Portrayal of the human blood transcriptome of 3,388 adults and its relation to ageing and health

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Abstract

Background: The blood transcriptome is expected to provide a detailed picture of organism’s physiological state with potential impact for applications in medical diagnostics and molecular and epidemiological research. We here present the first systematic analysis of blood specimen of 3,388 adult individuals collected in the Leipzig Research Center for Civilization Diseases, together with phenotype characteristics such as disease history, medication status, lifestyle factors and body mass index (BMI).

Methods: Multidimensional Self Organizing Maps-portrayal was applied to study transcriptional states on a population-wide scale. The method permits a detailed description and visualization of the molecular heterogeneity of transcriptomes and of their association with different phenotypic features.

Results: The diversity of transcriptomes is described by about one dozen modules of co-expressed genes of different functional context. We identify two major blood transcriptome types where type 1 accumulates more men, elderly and overweight people and it upregulates genes associating with inflammation and increased heme metabolism, while type 2 accumulates women, younger and normal weight participants and it associates with activated immune response, transcriptional, ribosomal, mitochondrial and telomere-maintenance cell-functions. We find a striking overlap of signatures shared by multiple diseases, ageing and obesity driven by an underlying common pattern, which reflects the increase in inflammatory processes.

Conclusions: Our portrayal framework provides a holistic view on the diversity of the human blood transcriptome. It provides a tool for comparative analyses of
transcriptional signatures and of associated phenotypes in population studies and medical applications.

1. Background

Blood is the pipeline of human organism’s physiology. The accessibility and minimal invasiveness during sampling made it the most feasible source in scientific research and clinical diagnostics as they could replace more invasive and risky tests [1]. Because of utility and simplicity, blood transcriptome investigations on genome-wide scales have gained in popularity over the past years. They were applied in medical context for characterizing diseases such as ischemic stroke [2], Alzheimer’s disease [3], epilepsy [4], sepsis [5-8]; in pharmacogenomics [9] and marker search [10]; and also in epidemiological investigations on ageing [11], obesity status [12, 13], lifestyle factors such as smoking and alcohol consumption [14], special nutrition [15] and in immune system characterisation [16] (see [17] and references cited therein for a broad literature survey).

Although relatively simple to extract, blood is not a simple tissue. It accounts for about 8% of body weight and composed of acellular fluid (plasma) and a mixture of multiple cell types at different stages of their life cycles, lineage and function. On transcript level, blood is a complex mixture where changes in transcript abundance can be attributed to either alterations of transcriptional regulation in the different cell types or to relative changes in composition of cell populations. Computational cell type deconvolution techniques such as CIBERSORT [18] (see [19] for an overview) attempt to deduce cellular composition from transcriptome data using statistical methodologies and cell-specific RNA-signatures, which are usually obtained independently in calibration experiments and are assumed to remain
invariant. This assumption is imprecise because blood cells can change their state, e.g. via transformation from naïve to specialized cell types involving biological processes such as chemotaxis, surface signalling and cell-cell adhesion, all governed by expression changes of associated genes [16]. Therefore, cell subset classification is to a greater or lesser extent artificial, reflecting our current ability to distinguish cells based on specific sets of available markers [11]. Since blood flows throughout the whole body it implements functions by recirculating between central and peripheral lymphoid organs as well as to and from different tissues, sites of inflammation and/or injury. The cells specifically recapitulate the influence of genetic, epigenetic, cellular and environmental factors, which can vary between individuals and between their particular constitutions over time and age. In conclusion, the blood transcriptome is expected to provide a detailed picture of organism’s physiological state with individual resolution. Most of the studies mentioned above focused on only one or a few diseases and/or environmental and lifestyle conditions and didn’t answered the question whether the identified gene expression signatures are condition-specific or more widely applicable. Moreover, factors contributing to transcriptome variability among nominally healthy individuals are relatively unexplored so far.

We here present the first systematic analysis of the transcriptomes obtained from whole peripheral blood specimen of more than 3,000 adult individuals collected in LIFE (-adult), the Leipzig Research Center for Civilization Diseases, together with a large collection of phenotype characteristics such as disease history, medication status, lifestyle factors and body mass index (BMI) [9](Table S 1). Our analysis aims (i), at characterizing the inter-individual variability of the blood transcriptomes in terms of a suited classification scheme; (ii), at describing the diversity of
transcriptome states using a collection of modules of co-expressed genes and at characterizing their biological functions; and (iii) at associating the transcriptome landscapes with a series of phenotype data collected in a participant-matched fashion. Overall, these issues are expected to provide a holistic view on essential properties of the blood transcriptome, a methodical framework for its analysis and its possible impact for future applications.

Previously, we have developed an omics ‘portrait’ methodology based on self-organizing maps (SOM) machine learning [20, 21]. It has been applied to a series of data types and diseases [22-26], among them a study about footprints of pneumonia in the blood transcriptome [8]. SOM-portrayal takes into account the multidimensional nature of gene regulation and pursues a modular view on co-expression, reduces dimensionality and supports visual perception by delivering ‘personalized’, case-specific transcriptome portraits. They enable a straightforward and intuitive interpretation and mutual comparison of whole transcriptome landscapes between cases and classes. By applying this method to the blood transcriptomes of thousands of individuals we aim at demonstrating that multidimensional SOM-portrayal permits a detailed description and visualization of the molecular heterogeneity of transcriptional states and of their association with different phenotypes with potential impact for applications in medical diagnostics and molecular and epidemiological research. Figure 1 provides a schematic overview of the design of this investigation.

Figure 1

2. methods
2.1. LIFE-adult study and phenotype characteristics

The LIFE (-adult) research project conducted one of the largest population studies in Germany focusing on extensive phenotyping of urban individuals from Leipzig city [27]. It included more than 10,000 participants in order to discover the interplay between molecular, environmental and lifestyle factors and their impact for the health status of the population. The study was approved by the ethics board of the Medical Faculty of the University of Leipzig. In this publication we analyzed transcriptomic data of whole peripheral blood (WPB) samples, which were obtained from 3,388 adult participants of the LIFE-adult study. They roughly divide equally into women and men covering an age range between about 20 and 80 years with a strong bias towards elderly persons. The LIFE-adult study overall collected a broad survey of lifestyle and health items (see [27] for details). We made use of selected lifestyle characteristics of the participants such as smoking behaviour and alcohol consumption, medication according to ATCs (Anatomical Therapeutic Chemicals) indexing and disease history of the participants collected via questionnaires, blood count data from clinical laboratory including selected serum markers and body mass index (BMI) (Table S 1).

2.2. Blood transcriptome sampling, microarray measurements and data preprocessing

We made use of pre-processed gene expression data extracted from WPB samples of individuals as provided by the LIFE data base. Participant’s recruitment, blood collection, storage and mRNA preparation, microarray measurements and primary data pre-processing was realized by different groups of the LIFE center [27]. WPB was collected in Tempus Blood RNA Tubes (ThermoFisher, Waltham, MA, USA) and
stored at -80 °C until further processing. RNA was isolated and then hybridized to Illumina HT-12 v4 Expression BeadChips (Illumina, San Diego, CA, USA) and measured on an Illumina HiScan device. Raw probe level data were extracted using Illumina GenomeStudio and then further pre-processed including outlier and missing value removal, log-transformation, quantile normalization and centralization of the expression value of each gene using an in-house pipeline (Supplementary File 1: supplementary methods). The final transcriptome data consists of more than 48,000 probe IDs including the expression values of 19,049 genes for each of the individuals.

2.3. Self-organizing maps (SOM) transcriptome portrayal

Pre-processed expression values were analyzed using the oposSOM pipeline, available as R-package “oposSOM” [28]. It uses SOM neuronal network machine learning to translate the high-dimensional expression data of N= 19,049 gene transcripts into K=10,000 metagene expression data per individual [20, 29]. Each metagene represents a ‘micro’-cluster of co-expressed genes showing mutually similar expression profiles across the samples. Metagenes were arranged in a 100x100 two-dimensional grid coordinate system and colored according to their expression level for each sample thus providing a ‘personalized’ image of the blood transcriptome of each individual studied (Figure S 1A). Mean portraits of transcriptome classes (see below) were calculated by averaging metagene expression values over all portraits of the respective group. Default color scale (red to blue for maximum to minimum expression, respectively) of the portraits uses log-expression values of the metagenes [20]. Diversity of sample portraits was visualized using a graph representation called ‘correlation network’ as implemented in ‘oposSOM’ [34].
2.4. ‘Spot’ clustering of co-expressed genes and stratification of samples

Metagenes of similar profiles cluster together forming ‘spot-like’ red and blue areas of over- and under-expression in the portraits due to the self-organizing properties of the SOM. Each of the spots represents a cluster of mutually correlated genes (Figure S 1B). The spots were detected using distance-metrics clustering, which determines each spot by a ‘halo’ of surrounding metagenes of local maximum distances to their neighbours [30]. The spot expression patterns obtained represents a characteristic fingerprint of each particular sample. The overall collection of spot-modules detected are major nodes of the co-expression network derived from the sample series (see the spot correlation and implication networks in Figure S 1B and C, respectively). Based on their transcriptome portraits we stratified the samples into appropriate groups. In a first step the portraits were divided into 33 so-called combinatorial pattern-types (cPATs), each defined by a certain unique combination of over-expressed spots [22] (Figure S 2A). Using the cPATs we estimated the tentative number of groups (Figure S 2A - C) and used them subsequently in a K-means clustering run, which stratifies the portraits into three major transcriptome types and nine subtypes (STs, Figure S 2A - C). The transcriptome strata were further characterized by detailed statistics about spot appearance (Figure S 2C - D).

2.5. Function mining

We applied gene set analysis to the lists of genes located in each of the spot modules to discover their functional context using right-tailed Fisher's exact test [36,37]. In addition, the gene set enrichment z-score (GSZ) was used to evaluate
the impact of the gene sets in the different transcriptomic strata [32,38]. The GSZ-metrics considers the mean expression of the gene set normalized by its variance, i.e. it provides high values for homogeneous gene sets reflecting activation of biological functions with high relevance for the respective transcriptional states. Gene set maps complement this analysis by visualizing the position of the gene of a set within the SOM grid. According to their degree of accumulation in or near the spots, one can deduce their potential functional context [20].

2.6. Phenotype portrayal

Phenotype information of the participants comprises their blood cell and marker counts, BMI and information about their lifestyle (smoking and alcohol consumption), medication and disease history (Table S 1). The enrichment of categorical phenotypic characteristics in each of the transcriptomic classes (types and subtypes) was estimated using one-tailed Fisher’s exact test and visualized as enrichment heatmaps. Phenotype-to-metagene correlation maps were generated by correlating each of the phenotype parameter-profiles over all participants with each of the metagene expression profiles. The matrix of correlation coefficients obtained was then visualized in the SOM-grid as ‘phenotype’ portraits using a red-to-blue (maximum-to-minimum correlation) color-code. The metagene of maximum correlation coefficient was marked in the SOM-grid of a phenotype overview map. Expression of each of the spots was fitted using multiple regression with the phenotype values of the participants of each of the categories as variables. Standardized regression coefficients were then visualized as heatmaps.

3. results

3.1. The blood transcriptome splits into three types
SOM analysis provides one portrait for each of the 3,388 LIFE-adult participant's WPB transcriptomes (Supplementary File 2 and Figure S1A). For an overview we performed unsupervised similarity analysis based on pairwise comparisons of these expression portraits using Pearson's correlation coefficients and their visualization as pairwise similarity heatmap (Figure 2A). The samples split into three major types where type 1 and type 2 show pronounced anti-correlated expression portraits while type M forms an intermediate group. The network presentation reveals that WPB transcriptomes of type 1 and type 2 split into separate clusters while type M samples overlap between them (Figure 2B). The functional context of activated genes was estimated using gene set analysis (Figure 2A, part below). Type 1 associates with functional categories related to oxygen transport, heme metabolism, neutrophil accumulation and repressed chromatin states of T-cells while type 2 relates to immune response, transcriptional activity, T-cell accumulation and active chromatin states (see below). Males and females were represented in all types. Higher percentage of men was noted in type 1 (29% versus 19% for women) and reverse relation was found for type 2 (percentage of women: 37% versus 51%; Figure 2C). Type 1 is higher among elderly persons compared with type 2; however, in the latter, the age-dependence is different between women and men (Figure 2D). The composition of types for women changes virtually monotonously with a steadily increasing percentage of type 1 in contrast to men, who show a maximum of type composition in the age range of 50 – 55 years. Note also that the age dependence of type M more resembles that of type 1 than that of type 2 which suggests functional correspondence between types M and 1 (see below). The type-composition of men and women is virtually independent of BMI (body mass index) except for very obese persons (BMI > 35 kg/m²) which seem to
accumulate more type 1 transcriptomes (Figure 2D).

Taken together, we identify two major blood transcriptome types and an intermediate type partly resembling type 1. Type 1 accumulates more men, elderly participants and it upregulates genes associating with inflammation and increased heme metabolism, while type 2 accumulates women and younger participants. It associates with activated immune response and transcriptional activity. The composition of types changes in a gender- and age-specific fashion.

Figure 2

3.2. A modular map of gene activation

Clusters of genes with correlated expression profiles appear as red spot-like areas in the transcriptomic portraits, which indicates their overexpression in the respective samples (Figure S 1A). Overall we identified 13 such major overexpression spots and labelled them with capital letters A – M (Figure 3A, for spot lists of genes see Table S 3 and Supplementary File 3). It roughly divides into two major areas containing spots predominantly upregulated either in type 1 (and partly also type M) or type 2 samples, respectively, and a third area with mixed spot assignment as illustrated by mean portraits of the transcriptomic types (Figure 3B), the spot profiles (Figure 3C and Figure S 3) and their correlation network (Figure 3D). Gene maps indicate the positions of genes taken from selected functional gene sets within the SOM grid of metagenes (Figure 3A). For example, genes upregulated in erythrocytes and platelets accumulate in spots C and N (up in type 1), respectively, while genes associated with mitochondrial function and RNA processing are found in spot E and G. Signature genes of T cells and of ribosomal function accumulate in and near spots I and J (up in type 2). Spot H accumulates the
signature of CD4 cytotoxic T lymphocytes (CTLs) including the marker genes GZMA and PRF1, which were recently found to associate with extreme longevity [31].

Genes with function in interferon (IFN) response accumulate in spot L without preferential upregulation in one of the three types. Typically, each of the individual sample portraits shows more than one spot, which reflects the parallel activation of different transcriptional programs and/or their mutual couplings. We subsume frequently observed combinations of expressed spots as so-called combinatorial pattern types (cPATs). Overall we identified 33 cPATs, which were then used to sub-stratify each of the major transcriptomic types into three subtypes (STs, annotated by 1.1, 1.2, 1.3, M.1, M.2, M.3 and 2.1, 2.2, 2.3, respectively) differing in their mean expression portraits (Figure 3D) and spot expression (Figure 3B and Figure S 2). Part of the spot profiles show marked expression differences between the STs (e.g. spots A, B, D, F) while others change continuously (e.g. spots H- J). Most of the spots upregulate either in type 1 or 2 samples. Interestingly, spot F enriching genes encoding ribosomal subunit S26 proteins shows a specific expression patterns with strong upregulation in part of STs without preference to either type 1 or type 2. Spot co-occurrence analysis indicates that adjacent spots are often observed together, but also spots from different areas can co-occur, especially in samples of type M, which supports their intermediate position between type 1 and type 2. Part of the STs are dominated by samples expressing only one spot while others, especially of type M, show a broader distribution owing to more heterogeneous expression patterns (Figure S 2C). The sample similarity net indicates that most samples of the different STs accumulate into well localized clouds reflecting their mutual similarity (Figure 3E and Figure S 2F). The ST-composition is virtually age-independent except ST 1.1, which collects
an increasing percentage of men and women at an age above 65 years (Figure S 4). In summary, the diversity of transcriptional states can be described by the combinatorics of about one dozen modules of co-expressed genes of different functional context which decompose each of the transcriptional types into three subtypes.

Figure 3

3.3. Footprints of functions: cellular programs, infections, telomeres and epigenetics

Next we performed functional analysis of the transcriptome strata using gene sets taken from the functional categories ‘biological process’ [33] (Figure 4A), ‘hallmarks of Cancer’ offering disease characteristics in a more general context [34] (Figure S 6), ‘telomere maintenance’ [35] and ‘epigenetic states’ (Figure 4A-E). Profiling of these signatures splits them into two major clusters either upregulated in type 1 (marked with green color in the figures) or type 2 (apricot color), respectively. Type 2 associates, for example, with activation of cell cycling, MYC-target genes, oxidative phosphorylation (oxphos) while inflammation, hypoxia, coagulation, reactive-oxygen species and pathway signaling of TNFalpha-, TGFbeta-, PI3K-Akt-MTOR-, IL6-JAK-Stat3 activate in type 1. A third cluster (blue color) accumulates signatures related to interferon (IFN) response, which eventually suggests association with viral infections. We analyzed expression signatures derived recently to differentiate between bacterial and viral infections [36-41] (Figure 4B and C, respectively). The former signatures associate with the
‘inflammatory’ spots A, O and, also M, which upregulate in type 1 samples. In contrast, viral signature genes accumulate strongly in the IFN-response spot L, which is found upregulated in about 10% of all samples.

We are also interested in expression profiles of genes involved in telomere length maintenance (TM) via activation of telomerase. Mean telomere length in human leukocytes is negatively correlated with lifespan and BMI [42, 43] and it associates with heart diseases, type 2 diabetes, cancer [44-46], lifestyle factors [47], diet [48] and psychological stress [49]. TM-genes are more active in type 2 transcriptomes, which suggests that they stronger counteracts telomere shortening in younger (and healthier) individuals (Figure 4D). TM expression associates with cell cycle activity, starvation, oxidative stress, ageing, DNA-methylation and other functions related to spots I and J this way indicating strong mutual coupling between TM and our transcriptome types (Figure S 7).

Next we analyzed the expression sets of genes assigned to distinct chromatin states in blood cells under healthy conditions, among them T-, B- and T-regulatory-cells [77] (Figure 4E and Figure S 8). States involving genes with active promoter (TssA) and completed transcription (Tx) and in repressed promoter states are expected to show high and low expression levels, respectively. This relation is indeed observed in type 2 transcriptomes, however it reverses in type 1. This reversal suggests de-repression of nominally repressed states and repression of active states in type 1 transcriptomes by epigenetic chromatin re-modelling. We recently demonstrated that differentiation and adjustment of cellular programs are governed by subtle cooperation of transcription factor (TF-) networks and epigenetics, e.g., via regulation of the polycomb repressive complex 2 (PRC2) and its targets[50]. We find that signatures, related to TF-networks regulate cell function requiring relatively
high expression levels of their major regulatory genes such as cell cycle, oxphos and transcription predominantly in type 2 transcriptomes (Figure S 9). On the contrary, repressive epigenetic signatures related to PRC2 function, repressive histone (H3K27me3) marks and DNA-methylation antagonistically change compared with those of the TF-networks. Interestingly, these profiles show moderate and low expression levels according to the accumulation of their signature genes in the central region of the map. In summary, type 2 transcriptomes associate with cell cycle, oxphos-metabolism, telomere maintenance and immune system activity regulated mainly via transcription factor networks, which become repressed in type 1 transcriptomes in parallel with epigenetic de-repression of inflammatory cellular programs including responses to infections.

**Figure 4**

3.4. Previous gene expression signatures of the blood transcriptome

Modules of co-regulated genes of a previous blood transcriptome study [53] well agree with our spot clusters and further specify functional interpretation in terms of associated blood compounds such as cytotoxic plasma-, T- and B-cells (up-regulated in type 2) and erythrocytes, platelets, neutrophils and cells of myeloid lineage (up in type 1) (**Figure 3A** and **Figure S 10**). Another study extracted ageing signatures of the blood transcriptome [11]. Genes of decreasing expression (‘age_dn’) accumulate near spots I and J (up in type 2) while genes of increasing expression (age_up) are found in wider areas around spots A, M and H (up in type 1) (**Figure S**
This asymmetry of the numbers of spots suggests that age_up involves a more heterogeneous collection of molecular mechanisms than age_dn (see below). Another set of signatures was obtained recently in a study of the blood transcriptomes collected from patients of sepsis framed with CAP (community acquired pneumonia) [8](Figure 4F). These signatures surprisingly correspond to signatures of nominally healthy individuals, e.g. patients with less severe CAP show signatures of type 2 transcriptomes, and while more severe CAP cases show type 1 transcriptomes associating partly with activation of inflammatory and endotoxin tolerance characteristics [8].

Next, we made use of a repertoire of 382 functionally annotated expression modules extracted from a recent meta-analysis of the blood transcriptomes of 16 disease and physiological states [52](Figure 4G and Figure S 12). Clustering of these signatures sub-stratifies them into three of type 1-like clusters which are strongly affected by spot O (C1 in Figure 4G), A (C2) or C (C3), respectively. Their profiles resemble those of the different severe CAP transcriptomes and can be interpreted as inflammatory signatures which are modulated by increased and decreased erythrocyte (spot C) and thrombocyte (spot N) activation patterns, respectively. Further, the 382 modules provide a rich repertoire of functional annotations, which support interpretation of our data (see example profiles in Figure 4G and Supplementary File 4 for the full set of profiles). For example, age_dn modules agree with DNA-methylation signatures in the blood. Methylation of CpG’s in the promoters or enhancers upon ageing obviously repress transcription of the respective downstream gene (see also Figure S 1), which is in agreement with the finding that altered methylation sites enrich in ageing genes [11]. Moreover, we find strong enrichment of 91 of these modules in at least one of our spots (Figure S
12A). Hence, the spots provide a sort of basis set of co-regulated genes, which further expands into a rich collection of functional annotations of different categories via a multitude of combinations as considered by our cPATs (see above). Correlation analysis of different previous blood signature sets [8, 11, 52, 53] and our spot profiles provides very similar patterns in support of this view on the modular structure of the blood transcriptome (Figure S 13). In summary, comparison of previous blood signatures with our data shows that our spot-modules represent a sort of minimum set describing co-expression of the blood transcriptome. It expands into a rich collection of functional annotations including molecular mechanisms, cellular programs, cell types but also lifestyle factors, diseases and ageing effects.

3.5. Blood cell signatures and seasonal effects

Gene sets implemented in blood cell deconvolution algorithms such as Cibersort [18] show the characteristic correlation patterns observed also in the other blood signatures (compare Figure 4H and Figure S 14). They link the expression patterns of 22 blood cell types with our spot profiles. Elevated expression (and cell fractions, Figure S 15) of monocytes, neutrophils and eosinophils is observed in type 1 transcriptomes while overall expression of T- and B-cells upregulates in type 2. Expression of M1 macrophages and dendritic cells associate with the IFN-response signature (spot L). Furthermore, signatures of monocytes, M0 and M2 macrophages are also enriched in spot L, however in combination with the inflammatory spot O. Recent studies report seasonal changes of gene expression of the blood transcriptome and of blood cell counts [54, 55]. We find a slight shift of transcriptome characteristics towards type 1 in winter compared with summer both for men and women (Figure S 16). It is characterized by increased expression levels of inflammation (spot A) and erythrocyte expression (spot C) and counts and
decreased levels of thrombocyte characteristics (spot N) and reticulocyte and eosinophil counts (Table S 5). Overall, the seasonal changes of type compositions are relatively small (less than 3% in men and 1% in women) and are not explicitly considered further.

3.6. Phenotype portrayal: Blood cell counts, lifestyle, medication and disease history

Previous blood transcriptome studies also extracted gene signatures which associate with health-related features such as BMI (body mass index) and smoking status and also with the development of different diseases such as heart failure [56], dental caries [57], schizophrenia and neoplasms [52]. We find that they predominantly upregulate in type 1 transcriptomes showing characteristics of ageing and/or inflammation (Figure S 17). The LIFE-adult study provided a series of features characterizing health and lifestyle of the participants in terms of so-called phenotypes (Table S 1). We associated them with the blood transcriptomes in a participant-matched fashion using phenotype portraits, which typically show areas of positive (colored in red) and negative (in blue) correlation between phenotype features and expression profiles in the transcriptome landscape with metagene resolution (Figure 5A, and for details Figure S 19 - Figure S 23). For example, phenotype associations with expression patterns of type 1 (red in the lower left part of the map) or type 2 (red in the upper right part) can be distinguished. In addition, overview maps were generated for each of the phenotype categories, which mark the metagene of maximum (and minimum) correlation for each of the phenotypes studied. Enrichment of phenotypes is evaluated in terms of the distribution of cases among the transcriptome types (Figure 5C, for enrichment significance evaluation
using Fishers exact test see Figure S 19D - Figure S 23D).

We find that most blood count data correlate either with type 1 (e.g. erythrocytes, reticulocytes, platelets, neutrophils) or type 2 (lymphocytes) transcriptomes in agreement with the blood cell transcriptomes analyzed above. Smokers, alcohol consumers (> 30 g/day), obese and elderly people, men and participants taking different categories of medication according to the ATC (Anatomical Therapeutic Chemicals) classification and also participants with different self-reported lifetime diseases show preferences for type 1 (and partly type M) transcriptomes while younger, under- and normal-weight participants, women and non-consumers of medication associate preferentially with type 2. The degree of correlation with metagene expression is markedly higher for blood counts compared with the other phenotypes (Figure 5C).

Part of the blood count portraits indicate fingerprint-like correlation patterns specific for the different blood compounds (Figure 5A, B, Figure S 18, Figure S 19 and Figure 4H). The portraits of the phenotypes of the other categories partly resemble those of blood counts, this way reflecting close association between them. For example, the ‘ageing’ portrait (visualizing the correlation between age and transcriptome) can be understood as superposition of the red blood cell (RBC)- and neutrophil (NE)-phenotype portraits indicating the increased levels of RBC and NE in elderly people (see next subsection). The ‘alcohol consumption’ portrait also resembles the RBC-portrait while smoking reveals an eosinophil (EO)-like patterns. Part of the medication and disease history portraits can be interpreted similarly reflecting, e.g., that part of medications and diseases are more prevalent in elderly people (see the mean age data of each of the phenotypes listed in Table S 1) and consequently associate with increased RBC- and NE-levels and decreased
lymphocyte (LY)-counts (Figure S 22-Figure S 23).

Other phenotype portraits, e.g. those of different age ranges (see next subsection) and of different medications, cannot be simply interpreted as composite of blood count portraits. For a more detailed view we performed correlation and multiple regression analysis to estimate the particular effect of phenotypes on spot expression (part C – F in Figure S 19 - Figure S 23). We find a close relationship between high correlation coefficients and significant contributions of phenotype-coefficients (log p <-6) especially for spots located in the lower left and upper right corners of the map. These refer first of all to age, obesity, gender, RBC and white blood cell (WBC) counts and LY, medications of the groups C (cardiovascular system) and B (blood forming organs) and the previous diseases HL (hyperlipidemia), DIA (diabetes), HT (hypertension) and CAN (cancer).

In summary, phenotype portrayal visualizes fine structures of the effect of health and lifestyle factors on the blood transcriptome. They reflect alterations of blood cell composition and presumably also the specifics of the transcriptional programs activated in the different cells. The transcriptome types (and subtypes) resolve the heterogeneity of blood transcriptomes while the spot modules provide a metrics for its quantification. Overall, the phenotype portraits enable an intuitive, perception-based interpretation in terms of function and mutual associations between the different features.

**Figure 5**

**3.7. Portrayal of ageing, obesity and of serum markers**

Ageing and alterations of the BMI are accompanied by changes of the composition of transcriptome types in a gender-specific fashion (**Figure 2D**). Functional analysis
shows that expression of type 1_up transcriptomes gains with age while expression of type 2_up decays on average (see the plots with age-ranked samples in Figure S 5 - Figure S 11). Plots of spot expression as a function of age and BMI reveal further details (Figure 6A): Spot expressions related to red blood (spot C) and platelet (spot N) characteristics increase as a function of age and BMI with differences between the mean LOESS-curves for men and women (compare the red and blue curves) in correspondence with the blood count data (Figure S 18). In turn, the expression curves of spots related to immunity (I and J) decay with age and BMI in a nearly sex-independent fashion. On the other hand, the curves show similar courses at different levels for the transcriptomic types which suggests type-independent ageing mechanisms. The ageing curves are partly non-linear where the slopes get steeper for ages above 55- 60 years (e.g. for spot A and I, indicative for inflammation and immune response, respectively) or above 65- 70 years (spot L, IFN response), which suggests altered mechanisms in elderly people above different age thresholds.

Gene maps of previous ageing signatures [11] reveal an asymmetrical distribution of ageing_up and ageing_dn genes (Figure 6B). The latter ones accumulate within a narrow area in and around spots I and J in the right upper corner of the map giving rise to strong correlation between signatures’ expression and that of these spots. Deactivation of associated cellular functions such as immune response, telomere maintenance and/or ribosomal and mitochondrial activities with age obviously proceeds homogenously, presumably driven via mechanisms such as DNA hyper-methylation (Figure S 12). In contrast, ageing_up genes distribute much more heterogeneously between different spot-regions where each of them shows a specific profile of expression gaining with age (see curves of spots A, O, N, M, L and
Ageing is obviously accompanied or even driven by the activation of a multitude of inflammatory mechanisms involving different molecular and cellular components (see spot characteristics), which combine in a patient specific fashion giving rise to a relatively heterogeneous ageing_up signature.

The mean ageing portrait (‘all ages’ in Figure 6C) corresponds to the distribution of ageing_up and ageing_dn genes of the ageing signature [11] (compare the respective gene set maps with the red and blue areas in Figure 6B, respectively). Moreover, the ageing portrait can be roughly interpreted by the superposition of increasing RBC- and NE-like (positive correlation in red) and decaying LY-like (negative correlation in blue) contributions (compare with the cell count portraits in Figure 6E) in agreement with the increase/decrease of the expression of the respective landmark spots C, O and I, J, respectively. Inspection of gender- and age (decade)-stratified portraits reveals that elderly women and men (> 60 years) are similarly affected by an increase of NE- and IFN-related (found especially for subtype M.3) characteristics while the RBC-like pattern (typical for subtype 1.3) is more pronounced for mid-aged men (40 – 60 years). The mean BMI-portrait (‘all BMI’ in Figure 6D) shows characteristics of type 1 transcriptomes without the NE-like patterns and the elevated expression of spot L observed in the respective ageing portrait. Interestingly, the BMI-stratified portraits ‘switch’ from type 2 into type 1 for obese women and men (BMI > 30 kg/m²), due to gained (positive) correlations between BMI and inflammatory (spot A), RBC- (spot C) and platelet (spot N) characteristics, on one hand, and decaying immune response (spots I, J) expression signatures on the other one. Interestingly, this behaviour possibly associates with the so-called obesity-paradox claiming that an intermediate BMI about 25 kg/m²
associates with minimum health risk [58] and thus with a switch from positive to negative effect of increasing BMI on health.

For further comparison, we generated phenotype (correlation) portraits of four selected serum protein markers (Figure 6E). The portraits of hsCRP (human serum C-reactive protein) and of cytostatin C reflect footprints of inflammation (spot O) and IFN-response (spot L) in the blood transcriptome associated with NE-like patterns of the blood counts. The portrait of ferritin closely resembles that of RBC reflecting correspondence between the level of stored iron and erythrocyte expression (spot C). The transferrin portrait reveals a different patterns associating with diminished spots O (inflammation) and especially L (IFN-response) and enhanced spot N (thrombocytes), possibly due to the role of platelets in iron transport [59]. In summary, ageing and obesity associate with characteristic alterations of the blood transcriptome reflecting a fine interplay between inflammatory and iron physiology as mediated by molecular (as IFN-response), cellular (e.g., WBC and RBC) and serum protein compounds.

Figure 6

4. discussion

We ‘portrayed’ the diversity of the blood transcriptome of a cohort of more than 3,000 nominally healthy adult individuals included into the Leipzig Health Study ‘LIFE-adult’ in terms of intuitive SOM-images and classified them into three major transcriptome types. The expression patterns were decomposed into a minimum set of modules of co-regulated genes. Their functional impact was interpreted based on
previous knowledge including the results of previous blood transcriptome studies. Finally, we associated the blood transcriptomes with a series of phenotype-features collected in LIFE-adult for the same participants such as age, obesity-status, blood cell count, disease history and medication by means of phenotype portraits. Overall our study provides a comprehensive characterization of the blood transcriptome taking into account the whole spectrum of transcriptional states on a population-wide scale.

4.1. SOM-portrayal reduces dimensions of the blood transcriptome

Dimension-reduction and feature extraction are important issues in high-throughput data analysis. Our machine-learning approach reduces the dimensionality of data (number of individuals x (number of genes + number of phenotypes)) into a handful transcriptome types and subtypes [60]. Their expression is governed by about one dozen expression (spot-) modules in close correspondence with a previous modularization of the blood transcriptome [53]. Moreover, data portrayal transforms high-dimensional data landscapes into easy-to-interpret images. Their visual inspection strongly supports analytic tasks on different levels of stratification ranging from individual ‘personalized’ to subtype- and type-averaged expression portraits. Our study thus provided a sort of album of transcriptomic ‘faces’ of the LIFE-participants (Supplementary File 2). Importantly, the phenotype portrayal projects low dimensional features such as age or BMI onto the high-dimensional transcriptome landscape, which generates highly granular correlation images serving as ‘fingerprint’ of the respective phenotype. Their mutual comparison helps to identify associations between them and also singular patterns.

4.2. Typing reveals parallels between a series of biological
functions and health phenotypes

The tree in Figure 7A illustrates the similarity relations between the subtype portraits. It reveals a virtually linear arrangement of subtypes along the backbone. The portraits at the left and right margins (type 1-versus-type 2) differ mainly in antagonistic expression of genes located in opposite corners of their portraits. Our analysis thus uncovered a striking simplicity of the transcriptome. It reflects a characteristic alteration of cell components, namely a decrease in signatures of myeloid-lineage cells and increase in signatures of lymphocytes from the left to the right. This basic pattern is superimposed by transcriptional footprints of erythrocyte and thrombocytes giving rise, e.g., to gender-specific differences; of cytotoxic CTL’s playing role in longevity and by patterns reflecting interferon-response, whose amplitude increases, on average, in elderly people (especially above 65 years). The transcriptional (spot-) modules diversify these basic patterns in a subtype-specific fashion (Figure 7C).

Transcriptome typing and modularization also paved an avenue to describe the effect of age and BMI on the blood transcriptome, and in a wider context, on human’s physiology. For example, the percentage of type 1 transcriptomes in the population reflecting more inflammatory characteristics gains with age and, to a less degree, BMI in a non-linear, gender-specific fashion. In agreement with previous results [61] we find a striking overlap of signatures shared by multiple diseases, ageing and obesity driven by an underlying common pattern. It reflects the increase of inflammation and decrease of immune-responsibility (Figure 7B).

Obesity is associated with leukocytosis and it may be regarded as a state of chronic low-grade inflammation [13, 62], which, in turn is considered a driver of many age-related disorders (inflammaging) [63].
Also mean leukocyte telomere length associates with age-related diseases [44, 45]. Telomeres are protective nucleoprotein structures that cap the ends of linear chromosomes. They shorten a bit after each cell division. In consequence, telomere length reflects cell ageing [64]. Telomere maintenance mechanisms counteract this process and thus their activation can be indicative for counteracting cell ageing [65, 66]. Expression of genes involved in telomerase-maintenance pathway [67] directly correlate with T-cell and immune response signatures suggesting that cell’s ability to maintain telomeres associates with better immune responsibility and overall health constitution observed especially in younger, non-obese, non-smoking and non-alcohol consuming people. Interestingly, decay of telomere length with age [68] partly resembles the decay of the amount of type 2 transcriptomes with age and women having a higher fraction of type 2 transcriptomes with activated telomere maintenance mechanisms possess on average longer telomeres than men [68, 69].

4.3. Parallels between health and disease and asymmetry of transcriptomic changes

Our recent study on sepsis framed by community acquired pneumonia [8], a high-grade inflammatory disease, identified three major axes of the variation of the blood transcriptome, namely the inflammatory axis (endotoxin tolerance, cytotoxic cells), a ‘blood-disturbance’ axis including mostly erythrocyte and thrombocyte characteristics and the IFN-response axis. These axes of variation were also found in the blood transcriptomes of nominally heathy subjects, however with a decreased amplitude of inflammatory expression changes. Part of our modules continuously change along the subtypes (e.g. I and J: immune response; H: cytotoxic cells), others show subtype-specific activations (C, N; erythrocytes, platelets), while a
third category spreads over almost all subtypes (L; IFN-response). Interestingly, also the sepsis signatures split into three type 1_up and only one type 2_up mechanism (Figure 4). They reflect a similar asymmetry of the heterogeneity of mechanisms as found between ageing_up and ageing_dn signatures (see above). It reflects multi-factorial activation mechanisms accompanying ageing and/or disease development (see [11] and Figure S 11). Also the phenotype portraits of ageing, obesity, disease history, medication, alcohol consumption and smoking reflect this asymmetry because the majority of them associates with type 1 showing more heterogeneous patterns than type 2 portraits.

We estimated ageing and obesity trajectories of spot expression by non-linear fits through our cross sectional data where stratification by types (and gender) reduces the variance of data points (Figure 6A). A recent longitudinal study revealed that individuals are more similar to their own expression profiles later in life than profiles of other individuals their own age [70]. Type-strata of the blood transcriptome thus eventually more adequately describe ageing effects. Longitudinal follow-up studies over different age ranges are required to study individual ‘life-courses’ of the blood transcriptome and their impact for lifetime-risk prediction.

4.4. Epigenetic background and ageing clocks

Our analysis also underlines the importance of epigenetic mechanisms, particularly of chromatin (re-)organization for changes the blood transcriptome. Using gene expression of nominally repressed and activated chromatin states as an indicator of gene activity we find a pronounced mutual switching between type 1 and type 2 transcriptomes suggesting that active states in type 2 become repressed in type 1 and that repressed states in type 2 become activated in type 1. Differences of
biological mechanisms behind transcriptomic [11] and the epigenetic predictors [71] of ageing or disease progression are not completely clear [11]. We recently reported that transcriptomic and epigenetic mechanisms partly decouple in cancer development [72, 73] and cell differentiation [50]. Thus, the expression changes observed might reflect changed chromatin organization leading to altered cell function in type 1 compared with type 2 as discussed, e.g., as epigenetic mechanisms accompanying ageing [74] and inflammation [75-78] and associating with changes of DNA-methylation and histone-marks governing gene activity. The search for reliable indicators of biological age, rather than chronological age, attracted large efforts in the last decades [79, 80]. DNA methylation-derived epigenetic clocks are currently better in estimating chronological age than transcriptomic or telomere length measures [81]. Currently it is not entirely clear how molecular clocks work, what aspect(s) of physiological or cellular aging they represent and whether age-related changes, such as telomere shortening or DNA-methylation contribute to the causes of ageing or are the results of it. There is only weak correlation between DNA-methylation and transcriptome age predictors meaning that the transcriptomic age and the epigenetic clock describes different aspects of biological aging [11]. DNA-methylation clock was assumed to reflect the function of the epigenetic maintenance system [71]. In support of this we found that DNA-methylation maintenance methyltransferase DNMT1 is part of spot gene cluster J (Table S 3) showing decaying expression with age (age_dn signature of [11]) and correlating with DNA-methylation signatures (Figure S 12). Hypomethylation accumulates with the number of cell divisions, due to insufficient re-methylation, and also inflammatory factors that increase cell turnover led to increased methylation loss, which in turn, disrupt DNA binding patterns of transcription factors
and modify their regulatory role in cell function as discussed above. Coupled transcription and DNA-methylation epidemiological studies are required to better disentangle the relation between epigenetic and transcriptomics of the blood [81].

**Figure 7**

5. conclusions

We have characterized the human blood in terms of a relatively simple scheme of transcriptome types and functional gene modules, which associate with health-, lifestyle and age-related phenotypes. The large sample size of our study and wide agreement with state of the art blood-transcriptome research let us conclude that our scheme reflects basic gene expression characteristics of the human blood in a wide spectrum of conditions. It provides interpretations for underlying biological mechanisms on cellular and molecular levels. It has impact for future applications for diagnosis and prognosis via the refinement of existing and the development of novel predictors for age, lifestyle and disease outcomes. The data provides a potential basis for the establishment of reference ranges for expression values and the identification of suited transcriptome candidate markers such as the expression of single genes or of sets of them, which can be derived from our spot-modules. The individual portrayal of transcriptomes and of their associations with phenotype features in terms of easy-to interpret images offers perspectives for visual-perception based personalized diagnostics. Large scale longitudinal studies and paired transcriptome-epigenome investigations are needed to better understand lifetime courses, causal relations and mechanisms of (epi-)genomic regulation.
6. abbreviations

A  Alimentary tract and metabolism
AP  Angina pectoris
ALC > 30  Participants consuming more than 30g alcohol per day
ALC ≤ 30  Participants consuming ess than 30g alcohol per day
ALC  Alcohol consumption
ART  Arthrosis
AST  Asthma
ATC  Anatomical Therapeutic Chemical Classification System of medication
B  Blood and blood forming organs
BA  Basophils absolute (10^9/l)
BAP  Basophils (%)
BD  Sepsis type „Blood disturbant“
BMI  Body mass index
BP  GO-term „Biological process“
C  Cardiovascular system
CAN  Cancer
CAP  Community Aquired Pneumonia
CAT  Cataract
cPAT  Combinatorial pattern type
CTL  Cytotoxic T lymphocytes
D  Dermatologics
DEP  Depression
DIA  Diabetes
| Abbreviation | Description |
|--------------|-------------|
| DNMT1        | DNA-methylation maintenance methyltransferase |
| EO           | Eosinophils absolute (10^9/l) |
| EOP          | Eosinophils (%) |
| EXSMO        | Ex-smoker |
| G            | Genitourinary system and sex hormones |
| GLA          | Glaucoma |
| GO           | Gene Ontology |
| GOU          | Gout |
| GSZ          | Gene set enrichment z-score |
| H            | Systemic hormonal preparations, excl. sex hormones and insulins |
| HA           | Heart attack |
| HCT          | Hematocrit (l/l) |
| HEP          | Hepatitis |
| HGB          | Hemoglobin (SI units, mmol/l) |
| HGBK         | Hemoglobin (conv. units, g/dl) |
| HL           | Hyper-lipidaemia |
| HS           | Sepsis type „High severity“ |
| hsCRP        | human serum C-reactive protein |
| HT           | Hypertension |
| HZO          | Hzoster |
| IFN          | Interferon |
| J            | Anti-infective for systemic use |
| L            | Antineoplastic and immunomodulating agents |
| LIFE(-adult) | Leipzig Research Center for Civilization Diseases |
| LOESS        | locally estimated scatterplot smoothing |
LS  Sepsis type „Healthy & Low severity“
LY  Lymphocytes absolute \((10^9/l)\)
LYP Lymphocytes (%)
M   Muscular-skeletal system
MCH Mean corpuscular hemoglobin (SI units, fmol)
MCHC Mean corpuscular hemoglobin concentration (SI units, mmol/l)
MCHK Mean corpuscular hemoglobin concentration (conv. units, g/dl)
MCHK Mean corpuscular hemoglobin (conv. units, pg)
MCV Mean corpuscular volume (fl)
MO  Monocytes absolute \((10^9/l)\)
MOP Monocytes (%)
MPV Mean platelet volume (fl)
MS  Sepsis type „Medium severity“
N   Nervous system
NE  Neutrophils absolute \((10^9/l)\)
NEP Neutrophils (%)
NONSMO Non-smoker
nwt Normal weight
ob  Obese
P   Antiparasitic products, insecticides and repellents
PLT Platelets \((10^9/l)\)
PRC2 Polycomb repressive complex 2
pre-ob Pre-obese
R   Respiratory system
7. Declarations

7.1. Ethics approval and consent to participate

The LIFE-adult study was approved by the ethics board of the Medical Faculty of the University of Leipzig. Written consent was obtained from all participants of LIFE-
7.2. Consent to publish

Written consent of the participants to publish results of LIFE-adult was obtained.

7.3. Availability of data and materials

The data that support the findings of this study are available from the LIFE centre but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of LIFE. Secondary data are available from ‘The Leipzig Health Atlas’ repository (https://www.health-atlas.de/; accession number will be available after acceptance of the manuscript).

7.4. Competing interests

All authors declare that they have no competing interests.

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7.6. Authors’ contributions

Conceived and wrote this paper: MS, HB; Performed analysis: MS, LH, HB, HLW; downstream analysis methods development: HLW, MS, AA; preprocessing of
transcriptomics data: HK; collection and curation of phenotype data: KW, CE, RB, KK; Coordinators of LIFE research centre: ML, JT. All authors read and approved the final manuscript.

7.7. Acknowledgements

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Figures

Figure 1
Schematic overview about portrayal approach applied in this study: Subject-matched transcriptome and 'phenotypic' features used for detailed pattern analyses. Phenotypic features analyzed in this study were listed in Table S1.

Figure 2
Stratification of the blood transcriptome into three types: (A) The pairwise correlation heatmap divides the three types into two clusters, with type 1 corresponding to the younger group and type 2 corresponding to the older group. The relative amount of type 1 men decreases with age while the relative amount of type 2 men increases with age.
A. Expression landscape and signature characteristics

Figure 3

The landscape of the blood transcriptome: (A) The spot overexpression summary
Figure 4

Functional characteristics and previous signatures of the blood transcriptome: (A)

Figure 5

Association of selected features (phenotypes) with the transcriptome landscape o

Figure 6

Ageing and BMI characteristics of the blood transcriptome: (A) Expression of sele

Figure 7

Portrayal of the blood transcriptome: (A) The similarity tree reflects a virtually lin

Supplementary Files

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