Very Early Onset Inflammatory Bowel Disease: NEOPICS

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State of the Art Lecture
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I have no financial relationships with any commercial entity to disclose

Summary

- **VEO-IBD**
  - Pathogenesis and Epidemiology
  - Clinical Features
  - Outcomes
  - Treatment Options
- **Cases – Personalized Medicine Approach to VEO-IBD**
  - Case I: IL10R and IL10 Defects
  - Case II: "Phenocopies" of IBD – NADPH oxidase Genes
  - Novel Approaches to Understand VEO-IBD

Definitions and Challenges

- **Definitions**
- Young children with IBD have a distinct disease with regards to disease location, extension over time, and are often challenging to treat
- Recent Paris Modification of the Montreal Classification system defined children diagnosed under 10 years of age as a distinct phenotype (A1a) – VEO-IBD
- Perhaps < 6 yo may be better?
- Children diagnosed under 1 year of age are also distinct and defined as infantile IBD

Sherlock et al (unpublished); Griffiths (2004) Best Pract Res Clin; Heyman et al (2005) J Ped; Ruemmele et al (2006) JPGN; Kappelman et al (2008) IBD; Levine et al (2010) IBD

Definitions and Challenges

- **Challenges**
- Diagnosis – very young age – need to rule out other causes of colitis – especially immunodeficiency.
- Treatment options – no treatment guidelines, and surgery may not always be a viable option with extension of disease.
- Long term outcomes including growth and cancer risk poorly understood.

Differential Diagnosis – VEO-IBD

Causes of Colitis in Young Children
Infections – including *Salmonella, Shigella, Yersinia, Campylobacter, Amoebias, Clostridium difficile, Giardia, Cytomegalovirus, tuberculosis,* HIV/AIDS
Allergic Colitis
Eosinophilic colitis
Benign lymphoid hyperplasia
Hemolytic uraemic syndrome (HUS)
Bechet's Disease
Primary Immunodeficiency including SCIDS, Wiscott-Aldrich Syndrome, CVID, CGD, IPEX, NEMO, GSD1b,
IL10R Defects
Hermanksy-Pudlak Syndrome
Autoimmune enteropathy
Hemophagocytic lymphohistiocytosis (HLH)

Laboratory Investigations

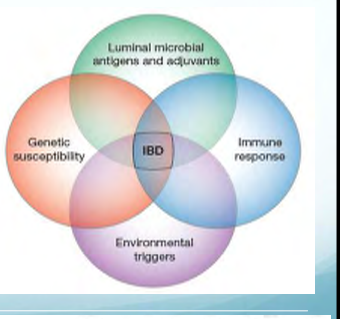
- **Classic "immunology labs" often will not help establish a diagnosis**
- Complete blood count with WBC Differential
- Quantitative Immunoglobulins – IgA, IgG, IgM
- B and T cell subsets by flow cytometry
- NBT/oxidative burst by flow cytometry

- **Clinical Symptoms may be useful**
- Hypoglycemia – GSD 1b
- Thrombocytopenia and eczema – WAS
- Albinism – Hermansky-Pudlak syndrome
- Ectodermal dysplasia, defective NK function – NEMO
- Recurrent abscesses and pulmonary infections – CGD

Diagnosis can only be made with a high index of suspicion and appropriate genetic testing.

What causes VEO-IBD?

- Complex Disease
  - Environmental Factors
  - Gut Bacteria
  - Abnormal Immune Response
  - Genetics
- VEO-IBD Patients
  - Generally thought that genetics play a more important role in VEO-IBD



Clinical Features of VEO-IBD

- VEO-IBD
  - Colon involved
    - 80% < 10 years of age
    - Decreases with age
  - Ileum involved
    - Rare at < 10 yrs of age
  - Positive FH – 40-50%
  - Stricturing – 20-46%
  - Surgery – up to 71%
  - Extension of disease – up to 40%
- Adult IBD
  - Colon only involved
    - < 20%
  - Ileum involved
    - Up to 80%
  - Positive FH – 14-20%
  - Stricturing – 29-40%
  - Surgery – up to 55%
  - Extension – up to 16%

Sherlock et al (unpublished); Griffiths (2004) Best Pract Res Clin; Heyman et al (2005) J Ped; Ruemmele et al (2006) JPGN; Kappelman et al (2008) IBD; Louis et al (2008) Gastro, van Limbergen et al (2008) Gastro, Vernier-Massouille (2008) Gastro

How do we treat VEO-IBD?

- No treatment guidelines available.
- Few small case studies.

* $P < 0.05$	Mild CD		Moderate-Severe CD		Mild UC		Moderate-Severe UC	
Age group (yrs)	<10	≥10	<10	≥10	<10	≥10	<10	≥10
Steroids (%)	28.2	35.9	74.6	73.6	43.5*	17.1*	53.1*	74.3*
5-ASA (%)	61.5	66.3	34.9	39.4	82.6	71.4	62.5	51.4
IM (%)	15.4	12.0	20.6	24.2	0	2.9	6.3	9.5
IFX (%)	0	0	4.8	2.6	0	0	3.0	0

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- Disease in very young is often severe at diagnosis.
- Cautious about surgery because extensive colonic disease has a tendency to extend to small bowel CD.
- Treatment may ultimately be based on genetic defects found in this patient group.

Markowitz et al (2006) JPGN, Pediatric IBD Collaborative Research Network

Incidence of Pediatric IBD in Canada

	USA (WI)[1]	UK[2]	Norway[3]	Netherlands[4]	BC, Canada[5]	NS, Canada[5]	ON, Canada[6]
Years of Study	2000-01	1998-99	1993-2004	2002-03	1998-2000	1998-2000	1994-2005
Sample Size	199	739	48	188	473 (prevalence)	473 (prevalence)	3169
Age (years)	<18	<16	<16	<18	<20	<20	<18
Inc CD (per 100k)	**4.6**	**3.0**	**2.8**	**2.1 – 4.1**	**5.4**	**12.0**	**6.2 – 7.0**
Inc UC (per 100k)	**2.4**	**2.2**	**2.8**	**1.6 – 2.6**	**3.2**	**5.7**	**4.4 – 4.8**

A 30% increase in incidence over a decade

Benchimol et al (2009) Gut 48:1490-97

[1]Kugathasan *et al.*, J Pediatr, 2003
[2]Sawczenko & Sandhu, Arch Dis Child, 2003
[3]Henriksen *et al.*, Inflamm Bowel Dis, 2006
[4]van der Zaag-Loonen, J Pediatr Gastroenterol, 2004
[5]Bernstein *et al.*, Am J Gastroenterol, 2006
[6]OCCC

Changes in IBD Incidence Rates By Age

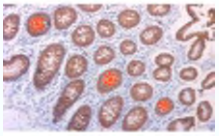
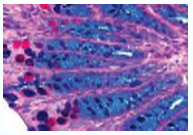
AGE	Change in Incidence Rate	95% CI	P-value*
0-4	+5.0% / year	0.5% - 10.5%	**0.032**
5-9	+7.6% / year	4.4% - 10.8%	**<0.0001**
10-14	+0.63% / year	-0.9% – 2%	**0.407**
15-17	-0.21% / year	-1.3% – 0.9%	**0.72**

*** By Poisson regression analysis, controlling for sex**

In Canada, VEO-IBD has an incidence of 4.37/100,000 and a prevalence of 14/100,000 (~ 0.3/2000) making it the highest in the world.

Benchimol et al (2009) Gut 48:1490-97; Benchimol et al (2011) IBD; Heyman et al (2005) J Ped; Ruemmele et al (2006) JPGN; Kappelman et al (2008) IBD

NEOPICS: interNational Early Onset Pediatric IBD Cohort Study

NEOPICS

NEOPICS

Mission Statement:

The interNational Early Onset Paediatric IBD Cohort Study (NEOPICS) brings together international Pediatric Gastroenterologists and Scientists from academic centers across the globe to work together to identify and investigate the causes, and develop new treatments for very young children and infants with IBD.

SickKids

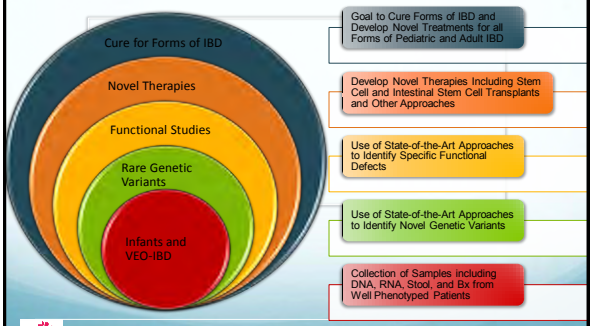
Boston Children's Hospital Early Childhood IBD Initiative

Care-for-Rare FOUNDATION

NEOPICS

- NEOPICS – expanded to 51 Centers on 5 continents with access to over 1000 VEO-IBD patients

NEOPICS: interNational Early Onset Pediatric IBD Cohort Study



Cases – Personalized Medicine Approach to VEO-IBD

NEOPICS

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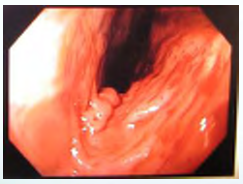
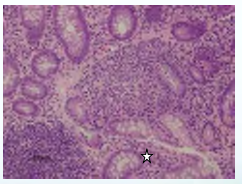
Case 1 – IL10R Pathway

- Patient BP – presented as infant
- 10 days of age presented with fever – full septic work-up
- All cultures negative treated with antibiotics
- During the first year of life – numerous febrile episodes requiring hospital admission
- Complete immunological work-up was negative including a normal 46 XX karyotype
- Screening for mutations in *JAK3*, *IPEX*, *RMRP*, *IL2RA*, *IRAK4*, and *TRAPS* genes as well as genetic mutations associated with chronic granulomatous disease was negative
- No Family history of IBD, parents not consanguineous

 Moran et al. IBD, 2012.

Case 1 – IL10R Pathway – Severe Pancolitis





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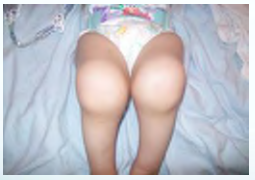
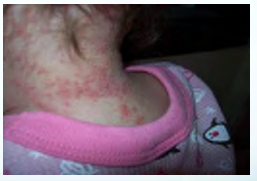
Case 1 – IL10R Pathway –Perianal Disease

Severe perianal disease with multiple fistulas

Case 1 – IL10R Pathway Extra-GI Manifestation of IL10R defects



Joint effusions

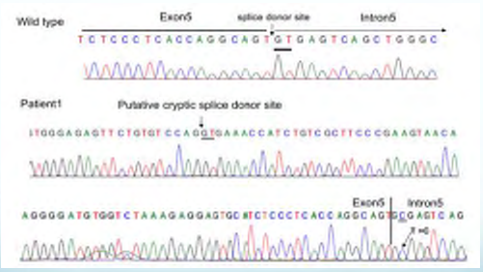


Folliculitis

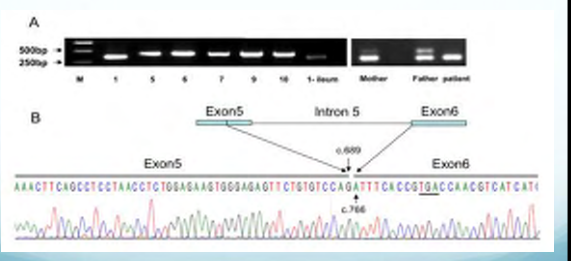
Case 1 – IL10R Pathway

- Antibiotics, intravenous immunoglobulins, steroids, anakinra, sulfasalazine and azathioprine were unsuccessful.
- Required diversion ostomy – issues with poor wound healing.
- Weekly Joint injections.

Identification of the IL10RA Mutation



Novel IL10RA Truncated Transcript

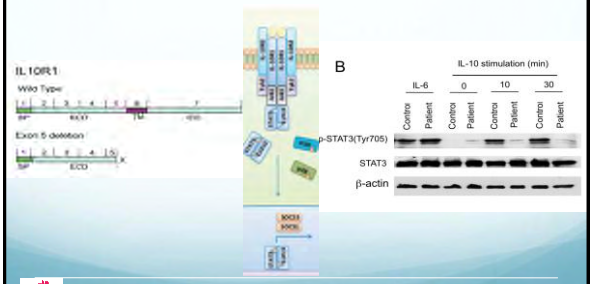


Case 1 – IL10R Pathway

- IL10 restricts excessive immune responses
- Secreted by wide variety of cells and inhibits the secretion of pro-inflammatory cytokines - TNFα, IL12, and IFNγ
- Receptor IL10R has two subunits:
  - Alpha (A / 1) – required for IL10
  - Beta (B / 2) – required for IL10, -22, -26
- Acts through JAK1, TYK2, and STAT3 (all identified in IBD GWAS)
- Defects in IL10R A/B and IL10 have been identified

Glocker et al (2009) NEJM; Glocker et al (2010) Lancet

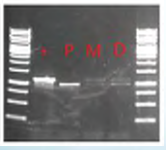
Functional Analysis of Putative IL10R1 Truncated Protein

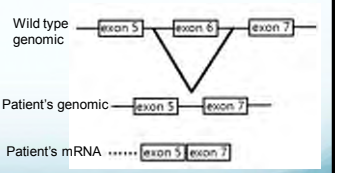


Moran et al. IBD, 2012.

Children National Medical Center Dr. Conklin

- Patient: 18 mos boy with infantile colitis, severe perianal disease requiring a diversion
- Poor would healing, not responsive to biologic treatments





unpublished

Case 1 – IL10R Pathway – Extension to VEO-IBD

- Collaboration with Scott Snapper (Harvard)
- Exon sequencing of 188 patients (from SickKids and Wisconsin) with early-onset IBD (EO-IBD < 18 yo) and 188 healthy controls
- Found at least 25 rare/novel polymorphisms in the EO-IBD and especially VEO-IBD population
- Two SNPs associated with VEO-UC – replicated in two independent cohorts (rs2228054 and rs2228055 - OR 3.08, P= 1.42 x 10^{-4}; and OR 2.93, P= 1.4 x 10^{-4} , respectively)
- Points to a broader role for the IL10R Pathway in the pathogenesis of VEO-IBD

Moran et al. IBD, 2012.

IL10/R Deficiency

- We have identified 2 more patients (3/20)
  - Gaza (Dr. Shteyer) – Homozygote IL10RA deletion exon 1-3
  - Washington DC (Dr. Conklin) – Homozygote IL10RB deletion resulting in a 224X truncation
- Kotlarz et al (Gastro 2012) – 16/66 very early onset IBD patients screened had IL10/R deficiency
- Engelhardt et al (JACI in press; includes one additional NEOPICS patient) – 9/40 very early onset IBD patients screened had IL10/R deficiency
- 9/31 patients worldwide have successful BMT

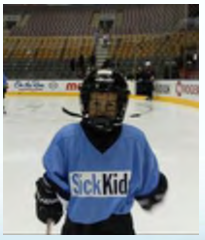
Case 1 – IL10R Pathway Lessons

- IL10R deficiency should be considered in all infants with severe colitis, perianal disease, folliculitis, +/- joint disease
- All patients so far (> 30) have had symptoms < 3 months of age
- IL10 deficiency should be considered in all infants with severe colitis +/- perianal disease that is non-responsive to standard IBD therapy
- Bone Marrow Transplant is required if a IL10/IL10R defect is identified
- A subset of VEO-IBD patients may have subtle defects in the IL10R pathway – candidates for treatment with IL10?

Case 1 – IL10R Pathway – An Update

- Had successful BMT (Dr. Roifman at SickKids)
- Doing very well – colitis and other auto-inflammatory issues now resolved

Case 2: Defective ROS Production



Case 2: Defective ROS Production

- Two months of age:
  - Blood in stool
  - Diagnosed - cow's milk protein allergy
- Blood in stool persisted - diagnosed < 1 yo with Crohn's colitis
- Developed perianal and small bowel disease < 2 years of age
- Has abnormal low normal ROS production.
- Does not have evidence of chronic infections or immunodeficiency
- No Family history of IBD, parents not known to be consanguineous

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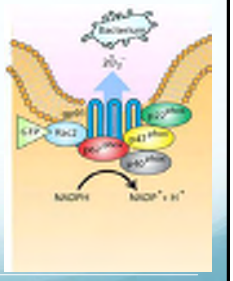
Muise, et al, Gut, 2011

Defects in NADPH oxidase Genes Results in CGD

- Chronic Granulomatous disease (CGD) is a genetic immunodeficiency – defects in NADPH oxidase complex genes
- Phagocytes are unable to kill certain bacteria and fungi as a result of reduced production of superoxide and hydrogen peroxide – reactive oxygen species (ROS)
- Patients with CGD develop:
  - recurrent and life-threatening infections
  - granulomatous inflammation of hollow viscera

NADPH Oxidase genes and CGD

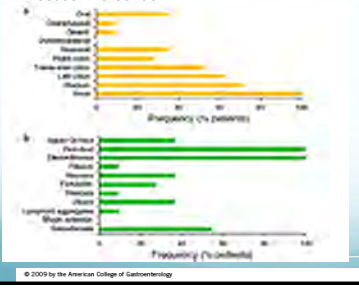
Gene	Inheritance	Frequency
CYBB: gp91phox	X-Linked Recessive	~65%
CYBA: p22phox	Autosomal Recessive	<5%
NCF1: p47phox	Autosomal Recessive	~25%
NCF2: p67phox	Autosomal Recessive	~5%
NCF4: p40phox	Autosomal Recessive	< 1%

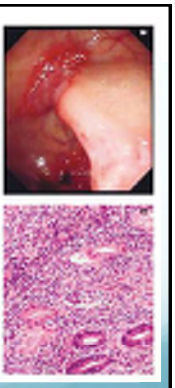


Muise, et al, Gut, 2011

CGD and IBD

- 40% of CGD Patients have Crohn's Disease-like Colitis



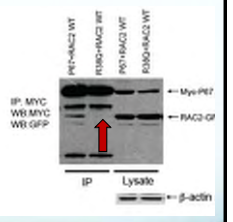


NADPH oxidase and IBD

- We hypothesize that defects in the NADPH oxidase pathway genes that do not cause CGD may still result in susceptibility to VEO-IBD
- Carried who directed exon sequencing of these genes in patients with very early onset IBD and low-normal ROS production to determine if this pathway played an important role in the pathogenesis of disease

Case 2: p67^{phox} R38Q

- Identified a novel *NCF2* variant - (c.113 G/A)
- Variant resulted in p67^{phox} R38Q
- Examined this mutation – 2 VEO-IBD cohorts
- **4% of VEO-IBD patients (11/268)**
- 0.3% of older IBD patients (1/330)
- 0.2% of healthy controls (1/480)
- $P < 1 \times 10^{-5}$, OR = 24 (3 – 142) (Fischer Exact Test)



NADPH oxidase Genes and VEO-IBD

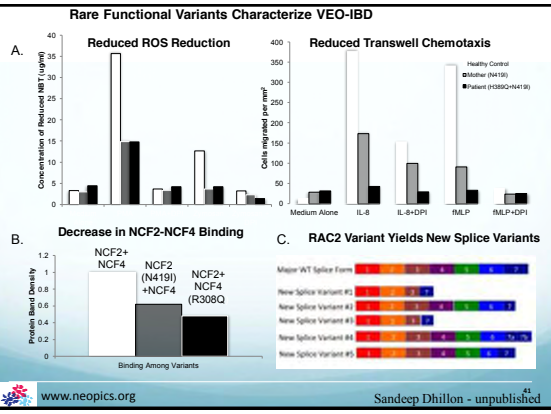
- Ongoing NADPH oxidase Studies.
  - Sequenced NADPH oxidase genes – 314 VEO-IBD patients compared to 4300 controls
  - Found novel and rare mutations in VEO-IBD patients - (replicated and validated)

SNP	Gene	Association	Combined p Value	OR (95% C.I.)
rs10951982	RAC1	VEO-IBD (Dominant)	**$2.12x10^{-3}$**	0.7 (0.57-0.9)
rs1476002	RAC2	VEO-IBD (Recessive) VEO-UC (Recessive)	**$2.16x10^{-4}$** **$7.77x10^{-7}$**	3.9 (1.8-8.6) 7.0 (2.9-17.0)
rs739041	RAC2	VEO-IBD (Additive)	**$5.34x10^{-3}$**	0.8 (0.6-0.9)
rs1883113	NCF4	VEO-IBD (Recessive)	**$7.65x10^{-3}$**	6.0 (1.3-27.0)

Novel and Rare SNPs

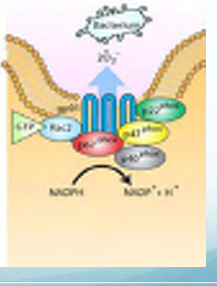
Gene	SNP	Protein Domain	Population Frequency	# of Patients (N=122)	Function
CYBB	G364R rs141756032	Ferric Reductase	0.005%* (Males)	1	Predicted Damaging
NCF1	**R90H rs13447**	**PX Domain**	**5.5%** (Hetero.)**	**12**	**Reduces ROS Production**
NCF2	G501R	SH3 Domain	0*	1	Predicted Damaging
NCF2	P454S rs55761650		0.005%*	1	Strong ESE Lost
NCF4	**R308Q**	**PB1**	**$8x10^{-5}$***	**1**	**Predicted Damaging**
RAC2	**Non-coding**	**Intron 5**	**0***	**1**	**hnRNP A1 site gained**

*Wash. U. Exome Variant Server, ESP **Olsson et al., Antioxid Redox Signal, 2012



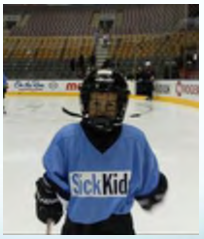
NADPH Oxidase genes and CGD

- > 35% of VEO-IBD patients have variants/mutations in the components of the NADPH oxidase complex compared to < 15% in controls
- **P = 8.90 x 10^{-24}, OR = 3.305 (95% CI - 2.569-4.250)**
- Variants/mutations predicted to cause disease – have functional consequences
- All involved in localization of the complex to the membrane



Lessons from Case 2

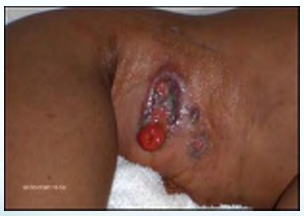
- Patient now 4 yo – treated with 5-ASA and antibiotics during flares – maintained good growth and symptom control
- Many VEO-IBD patient may have defects in ROS production
- May point to alternate therapies which have been shown to drive the non-infectious inflammation in CGD patients
  - Antibiotics
  - IL1R targeting - anakinra – IL1RA
  - GMCSF?

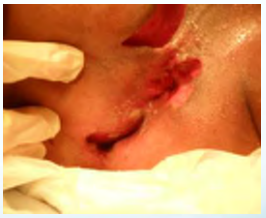


Case 3: Novel Approach to Understanding VEO-IBD Patients

- Family from Australia (Consanguineous Parents) – Drs. Lee and Stormon
- Colitis < 3 mos of age (negative for IL10R or IL10 Mutations)
- Severe perianal disease – did not respond to diversion or anti-TNFα
- Most likely to be the result of single gene mutation
- Therefore carried out whole exome sequencing (WES)

Case 3: Novel Approach to Understanding VEO-IBD Patients

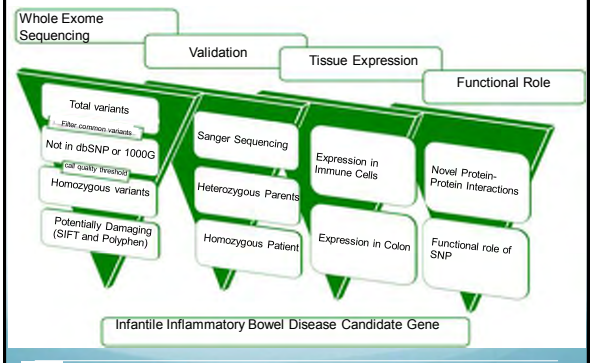




- Whole Exome Sequencing
- RNA Sequencing

Whole Exome Sequencing

- The "exome" - all the exons in the human genome (i.e. the transcribed region of the genome)
- Exons
  - short and functionally important sequences of DNA
  - represent the regions in genes that are translated into protein and untranslated region flanking them (UTR)
- 180,000 exons found in the human genome.
- Protein coding regions constitute about 1% of the human genome
- Translates to about 30 megabases (Mb) in length
- Protein coding regions - 85% of the disease-causing mutations
- Pitfalls – up to 30% error in sequencing



Preliminary Results - WES

Table 1 Categorization of variants

	Total (1000G and dbSNP)/novel count
High confindence variants	**38256 / 2839**
Genetic variants(variants within genes)	**33465/2113**
Insertions	**32/3**
Deletions	**47/8**
Other (substitutions, frame shift, splice site, stop, etc.)	**33386/2069**
Protein coding variants	**15590/938**
Insertions	**32/3**
Deletions	
87/35	**47/7**
Other (substitutions, frame shift, splice site, stop, etc.)	**15511/928**
Nonsynonymous variants	**7477/644**
Heterzygous	**3756/482**
Homozygous	**3721/162**
Homozygous patient with 2 heterozygous parents	**15**

Preliminary Results – Whole Genome Sequencing

- Validated – homozygote mutation in a candidate gene – predicted damaging
- Found mutations in candidate gene in patients from
  - Gaza (homozygous) - (Dr. Shteyer)
  - Montreal (compound heterozygote) - (Dr. Deslandres)
  - Toronto (compound heterozygote)- (Dr. Griffiths)
- All 4 patient have severe infantile onset colitis
- All have severe perianal disease non-responsive to surgical or medical therapy
- Functional studies are underway

Lucas Mastropaolo - unpublished

Final Conclusions

- Diagnosing children under the age of 10 years (VEO-IBD) is becoming more common
- These children most likely will have extensive colonic disease
- VEO-CD patients may have extension of colonic disease to ileal and perianal disease overtime
- Treatment algorithms are required for this ever increasing patient population to determine optimal therapy for long term outcomes such as growth and development

Final Conclusions

- Very young children and infants with IBD most likely have a genetic form of the disease that can be identified using current available genetic approaches
- Understanding the disease in this age group will lead to novel therapeutic approaches
  - In some cases bone marrow transplant
  - IL10, IL1RA, GMCSF….
  - And can lead to novel treatment strategies for all IBD patients

The Lab

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  - Sandeep Dhillon – CGD genes in VEO-IBD
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  - Athos Bousvaros
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- Or visit **www.NEOPICS.org**


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