

# TruSight One

「高効率・高感度な臨床研究を可能にする

TruSight Oneシーケンスパネル～ドライ編～」

Dec 19, 2014



長井 陽子

イルミナ株式会社

シーケンシングスペシャリスト

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# TruSight Oneシーケンスパネル

サンプル調製から解析までの一連の工程をサポート

BaseSpace®

ライブラリー調製

シーケンス

アライメント  
& 変異コール

アノテーション



TruSight One  
シーケンスパネル

2014年12月5日実施  
ウェビナー参照



Enrichment



VariantStudio



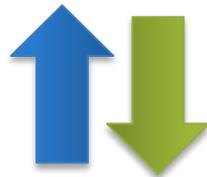
本ウェビナー内容

# イルミナ VariantStudioの流れ

バリエーションコールデータを入力、生物学的意味付けを出力

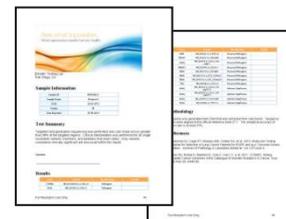
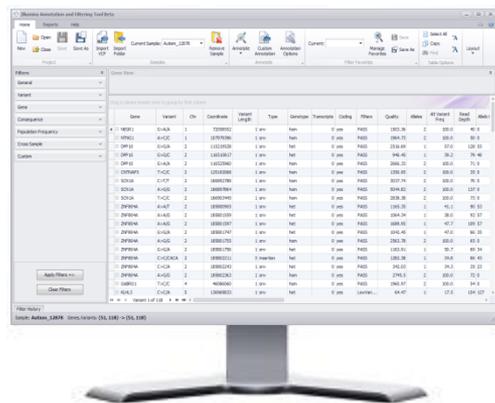


VariantStudio  
アノテーションデータベース



VCF または gVCF ファイルの入力

VCF: バリエーションコールフォーマット



解釈した変異をレポートとして出力

VariantStudio™

# アノテーション機能

様々なアノテーション情報を活用した生物学的意味付け

## ▶ 提供するアノテーション情報

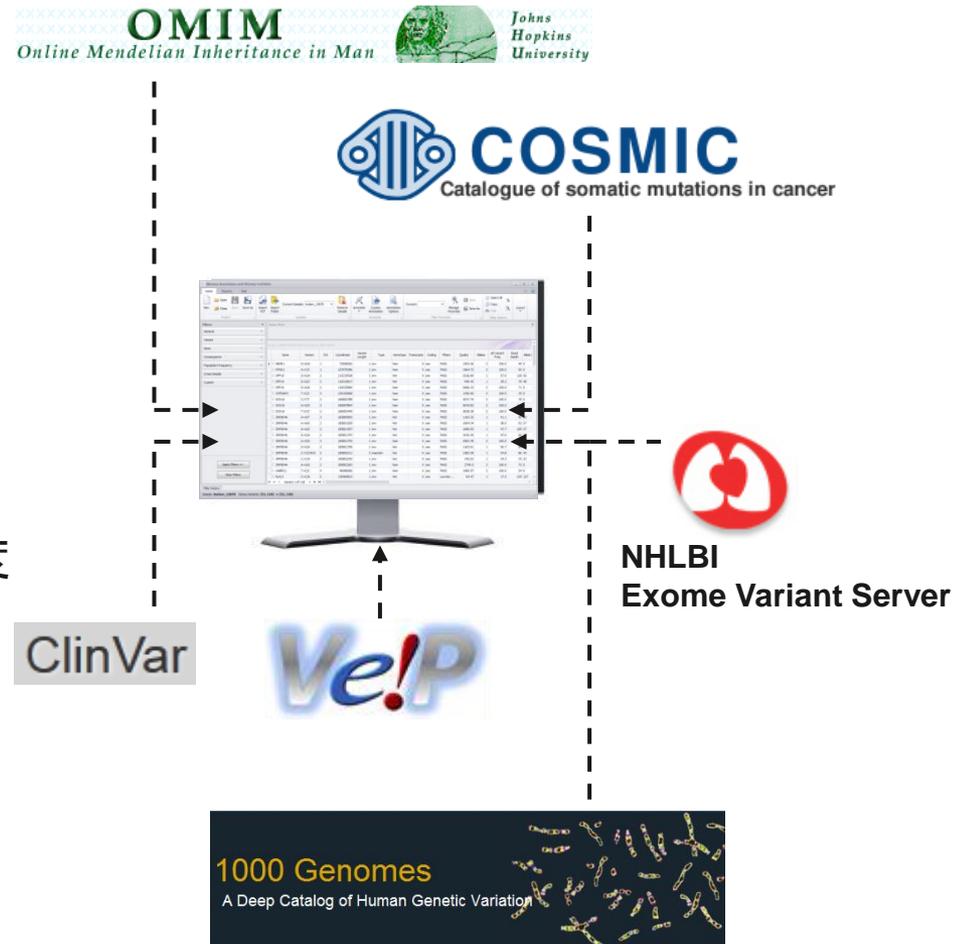
- 変異レベル
- 遺伝子レベル

## ▶ アノテーションに含まれる項目

- 転写産物名
- 関連する疾患や表現型
- 各種人類集団におけるアレル頻度
- 遺伝子機能への影響
- 文献検索
- データベースへのリンク

## ▶ 外部データ

- テキストファイルを読み込み



# VariantStudioによるバリエーションの絞り込み

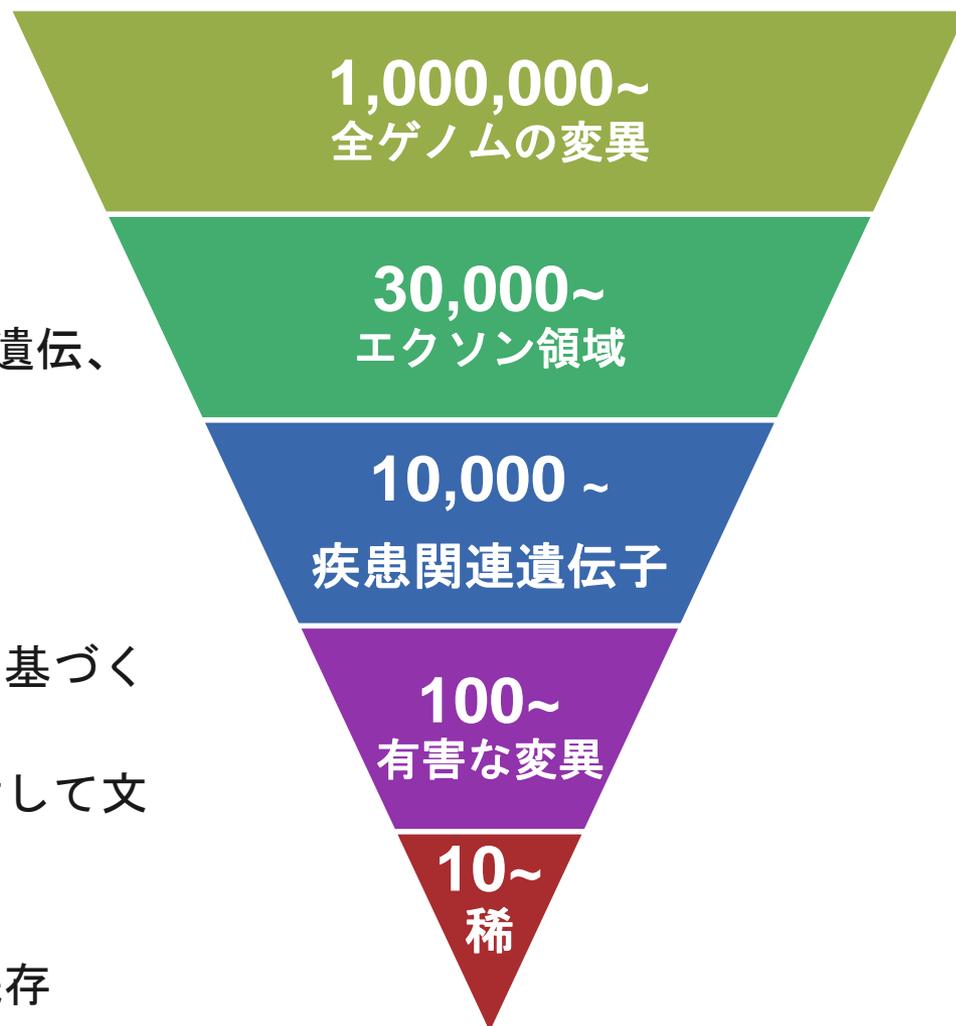
シーケンシングより得られた膨大な変異から疾患原因となる変異を同定

## ▶ 様々な研究に対応

- 腫瘍-正常ペアの体細胞変異解析
- 先天性疾患の疾患原因変異解析
- 親子トリオなどの家系解析
- X連鎖性劣性遺伝、常染色体劣性遺伝、常染色体優性遺伝、*de novo* 変異

## ▶ 柔軟で簡単な絞り込み機能

- 遺伝子名、疾患名、アレル頻度に基づく簡単な絞り込み
- 変異テーブルの全てのカラムに対して文字検索や並べ替えが可能
- 絞り込み条件の履歴を記憶
- 複雑な絞り込み条件をお気に入りに保存



# 家系解析の研究デザイン例

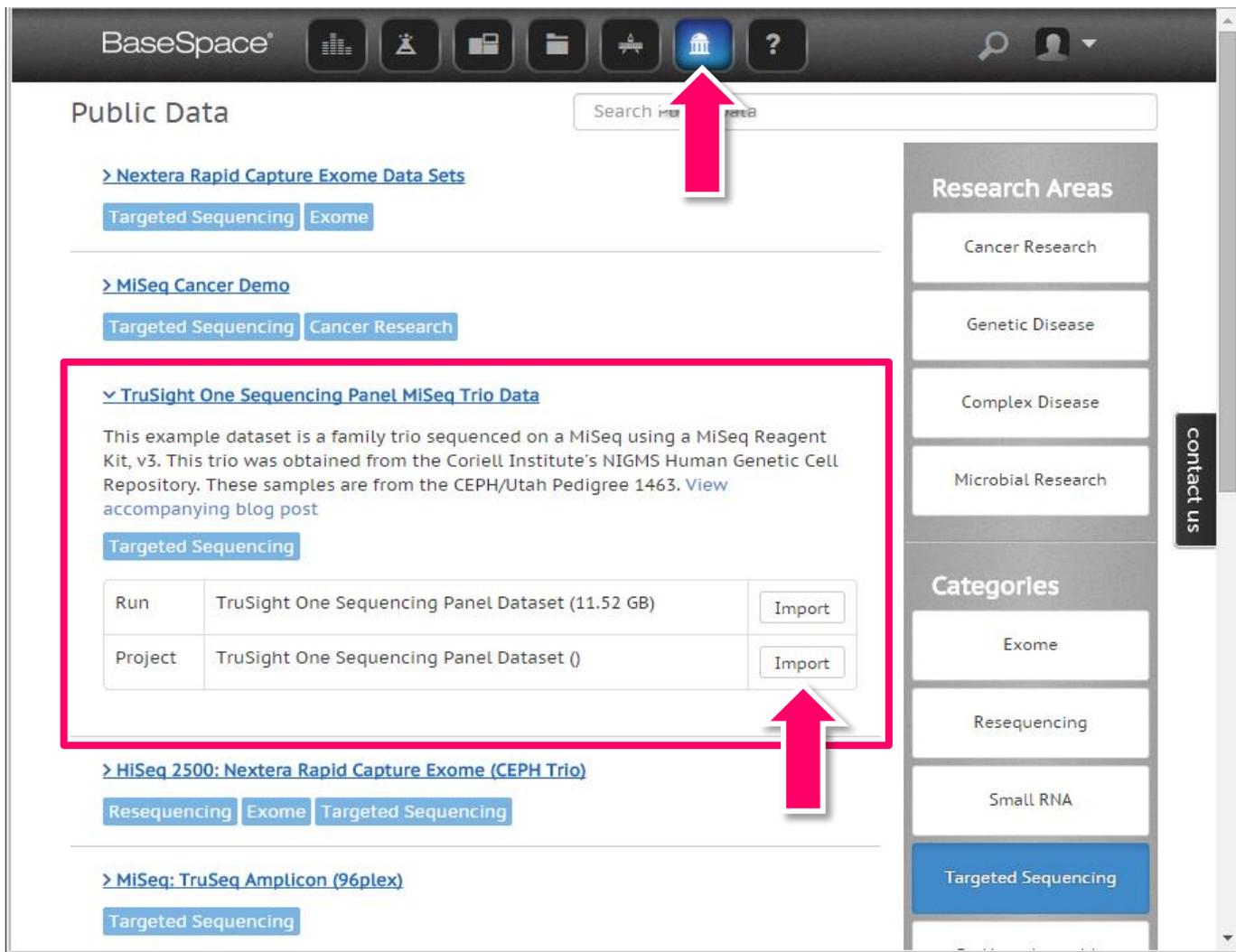
<p>患者情報</p>	<p>家族内集積がある 遺伝性疾患</p>	<p>家族内集積なし 孤発性疾患</p>
<p>仮説</p>	<p>常染色体劣性遺伝</p>	<p><i>De Novo</i> 変異</p>
<p>研究デザイン</p>	<p>スクリーニング：家族サンプル 検証：独立した複数家族</p>	<p>スクリーニング：家族サンプル 検証：独立した複数発症者</p>

# デスクトップ版とクラウド版VariantStudioの違い

	illumina VariantStudio 2.2	BaseSpace VariantStudio 2.2
特徴	デスクトップ版	クラウド版
ソフトウェア 起動	ローカルPCより起動	BaseSpaceより起動
VCF 読み込み	ローカルPCに保存された VCFファイル	BaseSpaceにアップロード/共有/ インポートされたVCFファイル
プロジェクト 保存	ローカルPCに保存	ローカルPCに保存
プロジェクト 読み込み	PCに保存されたプロジェクト	PCに保存されたプロジェクト

本ウェビナーでは、BaseSpaceパブリックデータのTruSightOne シーケンスパネルVCFファイルに独自に変異を導入したデモデータを用いて解析をします。

# BaseSpaceからパブリックデータの取得方法



The screenshot shows the BaseSpace Public Data interface. The top navigation bar includes the BaseSpace logo and several icons, with a red arrow pointing to the public data icon. Below the navigation bar, there is a search bar and a list of data categories. The 'TruSight One Sequencing Panel MiSeq Trio Data' section is highlighted with a red box, and a red arrow points to the 'Import' button for the 'Project' row in the table below it.

Public Data

Search

> [Nextera Rapid Capture Exome Data Sets](#)

Targeted Sequencing Exome

> [MiSeq Cancer Demo](#)

Targeted Sequencing Cancer Research

▼ [TruSight One Sequencing Panel MiSeq Trio Data](#)

This example dataset is a family trio sequenced on a MiSeq using a MiSeq Reagent Kit, v3. This trio was obtained from the Coriell Institute's NIGMS Human Genetic Cell Repository. These samples are from the CEPH/Utah Pedigree 1463. [View accompanying blog post](#)

Targeted Sequencing

Run	TruSight One Sequencing Panel Dataset (11.52 GB)	Import
Project	TruSight One Sequencing Panel Dataset ()	Import

> [HiSeq 2500: Nextera Rapid Capture Exome \(CEPH Trio\)](#)

Resequencing Exome Targeted Sequencing

> [MiSeq: TruSeq Amplicon \(96plex\)](#)

Targeted Sequencing

Research Areas

- Cancer Research
- Genetic Disease
- Complex Disease
- Microbial Research

Categories

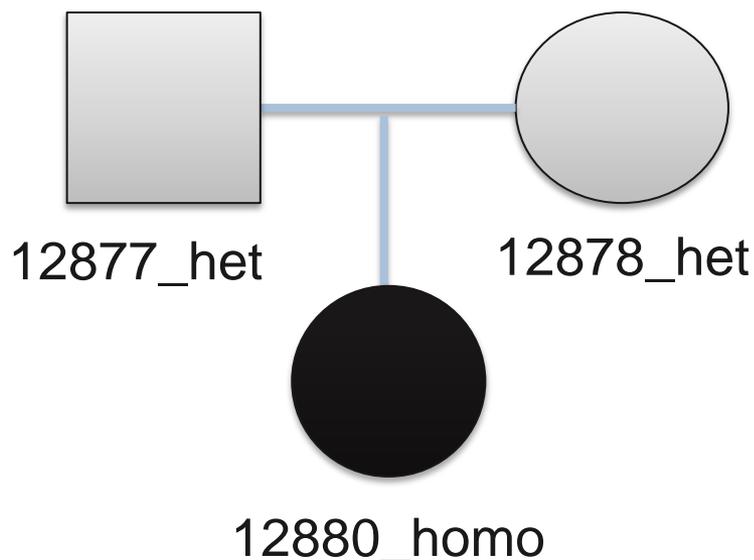
- Exome
- Resequencing
- Small RNA
- Targeted Sequencing

contact us

# 本ウェビナー デモ用親子トリオデータについて

## デモデータ

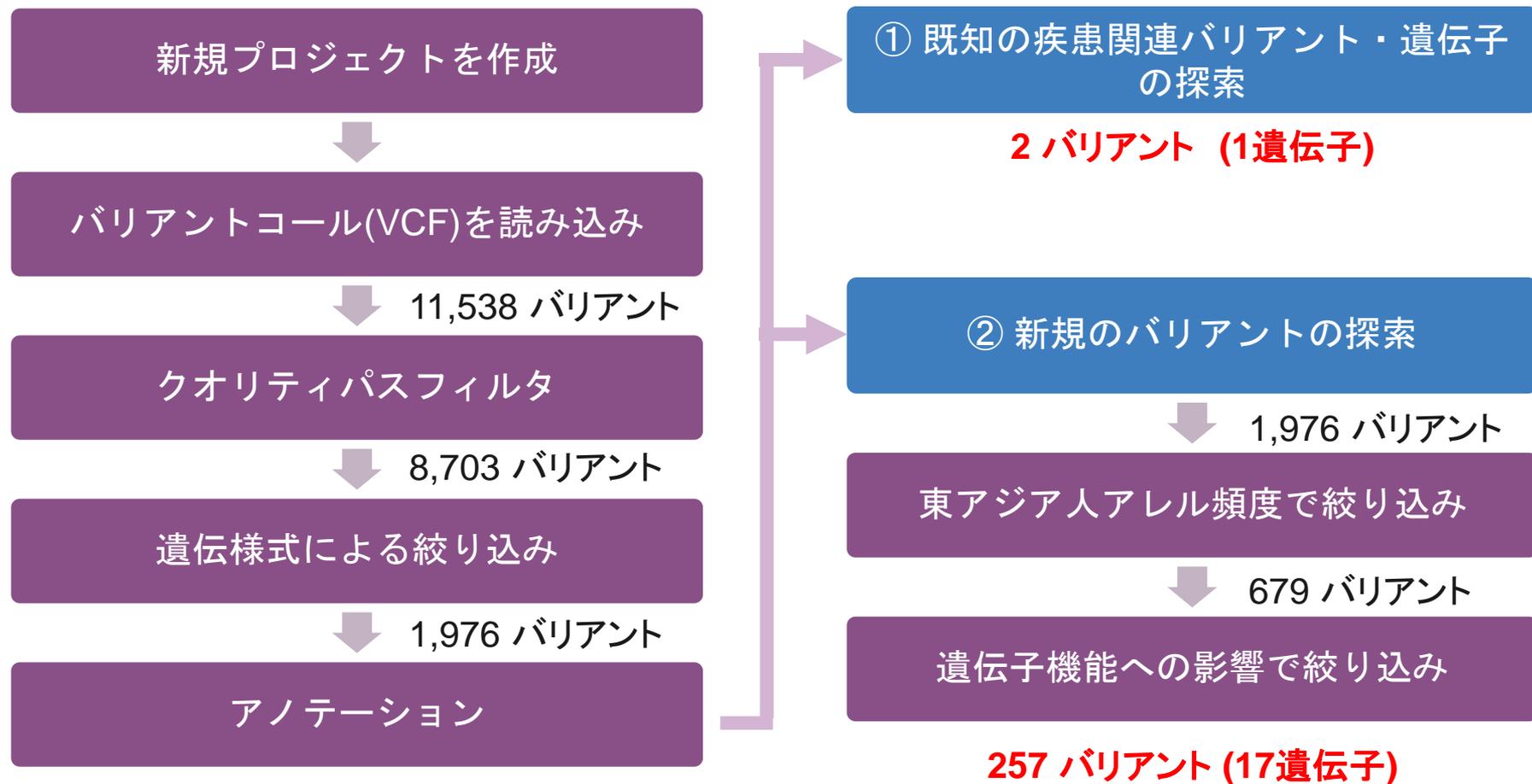
両親にヘテロ、子にホモの先天性疾患原因変異を導入



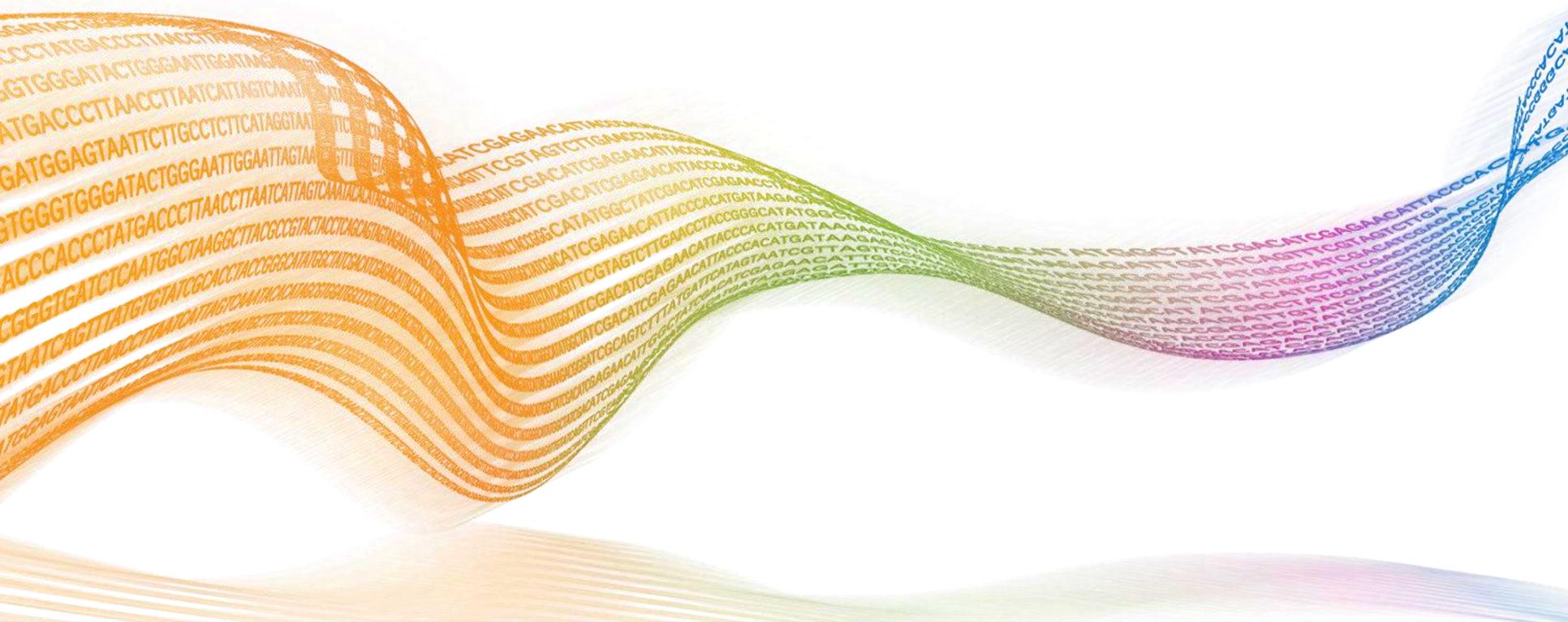
## トリオ解析の前提

1. 疾患は常染色体劣性遺伝と仮定。
2. 希少疾患で、原因変異はアレル頻度が低いと仮定。
3. 遺伝子上の変異で遺伝子機能に影響を与えると仮定。

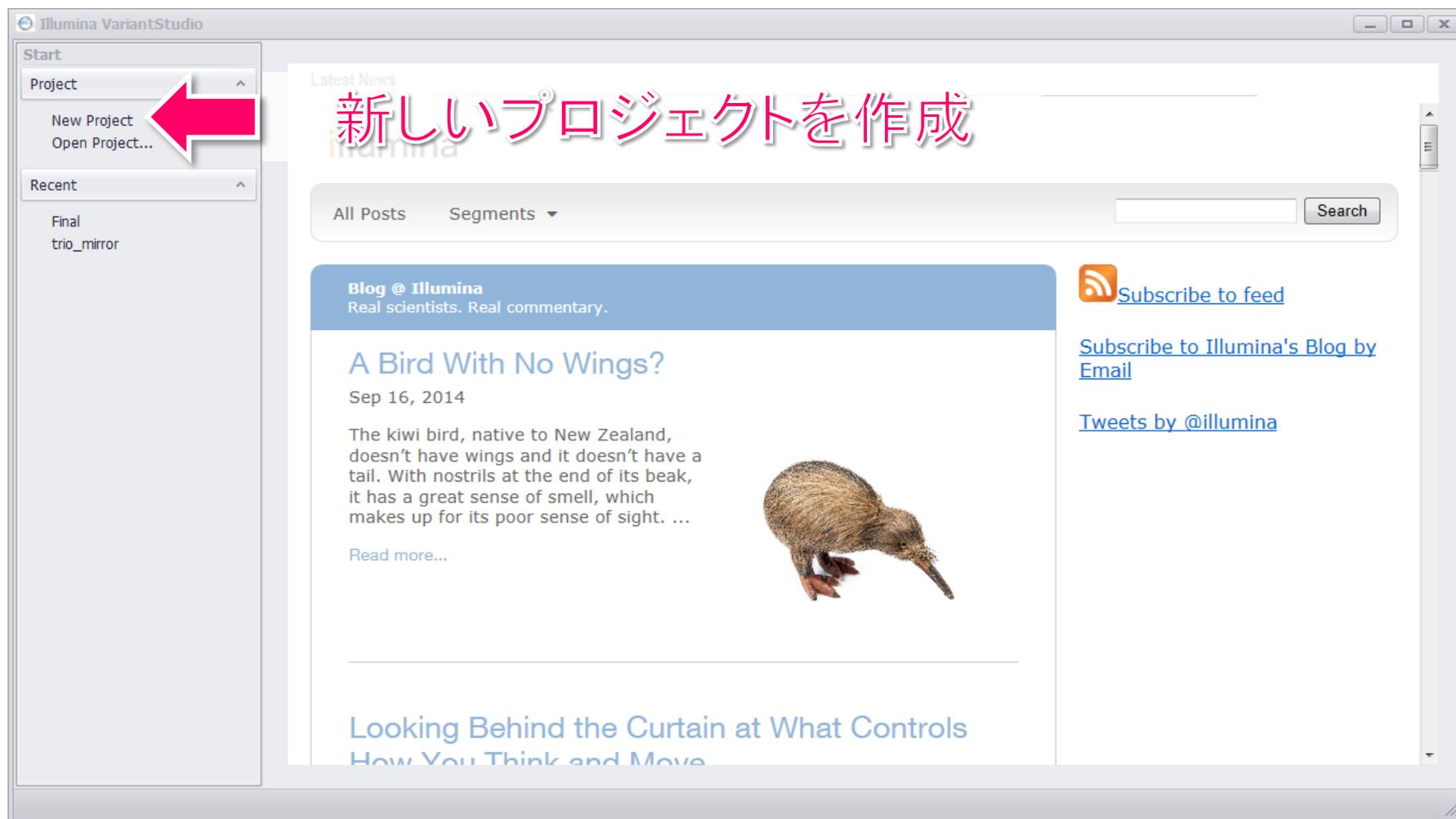
# VariantStudio解析ワークフロー例



# VariantStudioを使ってみよう



# 1. プロジェクトを作成する



Start

Project

New Project

Open Project...

Recent

Final

trio\_mirror

Latest News

## 新しいプロジェクトを作成

All Posts Segments

Search

**Blog @ Illumina**  
Real scientists. Real commentary.

### A Bird With No Wings?

Sep 16, 2014

The kiwi bird, native to New Zealand, doesn't have wings and it doesn't have a tail. With nostrils at the end of its beak, it has a great sense of smell, which makes up for its poor sense of sight. ...

[Read more...](#)



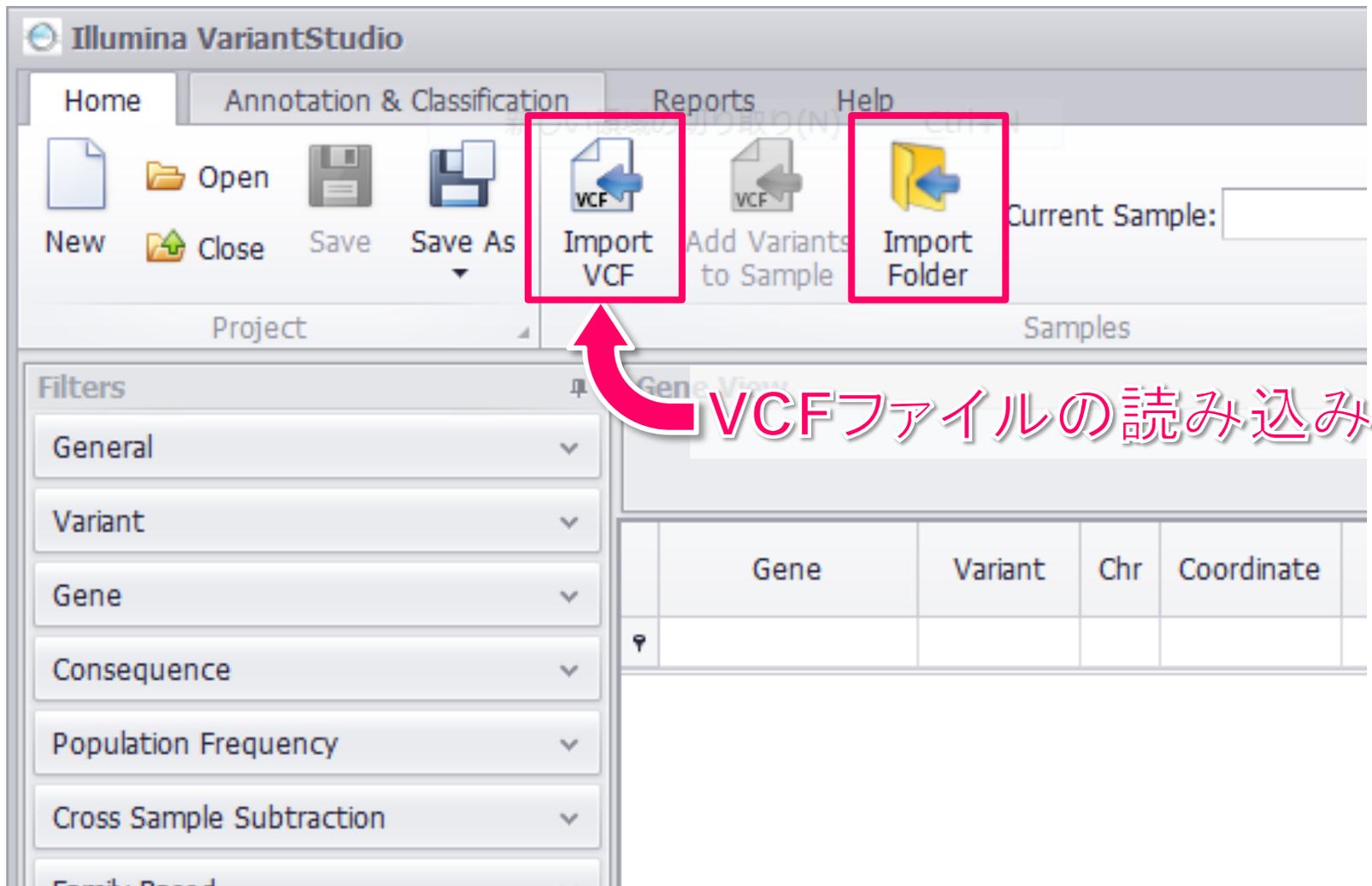
[Subscribe to feed](#)

[Subscribe to Illumina's Blog by Email](#)

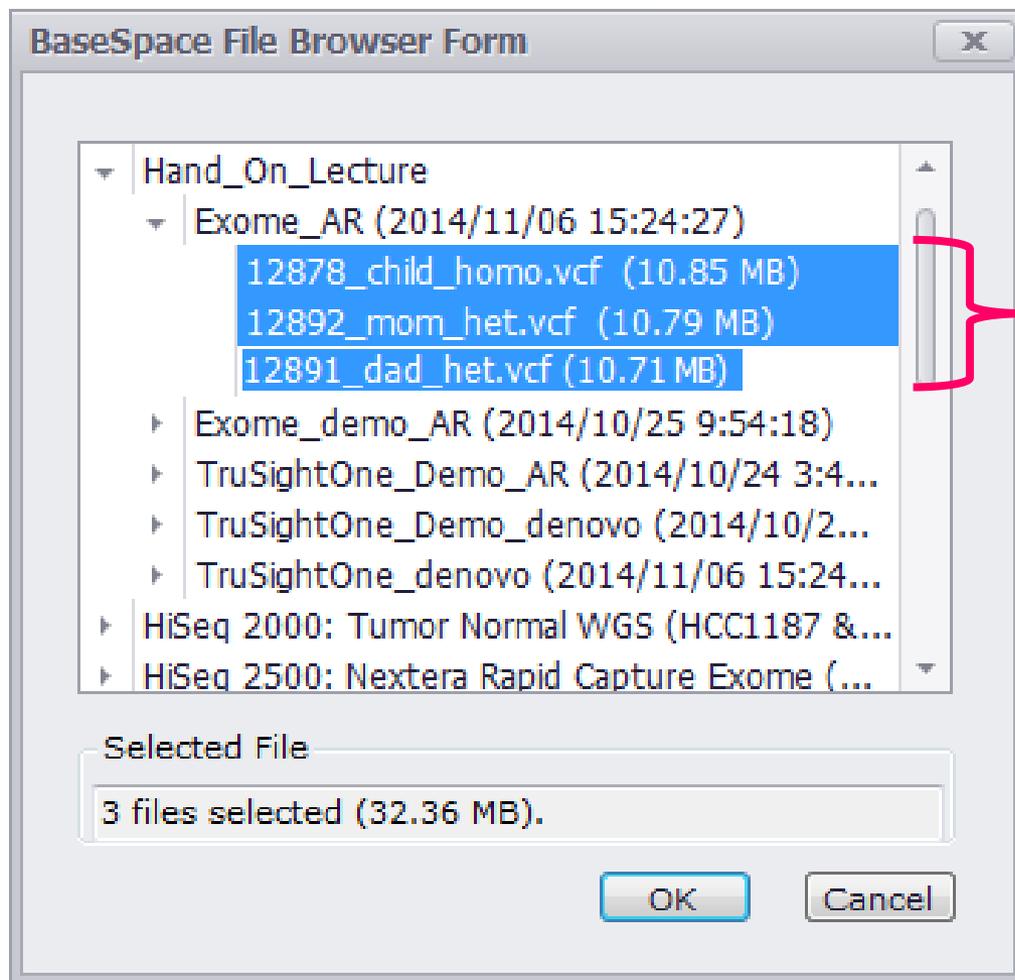
[Tweets by @illumina](#)

### Looking Behind the Curtain at What Controls How You Think and Move

## 2. サンプルVCFファイルの読み込み

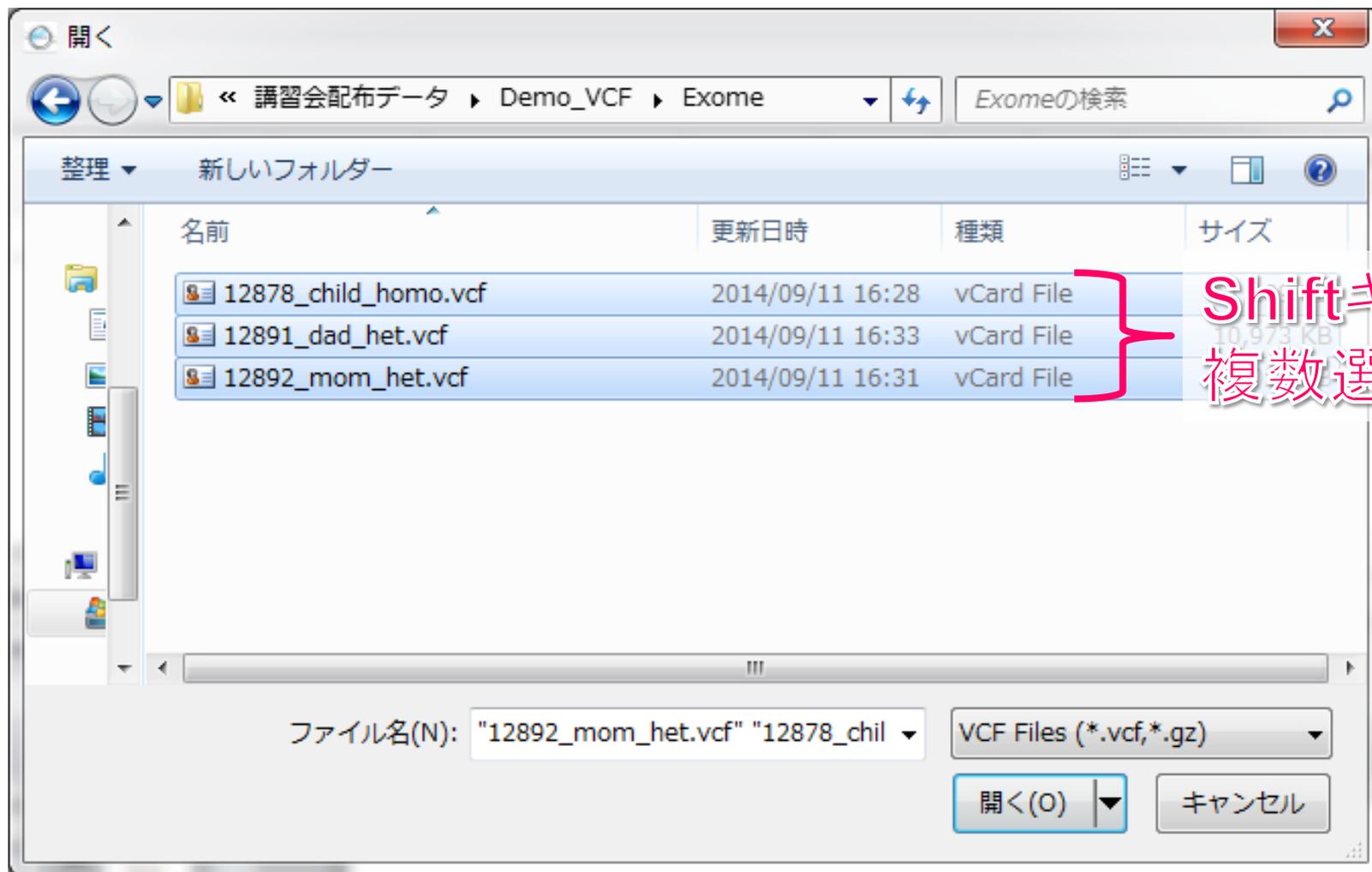


# クラウド版 : BaseSpaceからVCFファイルの読み込み



Shiftキーで  
複数選択可

# デスクトップ版 : VCFファイルの読み込み



## 2. サンプルVCFファイルの読み込み（オプション）

VCF Import Options

Please select which variants you would like to import from your VCF file(s)

All variants ← **全てのバリエーションを読み込む**

Variants in exons

Padding  bases

Variants in genes specified by gene list

Browse...

Variants in regions specified by BED file

Browse...

Load hom-ref positions

OK Cancel

## 2. 画面の見方

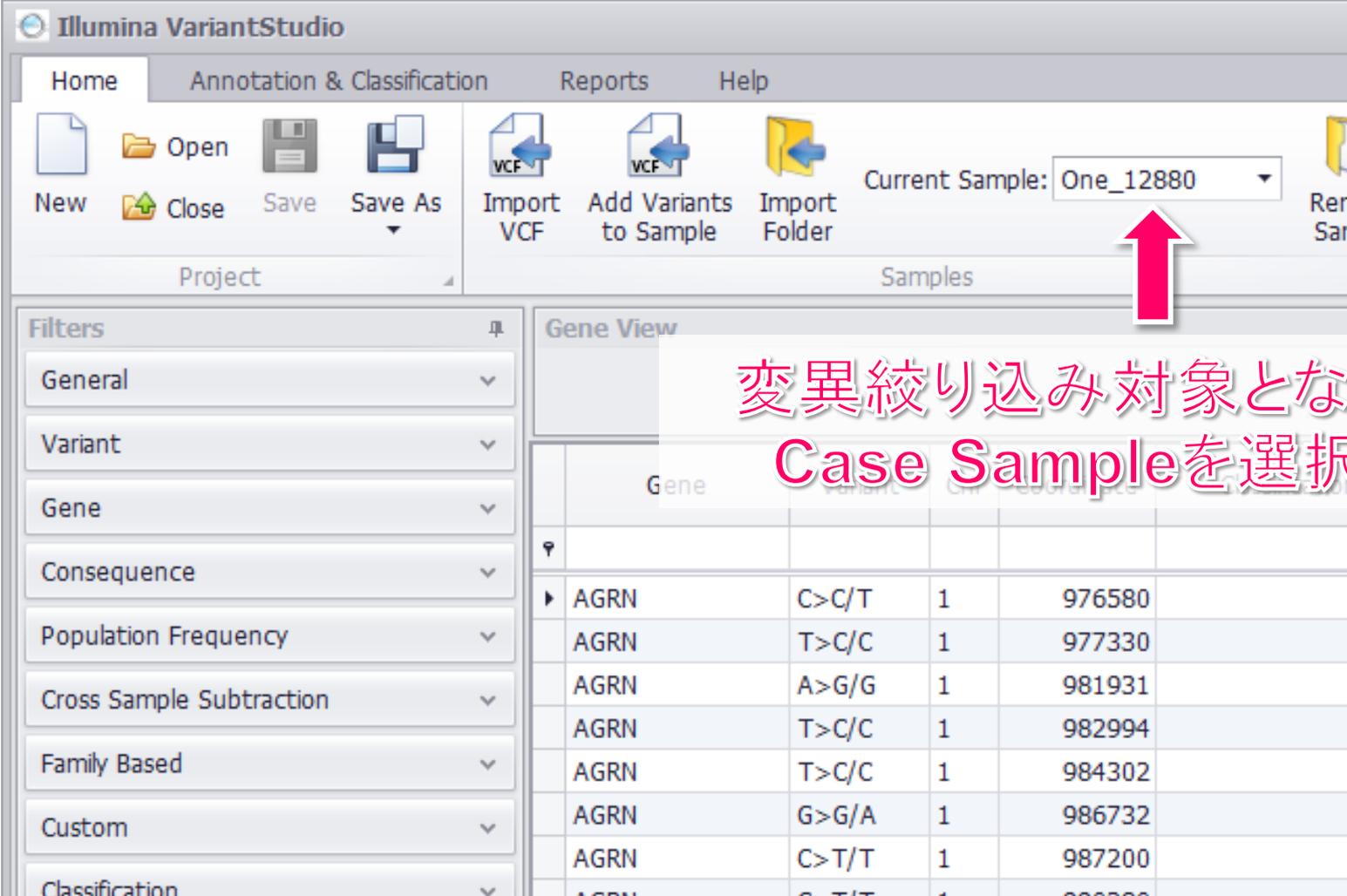
### プロジェクト管理、VCF読込、アノテーション、レポート作成

絞込みの設定

Gene	Variant	Chr	Coordinate	Classification	Type	Genot...	Exonic	Filters	Quality	GQX
AGRN	C>C/T	1	976580	...	snv	het	yes	PASS	345.29	99
AGRN	T>C/C	1	977330	...	snv	hom	yes	PASS	2880.59	99
AGRN	A>G/G	1	981931	...	snv	hom	yes	PASS	6800.75	99
AGRN	T>C/C	1	982994	...	snv	hom	yes	PASS	3549.67	99
AGRN	T>C/C	1	984302	...	snv	hom	yes	PASS	1659.51	95
AGRN	G>G/A	1	986732	...	snv	het	yes	PASS	1788.53	99
AGRN	C>T/T	1	987200	...	snv	hom	yes	PASS	3474.23	99
AGRN	C>T/T	1	990280	...	snv	hom	yes	PASS	3358.84	99
TNFRSF4	C>C/T	1	1147422	...	snv	het	yes	PASS	663.7	99
TAS1R3	G>G/A	1	1268987	...	snv	het	yes	PASS	802.16	99
TAS1R3	T>C/C	1	1269554	...	snv	hom	yes	PASS	4540.01	99
CDK11A	C>C/G	1	1647778	...	snv	het	yes	PASS	589.74	99
CDK11A	T>T/C	1	1647814	...	snv	het	yes	PASS	633.38	99
CDK11A	T>T/C	1	1647871	...	snv	het	yes	PASS	575.19	99

バリエント・遺伝子のリスト表示

## 2. サンプルごとのバリエーションリストを表示



The screenshot shows the Illumina VariantStudio interface. The 'Current Sample' dropdown menu is set to 'One\_12880', indicated by a red arrow. The 'Gene View' table displays the following data:

Gene	Variant	Count	Position
AGRN	C>C/T	1	976580
AGRN	T>C/C	1	977330
AGRN	A>G/G	1	981931
AGRN	T>C/C	1	982994
AGRN	T>C/C	1	984302
AGRN	G>G/A	1	986732
AGRN	C>T/T	1	987200
AGRN	C>T/T	1	990280

変異絞り込み対象となる  
Case Sampleを選択

## 2. サンプルごとのバリエーションリストを表示

Population Frequency  
Cross Sample Subtraction  
Family Based  
Custom  
Classification

Apply Filters =>  
Clear Filters

Filter History

Sample: One\_12880 Genes, Variants: (3034, 11536) -> (3034, 11536)

AGRN	C>C/T	1	976580
AGRN	T>C/C	1	977330
AGRN	A>G/G	1	981931
AGRN	T>C/C	1	982994
AGRN	T>C/C	1	984302
AGRN	G>G/A	1	986732
AGRN	C>T/T	1	987200
AGRN	C>T/T	1	990280
TNFRSF4	C>C/T	1	1147422
TAS1R3	G>G/A	1	1268987
TAS1R3	T>C/C	1	1269554
CDK11A	C>C	1	1647778
CDK11A	T>T	1	1647814
CDK11A	T>T	1	1647871

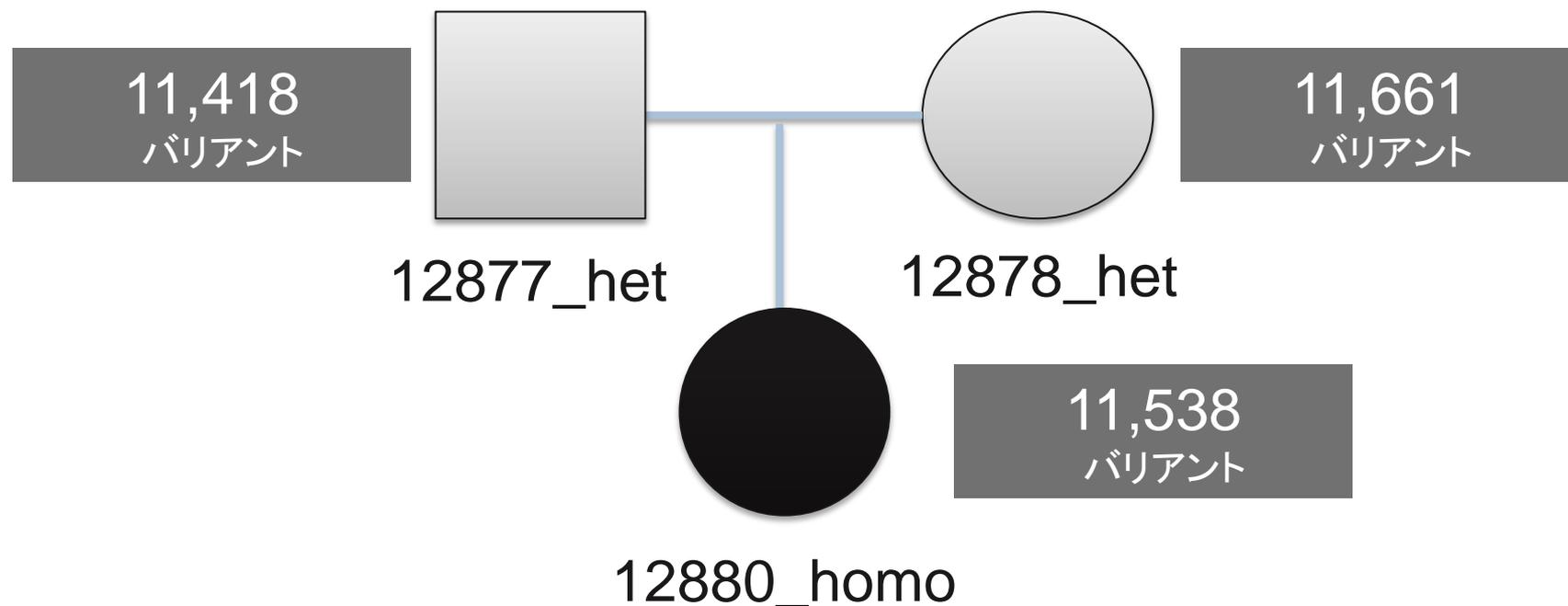
Variant 1 of 11536

Show Population Frequencies  Show Transcript Info

Variants Genes No-Call Regions

サンプルごとの遺伝子数、バリエーション数

# デモトリオサンプルのバリエーション数



Caseが有する11,538個のバリエーションから  
疾患原因候補遺伝子の絞り込みを行う

### 3. クオリティが良いバリアントの絞り込み

パスフィルタ  
バリアントを抽出

Gene	Variant	Chr	Coordinate	Classification	Type	Genot...	Exonic	Filters	Quality	GQX
ESPN	A>A/C	1	6505867	...	snv	het	yes	SB	5.39	99
ESPN	A>A/C	1	6505871	...	snv	het	yes	Low...	97.18	97
ESPN	A>A/C	1	6505872	...	snv	het	yes	SB	155.6	99
ESPN	T>T/C	1	6505874	...	snv	het	yes	Low...	12.41	32
ESPN	G>G/C	1	6505875	...	snv	het	yes	Low...	...	99
ESPN	A>A/C	1	6505881	...	snv	het	yes	Low...	...	95
TAS1R1	A>G/G	1	6635231	...	snv	hom	yes	PASS	1142.39	90
CAMTA1	C>C/T	1	7723588	...	snv	het	yes	PASS	910.21	99
CAMTA1	G>G/A	1	7723957	...	snv	het	yes	PASS	1451.6	99
CAMTA1	C>C/A	1	7724742	...	snv	het	yes	Low...	7.1	7
CAMTA1	A>A/G	1	7737799	...	snv	het	yes	PASS	561.81	99
PER3	T>C/C	1	7870048	...	snv	hom	yes	PASS	995.7	75
PER3	G>G/A	1	7887248	...	snv	het	yes	PASS	384.09	99

11,538 => 8,703 バリアント

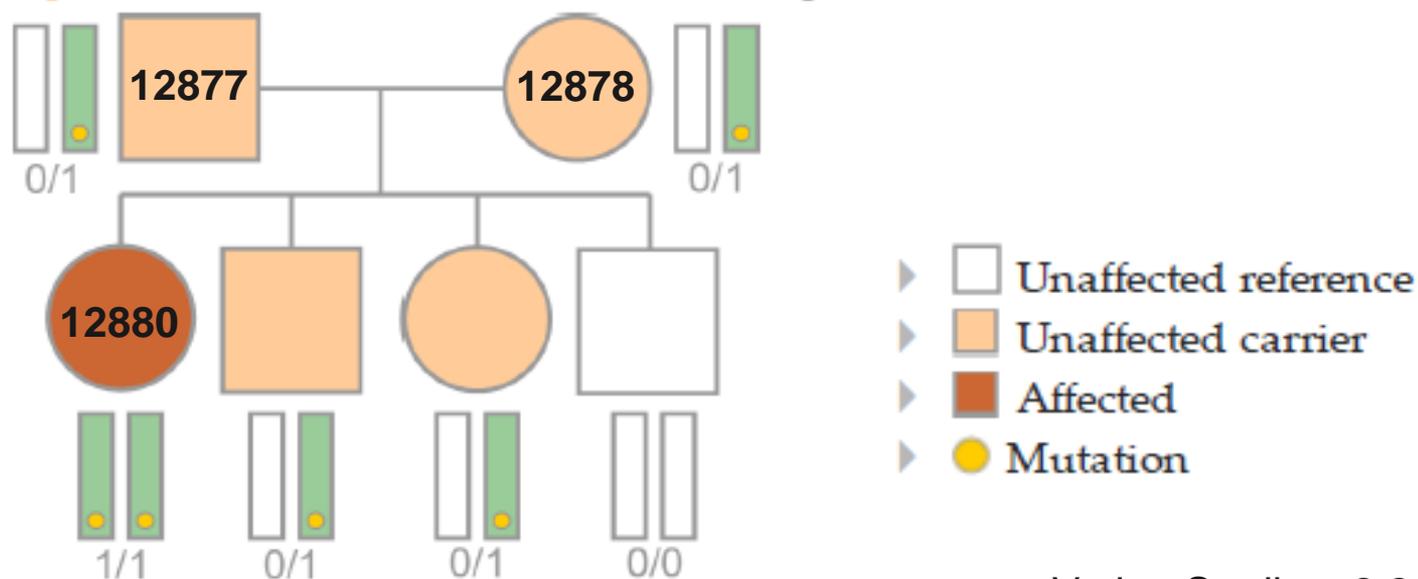
## 4. 遺伝様式での絞り込み（常染色体劣性遺伝）

### Autosomal Recessive Transmission Workflow

There are two possibilities for recessive transmission.

- 1 A single gene contains a variant that is:
  - Heterozygous (0/1) in the mother
  - Heterozygous (0/1) in the father
  - Homozygous (1/1) in the affected children

Figure 27 Autosomal Recessive Transmission Logic #1



VariantStudio v2.2  
Software User Guide

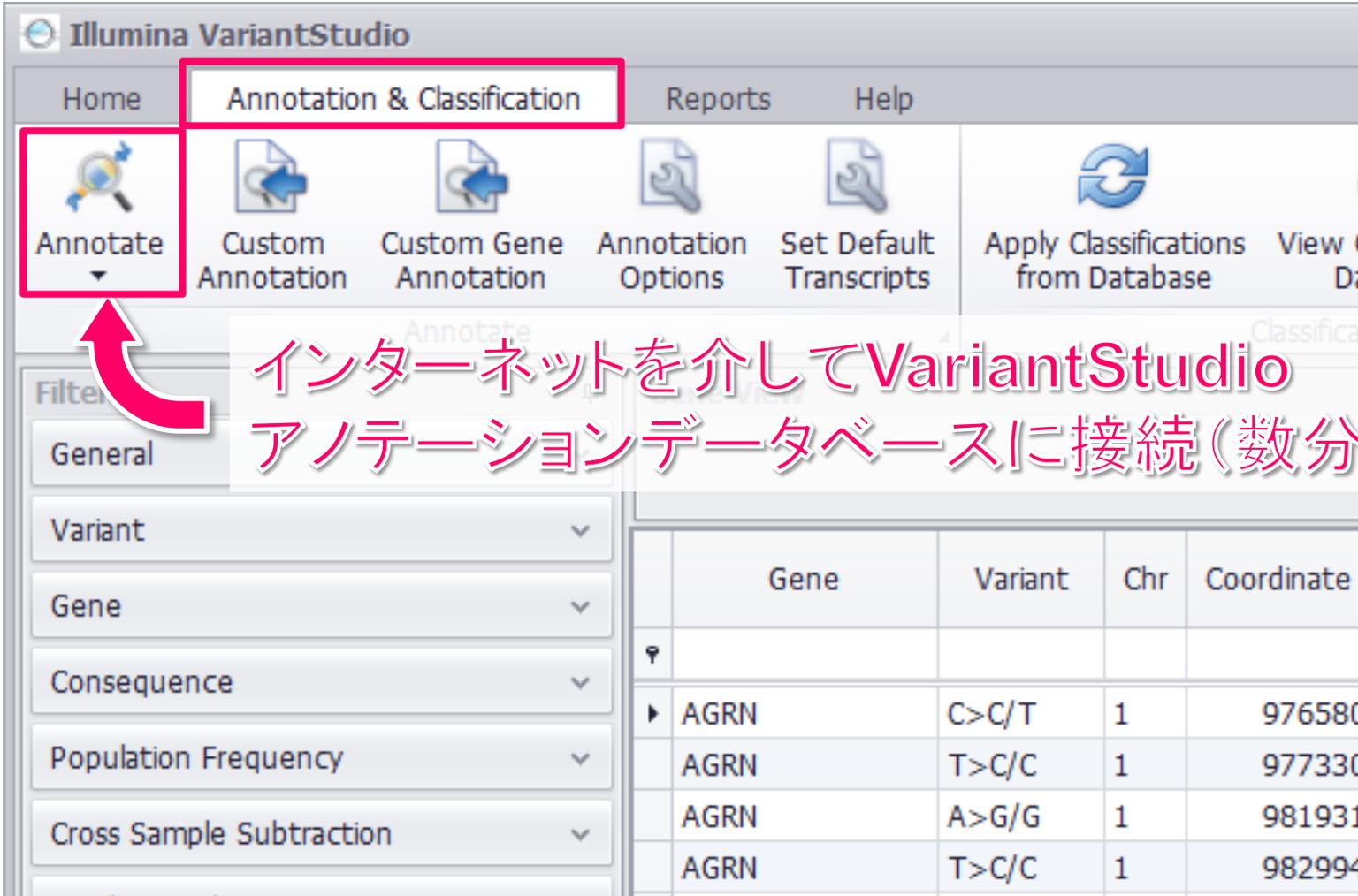
## 4. 遺伝様式での絞り込み（常染色体劣性遺伝）

“Autosomal Recessive”を選択  
父方、母方のサンプルIDを指定

Gene	Variant	Chr	Coordinate	Classification	Type	Genot...	Exonic	Filters	Qualit
AGRN	T>C/C	1	984302	...	snv	hom	yes	PASS	1659.
AGRN	G>G/A	1	986732	...	snv	het	yes	PASS	1788.
AGRN	C>T/T	1	987200	...	snv	hom	yes	PASS	3474.
AGRN	C>T/T	1	990280	...	snv	hom	yes	PASS	3358.
TNFRSF4	C>C/T	1	1147422	...	snv	het	yes	PASS	66:
TAS1R3	G>G/A	1	1268987	...	snv	het	yes	PASS	802.
TAS1R3	T>C/C	1	1269554	...	snv	hom	yes	PASS	4540.
CDK11A	C>C/G	1	1647778	...	snv	het	yes	PASS	589.
CDK11A	T>T/C	1	1647814	...	snv	het	yes	PASS	633.
CDK11A	T>T/C	1	1647871	...	snv	het	yes	PASS	575.

8,703 => 1,976 バリエント (445 遺伝子)

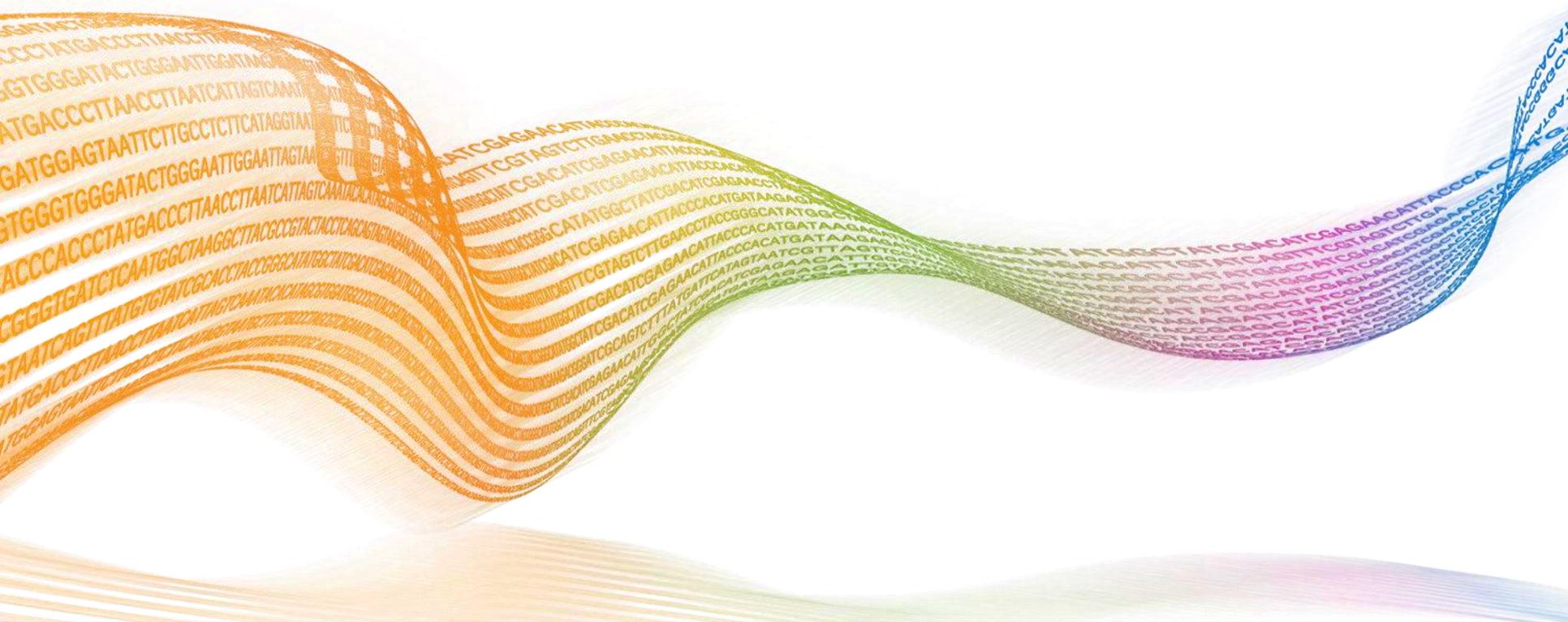
## 5. バリエーションへのアノテーション（意味付け）



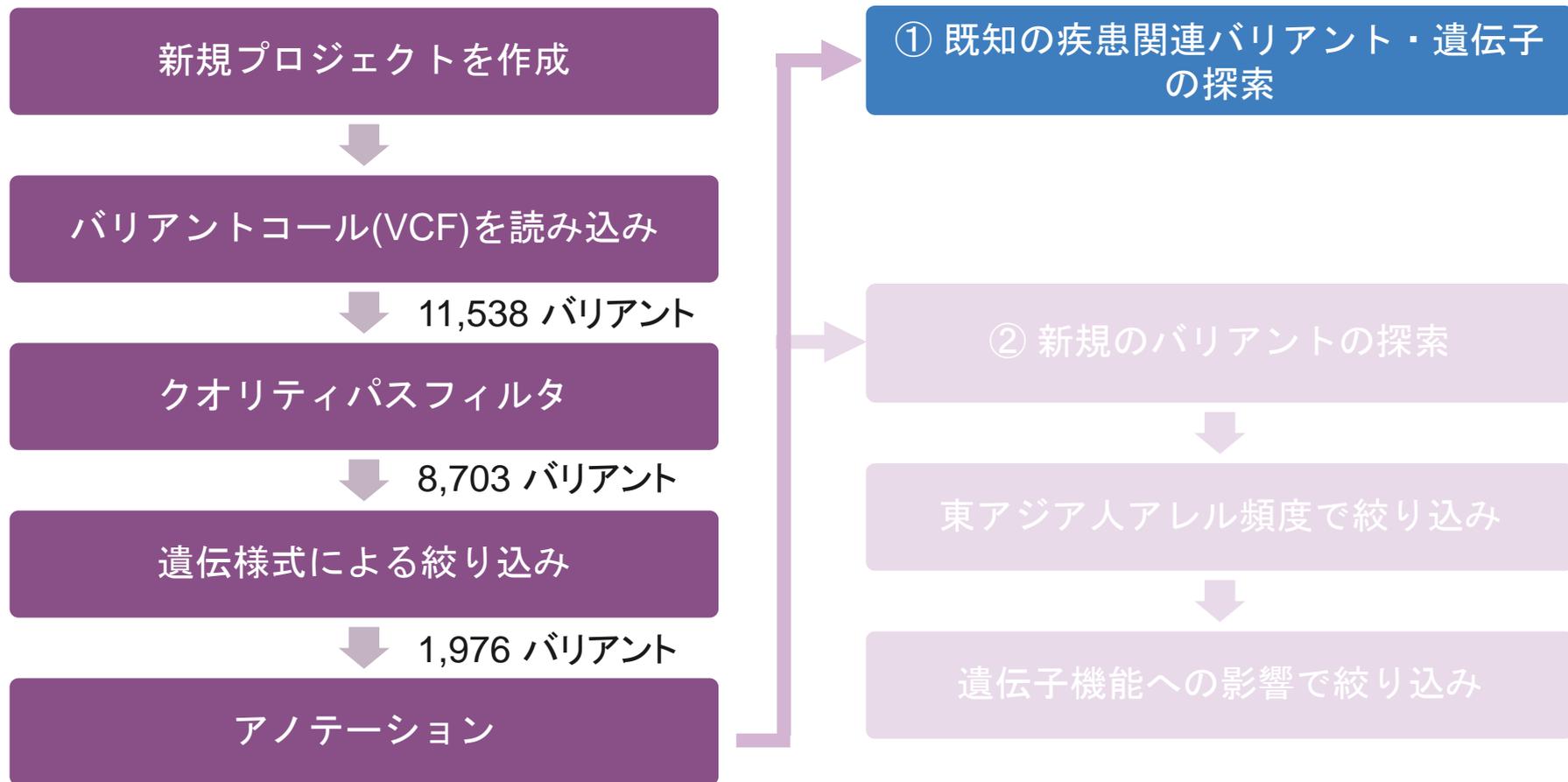
インターネットを介してVariantStudio  
アノテーションデータベースに接続(数分)

	Gene	Variant	Chr	Coordinate
▼				
▶	AGRN	C>C/T	1	976580
	AGRN	T>C/C	1	977330
	AGRN	A>G/G	1	981931
	AGRN	T>C/C	1	982994

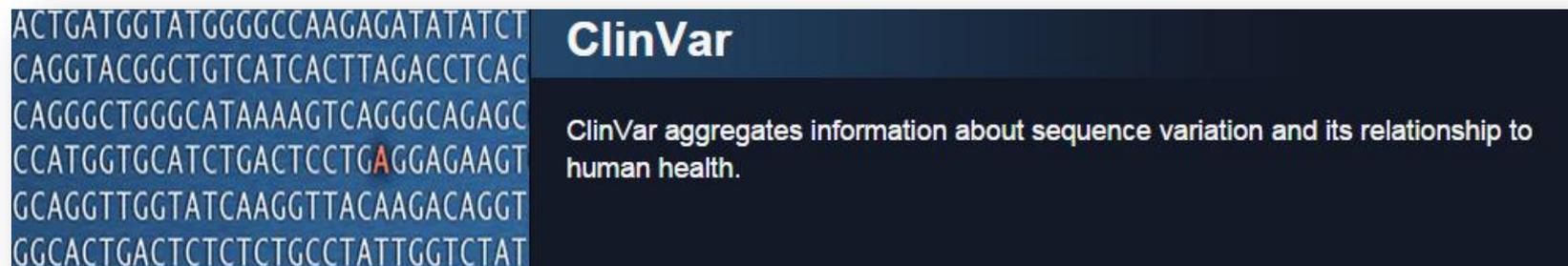
# 既知の疾患関連バリエーション・遺伝子の探索



# 既知の疾患関連バリエント・遺伝子の探索



# ClinVarを用いた疾患関連バリエーションの探索



**ClinVar**  
ClinVar aggregates information about sequence variation and its relationship to human health.

NIHのデータベース : <http://www.ncbi.nlm.nih.gov/clinvar/>

- ▶ 疾患と関連するバリエーションのデータベース
- ▶ CLIA承認あるいはISO1589のラボで実施された遺伝子研究として報告された臨床的に有意なバリエーション
- ▶ 研究プロジェクトの一部として、ヒトで同定されたバリエーション
- ▶ 論文から抽出した効果的なバリエーション（GWASは含まない）

# ClinVarに登録されるバリアントの絞り込み

Variant

Pass Filter

- Quality > 0
- Read Depth > 0
- Alt Variant Freq > 0

Show only variants:

- Inside genes
- In conserved regions
- Only variants without dbSNP ID
- Only variants with Cosmic annotation
  - where matches mutant allele
  - where not matches mutant allele
- Only variants with ClinVar annotation
  - where matches mutant allele
  - where not matches mutant allele

Apply Filters ==>

Gene	Variant	Chr	Coordinate	Classification	Type	Genot...	Exonic	Filters	Quality	GQX	Inherited From	Alt Var Freq
N/A	A>T/T	1	324822	...	snv	hom	no	PASS	2757.34	99	father	ε
N/A	A>T/T	1	187610852	...	snv	hom	no	PASS	3138.56	99	both	
N/A	A>G/G	1	187611003	...	snv	hom	no	PASS	2335.34	99	both	
N/A	T>G/G	1	202956340	...	snv	hom	no	PASS	2735.14	99	both	
N/A	T>C/C	1	219706428	...	snv	hom	no	PASS	1436.15	99	both	
N/A	A>G/G	2	3488502	...	snv	hom	no	PASS	1097.04	81	both	
N/A	G>C/C	2	10959475	...	snv	hom	no	PASS	1067.83	81	both	
N/A	A>G/G	2	131672950	...	snv	hom	no	PASS	2419.38	99	both	
N/A	C>T/T	2	131673316	...	snv	hom	no	PASS	431.14	36	both	
N/A	T>C/C	2	132229720	...	snv	hom	no	PASS	984.72	84	both	
N/A	C>G/G	3	136739406	...	snv	hom	no	PASS	588.87	45	both	
N/A	C>T/T	3	195467853	...	snv	hom	no	PASS	856.13	66	both	
N/A	A>G/G	3	5091384	...	snv	hom	no	PASS	1481.95	99	both	
N/A	T>C/C	4	5991384	...	snv	hom	no	PASS	5862.59	99	both	ε
N/A	T>C/C	4	5991384	...	snv	hom	no	PASS	1753.22	99	both	
N/A	A>G/G	9	10959475	...	snv	hom	no	PASS	1097.04	81	both	ε
N/A	A>G/G	9	10959475	...	snv	hom	no	PASS	1097.04	81	both	
N/A	A>G/G	9	10959475	...	snv	hom	no	PASS	1097.04	81	both	
N/A	G>A/A	6	32223632	...	snv	hom	no	PASS	1407.03	99	both	

ClinVarに登録される疾患関連バリアントを抽出

1,976 => 41 バリアント (20 遺伝子)

# ClinVarの定義によるバリエーションの分類

ASN.1 terms	ClinVar and VCF
0 – unknown	Uncertain significance
1 – untested	not provided (includes the cases where data are not available or unknown)
2 - non-pathogenic	Benign
3 - probable-non-pathogenic	Likely benign
4 - probable-pathogenic	Likely pathogenic
5 – pathogenic	Pathogenic
6 - drug-response	drug response
7 – histocompatibility	histocompatibility
255 - other	other
	confers sensitivity
	risk factor
	association
	protective

<http://www.ncbi.nlm.nih.gov/clinvar/docs/clinsig/>

- ▶ ClinVarの分類のうち”Pathogenic“（病原性の）バリエーションを絞り込む

# Pathogenicなバリアントの絞り込み

Gene View

Gene	Variant	Chr	Coordinate	ClinVar Allele Type	ClinVar Significance	ClinVar Disease Name
					pathogenic	
A2M	T>C/C	12	9232268	germline	other non-patho...	ALPHA-2-MACROGLOBULIN_POLYMORPHI.
ASPM	G>G/T	1	197070442		non-pathogenic	Primary_autosomal_recessive_microcephal.
ASPM	T>T/C	1	197070697		non-pathogenic	Primary_autosomal_recessive_microcephal.
ASPM	G>G/A	1	197070707		non-pathogenic	Primary_autosomal_recessive_microcephal.
ASPM	C>C/T	1	197070706		non-pathogenic	Primary_autosomal_recessive_microcephal.
ASPM	T>T/C	1	197072420		non-pathogenic	Primary_autosomal_recessive_microcephal.
ASPM	T>T/C	1	197072420		non-pathogenic	Primary_autosomal_recessive_microcephal.
ASPM	A>A/G	1	197097700		non-pathogenic	Primary_autosomal_recessive_microcephal.
ATP7B	C>C/T	13	52508979	germline	non-pathogenic	Wilson's_disease
ATP7B	G>G/A	13	52511606	germline	non-pathogenic	Wilson's_disease
ATP7B	A>A/G	13	52515354	germline	probable-non-pat...	Wilson's_disease
ATP7B	C>C/T	13	52520507	germline	non-pathogenic	Wilson's_disease

↑ ClinVar Significantカラム下に  
"Pathogenic"と入力

→ フィルターを編集

Contains([ClinVar Significance], 'pathogenic')

Variant 0 of 24

# Pathogenicなバリエアンの絞り込み

The image displays two overlapping windows of the 'フィルター エディタ' (Filter Editor) application. The top window shows a filter rule: 'And' followed by '[ClinVar Significance] Contains pathogenic'. A red arrow points to the word 'Contains'. The bottom window shows the same filter rule, but the operator has been changed to 'Equals', and the word 'Equals' is highlighted with a dashed border. A red box highlights the '適用(A)' (Apply) button at the bottom right of the bottom window. A red callout box with white text and a red arrow points to the 'Contains' operator in the top window, containing the text: '“Contains”をクリックして “Equals”に変更'.

フィルター エディタ

And

[ClinVar Significance] Contains pathogenic

フィルター エディタ

And

[ClinVar Significance] Equals pathogenic

OK(O) キャンセル(C) 適用(A)

“Contains”をクリックして  
“Equals”に変更

# Pathogenicなバリアントの絞り込み結果

Gene ▲	Variant	Chr	Coordinate	ClinVar Significance <sup>📶</sup>	ClinVar Disease Name	Inherited From
DHODH	G>G/A	16	72050942	pathogenic	Miller_syndrome	father
DHODH	C>C/T	16	72057435	pathogenic	Miller_syndrome	mother

✕  Contains([ClinVar Significance], 'pathogenic') ▼ フィルターを編集 ▼

 フィルタのキャンセルの仕方

41 => 2 バリアント (1 遺伝子)の絞り込みに成功

# 絞り込み履歴から過去の絞り込み条件を見る

The screenshot displays a software interface for filtering genomic data. On the left, there is a panel titled "Unaffected Siblings" with an empty text box and an "Edit Unaffected Siblings" button. Below this is a checkbox labeled "Use only passing variants in relatives" which is currently unchecked. Further down are two buttons: "Apply Filters =>" and "Clear Filters". At the bottom left of this panel, a button labeled "Filter History" is highlighted with a red rectangular box. To the right of the filter panel is a table with a list of gene symbols: CEP4, BARC, CDH1, EXO1, HBG1, SH3B, NPHS, MYO, FBP1, FUT6, ITGB, SPAT, AQP2, and PTC1. Below the table, there are navigation icons and the text "レコード 265 /". At the bottom of the interface, a status bar shows "Sample: One\_12880\_homo Genes, Variants: (3034, 11538) -> (445, 1976)".

Unaffected Siblings

Edit Unaffected Siblings

Use only passing variants in relatives

Apply Filters =>

Clear Filters

Filter History

Sample: One\_12880\_homo Genes, Variants: (3034, 11538) -> (445, 1976)

CEP4
BARC
CDH1
EXO1
HBG1
SH3B
NPHS
MYO
FBP1
FUT6
ITGB
SPAT
AQP2
PTC1

レコード 265 /

Variants Genes N

# 履歴の一覧から戻りたい絞り込み条件を選択

Filter History					
	Num Genes	Num Variants	Filter Name	Filter	Clear History
1	3034	11538		{}	View Apply
2	2821	8703		[ Filters.ToUpper == PASS ]	View Apply
3	445	1976		[ Recessive One_12878_het, One_12877_het AND Filters.ToUpper == PASS ]	View Apply
4	20	41		[ Recessive One_12878_het, One_12877_het AND Filters.ToUpper == PASS AND ClinVarID != ]	View Apply
5	1	2		[ GeneDisease contains 'Miller' AND Recessive One_12878_het, One_12877_het AND Filters.ToUpper == PA...	View Apply
6	445	1976		[ Recessive One_12878_het, One_12877_het AND Filters.ToUpper == PASS ]	View Apply

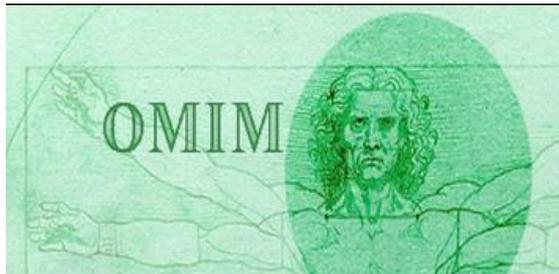
Filter History  
Sample: One\_12880\_homo Genes, Variants: (3034, 11538) -> (445, 1976)



Applyで過去の条件に戻る

3つ目レコードの「Apply」をクリックすることで  
アノテーション後の条件に戻る

# OMIMを用いた疾患関連遺伝子上のバリエーションの探索



**OMIM**

OMIM is a comprehensive, authoritative compendium of human genes and genetic phenotypes that is freely available and updated daily. OMIM is authored and edited at the McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, under the direction of Dr. Ada Hamosh. Its official home is [omim.org](http://omim.org).

NCBIのデータベース：<http://www.ncbi.nlm.nih.gov/omim>

Number of Entries in OMIM (Updated October 10th, 2014) :

Prefix	Autosomal	X Linked	Y Linked	Mitochondrial	Totals
* Gene description	13,963	683	48	35	14,729
+ Gene and phenotype, combined	88	2	0	2	92
# Phenotype description, molecular basis known	3,930	287	4	28	4,249
% Phenotype description or locus, molecular basis unknown	1,542	134	5	0	1,681
Other, mainly phenotypes with suspected mendelian basis	1,730	113	2	0	1,845
Totals	21,253	1,219	59	65	22,596

- ▶ 疾患や表現型と関連する遺伝子やゲノム情報のデータベース
- ▶ メンデル疾患、多因子疾患にも対応

# 遺伝子と関連する疾患名の表示

## OMIM由来アノテーション

The screenshot displays a table with columns for PubMed, GeneReviews, and Disease. The 'Disease' column is highlighted with a red box. Below the table, the 'Genes' tab is selected and highlighted with a red box and a red arrow. A red arrow also points from the text 'Genes' タブを選択' to the 'Genes' tab.

PubMed	GeneReviews	Disease
<a href="#">A2M</a>		Alzheimer's disease; Alzheimer disease, type 3; Alzheimer disease, type 4; Alz
<a href="#">ABCA12</a>	NBK1420	Autosomal recessive congenital ichthyosis 4B; Autosomal recessive congenita
<a href="#">ABCA3</a>		Disorder of lung; Pulmonary alveolar proteinosis; Respiratory distress; Congen
<a href="#">ABCA7</a>		
<a href="#">ABCB4</a>		Cholecystitis; Cholestasis of pregnancy; Jaundice, familial obstructive, of infan
<a href="#">ABCC9</a>	NBK1309	Atrial fibrillation familial; Primary dilated cardiomyopathy; Arrhythmogenic right
<a href="#">ABCG8</a>	NBK131810	Sitosterolemia; Gallbladder disease 4
<a href="#">ABO</a>		
<a href="#">ACACB</a>		
<a href="#">ACBD5</a>		
<a href="#">ACO2</a>		Infantile cerebellar-retinal degeneration
<a href="#">ACSF3</a>		Combined malonic and methylmalonic aciduria
<a href="#">ACVR1C</a>		
<a href="#">ADAMTS16</a>		
<a href="#">ADAMTS17</a>		Weill-Marchesani-like syndrome
<a href="#">ADAMTS2</a>		Ehlers-Danlos syndrome; Dermatosparaxis
<a href="#">ADARB1</a>		
<a href="#">ADCY9</a>		

Genes

Genes タブを選択

# 疾患名の入力による疾患関連遺伝子の絞り込み

The screenshot shows the Illumina VariantStudio interface. The 'Filters' panel on the left has a red box around the 'Gene' dropdown menu and a red arrow pointing to the 'Disease' input field, which contains the text 'Miller'. Below the input field are several filter options: 'Include List', 'Exclude List', 'Min Variant Alleles' (set to 2), and 'where custom gene annotation contains:'. The 'Gene View' table on the right displays a list of genes associated with the 'Miller' disease.

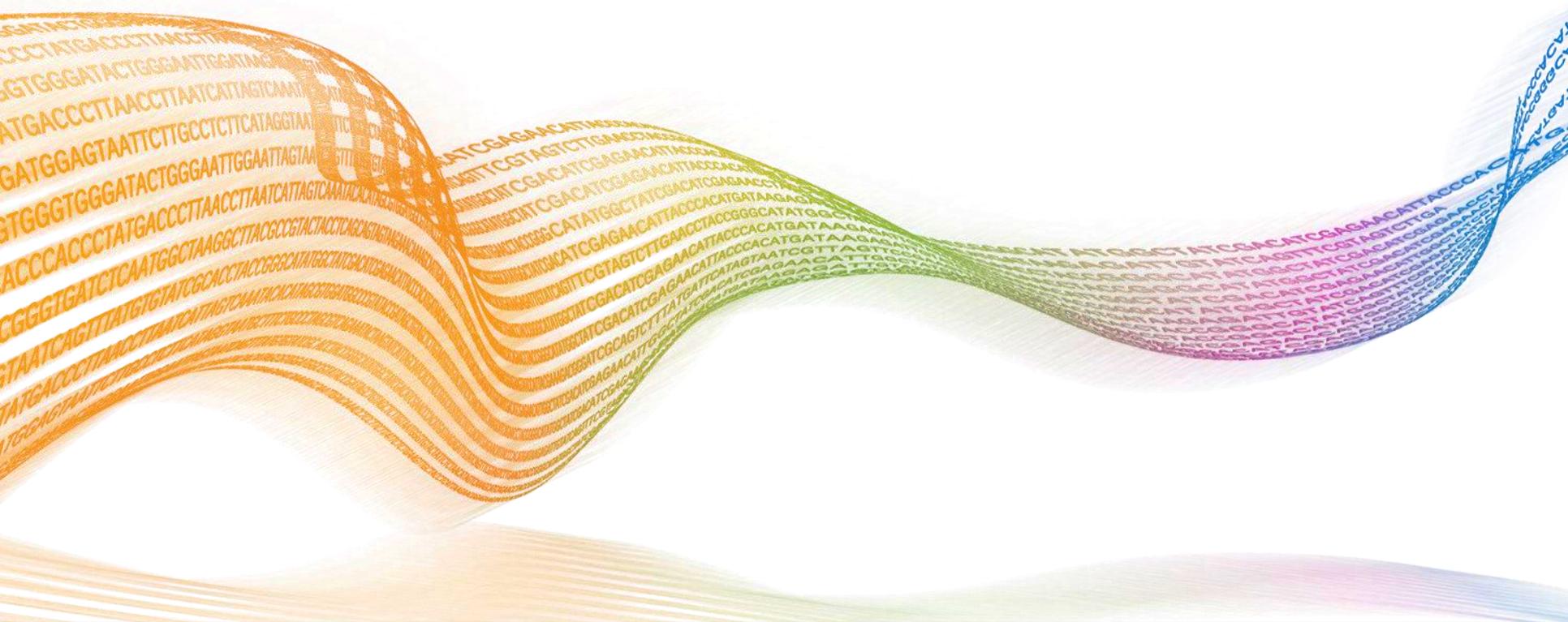
External Variants	Ambig... Variants	Custom Gene Annotation	PubMed	GeneReview
1	0		<a href="#">A2M</a>	
1	0		<a href="#">ABCA12</a>	NBK1420
1	0		<a href="#">ABCA3</a>	
1	5		<a href="#">ABCA7</a>	
2	0		<a href="#">ABCB4</a>	
1	0		<a href="#">ABCC9</a>	NBK1309
2	1		<a href="#">ABCG8</a>	NBK131810
1	0		<a href="#">ABO</a>	
1	0		<a href="#">ACACB</a>	
1	0		<a href="#">ACBD5</a>	
1	0		<a href="#">ACO2</a>	

# 既知の疾患関連遺伝子の絞り込み結果

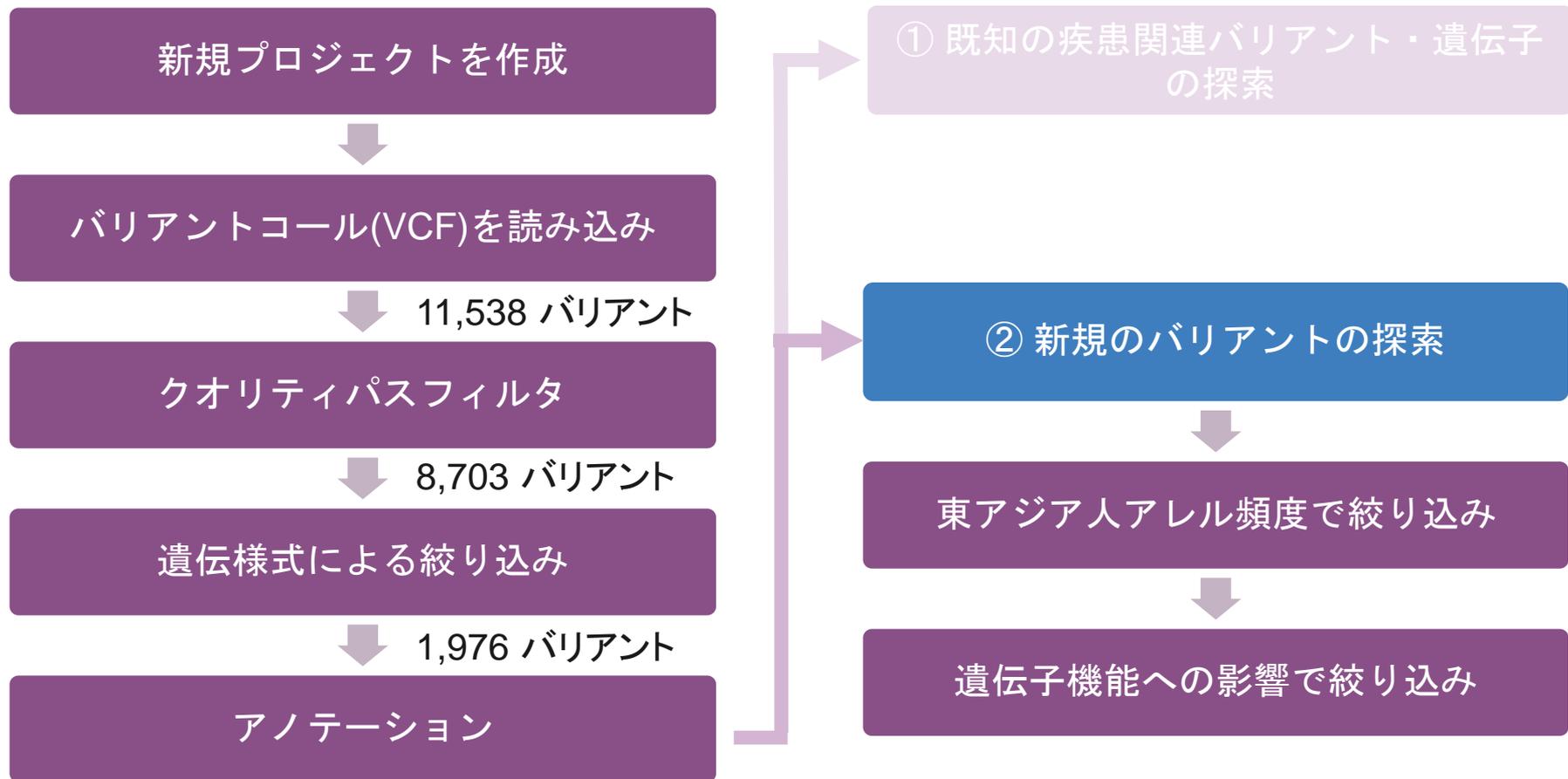
Gene ▲	Variant	Chr	Coordinate	ClinVar Significance <sup>?</sup>	ClinVar Disease Name	Inherited From
DHODH	G>G/A	16	72050942	pathogenic	Miller_syndrome	father
DHODH	C>C/T	16	72057435	pathogenic	Miller_syndrome	mother

1,976 => 2 バリエント (1 遺伝子)の絞り込みに成功

# 既知情報のない新規バリエーションの探索



## ② 既知情報のない新規バリエーションの探索



# 履歴の一覧から戻りたい絞り込み条件を選択

Filter History					
	Num Genes	Num Variants	Filter Name	Filter	Clear History
1	3034	11538		{}	View Apply
2	2821	8703		[ Filters.ToUpper == PASS ]	View Apply
3	445	1976		[ Recessive One_12878_het, One_12877_het AND Filters.ToUpper == PASS ]	View Apply
4	20	41		[ Recessive One_12878_het, One_12877_het AND Filters.ToUpper == PASS AND ClinVarID != ]	View Apply
5	1	2		[ GeneDisease contains 'Miller' AND RecessiveOne_12878_het, One_12877_het AND Filters.ToUpper == PA...	View Apply
6	445	1976		[ Recessive One_12878_het, One_12877_het AND Filters.ToUpper == PASS ]	View Apply

Filter History  
Sample: One\_12880\_homo Genes, Variants: (3034, 11538) -> (445, 1976)



Applyで過去の条件に戻る

3つ目レコードの「Apply」をクリックすることで  
アノテーション後の条件に戻る

## 6. アリル頻度が低いバリエーションの絞り込み

The screenshot shows the Illumina VariantStudio interface. The 'Filters' panel on the left has 'Population Frequency' selected in the 'Consequence' dropdown. The 'Global Frequency' is set to 100, 'Asian Pop Frequency' is set to 1, and 'EVS Frequency' is set to 100. The 'Apply Filters =>' button is highlighted with a red box. A red arrow points from the 'Asian Pop Frequency' dropdown to the text 'アジア集団1%以下'. The main table displays a list of variants with columns for Gene, Variant, Chr, Coordinate, Classification, Type, Genot..., Exonic, Filters, Quality, and GQX. The table shows 1976 variants before filtering and 679 variants after filtering. A dark grey box at the bottom of the screenshot contains the text '1,976 => 679 バリエーション'.

Gene	Variant	Chr	Coordinate	Classification	Type	Genot...	Exonic	Filters	Quality	GQX
N/A	A>T/T	1	324822	...	snv	hom	no	PASS	2757.34	9%
N/A	A>G/G	1	262956340	...	snv	hom	no	PASS	3138.56	9%
N/A	T>G/G	1	262956340	...	snv	hom	no	PASS	2735.14	9%
N/A	T>C/C	1	219706428	...	snv	hom	no	PASS	1436.15	9%
N/A	A>G/G	2	3488502	...	snv	hom	no	PASS	1097.04	8:
N/A	G>C/C	2	10959475	...	snv	hom	no	PASS	1067.83	8:
N/A	A>G/G	2	131672950	...	snv	hom	no	PASS	2419.38	9%
N/A	C>T/T	2	131673316	...	snv	hom	no	PASS	431.14	3%
N/A	T>C/C	2	132229720	...	snv	hom	no	PASS	984.72	8%
N/A	C>G/G	3	136739406	...	snv	hom	no	PASS	588.87	4%
N/A	C>T/T	3	195467853	...	snv	hom	no	PASS	856.13	6%
N/A	G>A/A	4	5990339	...	snv	hom	no	PASS	1481.95	9%
N/A	A>G/G	4	5990791	...	snv	hom	no	PASS	5862.59	9%
N/A	T>C/C	4	5991384	...	snv	hom	no	PASS	1753.22	9%

1,976 => 679 バリエーション

## 7. 遺伝子機能に影響を与えるバリエーションの絞り込み

- ▶ Polyphen: タンパク質の立体構造に影響を与えるインパクトが強いほど（疎水性など）Damagingとする。
- ▶ SIFT: タンパク質ファミリーで進化的に保存性が高いアミノ酸ほどDeleteriousとする。

# 7. 遺伝子機能に影響を与えるバリエントの絞り込み

保存性が高い領域あるいは立体構造に影響を与えるバリエントかどうか推定

Gene	Variant	Chr	Coordinate	Classification	Type	Genot...	Exonic	Filters	Quality	GQX
N/A									2757.34	9%
N/A								PASS	3138.56	9%
N/A								PASS	2335.34	9%
N/A								PASS	984.72	8%
N/A								PASS	856.13	6%
N/A								PASS	1705.04	5%
N/A								PASS	1171.69	6%
N/A								PASS	2212.65	9%
N/A	A>G/G	14	106610469	...	snv	hom	no	PASS	2889.52	9%
N/A	C>T/T	14	106610509	...	snv	hom	no	PASS	2252.86	9%
N/A	T>A/A	14	106610535	...	snv	hom	no	PASS	1835.49	8%
N/A	A>G/G	14	106791119	...	snv	hom	no	PASS	2861.8	9%
N/A	C>G/G	14	106815944	...	snv	hom	no	PASS	1078.98	8%
N/A	A>G/G	15	22482858	...	snv	hom	no	PASS	1779.39	9%
N/A	A>G/G	15	22483277	...	snv	hom	no	PASS	790.92	6%

679 => 257 バリエント (17遺伝子)



## 8. 原因バリエーションの絞り込みと検証例

### 1. 過去の文献や知見を調査

- 注目する遺伝子と関連する遺伝性疾患と類似性はあるか
- 代謝経路やシグナル伝達経路が疾患と関与ありそうか
- ノックアウトマウスの表現型が疾患と関連ありそうか

### 2. 技術的な検証

- サンガー法シーケンサーで検証

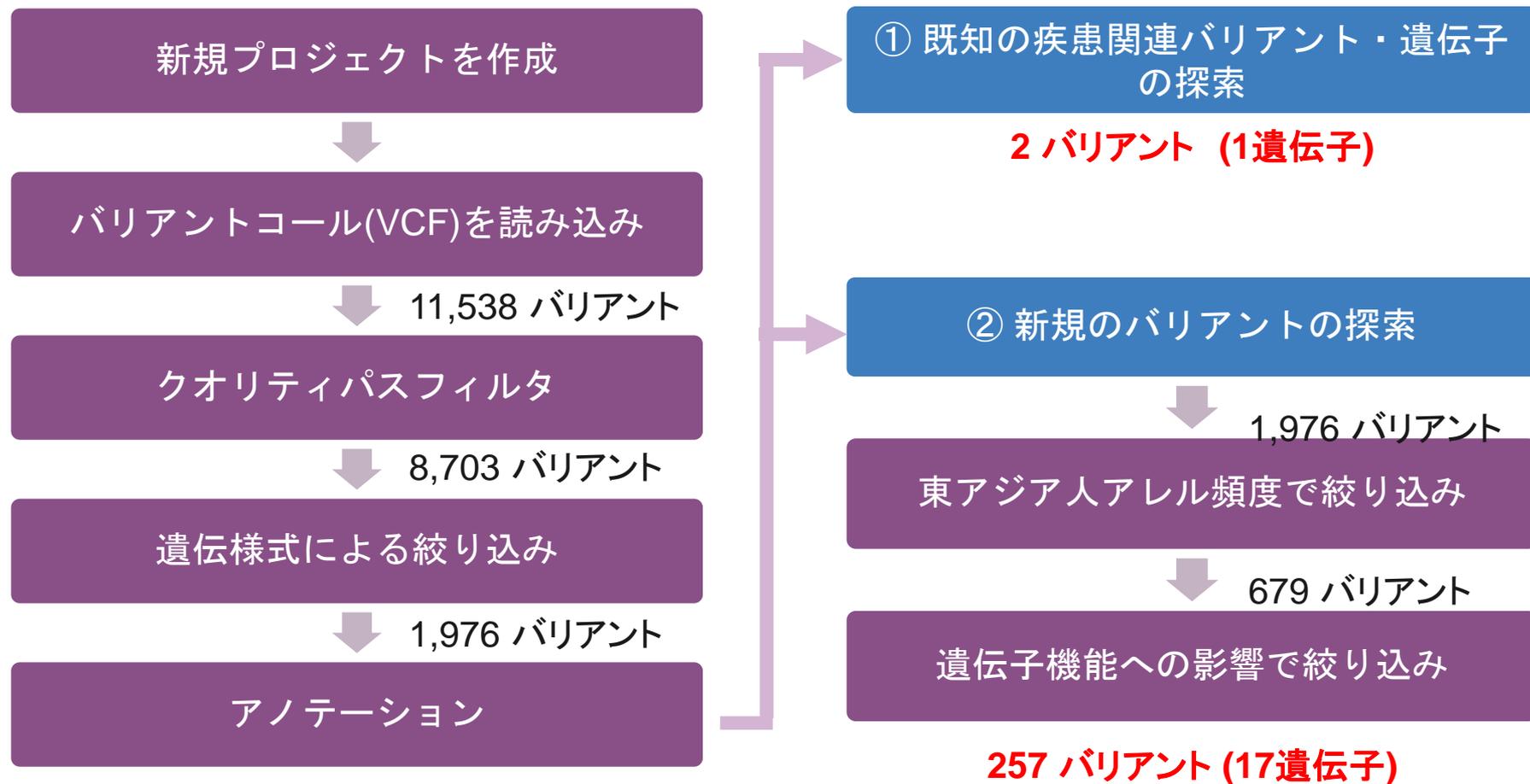
### 3. 再現性の検証

- 症例数を増やして再現性があるか検証

### 4. 生化学的実験による検証

- モデル生物を用いた遺伝子機能解析
- *in vitro* での遺伝子機能解析

# VariantStudio解析ワークフローまとめ



# 參考文獻一覽

- ▶ VEP v2.8 (Ensembl)
  - <http://asia.ensembl.org/info/docs/tools/vep/index.html>
  - McLaren W, Pritchard B, Rios D, Chen Y, Flicek P, Cunningham F. Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. **Bioinformatics** 26(16):2069-70(2010)
- ▶ PolyPhen
  - <http://genetics.bwh.harvard.edu/pph2/>
  - Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. **Nat Methods** 7(4):248-249 (2010).
- ▶ SIFT
  - <http://sift.jcvi.org/>
  - Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. **Nat Protoc.** 2009;4(7):1073-81.
- ▶ ClinVar Version September 5, 2013 (NCBI)
  - [www.ncbi.nlm.nih.gov/clinvar/](http://www.ncbi.nlm.nih.gov/clinvar/)
  - Landrum MJ *et al.* ClinVar: public archive of relationships among sequence variation and human phenotype. **Nucleic Acids Res.** 2014 Jan 1;42(1):D980-5.

# TruSight One その他 資料

- ▶ TruSight One User Guideなど  
[http://support.illumina.com/sequencing/sequencing\\_kits/trusight\\_one\\_kit/documentation.html](http://support.illumina.com/sequencing/sequencing_kits/trusight_one_kit/documentation.html)
- ▶ TruSight One Data Sheetなど  
[http://support.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/datasheet\\_trusight\\_one\\_panel.pdf](http://support.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/datasheet_trusight_one_panel.pdf)
- ▶ TruSight One 遺伝子一覧  
[http://products.illumina.com/content/dam/illumina-marketing/documents/products/gene\\_lists/gene\\_list\\_trusight\\_one.zip](http://products.illumina.com/content/dam/illumina-marketing/documents/products/gene_lists/gene_list_trusight_one.zip)
- ▶ TruSight One BaseSpace Public data  
<https://basespace.illumina.com/datacentral>

The screenshot shows the BaseSpace Public Data interface. The navigation bar includes Dashboard, Prep, Runs, Projects, Apps, Public Data, and Help. The main content area is titled 'Public Data' and features a search bar. Below the search bar, a list of data sets is displayed, each with a title and associated tags. Two entries are highlighted with red boxes:

- > **TruSight One Sequencing Panel MiSeq Trio Data**  
Targeted Sequencing
- > **NextSeq 500: TruSight One (CEPH Trio replicates)**  
Targeted Sequencing

ご清聴ありがとうございました!

本日セッション終了後のご質問は、  
[techsupport@illumina.com](mailto:techsupport@illumina.com)にお問い合わせください

テクニカルサポート直通のフリーダイヤルも  
ご利用くださいませ。

[0800-111-5011](tel:0800-111-5011)