Evaluation of Targeted Sequencing Technology to Screen 17 Genes for Actionable Conditions in Healthy Individuals

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Introduction
Screening programs, such as newborn screening, in healthcare serve a public health role in that they have the potential for early detection and prevention of diseases prior to clinical manifestation of symptoms. Advancements in next-generation sequencing (NGS) provide opportunities to implement genomic screening and “precision medicine” in the general population.

GeneScreen explores the feasibility and ethics of screening an adult population for 11 highly actionable conditions via targeted sequencing of 17 genes, mitigating ELSI concerns raised from genome-scale sequencing in healthy populations. As part of this work, we evaluated and compared targeted sequencing technologies that could provide a cost-effective alternative to genome-scale sequencing (GSS) approaches:

- Roche/Innolobogen Heat-Seq molecular inversion probes (MIPs)
- Integrated DNA Technologies xGen lock down hybridization capture probes

Methods
We targeted those 17 genes in a subset of 58 participants enrolled in the GeneScreen study, using Heat-Seq MIPs and xGen lock down probes. Our metrics assessed the performance on three aspects important for clinical sequencing:

- Gene-level “adequate” coverage
- Variant calling comparability

Category | Condition (gene) | Interventions
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Cancer | Familial adenomatous polyposis (APC) | Colonoscopy, endoscopy screening, thyroid ultrasound, surgery
| MUTH-associated polyposis (MUTH) | Colonoscopy, endoscopy
| Lynch syndrome (MLH1) | Colonoscopy, endoscopy, endometrial biopsy, possible surgery (prophylactic hysterectomy and salpingo-oophorectomy)
| Lynch syndrome (MSH2) | Colonoscopy, endoscopy
| Lynch syndrome (MSH6) | Colonoscopy, endoscopy
| Lynch syndrome (PMS2) | Colonoscopy, endoscopy
| Familial breast/ovarian cancer (BRCA1) | Breast imaging, prophylactic mastectomy and/or salpingo-oophorectomy
| Familial breast/ovarian cancer (BRCA2) | Breast imaging, prophylactic mastectomy and/or salpingo-oophorectomy
| MEN2A/2B (RET) | Prophylactic thyroidectomy, serum metanephrine blood test
| Long QT syndrome (KCNQ2) | Cardiology consultation, ECG, β-blocker medication if ECG is positive; implantable cardioverter-defibrillator if symptomatic
| Long QT syndrome (KCNQ2) | Cardiology consultation, ECG, β-blocker medication if ECG is positive; implantable cardioverter-defibrillator if symptomatic
| Long QT syndrome (SCNS5A) | Cardiology consultation, ECG, β-blocker medication if ECG is positive; implantable cardioverter-defibrillator if symptomatic
| Familial hypercholesterolemia (LDLR) | Lipid biochemical screening, pharmacotherapy if needed
| Marfan syndrome (FBN1) | Echocardiography, ophthalmologic screening
Cardiovascular | Malignant hyperthermia (Ryr1) | Avoidance of specific anesthetics
| Hereditary hemochromatosis (HFE) | Ferritin biochemical screening, phlebotomy
| α-1 Antitrypsin deficiency (SERPINA1) | Avoidance of exposure to smoke

Table 1. Characteristics of 11 screened conditions and 17 candidate genes. The GeneScreen Committee and Community Advisory Board reviewed and weighted these genes, when mutated, confer high risk of these potentially detectable and preventable disorders. 1-4

Variant detection
Both targeted probe technologies have their strengths and weaknesses:

- Roche Heat-Seq has G-C rich limitations, but cost-effective
- IDT Lockdown has extra variant noise, but effective for G-C rich regions and smaller panels

With further optimization, targeted genomic sequencing could be a feasible and ethical option of screening the general population as it not only promises lower cost than GSS but would avoid generating large numbers of variants in genes with unknown or non-clinical significance.

Conclusions & Future Implications
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