Populational landscape of INDELs affecting transcription factor-binding sites in humans

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Abstract
Background: Differences in gene expression have a significant role in the diversity of phenotypes in humans. Here we integrated human public data from ENCODE, 1000 Genomes and Geuvadis to explore the populational landscape of INDELs affecting transcription factor-binding sites (TFBS). A significant fraction of TFBS close to the transcription start site of known genes is affected by INDELs with a consequent effect at the expression of the associated gene.

Results: Hundreds of TFBS-affecting INDELs (TFBS-ID) show a differential frequency between human populations, suggesting a role of natural selection in the spread of such variant INDELs. A comparison with a dataset of known human genomic regions under natural selection allowed us to identify several cases of TFBS-ID likely involved in populational adaptations. Ontology analyses on the differential TFBS-ID further indicated several biological processes under natural selection in different populations.

Conclusion: Together, our results strongly suggest that INDELs have an important role in modulating gene expression patterns in humans. The dataset we make available, together with other data reporting variability at both regulatory and coding regions of genes, represent a powerful tool for studies aiming to better understand the evolution of gene regulatory networks in humans.

Keywords: Transcription factor, Transcription factor-binding site, INDEL, Population genetics

Background
Much has been debated about the evolutionary role of genetic alterations in the regulation of gene expression [1–7]. In that aspect, transcription factor binding sites (TFBS) have recently been studied both in humans and other animals [8–10]. Several genome-wide analyses have identified regions close to genes (usually enriched with TFBS) showing patterns of diversity in accordance with a model of positive selection [1, 10]. In a recent study, Arbiza et al. [1] found that TFBS are under weaker selection than protein-coding regions of genes although these authors could observe several instances of adaptation in TFBS. In a similar way, Vernot et al. [10] have found hundreds of variations that are adaptive.

Although these studies have shed some light on the evolutionary forces acting on TFBS and other regulatory elements, several issues remain poorly explored or even unexplored. One of them is the role of INDELs (insertion/deletion) as a source of genetic variability among TFBS. Most of the few populational studies in this area are biased towards single nucleotide variants (SNV) [3, 9, 11]. Based on that, we decided to explore this issue by using three types of data recently made public. First, whole-genome sequences of more than a thousand human individuals from the 1000 Genomes Project (TGP) [12] were used to identify polymorphic INDELs. Second, a genome-wide identification of TFBS for 148 transcription factors from the ENCODE (Encyclopedia of DNA Elements) Project [13] was used to generate a catalogue of TFBS in the human genome. Finally, expression data from a sub-set of individuals from the 1000 Genome Project [14] was used to evaluate the impact of TFBS-affecting INDELs (TFBS-ID) on the expression of the corresponding gene. Integration of all these data allowed us to show a high frequency of TFBS-ID in the human genome. Hundreds of TFBS-ID showed a differential frequency in human populations and ontology analyses of these cases suggested a role of natural selection and population history in their distribution.
Based on that, we argue that a TFBS-ID has been selected in Africans by down-regulating APIP (APAF1-interacting protein) and generating a better response to Salmonella infection. A comparative analysis with genomic regions, known to be under positive selection [15], revealed that a significant fraction of the TFBS-ID identified by us represent instances of adaptation in human populations.

Results and Discussion
Identification of TFBS-ID
Fig. 1 shows a schematic representation of the computational pipeline used in all analyses reported here. To build a catalogue of TFBS-ID, we first indexed all TFBS identified by the ENCODE project in the human reference genome (hg19 version). Data from the 1000 Genomes project regarding the position of INDELs in the reference genome was then compared to the position of TFBS and those cases in which an INDEL overlapped with a TFBS were selected. This strategy rendered us a total of 259,864 TFBS affected by at least one INDEL. Since a significant fraction of TFBS overlap at the sequence level, the non-redundant number of TFBS-ID in the above set was 100,182 (an average of 2.59 TFBS per INDEL). Due to the presence of long INDELs affecting many TFBS at once, we decided to limit our analysis to those INDELs shorter than 200 bp, which gave us a total of 99,642 TFBS-ID and 258,686 TFBS. Although the superior limit was set to 200 bp, the final set of 99,642 TFBS-ID is strongly biased

Fig. 1: Analysis overview. Schematic representation of the strategy used here to identify and analyse TFBS affected by polymorphic INDELs in human populations.
towards shorter indels. More than 99.8% of all indels were
equal or shorter than 20 bp. Next, TFBS-ID close (≤5 KB)
to the transcription start site (TSS) of known human
genes (as defined by the Reference Sequence set) were
selected. In total, 7,313 human genes had at least one TFBS
affected by a polymorphic INDEL in the 1000 Genomes
dataset. This set of 7,313 genes had a total of 38,339 TFBS
affected by INDELs and 10,528 TFBS-ID. A complete list
of this dataset is available at Additional file 1: Table S1.

Since many reports have also used a window that flanks
the TSS of known genes [16,17], we have also defined a
different window of same size (5 KB) now encompassing
2.5 KB in each side of a given TSS. For this window,
we found that 9,733 human genes had at least one
TFBS affected by a polymorphic INDEL in the 1000
Genomes dataset (with a total of 69,959 TFBS affected
by indels and 14,665 TFBS-ID). The complete dataset
found for the 5 KB window flanking TSS can be found
at Additional file 2: Table S2.

TFBS-ID showed a biased distribution in terms of lo-
cation within both 5Kbp windows proximal to the TSS
of known genes. As seen in Fig. 2, their distribution tend
to be closer to the TSS of genes (Fig. 2a) (the 3’ end
of the 5Kbp window upstream of the TSS) while in the
window with the TSS at center the distribution of TFBS-
ID is symmetrical with a slight higher frequency at the
upstream half of the window (Fig. 2b). When we split
the TFBS-ID per type of transcription factor, the same
biased distribution is observed for both windows, espe-
cially for some transcription factors (Additional file 3:
Figure S1).

We were also interested in knowing what types of TF
were more frequently interrupted by INDELs. A Monte
Carlo simulation was performed testing the enrichment
of specific TF within our TFBS final sets. Table 1 lists
the top 20 transcription factors enriched for binding
sites near genes (both 5 KB windows) and affected by
INDELs compared to all TF binding sites near genes.
Some of the TFs shown in Table 1 have already been
identified in other analyses. Yokoyama et al. [3], for
example, have recently shown that hominid-specific bind-
ing sites for GATA1 and CTCF are enriched near genes
related to sensory-related function and neurological
pathways. CTCF binding sites have also been shown to
be under positive selection in several Drosophila species
[18]. POL2 has also been studied in humans and chim-
panzees by Kasowski et al. [8] who found inter-species
divergence in the respective binding affinities.

**Evaluation of the effect of TFBS-IDs in the expression of
corresponding genes**

It has been shown that even small changes, like SNVs, in
TFBS affect the affinity of the corresponding transcrip-
tion factor and consequently the expression of the asso-
ciated gene [8]. Therefore, we wondered whether the
presence of an INDEL affecting at least one TFBS would
change the expression pattern of the corresponding
gene. RNA-Seq data for 465 individuals (all of them
from the 1000 Genomes project) from the Geuvadis ini-
tiative [14] was used to compare expression and geno-
type data for the same individual. A statistical analysis
was performed to identify those genes whose presence
of a TFBS-ID was associated to a change in its expres-
sion (comparing individuals according to their genotype:
homozygous for the absence of an INDEL, homozygous
for the presence of an INDEL and finally heterozygous
individuals). Out of the 7,313 genes with at least one
TFBS-ID in the 5 KB window upstream of TSS, 6,248
were informative for this expression survey. Out of these
6,248 genes we found that 18.5% (1,155 genes considering
q-value ≤0.05 as a threshold) had its expression affected
by the presence of a TFBS-ID (again by comparing in-
dividuals homozygous for absence of the TFBS-ID,
homozygous for the presence of the TFBS-ID and fi-
nally, heterozygous). This is significantly higher than
expected by chance (p-value < 10^-5; OR 1.16). For the
window flanking the TSS, we found that 1,804 genes
(q-value < 0.05) had its expression affected by the
presence of a TFBS-ID (18.4% of the total). Again, this
is significantly higher than what one expect by chance
(p = 0.04; OR 1.09). It is important to emphasize that
Table 1  Transcription factors enriched in the set of TFBS-ID close to the TSS of known human genes. "TF" refers to the name of the transcription factor; "Number of TFBS" refers to the number of binding sites for the respective TF within the TFBS-ID set; "p-value" refers to the degree of significance for the respective TF enrichment with the final TFBS set against all TFBS near genes.

| TF                | Number of TFBS | 5Kbp upstream | p-value | 5Kbp flanking | p-value |
|-------------------|----------------|---------------|---------|---------------|---------|
| Pol2              | N              | 1818          | <10^{-3}| 4505          | <10^{-4}|
| CTCF              | N              | 1368          | <10^{-3}| 1982          | <10^{-4}|
| TBP               | N              | 879           | <10^{-4}| 1941          | <10^{-4}|
| HA-E2F1           | N              | 684           | <10^{-3}| 1877          | <10^{-4}|
| NFkB              | N              | 666           | <10^{-4}| 1244          | <10^{-4}|
| ZNF263            | N              | 606           | <10^{-4}| 1238          | <10^{-4}|
| TCF4              | N              | 370           | <10^{-4}| 623           | <10^{-4}|
| AP-2alpha         | N              | 237           | <10^{-4}| 475           | <10^{-4}|
| Pol2(b)           | N              | 364           | 0.002   | 802           | <10^{-4}|
| YY1齐鲁(20)       | N              | 580           | 0.097   | 1134          | <10^{-4}|
| Max               | N              | 511           | 0.162   | 962           | <10^{-4}|
| CEBPB             | N              | 721           | 0.217   | 929           | <10^{-4}|
| Pol2-4h8          | N              | 1264          | 0.246   | 2532          | <10^{-4}|
| SP1               | N              | 534           | 0.315   | 1090          | <10^{-4}|
| TAF1              | N              | 905           | 0.577   | 2120          | <10^{-4}|
| USF-1             | N              | 538           | 0.668   | 872           | <10^{-4}|
| CCNT2             | N              | 385           | 0.752   | 915           | <10^{-4}|
| Elf1_(SC-631)     | N              | 693           | 0.993   | 1517          | <10^{-4}|
| c-Myc             | N              | 613           | 0.998   | 1164          | <10^{-4}|
| HEY1              | N              | 827           | 1.000   | 1523          | <10^{-4}|
| Sin3ak-20         | N              | 505           | 1.000   | 1292          | <10^{-4}|
| E2F6_(H-50)       | N              | 524           | 1.000   | 1026          | <10^{-4}|
| YY1              | N              | 434           | 1.000   | 872           | <10^{-4}|
| GATA-1            | N              | 432           | <10^{-4}| 747           | 1,000  |
| AP-2gamma         | N              | 351           | <10^{-4}| 684           | 1,000  |
| GATA-2            | N              | 373           | <10^{-4}| 546           | 1,000  |
| ELK4              | N              | 155           | <10^{-4}| 450           | 1,000  |
| KAP1              | N              | 280           | <10^{-4}| 444           | 1,000  |
| STAT1             | N              | 202           | <10^{-4}| 359           | 1,000  |
| ZZZ3              | N              | 28            | <10^{-3}| 41            | 1,000  |
| SETDB1            | N              | 193           | <10^{-4}| 246           | 0.494  |
| TR4               | N              | 97            | 0.001   | 225           | 1,000  |
| E2F4              | N              | 216           | 0.001   | 506           | 1,000  |
| eGFP-GATA2        | N              | 95            | 0.004   | 127           | 1,000  |

the effect of the INDEL in the expression of the corresponding gene is certainly underestimated by our analysis since only one cell type was evaluated regarding expression. If a given gene is not expressed in the lymphoblastoid cell lineage, no differential expression could be detected. The same is true regarding the expression of a given transcription factor whose binding site was affected by the INDEL.

What type of change is observed in the genes associated with a TFBS-ID? For the 5 KB window upstream of TSS, out of the 1,155 genes whose expression was changed, 654 were up-regulated and 553 were down-regulated in the individuals carrying a certain TFBS-ID, a significant difference from the null expectation (p-value < 0.01; OR 1.06). We could not observe any difference between the two datasets (up-regulated and down-regulated genes) regarding the type of transcription factors whose binding sites were affected by INDELs (q-value > 0.3). For the 5 KB window flanking the TSS, we found 990 and 912 up and down-regulated genes, respectively (a significant difference, p-value = 0.04). Like for the 5 KB window upstream of TSS, there was no enrichment of any specific transcription factor in either gene set (up or down-regulated – q-value = 0.6). In both situations, the sum of up and down-regulated genes does not match the total number of differentially expressed genes because few genes are present in both lists, due to their different behaviour depending on the composition of subjects with a given genotype.

TFBS-affecting INDELs with high differentiation between human populations

We next wondered whether we could identify in our set of TFBS-ID alleles that present a high differentiation between human populations represented in the 1000 Genomes Project. These frequency differences between populations are considered signatures of geographically restricted selection and have been used previously to identify regions under positive selection [13,19]. We restricted this analysis to a set of 911 individuals representing the three major continental groups: 246 Africans (AFR), 379 Europeans (EUR) and 286 Asians (ASN). To identify those INDELs with high differentiation between populations, we calculated the minimal frequency difference (δ) of the derived alleles between all pairs of populations and took into consideration all differences ≥ 20 % (δ ≥ 0.2). This threshold was based in statistical analysis of the distribution of all δ reported here, in which 20 % represents about two standard deviation from the mean (Additional file 4: Figure S2).

For the TFBS-ID identified in the 5 KB window upstream of TSS, this analysis generated a set of 1109, 507 and 663 TFBS-IDs that have a significant δ in AFR, EUR and ASN, respectively. When expression data is taken into consideration, 346, 149 and 132 TFBS-ID (out of the numbers above) seem to affect the expression of the corresponding genes in AFR, ASN and EUR, respectively. Table 2 reports the top 10 TFBS-ID with highest differentiation for all three populations. A
Table 2: TFBS-ID within the 5 KB window upstream of TSS and with highest δ in AFR, ASN or EUR.

| Population | dbSNP id  | Gene     | Type | Size | Population Frequency δ |
|------------|-----------|----------|------|------|------------------------|
| AFR        | rs113103282 CMAHP  | DEL | 1 | 0.88 | 0.71 |
|            | rs111659599 TMEM14C  | DEL | 6 | 0.73 | 0.70 |
|            | rs201685762 ATP1A1OS  | DEL | 3 | 0.75 | 0.69 |
|            | rs200228600 ATP1A1OS  | DEL | 2 | 0.83 | 0.68 |
|            | rs60963584 SAMD4B  | INS | 1 | 0.79 | 0.68 |
|            | rs34107968 MASP2  | DEL | 3 | 0.08 | -0.67 |
|            | rs3824212 MIR6805, RPL28, TMEM238  | DEL | 14 | 0.19 | -0.66 |
|            | rs201075641 ATP1A1OS  | DEL | 4 | 0.71 | 0.65 |
|            | rs60602324 IQCG  | INS | 1 | 0.88 | 0.65 |
|            | rs59484263 RESP18  | DEL | 1 | 0.89 | 0.64 |
|            | rs28366020 NCDN  | DEL | 3 | 0.06 | -0.62 |
|            | rs5840961 RPS-10049.1  | INS | 1 | 0.08 | -0.57 |
|            | - RPS-10049.1  | INS | 1 | 0.08 | -0.55 |
|            | rs34313783 CELA3B  | DEL | 1 | 0.62 | 0.53 |
|            | rs66822811 DUT  | DEL | 38 | 0.78 | 0.52 |
|            | rs5820777 FAM117A  | DEL | 1 | 0.66 | 0.50 |
|            | rs200692689 MRPL36  | INS | 2 | 0.88 | 0.49 |
|            | - MRPL36  | INS | 1 | 0.88 | 0.49 |
|            | rs199953326 MRPL36  | DEL | 1 | 0.88 | 0.49 |
|            | rs75077631 F12  | INS | 1 | 0.77 | 0.49 |
| EUR        | rs201884277 CCNL2  | DEL | 2 | 0.87 | 0.75 |
|            | rs75244934 MIR6808  | DEL | 2 | 0.83 | 0.69 |
|            | rs139938620 TAS1R3  | DEL | 13 | 0.79 | 0.68 |
|            | rs34692283 ADAT1  | DEL | 2 | 0.74 | 0.67 |
|            | rs55726149 EZR-AS1  | INS | 3 | 0.20 | -0.60 |
|            | rs77949675 PHLD1  | DEL | 2 | 0.78 | 0.60 |
|            | rs35231579 BHCC1  | DEL | 1 | 0.16 | -0.58 |
|            | rs139775692 ACAP3, PUSL1  | DEL | 11 | 0.79 | 0.58 |
|            | rs6107744 PYY  | INS | 1 | 0.70 | 0.57 |
|            | rs149947369 FLJ42351  | INS | 5 | 0.79 | 0.55 |

For the TFBS-ID identified in the 5 KB window flanking TSS, we found 1482, 679 and 885 that have a significant δ in AFR, EUR and ASN, respectively. A complete list for the TFBS-ID identified in the 5 KB window flanking TSS is presented at Additional file 6: Table S4.

One interesting gene found in our analysis is MCIR, known to be associated with skin pigmentation in humans [20,21]. A TFBS-ID (rs201097793) associated to this gene has a higher allelic frequency in AFR (0.70) and ASN (0.64) when compared to EUR (0.17). This supports the suggestion from Vernot et al. [10] that regulatory polymorphisms, under recent selection, have an influence in pigmentation phenotypes. Another gene reported to have a TFBS-ID with a differential frequency is VDAC3, a voltage-dependent channel essential for sperm mobility [22]. We found a TFBS-ID (rs145074200) with a higher frequency in AFR (δ = 0.26), as similarly reported by Colonna et al. [23] for a different polymorphism in the same gene.

Taste perception has been crucial in human evolution especially for the detection of toxins. Not surprisingly, bitter taste receptors have been show to be under positive selection in human populations [24]. Our analysis (Table 2) shows that a TFBS-ID associated with TAS1R3, a sweet receptor, shows a high δ in ASN. Shi and Zhang [25] concluded, based in a comparison of several vertebrate species, that both bitter and sweet receptors are under positive selection. TAS1R3 is also a component of the dimeric protein TAS1R1/TAS1R3, which is the umami taste receptor [26]. The umami taste is a common feature of many foods in Asia and is reasonable to speculate that this variant is being selected in Asians [27].

Response to parasites and microbes has been constantly subject to adaptations in human evolution [28,29]. We found a TFBS-ID (rs139999735) with a higher allelic frequency in AFR (0.34 compared to 0.11 in ASN and 0.12 in EUR). The gene associated with this TFBS-ID is APIP (APAF1-interacting protein) whose protein has been shown to be an inhibitor of pyroptosis and apoptosis, both a response to Salmonella infection [30]. Based on that it is predicted that the TFBS-ID would cause a decrease in APIP expression. Indeed, Fig. 3A shows that expression of APIP decreases significantly in individuals homozygous for the TFBS-ID (rs66822811) for a dimeric protein which is under positive selection in AFR due to a down-regulation of APIP and consequently a better response to Salmonella infection. Fig. 3B shows that a selective sweep analysis supports this proposal. Individuals homozygous for the presence of the TFBS-ID show a decreased genetic heterogeneity around the TFBS-ID position (vertical dashed line in Fig. 3B).

To gain further insights on what types of genes are associated with TFBS-ID showing a high differentiation between the three human populations, an ontology analysis was performed. Fig. 4 shows the major GO categories enriched (using a threshold of p ≤ 0.01) in the dataset of genes associated to TFBS-ID for each of the three populations used in this study (5 KB window upstream of TSS). Two GO categories were enriched in all three populations: “Regulation of Transcription” and “Histone 3’ end mRNA processing”. “Urea transport” is enriched in both ASN and EUR. All the other categories are enriched only in one population, as seen in Fig. 4. Overall, there are a large number of categories related to
immunological response. Interesting categories enriched in Africans and Asians are “Response to protozoans” and “Response to biotic stimulus”, respectively. In Europeans one enriched category is “UV protection”, known to be under positive selection in this population [31]. For the 5 KB window flanking the TSS, some of the categories seen for the 5 KB window upstream of TSS are still present (Additional file 7: Figure S3) although several categories clearly linked to recent selection in humans are missing.

**TFBS-ID match regions known to be under positive selection in the human genome**

In the last few years, several genome-wide strategies have been used to identify regions in the human genome that are under positive selection [15,28,29,32,33]. The recent availability of data from the 1000 Genomes project has catalysed such approach and hundreds of regions have been identified. To evaluate whether our set of TFBS-IDs correspond to genetic units that are under selection, a comparison was made with one of the most complete, in terms of the number of metrics used, of such studies [15].

When we compared our total set of 10,520 TFBS-ID close to the 7,313 human genes (5 KB window upstream of TSS), we found that 3,499 (33.2 %) matched regions under selection as defined by Pybus et al. [15] within a 95 % confidence interval. With a 99 % confidence interval, we found 797 TFBS-IDs (7.5 %) that matched genomic regions under selection. For the 5 KB window flanking TSS, we found that 4,747 (32.3 %) TFBS-ID match regions under selection within a 95 % confidence interval. With a 99 % confidence interval we found 1,061 (7.2 %) TFBS-ID matching regions under selection. Fig. 5 shows the results for a gene ontology enrichment analysis (p ≤ 0.01) with the set of 797 TFBS-ID (5 KB window upstream of TSS) that matched genomic regions under selection. Three major categories are evident: ontologies associated with immunological responses, response to radiation and haematological/cardiac processes. All these processes have been shown to be under recent positive selection in humans [15, 23, 28, 31, 32, 34]. For the set of 1,061 TFBS-ID matching genomic regions under selection and within the 5 KB window flanking the TSS, we found while some categories are still present, when compared to the 5 KB window upstream of TSS, several differences exist (Additional file 8: Figure S4). Overall, the gene ontology analysis presented here (Figs. 4, 5, Additional file 7: Figure S3 and Additional file 8: Figure S4) suggests that the inclusion of a region downstream of TSS diluted the selection signal observed for the 5 KB window upstream of TSS. This in accordance with a recent finding from the GTEx Consortium about a higher frequency of eQTLs located upstream of TSS [17].

**Conclusion**

By integrating different types of data, we provide a comprehensive catalogue of polymorphic INDELs affecting TFBS in the human genome. Overall, our findings
support the notion that regulatory variation has been important during human evolution. Some of the genes associated with these TFBS-affecting INDELs have been previously identified as targets of positive selection in human populations. The remaining set of genes and INDELs, however, represents a rich source of new information related to human evolution. We envisage that this dataset, together with the ones previously reported, will catalyse a series of new investigations on how recent human evolution has shaped gene regulatory networks.

Methods

Data Sources

Data from several projects were gathered in a local processing server for further analysis. This data included: (i) genome coordinates of all TFBS peaks from the ENCODE project [13] release 2 obtained from http://genome.ucsc.edu/encode; (ii) phase 1 genotype data from the 1000 Genomes Project Consortium [12] obtained from http://www.1000genomes.org; (iii) gene expression quantified by the Geuvadis project [14] obtained from http://www.geuvadis.org; (iv) the genome-wide selection measures of CLR [34], Fay and Wu's H [35], Fu and Li's D [36], R2 [37] and Tajima's D [38] calculated by Pybus et al. [15] obtained from http://hsb.upf.edu/; and (v) genome coordinates of the largest transcript of each known human gene from RefSeq release 64 obtained from http://genome.ucsc.edu/.

All the data from humans was obtained from public sources. All ethical considerations were dealt in the original publications.
To identify TFBS peaks, ENCODE project analysed ChiP-seq of 145 TF's antibodies among 95 cell lineages employing a pipeline developed by Landt et al. [39]. This pipeline uses multiple peak calling software (e.g. MACs, SPP and PeakSeq) and analyses replicates variance to further improve the peak calling sensitivity [13,39]. The employed procedure is detailed at the ENCODE project guideline page (http://genome.ucsc.edu/ENCODE/experiment_guidelines.html).

This study is exempted from ethical approval since all human data used here is publicly available in an anonymized fashion.

Data Filtering and Annotation
Using GATK v.2.6 [40] (Genome Analysis Toolkit) we first filtered all INDEL variants shorter than 200 bp reported by TGP that overlapped an autosomal TFBS described by ENCODE. It is important to mention that our pipeline establishes as a rule that the beginning of a given indel had to be inside a TFBS, precluding therefore that a whole TFBS be removed by an indel. This set was then annotated and only those TFBS-ID near any known gene (up to 5Kbp upstream of TSS) were selected using snpEff v.3.5 [41]. This procedure and the number of elements at each step of the pipeline are illustrated in Fig. 1. The results were organized in a local MySQL v.5.5 (Oracle Corporation) database for easy access and manipulation.

Statistical Analysis
All statistical analysis and plotting were performed with R package v.3.1 [42]. Multiple analyses were corrected by Benjamin-Hockberg method (or False Discovery Rate - FDR).

Population Differentiation
To identify differentiated alleles among the European, Asian and African populations from the TGP (376, 286...
and 246 individuals respectively), the minimum allele frequency difference ($\delta$) for each mutation per population was calculated according to the following equation.

$$\delta(i,j) = \min\left(\left|f_{ijk} - f_{ij}\right|\right) \forall k \in \{P - j\}$$

Where $\delta(i,j)$ is the minimum allele frequency difference of the variant $i$ in the population $j$; $f_{ij}$ is the allele frequency of the variant $i$ in the population $j$; $f_{ik}$ is the allele frequency of the variation in the population $k$ and $P$ is a representation of all populations investigated (in this case EUR, ASN and AFR). This analysis did not include the American samples from the TGP due to their admixed nature.

**Gene expression association**

The Spearman correlation test was used to evaluate any putative association between genotype data from TGP and gene expression data from Geuvadis of all TFBS-ID associated to the respective genes. The number of variant copies was assumed as dependent variable (therefore $0$ for reference homozygous, $1$ for the heterozygous and $2$ for mutant homozygous). The same is true for the gene expression measured in FPKM (Fragments per Kilobase of Transcript per Million Mapped Reads). To interactively perform this test, a Python v2.6 (Python Software Foundation) script was developed using SciPy v0.14 [43] statistics library to calculate the correlation. The result was later filtered for non-quantified genes and non-variable genotypes among the Geuvadis samples.

The spearman correlation coefficient was calculated using the following formula $(r_s)$, where $n$ is the sample size, $r_{variants}$ is variant number rank and $r_{fpkm}$ is fpkm rank. Rank ties were resolved using rank tie mean value. The correlation $p$-value was obtained by approximation to a $t$ distribution and multiple testing was corrected by Benjamin-Hockberg method. The correlation was considered significant on q-value $\leq 0.05$.

$$r_s = 1 - \left[6 \sum \left(r_{variants} - r_{fpkm}\right)^2 / (n^3 - n)\right]$$

**Gene ontology category enrichment**

To evaluate potential functional aspects within the set of investigated genes, we analysed gene ontology enrichment by two strategies. The first one employed Cluster-Profiler v2.0 [44] from the R package to search for overrepresented categories on the subset of investigated genes based on hypergeometric distribution. The second strategy employed a Monte Carlo method to evaluate the probability of ontology enrichment using 10,000 random simulations.

During each Monte Carlo simulation, a random gene set was generated with the same size of the investigated set and its ontology annotated. The ontology $p$-value was obtained from the simulated distribution of annotated genes.

**Positive selection sites identification**

To identify genomic sites under positive selection, we gather data of five statistical measures of positive selection (CLR, Fay and Wu's H, Fu and Li's D, R2, and Tajima's D) [34–38] from three populations (Utah Residents with Northern and Western European ancestry; Han Chinese from Beijing, China; and Yoruba from Ibadan, Nigeria representing EUR, ASN and AFR) respectively calculated by Pybus et al. [15]. All five measures are common positive selection score of the literature further explained on [15,34–38]. The authors computed a ranked $p$-value according to each measure genome-wide distribution to access the value statistical significance. The ranked $p$-value was obtained by sorting the measures genome-wide scores and computing the fraction of higher scores, further explained on Pybus et al. [15]. Since each measure considers different selection parameters, any given site was considered under selection with at least 95 % confidence interval ($p$-value $< 0.05$) to any measure or population.

**Additional files**

Additional file 1: Table S1. TFBS-ID description and associations for 5 KB window upstream of TSS.
Additional file 2: Table S2. TFBS-ID description and associations for 5 KB window flanking TSS.
Additional file 3: Figure S1. Distribution of TFBS-ID within both 5 KB windows split by types of transcription factor.
Additional file 4: Figure S2. TFBS-ID minimum frequency difference ($\delta$) distribution. The black curve represents the observed $\delta$ distribution and the red curve represents a normal curve of same mean and standard deviation. The dashed lines indicate two standard deviation ($\pm 0.2$) from average (regarding the observed $\delta$ distribution) on both sides.
Additional file 5: Table S3. TFBS-ID showing $\delta > 0.2$ between populations (5 KB window upstream of TSS).
Additional file 6: Table S4. TFBS-ID showing $\delta > 0.2$ between populations (5 KB window flanking TSS).
Additional file 7: Figure S3. Ontology analysis for genes associated to TFBS-ID with $\delta = 0.2$ in the respective population (5 KB window flanking TSS). Color of the circle refers to the $p$-value of the enrichment while size of the circle refers to the numbers of genes within that GO category.
Additional file 8: Figure S4. GO enrichment analysis for TFBS-ID matching regions known to be under selection in the human genome (5 KB window flanking TSS). Color of the bars refers to the $p$-value of the respective enrichment. Length of the bar refers to the number of genes within the respective GO category.

**Abbreviations**

AFR: African population from 1000 Genomes Project; ASN: Asian population from 1000 Genome Project; ENCODE: Encyclopedia of DNA Elements; EUR: European population from 1000 Genomes Project; INDEL: Insertion / Deletion; SNV: Single Nucleotide Variant; TF: Transcription Factor; TFBS: Transcription Factor Binding Site; TFBS-ID: Transcription Factor Binding Site affecting INDEL; TGP: 1000 Genomes Project; TSS: Transcription Start Site.
Competing interests
The authors have read BioMed Central's guidance and declare no competing interests.

Authors' contributions
AMRS carried out the data acquisition, organization and analysis, and drafted the manuscript. VLS carried out gene ontology enrichment and drafted the manuscript. JESS assisted in data analysis and participated in the study design. SJS conceived the study, participated in its design and coordination and helped draft the manuscript. All authors read and approved the final manuscript.

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