina	Jun - Jul 1998	151	142	1,5,6,7,9,10	ACE, NOS1, EOT, Gal-3, MCP-4, IL-18, CatS, NOS3
3	H97-10 Warfighter Physiologic Status Monitoring: Body Core Temperature, Blood Oxygen Saturation and Environmental Symptoms during an Expedition to Mt. Logan, Canada	Muza, TMD	Mt. Logan, Canada	May - Jun 1999	13	13	1,2,3,4,9	ACE, EOT, Gal-3, MCP-4, IL-18, CatS, NOS3
4	H98-09 Effect of Residence at Low and Moderate Altitudes on Arterial Oxygen Saturation at Moderate-to-High Altitudes	Muza, TMD	Pike's Peak, Colorado	Jun 1999	40	40	1,2,4,8,9	EOT, Gal-3, MCP-4, IL-18, CatS, NOS3
5	H99-12/A-9212 Role of Exercise During Intermittent Exposures to Hypobaric Hypoxia on Acclimation to 4300 m	Beidleman, TMD	USARIEM	Oct 1999	8	8*	1,2,4	EOT, Gal-3, MCP-4, IL-18, CatS, NOS3
6	H99-03 Role of Leukotrienes in High Altitude Illness	Muza, TMD	USARIEM	Jan - Feb 2000	9	8*	1,2,9	Gal-3, MCP-4, IL-18, CatS, NOS3
7	5-Lo Immortalized Lymphocytes of Phenotyped Asthmatics and Non-Asthmatics.	Lilly, Pulmonary	BWH	April 2001	56	56	1,3,4	C3a

*(One subject participated in both studies and is only counted once for purposes of the EMGB.)

Table 1 (Continued)

Key to Available Phenotypic Information

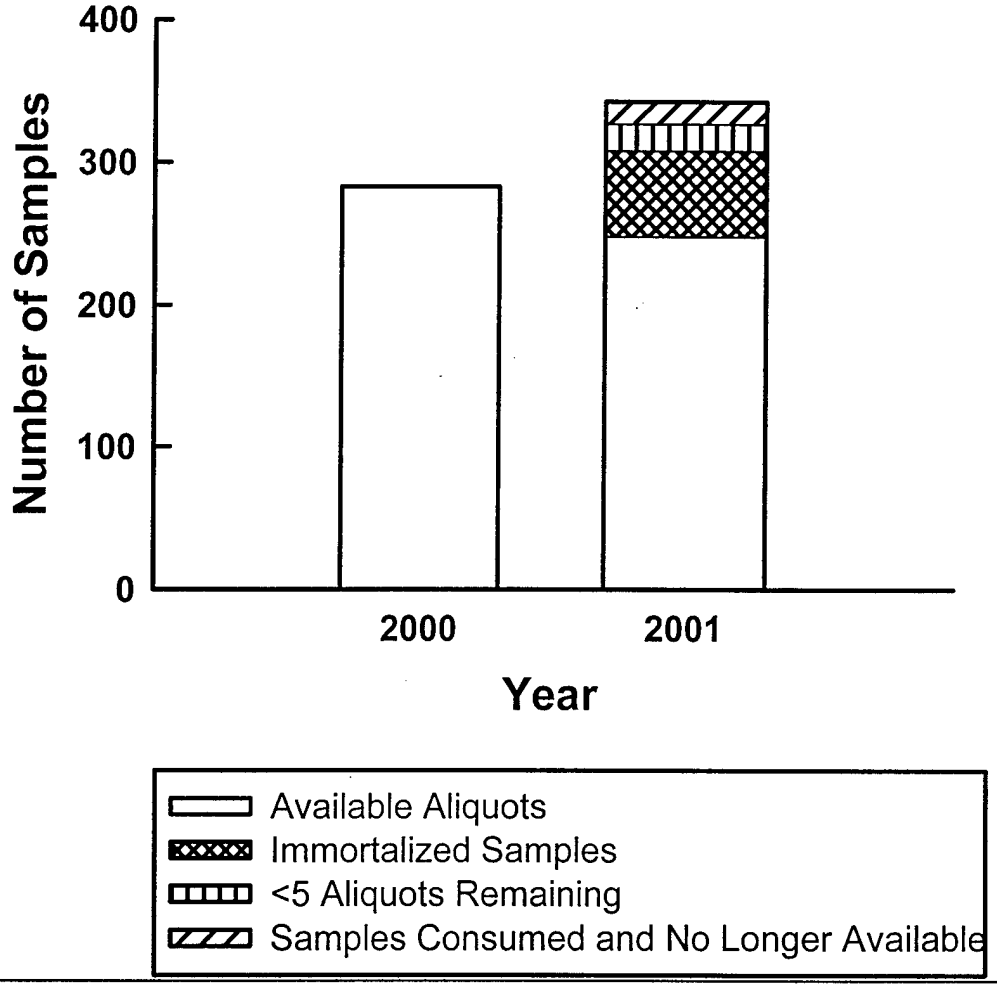
1. Age, race and gender
2. Smoking status
3. History of asthma or exercise-induced bronchospasm
4. Spirometry data
5. Spirometry before and after exercise
6. Army Physical Fitness Test scores
7. Army Physical Fitness Test scores before and after basic training
8. Oxygen saturation with increasing altitude
9. Height and weight

Key to Genotypes

ACE: Angiotensin Converting Enzyme Insertion/Deletion Polymorphism, Intron 16
NOS1: Neuronal Nitric Oxide Synthase CA repeat Polymorphism, Exon 29
EOT: Eotaxin 23ALA → 23 THR Polymorphism
MCP-4: Monocyte Chemoattratctant Protein 4, Chromosome 17q11.2 promoter mutation
Gal-3: Galectin 3, Chromosome 14, Exons 3 and 6
CatS: Cathepsin S, Chromosome 1
NOS3: Nitric Oxide Synthase 3, Chromosome 7
IL-18: Interleukin 18, Promoter Region, Chromosome 2q12
C3a: Complementary Cascade Protein

Figure 1. Sample Availability in the EMGB.

15 Samples have been completely consumed. 19 Samples have five or less aliquots before being completely consume 60 Immortalized samples have been added in 2001. There were 283 samples in 2000.



In the year 2000, the Angiotensin Converting Enzyme (ACE), nitric oxide synthase CA (NOS1), and the eotaxin (EOT) genotypes have been tested for population studies using the EMGB. The Angiotensin Converting Enzyme I/D polymorphism in intron 16 has been implicated by some as a marker of physical performance (5;7;9), though others have questioned this association (11;12). We concluded that ACE genotype does not have a strong effect on aerobic power or muscular endurance in healthy, young American adults drawn from an ethnically and geographically diverse population (13). The neuronal NOS1 CA repeat polymorphism in exon 29 has been found to be associated with asthma, in a study to which the EMGB contributed samples

(6). The EMGB was also used to determine the frequency of a novel mutation in the eotaxin gene that limits eotaxin secretion (10).

DEMOGRAPHIC INFORMATION

A summary of the ages, races and genders of the subjects for whom current samples exist in the EMGB is given in Table 2.

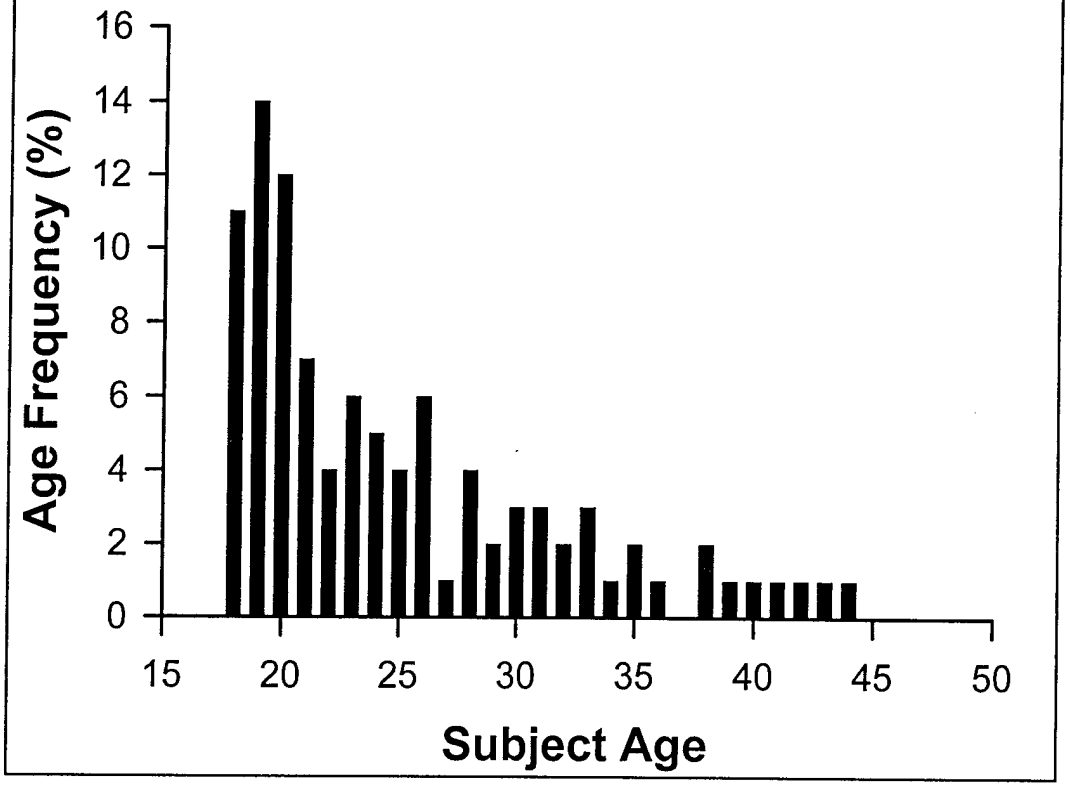
Table 2. Demographic Characteristics of the EMGB.
This table only includes sample numbers that still have DNA available for further experimentation.

Demographic	N =	%
Gender		
Male	175	56%
Female	123	44%
Ethnic Origin		
Asian	10	4%
African	51	19%
Caucasian	183	67%
Hispanic	24	9%
Native American	3	1%
Other	1	<1%
N = 298	Median	Interquartile Range
Age	23	19.25 - 29

The distribution of the ages of the donors at the time of sample collection is illustrated in Figure 2. The median age of the subjects who have donated to the EMGB is 23 (interquartile range, 19.25 - 29). Slightly more than half (59%) of the subjects are male. Subjects of ethnic backgrounds other than Caucasian donated about a third of the samples; subjects of African-American origin donated 19% of the samples. Homes of origin are known for 59% of the subjects and include 44 different U.S. states, two U.S. territories, and four foreign nations.

Figure 2. EMGB Age Distribution.

Distribution of subject ages, at the time of donation, who have donated samples to the EMGB. The histogram only includes samples currently available.



DISCUSSION

The EMGB consists of DNA samples obtained from an ethnically diverse and geographically dispersed population of subjects. This diversity makes the EMGB a valuable resource for several types of genetic studies. At present, we envision three principal uses for the bank. First, given a gene known or suspected to be of interest to environmental medicine, the EMGB can be used to identify new polymorphisms in this gene and to obtain an estimate of the frequency of these polymorphisms in young, healthy U.S. adults. Because the information collected in the EMGB includes both ethnic origin and gender, it is also possible to compare allele frequencies across important demographic subgroups. Second, the EMGB is a source of control material for genetic studies of human diseases, such as asthma. Third, some of the donor phenotypes in the EMGB (particularly those from study #2) are characterized well enough to allow genetic association studies.

Previously, one significant limitation of the EMGB was that samples were not easily renewed. Now that techniques have been adopted to immortalize lymphocytes, anonymous samples from individual donors can provide a renewable source of DNA.

In summary, the current heterogeneity of the EMGB makes it a valuable resource of anonymous samples for genetic research. It has already proven to be of value in collaborative studies of human disease (6;10) and used to examine the genetic basis of physical performance (13). The addition of a technology to allow source DNA to be renewed has greatly enhanced the longevity and potential value of this resource.

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