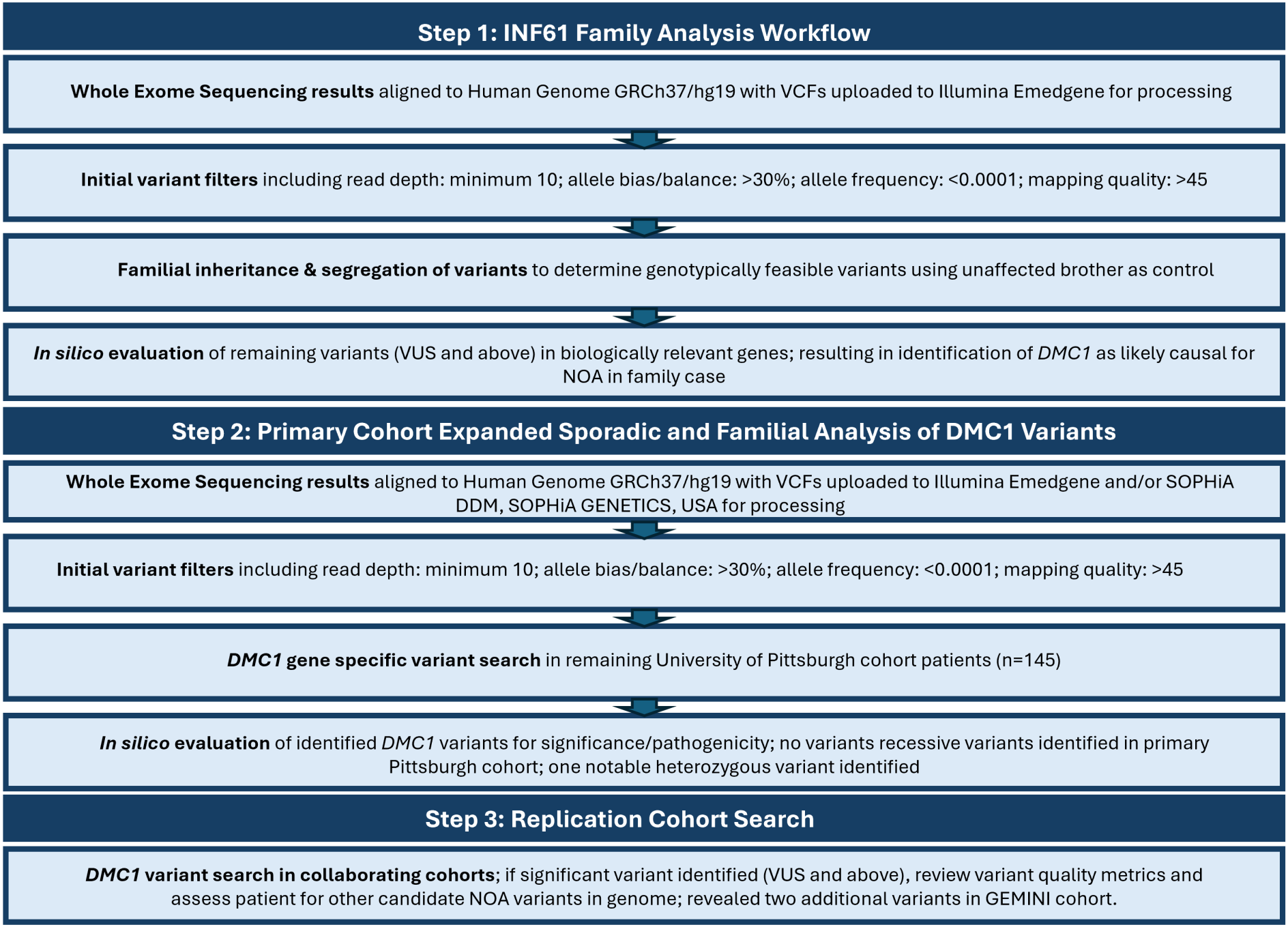
Step 1: INF61 Family Analysis Workflow
Whole Exome Sequencing results aligned to Human Genome GRCh37/hg19 with VCFs uploaded to Illumina Emedgene for processing
Initial variant filters including read depth: minimum 10; allele bias/balance: >30%; allele frequency: <0.0001; mapping quality: >45
Familial inheritance & segregation of variants to determine genotypically feasible variants using unaffected brother as control
In silico evaluation of remaining variants (VUS and above) in biologically relevant genes; resulting in identification of DMC1 as likely causal for NOA in family case
Step 2: Primary Cohort Expanded Sporadic and Familial Analysis of DMC1 Variants
Whole Exome Sequencing results aligned to Human Genome GRCh37/hg19 with VCFs uploaded to Illumina Emedgene and/or SOPHiA DDM, SOPHiA GENETICS, USA for processing
Initial variant filters including read depth: minimum 10; allele bias/balance: >30%; allele frequency: <0.0001; mapping quality: >45
DMC1 gene specific variant search in remaining University of Pittsburgh cohort patients (n=145)
In silico evaluation of identified DMC1 variants for significance/pathogenicity; no variants recessive variants identified in primary Pittsburgh cohort; one notable heterozygous variant identified
Step 3: Replication Cohort Search
DMC1 variant search in collaborating cohorts; if significant variant identified (VUS and above), review variant quality metrics and assess patient for other candidate NOA variants in genome; revealed two additional variants in GEMINI cohort.