Identification of Genetic and Epigenetic Marks Involved in Population Structure

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Abstract

Population structure is well known as a prevalent and important factor in genetic studies, but its relevance in epigenetics is unclear. Very little is known about the affected epigenetic markers and their connections with genetics. In this study we assessed the impact of population diversity on genome wide single nucleotide polymorphisms (SNPs) and DNA methylation levels in 196 participants from five ethnic groups, using principle and independent component analyses. Three population stratification factors (PSFs) were identified in the genomic SNP dataset, accounting for a relatively large portion of total variance (6%). In contrast, only one PSF was identified in genomic methylation dataset accounting for 0.2% of total variance. This methylation PSF, however, was significantly correlated with the largest SNP PSF (r = 0.72, p = 1E-23). We then investigated the top contributing markers in these two linked PSFs. The SNP PSF predominantly consists of 8 SNPs from three genes, SLC45A2, HERC2 and CTNNAL2, known to encode skin/hair/eye color. The methylation PSF includes 48 methylated sites in 44 genes coding for basic molecular functions, including transcription regulation, DNA binding, cytokine, and transferase activity. Among them, 8 sites are either hypo- or hyper-methylated correlating to minor alleles of SNPs in the SNP PSF. We found that the genes in SNP and methylation PSFs share common biological processes including sexual/multicellular organism reproduction, cell-cell signaling and cytoskeleton organization. We further investigated the transcription regulatory network operating at these genes and identified that most of genes closely interact with ID2, which encodes for a helix-loop-helix inhibitor of DNA binding. Overall, our results show a significant correlation between genetic and epigenetic population stratification, and suggest that the interrelationship between genetic and epigenetic population structure is mediated via complex multiple gene interactions in shared biological processes, through possibly, SNP-dependent modulation and ID2 repressor function.

Introduction

Genetic association studies analyze the connection between phenotypes (e.g. symptom of disease) and genotypes (e.g. single nucleotide variation) to identify genetic effects on the prevalence of diseases or other traits of interest. Such a study usually investigates large samples [1,2], possibly from more than one population [3,4,5]. Additionally, the availability of genome wide genotyping has led to a rapid increase in the number of genome wide association studies (GWAS), which can analyze simultaneously over a million genetic loci in one study. The confounding effect of population diversity on allelic frequencies may alter the results and inflate false positives [6,7,8]. Thus it is critical to understand the population structure in a given sample set and account for it before performing association analyses with other factors.

A similar situation holds true for epigenetic studies, i.e. studies of heritable phenomena that regulate gene expression and hence genetic function without altering the DNA sequence. Epigenetic inheritance has been documented in a substantial number of animal studies and human studies [9,10,11,12]. DNA methylation, one of the epigenetic mechanisms, is essential for normal cellular differentiation, parental imprinting and X inactivation, and is also prone to perturbation by environmental factors. A number of DNA methylation studies have identified methylation changes with diet, smoking, drinking, age, gender and lifestyle [13,14,15,16,17,18]. DNA methylation changes are also associated with carcinogenesis where genome-wide hypomethylation and some gene specific hypermethylation were observed in cancer cells [9]. However, the relationship between the diversity of population and the diversity of epigenetic marks is poorly understood. Only a couple of studies have demonstrated ethnic difference in the overall DNA methylation level [19] or methylation of some specific genes involved in cancer [20]. Furthermore, Nielsen et al. have observed the ethnic diversity of OPRM1 gene methylation in heroin addicts [21]. Ultimately, the identification of the population structure embedded in epigenetic data will be critical for understanding the epigenetic influence on disease processes and the interaction between epigenetics and environmental factors.

Additionally, the relationship between genetic and epigenetic marks in terms of population structure remains unknown. To
address this issue in this study we sought to answer the following questions: Does a similar population structure exists in genetic and epigenetic datasets? Do the same genetic or biological pathways contribute to population structure? From a population stratification perspective, how strongly are epigenetic and genetic marks correlated? When genome-wide SNP genotypes and DNA methylation are obtained from the same participants, we are interested in determining if there is a correlation? When genome-wide SNP genotypes and DNA methylation data are available from the same participants, are they correlated? When genome-wide SNP genotypes and DNA methylation data are obtained from the same participants, do we have the same genes or biological pathways contributing to population structure? From a population stratification perspective, how strongly are epigenetic and genetic markers correlated? When genome-wide SNP genotypes and DNA methylation data are obtained from the same participants, are they correlated? When genome-wide SNP genotypes and DNA methylation data are obtained from the same participants, do we have the same genes or biological pathways contributing to population structure?

In this study, DNAs derived from saliva DNA of 196 participants were analyzed in Illumina Human IM-Duo Single Nucleotide Polymorphism (SNP) arrays and Illumina Infinium 27K methylation arrays. Based on self-report ethnicity, the participants were comprised of Caucasian, Latino, African American, Native American, Asian and mixed group. We first examined the population structure in the SNP array and methylation data separately, and then studied the inter-relation between them.

### Results

1. **SNP population stratification factors (PSF)**

A total of 195 principle component (PC) factors were extracted from genomic SNP array and 3 of them were identified as PSFs by correlation with ethnicity, which are the 1st, 2nd, and 6th PCs. These three PCs account for 10% of genome-wide SNP variance, with the 1st one alone accounting for 4%. ANOVA test results in Table 1 show how each PSF differs in ethnic groups, indicated by the p value of group difference and percentage variance explained by each ethnic group. Based on a |Z| >4 threshold, the top contributing SNPs for each PSF are also listed; eight SNP loci are identified for the 1st PSF, 13 SNP loci for the 2nd PSF, and 15 SNP loci for the 3rd PSF (genes associated are provided in the supporting Table S1). To present more clearly the population structure in the three PSFs, we plotted the distributions of relative weights of the PSFs in all ethnic groups, as well as the top SNP genotype distribution in the population (Figure 1). Relative weights were projected values calculated in a factorization method, showing how the data present in a particular direction. Here the direction is the PSF derived from linear combination of SNPs genotype, and weights show how the PSF reveals itself in each subject. The 1st PSF is able to differentiate Caucasian, Latino and Native American groups, but it works better for Caucasians and Native Americans, each explaining about 40% of the PSF variance. The top SNP rs16891982 (SLC45A2) demonstrates a varying allelic frequency from Caucasian to African American, where Caucasians have a marked high frequency of genotype BB (i.e. homozygous for major allele G), Native Americans and African Americans have marked high frequencies of genotype AA (i.e. homozygous for minor allele C), and Latinos have approximately same frequency of genotype BB and AB. The 2nd PSF only differentiates African Americans from others with African American group explaining 68% of this PSF variance. SNP rs3550788 (MOBP) shows that only African Americans have a remarkable high frequency of genotype AA (allele C). The 3rd PSF can differentiate Caucasian, Latino and Native America, but mostly for Latino, which explains 36% of this PSF variance. SNP rs13013484 shows that all ethnic groups excluding Asian and Latinos, express the highest frequency of genotype BB (allele A), whereas the most frequent genotype in Latinos is AB, with the same rate for allele A and G.

2. **Methylation PSF**

A total of 35 independent components (ICs) were extracted by independent component analysis in methylation data and only one, the 22nd IC was identified as a methylation PSF, accounting for 0.2% of total genomic methylation variance. In this PSF, as reported in Table 1, three groups, Caucasian, Latino and Native American show significant differences, and altogether they explain 69% of variance of the PSF. The top 48 methylated sites (|Z| >6) in 44 genes, 18 Chromosomes are selected as the prominent contributing sites for this PSF (see Supporting Table S2 for the details). Figure 2A presents this PSF relative expression weight per subject in each of the 5 groups. Caucasians show a relative high expression, then Latinos, followed by African Americans and Native Americans. Among the 48 sites, some show methylation patterns similar to the PSF, as exemplified in Figure 2B using methylation β values of the PM20D1 gene. The mean and standard deviation (SD) of methylation levels in each ethnic group is also plotted, which shows that Caucasians present the highest methylation level, followed closely by Latinos and the mixed group, while native Americans and African Americans have similar low methylation levels. Others show methylation patterns with an opposite-direction population structure, negatively contributing to the PSF. Twenty three of these PSF methylation sites are in CpG islands, resulting a 48% CpG island rate. Compared with the overall 64% CpG island rate in the methylation data, the PSF methylation sites tends to have a lower probability of being located in CpG islands.

### Table 1. PSFs and their differences in ethnic groups.

| PC/IC Number | Group difference* | P value & Variance explained (|Z|) | Top contributing sites |
|---------------|------------------|---------------------------------|------------------------|
| 1st | Caucasian: p<1E-23; η² = 0.40, Latino: p<1.61E-5; η² = 0.09, Native American: p<1E-23; η² = 0.41. | rs16891982, rs35389, rs35407, rs35391, rs35412, rs28117, rs3755095, rs12913832 | |
| 2nd | Caucasian: p<6E-4; η² = 0.06, African American: p<1E-23; η² = 0.68. | rs535878, rs326946, rs594624, rs11709533, rs6090442, rs1708000, rs1606473, rs2909679, rs13066103, rs4725537, rs11129837, rs16891982 | |
| 6th | Caucasian: p<1.6E-8; η² = 0.15, Latino: p<1.61E-5; η² = 0.36, Native American: p<4.41E-11; η² = 0.20. | rs13013484, rs9677663, rs9967838, rs6080826, rs13075278, rs3792252, rs3736594, rs4814656, rs10829792, rs11708297, rs10762401, rs1966963, rs334543, rs12147353, rs2150392 | |
| Methylations: Top contributing genes (|Z| >6) | Caucasian: p<1E-23; η² = 0.32, Latino: p<4.6E-5; η² = 0.08, Native American: p<5.5E-16; η² = 0.29. | 48 sites in 44 genes from 18 chromosomes | |
3). Relationship between genetic and epigenetic PSFs

Pair wise correlation tests between SNP PSFs and the methylation PSF show that only one pair of PSFs, the first SNP PSF and the methylation PSF, are significantly associated with a correlation coefficient R of 0.72 (p value <1E-23). We further analyzed the top contributing sites in the linked PSFs. Of the top 8 SNPs, rs16891982, rs35389, rs35407, rs35391, rs35412 and rs28117 are in the SLC45A2 gene (the solute carrier family 45, member 2 encoding a transporter protein that mediates melanin synthesis). SNP rs3755095 is in CTNNA2 (encoding alpha 2 catenin). SNP rs12913832 is in HERC2 (encoding the HECT domain and RCC1-like domain-containing protein 22). The top 48 methylation sites are in 44 genes covering a broad spectrum of biological/molecular functions. To better understand the potential association of SNPs on methylation levels, we tested the correlation between the 8 SNPs’ genotypes and the 48 methylation sites. After Bonferroni correction (P value <4E-4), 8 methylation sites were significantly correlated with the SNPs listed in Table 2. A positive correlation implies that the minor allele of a SNP is associated with hypomethylation of a methylated site, while negative correlation indicates a hypermethylation association. All 8 SNPs are significantly associated with the methylation of one or more methylated sites. Two significant associations (one positive, one negative) are shown in Figure 3. SNP rs16891982 (along with all other 5 SNPs in SLC45A2, not plotted) shows a significant positive correlation with the methylation β values of PM20D1; i.e., the minor allele is associated with lower β values of PM20D1. Besides PM20D1, SNP rs16891982 also correlates with methylation of CDC42BPA, IL6 and SERPINB3, but negatively. In Figure 3B, SNP rs12913832 in HERC2 shows a significant negative correlation with SLC44A4 methylation. Moreover, rs12913832 is also negatively associated with methylation of CDC42BPA, FAM181A and SERPINB3, and positively associated with HLA-DRA (not plotted but listed in Table 2). To further ensure the associations of SNPs and methylation level are real, we tested the top 8 SNPs from all 195 SNP principle components. From a total of 1552 SNPs excluding the 8 SNPs we identified, 5% were correlated with the methylation of PSF methylated sites, reflecting the 5% false positive control.

Of the total 47 genes from both SNP and methylation PSFs, we identified 13 shared functional clusters based on functional categories, gene ontology (GO) and pathway information. These 13 clusters (see Supporting Table S3) focus on functions of, 1) taxis/chemokine/cytokine activity with emphasis on inflammatory/immune/wound/defense response, 2) extracellular matrix, 3) actin cytoskeleton organization, 4) sexual reproduction and multicellular organization reproduction, 5) cell morphogenesis and cellular component morphogenesis, 6) macromolecule cata-
bolic process and protein catabolic process, 7) zinc-finger, zinc ion binding, 8) phosphorus metabolic process and intracellular signaling cascade, 9) cation/ion/metal ion binding, 10) purine ribonucleotide/adenyl ribonucleotide/ATP binding, 11) intracellular organelle lumen, organelle lumen and nuclear lumen, 12) transmembrane region, intrinsic to member and integral to membrane, and 13) transcription regulation. Importantly, 7 clusters (Clusters 1, 4–7, 9, 12) include biological processes/pathways where both SNP and methylation PSF genes are involved (these pathways are highlighted using * in the Supporting Table S3). Furthermore, clusters 1, 7 and 9 include processes in which direct associations of SNPs with hypo- or hyper-methylation were identified (see Table 2).

To further investigate the relationship of the SNP and methylation PSFs, a network focusing on the transcription factors among all the 47 genes was extracted using the shortest path of 2 steps tool in MetaCore™ software. In order to represent the main network clearly, we centered the network at the transcriptional repressor, removed the extended connections (2 steps away from ID2) (Figure 4). Nineteen genes in the SNP and methylation PSFs are connected to ID2 through 1 node, frequently involving a transcription factor. Additional connections exist between these gene products, but, for display purposes, we only show those related to transcriptional regulation.

Discussion

As expected for genomic SNP array data, clear population structure information was extracted, resulting in three PSFs, which are ranked as the 1st, 2nd, and 6th PC in a descending order of variance. These three PSFs accounted for a relatively large portion of total variance in the whole SNP array (6% of total 195 PCs), reinforcing the importance of defining population stratification in GWAS. The three PSFs comprise different SNPs, which differentiate each of the ethnic groups in a unique manner. The first PSF reveals positive weights in Caucasians, close to zero in Latinos and...
African Americans, and negative weights in Native Americans and Asians, with mixed group lying between Caucasians and Latinos. Considering the uncertainty of self reporting individual ethnicities, it is not surprising to see some participants showing expression deviating from their own ethnic group. Overall, this PSF allows separation in all ethnic groups. The 2nd and 3rd PSFs are more focused on one specific ethnic group, African American and Latino respectively. It is possible there are more PSFs that can be identified, but given we do not have a large subject sample size, we use a stringent criterion of $p<1E^{-4}$.

Only one PSF was identified in methylation ranked as the 22nd IC, accounting for much less variance (0.2% of total methylation variance) compared to the PSFs identified in the SNPs, which implies that population structure does affect methylation but is not the largest influencing factor. This PSF shows decreasing weights from Caucasians to Latinos and African Americans, and then to Native Americans and Asians. This trend can also be seen in the example of gene \textit{PM20D1} methylation (Figure 2B), though with large deviations. It again indicates that ethnicity affects methylation, but large portion of methylation variation is induced by other factors. Since epigenetics is prone to environmental influence, we are not surprised to see that ethnicity only plays a relatively small role in methylation variation.

Three genes, 8 SNP loci, contribute most to the 1st PSF in SNPs (we used a conservative empirical threshold to select the top contributing loci and hence might have missed SNPs contributing less to the PSF). \textit{SLC45A2} encodes a transporter protein that is involved in melanin synthesis. Melanin serves predominantly as a pigment in humans, and is the primary determinant of skin, hair and eye color. This is consistent with the differences in skin pigmentation seen among different ethnic groups. 

\textit{HERC2} belongs to the \textit{HERC} (HECT domain and RCC-1 domain) gene family and it is thought to encode an E3 ubiquitin-ligase. Genetic variations in this gene has been reported to be associated with hair color, eye color and skin color [5,22,23] and type 1 diabetes [24]. In particular, the top SNP we identified in this study rs12913832 is repeatedly identified to determine eye color. [23,25,26].

\textit{CTNNA2} encodes alpha N-catenin, a protein that links the cadherins adhesion receptor to the neuronal cytoskeleton and is expressed mainly in the nervous system. Animal studies show that \textit{CTNNA2} is essential for the stability of dendritic spines and synaptic contacts [27], and also for normal cerebellar and hippocampal morphology.
[28]. However, there is no information about its connection with population structure as yet. Forty-four genes are identified as the top contributing sites to the methylation PSF (Supporting Table S3). These 44 genes cover a broad spectrum of molecular functions in many biological processes. To the best of our knowledge, they encode proteins responsible for very basic functions such as DNA transcription and processes. To the best of our knowledge, they encode proteins involved in a broad spectrum of molecular functions in many biological processes.

Figure 4. Biological network linking SNP PSF genes with methylation PSF genes. Nineteen gene products encoding various proteins are one node away from ID2, mostly connected via a transcription factor. Among these are the 3 genes in the SNP PSF (HERC2, CTNNA2 and SLC45A2) and 16 genes in the methylation PSF. doi:10.1371/journal.pone.0013209.g004

Forty-four genes are identified as the top contributing sites to the methylation PSF (Supporting Table S3). These 44 genes cover a broad spectrum of molecular functions in many biological processes. To the best of our knowledge, they encode proteins responsible for very basic functions such as DNA transcription and regulation (ID2, LASS3, TFP2E, KLF17, ZNF205), DNA binding and protein binding (TDFG, ID2, ZNF205, DNAJB7, NLRP5, CDC42BPA, CDS2, KLF17, MGCG207, PSMD5, STK38), metal ion binding and calcium ion binding (CAPN9, SUSDI1, CHFR, CYP2F1, PAZ2D1), hydrolyase activity and metalloproteinase activity (PM20D1, DUSP22D), cytokine activity and chemokine activity (CCL4, CCL26, IL6, CCL4L2), and sulfotransferase activity and transferase activity (PPP4R2, CHST11, SULT1C1, GSTM5, GSTM1, UGT2B17). More clearly, the 13 overrepresented functional clusters confirm the main biological processes.

Though there is no overlap between genes contributing to the SNP PSF and methylation PSF, we did identify the association of SNPs in the SNP PSFs with the DNA methylation of sites in methylation PSF. Specifically, a remarkable similarity was found between the 1st SNP PSF and the methylation PSF manifesting as a correlation of 0.72 (P<1E-23), which strongly suggests the existence of a population structure linkage between genetics and epigenetics. As we show in two examples in Figure 3, the SNPs (rs16891982 and rs12913832), known to encode pigmentation in humans [23,25,29], appear to modulate the methylation level of other genes (PAZ2D1 and SLC44A4). These modulations were further confirmed by testing all possible SNP components, where 5% of SNPs in general showed correction, yet 100% of the top 8 SNPs in the SNP PSF showed significant correlations. Given that there are no direct associations of the genes containing these 8 SNPs with any known DNA methylases, this implies that the genetic influence on the epigenetic population structure is more complex, possibly through interactions involving multiple genes. This implication can be further strengthened by the identified 7 functional pathway/biological process clusters, where both genetic and methylation PSF genes are involved. In particular, we found SNP to hypo- or hyper-methylation associations (Table 2), which participate three functional clusters. Finally, we identified a biological network with multiple-gene interactions, where most genes are closely connected to the ID2 protein via a transcription factor. This protein belongs to the inhibitor of DNA binding (ID) family. Members of the ID family inhibit the functions of basic helix-loop-helix (bHLH) transcription factors in a dominant-negative manner by suppressing their heterodimerization partners through the HLH domains. Even though the detailed mechanism is unknown, ID2, known to down- or up-regulate gene expression in cell differentiation and proliferation, could potentially work as a master regulator coordinating the expression of the genes in the SNP and methylation PSFs described in our study. The hypothesis of shared biological process bridging the population structure in genetics and epigenetics, suggested by our results, needs a detailed molecular level investigation.

In summary, remarkably similar population stratification patterns were discovered in the genetic and epigenetic data. While the bases for this are presently unclear, our results suggest that 1) the genetic population structure is determined by a small set of focused genes; 2) in contrast, multiple genes coding for basic molecular functions are regulated by the population structure to a small extent in DNA methylation data; 3) the interconnection between genetic and epigenetic PSFs is more likely achieved through complex multiple gene interactions involved in shared biological processes, possibly through specific SNP to methylation modulation, and which in turn could be regulated by ID2. Further in-depth studies are needed to test this hypothesis.

Materials and Methods

1). Subjects

The study was conducted according to the principles expressed in the Declaration of Helsinki, approved by the Institutional Review Board of University of New Mexico. All participants provided written informed consent for the collection of samples and subsequent analysis. One hundred ninety-six participants including 53 females with age 32.21 ± 10.74 and 143 males with age 32.22 ± 9.70 were investigated in this study, which is a subsample of an on-going study designed to investigate genetic/epigenetic prediction for substance dependence. Subjects between age 21 and 55 (right handed) with a minimum alcohol consumption of a regular pattern of two binge drinking episodes per week, otherwise healthy (no maximum alcohol consumption limit, but no history of severe brain injury or brain related medical problems, no symptoms of psychosis during a diagnostic interview), were included.

The demographic information and alcohol dependence level were assessed through questionnaires. We gave the alcohol use disorder identification test (AUDIT) [30] to participants and obtained a score for alcohol dependence level. The self report ethnicity shows that all participants are comprised of five ethnic groups listed in Table 3. There is no significant (p<0.05) difference in age, gender, or AUDIT score between ethnic groups (no test was performed on the Asian group).

2). SNP Genotyping

Participants were instructed to deliver 5 ml of saliva into a sterile 50 ml conical centrifuge tube. DNA was then extracted,
purified and ready for genotyping based on Illumina human 1M-duo SNP assay recommendation. 1,199,187 loci were genotyped. A quality control was set to select readings with a call rate above 99%. Loci with more than 5 missing values were excluded, otherwise filled using the most common genotype in the given dataset. We code the three genotypes, AA, AB and BB, as 1, 0, and -1 without assuming dominate or recessive model. Preprocessing of SNP array was performed, including removing loci with minor allele frequency less than 0.01, redundant loci with correlation -1 without assuming dominate or recessive model. A total of 541,204 allele frequency less than 0.01, redundant loci with correlation -1 without assuming dominate or recessive model. A total of 541,204

| Ethnicity         | Number | Gender (m/f) | Age  | AUDIT |
|-------------------|--------|--------------|------|-------|
| Caucasian         | 90     | 64/26        | 32.4±10.2 | 18.9±7.6 |
| Latino            | 52     | 36/16        | 32.8±10.3 | 19.1±7.5 |
| African American  | 4      | 4/0          | 34.0±5.0  | 21.8±6.1 |
| Mixed             | 37     | 28/9         | 30.1±9.5  | 18.7±8.0 |
| Native American   | 12     | 10/2         | 35.3±10.1 | 21.5±6.8 |
| Asian             | 1      | 1/0          | 24    | 11    |

From the factorization viewpoint, ICA can be categorized as an extension of PCA, using more strict (in some cases additional) criteria to refine the components. For some components (most likely the components capturing more variance), there is no obvious difference between ICA and PCA results. That is the case when we applied ICA and PCA on the SNP data in this study; similar PSF components were extracted. But to compare with the literature where PCA has been used to extract genetic population structures, we also applied PCA onto the SNPs. For methylation data ICA produced a stronger PSF component, likely due to the relatively weak population diversity reflected in methylation.

4.3). ANOVA tests are performed for each PC or IC extracted from SNPs or methylation data separately, to examine the existence of ethnic group differences (except for Asian). The PC(IC) showing significant group differences (p value<1E-4) presents, to an extent, the population structure embedded in genetic or epigenetic data. Thus we termed the PC (IC) a PSF. For each PSF, we also calculated the percentage of variance explained by each ethnic group, which provides additional information about the population structure.

4.4). Pearson correlation tests were performed on all pairs of SNP PSFs and methylation PSF. A significant correlation (passing multiple comparison correction) indicates the presence of interconnection between genetic and epigenetic population structure. We then identified the loci contributing to the linked PSFs, by Z transforming the PSFs' projection coefficient or source (removing the mean and dividing by the standard deviation), and selecting the loci with Z score higher than an empirical threshold (4 for SNPs, and 6 for methylation based on a subjectively observed break point in the Z score distribution). We subsequently correlated directly the genotypes of each identified contributing SNP with the methylation values of each identified methylation site, and by doing so we were able to test the potential modulation function of SNP genotypes on methylation levels.

4.5). To localize the shared biological functions or involved pathways of the contributing genes from both SNP and methylation PSFs, we used the gene functional annotation clustering tool, built into the database for annotation, visualization and integrated discovery (DAVID, http://david.abcc.ncifcrf.
This tool identified clusters (similarity) of many gene functions. The clusters were extracted using default clustering criteria, including functional categories, gene ontology and pathway information, and the classification stringency was set as high. We further investigated biological networks among all the PSF genes focusing on transcription regulation factors, using MetaCore™ from GenoGo Inc. [http://www.genego.com/metacore]. The network was built via the shortest path of maximum 2 steps, and using only curated interactions and functional and binding interactions.

Supporting Information

Table S1 8 SNPs in the 1st genetic PSF
Found at: doi:10.1371/journal.pone.0013209.s001 (0.05 MB DOC)

Table S2 Forty-eight sites in the methylation PSF

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