

The Saliva Exposome for Monitoring of Individuals' Health Trajectories

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BACKGROUND: There is increasing evidence that environmental, rather than genetic, factors are the major causes of most chronic diseases. By measuring entire classes of chemicals in archived biospecimens, exposome-wide association studies (EWAS) are being conducted to investigate associations between a myriad of exposures received during life and chronic diseases.

OBJECTIVES: Because the intraindividual variability in biomarker levels, arising from changes in environmental exposures from conception onwards, leads to attenuation of exposure–disease associations, we posit that saliva can be collected repeatedly in longitudinal studies to reduce exposure–measurement errors in EWAS.

METHODS: From the literature and an open-source saliva–metabolome database, we obtained concentrations of 1,233 chemicals that had been detected in saliva. We connected salivary metabolites with human metabolic pathways and PubMed Medical Subject Heading (MeSH) terms, and performed pathway enrichment and pathway topology analyses.

RESULTS: One hundred ninety-six salivary metabolites were mapped into 49 metabolic pathways and connected with human metabolic diseases, central nervous system diseases, and neoplasms. We found that the saliva exposome represents at least 14 metabolic pathways, including amino acid metabolism, TCA cycle, gluconeogenesis, glutathione metabolism, pantothenate and CoA biosynthesis, and butanoate metabolism.

CONCLUSIONS: Saliva contains molecular information worthy of interrogation via EWAS. The simplicity of specimen collection suggests that saliva offers a practical alternative to blood for measurements that can be used to characterize individual exposomes. <https://doi.org/10.1289/EHP1011>

Introduction

Because genetic factors typically account for only about 18% of chronic disease risks, it is reasonable to infer that nongenetic factors (i.e., exposures) are major causes of chronic diseases (Rappaport 2016). Given the myriad exposures from both exogenous and endogenous sources that an individual experiences during life [the “exposome” (Wild 2005)], investigators are performing exposome-wide association studies (EWAS) that interrogate levels of chemicals in biospecimens to discover causes of chronic diseases (Patel et al. 2010; Rappaport 2012; Wild et al. 2013). By measuring entire classes of chemicals (e.g., small molecules, protein modifications, antigens) in archived biospecimens from incident disease cases and matched controls, EWAS can pinpoint discriminating features that then generate hypotheses for targeted follow-up studies (Rappaport 2011; Rappaport et al. 2014). For example, Hazen and coworkers employed this avenue to implicate joint microbial/human metabolism of the nutrient choline as a potentially major cause of coronary heart disease (Wang et al. 2011; Tang et al. 2013; Koeth et al. 2013).

An important challenge to designing EWAS is the intraindividual variability in levels of circulating molecules arising from changes in diet, lifestyle factors, and sources of pollutants during decades of life that precede disease onset. This within-person variability in biomarker levels leads to exposure measurement errors that attenuate causal signals and obscure disease associations (Lin et al. 2005; Sampson et al. 2013). One way to circumvent such measurement errors is to perform longitudinal studies with repeated biospecimens, collected from subjects during critical stages of life (Rappaport 2011; Robinson and Vrijheid 2015).

The most logical approach for doing this relies on prospective cohorts that archived blood or other biospecimens repeatedly from the same subjects. However, such cohorts are rare and repeated collection of blood, the main archival specimen, is difficult to perform (Hansen et al. 2007; Randell et al. 2016).

Saliva (also referred as oral fluid) is a natural filtrate of blood that contains omic features (small molecules, metals, proteins, and DNA) worthy of interrogation via EWAS. Because collection is “stress-free,” repeated specimens of saliva are routinely obtained for determination of steroid hormones in psychobiological studies (Hjortskov et al. 2004; Kajantie and Phillips 2006; Hunter et al. 2011). Sampling of saliva is straightforward and protocols are available that allow subjects to collect their own samples and ship them to a laboratory or repository.

Metabolomics is recognized as a powerful top-down approach for detecting small molecules in biological matrices (Nicholson and Wilson 2003; German et al. 2005). These small molecules can be either substrates or end products of cellular metabolism and can originate from exogenous sources via inhalation, ingestion and dermal absorption, or from endogenous processes including human and microbial metabolism. Adductomics is another top-down technique that employs modifications of blood proteins like hemoglobin or human serum albumin (HSA) to characterize exposures to reactive electrophiles that are inherently toxic but cannot be measured directly in biospecimens (Rubino et al. 2009; Li et al. 2011; Carlsson et al. 2014; Grigoryan et al. 2016). Because blood is in equilibrium with the tissues and saliva is in equilibrium with blood, both blood and saliva represent dynamic reservoirs of small molecules that are present in the body at a given time. Given the potential utility of saliva as a biospecimen for EWAS, we will evaluate the linkages between salivary metabolites and human metabolic pathways, as well as those between these pathways and chronic diseases. We will also consider methods for collection and analysis of saliva via untargeted metabolomics and adductomics.

Methods

Saliva Metabolome

Salivary metabolites ($n = 1,233$) were obtained from the saliva metabolome database (<http://www.salivametabolome.ca/>) that was recently integrated into the Human Metabolome Database

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(HMDB) (Wishart et al. 2013; Dame et al. 2015). This database compiles important physical, chemical and biological information of metabolites derived from peer-reviewed articles, including chemical structure, chemical class, origin, biological function, and cellular location. Normal and abnormal concentrations are also reported for metabolites previously quantified in humans as well as standard deviations of the measurements that can be used in some cases to estimate the intra- and interindividual variability. It is important to note that this database was built from a recent study by Dame et al. who combined contemporary measurements of salivary metabolites in healthy subjects with literature data derived from the healthy and/or diseased subjects (Dame et al. 2015). Those investigators measured 308 salivary metabolites in 16 healthy adult subjects sampled once and two subjects sampled three times in a day (morning, midday, and afternoon/evening). They also reported levels of an additional 708 metabolites from peer-reviewed articles. All metabolites included in the saliva metabolome database were used for analyses.

Visualization of Human Metabolic Pathways

Salivary metabolites were connected to their human metabolic pathways using the Metscape 3.1 App (Karnovsky et al. 2012) for Cytoscape 3.2.1. (Shannon et al. 2003). The network of metabolites and reactions was built using the internal Metscape database that integrates metabolomics and gene-expression data derived from all available biospecimens, and compiled in the Kyoto Encyclopedia of Genes and Genomes (KEGG) and the Edinburgh Human Metabolic Network (EHMN) (Karnovsky et al. 2012). Nodes (i.e., metabolites) were colored according to their source (e.g., host, microbial). Edges connecting nodes represent KEGG and EHMN biochemical reactions. Only salivary metabolites with a KEGG IDs (196 metabolites) were retained with “human” as the model organism. The network type “compound” was used to map metabolic connections between small molecules.

Pathway Enrichment and Pathway Topology Analysis

Pathway enrichment and topology analyses of the saliva metabolome were performed using MetaboAnalyst 3.0 (Xia et al. 2015) with “Homo sapiens” as the model organism. Pathway enrichment analysis was conducted using the hypergeometric test to determine whether saliva metabolites were represented in a particular KEGG metabolic pathway more than expected by chance. p -Values ≤ 0.05 indicate statistically significant representations of metabolic pathways. Pathway topology analysis was performed using the “relative-betweenness” centrality measure to estimate the importance of saliva metabolites relative to the biological pathway structure. Metabolite centrality is reported on a scale from 0 (isolated metabolite) to 1 (key metabolite).

Connections between the Saliva Metabolome and Human Chronic Diseases

Salivary metabolites were connected with human chronic diseases using the MetDisease App for Cytoscape 3.2.1 (Duren et al. 2014). MetDisease is a text-mining App that queried metabolites previously reported in any biospecimen as associated with PubMed Medical Subject Heading (MeSH) terms in peer-reviewed human and/or animal studies. For each disease, the total number of salivary metabolites, associated with a particular MeSH term, was determined. Salivary metabolites were queried using their KEGG IDs as identifiers and their shared names as select attributes. MeSH terms related to both chronic and non-chronic diseases (e.g., bacterial infections) were included in the network. The network of MeSH terms was built with Cytoscape 3.2.1

(Shannon et al. 2003) using the organic layer. The number of salivary metabolites associated with MeSH terms was used as the node attributes. Edges connecting nodes represented interconnections between MeSH terms.

Results and Discussion

The Saliva Metabolome

Saliva is a mixture of fluids originating mainly from the parotid, submandibular, sublingual, and minor salivary glands, and to a lesser extent from oral and nasal mucosa (de Almeida et al. 2008). Saliva consists of approximately 99% water with the remaining 1% comprised of electrolytes, mucus, cellular debris, proteins, and small molecules (Humphrey and Williamson 2001; de Almeida et al. 2008). Transfer of small molecules from blood to saliva relies on passive diffusion of lipophilic compounds and ultrafiltration of hydrophilic compounds of low molecular weight (<300 – $1,900$ Da) (Gallardo and Queiroz 2008; de Almeida et al. 2008; Thieme 2012; Higashi 2012). The pK_a of a molecule plays an important role in transfer from blood to saliva. For example, basic molecules accumulate in saliva due to ion-trapping phenomena associated with their transfer from neutral blood (pH 7.4) to acidic saliva (pH 6) (Thieme 2012). Also, several factors including disease states, diet, drug consumption, and physical activity can significantly affect saliva excretion and saliva pH can be influenced by variation in bicarbonate concentrations (Aps and Martens, 2005).

Several studies have evaluated the correlation between saliva and blood concentrations for compounds with diverse physical and chemical properties, as summarized in Table 1. The median value of the Pearson correlation coefficients was 0.92 with a range of $0.30 \leq r \leq 0.98$. High correlations were observed between blood and saliva concentrations for neutral molecules that are not affected by changes in saliva pH (Hill et al. 2001; Sakaguchi and Hasegawa 2005; Juniarto et al. 2011; Gunnala et al. 2015). Lower correlations for acidic and basic compounds have been attributed to intraindividual variation in saliva pH as well as buccal contamination (for nicotine) (Fisher et al. 2013). However, these studies have been conducted only on a small subset of compounds (mainly drugs and pollutants). Because the blood–saliva transfer of small molecules relies on physical and chemical processes, more in-depth studies are needed to determine effects of pK_a , polarity, physical activity, diet, and disease state.

Dame et al. (2015) recently combined results from metabolomic analyses of human saliva with a literature review of salivary metabolites. This characterization of the saliva metabolome included 1,233 small molecules, metals and ions, which is about one-fourth of the 4,549 metabolites that have been reported in human blood (Wishart et al. 2013). The difference in coverage of the two metabolomes reflects the lower metabolite concentrations observed in saliva (nM to low μ M) compared with blood (μ M to mM). We classified the sources of these salivary molecules and metals as follows: *a*) host endogenous (879), *b*) microbial endogenous (52), *c*) food (225), *d*) drugs (15), *e*) pollutants (25), and *f*) metals (37) (Figure 1).

Using Metscape, we mapped the 1,233 salivary metabolites into biological pathways (Figure 2). Metscape uses an internal database that integrates metabolomic and gene-expression data from KEGG and EHMN (Karnovsky et al. 2012). Because only 196 of the 1,233 (16%) metabolites detected in saliva were included in these databases, an additional 529 metabolites were added to the network through linkages (i.e., direct neighbor in metabolic pathways) with the 196 salivary metabolites through 49 recognized pathways. Most of these additional 529 metabolites

Table 1. Blood:saliva concentration ratios and correlations for various classes of small molecules.

Compound	Log P^{ac}	pK_a	Blood: saliva ratio (mean)	Correlation coefficient (r)	Reference
<i>Therapeutic drugs</i>					
Clozapine	3.7	15.9	3.6 ^b	0.728	Fisher et al. 2013
Norclozapine	3.2	15.9	3.6 ^b	0.806	Fisher et al. 2013
Quetiapine	2.9	15.1	3.0 ^b	0.843	Fisher et al. 2013
Risperidone	3.4	8.8	2.6 ^b	0.954	Fisher et al. 2013
9-hydroxyrisperidone	2.3	13.7	2.5 ^b	0.640	Fisher et al. 2013
Alprazolam	2.2	18.3	2.3	ND	Gjerde et al. 2014
Clonazepam	2.8	11.9	7.1	ND	Gjerde et al. 2014
Diazepam	2.6	2.9	27.0	ND	Gjerde et al. 2014
Nordiazepam	2.8	12.3	22.0	ND	Gjerde et al. 2014
Busulfan	-0.9	NA	0.92 ^b	0.980	Rauh et al. 2006
Methylphenidate	1.5	8.9	0.5 ^b	ND	Seçilir et al. 2013
Mycophenolic acid	2.4	3.6	NR ^b	0.922	Shen et al. 2009
Voriconazole	1.6	12.7	2.04 ^b	0.943	Vanstraelen et al. 2015
<i>Illicit drugs</i>					
Cocaine	1.9	8.8	0.4 ^b	ND	Moolchan et al. 2000
Benzoylcegonine	1.7	3.1	5 ^b	ND	Moolchan et al. 2000
Ecgonine methyl ester	0.1	14.6	1 ^b	ND	Moolchan et al. 2000
<i>Pollutants</i>					
Nicotine	0.9	8.9	NR ^b	0.300	Shin et al. 2002
Cotinine	0.4	4.8	2.3 ^b	0.980	Shin et al. 2002
Perchlorate	-0.9	-6.9	0.07 ^c	0.927	Oldi et al. 2009
<i>Endogenous</i>					
Cortisol	1.8	12.6	0.87	0.933	Gunnala et al. 2015
7-HydroxyDHEA	1.8	18.2	4.5 ^c	0.975	Hill et al. 2001
Androstenedione	2.9	19.0	NR ^b	0.781	Juniarto et al. 2011
17-Hydroxyprogesterone	2.9	12.7	NR ^b	0.964	Juniarto et al. 2011
Testosterone	2.9	19.1	NR ^c	0.843	Sakaguchi and Hasegawa 2005

Note: ND, not determined.

^aFrom *in silico* prediction using ALOGPS 2.1 (<http://www.vcclab.org/lab/alogs/>).

^bPlasma:saliva ratio.

^cSerum:saliva ratio.

were either phase-II conjugates or compounds tightly bound to proteins. In saliva, metabolites are mainly present in the free form (i.e., unbound fraction) due to blood-saliva transfer processes. Phase-II conjugation increases the molecular weight, acidity, and hydrophilicity of each parent compound (Figure 2A), whereas protein binding increases the size of the compound. Both of these processes limit the efficiency of blood-saliva transfer. For compounds tightly bound to proteins, such as eicosanoids (markers of inflammation) (Figure 2A), the free form represents only about 1% of the total concentration in the systemic circulation (Brodersen et al. 1990; Fujiwara and Amisaki 2013; Bessonneau et al. 2015b). Salivary levels of these compounds are very low (nM) and, therefore, difficult to detect with untargeted analyses.

Next, we performed pathway enrichment and pathway topology analyses to identify the most significant pathways in the saliva exposome. Table 2 summarizes the 14 most significant

metabolic pathways ($p \leq 0.05$), which prominently includes amino acid metabolism, TCA cycle, gluconeogenesis, glutathione metabolism, pantothenate and CoA biosynthesis, and butanoate metabolism.

Connections between the Saliva Metabolome and Human Diseases

In order to provide additional biological context for the saliva metabolome, we evaluated associations between salivary metabolites and MeSH terms using MetDisease. The resulting network (Figures 3 and 4) indicates that salivary metabolites were associated with most human diseases (Table 3). For example, substantial evidence from breast cancer epidemiology shows the influence of hormones at different stages of women's development. A nested case-control study found that premenopausal concentrations of testosterone were associated with breast cancer risk (Zeleniuch-Jacquotte et al. 2012). Another case-control study reported lower salivary testosterone levels in women with breast cancer, suggesting a protective effect of testosterone (Dimitrakakis et al. 2010). The carboxylic acid ester phenylacetate and its analogs have also been linked to breast neoplasms (Sawatsri et al. 2001) due to their antiproliferative activities on human breast cancer cells. Recently, targeted metabolomics of salivary levels of polyamines such as spermidine has discriminated breast cancer patients from healthy subjects (Takayama et al. 2016). Polyamines are essential molecules for normal cell growth and regulate gene expression by modulating ligand-receptor interactions, including estradiol binding to estrogens receptors (Cervelli et al. 2014). Previous studies have attributed the proliferation of highly invasive breast cancer tumor cells to the upregulation of polyamine metabolism (Cervelli et al. 2014).

There is also growing evidence that the human gut microbiome and its complex interactions with exogenous exposures

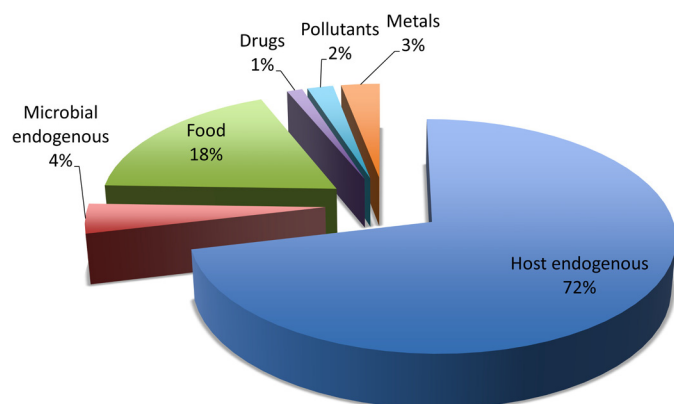


Figure 1. Source category of salivary metabolites compiled from the saliva metabolome database (Dame et al. 2015).

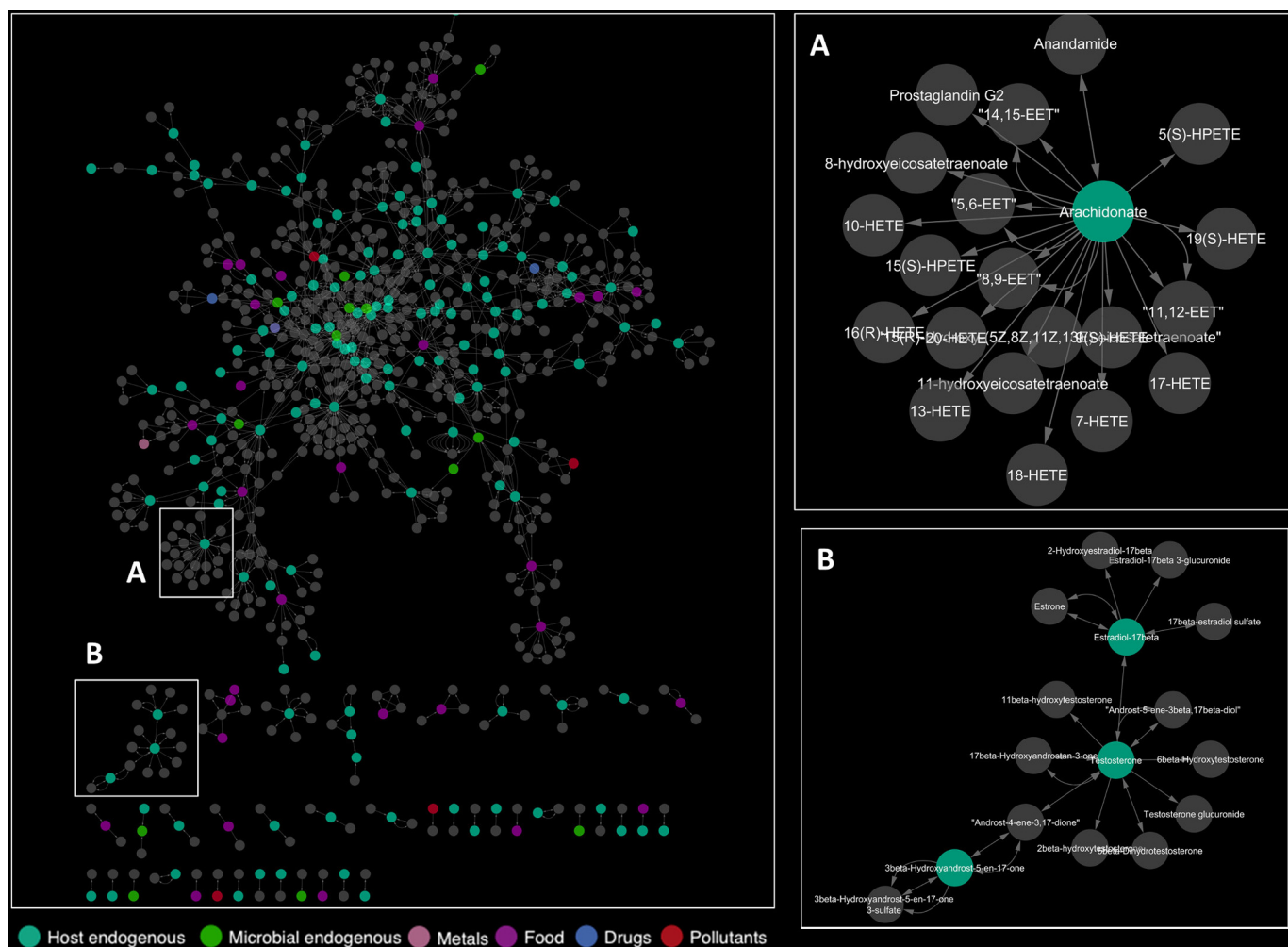


Figure 2. Network of metabolic pathways reconstructed from metabolites detected in human saliva. Gray nodes represent metabolites that had not previously been detected in saliva but have direct neighbors in metabolic pathways. Edges represent biochemical connections between metabolites.

play roles in disease processes (Jiménez et al. 2008; Orešič et al. 2008; Nicholson et al. 2012). Of the 14 metabolites of known microbial origin, 12 were associated with congenital, hereditary, and neonatal diseases, 11 with nervous system diseases, 10 with nutritional and metabolic diseases, eight with digestive system

diseases, and seven each with neoplasms and urogenital and pregnancy complications.

The above analyses suggest that the saliva metabolome captures a biologically meaningful fraction of exposures that are associated with human diseases. Thus, any discriminating

Table 2. Metabolic pathways represented by the saliva metabolome (metaboanalyst – homosapiens) for pathways having p -values < 0.05 from pathway enrichment analysis. Impact values are derived from pathway topology analysis.

Pathway	Total metabolites ^a	Hits ^b	p -value ^c	Impact ^d
Alanine, aspartate, and glutamate metabolism	24	13	$1.4e^{-4}$	0.88
beta-Alanine metabolism	28	13	$9.8e^{-4}$	0.38
Phenylalanine metabolism	45	18	$9.9e^{-4}$	0.45
Arginine and proline metabolism	77	26	$1.7e^{-3}$	0.73
Nitrogen metabolism	39	15	$4.0e^{-3}$	0.22
Citrate cycle (TCA cycle)	20	9	$7.7e^{-3}$	0.43
Glycolysis or gluconeogenesis	31	12	$9.3e^{-3}$	0.32
D-Glutamine and D-glutamate metabolism	11	6	$9.7e^{-3}$	0.64
Glycine, serine, and threonine metabolism	48	16	$1.4e^{-2}$	0.56
Glutathione metabolism	38	13	$2.1e^{-2}$	0.35
Pantothenate and CoA biosynthesis	27	10	$2.4e^{-2}$	0.35
Valine, leucine, and isoleucine biosynthesis	27	10	$2.4e^{-2}$	0.36
Butanoate metabolism	40	13	$3.3e^{-2}$	0.21
D-Arginine and D-ornithine metabolism	8	4	$5.0e^{-2}$	0.50

^aTotal number of metabolites involved in the pathway.

^bNumber of salivary metabolites involved in the pathway.

^cFisher's exact test was used to measure the association between input metabolites (i.e., salivary metabolites) and pathways. p -Values $\leq 5.0e^{-2}$ indicate that associations are less likely due to random chance.

^dImpact represents, on a scale from 0 to 1, the centrality of salivary metabolites in their respective pathways.

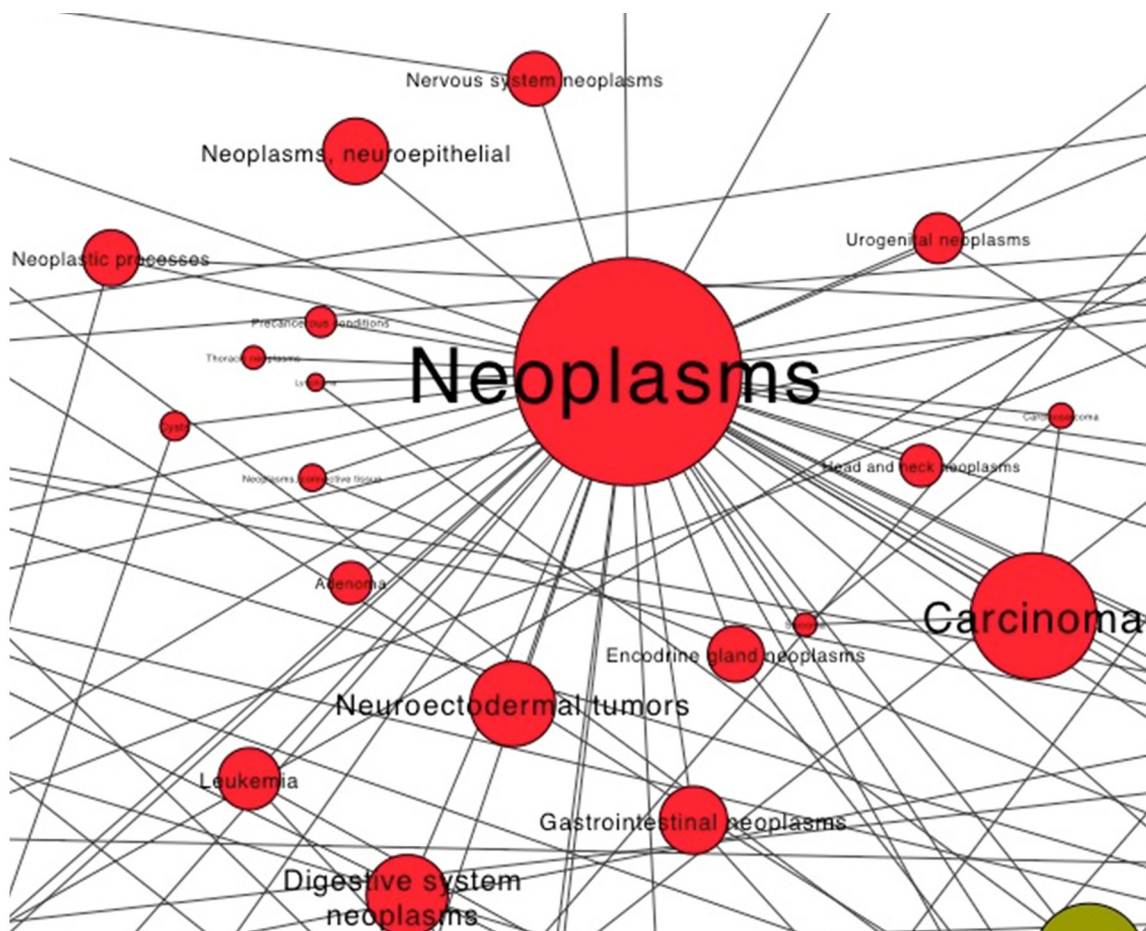


Figure 4. Subnetwork of neoplasm-related PubMed Medical Subject Headings (MeSH) terms reported associated with salivary metabolites. The size of a node and text reflects the number of metabolites associated with MeSH terms. Edges represent links between MeSH terms.

(Gröschl et al. 2008; Lund et al. 2011). This approach is often used to increase the volume of saliva collected and to control the pH so as to standardize the transfer of acidic and basic molecules. Unstimulated saliva can be collected passively with a collection tube or an oral swab (Drummer 2008). Collection devices should be selected carefully because some media, including cotton and polypropylene pads, can nonspecifically bind small molecules (Gallardo and Queiroz 2008; de Almeida et al. 2008; Gröschl et al. 2008; Higashi 2012). Given that the buccal cavity can be contaminated by components originating from previous oral ingestion (Shin et al. 2002), it is useful for subjects to refrain from eating and drinking for a few hours prior to saliva collection. Once collected, saliva samples can be easily transported by

mail to the laboratory for analysis. Because a large number of salivary metabolites such as amino acids, steroids, or fatty acids are under circadian control (i.e., high diurnal variation) (Dallmann et al. 2012; Dame et al. 2015), it is important to collect repeated samples at similar times (e.g., all samples collected during early morning). Saliva is also an attractive alternative matrix for pediatric populations, due to the noninvasiveness of the sample collection. Although several collection devices have been specifically developed for children 6 mo–6 y old, more studies are required to address the safety of saliva sampling devices and procedures in young children.

Several studies have demonstrated that various classes of compounds, including illicit drugs (Lund et al. 2011), therapeutic drugs (Mendonza et al. 2006; Moore et al. 2007; Ogawa et al. 2014), pollutants (Bentley et al. 1999; Wang and Lu 2009; Gherardi et al. 2010), and endogenous steroids (Higashi et al. 2011; Alvi et al. 2013) are stable in saliva for a few days at room temperature and for at least 1 year when stored at low temperature ($\leq -20^{\circ}\text{C}$).

Sample Preparation

The strategy for preparing samples plays an important role in the quality of metabolomics data. Issues related to the quenching of metabolism prior to analysis, ion suppression during mass spectrometry and metabolite instability can adversely impact the interpretation of data from untargeted analysis. The most common techniques for preparing saliva samples for mass spectrometry are liquid–liquid extraction (LLE), protein precipitation (PP),

Table 3. Summary of the most important human chronic diseases associated with salivary metabolites.

Human chronic disease	Number of salivary metabolites
Congenital, hereditary, and neonatal diseases and abnormalities	145
Nutritional and metabolic diseases	144
Central nervous system diseases	138
Neoplasms	118
Digestive system diseases	105
Urogenital diseases	103
Cardiovascular diseases	101
Immune system diseases	76
Respiratory tract diseases	69
Endocrine system diseases	64

and solid-phase extraction (SPE) (Higashi 2012). Although LLE and PP provide exhaustive extraction of small molecules, such procedures are labor intensive, difficult to automate, require multistep sample handling, and are prone to suppression or enhancement of ionization in mass spectrometric analysis. Most of metabolites in the saliva metabolome database have been obtained through LLE or PP of saliva samples. These analytical techniques often used an internal standard in order to correct for possible loss and/or degradation during sample preparation and for matrix effects during analysis.

In contrast to LLE and PP, solid-phase microextraction (SPME) offers a simple, fast, and sensitive technique for preparing saliva and other biological fluids for metabolomics analysis (Bojko et al. 2014; Bessonneau et al. 2013, 2015a). In fact, when coupled to the Concept-96 system (employing 96-well plates), thin-film SPME (TF-SPME) motivates automation of all preconditioning, extraction, rinsing, and desorption steps (Jiang and Pawliszyn 2012). For example, preparation for saliva via TF-SPME can be achieved in less than 2 min per sample and prevents matrix effects and adsorption of macromolecules compared with LLE (Bessonneau et al. 2015a).

The small size and biocompatibility of SPME materials also permits *in vivo* sampling of saliva (Bessonneau et al. 2015a). By placing the SPME probe in the mouth for a short period of time, this technique can be used for rapid extraction and stabilization of metabolites for analysis (Bessonneau et al. 2015b). For example, SPME coatings were able to rapidly stabilize highly reactive metabolites (i.e., eicosanoids) formed *in vivo* and prevented their autooxidation during storage (Bessonneau et al. 2015b). Although promising, *in vivo* sampling of saliva with SPME is a new technique that will require substantial validation to address variability arising from salivary flow rate, pH, sample volume, and agitation.

The Saliva Adductome

Biotransformation of dietary chemicals and pollutants leads to the formation of reactive electrophiles, including reactive oxygen and nitrogen species, aldehydes, oxiranes, and quinones that can modify DNA, proteins, and lipids and can lead to dysregulation of homeostasis (Farmer and Davoine 2007; Fritz and Petersen 2013). Because reactive species are unstable, it is difficult to measure them in their free-circulating form. However, one can detect adducts formed from reactions between reactive electrophiles and nucleophilic residues on DNA and proteins. Several targeted studies have reported correlations between adducts of reactive electrophiles from endogenous and exogenous sources with DNA (Sturla 2007,) and prominent blood proteins, that is, hemoglobin (Hb) and human serum albumin (HSA) [reviewed by Törnqvist et al. 2002; Rubino et al. 2009].

Both DNA and proteins are also present in saliva, albeit at lower concentrations than in blood. For example, HSA is found in saliva at concentrations of about 0.5 mg/mL compared with 35–50 mg/mL in serum (Wang et al. 2012; Shaila et al. 2013; Metgud and Patel 2014; Nam et al. 2015); whereas Hb is present at a 50,000-fold lower concentration in saliva (3.3 µg/mL) compared with blood (150 mg/mL) (Nomura et al. 2012). For DNA, mean salivary levels (12 µg/mL) are 2-fold lower than those in blood (26 µg/mL) (Abraham et al. 2012). Although the analysis of salivary adducts is still in its infancy, targeted studies have reported adducts of salivary DNA with electrophiles from smoking and dietary sources (Bessette et al. 2010; Chen and Lee 2014; Chen and Lin 2014), and of HSA adducts in nasal lavage fluid from workers exposed to reactive electrophiles (Kristiansson et al. 2004; Jeppsson et al. 2009).

Untargeted analysis of adducts of DNA, HSA, and Hb has motivated adductomics for untargeted characterization of exposures to reactive electrophiles (Rappaport et al. 2012). That is, an adductome represents the totality of adducts generated from reactions between reactive electrophiles and a particular nucleophilic locus on one of these molecules. By characterizing a complete adductome, it is possible to map systemic exposures that occurred over the *in vivo* residence time of the nucleophile, which can range from hours to months. Particular attention has been paid to mass spectrometric characterization of DNA adductomes in human tissue samples (reviewed by Balbo et al. 2014) and the blood proteins, HSA (Li et al. 2011; Grigoryan et al. 2016) and Hb (Carlsson et al. 2014).

Here, we posit that salivary HSA and Hb residence times would be similar to those in blood (i.e., 30 days for HSA and 60 days for Hb), which are much longer than those for DNA (a few days). Consequently, by performing untargeted mass spectrometry of HSA and Hb adducts in saliva samples, it should be possible to characterize systemic exposures to reactive electrophiles during 1–2 mo prior to specimen collection. With repeated saliva sampling every few years, one can construct an individual exposure history of relevance to a person's health trajectory.

Conclusions

Saliva contains a rich set of molecular information for circulating chemicals that can be interrogated via EWAS, with metabolomics and adductomics, to discover exposure–risk factors for chronic diseases. Although the number of metabolites detected in saliva is smaller than that in blood, we can anticipate that analytical improvements will uncover many additional salivary metabolites, present at low concentrations.

Given the simplicity and noninvasiveness of specimen collection, saliva offers a practical alternative to blood for longitudinal measurements of individual's exposomes. Several studies have shown that participants are more willing to donate saliva specimens than venous blood, and saliva can be collected by the subjects themselves. This would eventually improve participation rates in cohort studies and, therefore, generate larger sets of biospecimens.

Data-driven studies that utilize repeated omic measurements from individuals at different stages of life should reduce exposure measurement errors and thereby increase power to discover unknown causes of chronic diseases. Given the ease of collection, saliva could well be the specimen of choice for obtaining repeated samples to profile small molecules, DNA, and proteins.

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