

Association Analysis using PLINK

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File formats: VCF

Tab delimited text file with a header section

```
##fileformat=VCFv4.2
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,assembly=B36,md5=f126cdf8a6e0c7f379d618ff66beb2da,species="Homo sapiens",taxonomy=x>
##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT NA000001 NA000002 NA000003
20 14370 rs6054257 G A 29 PASS NS=3;DP=14;AF=0.5;DB;H2 GT:GQ:DP:HQ 0/0:48:1:51,51 1/0:48:8:51,51 1/1:43:5:..
20 17330 . T A 3 q10 NS=3;DP=11;AF=0.017 GT:GQ:DP:HQ 0/0:49:3:58,50 0/1:3:5:65,3 0/0:41:3
20 1110696 rs6040355 A G,T 67 PASS NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ 1/2:21:6:23,27 2/1:2:0:18,2 2/2:35:4
20 1230237 . T . 47 PASS NS=3;DP=13;AA=T GT:GQ:DP:HQ 0/0:54:7:56,60 0/0:48:4:51,51 0/0:61:2
20 1234567 microsat1 GTC G,GTCT 50 PASS NS=3;DP=9;AA=G GT:GQ:DP 0/1:35:4 0/2:17:2 1/1:40:3
```

May be compressed and indexed using tabix

File formats: VCF

Each variant is described by **8 fields**

- 1 CHROM: chromosome
- 2 POS: position
- 3 ID: name
- 4 REF: reference base(s)
- 5 ALT: non-reference alleles
- 6 QUAL: quality score of the calls (phred scale)
- 7 FILTER: PASS / filtering_tag
- 8 INFO: additional information

Genotype data for several samples may be included in a batch of additional columns (one for each sample) preceded by a FORMAT column which describes their format.

File formats: VCF INFO column

May include several semicolon separated fields containing information about the variants coded in key value style:

`<key>=<data>[,data]`

Some reserved (but optional) keys are:

- AA ancestral allele
- AC allele count in genotypes, for each ALT allele, in the same order as listed
- AF allele frequency
- CIGAR cigar string describing how to align an alternate allele to the reference allele
- DB dbSNP membership
- MQ RMS mapping quality, e.g. MQ=52
- MQ0 Number of MAPQ == 0 reads covering this record

File formats: PED & MAP

Classic format to represent genomic variants for several individuals

<---- normal.ped ---->

```
1 1 0 0 1 1 A A G T
2 1 0 0 1 1 A C T G
3 1 0 0 1 1 C C G G
4 1 0 0 1 2 A C T T
5 1 0 0 1 2 C C G T
6 1 0 0 1 2 C C T T
```

<--- normal.map --->

```
1 snp1 0 5000650
1 snp2 0 5000830
```

would be represented as TPED/TFAM files:

<----- trans.tped ----->

```
1 snp1 0 5000650 A A A C C C A C C C C C
1 snp2 0 5000830 G T G T G G T T G T T T
```

<- trans.tfam ->

```
1 1 0 0 1 1
2 1 0 0 1 1
3 1 0 0 1 1
4 1 0 0 1 2
5 1 0 0 1 2
6 1 0 0 1 2
```

File formats: PED & MAP

PED file

- 1 Family ID
- 2 Individual ID
- 3 Paternal ID
- 4 Maternal ID
- 5 Sex (1=male; 2=female; other=unknown)
- 6 Phenotype (1=unaffected; 2=affected; 0 missing; -9=missing)
- 7 ... genotypes ...

MAP file

- 1 chromosome (1-22, X, Y or 0 if unplaced)
- 2 rs... or SNP identifier
- 3 Genetic distance (Morgans)
- 4 Base-pair position (bp units)

Association to Disease / Phenotype



	AA	Aa	aa
Affected	AA Affected	Aa Affected	aa Affected
Unaffected	AA Unaffected	Aa Unaffected	aa Unaffected

	AA	Aa	aa
Affected	15	8	4
Unaffected	5	3	2

30+8 | 8+8

10+3 | 3+4

Association to Disease / Phenotype



Affected



Unaffected

	A	a
Affected	A , Affected	a , Affected
Unaffected	A , Unaffected	a , Unaffected

	A	a
Affected	38	16
Unaffected	13	7

Statistical tests

- Chi-squared test (χ^2)
- Fisher's exact
- Logistic regression models
- ...

Multiple-test correction is generally needed.

Many software available R, PLINK ...

<http://pngu.mgh.harvard.edu/~purcell/plink/anal.shtml>