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Title:
Leveraging genomic data in smoking cessation trials in the era of Precision Medicine: Why and how

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Abstract (WC=249)

In an era of Precision Medicine it is vital to collect biological data within clinical trials and to integrate their analysis within the outcomes of the trial. The identification of genomic biomarkers that affect treatment response to smoking cessation treatment, both pharmacological and behavioral, or susceptibility to medication-related adverse reactions, holds real promise to improving treatment efficacy and to tailoring the treatment approach to the individual. However, a clear challenge in identifying reliable biomarkers is in obtaining adequate sample sizes. Consortium-based approaches will likely be necessary to yield real successes. Thus, meta-analyses of data from individual smoking cessation trials will become crucial and will be facilitated by standardized trial designs, assessments, and outcomes, and harmonizable measures. To foster increased collection of high quality genetics data in clinical trials, we discuss 1) Genetically informed trial design, 2) Biological samples (collection requirements, storage, and analysis with a focus on genomic data) and genetics consortia, 3) Participant consent and data sharing requirements for Institutional Review Board (IRB) approvals, and 4) Information on phenotype characterization, and meta-analysis. This work aligns with the objectives of the Precision Medicine Initiative, and offers guidance for integrating treatment research and genetics/genomics within the nicotine and tobacco research community. It is intended to promote the collection and genotyping of biosamples in existing subject samples, as well as the integration of genetic research elements into future study designs. This paper cross-references a companion paper in this issue that reviews current evidence on genetic and epigenetic markers in cessation trials.

Keywords: Addiction, Cessation, Genetic Research, Treatment and intervention.

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Implications

This paper outlines a framework for the consistent integration of biological data/samples into smoking cessation pharmacotherapy trials, aligned with the objectives of the recently unveiled Precision Medicine Initiative. Our goal is to encourage and provide support for treatment researchers to consider biosample collection and genotyping their existing samples, as well as integrating genetic analyses into their study design in order to realize precision medicine in treatment of nicotine dependence.

1. INTRODUCTION

Smoking is a major risk for preventable death and disability,[1-4] and smoking cessation reduces the risk of mortality.[5] However, cessation failure is common despite clinical practice guidelines[6] and available cessation medications, which are associated with different efficacies, side effects, adherence, use constraints, and costs.[7] Cessation treatment, pharmacological or behavioral, may be improved via precision medicine: i.e., optimizing treatments to maximize efficacy and minimize side effects.[8,9] Smokers vary greatly in the benefit they derive from particular pharmacotherapies, and biomarkers can predict a smoker's response to a specific pharmacotherapy.[10-14] Because the health cost of cessation failure is high, there is a need to identify treatments that are most likely to be effective for smokers who want to quit and to maintain long term abstinence.

An important initiative of the NIH is to develop precision medicine to improve care.[15] The initiative will increase our ability to characterize smokers and predict their responses to cessation pharmacotherapies. Such studies will help optimize treatments for enhanced efficacy and medication adherence, and to reduce side effects. In recent years, scientists have gained extensive knowledge on how personal factors (including genetics) can be used to predict important health outcomes.[16] The concept of precision medicine is not new; clinical history of allergic reactions to medication, for instance, has been used to guide medication choice for more than a century.[17] However, the assessment of individual variation has been dramatically improved by the recent development of large-scale biologic databases (i.e., the human genome sequence), and powerful methods for characterizing patients (i.e., proteomics, transcriptomics, epigenomics, metabolomics, and genomics). What is needed now is to leverage biological samples, test them rigorously, and ultimately use them to build the evidence-base needed to guide clinical practice. This will enable more accurate diagnoses, more rational disease

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prevention strategies, better treatment selection, and the development of novel therapies, including ones for nicotine dependence and the multitude of tobacco-related diseases.

The promise of precision medicine has already been fulfilled in some areas of medicine. For example, underlying causal genotypes are used to personalize cancer treatment.[18] The application of genetic discoveries to clinical decision making and treatment decisions is occurring in a variety of medical specialties. For example, genetic screening has identified a specific molecular subset of non-small cell lung cancer patients, where patients positive for a specific oncogene were more likely to be young never-smokers or light smokers, compared to older, heavier smokers.[19] In addition, guidelines from the College of American Pathologists and the International Association for the Study of Lung Cancer, recommend testing for two well-characterized genetic biomarkers in patients newly diagnosed with non-small cell lung cancer: epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) for treatment guidance.[20] The current clinical approach of using molecular and genetic phenotyping to guide clinical care of patients with lung cancer is a welcome addition to traditional therapy that has markedly limited effectiveness. In the field of addiction, genetic variants in the nicotinic receptor subunit gene *CHRNA5*, variants in the nicotine metabolism gene *CYP2A6*, and the nicotine metabolite ratio genotypes showed promise as a marker for smoking cessation pharmacotherapy selection. [21-25]

We are beginning to understand how to optimize therapies for other diseases based on different genetic polymorphisms.[26,27] Thus, genetic variables are used to optimize drug selection and dosing;[28] e.g., individual genetic profiles are used to avoid medications likely to cause serious adverse effects such as abacavir, carbamazepine and thiopurine.[29-31] Finally, with individual whole genome or exome sequencing, we can now not only better classify diseases, but also

diagnose patients with previously undiagnosed genetic diseases.[32,33] The Evaluation of

Genomic Applications in Practice and Prevention[34] Initiative, established by the National Office

of Public Health Genomics at the Centers for Disease Control and Prevention, supports the

development and implementation of a rigorous, evidence-based process for evaluating genetic

tests and other genomic applications for clinical and public health practice in the United

States.[34]

Based on the utility of individual biological variability for clinical care, we propose that, ideally,

examination of biological samples should be integrated into clinical trials to address issues that

are central to study aims, rather than as a data collection procedure merely affixed to the study.

We encourage the next generation of scientists to develop creative new approaches for

detecting, measuring, and analyzing a wide range of biomedical information including molecular,

genomic, cellular, clinical, behavioral, physiological, and environmental parameters. The NIH's

Precision Medicine Initiative plans to recruit a longitudinal cohort of 1 million or more Americans

to give consent for extensive characterization of biologic specimens (cell populations, proteins,

metabolites, RNA, and DNA — including whole-genome sequencing, when costs permit) and

behavioral data, all linked to their electronic health records as summarized in Figure 1.[15] Blood

specimens will be collected and processed using a standard CLIA compliant procedure,

ensuring quality control and comparability, and sent to a local or central biorepository, that will

support collection, processing, storage, retrieval and biochemical analysis and/or shipment to

analytic laboratories, in addition to a wide range of phenotypic data including mobile health

measures. Understanding the biological basis of complex traits will be informed by these

technological advances in data generation from multiple levels of biological systems — including

DNA sequencing,[35] RNA expression,[36,37] methylation patterns,[38] other epigenetic markers,[39]

proteomics[40] and metabolomics[41] (see Figure 1). Corresponding actions are being undertaken in many other countries as part of their national genome strategies.

Today, much treatment research is designed to develop and evaluate treatments that are expected to benefit the population as a whole, based on the expected response of a "typical" patient. However, individual patients can have markedly variable responses to therapy, ranging from highly efficacious outcome, to no effect, to deleterious outcome (see Figure 2). The roots of this variability likely include unrecognized differences in disease pathophysiology, environmental exposures, social and behavioral factors, and genetic factors. Prior research has, of course, used moderator variables to uncover person by treatment interactions. However, the new era will advance this effort via a much more precise and comprehensive assessment of individual biological characteristics. Thus, an important overarching goal is to determine whether and how state-of-the-art genomic biomarkers can be used to optimize smoking cessation pharmacotherapy to enhance efficacy and medication adherence, and to reduce side effects.

To promote incorporation of genetics data into smoking cessation treatment research, in this review we discuss 1) study design considerations (e.g., genetically informed trial), 2) practical considerations of biological samples collection and participant consent for genetic data sharing requirements, 3) development of genetic consortia and meta-analysis to obtain adequate sample sizes for robust pharmacogenetics analyses, and 4) Information on phenotype characterization and outcome harmonization for cross-study comparisons.

Key concepts and glossary
This review seeks to inform a broad medical readership about the current status of how biological samples can be used in smoking cessation trials and to highlight the rationale, study

design, practical considerations, and opportunities for nicotine and tobacco researchers. Views are still evolving about several issues such as the clinical validity of potential genomic biomarkers. Current findings on biological markers for smoking cessation and a glossary for key genetic and "omic" terms are presented in a companion paper by Saccone et al.

2. STUDY DESIGN CONSIDERATIONS AND EXAMPLES

Here we present examples on how researchers can consider biosample collection and genotyping their existing samples, as well as integrating genetic analyses into their study design. Genetic data collection is easier compared to collection of therapeutic drug level or proteins, which may be more sensitive to temperature or light with more restrictive collection and storage procedures.

A. Collecting biomarkers: Biomarkers relevant to nicotine and tobacco cessation research generally fall into three categories:[42,43] (1) diagnostic biomarkers for patient selection; (2) pharmacodynamic biomarkers for optimal dosing; (3) predictive biomarkers for therapeutic efficacy, which may include pharmacodynamic (e.g.,genotypes at specific genetic variants, electroencephalogram or functional connectivity) and pharmacokinetic (e.g., nicotine metabolite ratio) biomarkers. Additional details on definitions of biomarkers and the Institute of Medicine's proposed three-part framework for biomarker development (analytical validation, qualification/context of use and utilization)[44] are described in more detail elsewhere.[43]

Most biomarkers for omic research may be collected from whole blood or saliva. The timing of sample collection needs to be determined by the type and context of the research questions being addressed. For example, for germline DNA analyses, biosamples can be collected at any time prior to, during or after the study. However, gene expression and epigenetic studies are

often timed in relation to an exposure such as before and after drug administration or before and after smoking cessation, requiring careful adherence to the timing of a study's primary endpoints in relevant tissue types. For example, some epigenetic markers are sensitive to smoking cessation and will revert to unexposed levels ranging from weeks to years after smoking cessation depending on specific markers. [45-47] Consent forms should be thorough and specific enough to include whatever type of biomarkers one intends to collect. Furthermore, informed consent documents would need to be modified any time a novel omic test is added to an extant research plan. Trained phlebotomists, who could be research assistants, that receive special training and meet CLIA-requirements, should collect whole blood samples. Saliva sample collection can be easily performed by study participants according to manufacturer's protocols and clear participant instructions.

For DNA collection, saliva sample collection can be performed feasibly by mail using pre-addressed, return envelopes and collection kits available from multiple manufacturers.[48,49] In contrast, collection of other omics data may be more restrictive such that all samples should be frozen in -80°C or colder freezers as rapidly as possible after collection in appropriate containers to maintain the specific omic features. Investigators designing a biomarker study without previous experience can often contact their institutions' IRB for standard language required for informed consent documents and contact experienced investigators for sample collection, storage and processing protocols. We have also provided template language in Supplementary Table 1.

B. Biomarker-based randomization: There are at least three general types of randomized controlled trial (RCT) designs for pharmacogenomic investigations of smoking cessation, as illustrated in Figure 3 – according to a presentation by Dr. Caryn Lerman at an Institute of

Medicine Roundtable on Translating Genomic-Based Research meeting on evidence generation

for genomic diagnostic test development.[50] Most pharmacogenetics studies of smoking

cessation are analyses of genetic data from existing treatment studies (e.g., a single

polymorphism, multiple polymorphisms, additive genetic risk scores or metabolite proxies for

polymorphisms), and is called a **Retrospective** design. That is, analyses are conducted after

completion of the RCT that relate patient biological variables such as genotype or metabolite

status (e.g., normal vs. slow nicotine metabolizers) to targeted clinical outcomes: e.g., efficacy

of the drug for smoking cessation or the reduction of nicotine withdrawal symptoms, smoking

urges, drug dosing, or side effects (Figure 3). Retrospective trials are useful when the clinical

utility of such markers is unknown or not well established at the time of trial initiation and can

inform hypothesis generation, replication and independent validation. Retrospective designs

have several limitations, such as unbalanced groups (status on a biomarker might be unevenly

distributed across groups), reduced power resulting from either unbalanced groups or highly

skewed biomarker distributions due to base rates of the biomarker status, and missing data

because not all patients consented to provide biosamples.

Prospective pharmacogenomic trials can be divided into two types: prospective stratified and

prospective screened.[50] **Prospective stratified** trials conduct testing of a biomarker prior to trial

entry and define a biomarker as 'positive' or 'negative'. An advantage of this design is that the

trial is hypothesis-driven, taking into account prior knowledge about a biomarker and members

of the test population. Another advantage of this design is that it permits enrichment of the less

common genotype or biomarker by oversampling during the screening process – in order to

achieve balanced groups. For example, Lerman and colleagues tested smokers for the nicotine

metabolite ratio (NMR; 3'-hydroxycotinine/cotinine) and set an initial cut-off based upon prior

knowledge[51] to define 'normal metabolizers' and 'slow metabolizers'.[52] Marker positive and

marker negative smokers were then independently randomized to either nicotine replacement

patch or placebo patch or varenicline, resulting in balanced groups by biomarker status and

drug. Roughly 20% of patients, depending on ancestry, were slow metabolizers. Thus,

oversampling of slow metabolizers was required in this prospective stratified study and resulted

in excluding many patients who tested positive for normal metabolizer status. In another

ongoing study by Chen and colleagues, participants receive prospectively stratified treatment

randomization by the individual's cessation-relevant genotypes such as the *CHRNA5* D398N

gene variant [21,23] in order to yield balanced groups for testing the relation of genotype status

with medication efficacy and adverse effects. [22]

A third type of pharmacogenomic trial is the **prospective screened** design in which patients are

randomized to receive biomarker-guided treatment or usual care. In the biomarker-guided

treatment group, patients are tested for genotype or metabolite status and assigned to a

treatment based on a hypothesized association of the marker with the efficacy of a particular

drug (Figure 3). Assume for example that genotype AA (marker positive) for a given

polymorphism predicts enhanced efficacy of nicotine replacement therapy (NRT), but not

varenicline efficacy. Genotype GG (marker negative) on the other hand, predicts enhanced

efficacy of varenicline, but not NRT efficacy. Therefore, in the genotype-guided group patients

with genotype AA would receive NRT and patients with genotype GG would receive varenicline.

Patients in the usual care group would receive either a pre-defined standard medication, or they

might be randomized to either of the drugs, or their physician might prescribe their medication

based on usual practice. The results of the genotype-guided group would then be compared

with the usual care (non-guided) group. An advantage of the prospective screened design is its

potential for high ecological validity, offering evidence of whether or not a genotype- or

metabolite-driven therapy provides improved effectiveness over non-guided therapy in non-

research settings.[50]

In addition to these three general designs for pharmacogenomics RCTs, other designs are more fitting for enabling clinical implementation and patient-centered effectiveness, such as pragmatic trial designs that evaluate metrics germane to real-world clinical practice such as cost-effectiveness, patient satisfaction, clinical outcomes and feasibility.[50,53,54] In addition, it may be advantageous for researchers to use factorial designs to explore pharmacogenetic relations. This is, in part, because of the efficiency of such designs, as they permit experimental analysis of multiple, discrete intervention components.[55,56] Thus, the researcher might investigate the efficacies of both multiple pharmacotherapies, and at different levels of counseling intensities. Another advantage of such designs is that they provide relatively good power; when each factor comprises two levels, all subjects in the design contribute to estimation of the effects of each factor. Also, they uniquely permit estimation of interaction effects. For instance, they might reveal that genetically determined differential response to a medication might be neutralized by more intense counseling, or by the conjoint use of two medications. For more details about trial design, we refer readers to these reports.[43,50,55,56]

3. PRACTICAL CONSIDERATIONS

The benefits and challenges of various biosampling options regarding biospecimen requirements, storage and analysis are summarized in Table 1.

Collection of appropriate participant consent

Collection of informed written consent from participants to use their biological samples for the purposes of genetics testing is critical to the ethical conduct of genetics research. Research participants need to know what data will be studied, who will have access to their genetics data,

what protection will be in place to ensure the anonymity of their genetic information is maintained, and any other study-specific information.

For some clinical trials it may be necessary for the genetic testing to be a mandatory component of participation in the research. If this is the case, then consent for genetic testing should be included as part of the informed consent for the study as a whole. However for some studies, participating in the genetics part is not essential. In this case, the genetics part of the research can be presented to the participant as an optional 'sub-study', and a separate informed written consent specifically for genetic or other omics participation should be completed. In our experience, majority of participants consent to give biological samples (e.g. blood or saliva) for genetic studies [57], whether these results can generalized to individuals who decline biological samples needs to be examined in future research.

Participants must be advised of the potential privacy risks associated with donating a DNA sample for research. The consent form should provide instruction to participants regarding the importance of actively protecting their own privacy. Participants should be informed about the Genetic Information Nondiscrimination Act (GINA), which makes it illegal for health insurance companies, group health plans, and most employers to discriminate against people based on their genetic information. The consent form should also describe the protections taken by the study to protect the privacy of participants. These may include assignment of unique numerical identifiers that are used to label all samples and genotypic data, procedures for securely storing hard copy records and electronic data, and attainment of a Certificate of Confidentiality from the Department of Health and Human Services. Supplementary Table 1 provides research elements and example consent languages for study purpose, risks/benefits, confidentiality, sample and information on storage and destruction. We acknowledge that there are clinical trial situations in

which collection of biosamples may not be feasible due to specific concerns (e.g., certain vulnerable population, costs)

NIDA Genetics Consortium (NGC), NIDA Genetics Study Center (Biorepository), and NIH Resource Sharing Guidelines

As described earlier, consortium-based biorepositories are important to enable evidence needed for translation. The NIDA Genetics Consortium was created in 1999 to identify human genes for drug addiction, create a repository of data, generate a database on genetics of drug use and related phenotypes, and establish a consortium of collaborating scientists.[58] In particular, the NIDA Center for Genetics Studies (NCGS Biorepository) is a NIDA-funded scientific resource for informing the human molecular genetics of addiction. The Biorepository will produce, store, and distribute clinical data and biomaterials (DNA samples and cell lines) available in the NIDA Genetics Initiative.[58] The Biorepository collects high-quality DNA, plasma (if consented), and cryopreserved lymphocytes (CPLs) on all whole blood samples submitted by NGC members. The sharing of data in the NCGS is done in strict accordance with the informed consent provided for each research subject. Many genotyping arrays are available including a NIDA-funded custom genotyping array for studying the genetics of addiction and treatment.[59]

Support outside of United States

Most high-income countries in Europe and elsewhere in the world have equivalent ethical and data protection procedures and legislation as in the U.S., but details vary. European data protection regulations are more restrictive than in the U. S., and genetic study data are typically deposited at the European Bioinformatics Institute (EMBL-EBI) (www.ebi.ac.uk). Clinicians planning to undertake such studies outside the U.S. should refer to national ethics boards and data protection agencies for guidance as needed.

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4. META-ANALYSIS AND HARMONIZATION

The research is growing on treatment effect in the context of differences in participants, clinical trial designs and treatments using systematic reviews, traditional meta-analyses and more recent methods such as Bayesian, multiple treatment, multiple outcome, and network meta-analysis. Clinical and statistical experts have estimated effect sizes of participant, clinician, and treatment factors on prospective abstinence using meta-analyses of randomized clinical trials of smoking cessation.[6,60] Meta-analysis goals include utilization of the retrospective evidence base (e.g. based on published literature) to (1) provide guidance to patients and clinicians,[61] (2) evaluate effect modification, e.g., of nicotine dependence[62] or genetic variants,[21] on outcomes, and (3) develop clinical trial hypotheses for design and analysis.[63] In addition, *de novo* collaborative meta-analyses, which utilize new analyses of existing data, usually with harmonized phenotypes and uniform analytic models, have been highly effective in aggregating evidence for human genetic associations, including for smoking behavioral traits.[64-67]

Development of databases for pursuing meta-analysis of smoking cessation clinical trials involves identifying participant, treatment, and outcome measures from existing RCT datasets, comparing assessments used to obtain these data, and reviewing coding. Harmonization approaches to render clinical trials suitable for data analysis include expert opinion, regression analyses, and multiple imputation methods. The degree of harmonization between phenotypes in two clinical trials can be assessed if at least one of those trials contains all the information required to estimate the phenotype in the other trial. The degree of agreement between those phenotype estimates is a measure of harmonization. Multiple imputation is applicable to target phenotypes that are missing by design, i.e., that can be considered to be missing completely at random; when missing values are not missing at random, the resulting imputation of the target

phenotype might be biased. This situation may arise for abstinence when the subject fails to report abstinence because they have relapsed. The usual approach is to assume that all non-reporting individuals have relapsed. However, this approach ignores the accessible factors that are related to missing data and the outcome. In such cases, it may be better to apply a principled method to account for the effects of the accessible mechanism.[68,69] Harmonization of prospective abstinence outcome measures has been discussed by clinical experts.[70] Interval-censored regression is an indirect method of harmonization applicable if response categories differ for a group of phenotypes that are otherwise consistently measured. On the other hand, pharmacogenomic allele nomenclature standardization (to human reference sequence assemblies) contributes to the ongoing nicotine metabolism biomarker and genotype metric harmonization efforts.[71]

Multiple treatment comparison meta-analysis is a form of integrated data analysis that includes the more familiar meta- and mega- analysis approaches and enables both direct and indirect comparisons of treatment effects.[63,72] Direct comparisons of treatment effects take place when individuals are randomized to different treatments; indirect comparisons of treatment effects take place when analyses rely upon multiple direct comparisons to estimate the indirect comparison via a network. Analysis of both direct and indirect treatment effect comparisons increase the total sample size of the treatment comparisons and may, as in conventional meta-analyses, identify heterogeneity between randomization arms, or between directly and indirectly estimated effects. Examination of modeling assumptions through simulation, sensitivity analyses and collaborative standardized approaches will be necessary to integrate multiple related patient and environmental information and extract guidance from analyses of clinical trials.

5. INFORMATION ON PHENOTYPE ASSESSMENT AND CHARACTERIZATION

Phenotype Assessment

The key conceptual and practical considerations for phenotyping overlap considerably with issues surrounding assessment of treatment efficacy. Thus, existing conventions established to promote the rigor and comparability of smoking cessation studies also provide useful guidance for phenotype assessment.[70,73]

A common primary endpoint for cessation studies is the attainment of an extended period of abstinence from smoking at a distal follow-up after the quit date (typically 6 or 12 months). Individuals who meet these benchmarks remain at risk for relapse,[74] however a lengthy period of sustained abstinence is the best available indicator of lifelong abstinence, the typical treatment goal and the outcome expected to yield the maximal health benefit. The SRNT workgroup on outcomes in clinical trials recommended using a "prolonged abstinence" standard, defined as a period of sustained abstinence following a short (i.e., 2 wk) initial grace period,[70] with point prevalence of 7-day abstinence as a secondary measure. A proposed alternative, the "Russell Standard Abstinence" definition, requires the conjunction of a self-report of smoking 5 or fewer cigarettes since the quit date and a negative biochemical test at the follow-up.[73] Both definitions incorporate allowances for a limited amount of smoking after the target quit date, recognizing that smoking cessation is a difficult process and temporary setbacks do not necessarily preclude long-term success, and that treatment delivery generally continues beyond the quit date. However, the various definitions are differentially sensitive to post-quit lapsing that occurs relatively late in the follow-up period, but that still may be effectively treated by continued treatment.[75] In essence, the researcher must try to adopt an outcome definition that is clinically meaningful, mergeable across other studies, and that provides a sensitive signal of targeted treatment effects.

These conventions provide important information about ultimate clinical outcomes, but they offer little insight into the process of cessation or the mechanisms through which treatments exert their effects. One complementary approach is to focus on pivotal clinical milestones in the cessation process, such as the establishment of an initial period of abstinence, the occurrence of the first smoking lapse, and the transition from a lapse to full relapse.[76] In treatment evaluation research, a series of survival analyses can be used to test whether treatment condition influences the time to each milestone, providing clues as to how effective treatments work.[76,77] An assumption here is that these milestones are differentially sensitive to medication effects and their relations with omic determinants. For instance, there is some evidence that the effects of medication early in the quit attempt (e.g., on initial abstinence) are especially sensitive to medication benefit.[55,64,78]

Another fruitful approach is to measure presumed mediators of treatment effects.[78,79] The most relevant mediators can differ as a function of the treatment being evaluated, but a host of common barriers to cessation have been identified by theory and empirical investigations of lapse antecedents. These include urge/craving, withdrawal symptoms, exposure to tobacco cues, stressors, alcohol use, and reactions to lapse events[80,81] In pharmacotherapy studies, medication compliance and drug side effects may represent important mediators of treatment outcome.[82,83] Investigating whether treatment allocation influences these barriers to cessation, and testing whether group differences in long-term outcomes are mediated through effects in these domains, can help to refine our understanding of treatment mechanisms. There is also the prospect that medication effects on sensitive mediators (e.g., craving suppression) provide especially sensitive indices of medication benefit.[84]

Genetic studies of smoking cessation will benefit from incorporating clinical phenotypes rooted in each of these approaches. Long-term abstinence endpoints seem strongly indicative of the public health benefit of treatment, and thus clearly relevant to the development of precision medicine protocols. Investigating how candidate genetic markers influence clinical milestones and treatment mediators in addition to the traditional outcome of end of treatment abstinence may lead to a better understanding of their functional significance (e.g., due to differential contribution of error).[78,85] Use of multiple outcomes may be especially valuable for positional candidates discovered via genome-wide scans that are not anticipated by theory and for which knowledge of biological function is lacking. On the other hand, increasing biological knowledge in genomic databases speeds the discovery process to link genes, functions, and clinical outcomes. Of note, is that, the causal gene and variants are not necessarily the ones closest to the genetic marker identified in a genome-wide screen.

Anticipation of genetic analyses may encourage investigators to alter their assessment plans when designing cessation trials. Pooling or meta-analyzing data from many trials represents a powerful method for exploring genetic influences on smoking cessation. This encourages the use of broad and flexible assessment strategies. Ideally, clinical studies would incorporate detailed assessments of smoking behavior with good resolution of timing, amount, antecedents, and consequences of post-cessation cigarette use. Examples include calendar-based methods or intensive longitudinal assessments.[86,87] This would allow outcomes to be scored according to multiple criteria (e.g. various grace periods or thresholds for progression to the relapse milestone), facilitating cross-trial harmonization and pooled analyses.

When designing a stand-alone trial, it may make sense to assess a small set of targeted mediators based on a working knowledge of the treatment under study, i.e., how the tested

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treatment is thought to work. However, the possibility of future, pooled genetic analyses should encourage clinical investigators to cast a wider net when it comes to assessing possible mediators. This alternative approach is to assess mediators based on the outcome model – what important factors may influence outcomes. One reason is that there is often uncertainty about which genetic variant(s) may eventually be tested in secondary analyses, and therefore the important mediator(s) may not be knowable in the trial-planning phase. A second consideration is that mediators thought to be irrelevant in an individual trial might be very important in a pooled or aggregated analysis.

Increasing need for interdisciplinary collaboration and data sharing has led to initiatives such as the PhenX Toolkit[88] and the PROMIS system[89,90] designed to encourage use of consensus measures in health research. Going forward, the smoking cessation field might benefit from development and dissemination of a comparable set of standardized, flexible assessment tools designed to gather information on post-cessation smoking patterns, common barriers to cessation, variables that may mediate of treatment effects, and potentially useful intermediate phenotypes for genetic research.

6. CONCLUSION

In the era of 'Precision Medicine', it is becoming increasingly important that investigators collect biological samples within clinical trials and integrate their analysis and interpretation with the goals of the trial. The identification of genomic markers that affect response to smoking cessation pharmacotherapies, or susceptibility to adverse reactions to such drugs, holds real promise to improve smoking cessation treatment efficacy through tailored treatment interventions, pharmacological or behavioral. A major concern for trial design is the timing of genomic assessment. Available genomic data before treatment randomization will allow gene-

based stratified randomization or experimental testing of gene-based personalized treatment, while collection of any biosamples at any time in the trial for subsequent genotyping is still beneficial. Another challenge in identifying such genomic biomarkers will be to obtain adequate sample sizes. Consortium-based approaches will likely be necessary to yield real successes, as we have seen from previous genome-wide association studies of complex traits including smoking behavior.[64-66,91-93] Thus for pharmacogenomic studies, meta-analysis of data from individual smoking cessation trials will be crucial and will require comparable trial designs and outcomes.[21,94,95] Other related topics such as the genetic effects on smoker response to non-pharmacologic smoking cessation interventions are not included in this review.

In this paper, we outline a framework for the consistent integration of biological data/samples into smoking cessation pharmacotherapy trials. This work aligns with the objectives of the recently unveiled Precision Medicine Initiative, and addresses a call for practical advice to guide the integration of treatment and genetics research within the nicotine and tobacco research community. Our goal is to encourage treatment researchers to consider biosample collection and genotyping their existing samples, as well as integrating genetic analyses into their study design. Of course, identifying an optimal pharmacogenetic strategy is highly complex, as treatment trials vary in study designs, the type and intensity of the counseling treatment provided to all groups including the placebo arm, subject inclusion/exclusion criteria, and other experimental methods. Still, progress is underway, as reviewed in the companion paper by Saccone et al. In summary, this work encourages and provides support for study designs that are needed in order to realize precision medicine in treatment of nicotine dependence.[96]

Figure Legend:

Figure 1.

Title: Biological systems multi-omics from the genome, epigenome, transcriptome, proteome and metabolome to the phenome

Legend: single-nucleotide polymorphism (SNP), copy number variation (CNV), micro RNA (miRNA).

Figure 2.

Title: Example: Benefits of nicotine replacement therapy may vary by genetic marker

Legend:

Blue: patients who benefit; Clear: patients who fail to benefit,[21,97] both studies of European Ancestry.

Figure 3.

Title: Pharmacogenomic trial designs, including retrospective, prospective stratified, and prospective screened. Source: Adapted from Lerman, IOM workshop presentation on November 17, 2010

DECLARATION OF INTEREST

LJB is listed as an inventor on Issued U.S. Patent 8080371 "Markers for Addiction" covering the use of certain SNPs in determining the diagnosis, prognosis, and treatment of addiction, and served as a consultant for Pfizer in 2008. The spouse of NLS is also listed as an inventor on the above patent. RT has consulted for Apotex. JWB is an owner and employee of BioRealm and AWB is an employee of BioRealm, which offers commercial services related to the Smokescreen Genotyping Array. SPD has consulted for BaseHealth, Inc., which develops predictive platforms for population health management. JK has consulted for Pfizer. RT has consulted for the pharmaceutical company Apotex. The remaining authors declare no conflict of interest.

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Table 1. Pros and Cons of Biological Samples

Type of biosample	Primary Use	Storage	Pros	Cons
Whole blood	Generate subfractions [plasma, serum and cells (for extraction, viable storage, or transformation)], and isolate nucleic acids, proteins, and metabolites.	Ultra-low temperature with some alternative storage approaches.	Wide variety of fractions and analytes. Costs proportional to the number/diversity of tubes drawn and subsequent processing steps.	Requires access to -80C freezer. Need access to phlebotomist
Saliva	Isolate nucleic acids and proteins from host and from the meta-genome.	Room temperature for saliva possible; ultra-low temperature for analytes.	Ease of collection. Can be done remotely and mailed in.	Lack of clinical observation during collection results in minor rate of biospecimen substitution. Quality/quantity of DNA lower than for blood. Contamination of DNA from food etc.
Urine	Isolate metabolites.	Ultra-low temperature.	24 hour urine collection is standard but processing urine volumes can be challenging.	Requires access to -80C freezer. No DNA.
Buccal Cells	Isolate nucleic acids	Ultra-low temperature.	One tissue type exposed to the environment highly relevant to smoking/vaping behaviors.	Care in selecting buccal sampling protocol for comparability.