Genome-wide association study provides insights into the etiology and epidemiology of intracranial germ cell tumors.

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COI Disclosure Information

Lead Presenter: Kyuto Sonehara

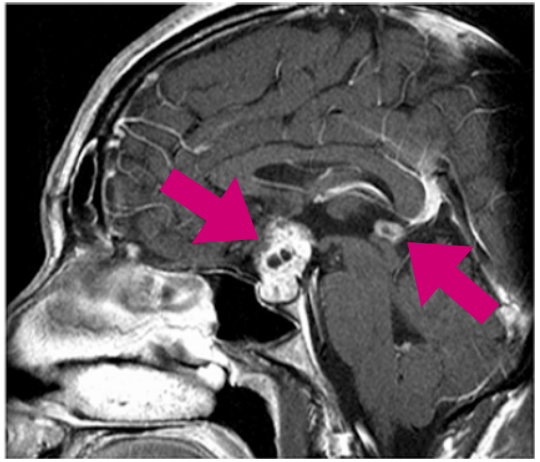
Principal Researcher: Ryo Nishikawa, Yukinori Okada, Keita Terashima

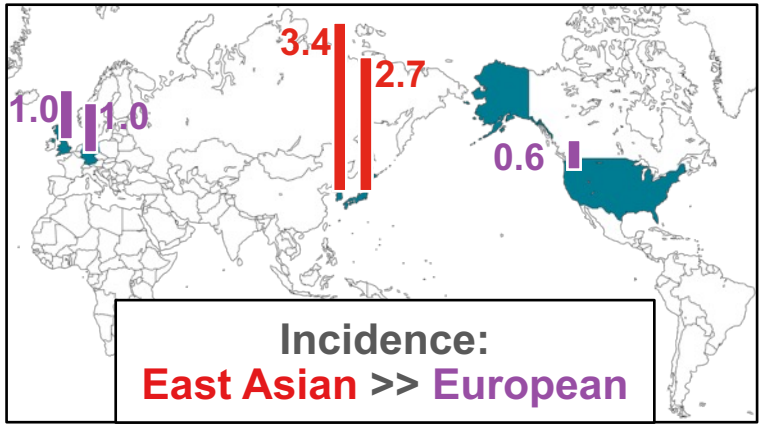
We have no financial relationships to disclose.

Intracranial Germ Cell Tumors: IGCTs

- The **second most common** (~12%) pediatric brain tumor in Japan.
- **High incidence in East Asia**.
  (e.g., Japan, 2.7/million/year
  U.S., 0.6/million/year)
- **The low incidence** has limited basic research on this rare disease.

Elucidation of IGCTs etiology is an important challenge especially **for East Asia including Japan**.

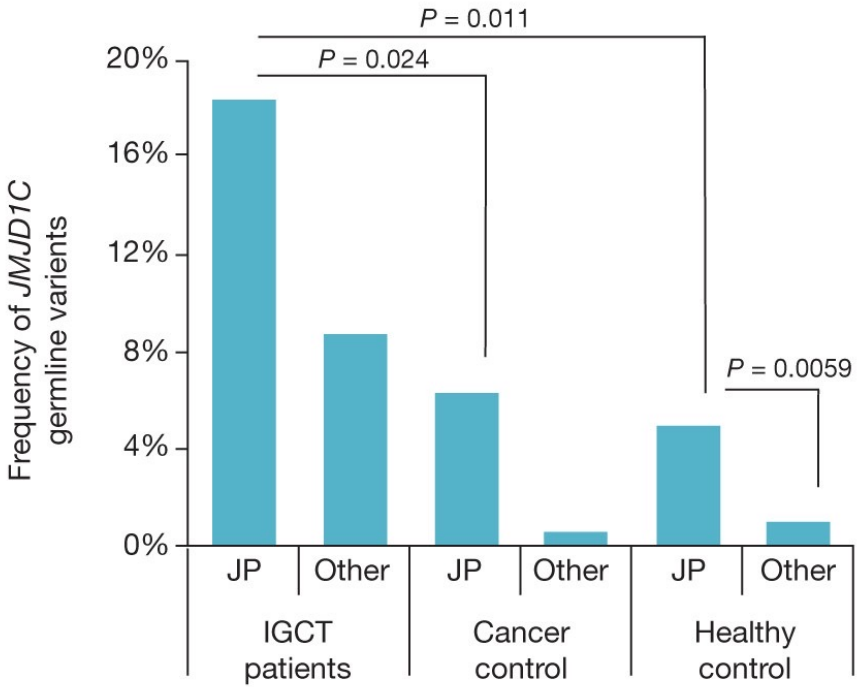




modified from Kanamori M et al. *Neuro Oncol* 2021

Intracranial Germ Cell Tumors: IGCTs

- The largest earlier study on IGCTs germline genetics involved whole-exome sequencing on 62 cases.
- Rare coding variants of *JMJD1C* were implicated in the susceptibility.
- Genome-wide screening of susceptibility genes has never been conducted.

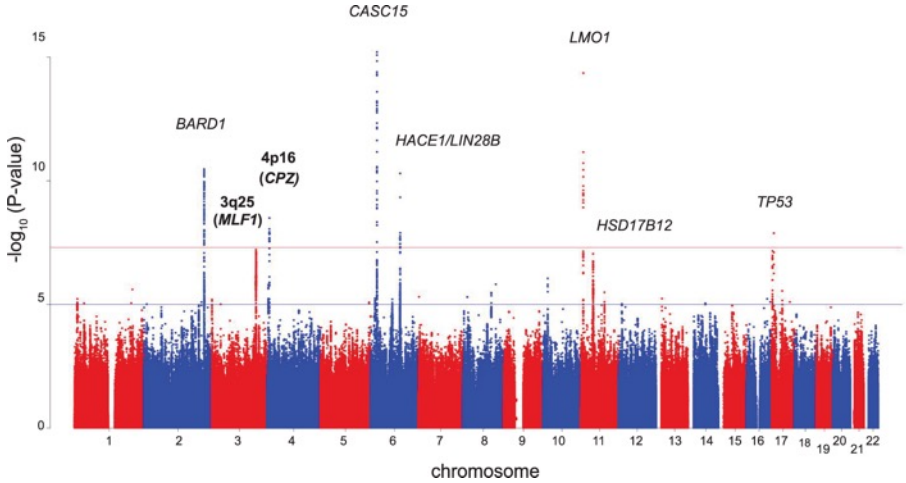


Wang L et al. ***Nature*** 2014

Common genetic variants and pediatric tumors

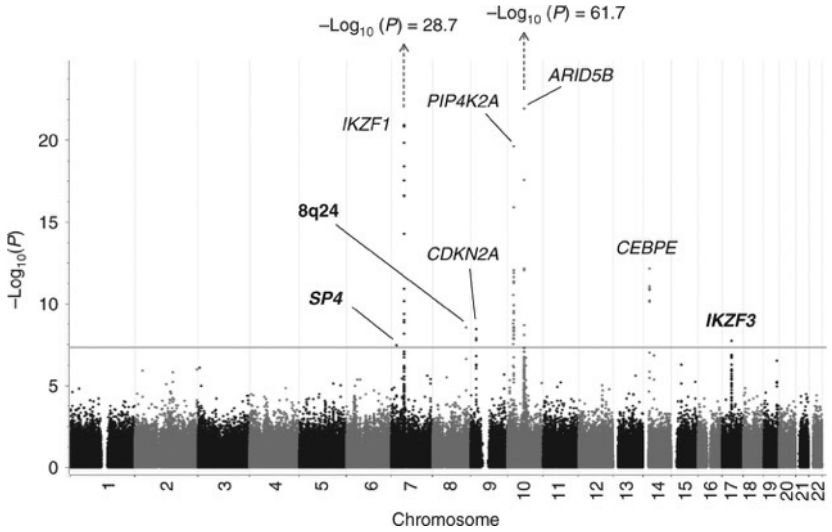
- Genome-wide association studies reveal that pediatric tumors without apparent familiality also have genetic predisposition.

Neuroblastoma



Diskin SJ et al. ***Nat Genet*** 2012
McDaniel LD et al. ***PLoS Genet*** 2017

Childhood acute lymphoblastic leukemia



Wiemels JL et al. ***Nat Commun*** 2018

Study Design

- We designed **an initial genome-wide association study** on IGCTs with **the largest case sample size** ever reported.

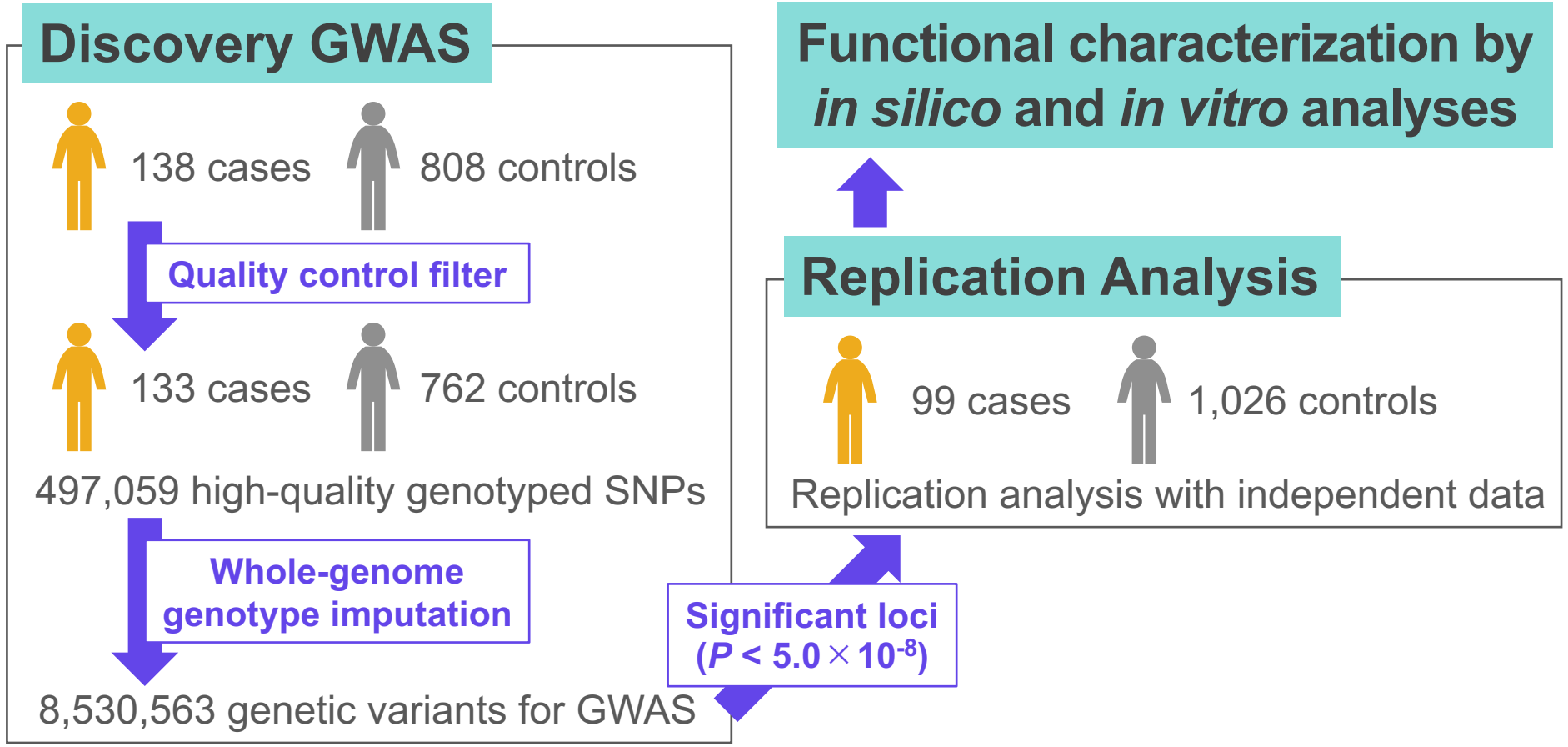
- **138 IGCTs cases**: from the National Center for Child Health and Development and seven other hospitals throughout Japan.

- **808 Healthy controls**: from the University of Tokyo, Osaka University, and affiliated institutes.

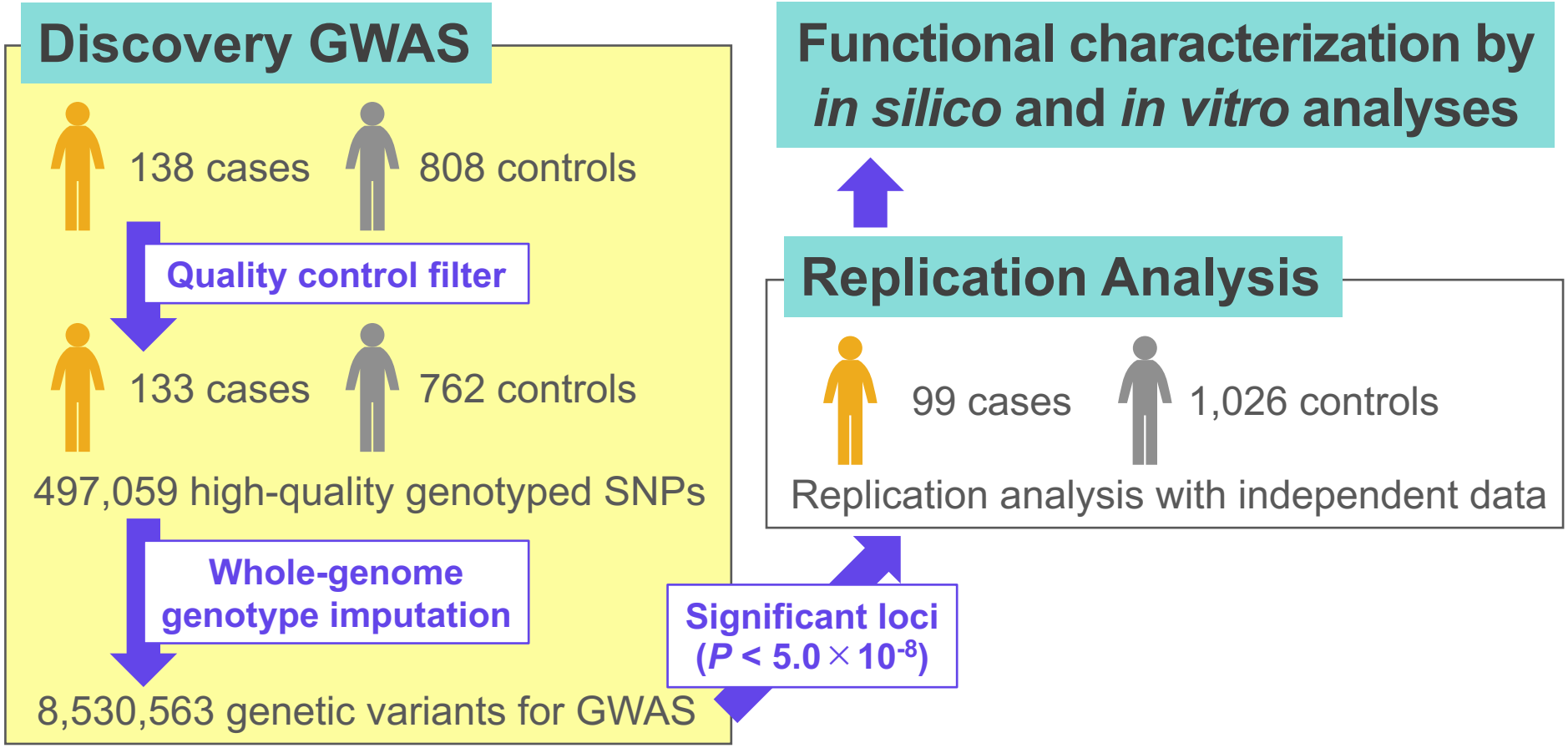
- In addition, **99 IGCTs cases** and **1,026 controls** were available as a replication dataset.

(from Ichimura K et al. ***Acta Neuropathol*** 2016 and Okada Y et al. ***Nat Commun*** 2018)

Study Design

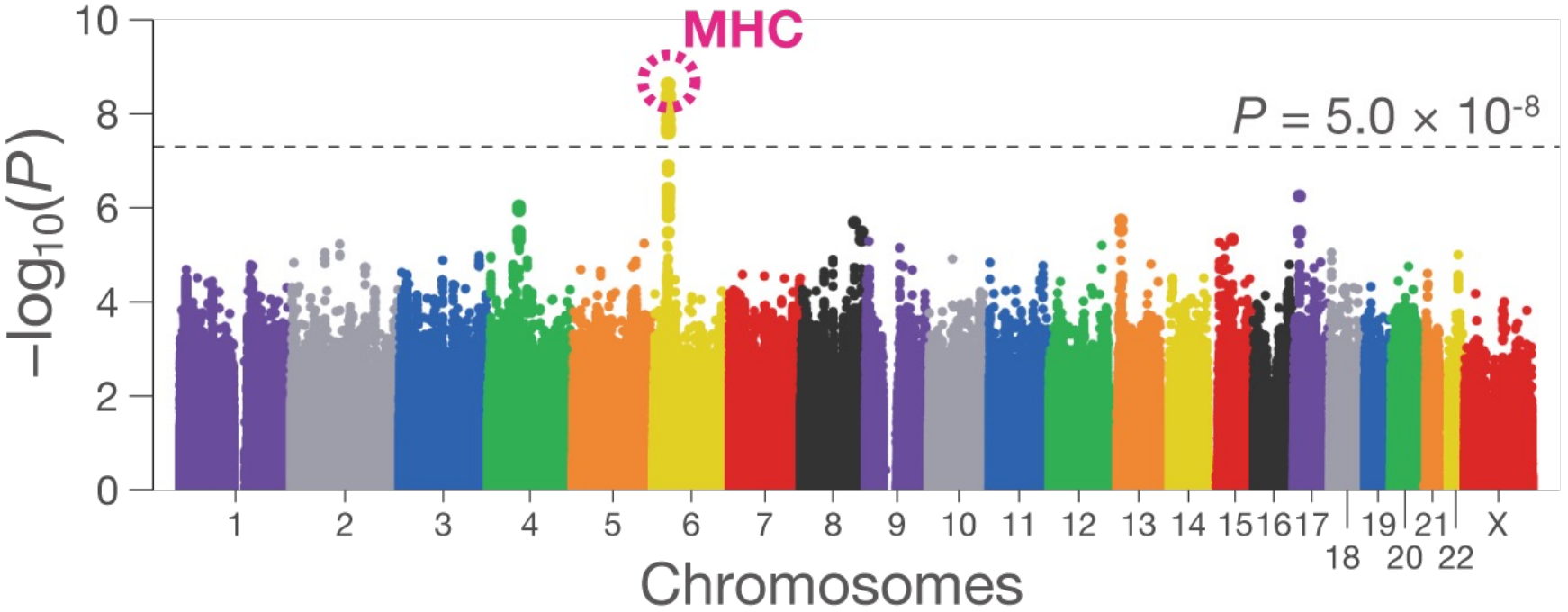


Study Design



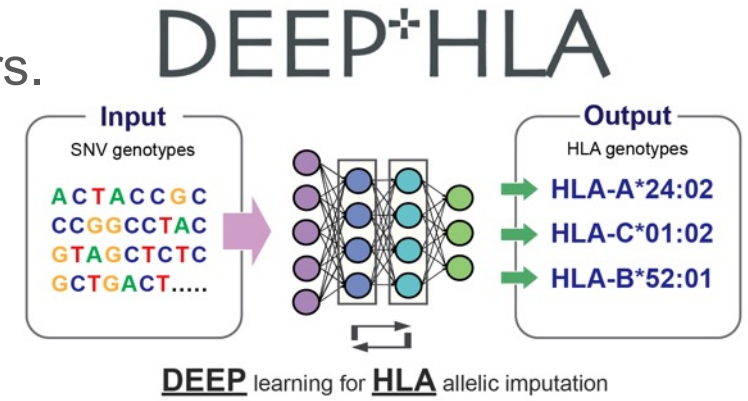
Genome-Wide Association Study

Genome-wide significant variants were found at the **major histocompatibility complex region (MHC; $P = 2.4 \times 10^{-9}$)**.

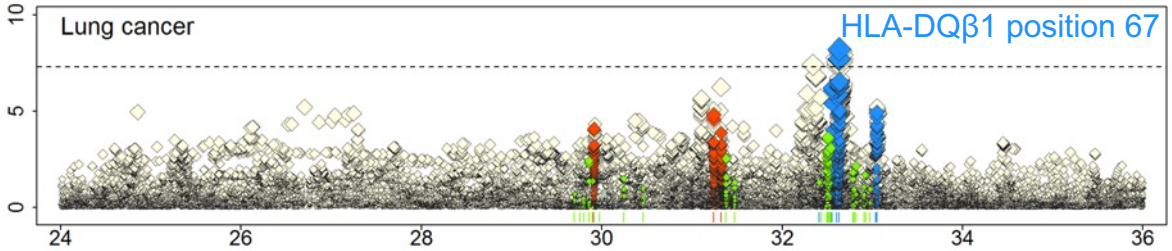


HLA imputation analysis

- Human leukocyte antigen (HLA) variants in MHC have been implicated in various cancers.
- Typically, MHC associations are fine-mapped to HLA variants (e.g., HLA-DQβ1 with lung cancer).
- We computationally inferred HLA variant genotypes based on the surrounding SNPs using a convolutional deep learning method.



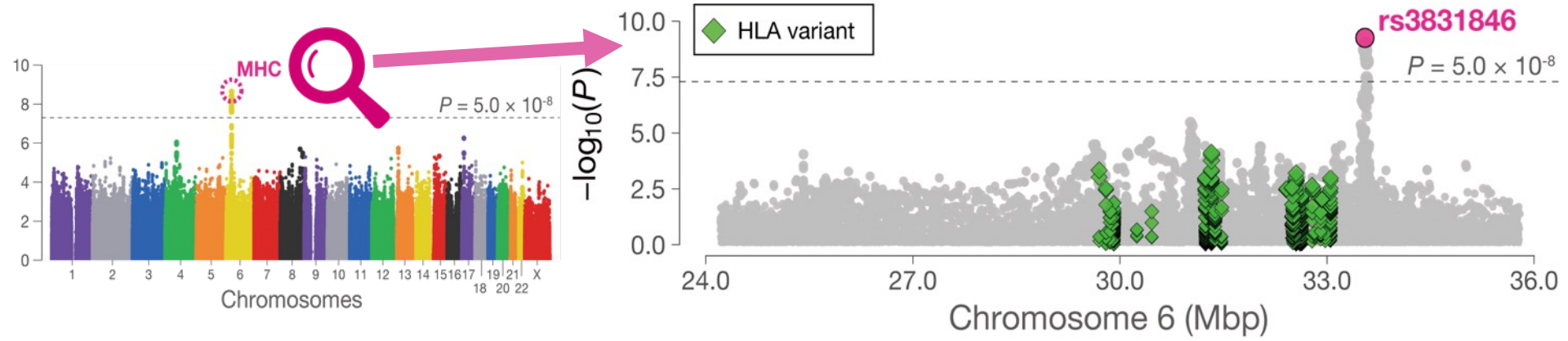
Naito T et al. ***Nat Commun*** 2021



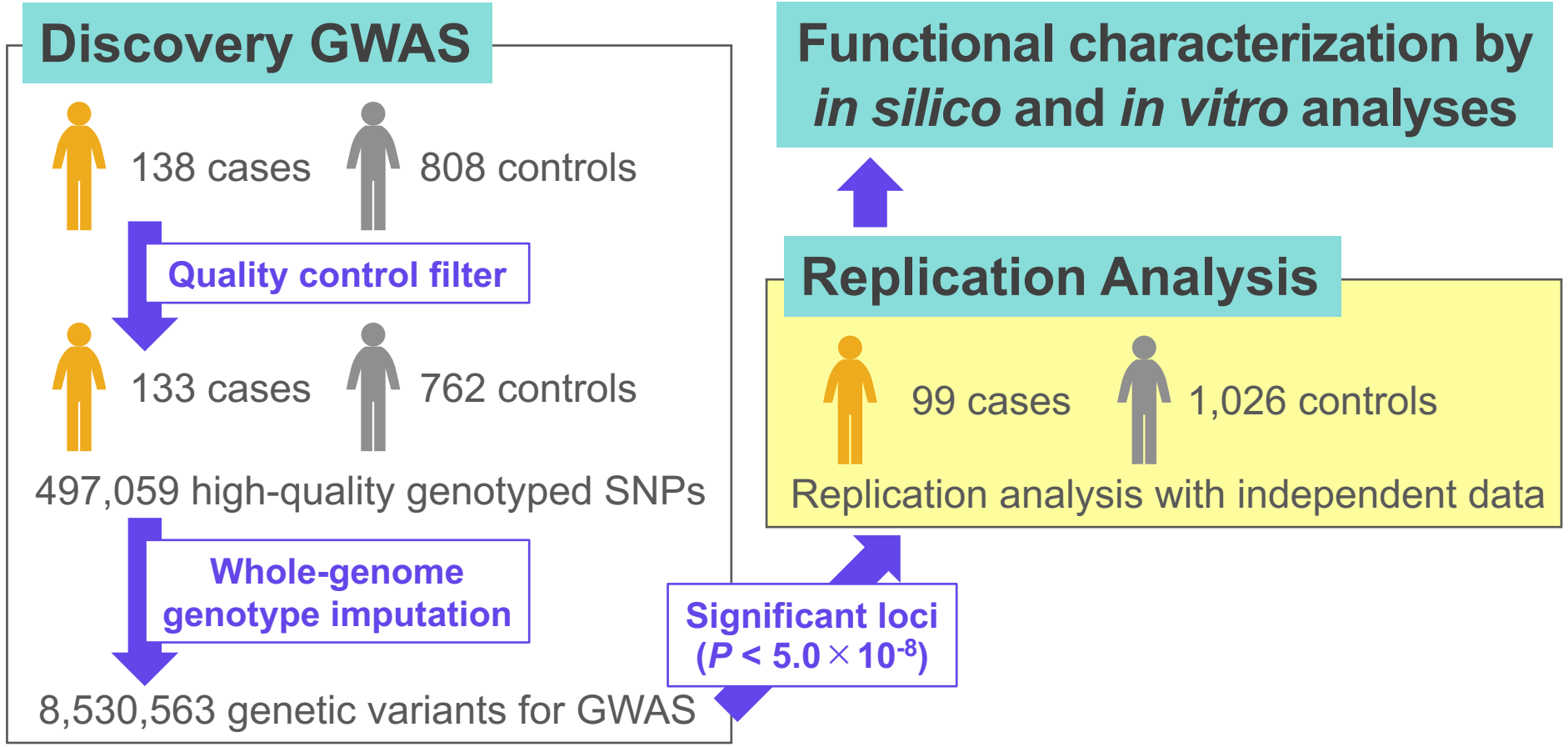
Hirata J et al. ***Nat Genet*** 2019

No association with HLA variants

- Performing HLA imputation, we analyzed HLA variants association.
- Unexpectedly, **no HLA variants showed association**.
- The lead variant was **rs3831846**, a **4-bp deletion**.



Study Design

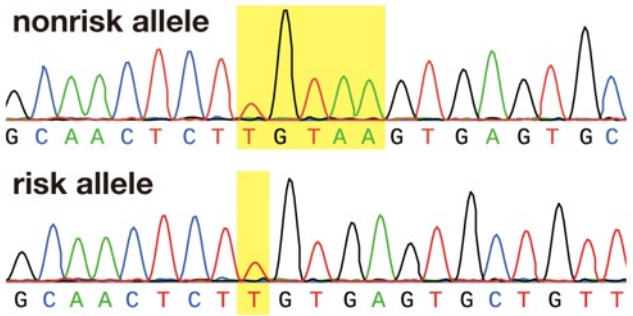


Validating Deletion and Replicating Association

Discovery Dataset (133 cases vs 762 controls)

SNP	Chr	Position	Alleles	Risk Allele	Freq. in Cases	Freq. in Controls	Odds Ratio (95% CI)	*P*-value
rs3831846	6	33,548,346	TGTAA/T	T	0.63	0.43	2.46 (1.83–3.31)	2.4×10^{-9}

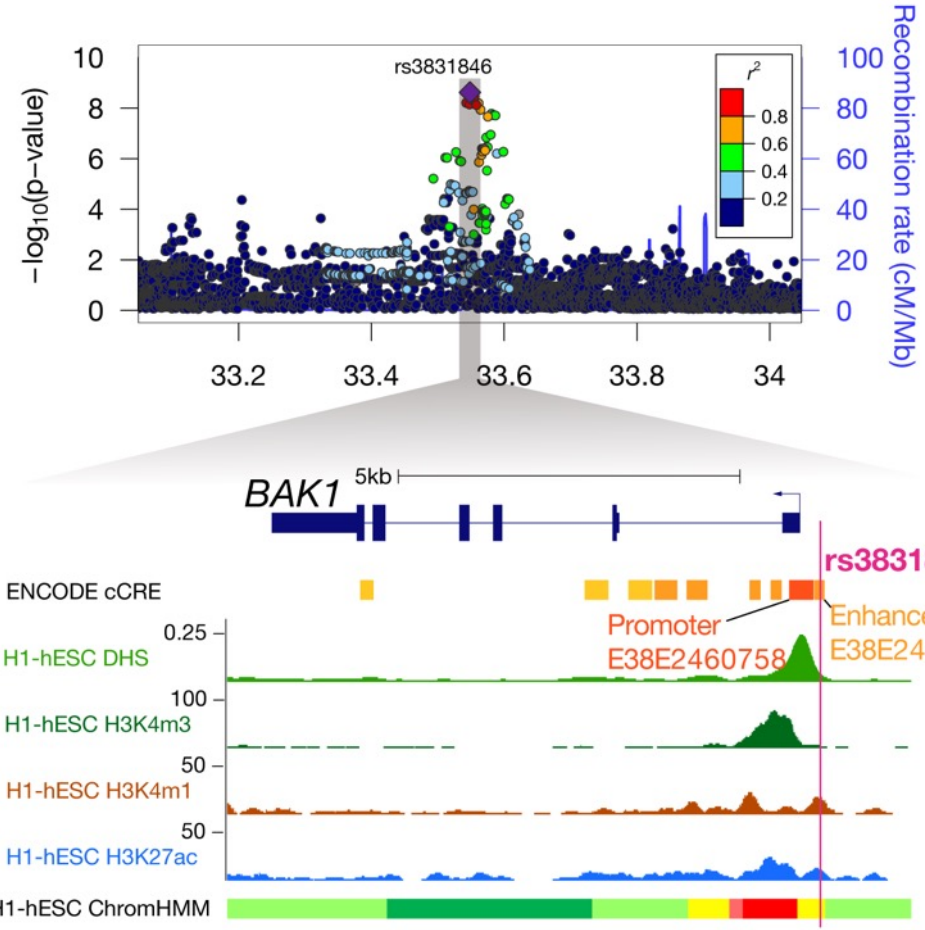
Sanger sequencing validation



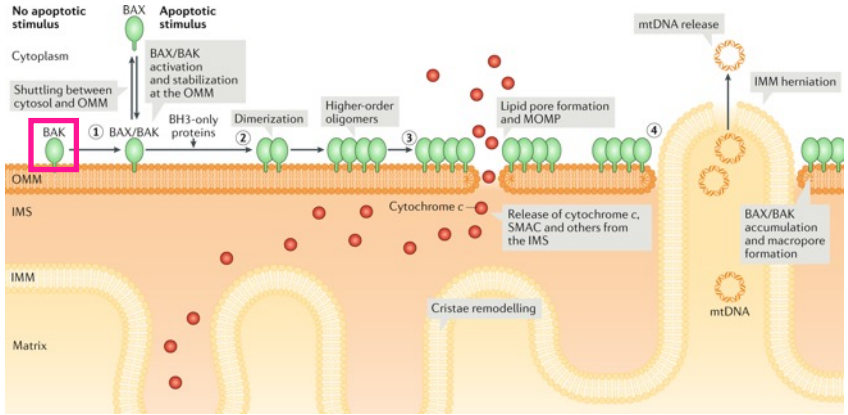
Replication Dataset (99 cases vs 1,026 controls)

Freq. in Cases	Freq. in Controls	Odds Ratio (95% CI)	*P*-value
0.62	0.42	2.22 (1.63–3.03)	1.7×10^{-7}

rs3831846 is in *BAK1* enhancer

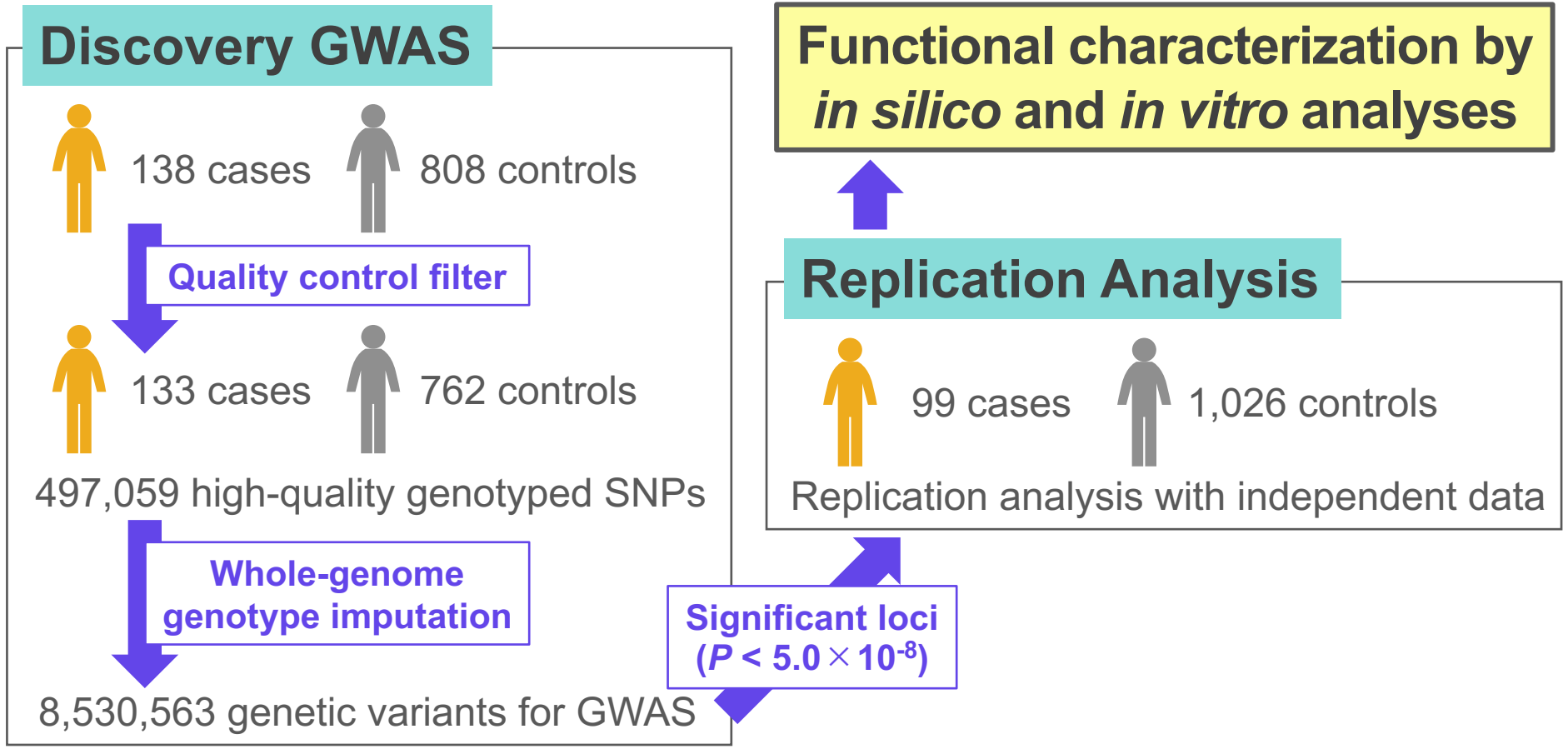


- Rs3831846 resides in an **enhancer** element adjacent to the ***BAK1* promoter**.
- ***BAK1*** (BCL2 Antagonist/Killer 1): an **apoptosis-inducer** of the BCL2 protein family, the apoptosis regulators.



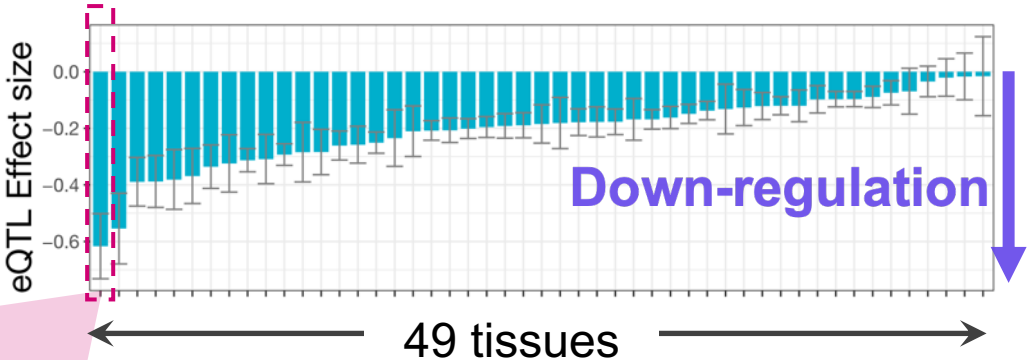
Bock F J and Tait S W. ***Nat Rev Mol Cell Biol*** 2020

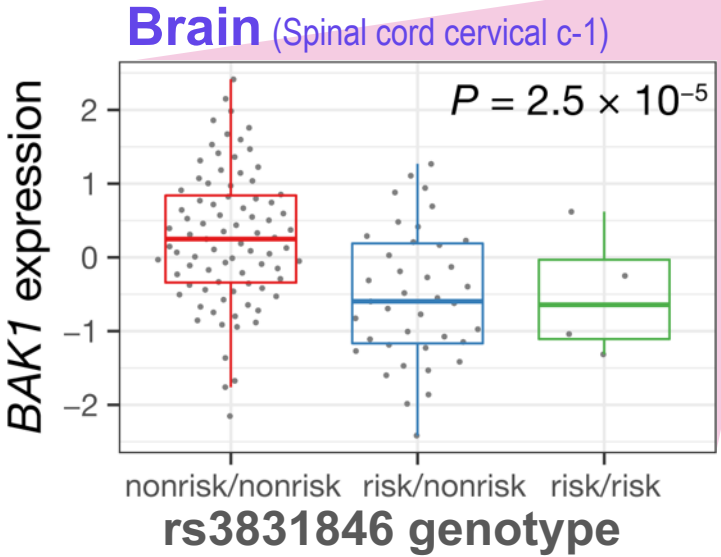
Study Design

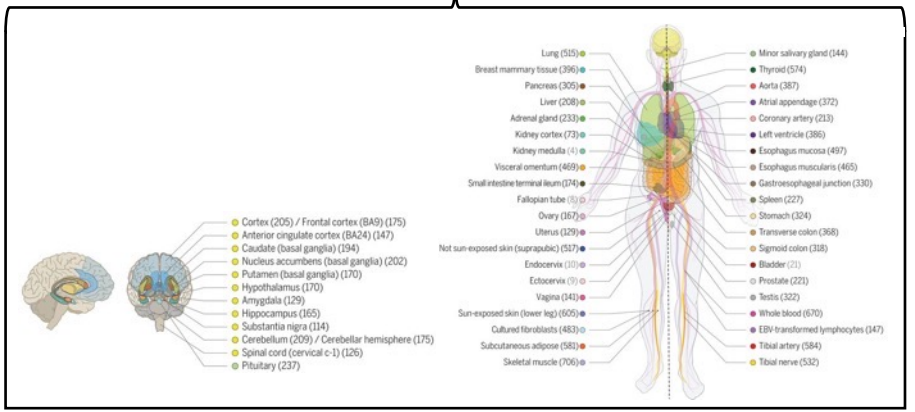


rs3831846 has eQTL effect on *BAK1*

Expression quantitative trait locus (eQTL) analysis revealed **the risk allele down-regulating *BAK1*** in a broad range of tissues.



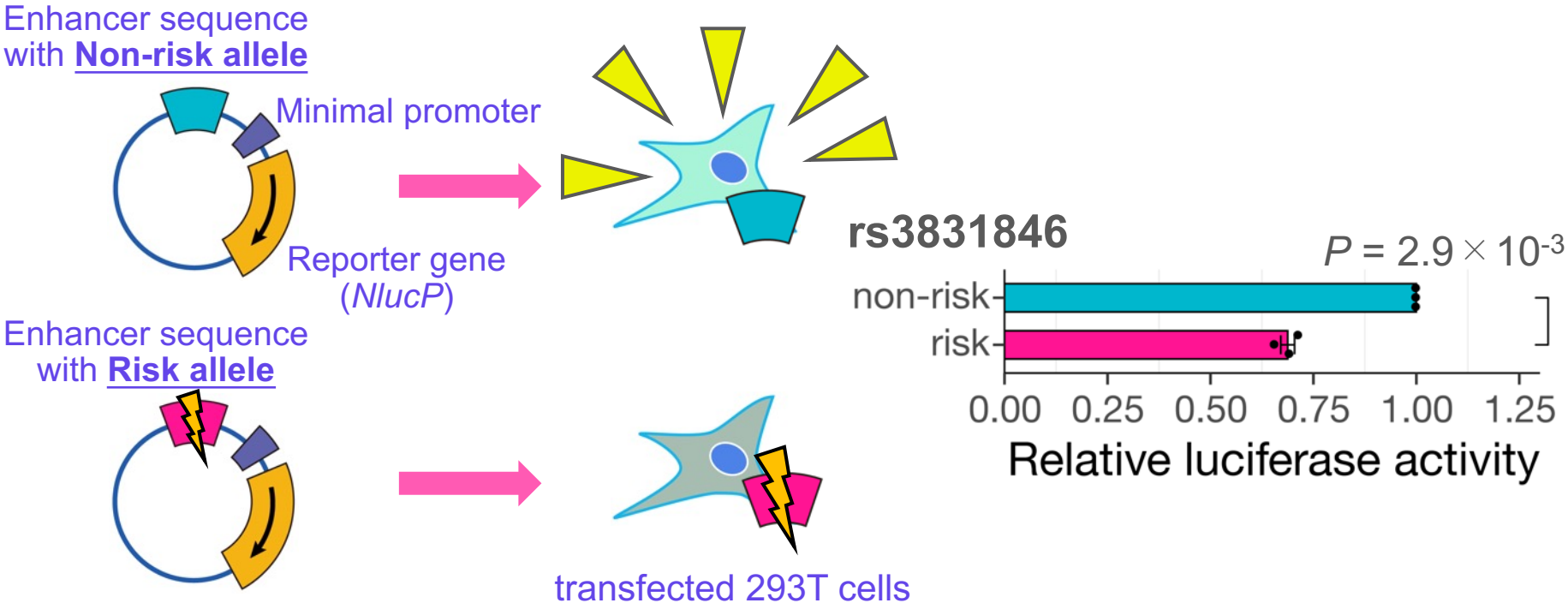




GTEx Consortium. ***Science*** 2020

rs3831846 reduces *BAK1* enhancer activity

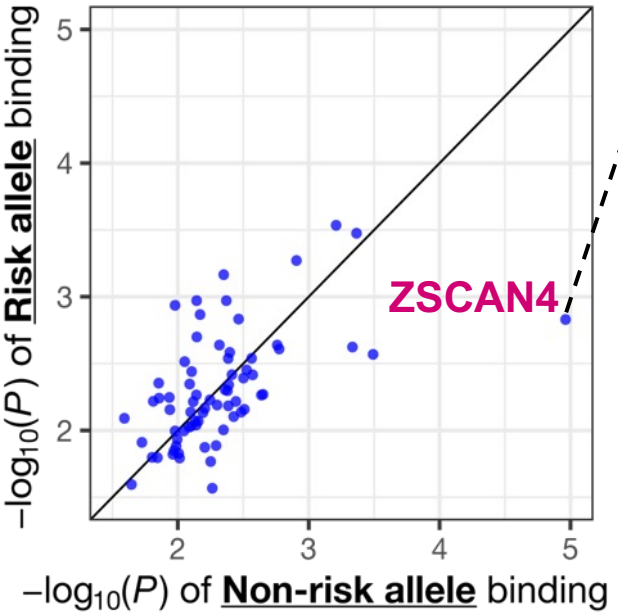
In vitro reporter assay experimentally validated
the risk allele of rs3831846 attenuating the *BAK1* enhancer activity.

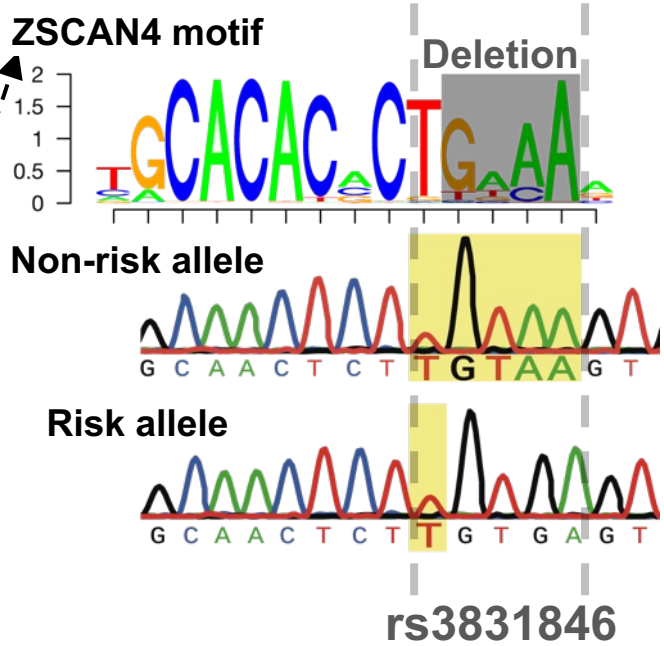


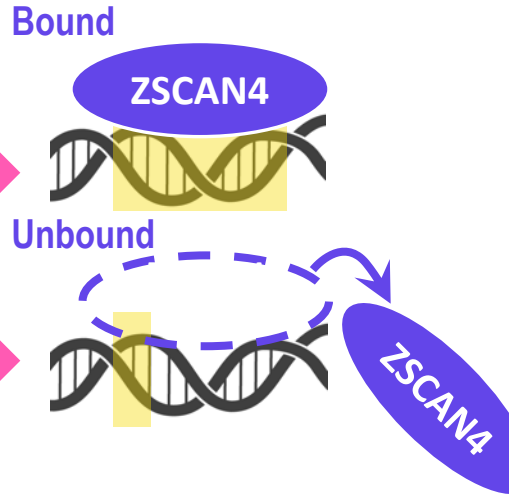
Disrupting Impact on TF Binding Motif

We evaluated transcription factor binding score using the JASPAR database.

The deletion has **disrupting impact on the binding motif in the enhancer**.







Disrupting Impact on TF Binding Motif

We evaluated transcription factor binding score using the JASPAR database.

The

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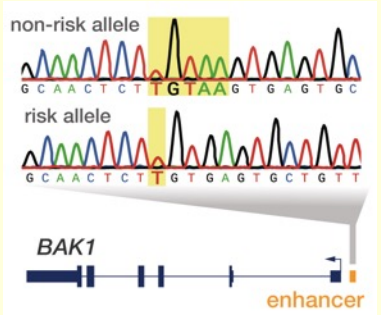
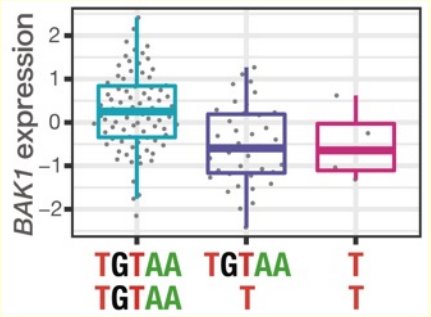
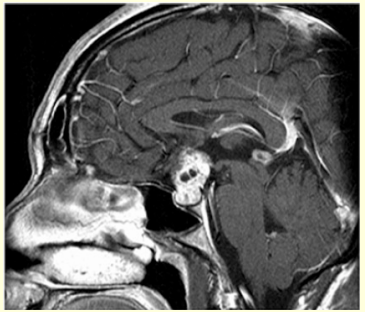
Molecular mechanism behind the risk association



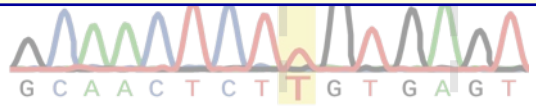
Disrupted enhancer activity



***BAK1* down-regulation**



Tumor development

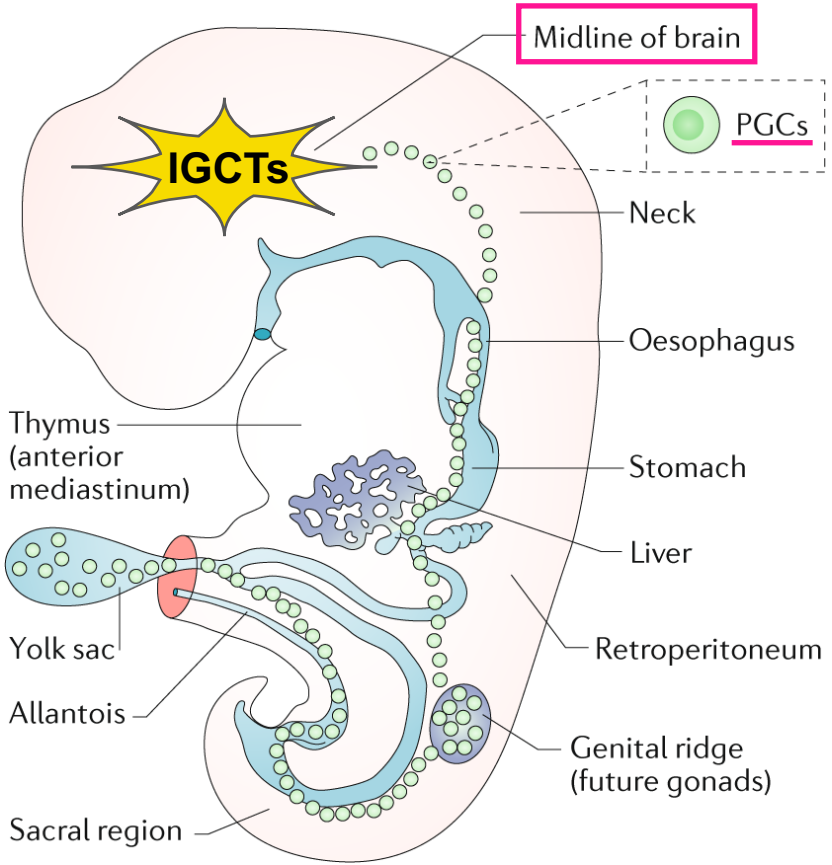


rs3831846

ZSCAN4

BAK1 in IGCTs Etiology (from embryology)

- IGCTs are considered originated from the embryonic precursor of sperm and egg (**primordial germ cells: PGCs**).
- During embryogenesis, some PGCs can mis-migrate into the brain, **physiologically removed by apoptosis**.
- The down-regulation of *BAK1* may allow **those mis-migrated PGCs to escape the removal and form IGCTs**.

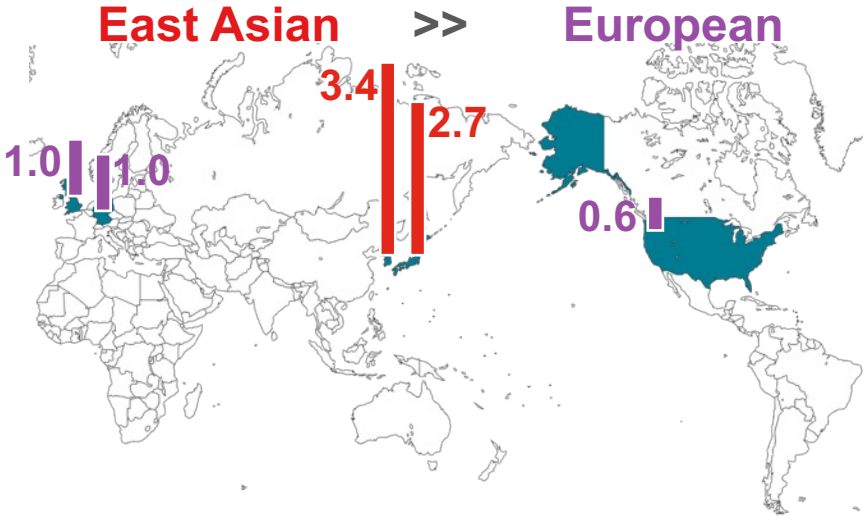


modified from Oosterhuis J W and Looijenga L H. *Nat Rev Cancer* 2019

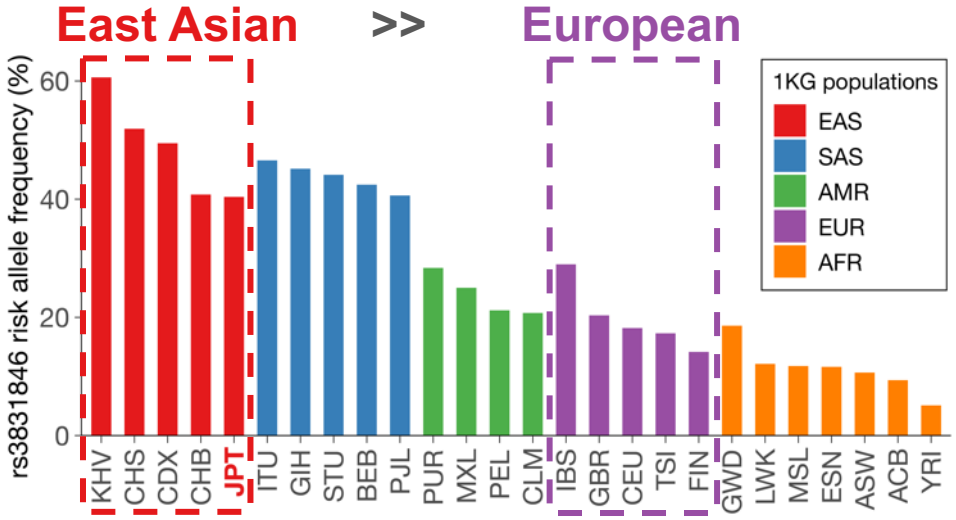
BAK1 rs3831846 in IGCTs Epidemiology

- The risk allele frequency of rs3831846 is **48.5% in East Asian** and **19.9% in European**.
- The proportion of the risk allele carriers may contribute to the geographical difference in incidence.

Incidence (/million/year)

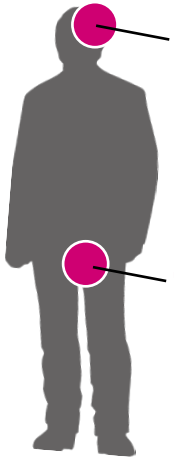


Risk allele frequency



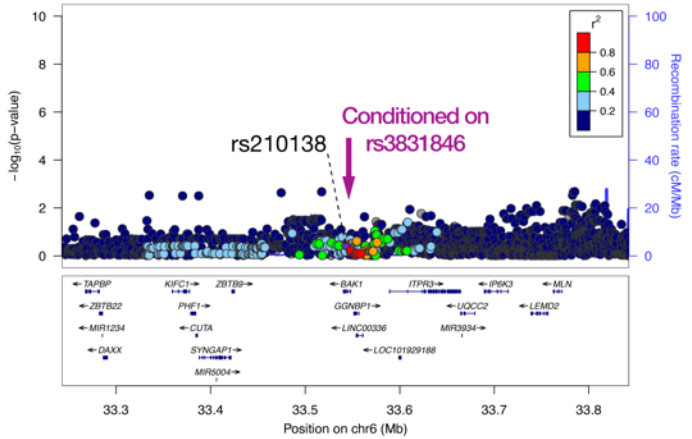
Shared Genetic Basis with Testicular GCTs

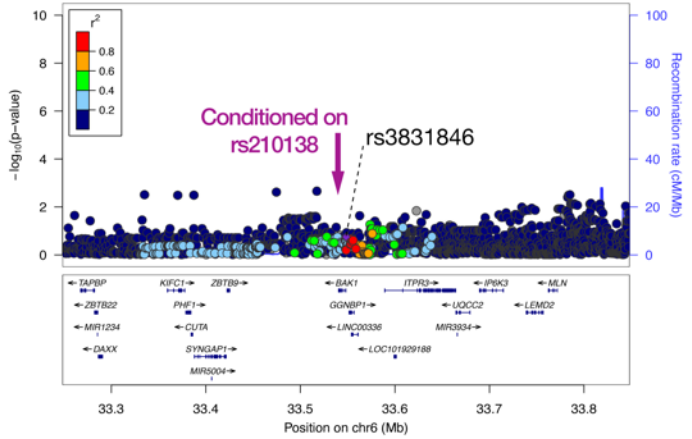
- **Testicular GCTs (TGCTs)** are major testicular cancer histologically similar to IGCTs.
- Rs3831846 is in strong linkage disequilibrium with TGCTs risk SNP rs210138 (r^2 = 0.98 in both European and East Asian).



Intracranial GCTs

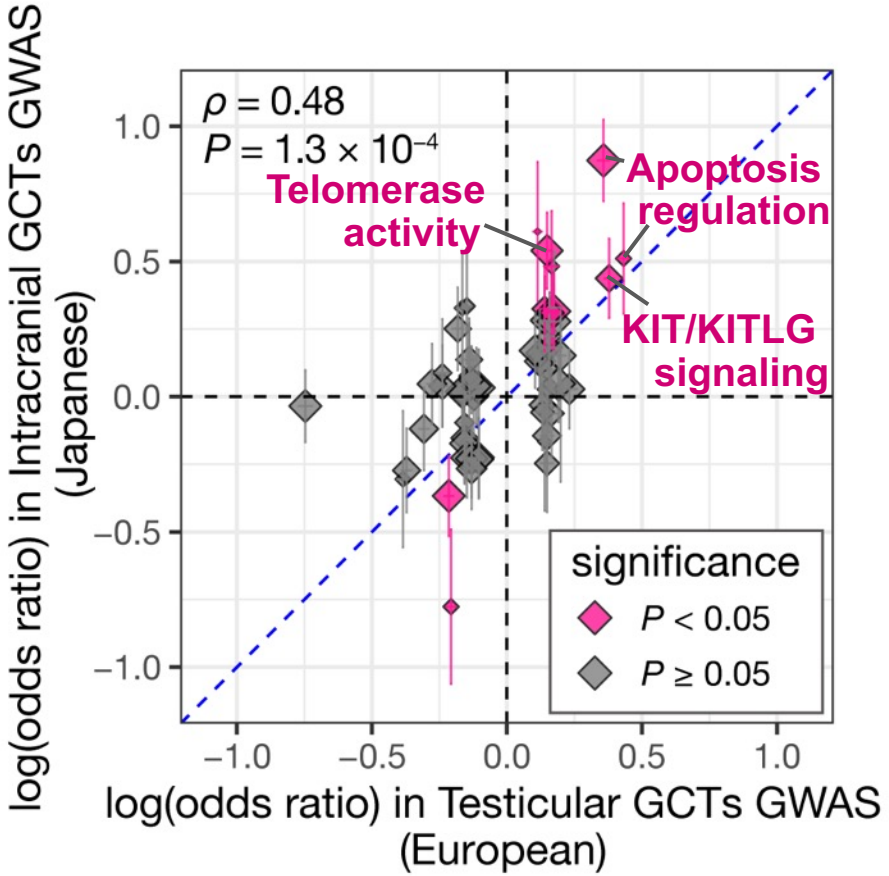
Testicular GCTs





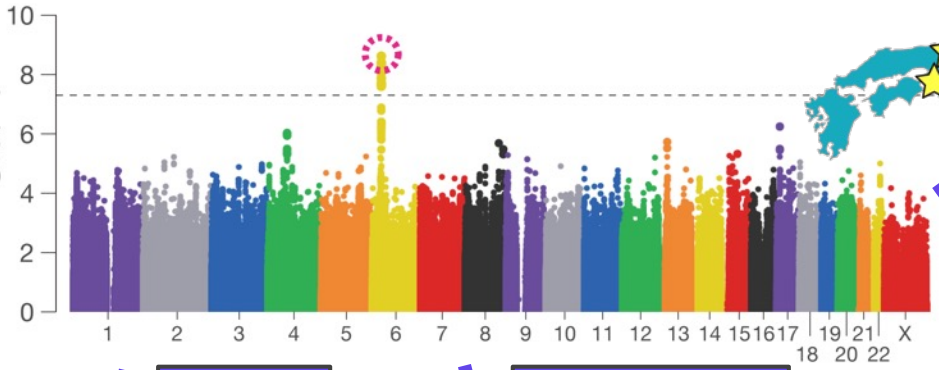
Shared Genetic Basis with Testicular GCTs

- Among 57 TGCTs-risk loci available in our GWAS, **11 loci showed *P* < 0.05 for IGCTs risk**, with concordant risk alleles.
- These significant loci were implicated in multiple biological pathways, such as:
  - *BAK1* and *SPRY4* from KIT/KITLG signaling
  - *CLPTM1L* from apoptosis regulation
  - *PITX1* from telomerase activity

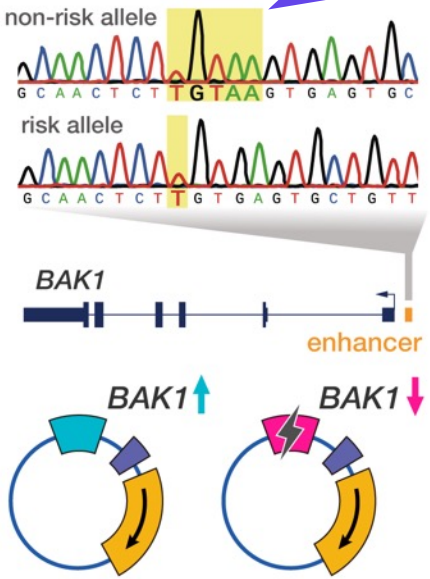


Summary

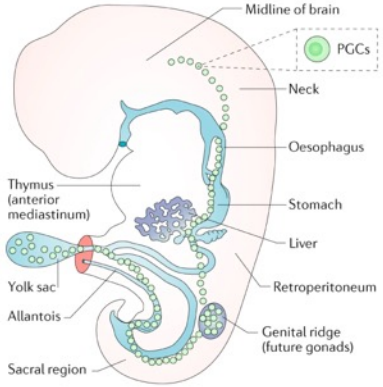
The first IGCTs GWAS



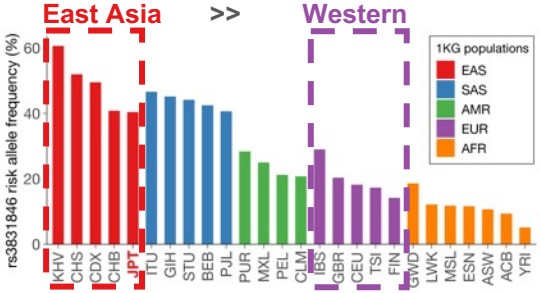
Molecular mechanism



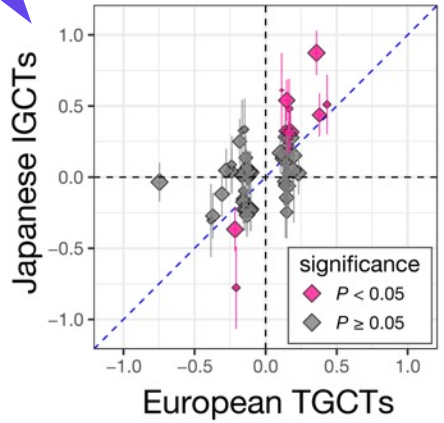
Etiology



Epidemiology



Shared genetic basis



Sonehara K et al. ***Nat Commun*** 2022

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