GWAS of Canker Sores
Implicates Th-1 Pathway and Shared Genetic Architecture with Immune-Mediated Disease

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23andMe, Inc.
ASHG ‘17
Canker Sores

- Mouth ulcers or recurrent aphthous stomatitis.
- One of the most prevalent immune-mediated conditions:
  - Prevalence: 5-25% (Ślebioda et al. 2014)
  - Incidence: 34% per year (23andMe)
  - Lifetime cumulative incidence: 73% (23andMe)
- Range in severity from irritating to debilitating.
- Comorbidity with several immune-mediated diseases:
  - Inflammatory bowel disease (Katsanos et al. 2015)
  - Celiac disease (Aydemir et al. 2004)
  - Behçet's disease (Yilmaz and Cimen 2010)
- A common side effect of medication such as chemotherapy drugs (mucositis).
Genetic Contribution

- 24-46% of severe cases have family history (Scully and Porter 2008).

- Genetic studies have been limited to the targeted gene approach with small sample sizes (<100 cases) and limited success (Bazrafshani et al. 2002, Kalkan et al. 2013, Alkhateeb et al. 2013).

The 23andMe Database

2M+
genotyped customers

~85%
of our customers opt in to participate in research

700M+
phenotypic data points collected
Canker Sores GWAS

Question

Have you ever had a canker sore (an open sore on the soft tissue inside the mouth)?
Yes / No / Not sure

Demographic

<table>
<thead>
<tr>
<th>Group</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>case</td>
<td>178,409</td>
<td>71,649 (69.8%)</td>
<td>106,760 (75.0%)</td>
</tr>
<tr>
<td>control</td>
<td>66,603 (27.2%)</td>
<td>30,943 (30.2%)</td>
<td>35,660 (25.0%)</td>
</tr>
</tbody>
</table>
Canker Sores GWAS

178,409 cases (59.8% female); 66,603 controls

47 loci significantly associated at p<5E-8; 75 loci at p<1E-6
UK Biobank “Mouth Ulcers” from Neale Lab

34,398 cases; 301,740 controls

27 loci at p<5E-8, 21 overlap with 23andMe, and all are validated by 23andMe at p<0.05.
Of the 47 loci, 21 overlap with UK Biobank, and 43 are replicated at p<0.05.
**IL12A Locus**

rs17753641 is in a regulatory region upstream of *IL12A*.
- TF binding sites
- DNAse hypersensitivity site

Conditional analysis shows an additional independent association in this region.
**IL10 Locus**

rs1518110 is an eQTL for *IL10* in GTEx whole blood (p=2.5E-9).
All associations are significant at p<1E-6
Relationship to Other Immune-Mediated Diseases

LD score regression **heritability estimate** (liability scale): 9.62%, which is comparable to most other autoimmune diseases (median 8.05%; max 19.56%).

**Genetic correlation** highlights shared genetic architecture with other infectious diseases (strep throat, common cold) as well as inflammatory diseases (IBD, eczema).
Adaptive Immunity: Th-1

Canker sore GWAS hits are enriched for genes in the Th-1 pathway ($p=9.0\times10^{-6}$) and the interferon gamma signaling pathway ($p=4.0\times10^{-6}$).

This is in line with previous gene expression experiments (Buno et al. 1998, Borra et al. 2004, Gallo et al. 2012).
Innate Immunity: Lysozyme

*LYZ* gene encodes human **lysozyme**, whose natural substrate is the bacterial cell wall peptidoglycan. [NCBI]

Missense variant rs1800973 in *LYZ* is associated with canker sores.
Innate Immunity: Microbial Recognition

Toll-like receptor signaling

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Alleles</th>
<th>MAF</th>
<th>OR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIRAP</td>
<td>rs8177399</td>
<td>C/T</td>
<td>0.020</td>
<td>1.155</td>
<td>2.84E-09</td>
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<tr>
<td>TICAM1</td>
<td>rs9749105</td>
<td>G/T</td>
<td>0.213</td>
<td>0.950</td>
<td>2.32E-10</td>
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<tr>
<td>IRAK2</td>
<td>rs358849</td>
<td>C/T</td>
<td>0.467</td>
<td>0.961</td>
<td>4.08E-09</td>
</tr>
</tbody>
</table>

NOD2 signaling

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Alleles</th>
<th>MAF</th>
<th>OR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIPK2</td>
<td>rs34970073</td>
<td>A/C</td>
<td>0.162</td>
<td>1.091</td>
<td>1.99E-21</td>
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<tr>
<td>NOD2</td>
<td>rs2066847</td>
<td>-/C</td>
<td>0.025</td>
<td>0.803</td>
<td>1.98E-19</td>
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<td>NOD2</td>
<td>rs2066844</td>
<td>C/T</td>
<td>0.016</td>
<td>0.877</td>
<td>2.20E-15</td>
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<tr>
<td>NOD2</td>
<td>rs2066845</td>
<td>G/C</td>
<td>0.045</td>
<td>0.875</td>
<td>3.72E-07</td>
</tr>
</tbody>
</table>

NOD2 loss-of-function variants are protective against canker sores.
Smoking protects against canker sores but increases risk of Crohn’s Disease

Relief of Canker Sores on Resumption Of Cigarette Smoking

RALPH BOOKMAN, M.D., Beverly Hills

Although aphthous stomatitis—“canker sores”—usually takes the form of lesions of the oral mucosa or tongue that heal spontaneously, sometimes the lesions are multiple, confluent, recurrent and resistant to therapy, in that form the disease can torment the patient and dismay the physician who treats him.

The etiology of recurrent aphthae is obscure. Various observers have inculpated viral infection, allergic sensitivity to foods, hormonal influence (with exacerbation related to menses) and trauma from weak organic acids but proof of any single cause is lacking.

Submitted May 4, 1960.
Gene x Environment Interaction between Smoking and NOD2 LoF

NOD2 LoF is a combination of three independent NOD2 LoF variants:
- Leu1007fsX1008 [rs2066847]
- G908R [rs2066845]
- R702W [rs2066844]
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- R702W [rs2066844]
Cigarette Smoking Affects NOD2 Pathway

Smoking → NOD2 LoF → Crohn’s | Canker Sore
Conclusion

- Canker sore biology involves both innate and adaptive immune system:
  - Innate immunity: lysozyme, TLR signaling, NOD2 signaling
  - Adaptive immunity: Th-1 pathway
- Benign, common conditions such as canker sores share genetic architecture and molecular mechanism with more serious, rare diseases such as IBD and susceptibility to various infections.
- Cigarette smoke affects the risk of canker sores and Crohn’s disease through modulating NOD2 expression.
- Canker sores can be used as a phenotype to study other autoimmune disease such as IBD and eczema.
Thank you

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23andMe Research Team and employees
23andMe research participants