Development and validation of a high throughput next generation sequencing assay from buccal cell DNA as a cost-effective screening method for celiac genetic risk

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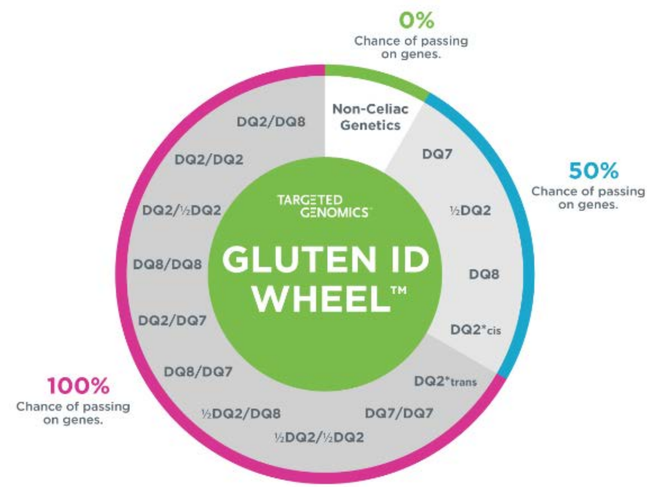
Background

An estimated 1% of the global population has been diagnosed with celiac disease (CD), an autoimmune condition triggered by dietary gluten in individuals who carry HLA-DQ2 and/or HLA-DQ8 (DQ2/DQ8) celiac risk alleles. Clinical diagnosis of active CD is based on symptoms, serologic tissue transglutaminase (tTG) antibody testing, and characteristic histopathologic changes identified by small intestinal tissue biopsy. The presence of DQ2/DQ8 genes is necessary but not sufficient for development of CD, and the negative predictive value (NPV) of negative DQ2/DQ8 test results is > 90%. Approximately 30-40% of the general population carries specific allelic combinations for DQ2/DQ8 that would place them on a spectrum of risk for CD. However, despite the high NPV of DQ2/DQ8 testing, routine screening of celiac family members and asymptomatic individuals has traditionally been performed by tTG serologic testing and/or small intestinal biopsy. Disadvantages to this approach include false negative results in individuals who are not consuming dietary gluten, and unnecessary healthcare costs associated with repeated testing of asymptomatic individuals. In the current study we developed and validated a low cost, non-invasive, buccal cell DNA-based next generation sequencing (NGS) population screening method for the DQ2/DQ8 alleles.

Materials and Methods

Buccal cell DNA was collected from 98 healthy individuals (including multi-generation family members) and analyzed using next generation sequencing (NGS) technology to amplify and sequence across HLA-tagging celiac risk SNPs for DQ2.5, DQ8, DQ2.2, and DQ7 at a minimum of 40X coverage. Sequencing data was analyzed in Galaxy, where the fastq files were aligned, variants called, and SNP's identified.

Haplotypes identified by the celiac genetic health risk screening assay with associated chance of inheritance



Haplotype-based risk for development of celiac disease

Population Risk For Celiac Disease By Gluten ID

Gluten ID	Risk
DQ2/DQ8	1:7 (14.3%)
DQ2/DQ2	1:10 (10%)
DQ2/½DQ2	1:10 (10%)
DQ8/DQ8	1:12 (8.4%)
DQ8/½DQ2	1:24 (4.2%)
½DQ2/½DQ2	1:26 (3.8%)
DQ2/DQ7	1:35 (2.9%)
DQ2	1:35 (2.9%)
DQ8/DQ7	1:89 (1.1%)
DQ8	1:89 (1.1%)
½DQ2	1:210 (0.5%)
DQ7*	1:1842 (0.05%
Non-celiac genetics (NCG)	1:2518 (< 0.04%)

Celiac Disease Risk

*Recent evidence suggests DQ7 may be associated with higher risk [3]

Results

All possible heterozygous and homozygous combinations of celiac risk alleles were identified in the study population including non-celiac genetics (NCG), DQ2(cis), DQ2(trans) DQ8, and DQ7. Allelic inheritance could be clearly traced through multiple generations of families. A subset of 20 samples with known DQ2/DQ8 status performed by an outside CAP/CLIA certified clinical laboratory was used for qualitative analytical validation of the assay which showed 100% concordance with known DQ2/DQ8 results.

Conclusions

DQ2/DQ8 typing by NGS is a cost-effective screening method for celiac genetic risk in individuals, families, and populations. The assay identifies those with high-risk genetics who would benefit from annual serologic and/or small biopsy testing while allowing NCG low risk, asymptomatic individuals to forgo further screening for celiac disease.

References

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