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Newborn Screening for Hermansky-Pudlak Syndrome Type 3 in Puerto Rico

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Abstract

Background—Hermansky-Pudlak Syndrome (HPS) is an autosomal recessive disorder characterized by albinism, mucocutaneous bleeding, and storage of ceroid material in macrophages[1]. Patients that are not easily identified by physical characteristics (mostly HPS-3 patients) may have hemorrhagic complications with trauma or surgery.

Objective—To determine the prevalence of HPS-3 in Puerto Rican newborns using DNA pooling technique.

Design/Methods—Twelve percent of annual Puerto Rican births were tested randomly by PCR for the HPS-3 mutation, using pooled DNA extracted from dried blood samples.

Results—HPS-3 mutation was detected in 75 samples. Two newborns were found to be homozygous. Carrier frequency was 1:85 (1.18%).

Conclusions—The HPS-3 carrier frequency found (1.18%) justifies universal newborn screening in Puerto Rico. DNA pooling reduces time and labor in newborn screening thus facilitating early diagnosis and treatment of children with HPS-3 and the provision of genetic counseling to parents and relatives.

Keywords

Hermansky-Pudlack Syndrome; bleeding; albinism; platelets; DNA pooling

Introduction

Hermansky-Pudlak Syndrome (HPS) (MIM #203300) is a genetically heterogeneous disorder in which mutations in one of several genes affect melanosomes biogenesis, platelet

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dense bodies, and lysosomes[1]. A defect in the gene products required for the formation of these organelles can result in HPS[2]. The disease is characterized by oculocutaneous albinism, bleeding, and ceroid storage in tissues [3].

Albinism results from inability of cutaneous and ocular melanocytes to produce fully pigmented melanosomes due to melanogenic proteins mistrafficking[2]. The bleeding results from platelet dysfunction due to deficiency of its dense bodies[4]. A lysosomal storage defect is responsible for the development of granulomatous colitis and pulmonary fibrosis[5,6] in some patients. Eight types of HPS have been described in humans, each one associated with mutations in eight different genes and with characteristic clinical features[7,8]. HPS is a rare disease worldwide, but is a common single gene disorder among Puerto Ricans[9].

Newborn screening represents one of the major advances in child health care since the 1970's. Approximately 4.1 million infants are screened annually in the US for genetic disorders[10]. Each state is responsible for its own newborn screening program. When the gene responsible for a condition has been identified and mutations within a gene have been characterized, direct mutation analysis can be used to identify such a condition[11]. These techniques are specific identifying the mutations, and do not require testing of parents or relatives. The HPS phenotype is so variable that patients might not be easily recognized on routine examination, thus many of them take years to be identified and receive treatment.

Since a 3,904 bp deletion in the HPS3 gene is a founder mutation that accounts for a large proportion of HPS cases in Puerto Rico (PR) and its diagnosis of albinism is often missed, we conducted a prospective study to determine the prevalence of this genetic disorder in Puerto Rican newborns. Although genetic testing is an expensive alternative for large scale studies, DNA pooling has been used to reduce cost and time using fewer PCR reactions[12]. Therefore, determining the viability of using pooled DNA to reduce cost, by allowing multiple samples to be tested at once, was also an objective in this project. Cost reduction is an important factor to consider for the addition of *HPS3* genetic testing to a newborn screening panel.

Materials and Methods

A random sample of 6,164 infants born in PR in 2005 (12% of newborns for that year) was tested for the 3,904 bp deletion in an *HPS3* gene. The samples were coded to protect the subject's identity. Geographic data was available for each sample given by the mother's hometown.

This study was approved as a full review by the University of PR Medical Sciences Campus Institutional Review Board (Protocol #2060199). Dried blood specimens were collected on Guthrie cards. Extraction was carried out using 5% Chelex-100 resin as described[13]. To validate the PCR, we isolated DNA from 40 samples with known results from a reference research laboratory (UPR MSC RCMI Molecular Genetics Lab). We measured the DNA extracted from these samples finding that their concentrations varied from 2.0 to 500 ng/μl. A mutation-specific multiplex PCR for the first exon and 5′flanking DNA of the human *HPS3* gene was performed as described[14] and results were compared to those of the reference laboratory. The criteria to determine the optimal number of samples to be pooled together were: the quality of the PCR product (intensity of the band), the sensitivity, reproducibility and cost effectiveness. Pools of 2, 4, 5 and 10 samples were prepared using equal volumes of each sample (10 μl). The DNA concentration was not measured since a concentration of only 2ng/μl is sufficient to get adequate amplification, and the average DNA concentration obtained using extraction with 5% Chelex resin was 48.74ng/μl. Based

on the established criteria while keeping the DNA dilution to a minimum possible, we selected a maximum pool size of 5 samples.

To validate the DNA pooling technique we analyzed 125 samples individually and in 25 pools. Fourteen of these samples were known heterozygous; all other samples were known negatives. We added the 14 heterozygous samples randomly to 14 pools. The results of single sample analysis were compared to the pooled samples results for reproducibility. Additionally we tested the first 1,500 samples of the study individually and also in 5-sample pools for a total of 300 pools.

Results

PCR amplification of control samples from 40 individuals matched in 100% of the cases, obtaining a robust amplification product even when 2 ng/μl of DNA was used in the reaction, thus validating the PCR conditions in our laboratory.

For the purpose of this study, the island of PR was divided in three sections considering the distribution pattern of positive samples for the HPS3 mutation (Figure1). The resulting sections were designated as Western, Central, and Eastern regions. A total of 75 (1.22%) samples were positive for the mutation, 73 samples were heterozygous and 2 resulted homozygous for the HPS3 mutation (Table 1). 53 of the positive samples were from Puerto Rico's Central region, including the two homozygous. The carrier frequency in the Western region was 0.39% (6 carriers); 0.97% (16 carriers) for the Eastern region; and 1.7% (51 carriers and 2 homozygous) in the Central region. The mutant allele frequency (q) in the Central region, calculated from the number of heterozygous individuals obtained, is 0.009, whereas when q is calculated from the number of homozygous individuals, it equals 0.026. Chi-square analysis of the Central region samples showed that there is significant difference between the observed and expected allele frequencies ($\chi^2 = 12.33$, $P < 0.0005$), mainly due to an excess of homozygotes. Genetic studies of the admixed Puerto Rican population evaluating ancestry informative markers have shown that the population is heterogeneous,[15,16,17] which may explain the differences in allele frequencies seen in our results. Nevertheless, Hardy-Weinberg equilibrium was not met in the central region possibly due to population substructure and/or occult inbreeding. Within that region we have towns that have a high carrier frequency ranging from 7% in Barranquitas, 6.4% in Orocovis to 6.0% in Ciales, and two HPS3 cases detected in towns with lower but significant carrier frequencies, Cayey (2.4% carrier frequency) and Coamo (2.1% carrier frequency).

When testing using a pooled-sample strategy, if a sample positive for the mutation falls within the group of samples in the pool, all samples in that tube have to be tested individually (Figure 2). Given a carrier frequency of approximately 1.5 over a base of 100 samples, 20 five-sample runs plus ten individual repetitions, versus running these samples individually will provide a 70 percent test reduction. This makes 5-sample pools cost-efficient in terms of reagent consumption and labor intensity.

The pooling technique validation resulted in 100% reproducibility and all added positive samples were identified. Verification was carried out and all heterozygote samples detected individually were also unequivocally identified as heterozygotes when tested in pools.

Discussion

Two founder mutations in two different HPS genes (*HPS1* and *HPS3*) account for most of the HPS cases in PR[18]. The *HPS1* gene 16 bp duplication in exon 15 is widely prevalent in the Northwestern region[19], and the 3,904 bp deletion in the *HPS3* gene was found in patients from a small mid-central region of the island[14] (figure 3). HPS-3 is caused by mutations in

the *HPS3* gene, which consist of 17 exons, and has been mapped to chromosome 3q24. Two founder mutations of the HPS3 gene have been discovered worldwide; the 3,904-bp deletion discovered in central PR, and 1303+1G→A splice-site mutation that causes skipping of exon 5 in Ashkenazi Jews[20].

In PR, the founder mutation causing HPS-3 was identified in a study by Anikster et al. Six families from the towns of Aibonito, Naranjito and Barranquitas were examined. Based on their medical history and clinical findings 13 individuals from these families were identified and studied [14].

HPS-3 patients usually present mild skin hypopigmentation, thus, diagnosis of albinism is often missed. These patients are usually identified by an ophthalmologist while evaluating children for nystagmus or visual acuity problems, or by the hematologist evaluating for bleeding or an abnormal coagulation profile. Spontaneous bleeding is not common in HPS-3, but hemorrhagic complications associated with surgical or dental procedures occur. Disturbances with visual acuity are often detected late in infancy or childhood. Delayed diagnosis and treatment usually results in poor academic performance and late genetic counseling for their parents.

The findings in our study correlate with findings from previous studies[14,18] in which the central region was identified as a “hot spot” area for HPS-3 due to a founder effect. The founder mutation described by Anikster et al has spread over time throughout the rest of the island. Apparently this is the result of increased mobility of the PR population during the last decades. Our data also suggests that the relatively high island-wide carrier frequency of the *HPS3* mutation (1.18% of the 6,164 samples tested) in newborns justifies screening for this mutation, specially in the central region of the island of PR, where the HPS3 mutation carrier frequency is 1.7%, which is of more significance in some towns within the region where the carrier frequency is as high as 7.0% (1:14 and a probability of 1 homozygote for each 816 births). The higher than expected prevalence of HPS-3 seen in two of these towns (Coamo and Cayey) could be the result of population structure and/or occult inbreeding.

Based on these findings the PR newborn screening program will begin screening for HPS-3 using DNA pooling. This will help in the early diagnosis and treatment of children with this condition and the provision of genetic counseling to patient’s parents and relatives early in life.

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Figure 1.

Map of Puerto Rico indicating the municipalities where the HPS3 gene 3.9Kb deletion mutation was detected in newborn dried blood samples. The number of circles within the municipalities correspond to the number of samples with a given genotype. Heterozygous individuals in municipalities with a carrier frequency >2.0% are identified with red circles, while those from regions with carrier frequencies <2.0% are indicated by green circles. Individuals homozygous for the HPS3 gene deletion are indicated in blue.

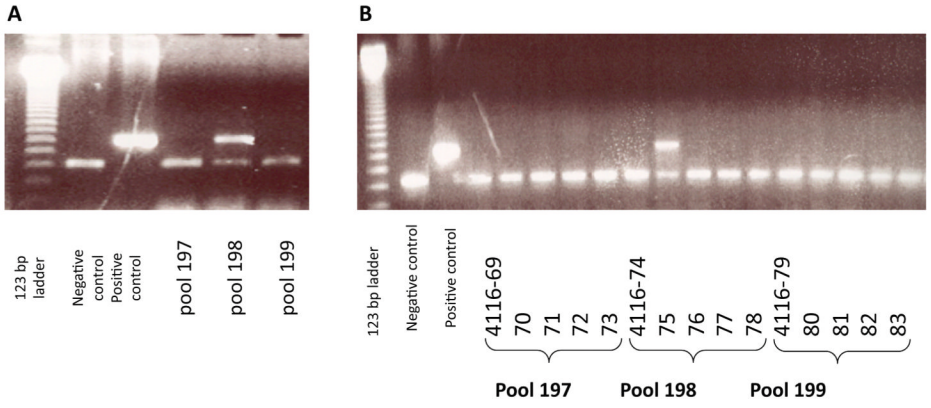

Figure 2.

PCR Analysis for the HPS3 gene 3.9Kb deletion mutation using DNA pooling. A) DNA analysis of control and pooled DNA samples; B) DNA analysis of control and dried blood samples contained in pool 197, 198 and 199 amplified individually.

Figure 3.

Map of Puerto Rico showing the area where HPS-1 and HPS-3 were reported to be most prevalent. Adapted from Figure 1 in Anikster et al, 2001. The municipalities with high prevalence of HPS-1 are located in the Northwest (shaded in grey), while the ones with high prevalence of HPS-3 are located close to the Center of the island (shaded in light blue).

Table 1

Results of DNA testing of Puerto Rican Newborns for the 3,904 bp deletion in the *HPS3* gene

HPS-3 3,904 bp deletion genotype								
Region	**NN**	**NM**	**MM**	**Total**	**p**	**q**	**Estimated population frequency↑**	**Carrier Frequency**
Western	1,518	6	0	1524	0.998	0.002	1:250,000	0.39
Central Expected←	2,945 (2943)	51 (54.5)	2 (0.25)	2998	0.991 0.974*	0.009 0.026*	1:12,345 1:1,515*	1.80 5.0*
Eastern	1,626	16	0	1642	0.995	0.005	1:40,000	0.97
Total	6089	73	2	6164	0.994	0.006	1:27,777	1.18

*These values were calculated using a mutant allele frequency calculated from $\sqrt{q^2} = \sqrt{2\ 7\ 2\ 9\ 9\ 8} = 0.0258$.

←χ^2 =12.33 with one degree of freedom

↑Based on population for the island of Puerto Rico reported by the USA Census Bureau for the year 2000.

TABLE 2

Pilot Study for the Detection of Hermansky-Pudlak Syndrome

Population and Births Data for 2005					Study Data	
Municipality	**Population**	**Percent Population (%)**	**Births**	**Births/Total Births**	**Test Run Per Municipality**	**Positive Results**
Adjuntas	18,719	0.48	269	0.53	25	
Aguada	44,173	1.13	499	0.99	65	1
Aguadilla	66,457	1.70	784	1.55	108	
Anasco	29,649	0.76	352	0.70	59	1
Arecibo	101,876	2.60	1166	2.31	139	
Barceloneta	22,651	0.58	369	0.73	53	1
Cabo Rojo	51,184	1.31	593	1.17	74	
Camuy	38,411	0.98	432	0.85	48	1
Florida	14,645	0.37	186	0.37	35	
Guanica	22,673	0.58	288	0.57	32	
Guayanilla	23,567	0.60	332	0.66	31	
Hatillo	41,788	1.07	476	0.94	55	1
Hormigueros	17,288	0.44	191	0.38	31	1
Isabela	46,719	1.19	547	1.08	57	
Lajas	27,333	0.70	330	0.65	34	
Lares	36,978	0.94	355	0.70	42	
Las Marias	11,957	0.31	121	0.24	17	
Maricao	6,552	0.17	72	0.14	9	
Mayaguez	95,862	2.45	1064	2.10	161	
Moca	43,047	1.10	544	1.08	66	
Penuelas	28,774	0.74	446	0.88	30	
Quebradillas	27,341	0.70	348	0.69	41	
Rincon	16,099	0.41	200	0.40	19	
Sabana Grande	27,272	0.70	366	0.72	47	
San German	37,768	0.96	438	0.87	59	
San Sebastian	46,615	1.19	580	1.15	60	
Utuado	34,967	0.89	399	0.79	63	
Yauco	48,139	1.23	583	1.15	64	
Western Region A Sub-total	1,028,504		12,330	24.39	1,524	6
Aguas Buenas	30,711	0.78	374	0.74	41	1
Aibonito	26,942	0.69	444	0.88	55	1
Barranquitas	30,397	0.78	453	0.90	57	4
Bayamon	222,512	5.68	2735	5.41	381	4
Catano	27,720	0.71	351	0.69	54	1
Cayey	47,076	1.20	692	1.37	84	2
Ciales	20,515	0.52	296	0.59	50	3
Cidra	46,388	1.19	648	1.28	69	3
Coamo	38,936	0.99	537	1.06	47	1

Population and Births Data for 2005					Study Data	
Municipality	**Population**	**Percent Population (%)**	**Births**	**Births/Total Births**	**Test Run Per Municipality**	**Positive Results**
Comerio	19,662	0.50	261	0.52	41	
Corozal	38,457	0.98	555	1.10	85	1
Dorado	35,371	0.90	565	1.12	81	3
Guaynabo	102,376	2.62	1236	2.44	147	3
Jayuya	18,067	0.46	262	0.52	22	
Juana Diaz	52,558	1.34	749	1.48	83	
Manati	48,355	1.24	664	1.31	84	2
Morovis	32,112	0.82	526	1.04	71	2
Naranjito	30,248	0.77	413	0.82	70	3
Orocovis	25,010	0.64	307	0.61	31	2
Ponce	182,661	4.67	2438	4.82	204	2
Salinas	32,037	0.82	467	0.92	55	1
San Juan	423,453	10.82	5326	10.53	660	2
Santa Isabel	26,253	0.67	394	0.78	37	1
Toa Alta	75,932	1.94	939	1.86	112	3
Toa Baja	94,928	2.43	1282	2.54	144	3
Vega Alta	39,182	1.00	516	1.02	64	1
Vega Baja	64,009	1.64	927	1.83	122	3
Villalba	30,027	0.77	388	0.77	47	1
Central Region B Sub-total	1,861,895		24,745	48.94	2,998	53
Arroyo	19,039	0.49	288	0.57	21	1
Caguas	141,872	3.62	1978	3.91	245	1
Canovanas	46,226	1.18	769	1.52	119	
Carolina	187,995	4.80	2258	4.47	302	4
Ceiba	18,279	0.47	185	0.37	25	1
Culebra	2,050	0.05	16	0.03	2	
Fajardo	42,366	1.08	539	1.07	56	
Guayama	44,989	1.15	654	1.29	62	2
Gurabo	40,812	1.04	581	1.15	52	1
Humacao	60,317	1.54	876	1.73	111	1
Juncos	39,135	1.00	547	1.08	62	2
Las Piedras	37,855	0.97	525	1.04	69	2
Loiza	33,997	0.87	398	0.79	58	
Luquillo	20,455	0.52	275	0.54	32	
Maunabo	12,866	0.33	167	0.33	18	
Naguabo	24,041	0.61	371	0.73	34	1
Patillas	20,278	0.52	242	0.48	36	
Rio Grande	55,420	1.42	724	1.43	78	
San Lorenzo	43,557	1.11	506	1.00	61	
Trujillo Alto	83,082	2.12	927	1.83	125	

Population and Births Data for 2005					Study Data	
Municipality	**Population**	**Percent Population (%)**	**Births**	**Births/Total Births**	**Test Run Per Municipality**	**Positive Results**
Vieques	9,291	0.24	149	0.29	7	
Yabucoa	40,175	1.03	511	1.01	67	
Eastern Region C Sub-total	1,024,097		13,486	26.67	1642	16
Total	3,914,496	100.00	50,561	100.00	6164	75