Ancestry patterns inferred from massive RNA-seq data

RUTH BARRAL-ARCA,1,2 JACOBO PARDO-SECO,1,2 XABI BELLO,1,2 FEDERICO MARTINÓN-TORRES,2 and ANTONIO SALAS1,2

1Unidade de Xenética, Instituto de Ciencias Forenses (INCIFOR), Facultade de Medicina, Universidade de Santiago de Compostela, and GenPoB Research Group, of the Instituto de Investigación Sanitaria de Santiago (IDIS), Hospital Clínico Universitario de Santiago (SERGAS), 15706 Galicia, Spain
2Translational Pediatrics and Infectious Diseases Unit, and GENVIP Research Group (www.genvip.org) of the Instituto de Investigación Sanitaria de Santiago (IDIS), Hospital Clínico Universitario de Santiago (SERGAS), 15706 Galicia, Spain

ABSTRACT

There is a growing body of evidence suggesting that patterns of gene expression vary within and between human populations. However, the impact of this variation in human diseases has been poorly explored, in part owing to the lack of a standardized protocol to estimate biogeographical ancestry from gene expression studies. Here we examine several studies that provide new solid evidence indicating that the ancestral background of individuals impacts gene expression patterns. Next, we test a procedure to infer genetic ancestry from RNA-seq data in 25 data sets where information on ethnicity was reported. Genome data of reference continental populations retrieved from The 1000 Genomes Project were used for comparisons. Remarkably, only eight out of 25 data sets passed FastQC default filters. We demonstrate that, for these eight population sets, the ancestral background of donors could be inferred very efficiently, even in data sets including samples with complex patterns of admixture (e.g., American-admixed populations). For most of the gene expression data sets of suboptimal quality, ancestral inference yielded odd patterns. The present study thus brings a cautionary note for gene expression studies highlighting the importance to control for the potential confounding effect of ancestral genetic background.

Keywords: RNA-seq; gene expression; transcriptomics; biogeographical ancestry; SNPs; genomics

INTRODUCTION

There is growing body of evidence indicating that ethnicity can impact gene expression. Human populations show millions of DNA polymorphisms and variable patterns of allele frequencies, and a number of these markers fall at regulatory DNA positions (Spielman et al. 2007), which could ultimately lead to differential gene expression patterns. The study by Spielman et al. (2007) based on the analysis of >4000 genes indicated that genetic variation among populations contributes to differences in gene expression phenotypes; most of the variation observed was due to allele frequency differences at cis-linked regulators. The subsequent study by Price et al. (2008) on “African-American” cell lines, however, indicated that both cis and trans markers have highly significant effects, although they estimated that ~12% of all heritable variation in human gene expression was due to cis variants. The large-scale study by Stranger et al. (2007) confirmed that gene expression levels are hereditable and indicated an abundance of cis-regulatory variation in the human genome, whereas Storey et al. (2007) estimated that ~17% of genes are differentially expressed among populations. Most recently, Serrano-Gómez et al. (2017) found five genes modulated by genetic ancestry in breast tumors from Colombian women.

A major hindrance for gene expression studies is that the most of them do not consider ethnicity or ancestral background to be a variable of interest in their analysis. There are only very few attempts at monitoring ancestry in transcriptomic studies; see, for example, Serrano-Gómez et al. (2017) on breast cancer patients and Barral-Arca et al. (2018) in an infectious disease context.

In contrast, procedures to infer biogeographical ancestry (BGA) from DNA data are very popular in human population genetics at continental or regional geographical scale (Galanter et al. 2012; Reich et al. 2012; Lazaridis et al. 2014; Pardo-Seco et al. 2014a, 2016), and in forensic genetics (e.g., investigation of crime scene evidence; Sánchez et al. 2006; Phillips et al. 2009; Pardo-Seco et al. 2014b). Usually, ancestry can be efficiently inferred by...
using dense single nucleotide polymorphism (SNP) data derived from genotyping studies (Reich et al. 2012; Pardo-Seco et al. 2014a, 2016) or massive sequencing studies (Lazaridis et al. 2014; Salas et al. 2017), but also by genotyping panels of ancestry informative markers (AIMs) (Sánchez et al. 2006; Phillips et al. 2009) or a reasonable number of random SNPs uniformly distributed along the genome (Pardo-Seco et al. 2014b).

At the same time, although DNA polymorphisms are generally genotyped/sequenced, there are now bio-informatic procedures that allow inferring DNA variation indirectly from RNA data obtained from large-scale sequencing projects (RNA-seq). This strategy has two main advantages: It is inexpensive, and it can be applied when no DNA data is available (e.g., using RNA data sets retrieved from public repositories). However, these procedures are still uncommon in the field of gene expression studies. This might be due to a variety of factors, including: (i) the very little effort made to date at evaluating the efficiency of these procedures, and/or (ii) the trend to believe that ethnicity does not impact gene expression patterns, despite the existing evidence against this belief. To the best of our knowledge, our recent study, Barral-Arca et al. (2018) constitutes the only one where inferred DNA variation was used to estimate genome ancestry in a gene expression context. This study explored a 2-transcript host cell signature to distinguish viral from bacterial infections (Barral-Arca et al. 2018) in a RNA-seq Mexican data set (see also Herberg et al. 2016).

The aim of the present study is to formally test the preliminary procedures used in Barral-Arca et al. (2018) by way of exploring a broader set of RNA-seq data sets representing worldwide populations. In addition, we show new evidence clearly indicating the impact of ethnicity on modulating gene expression.

RESULTS

Characteristics of the data sets and quality control

The quality of the gene expression data sets can have an important impact on the inference of genome ancestry variation. Of the 25 data sets initially explored (Fig 1A; see Materials and Methods), only eight (32%) passed the default FastQC quality filters (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) (Supplemental Fig. S1; see also Conesa et al. 2016), while as many as 17 (68%) did not (Supplemental Fig. S2).

The eight data sets that met all the quality requirements (Supplemental Fig. S1) represent populations from different continental regions and provided a high number of SNPs (>4000 after applying additional genome filters indicated above; Table 1; Supplemental Table S1). The small number of SNPs shared by all of these data sets (Fig. 1B; Supplemental Table S3) makes it inviable to process all the data sets for a common set of SNPs. Therefore, all the analyses were carried out by comparing each of the eight data sets individually against the reference continental populations. Figure 1C shows the distribution of the SNPs in chromosomes in the eight data sets.

We further explored the data sets that did not satisfy the quality filters with the aim of evaluating the impact of low quality RNA-seq data on ancestry inference. Although the quality of the data was suboptimal for variant calling in 17 data sets, four of them still yielded patterns of ancestry according to expectations (see section below).

Ethnicity correlates with gene expression patterns

In contrast to genomic studies where matched ethnic samples are generally required, e.g., case-control studies (Martinón-Torres et al. 2016; Aung et al. 2017), only a few gene expression studies reported the ethnicity of the donors. For the sake of illustrating the role of the ancestral background in gene expression, we selected two studies on tuberculosis (TB) where ethnicity for individual samples was declared in the GEO database, and where more than one ethnic group was considered in the same study (hence using the same methodology).

The study by Berry et al. (2010) investigated the immune response of patients infected by Mycobacterium tuberculosis (GEO: PRJNA422129). The MDS plot shows expression patterns of the active TB cohort (Fig. 2A); its Dimension 1 (accounting for ∼20% of the variation) is responsible for the major separation between South African and UK expression patterns, while Dimension 2 (which explains a notable 13% of the variation) separates a few UK cases from the rest. Their latent cohort of TB patients (Fig. 2B) was also ascribed to two well differentiated clusters in its Dimension 1 (accounting for ∼18% of the variation), which again clearly separates their gene expression patterns according to their different geographic origins.

In their follow-up study on TB infected patients (GEO: PRJNA422130), Singhania et al. (2018), used a similar sampling scheme, with some overlapping of samples with their previous study (Berry et al. 2010). There is a marked sub-structure of expression patterns in the control female group in the Singhania et al. (2018) study (Fig. 2C), which perfectly allocates the female controls in the vertex of an equilateral triangle according to ethnicity. In control males (Fig. 2D), the expression patterns of South Asians are much more dispersed than in Kenians and Indians. Their male cases included donors from Afghanistan, South Asia, Sudan and Tanzania. Dimension 1 (∼26% of the variance) differentiates these clusters by main continental ancestries; one pole is dominated by the African samples (Sudan and Tanzania), while Asian ones (Afghanistan and South Asia) plot on the opposite (Fig. 2E). Dimension 2 (∼14% of the variation) further distinguishes the two African samples and indicates a higher dispersion for the South Asian donors.
FIGURE 1. (Legend on next page)
Finally, the effect of ethnicity is not only visible in RNA-seq data. For the sake of illustrating the same issue in expression arrays, we examined the study by Obermoser et al. (2013) in regard to influenza and pneumococcal vaccines. The effect of ethnicity is clear when examining (i) their control females, with a clear differentiation between “African-American” and “Caucasian” in Dimension 2 (∼8%; Supplemental Fig. S3A), (ii) their control males, with a similar pattern (Dimension 2; ∼11%) but this time separating Asians from “Caucasians” (Supplemental Fig. S3B), and (iii) their Pneumovax vaccinated males (Supplemental Fig. S3C) and females (Supplemental Fig. S3D), where Dimension 2 completely separates their “Caucasian” from their Asian donors again.

It is most remarkable that, although ethnicity information is clearly specified in the GEO repository, this information is not used at all, or not even mentioned in the main text of the associated publications.

**Functional characteristics of the SNPs inferred from RNA-seq data**

Eight population sets passed through all the quality filters specified above. We examined the functional characteristics of these data sets (Table 2). Since these SNPs were inferred from RNA-seq data, one could expect the main proportion of them to be located at exonic regions. Surprisingly, when considering all the SNPs from all the eight data sets together, 25.3% were intergenic, and only 3.1% fell at intronic regions. Furthermore, a total of 74% of them do not have exonic function. These percentages vary considerably depending on the data set considered

---

**TABLE 1.** RNA-seq data sets used in the present study and characteristics of the inferred SNPs

| ID no. | ID in figures | GEO acc. no. | Tissue | Country | No. SNPs |
|-------|---------------|--------------|--------|---------|----------|
| 1     | Afghanistan   | PRJNA422130  | Blood  | Afghanistan | 22,375   |
| 2     | Burkina-Faso  | PRJNA392116  | Blood  | Burkina-Faso | 58,841   |
| 3     | China_1       | PRJNA296108  | Blood  | China    | 7807     |
| 4     | China_2       | PRJNA412314  | Tumor  | China    | 39,198   |
| 5     | China_3       | PRJNA134241  | Tumor  | China    | 5591     |
| 6     | Colombia_1    | PRJNA395937  | Tumor  | Colombia | 229      |
| 7     | Colombia_2    | PRJNA279199  | Blood  | Colombia | 4214     |
| 8     | A.-American   | PRJNA341854  | CD4+T cells | “African-American”; USA | 6703     |
| 9     | “Hispanic”    | PRJNA341854  | CD4+T cells | “Hispanic”; USA | 6703     |
| 10    | Kenya         | PRJNA422130  | Blood  | Kenya    | 24,171   |
| 11    | Malaysia      | PRJNA238241  | Adipose tissue | Malaysia | 39,576   |
| 12    | Mexico        | PRJNA285798  | Blood  | Mexico   | 18,180   |
| 13    | Russia        | PRJNA350714  | Paraffin-embedded tumor | Russia | 558      |
| 14    | S. Africa_1   | PRJNA422129  | Blood  | South Africa | 51,763   |
| 15    | S. Africa_2   | PRJNA309415  | Innate lymphoid cells | South Africa | 181      |
| 16    | S. Korea_1    | PRJNA218851  | Tumor  | South Korea | 44,063   |
| 17    | S. Korea_2    | PRJNA163279  | Tumor  | South Korea | 7        |
| 18    | Spain         | –             | Blood  | Spain    | 43,681   |
| 19    | Sudan         | PRJNA422130  | Blood  | Sudan    | 2029     |
| 20    | Sweden        | PRJNA354367  | Blood  | Sweden   | 27,703   |
| 21    | Taiwan        | PRJNA318782  | Tumor  | Taiwan   | 7        |
| 22    | Tanzania      | PRJNA422130  | Blood  | Tanzania | 10,944   |
| 23    | Uganda        | PRJNA422130  | Blood  | Uganda   | 17,618   |
| 24    | UK            | PRJNA294293  | Tumor  | United Kingdom | 10,210   |
| 25    | Zambia        | PRJNA392660  | Tumor  | Zambia   | 18,536   |

Extended information on this table is provided in Supplemental Table S1. ID code: used in map of Figure 1A.
FIGURE 2. (Legend on next page)
(Table 2). To illustrate this phenomenon, we allocate the reads for the eight different studies corresponding to the housekeeping gene CHM02A (charged multivesicular body protein 2A). Different studies have different coverages outside the exon regions (Supplemental Fig. S4). The same patterns are observed for other genes (data not shown).

Inference of ancestry from RNA-seq data

Figure 3 shows MDS plots and ancestry analysis for the eight populations that passed through all the quality filters specified above. Three European ancestry population sets, namely Spain (Salas et al. 2016), Sweden (GEO: PRJNA354367; Shchetynsky et al. 2017), and UK (GEO: PRJNA294293; Wood et al. 2016), are represented in Figure 3A–C. The three data sets fall within the cluster defined by the European populations in The 1000 Genomes Project (1000G) reference populations. In the case of Sweden, there is one individual that shares a visible proportion of ancestry membership with the East Asian population.

Three additional data sets represent gene expression data of patients from Asia, two of them from China (Fig. 3D–F), and one from Korea (Fig. 3G). In all three cases, the profiles fall within the East Asian clade defined by the reference 1000G populations. The plot built on data set China_1 consists of only 7807 SNPs (GEO: PRJNA296108; Yu et al. 2017); this explains a subtle but visible dispersion of the plots compared to, e.g., China_2 (a plot built on 39,198 SNPs; GEO: PRJNA412314 [Hong et al. 2018]). Korean profiles (GEO: PRJNA218851; Kim et al. 2014) also fall entirely within the Asian cluster.

Two additional data sets represent admixed populations: Colombia (GEO: PRJNA279199; Rojas-Peña et al. 2015) and Mexico (GEO: PRJNA285798; DeBerg et al. 2018). These two sample sets provide the opportunity to calibrate the method for the detection of complex patterns of admixture from gene expression data. From the MDS plot it is clear that the Colombians samples include patient profiles of different ancestries (Fig. 3G). For instance, three individuals have a clear African ancestry, which is not unusual given the impact of the transatlantic slave trade in America (Salas et al. 2004, 2005), and in this country in particular (Salas et al. 2008). The other individuals display a variable admixture contribution from a European and a Native American component (East Asia is used here as a surrogate ancestral population of Native Americans). Again, this pattern of admixture is common in South American populations (Reich et al. 2012). The Mexican data set has a different population admixture pattern (Fig. 3H). Thus, Mexican samples have a main Native American component (much higher than the Mexican sample from 1000G [MXL]), with a minor dispersion (of some of the samples) toward the European pole; this pattern is also expected according to different studies indicating an important Native American background in Mexican genomes with variable admixture with Europeans and minor sub-Saharan African influence (Sandoval et al. 2009).

Figure 4 summarizes the main ancestries observed in these eight data sets. The three European data sets show a main European ancestry, while the four Asian data sets show a predominant Asian ancestry. Conversely, the two American samples show both differential proportions of ancestry in agreement with their different patterns of admixture.

Four data sets did not pass the quality filters, but the patterns of ancestry could also be inferred (Supplemental Fig. S5). The Russian sample set (GEO: PRJNA350714; Supplemental Fig. SSA; Nikitina et al. 2017) only yielded 558 SNPs but still all the samples fall within the European cluster; the fact that this MDS plot is built on a few hundred SNPs explains the global scattering of the data points on the plot compared to other plots (e.g., in Fig. 3). Supplemental Figure S5B indicates patterns of ancestry for “Hispanic” and “African-American” data sets (GEO: PRJNA341854; Rastogi et al. 2018). Whatever the pseudo-ethnic category “Hispanic” means from the biological point of view (Salas et al. 2007), their patterns of genetic variation fit well with expectations: Most individuals have a three-way continental admixture pattern, with significant membership within the European cluster. On the other hand, some of the “African-American” samples fall within the African reference set, but others have a more variable and complex pattern of ancestry; it is also remarkable that one “African-American” profile falls neatly in the Asian pole of the plot. The South African sample (GEO: PRJNA422129; Supplemental Fig. S5C; Berry et al. 2010) falls entirely within the African cluster. The sample set from China (GEO: PRJNA134241; Huang et al. 2011) also fit completely within the East Asian cluster.

Finally, inferring ancestry from data sets that did not pass all the quality filters can lead to artifacts that are difficult to interpret from a genetic point of view. Supplemental Figure S6 displays all the MDS plots and ancestry barplots for 13 suboptimum data sets. Compared to expected patterns...
| Func refGene | All       | China 1     | China 2     | Colombia    | Mexico      | Korea 1     | Spain       | Sweden      | UK         |
|--------------|-----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|------------|
|              | All       | China 1     | China 2     | Colombia    | Mexico      | Korea 1     | Spain       | Sweden      | UK         |
| **UTR3**     | 47,115 (28.7%) | 5425 (56.3%) | 21,700 (34.9%) | 2944 (55.1%) | 10,760 (47%) | 25,458 (38.5%) | 24,121 (29.4%) | 15,202 (37.2%) | 6025 (48.9%) |
| **Exonic**   | 44,406 (27%)    | 3412 (35.4%) | 15,701 (25.2%) | 1858 (34.8%) | 8401 (36.7%) | 23,771 (36%) | 23,181 (28.2%) | 12,552 (30.7%) | 5201 (42.2%) |
| **Intergenic** | 41,485 (25.3%) | 14,631 (23.5%) | 161 (3%) | 1358 (5.9%) | 5654 (8.6%) | 15,818 (19.2%) | 6837 (16.7%) | 423 (3.4%) | 176 (1.4%) |
| **ncRNA_exonic** | 11,765 (7.2%) | 334 (5.4%) | 91 (1.7%) | 740 (3.2%) | 4164 (6.3%) | 7961 (9.7%) | 2035 (5%) | 160 (1.3%) |
| **ncRNA_intronic** | 5611 (3.4%) | 2202 (3.5%) | 89 (1.7%) | 705 (3.1%) | 2241 (3.4%) | 3271 (4%) | 1376 (3.4%) | 2035 (5%) | 160 (1.3%) |
| **Intronic** | 5152 (3.1%) | 1751 (2.8%) | 87 (1.6%) | 393 (1.7%) | 2059 (3.1%) | 2750 (3.3%) | 1094 (2.7%) | 153 (1.2%) |
| **UTR5**     | 4583 (2.8%) | 1586 (2.6%) | 78 (1.5%) | 380 (1.7%) | 1470 (2.2%) | 2452 (3%) | 1049 (2.6%) | 139 (1.1%) |
| **Downstream** | 2845 (1.7%) | 947 (1.5%) | 27 (0.5%) | 102 (0.4%) | 694 (1.1%) | 1956 (2.4%) | 434 (1.1%) | 25 (0.2%) |
| **Exonic;splicing** | 1001 (0.6%) | 235 (0.4%) | 4 (0.1%) | 32 (0.1%) | 466 (0.7%) | 525 (0.6%) | 187 (0.5%) | 4 (0%) |
| **Upstream** | 151 (0.1%) | 26 (0%) | 3 (0.1%) | 15 (0.1%) | 36 (0.1%) | 97 (0.1%) | 30 (0.1%) | 2 (0%) |
| **UTR5;UTR3** | 42 (0%) | 15 (0%) | 2 (0%) | 11 (0%) | 29 (0%) | 24 (0%) | 15 (0%) | 2 (0%) |
| **Splicing** | 39 (0%) | 12 (0%) | 1 (0%) | 7 (0%) | 19 (0%) | 10 (0%) | 13 (0%) | 1 (0%) |
| **ncRNA_exonic;splicing** | 13 (0%) | 11 (0%) | 1 (0%) | 4 (0%) | 9 (0%) | 8 (0%) | 11 (0%) | 0 (0%) |
| **ncRNA_splicing** | 4 (0%) | 1 (0%) | 0 (0%) | 0 (0%) | 2 (0%) | 2 (0%) | 1 (0%) | 0 (0%) |
| **Upstream;downstream** | 2 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 2 (0%) | 0 (0%) | 0 (0%) |
| **No exonic function** | 12,276 (74.7%) | 6226 (64.6%) | 46,480 (74.7%) | 3486 (65.2%) | 14,500 (63.3%) | 42,293 (64%) | 66,352 (80.7%) | 28,273 (69.2%) | 7108 (57.7%) |
| **Synonymous SNV** | 22,942 (14%) | 1981 (20.5%) | 8729 (14%) | 1055 (19.7%) | 4809 (21%) | 13,125 (19.9%) | 8887 (10.8%) | 7135 (17.5%) | 3076 (25%) |
| **Nonsynonymous SNV** | 18,028 (11%) | 1396 (14.5%) | 6779 (10.9%) | 792 (14.8%) | 3492 (15.2%) | 10,351 (15.7%) | 6769 (8.2%) | 5272 (12.9%) | 2092 (17%) |
| **Unknown** | 406 (0.2%) | 169 (0.3%) | 6 (0.1%) | 72 (0.3%) | 251 (0.4%) | 136 (0.2%) | 124 (0.3%) | 24 (0.2%) |
| **Stopgain** | 106 (0.1%) | 30 (0%) | 4 (0.1%) | 29 (0.1%) | 47 (0.1%) | 30 (0%) | 27 (0.1%) | 9 (0.1%) |
| **Stoploss** | 16 (0%) | 5 (0%) | 3 (0.1%) | 6 (0%) | 6 (0%) | 4 (0%) | 5 (0%) | 2 (0%) |

The values are shown for the eight data sets that were finally retained to infer ancestry and passed all quality filters specified in the main text. See Supplemental Table S1 for codes for populations.
FIGURE 3. (Legend on next page)
of variation (Fig. 3), these plots lack structure (e.g., Supplemental Fig. S6A,B) or the samples form outlier clusters clearly separated from the three main continental groups (e.g., Supplemental Fig. S6E–G) denoting artificial patterns of variation. Some data sets might represent real variability, as is the case of the samples from Malaysia (Supplemental Fig. S6K) or Afghanistan (Supplemental Fig. S6L), but the fact that the original RNA-seq data sets are in suboptimal conditions calls for caution with these patterns.

In general, it may be argued that these genetic/ancestry analyses themselves could also serve as an additional quality filter per se.

**DISCUSSION**

We provide new evidence suggesting that ancestral background can impact gene expression patterns. Notably, this evidence stands in contrast with the fact that only a very small number of gene expression studies control for ancestral background information in their experiments; when this information is available, it is not used at all. Thus, there are studies where cases entirely represent a given continental ancestry while their control group was recruited from another continental ancestral background. For instance, in the very recent study by Tian et al. (2017), the authors investigated the immune response of patients infected by dengue virus. Their patients were recruited in Sri Lanka, whereas their controls were sampled in San Diego (USA). The MDS plot in Supplemental Figure S7 indicates that these two groups differentiate clearly in Dimension 2 (23%). The extent to which the differences between cases and controls were due to ethnicity instead of the presence of the pathogen remains to be investigated.

Here we demonstrate that it is possible to infer DNA variation from RNA-seq data, and that this variation can be used to estimate ancestry background of patients. Although the DNA information from samples represents mainly coding region variation (which shows more evolutionary constraints and therefore less variation than non-coding region variants), the procedure performs well with only a few thousand SNPs available or even a few hundred, a finding that is consistent with those advanced by Salas (2019). Moreover, the procedure performs also correctly when analyzing populations with complex patterns of admixture, as is the case of many American populations.

A remarkable finding of the present study is that an important proportion of the investigated RNA-seq data sets shows deficiencies in terms of data quality. Exploring the consequences of this finding within the framework of each individual study is beyond the scope of the present study, but the issue deserves further attention in future investigations. We observed that low quality RNA-seq data produce unusual patterns of ancestry; this therefore led to the conclusion that unusual patterns of genetic variation inferred from RNA-seq data can be used as an additional quality control of the data.

Ancestral background is usually inferred in cases and controls by directly genotyping a set of SNPs (e.g., AIMs). However, here we demonstrate that ancestry can be deduced from RNA-seq data. This has several advantages for future gene expression studies. On the one hand, it avoids the need of obtaining ad hoc samples for DNA genomic analyses from donors (there could be many situations where only a RNA sample can be obtained). On the other hand, once the RNA-seq data have been generated, inferring DNA variation from expression patterns is inexpensive; it only requires the use of a few computational tools already available in the public domain.

**MATERIALS AND METHODS**

**RNA-seq data sets and evaluations of data quality**

Figure 5 summarizes the process followed in the present study to infer ancestry proportions from RNA-seq data. First, gene expression data were retrieved from the GEO (Gene Accession...
Omnibus; https://www.ncbi.nlm.nih.gov/geo/) database (Edgar et al. 2002). The vast majority of RNA-seq studies do not record the population background of their samples. We manually searched for RNA-seq data sets that have information on ethnicity or sampling location available and rejected studies that used inaccurate ethnic terminology (e.g., “latini” people) (Salas et al. 2007). Finally, the databases were chosen with the idea to represent all the continents and different tissues. We initially collected data from 25 RNA-seq data sets (corresponding to 20 independent studies) representing populations from different continental regions (Table 1; Fig. 1A; Supplemental Tables S1, S2). Two out of 20 studies (Berry et al. 2010; Singhania et al. 2018) examined expression patterns of groups of individuals from different ethnicities at the same time; therefore, their sampling design allows us to evaluate the impact of ethnicity in gene expression patterns.

For variant calling analyses, it is particularly relevant to initially evaluate the quality of the data. We examined the quality of the RNA-seq data by way of exploring per base sequence quality plots and using FastQC (Brown et al. 2017) and MultiQC (Ewels et al. 2016) software. Quality score plots show the distribution of base quality values for each position in the input sequence.

A medium level of duplication might be unavoidable because some sequences occur more frequently than others. Therefore, to detect transcripts with extremely low expression level, it is necessary to over-sequence the library, which could generate large amounts of the most common sequences such as housekeeping genes. FastQC and MultiQC were used to produce duplication level plots. These plots show for each sample set the fraction of reads observed at different duplication levels. This procedure allows the identification of fragments of adapters remaining on the reads, contamination, or any kind of enrichment bias (due to, e.g., PCR over amplification). The software raises a warning (orange line) if duplicated sequences constitute more than 20% of the total library and it will report an error (red line) if they represent more than 50% of the total (Supplemental Figs. S1 and S2).

RNA-seq reads prior to variant calling were processed using Opossum (Oikkonen and Lise 2017). Although the Opossum software deals with duplicate sequences, we have empirically observed that when the duplication level is too high (>50% of the total), it yields weak results in terms of the SNPs inferred. This could be caused by: (i) Removal of duplicates from the library may lead to an extremely poor library, hence the SNPs inferred are very few, biased and not evenly distributed across the genome, and/or (ii) overamplification of the library increases the chances of PCR errors and artifacts.

Annotating DNA variation from RNA-seq data sets and statistical treatment of SNP data

Variants were inferred from the processed RNA-seq data using the variant caller software Platypus (Rimmer et al. 2014). The number of annotated markers inferred from the different data sets varies from a few hundred (PRJNA318782; Supplemental Table S1) to more than 4000K variants (PRJNA163279).

SNPs without rs code, low genotyping rate (below 10%), and in LD (r^2 > 0.75) were removed from the analysis. Eliminating LD allows us to mitigate the effect of ascertainment bias which could be particularly important in genome data inferred from gene expression patterns.

SNP data from continental reference populations were retrieved from 1000G (http://www.internationalgenome.org). Reference genome data from 1000G were processed as per previous studies (Pardo-Seco et al. 2014a, 2016). Subsequently, each SNP population data set was individually intersected with SNP data from the 1000G reference populations.

Next, we computed identity-by-state (IBS) values between each population data set and the reference populations using PLINK (Purcell et al. 2007). Multidimensional scaling (MDS)
analysis using this matrix of IBS values was used to represent the clustering gene expression patterns and geographical affinities between the test data and the reference continental populations. MDS plots were built using the function cmdscale (library stats) from R (http://www.r-project.org).

We additionally investigated admixture patterns for each data set using populations in 1000G as reference data sets representing main continental regions. Admixture proportions were obtained by using a maximum likelihood estimation of individual ancestries from multilocus SNP data and using the ADMIXTURE software (Alexander et al. 2009), and fixing the number of predefined clusters (K) to the number of continental regions considered for each analysis.

SUPPLEMENTAL MATERIAL
Supplemental material is available for this article.

ACKNOWLEDGMENTS
A.S. received support from the Instituto de Salud Carlos III (Proyecto de Investigación en Salud, Acción Estratégica en Salud: project GePEM ISCIII/PI16/01478/Cofinanciado FEDER). F.M.T. received support from project ReSYVinet ISCIII/PI16/01569/Cofinanciado FEDER; Conselleria de Sanidade, Xunta de Galicia (PR107/2-intensificación actividad investigadora, P509749 and 10PXIB1918184PR), Instituto de Salud Carlos III (Intensificación de la actividad investigadora 2007–2012, PI16/01569), Fondo de Investigación Sanitaria (FIS; PI1000699/PI1000000) of the plan nacional de I+D+i and “fondos FEDER.” A.S. and F.M.T. received support from 2016-PG071 Consolidação e Estructuración REDES 2016GI-1344 G3VIP (Grupo Gallego de Genética Vacunas Infecciones y Pediatría, ED341D R2016/021). We would also like to acknowledge CESGA (Supercomputing Centre of Galicia, Santiago de Compostela, Spain) for the availability of supercomputing resources, web hosting, and support.

Received December 18, 2018; accepted April 16, 2019.

REFERENCES
Alexander DH, Novembre J, Lange K. 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome Res 19: 1655–1664. doi:10.1101/gr.094052.109
Aung T, Ozaki M, Lee MC, Schlötzer-Schrehardt U, Thorleifsson G, Mizoguchi T, Igo RP Jr, Haririya A, Williams SE, Astakhov YS, et al. 2017. Genetic association study of exfoliation syndrome identifies a protective rare variant at LOXL1 and five new susceptibility loci. Nat Genet 49: 993–1004. doi:10.1038/ng.3875
Barral-Arca R, Pardo-Seco J, Martínón-Torres F, Salas A. 2018. A 2-transcript cell signature distinguishes viral from bacterial diarrhea and it is influenced by the severity of symptoms. Sci Rep 8: 8043. doi:10.1038/s41598-018-26239-1
Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, Oni T, Wilkinson KA, Banchereau R, Skinner J, Wilkinson RJ, et al. 2010. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature 466: 973–977. doi:10.1038/nature09247
Brown J, Pirung M, McCue LA. 2017. FQC Dashboard: integrates FASTQ quality control tool. Bioinformatics 33: 3137–3139. doi:10.1093/bioinformatics/btx373
Conesa A, Madrigal P, Tarazona S, Gomez-Cabrero D, Cervera A, McPherson A, Szcześniak MW, Gaffney DJ, Elo LL, Zhang X, et al. 2016. A survey of best practices for RNA-seq data analysis. Genome Biol 17: 13. doi:10.1186/s13059-016-0088-1
DeBerg HA, Zaidi MB, Altman MC, Khaemam P, Gersuk VH, Campos FD, Perez-Martinez I, Meza-Segura M, Chausabel D, Banchereau J, et al. 2018. Shared and organism-specific host responses to childhood diarrheal diseases revealed by whole blood transcription profiling. PLoS One 13: e0192082. doi:10.1371/journal.pone.0192082
Edgar R, Domrachev M, Lash AE. 2002. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 30: 207–210. doi:10.1093/nar/30.1.207
Ewels P, Magnusson M, Lundin S, Käller M. 2016. MultQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics 32: 3047–3048. doi:10.1093/bioinformatics/btw354
Galanter JM, Fernández-López JC, Gignoux CR, Bamholtz-Sloan J, Fernández-Rozadilla C, Via M, Hidalgo-Miranda A, Contreras AV, Figueroa LU, Raska P, et al. 2012. Development of a panel of genome-wide ancestry informative markers to study admixture throughout the Americas. PLoS Genet 8: e1002554. doi:10.1371/journal.pgen.1002554
Herberg KA, Kaforou M, Wright VJ, Shailes H, Eleftherohorinou H, Hoggart CJ, Cebey-López M, Carter MJ, Janes VA, Gormley S, et al. 2016. Diagnostic test accuracy of a 2-transcript host RNA signature for discriminating bacterial vs viral infection in febrile children. JAMA 316: 835–845. doi:10.1001/jama.2016.11236
Hong W, Zhang CZ, Lu SX, Zhang MF, Liu LL, Luo RZ, Yang X, Wang CH, Chen SL, He YF, et al. 2018. A CCDC50 splice variant is modulated by SRSF3 and promotes hepatocellular carcinoma via the Ras signaling pathway. Hepatology 69: 179–195
Huang Q, Lin B, Liu M, Ma X, Mo F, Yu W, Li L, Li H, Tian T, Wu D, et al. 2011. RNA-seq analyses generate comprehensive transcriptomic landscape and reveal complex transcript patterns in hepatocellular carcinoma. PLoS One 6: e26168. doi:10.1371/journal.pone.0026168
Kim SK, Kim SY, Kim JH, Roh SA, Cho DH, Kim YS, Kim JC. 2014. A nineteen gene-based risk score classifier predicts prognosis of colorectal cancer patients. Mol Oncol 8: 1653–1666. doi:10.1016/j.molonc.2014.06.016
Lazaridis I, Patterson N, Mitnik A, Renaud G, Mallick S, Sirksanow K, Sudmant PH, Schraiber JG, Castellano S, Lipson M, et al. 2014. Ancient human genomes suggest three ancestral populations for present-day Europeans. Nature 513: 409–413. doi:10.1038/nature13673
Martínón-Torres F, Png E, Khor CC, Davila S, Wright VJ, Sim KS, Vega A, Fachal L, Inwald D, Nadel S, et al. 2016. Natural resistance to meningococcal disease related to CFH loci: meta-analysis of genome-wide association studies. Sci Rep 6: 35842. doi:10.1038/srep35842
Nikitina AS, Sharova EI, Danilenko SA, Butusova TB, Vasilev AO, Govorov AV, Prilepskaya EA, Pushkar DY, Kostryukova ES. 2017. Novel RNA biomarkers of prostate cancer revealed by RNA-seq analysis of formalin-fixed samples obtained from Russian patients. Oncotarget 8: 32990–33001. doi:10.18632/oncotarget.16518
Obermoser G, Presnell S, Domico K, Xu H, Wang Y, Anguiano E, Thompson-Snipes L, Ranganathan R, Zeitner B, Bjork A, et al. 2013. Systems scale interactive exploration reveals quantitative and qualitative differences in response to influenza and pneumococcal vaccines. Immunity 38: 831–844. doi:10.1016/j.immuni.2012.12.008
