Association of rare variants in genes of immune regulation with pediatric autoimmune CNS diseases

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Disclosures

- Drs. Jafarpour, Banerjee, Ahsan, and Mitchell report no conflicts of interest.
- Ms. Paulsen reports no conflicts of interest
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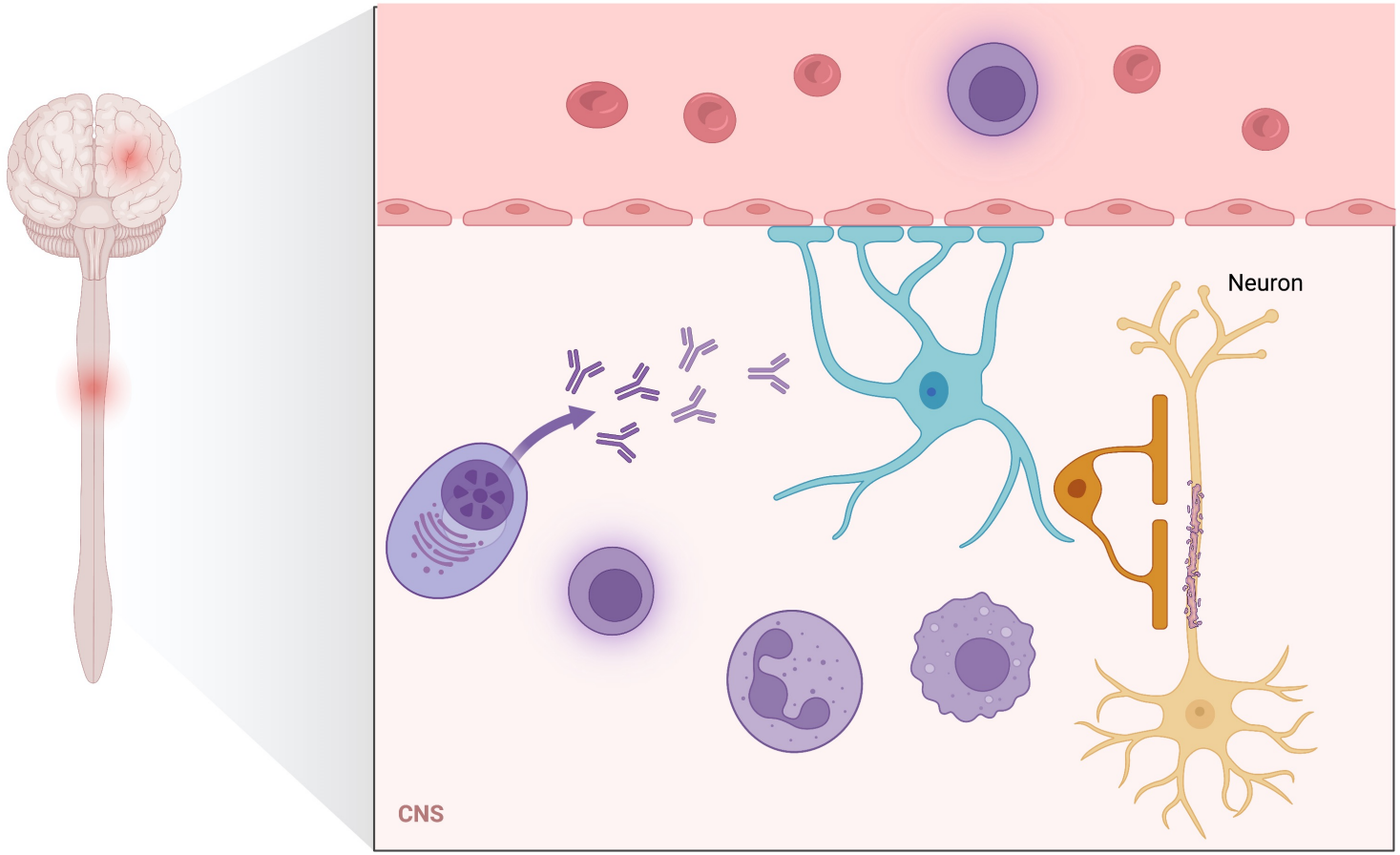
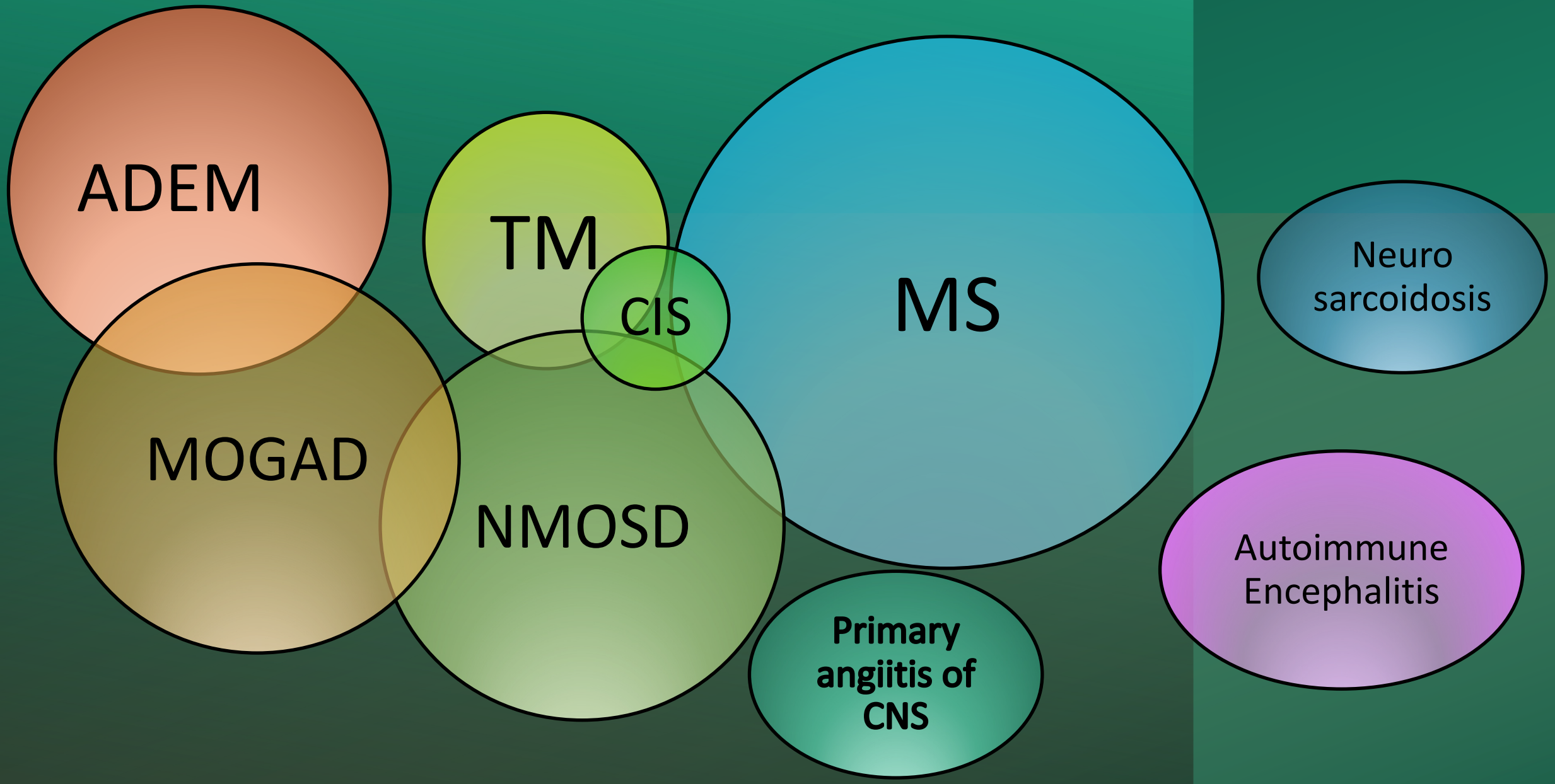
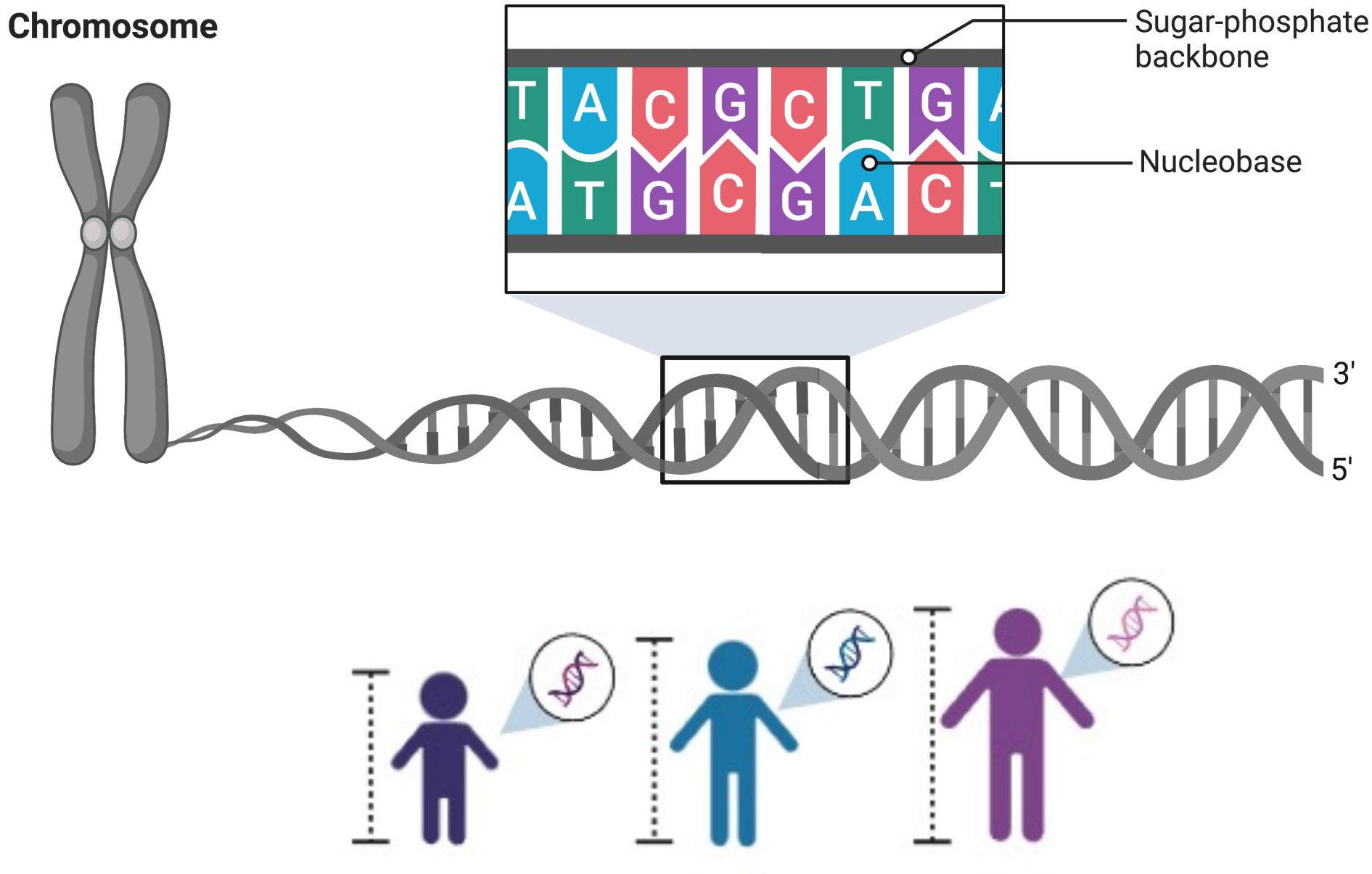
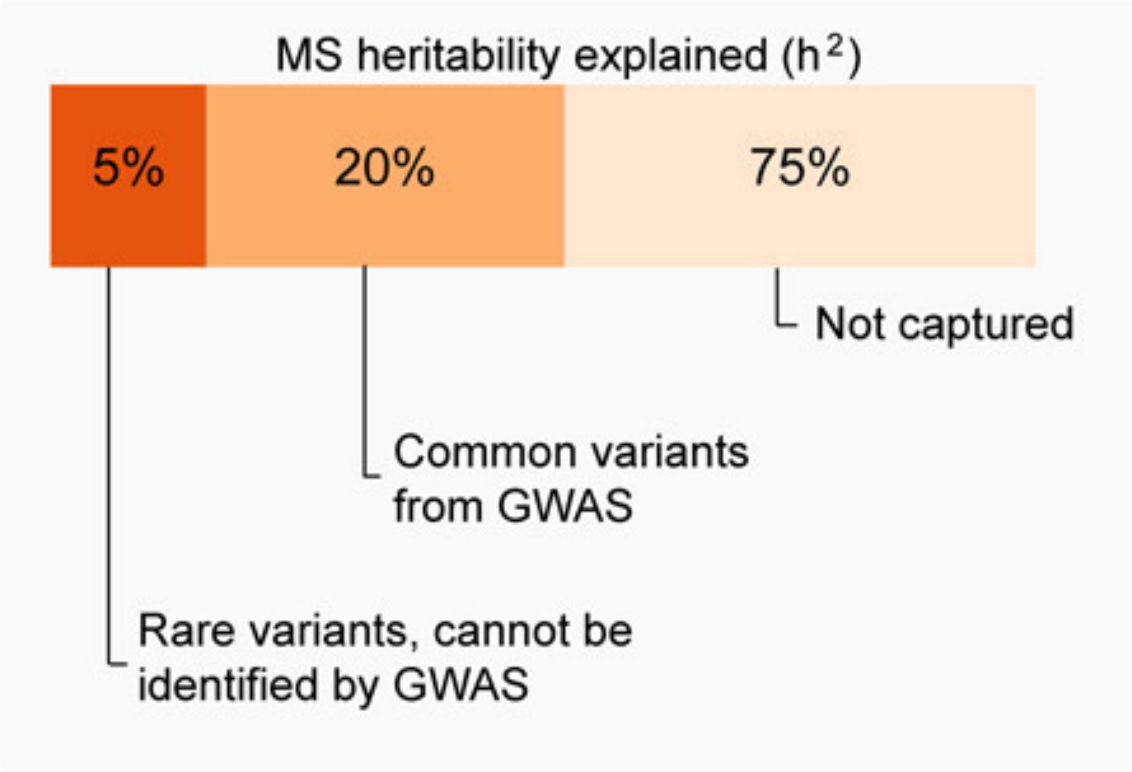
Autoimmune CNS Disorders



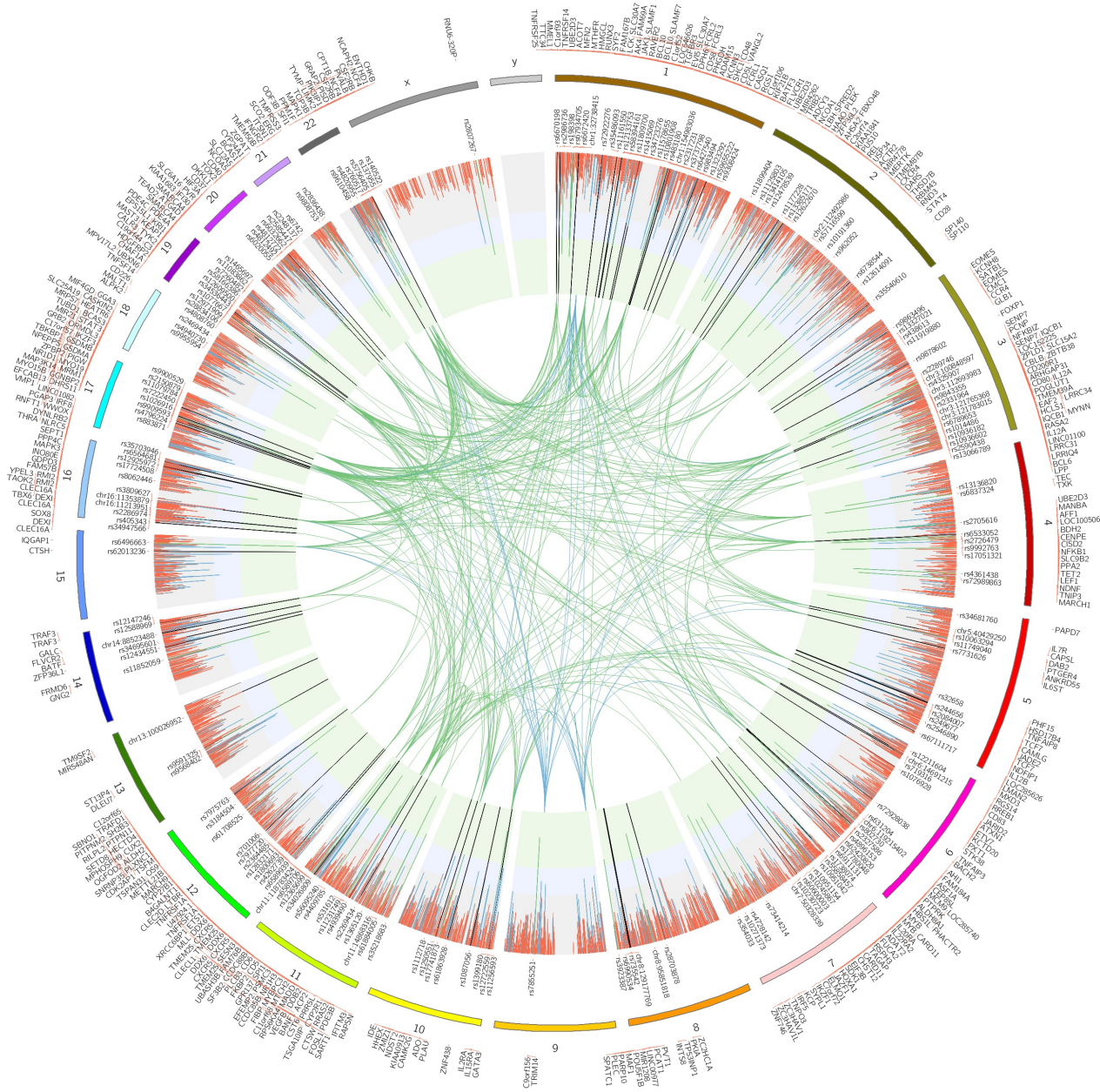

ADEM
TM
CIS
MS
Neuro sarcoidosis
MOGAD
NMOSD
Autoimmune Encephalitis
Primary angiitis of CNS


Chromosome
Sugar-phosphate backbone
Nucleobase
T A C G C T G
A T G C G A C
3'
5'

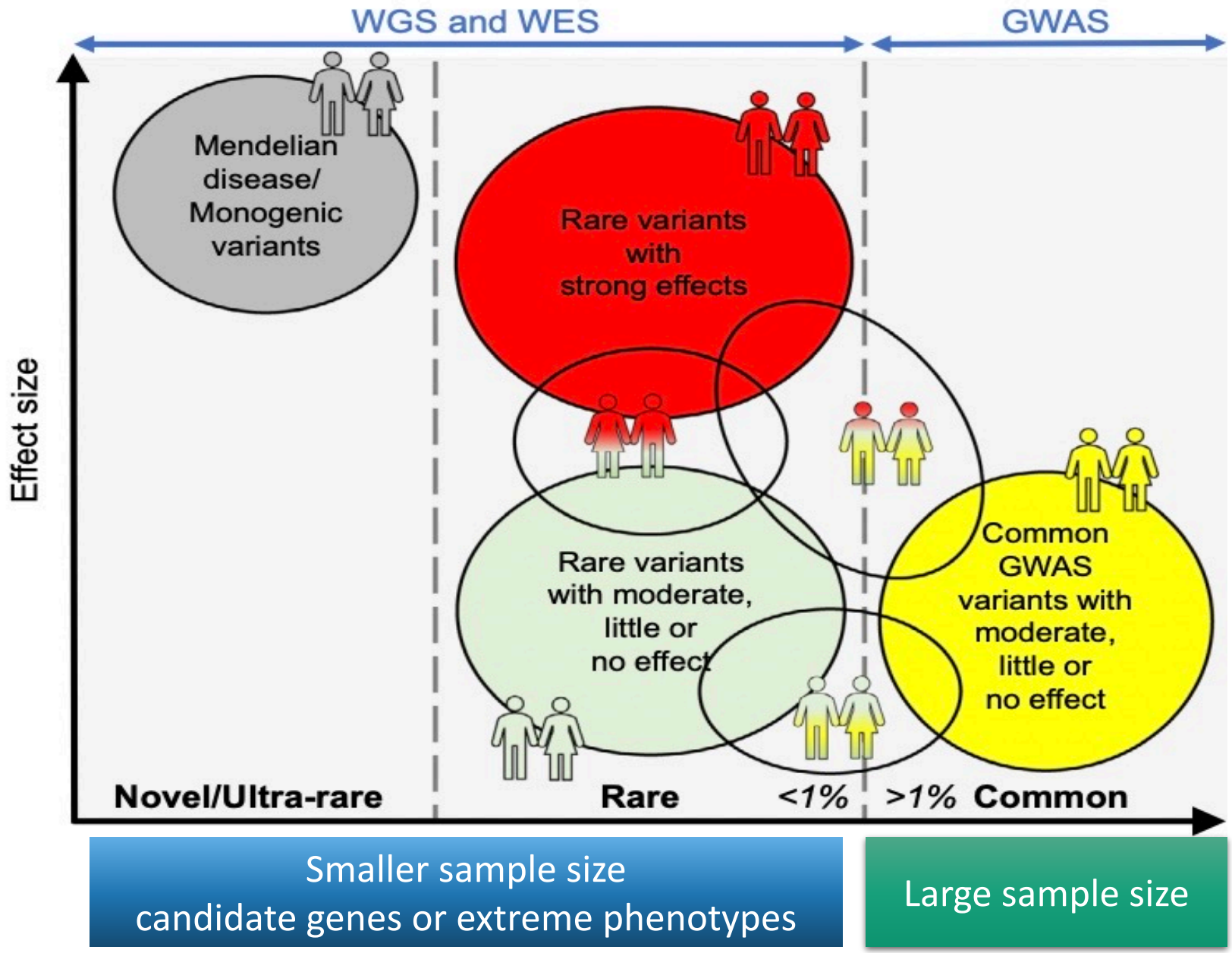
Genetics of autoimmune CNS disorders



Mitrovič M, et al, *Cell,* 2018



International Multiple Sclerosis Genetics, et al, *bioRxiv,* 2017:143933



Jiang SH, et al, *Immunology & Cell Biology*, 2020

Are rare variants of immune regulation genes associated with pediatric autoimmune and inflammatory CNS disorders?

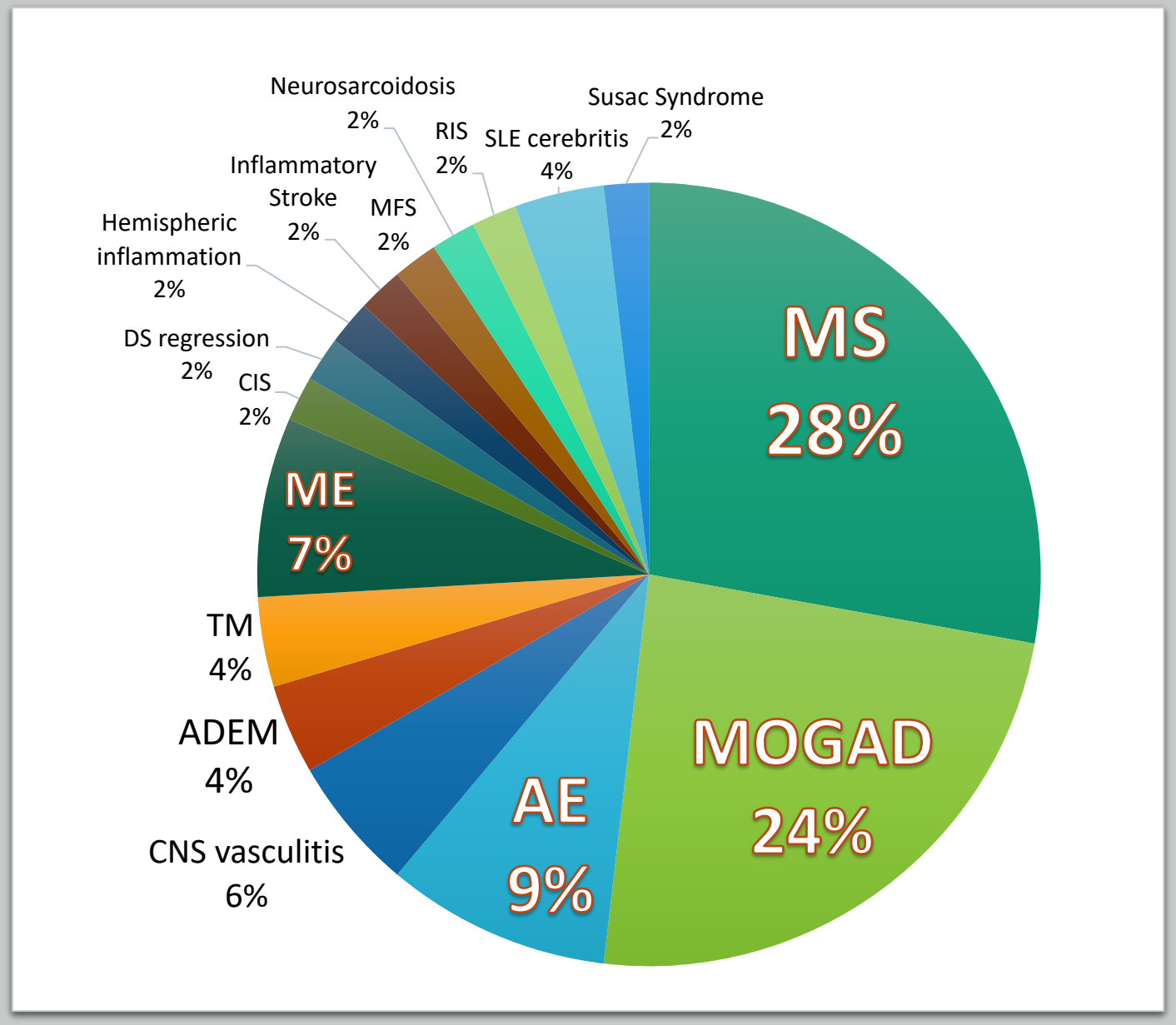
Characteristics

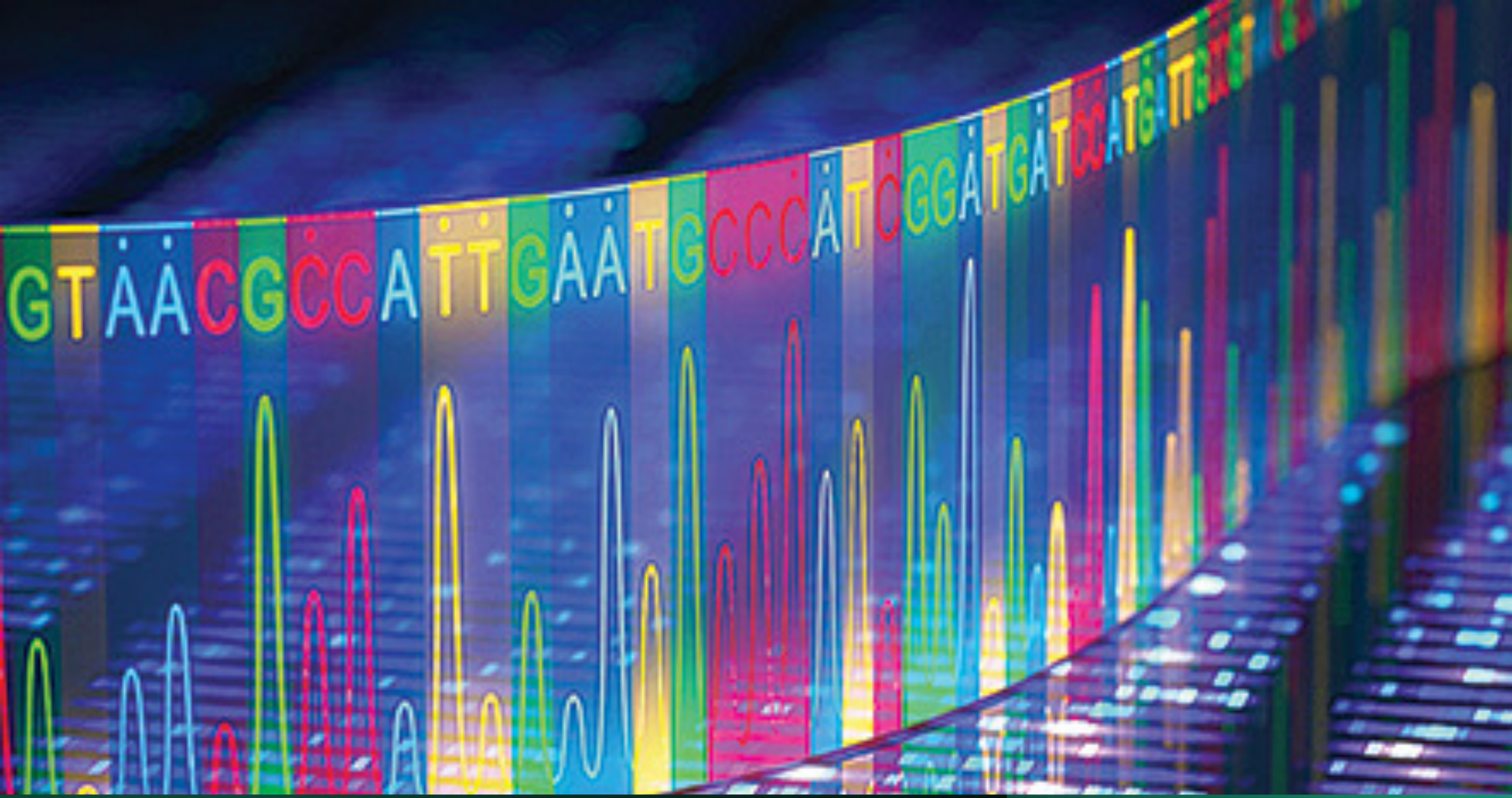
N=54

Age 13.4 ± 5.3 yr

55.6% Female

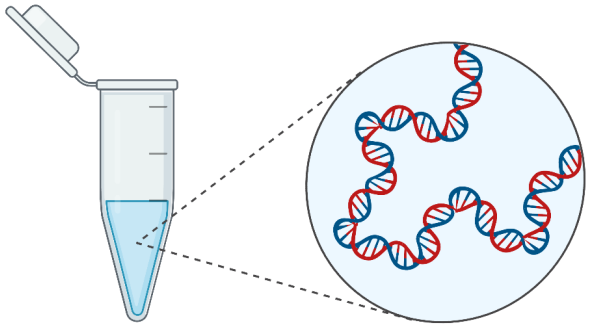
50% Hispanic/Latino



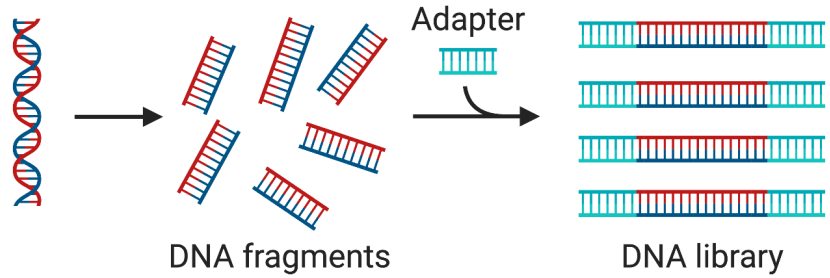

GTAACGCCATTGAATGCCCATCGGATGATCCATG

Next Generation Sequencing Workflow

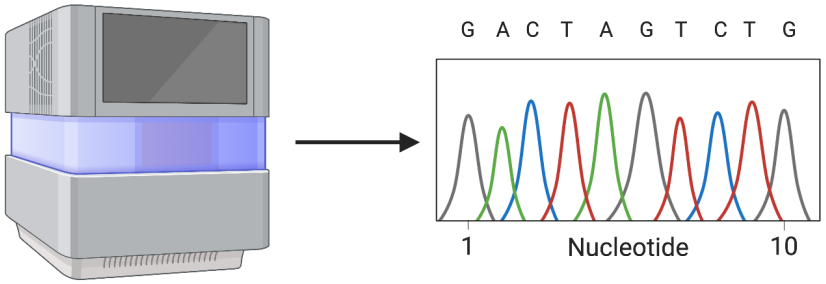
Step 1:
DNA extraction



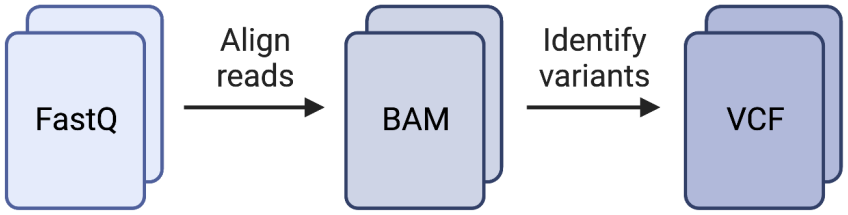
Step 2:
Library preparation



Step 3:
Sequencing

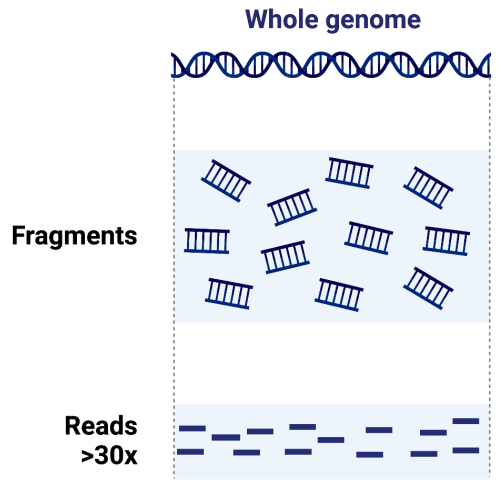


Step 4:
Analysis



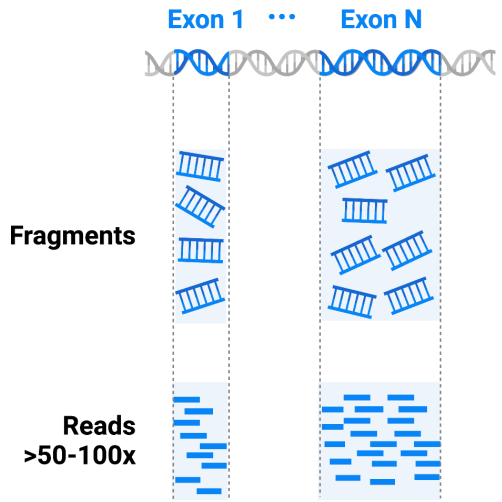
Next Generation Sequencing

Genome Sequencing



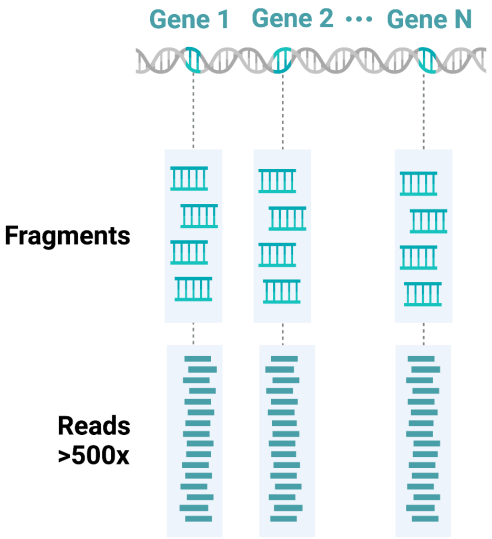
Coverage: All genes and non-coding DNA
Acuracy: Low
Time: Longest turnaround time
Cost: Most expensive
Depth: >30X

Exome Sequencing



Coverage: Entire exome (20-25k genes)
Acuracy: Good
Time: Long turnaround time
Cost: Cost-effective
Depth: >50-100X

Targeted Gene Panel

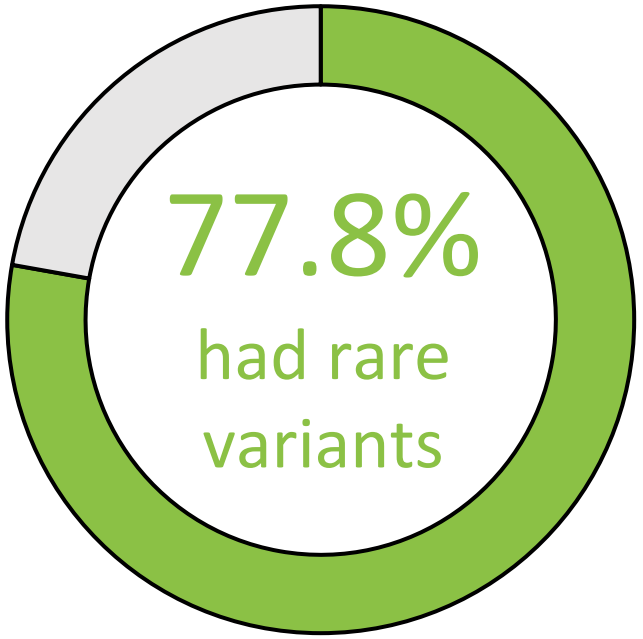


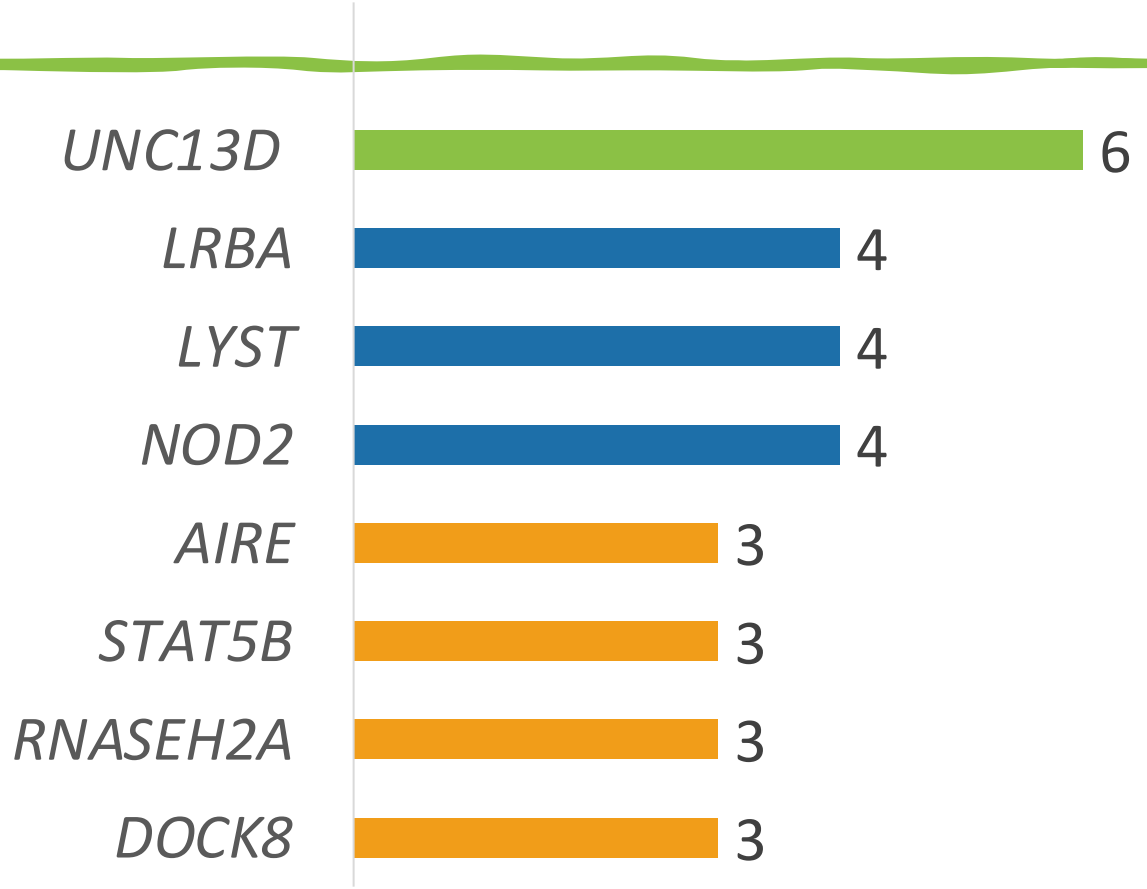
Coverage: 10-500 genes
Acuracy: High
Time: Rapid turnaround time (few days)
Cost: Most cost-effective
Depth: >500X

Template adapted from: *Dr. Roshini Abraham*
Clinical Immunologist at Nationwide Children's Hospital

Identification

Identification





- 88 unique variants of 55 genes
- 2 increased risk, the rest VUS
- 90% ExAC AF <0.1%, , 45% ≤0.01%

Table 1. Genes harboring variants categorized by clinical diagnosis	
Diagnosis (Phenotype)	**Gene**
ADEM	*ADAR, AIRE, DEF6, ITGB2, NOD2*
Autoimmune Encephalitis	*AIRE, IL21R, RNASEH2A, STAT1, TNFRSF1A, XIAP*
CIS	*CYBA*
CNS vasculitis	*DOCK8, IL21, SLC7A7, UNC13D*
Down Syndrome Regression Disorder	*CTLA4, IRF7, LYST, SMARCAL1*
Hemispheric Inflammation	*RBCK1, UNC13D*
Meningoencephalitis of Unknown Etiology	*CARD14, CYBA, DOCK8, PLCG2, PSTPIP1, RMRP, STAT5B, TNFRSF13B, TNFSF12, TREX1*
MOGAD	*ACP5, ADA2, AIRE, CTLA4, IFIH1, LRBA, MEFV, NOD2, RAG1, RBCK1, STAT5B, STIM1, STXBP2, TNFRSF13B, UNC13D, ZAP70*
MS	*ACP5, ADAM17, BACH2, CARD14, DOCK8, DUOX2, G6PC3, IL10, IL1RN, LRBA, LYST, NLRC4, NOD2, ORAI1, RAB27A, RFXANK, RMRP, SH3BP2, STAT5B, STIM1, TBX1, TNFAIP3, UNC13D*
Neuropychiatric SLE	*SLC29A3*
Neurosarcoidosis	*RNASEH2A*
Inflammatory Stroke	*NOD2, RTEL1*
RIS	*NOD2, TTC7A*
SLE Cerebritis	*CARD8, LYST, NOD2*
Susac Syndrome	*DCLRE1C*
Transverse Myelitis	*IFIH1, RNASEH2A*

Functional Effect

Combined Annotation Dependent Depletion (CADD)

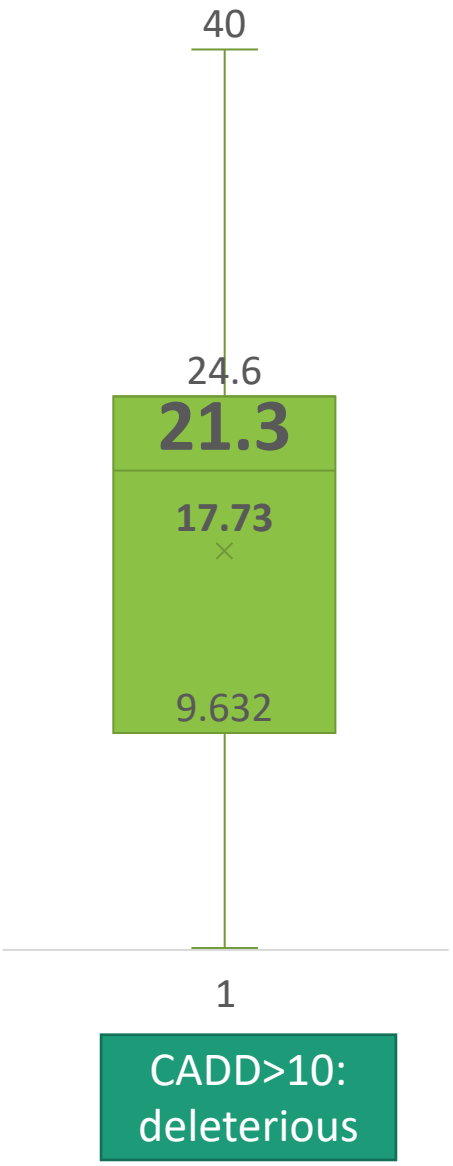


Table 2. Variants that were predicated detrimental based on in silico predictions.

Gene	Variant	ExAC AF	PolyPhen-2	SIFT	Conservation	CADD
ACP5	c.249C>G (p.Asp83Glu)	0.0001	Probably damaging	Deleterious	High	**24.3**
ADAR	c.577C>G (p.Pro193Ala)	0.003	NA	NA	Mod	**23.5**
DEF6	c.1745T>A (p.Leu582Gln)	0.0001	Possibly damaging	Deleterious	High	**27.7**
LYST	c.2465C>T (p.Thr822Ile)	0.0003	Probably damaging	Deleterious	Mod	**26.4**
NLRC4	c.443G>T (p.Arg148Leu)	NA	Possibly damaging	Deleterious	High	**15.23**
NOD2	c.1295C>T (p.Ala432Val)	0.0002	Probably damaging	Deleterious	High	**16.34**
NOD2	c.2722G>C (p.Gly908Arg)	0.014	Probably damaging	Deleterious	Mod	**29.7**
RAB27A	c.543A>G (p.Ile181Met)	0.0001	Possibly damaging	Deleterious	High	**22.4**
RFXANK	c.661G>A (p.Asp221Asn)	NA	Possibly damaging	Deleterious	Mod	**NA**
RNASEH2A	c.871C>T (p.Arg291Cys)	0.00006	Probably damaging	Deleterious	High	**24.6**
RNASEH2A	c.101A>G (p.Asp34Gly)	0.001	Probably damaging	Deleterious	High	**27.5**
SLC7A7	c.187C>T (p.Leu63Phe)	NA	Probably damaging	Deleterious	High	**26**
TTC7A	c.563G>A (p.Arg188His)	0.0002	Probably damaging	Deleterious	Mod	**25.2**
UNC13D	c.652G>T (p.Gly218Trp)	0.00001	Possibly damaging	Deleterious	Mod	**26.1**
UNC13D	c.419T>C (p.Ile140Thr)	NA	Probably damaging	Deleterious	Mod	**25.9**
XIAP	c.844G>C (p.Glu282Gln)	NA	Probably damaging	Deleterious	High	**37**

Table 3. Variants that were predicated detrimental based on in silico predictions.

Gene	Associated immune dysregulation condition	Neurologic manifestations	Dx
ACP5	Spondyloenchondrodysplasia with immune dysregulation, monogenic SLE, Sjögren's syndrome, inflammatory myositis, Raynaud's disease, vitiligo	Childhood-onset spastic diplegia, developmental delay, calcification of the basal ganglia	MOGAD
ADAR	AGS 6, Dyschromatosis symmetrica hereditaria	AGS, Torsion dystonia, Bilateral striatal necrosis and spastic paraplegia	ADEM
IFIH1	AGS 7	AGS, rapid neuroregression, spastic-dystonic syndrome, spastic paraparesis	TM
LYST	Chediak-Higashi syndrome	Learning difficulties, cerebellar deficits, polyneuropathies, spasticity, cognitive decline, and parkinsonism	MS
NOD2	Increased risk Crohn's disease, granulomatous diseases (Blau syndrome, early onset sarcoidosis)	Rasmussen syndrome with CNS granulomatosis, Multiple system atrophy	MS, RIS, IS
RAB27A	Griscelli syndrome type 2 (affecting skin, hair, immune system)	Developmental regression, seizure	MS
RNASEH2A	AGS4	AGS, Developmental delay, intellectual disability, seizures and epileptic encephalopathy	AE, Neurosarcoidosis
STXBP2	FHL5	Neuro HLH	MOGAD
TNFAIP3	Familial Behcet-like autoinflammatory syndrome	Relapse biomarker in MOGAD, Neuropsychiatric SLE, granulomatous neuroinflammatory disorder, NMO	MS
TREX1	AGS1, susceptibility to SLE, Retinal vasculopathy with cerebral leukodystrophy	AGS, white matter ring-enhancing lesions, stroke	CNS vasculitis
TTC7A	Gastrointestinal defects and immunodeficiency syndrome	Perisylvian polymicrogyria, cerebellar hypoplasia and arthrogryposis, severe microcephaly, refractory epilepsy, developmental delay, hypomyelinating leukodystrophy	RIS
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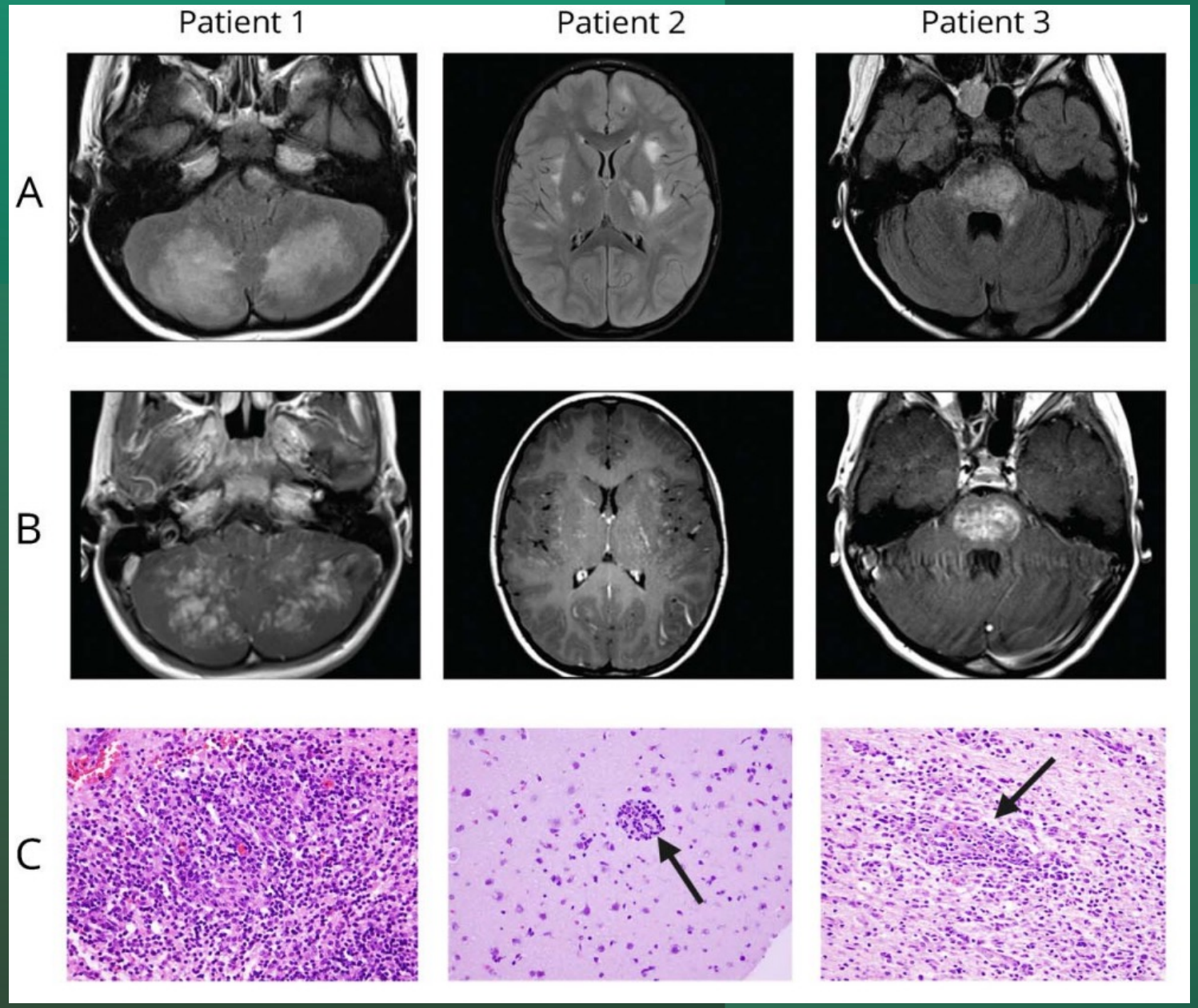
Familial Hemophagocytic Lymphohistiocytosis



Benson et al, *Neurol Neuroimmunol Neuroinflamm*, 2019

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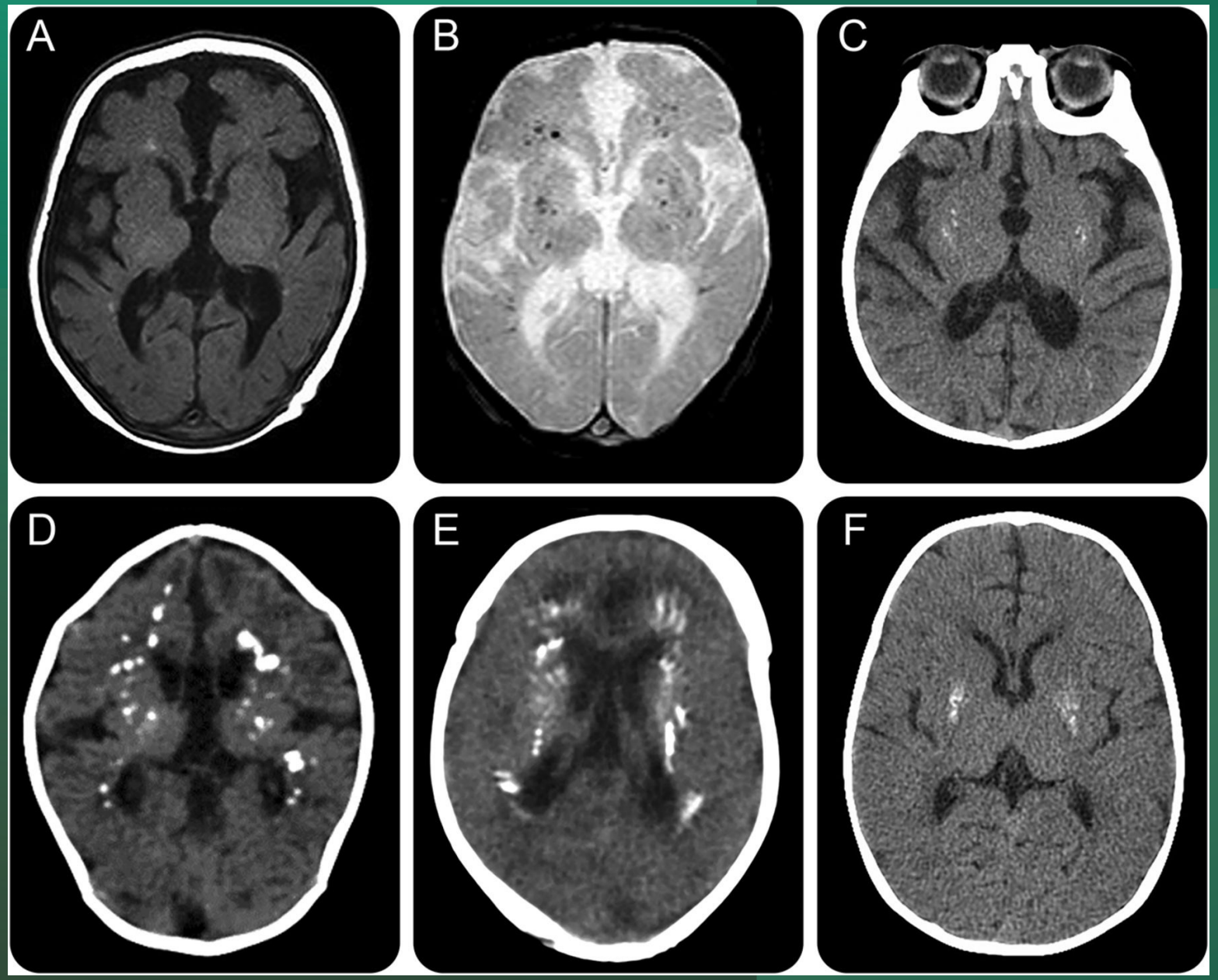
Aicardi-Goutières Syndrome



La Piana, et al, *Neurology*, 2016

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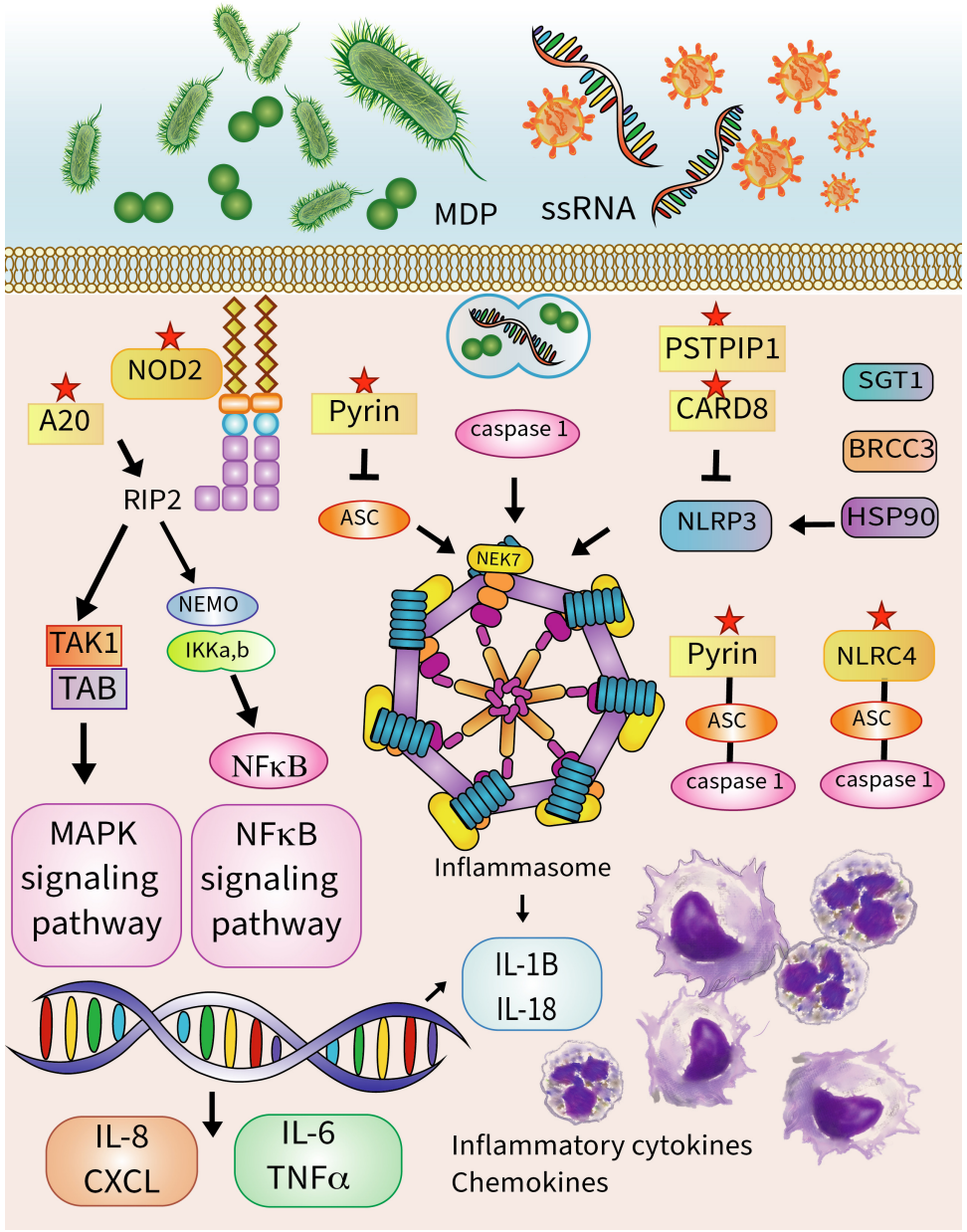
Pathways

NOD-like signaling pathway



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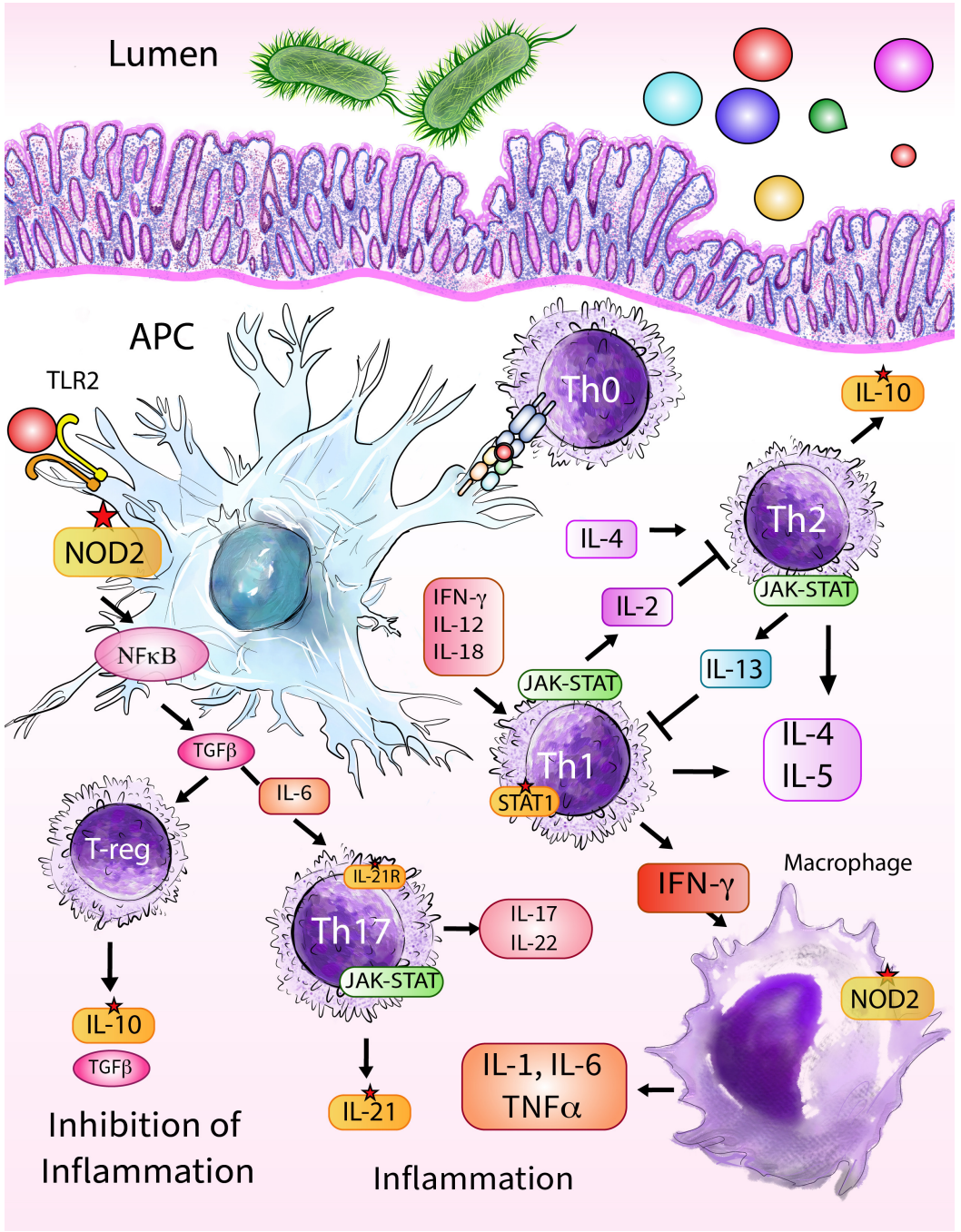
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IL-18
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Th1
STAT1
IL-4
IL-5
IL-2
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IL-10
IL-4
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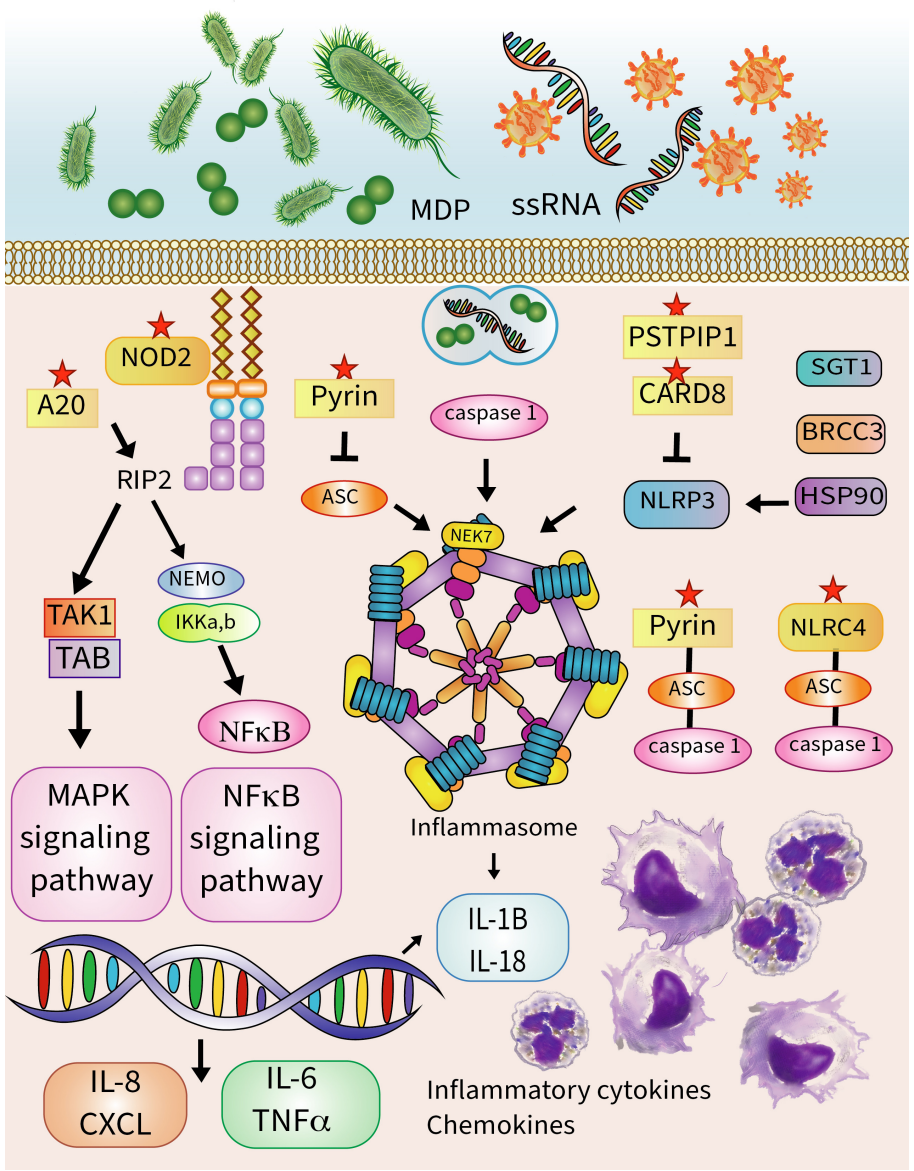
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caspase 1
NEK7
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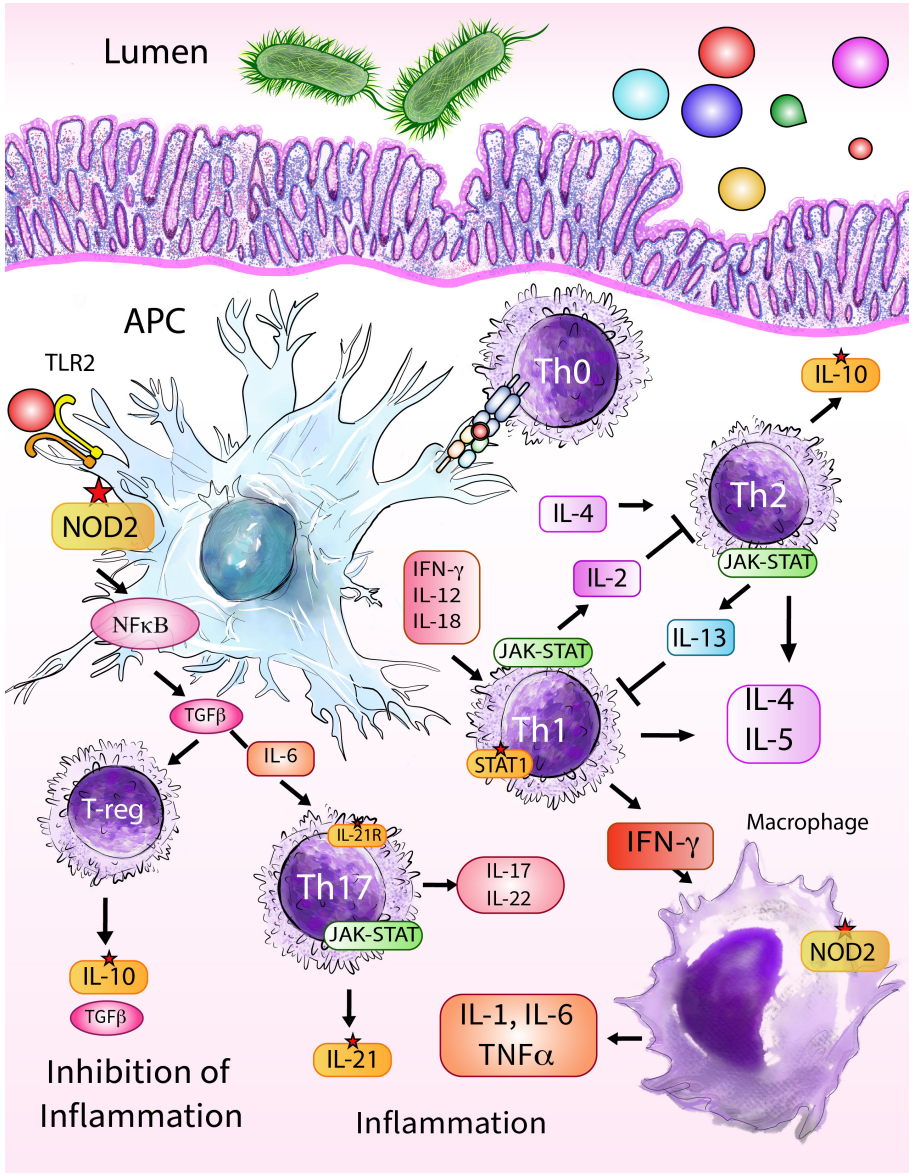
CARD8
NLRP3
SGT1
BRCC3
HSP90
Pyrin
ASC
caspase 1
NLRC4
ASC
caspase 1
IL-1B
IL-18
IL-8
CXCL
IL-6
TNFα
Inflammatory cytokines
Chemokines


Lumen
APC
TLR2
NOD2
NFκB
TGFβ
IL-6
T-reg
IL-10
TGFβ
Inhibition of Inflammation
Th0
IL-4
Th2
JAK-STAT
IL-10
IL-2
IL-13
IFN-γ
IL-12
IL-18
JAK-STAT
Th1
STAT1
IL-4
IL-5
IFN-γ
Macrophage
NOD2
IL-21R
Th17
JAK-STAT
IL-17
IL-22
IL-21
IL-1, IL-6
TNFα
Inflammation

NOD2 signaling may be an attractive candidate for further investigation and targeting in pediatric autoimmune and inflammatory CNS conditions.

Summary

- The genetic basis of autoimmune and inflammatory CNS disorders remains largely unknown.
- We observed a high rate (77.4%) of identification of rare and low-frequency variants in immune regulation genes.
- We identified 88 unique variants of 55 genes, including *UNC13D, LRBA, LYST, NOD2, DOCK8, RNASEH2A, STAT5B, and AIRE*.
- Pathway analysis revealed an enrichment of NOD2-receptor signaling within this patient cohort.
- These findings may shed light on pathogenesis of autoimmune CNS disorders and have therapeutic implications.

Acknowledgments

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Questions?

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