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# The Genetic Landscape of Moyamoya Disease in a Multi-Ethnic Cohort

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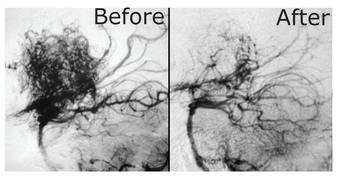
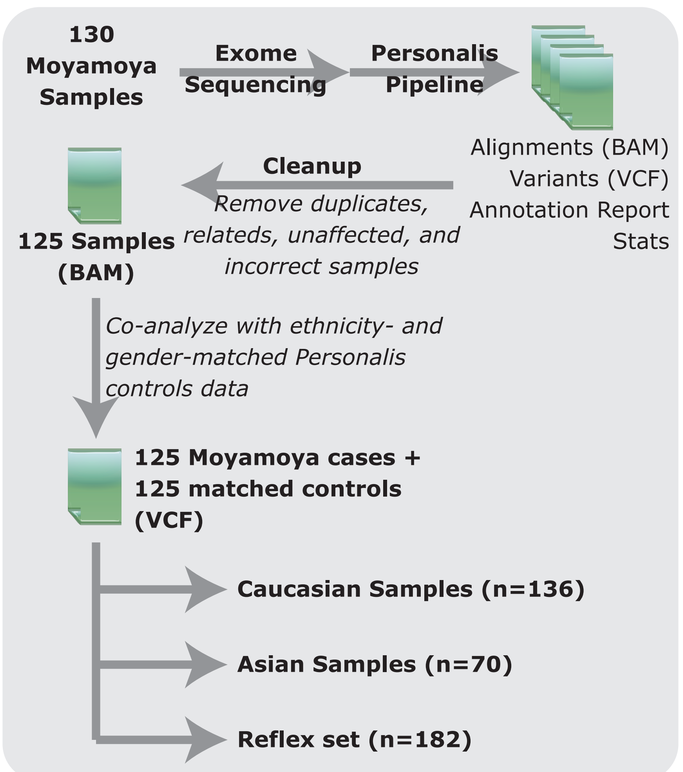
## What is Moyamoya Disease?




Moyamoya Disease (MMD) is a rare cerebrovascular disease typically displaying a dominant low-penetrance mode of inheritance. "Moyamoya" means "puff of smoke" in Japanese, and in the context of MMD, it refers to abnormal vessels, which appear as a cloud of smoke in cerebral angiograms, resulting from progressive occlusion of the vessels of the Circle of Willis . The Moyamoya vessels are prone to hemorrhage and aneurism, and can lead to transient ischemic attacks, devastating strokes, headaches, paralysis, and other neurological symptoms. MMD is treatable by direct revascularization (STA-MCA bypass), which has greater than 95 percent graft patency and excellent long-term outcomes.

## Personalis Genomic Interpretation Service Case Control Study

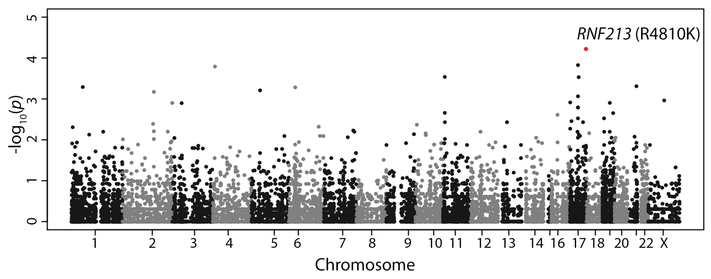



High-depth whole exome sequencing was performed on 125 ethnically diverse, unrelated, non-familial MMD patients and 125 sex- and ethnicity-matched controls from the Personalis Ethnic Controls Database. Samples were divided into two large ethnic subsets based on self-declared ethnicities: an Asian subset (including East Asian, Southeast Asian and Pacific Islander samples, n=70) and a Caucasian subset (including individuals declaring ethnicity from all over Europe, n=136). After identifying a founder mutation in a subset of samples, a third panethnic subset was created consisting of cases lacking the founder mutation and matched controls (n=182).

## Personalis Large-Scale Comparative Genomic Analysis

Each of the three matched case-control studies was analyzed independently in order to determine variant and gene associations with MMD. Due to variance in coverage around targets in exome sequencing, we removed variants if they had low quality according to our variant calling algorithm or if the variant could not be called in more than 30% of the samples. Filtered variants were assessed by Fisher's Exact Test and then further filtered for effect on protein coding, minor allele frequency (<5%), and enrichment in cases.

## A Founder Mutation in the Asian Population

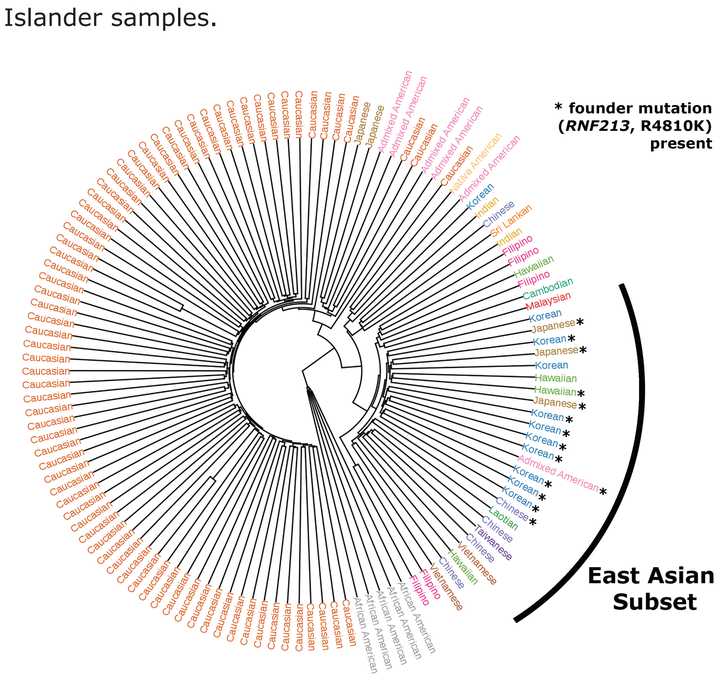



Among Asian cases, the most significant variant was found in the *RNF213* gene ($p=6.01 \times 10^{-5}$). This mutation, rs112735431, causes a nonsynonymous coding change, R4810K. This finding validates previous work by Kamada et al. (2011), who had previously identified the same variant as a founder mutation in the Japanese population, and which had subsequently been found in Chinese and Korean individuals with Moyamoya disease as well.

The *RNF213* (R4810K) mutation was observed in 73% of self-declared Korean, 60% of self-declared Japanese, and 17% of self-declared Chinese cases. It was not identified in any of the 125 controls samples. However, we also observed the mutation in one self-declared Hawaiian and one self-declared Admixed American (Hispanic) case.

## Ethnic Breakdown of Cases Reveals Founder Mutation Specific to East Asians

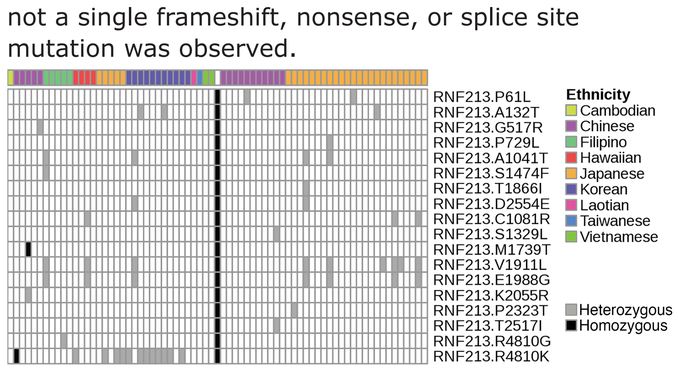
Previous studies of the *RNF213* (R4810K) founder mutation were performed in East Asia and found the variant highly enriched in MMD individuals of East Asian descent. Our study was performed in the United States on a cohort of various ethnicities. Because we found an Admixed American and Hawaiian sample with the *RNF213* (R4810K) founder mutation we asked whether it was possible these individuals were genetically more similar to East Asians (specifically Chinese, Japanese, Korean, and Taiwanese) than to other Admixed American or Pacific Islander samples.




We clustered all 125 case samples based on genetic similarity. Generally, samples with the same self-declared ethnicity or ethnic backgrounds from the same geographic region were more genetically similar. We observed that all samples bearing the *RNF213* founder mutation tightly clustered in a block containing most of the self-declared East Asian samples, and including two Hawaiian and one Admixed American sample. The founder mutation was not present in any samples outside this East Asian block. Of particular note, none of the 74 Caucasians, 5 African Americans, 4 non-Asian Admixed Americans or 3 South Asians had the founder mutation either.

## A Survey of *RNF213* Mutations in Moyamoya Disease

In total, we identified 142 mutations in the protein coding sequence of *RNF213* across 125 MMD cases. Only 12 of those mutations have been reported previously in Moyamoya studies, 44 of them had not been observed by the 1000 Genomes Project, and 29 of them were absent from dbSNP137. We identified 67 rare (<5% population frequency), protein-coding variants. Interestingly, all 81 nonsynonymous *RNF213* variants in MMD cases were missense mutations and not a single frameshift, nonsense, or splice site mutation was observed.




In the above figure, we show missense mutations in *RNF213* detected in our Asian subset. Note that R4810K is the founder mutation and that we did capture a homozygous individual of Chinese descent with this mutation. Also of interest, a Filipino individual had a different mutation at the same position (R4810G). No mutations at this position were detected in any other individuals, and no other founder mutations were observed in any other population.

## Moyamoya-Associated Mutations and Genes

| Asian Subset | Caucasian Subset | Reflex Subset |
|---|---|---|
| (N=70) | (N=136) | (N=182) |
| *Filtering* | *Filtering* | *Filtering* |
| **607,084 variants** | **738,220 variants** | **904,223 variants** |
| *Fisher p<0.0001* | *Fisher p<0.0001* | *Fisher p<0.0001* |
| **519** | **1,109** | **1,589** |

Our variant enrichment analysis using Fisher's Exact test with a p-value cut-off of p<0.0001 distilled down each candidate variant list to less than 0.2% of total variants. One variant each in the Asian and Caucasian sets reached genome-wide significance. The *RNF213* founder mutation was the variant in the Asian set. In the Caucasian set, it was a previously undescribed variant in the *ZXDC* gene. However, population frequency and a lower overall represenation in cases suggests that it is much lower penetrance than the *RNF213* founder mutation if it is indeed causative.

| Asian Subset | Caucasian Subset | Reflex Subset |
|---|---|---|
| 607,084 variants | 738,220 variants | 904,223 variants |
| *Filtering & Collapsing* | *Filtering & Collapsing* | *Filtering & Collapsing* |
| **24 Genes** | **25 Genes** | **35 Genes** |

We also used the Combined Multivariate and Collapsing (CMC) method to collapse variants into genes and look for genes enriched for mutation in our case/control sets. While the Asian population was enriched for *RNF213* mutations, this was due to the presence of the founder mutation. Removing the samples with the founder mutation also dropped *RNF213* from the candidate list, calling into question what its role is in the development of Moyamoya Disease.

### Other Personalis Posters

Chandratillake et al., **2639W**, Weds., Oct. 23
Li et al., **1642T**, Thurs., Oct 24
Garcia et al., **1550F**, Fri., Oct 25
Pratt et al., **2608F**, Fri., Oct 25