ROJETECHNOLOGIES

YDS

Come my friend. Let’s forget the cares of tomorrow

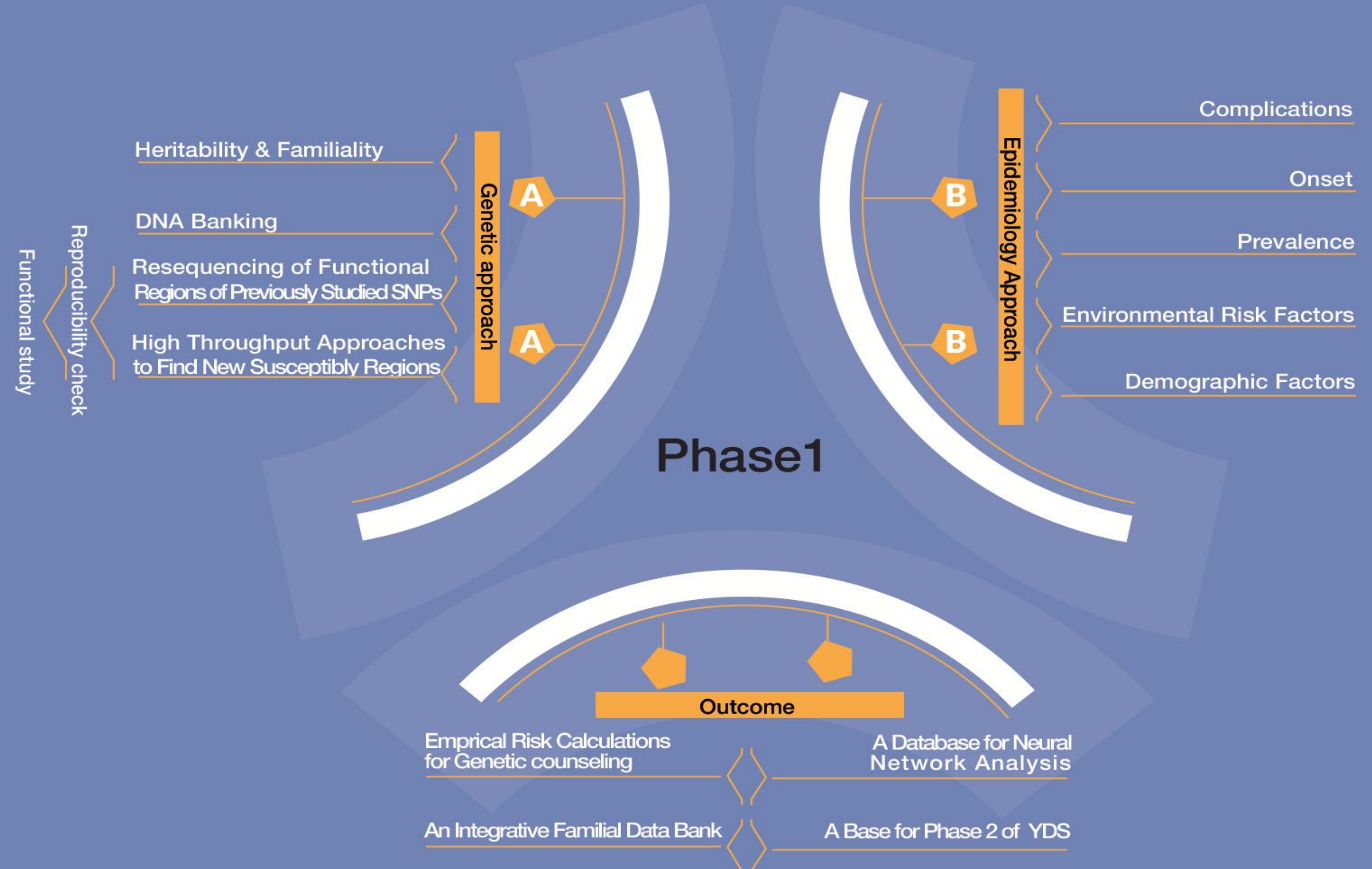
And instead enjoy this moment of our life

For tomorrow when we have left this ancient abode

We shall be equal to all the seven thousand year olds

YDS

Yazd Diabetes Study



Phase1

Genetic approach

- Heritability & Familiality
- DNA Banking
- Resequencing of Functional Regions of Previously Studied SNPs
- High Throughput Approaches to Find New Susceptibly Regions

Reproducibility check

Functional study

Epidemiology Approach

- Complications
- Onset
- Prevalence
- Environmental Risk Factors
- Demographic Factors

Outcome

- Emprical Risk Calculations for Genetic counseling
- A Database for Neural Network Analysis
- An Integrative Familial Data Bank
- A Base for Phase 2 of YDS

Resequencing of KLF14 Gene in a Population Based Family Study With Type 2 Diabetes

Presented by: Ensieh Shahvazian

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Introduction

Resequencing Study

- The loci identified in GWAs range from 10 to 100 kb,
- It does not cover causal variants and genes
- To find new susceptibility associated with T2D

Population based family Study

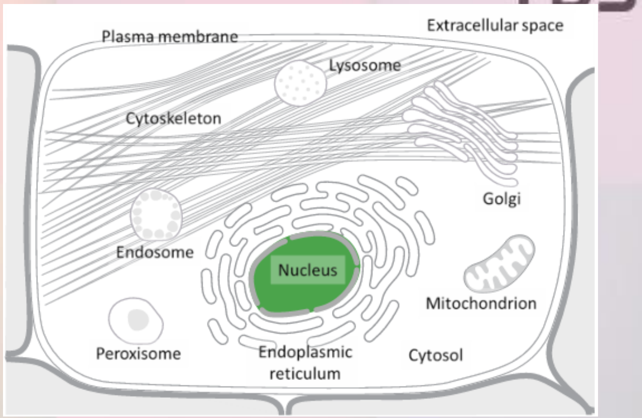
- Robustness to the influence of population stratification,
- Detectable genotyping error,
- Checking the effect of imprinting gene on phenotypes
- Determining the inherited or *de novo* Allele

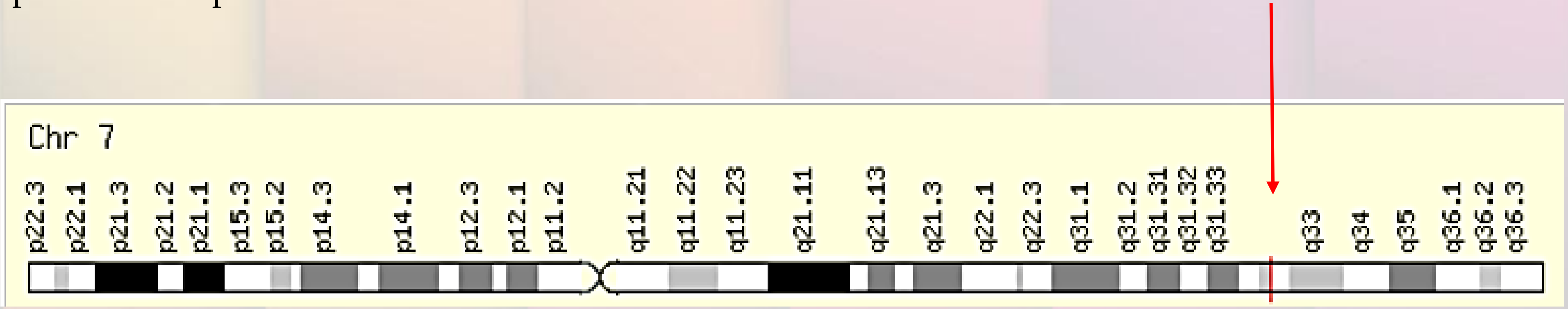
KLF14 Locus at other studies

rs972283	Associated with T2D China, Pakistan, United state (African –American population), Europe, Japan,
rs13234269	African- American
rs4731702	No significant results
rs1562398	Associated with TG in women
rs3800561	No significant results
rs3800562	No significant results
rs3800563	No significant results
rs3800564	No significant results
rs3807137	No significant results
rs3807139	No significant results

KLF14 Gene card

- 7q23.3
- 1480bp
- Intron less
- BTEB5
- GC content around 75%
- Master Transcription factors of fat tissue
- Transcriptional co-repressor & co activator

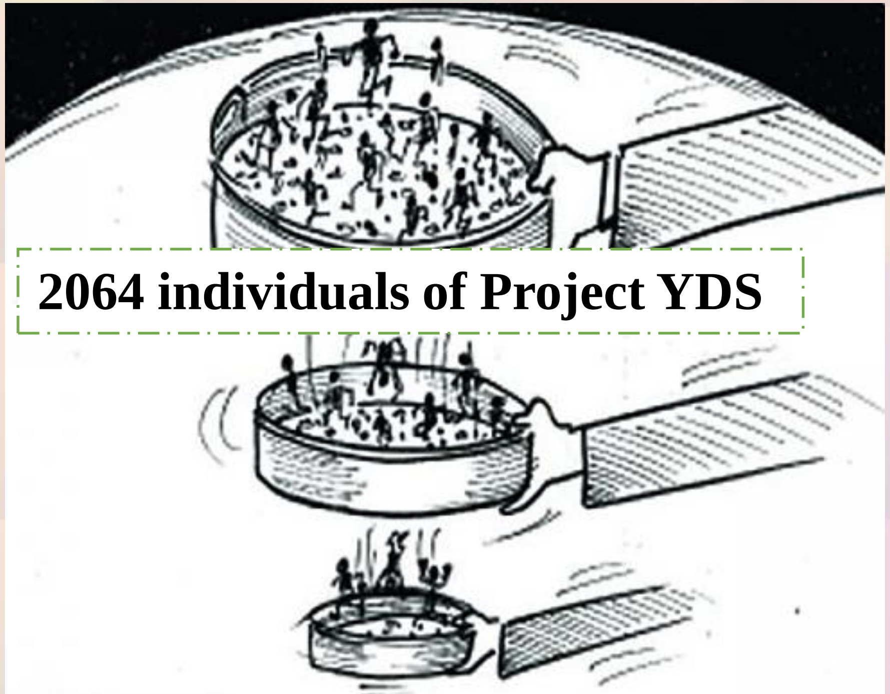




Material & Methods

Sample collection

>35



2064 individuals of Project YDS

150 Individuals

Nuclear family

- At least one parent
- Probands
- Siblings (if available)
- At least one control

DiagnosingT2D

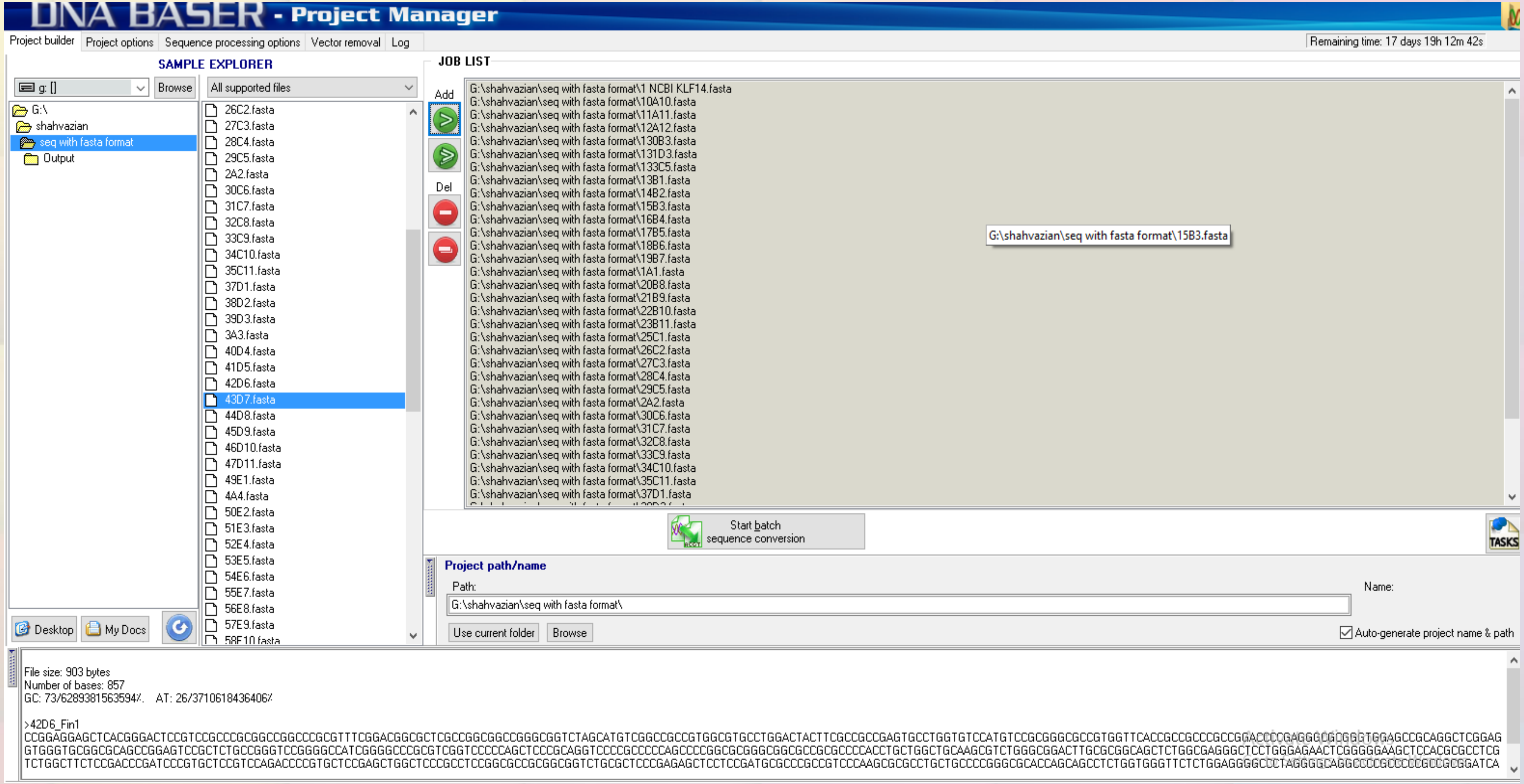
- fasting glucose≥126 mg/dL
- 2-h oral glucose tolerance test glucose≥ 200 mg/dL
- random glucose ≥200 mg/dL
- HbA1C ≥6

KLF14 Amplifying

First Part of KLF14 genes, PCR Program		
No	Description	Cycles
Step 1	95°c, 7 min	1
Step 2	95°c,1min 63°c, 30 Sec 72°C, 1min & 50 Sec	35
Step 3	72°C, 1min 4°C	1

Second Part of KLF14 genes, PCR Program		
No	Description	Cycles
Step 1	95°c, 7 min	1
Step 2	95°c,1min 61°c, 30 Sec 72°C, 1min & 50 Sec	35
Step 3	72°C, 1min 4°C	1

1 Sequencing results are converted to Multi-FASTA format



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G:\shahvazian\seq with fasta format\10A10.fasta
G:\shahvazian\seq with fasta format\11A11.fasta
G:\shahvazian\seq with fasta format\12A12.fasta
G:\shahvazian\seq with fasta format\130B3.fasta
G:\shahvazian\seq with fasta format\131D3.fasta
G:\shahvazian\seq with fasta format\133C5.fasta
G:\shahvazian\seq with fasta format\13B1.fasta
G:\shahvazian\seq with fasta format\14B2.fasta
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G:\shahvazian\seq with fasta format\19B7.fasta
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G:\shahvazian\seq with fasta format\37D1.fasta
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Path: G:\shahvazian\seq with fasta format\

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Number of bases: 857
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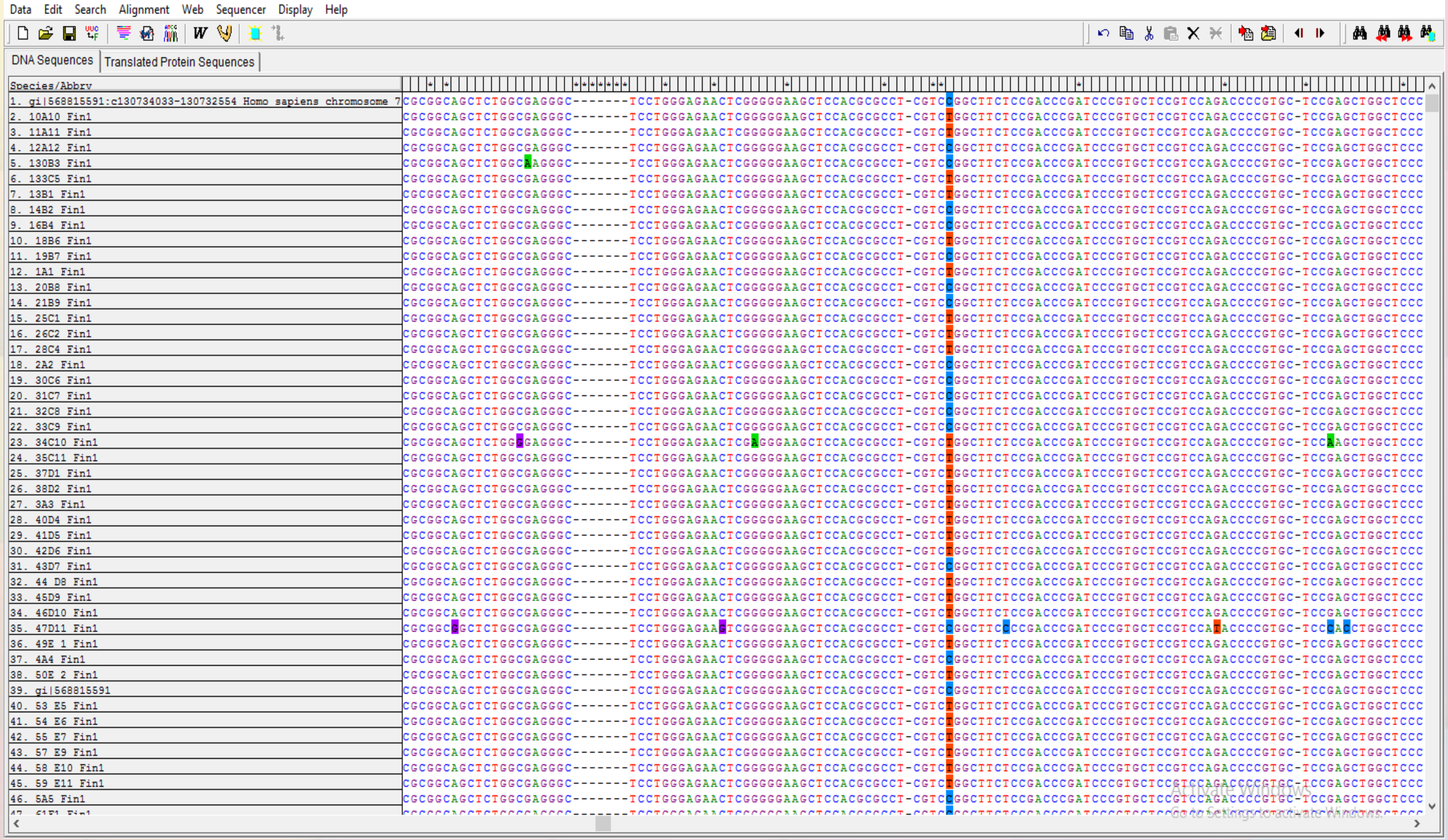
② Alignment by ClustalW

③ Togle conserved sites



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3. 11A11 Fin1   CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCTGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
4. 12A12 Fin1   CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCCGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
5. 130B3 Fin1   CGCGGCAGCTCTGGCAAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCCGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
6. 133C5 Fin1   CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCTGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
7. 13B1 Fin1    CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCTGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
8. 14B2 Fin1    CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCCGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
9. 16B4 Fin1    CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCCGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
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11. 19B7 Fin1   CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCCGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
12. 1A1 Fin1    CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCTGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
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18. 2A2 Fin1    CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCCGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
19. 30C6 Fin1   CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCCGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
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21. 32C8 Fin1   CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCCGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
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25. 37D1 Fin1   CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCTGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
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27. 3A3 Fin1    CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCTGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
28. 40D4 Fin1   CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCTGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
29. 41D5 Fin1   CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCTGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
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31. 43D7 Fin1   CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCCGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
32. 44 D8 Fin1  CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCTGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
33. 45D9 Fin1   CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCTGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
34. 46D10 Fin1  CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCTGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
35. 47D11 Fin1  CGCGGCGGCTCTGGCGAGGGC-------TCCTGGGAGAAGTCGGGGGAAGCTCCACGCGCCT-CGTCCGGCTTCCCCGACCCGATCCCGTGCTCCGTCCATACCCCGTGC-TCCCACCTGGCTCCC
36. 49E 1 Fin1  CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCTGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
37. 4A4 Fin1    CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCCGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
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40. 53 E5 Fin1  CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCTGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
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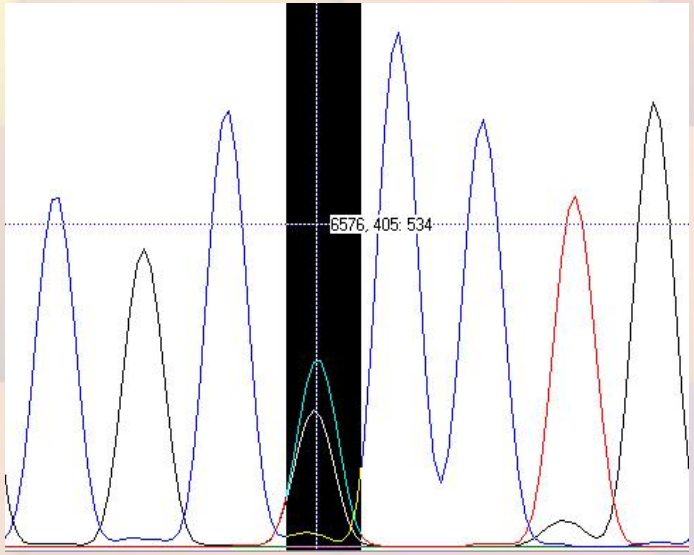
43. 57 E9 Fin1  CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCTGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
44. 58 E10 Fin1 CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCTGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
45. 59 E11 Fin1 CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCTGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
46. 5A5 Fin1    CGCGGCAGCTCTGGCGAGGGC-------TCCTGGGAGAACTCGGGGGAAGCTCCACGCGCCT-CGTCCGGCTTCTCCGACCCGATCCCGTGCTCCGTCCAGACCCCGTGC-TCCGAGCTGGCTCCC
```

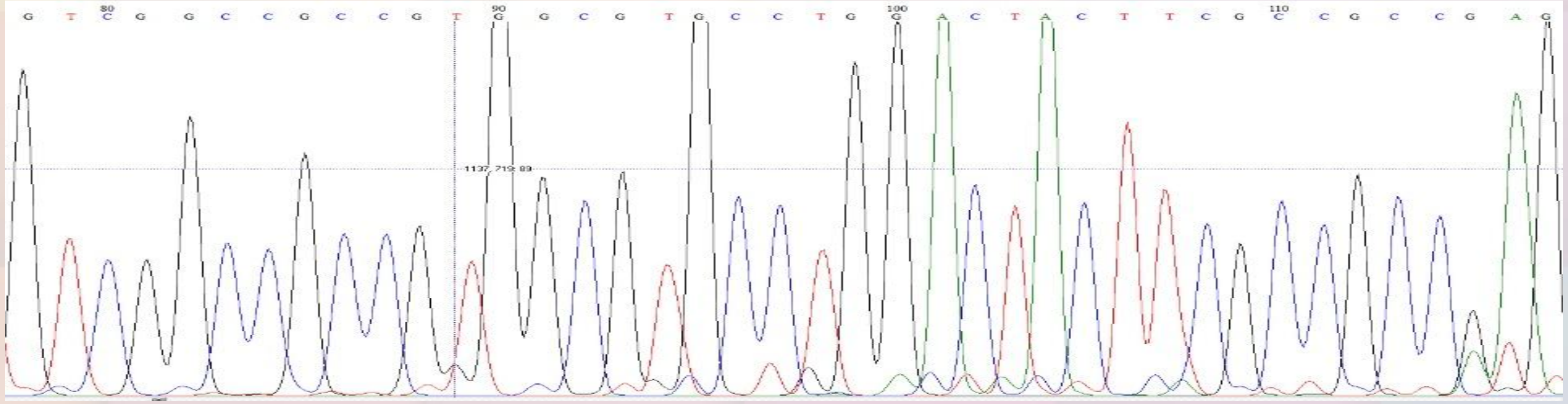
Statistical analysis

- Applying an exact test for deviation from Hardy–Weinberg equilibrium (HWE)
- The proportions of genotypes or alleles were compared by the chi square (χ^2) test.
- SNP× T2D associations were assessed using odds ratios and 95% confidence intervals derived from logistic regression models including all members, adjusting for age and sex.

Results

Sequencing results of KLF14 by bioedith Software



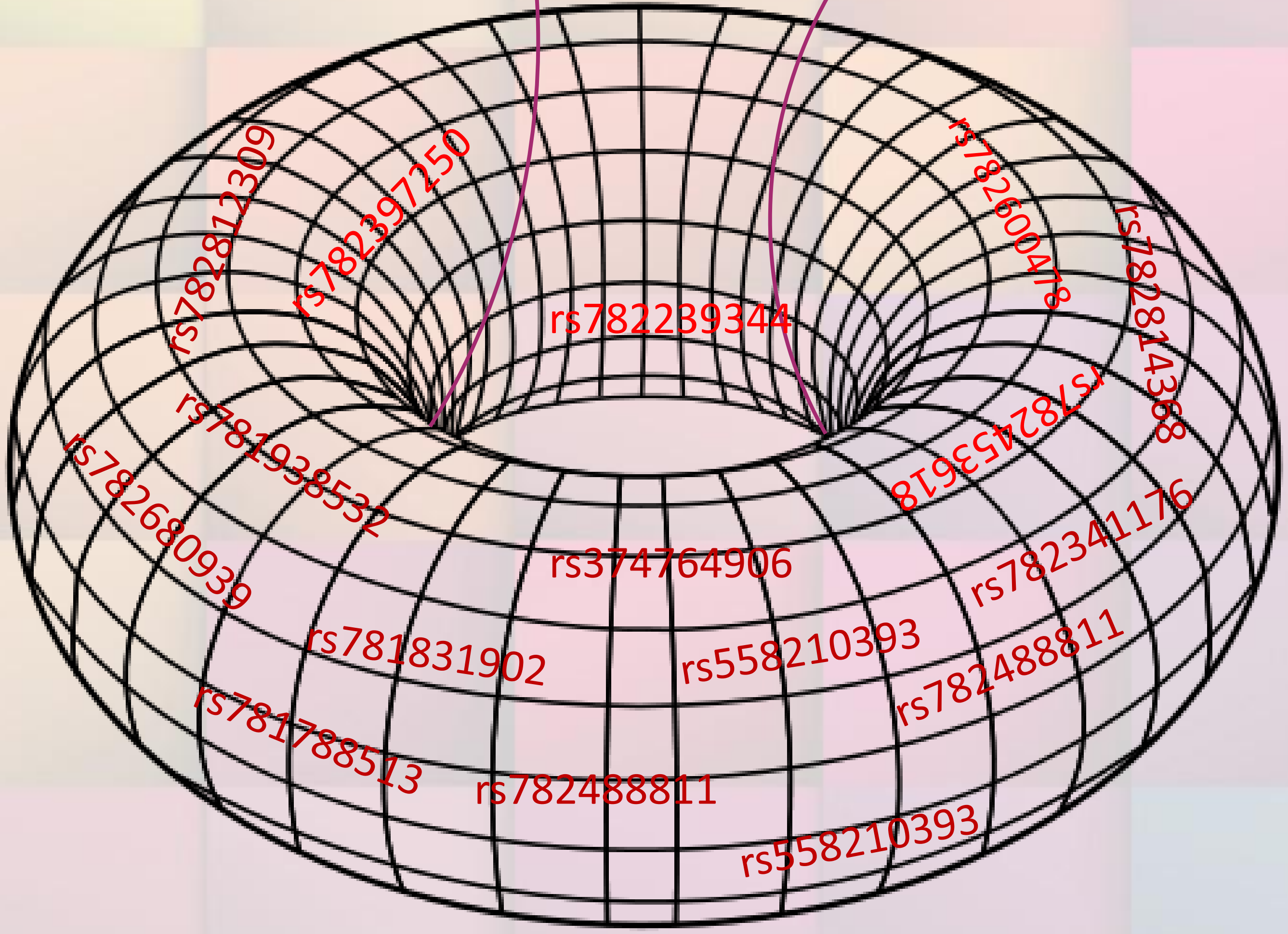


rs76603546 ✓

rs111731678

rs782564043

Diversity in variants



220 variants

Hardy-Weinberg equilibrium Results

SNP	Chi Square	P-value
rs76603546	3.84	0.04
rs111731678	0.48	0.48
rs782564043	2.2	0.13

Genotyping and Allele frequencies of rs76603546

Genotype	Case	Control	OR (95%CI)	P-value
CC	0.5	0.33		
TT	0.18	0.37	0.33(0.1-1.1)	0.06
TC	0.32	0.27	0.72(0.22-2.29)	0.57
Allele				
C	0.66	0.48	2.15 (1.08- 4.30)	0.02
T	0.34	0.518		

Genotyping and Allele frequencies of rs111731678

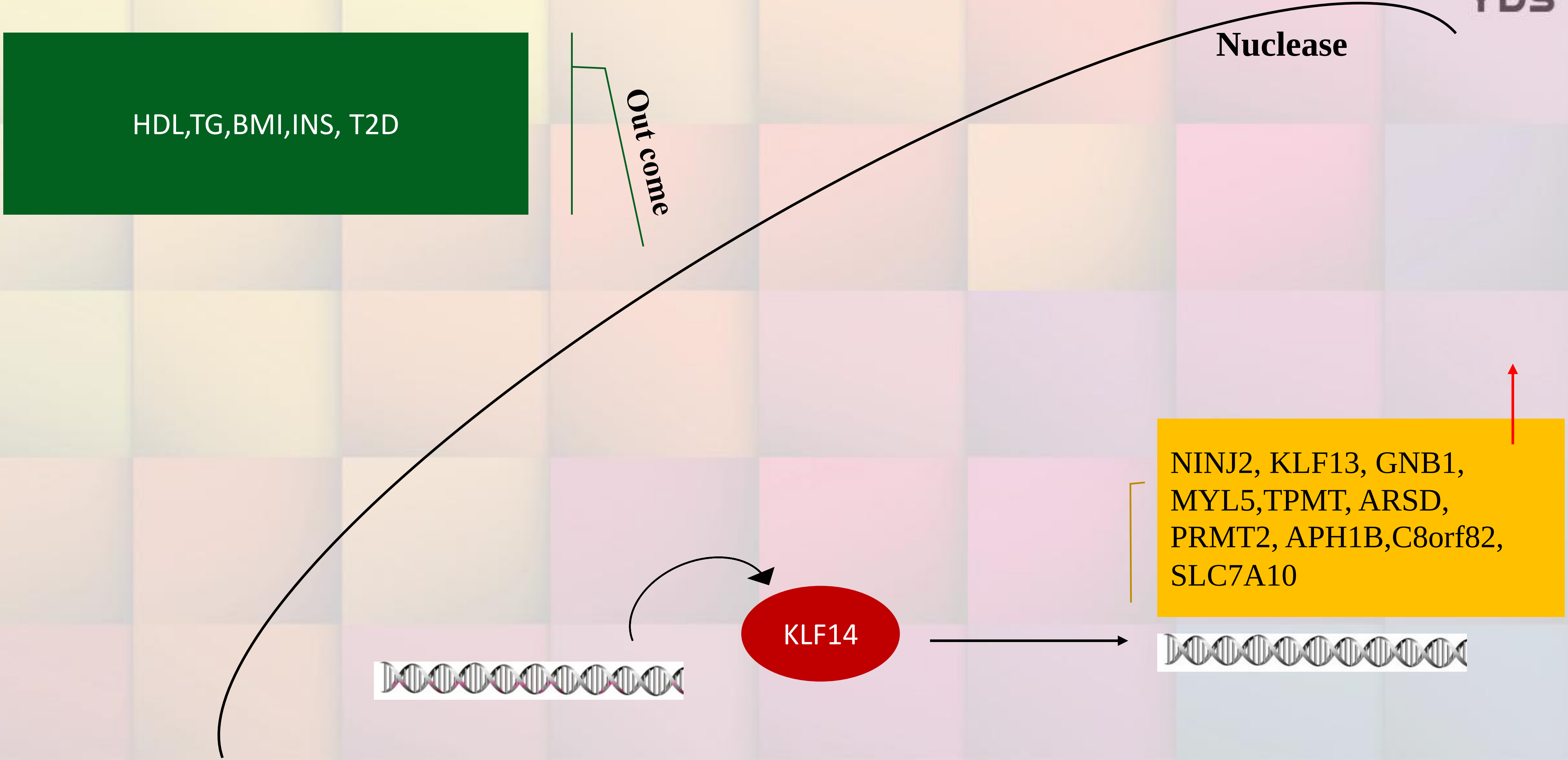
Genotype	Case	Control	OR (95%CI)	P-value
AA	0.73	0.78		
TT	0.043	0	---	
AT	0.23	0.21	1.15	0.82
		Allele		
A	0.84	0.89	0.63(0.29 -1.77)	0.38
T	0.16	0.11		

Genotyping and Allele frequencies of rs782564043

Genotype	Case	Control	OR (95%CI)	P-value
GG	0.45	0.57		
AA	0.	0	---	----
GA	0.54	0.43	1.6(0.62-4.16)	0.33
	Allele			
G	0.73	0.78	0.7(90.33 - 1.61)	0.43
A	0.27	0.21		

Discussion

Adipocyte



HDL,TG,BMI,INS, T2D

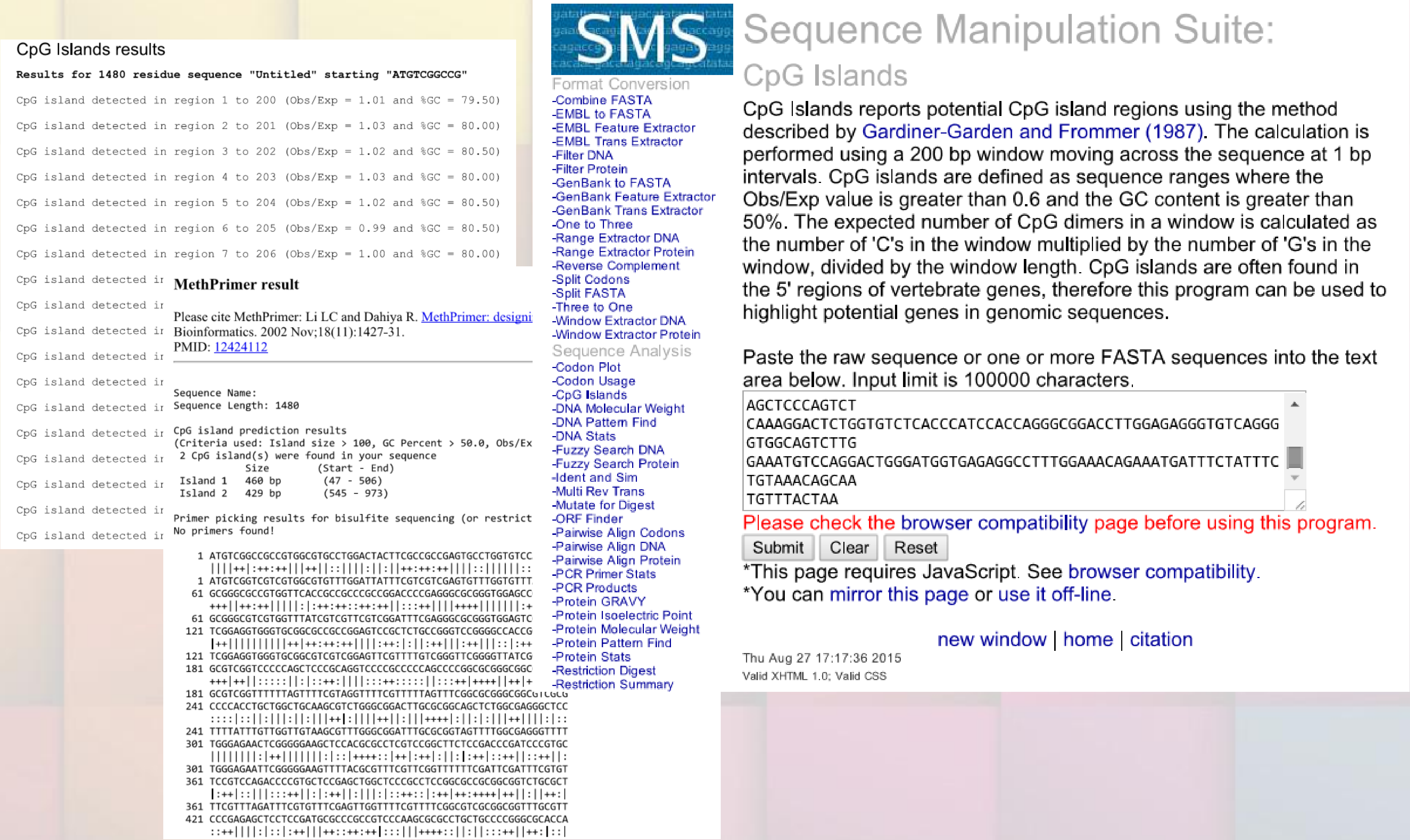
Out come

Nuclease

KLF14

NINJ2, KLF13, GNB1, MYL5,TPMT, ARSD, PRMT2, APH1B,C8orf82, SLC7A10

rs76603546



CpG Islands results

```
Results for 1480 residue sequence "Untitled" starting "ATGTCGGCCG"

CpG island detected in region 1 to 200 (Obs/Exp = 1.01 and %GC = 79.50)

CpG island detected in region 2 to 201 (Obs/Exp = 1.03 and %GC = 80.00)

CpG island detected in region 3 to 202 (Obs/Exp = 1.02 and %GC = 80.50)

CpG island detected in region 4 to 203 (Obs/Exp = 1.03 and %GC = 80.00)

CpG island detected in region 5 to 204 (Obs/Exp = 1.02 and %GC = 80.50)

CpG island detected in region 6 to 205 (Obs/Exp = 0.99 and %GC = 80.50)

CpG island detected in region 7 to 206 (Obs/Exp = 1.00 and %GC = 80.00)

CpG island detected in

CpG island detected in

CpG island detected in

CpG island detected in

CpG island detected in

CpG island detected in

CpG island detected in

CpG island detected in

CpG island detected in

CpG island detected in

CpG island detected in
```

MethPrimer result

Please cite MethPrimer: Li LC and Dahiya R. MethPrimer: designi
Bioinformatics. 2002 Nov;18(11):1427-31.
PMID: 12424112

```
Sequence Name:
Sequence Length: 1480

CpG island prediction results
(Criteria used: Island size > 100, GC Percent > 50.0, Obs/Ex
 2 CpG island(s) were found in your sequence
            Size        (Start - End)
 Island 1   460 bp       (47 - 506)
 Island 2   429 bp       (545 - 973)

Primer picking results for bisulfite sequencing (or restrict
No primers found!

      1 ATGTCGGCCGCCGTGGCGTGCCTGGACTACTTCGCCGCCGAGTGCCTGGTGTCC
        ||||++|:++:++|||++||::||||:||:||++:++:++||||::||||||::
      1 ATGTCGGTCGTCGTGGCGTGTTTGGATTATTTCGTCGTCGAGTGTTTGGTGTTT
     61 GCGGGCGCCGTGGTTCACCGCCGCCCGCCGGACCCCGAGGGCGCGGGTGGAGCC
        +++||++:++|||||:|:++:++::++:++||:::++||||++++||||||||:+
     61 GCGGGCGTCGTGGTTTATCGTCGTTCGTCGGATTTCGAGGGCGCGGGTGGAGTC
    121 TCGGAGGTGGGTGCGGCGCCGCCGGAGTCCGCTCTGCCGGGTCCGGGGCCACCG
        |++||||||||||++|++:++:++||||:++:|:||:++|||:++|||::|:++
    121 TCGGAGGTGGGTGCGGCGTCGTCGGAGTTCGTTTTGTCGGGTTCGGGGTTATCG
    181 GCGTCGGTCCCCCAGCTCCCGCAGGTCCCCGCCCCCAGCCCCGGCGCGGGCGGC
        +++|++||:::::||:|::++:||||:::++:::::||:::++|++++||++|+
    181 GCGTCGGTTTTTTAGTTTTCGTAGGTTTTCGTTTTTAGTTTCGGCGCGGGCGGCGTCGCG
    241 CCCCACCTGCTGGCTGCAAGCGTCTGGGCGGACTTGCGCGGCAGCTCTGGCGAGGGCTCC
        ::::|::||:|||:||:|||++|:||||++||:|||++++|:||:|:|||++||||:|::
    241 TTTTATTTGTTGGTTGTAAGCGTTTGGGCGGATTTGCGCGGTAGTTTTGGCGAGGGTTTT
    301 TGGGAGAACTCGGGGGAAGCTCCACGCGCCTCGTCCGGCTTCTCCGACCCGATCCCGTGC
        ||||||||:|++|||||||:|::|++++::|++|:++|:||:|:++|::++||::++||:
    301 TGGGAGAATTCGGGGGAAGTTTTACGCGTTTCGTTCGGTTTTTTCGATTCGATTTCGTGT
    361 TCCGTCCAGACCCCGTGCTCCGAGCTGGCTCCCGCCTCCGGCGCCGCGGCGGTCTGCGCT
        |:++|::|||:::++||:|:++||:|||:|::++::|:++|++:++++|++||:||++:|
    361 TTCGTTTAGATTTCGTGTTTCGAGTTGGTTTTCGTTTTCGGCGTCGCGGCGGTTTGCGTT
    421 CCCGAGAGCTCCTCCGATGCGCCCGCCGTCCCAAGCGCGCCTGCTGCCCCGGGCGCACCA
        ::++||||:|::|:++|||++::++:++|:::|||++++::||:||:::++||++:|::|
```

SMS

Sequence Manipulation Suite:

CpG Islands

Format Conversion
- -Combine FASTA
- -EMBL to FASTA
- -EMBL Feature Extractor
- -EMBL Trans Extractor
- -Filter DNA
- -Filter Protein
- -GenBank to FASTA
- -GenBank Feature Extractor
- -GenBank Trans Extractor
- -One to Three
- -Range Extractor DNA
- -Range Extractor Protein
- -Reverse Complement
- -Split Codons
- -Split FASTA
- -Three to One
- -Window Extractor DNA
- -Window Extractor Protein

Sequence Analysis
- -Codon Plot
- -Codon Usage
- -CpG Islands
- -DNA Molecular Weight
- -DNA Pattern Find
- -DNA Stats
- -Fuzzy Search DNA
- -Fuzzy Search Protein
- -Ident and Sim
- -Multi Rev Trans
- -Mutate for Digest
- -ORF Finder
- -Pairwise Align Codons
- -Pairwise Align DNA
- -Pairwise Align Protein
- -PCR Primer Stats
- -PCR Products
- -Protein GRAVY
- -Protein Isoelectric Point
- -Protein Molecular Weight
- -Protein Pattern Find
- -Protein Stats
- -Restriction Digest
- -Restriction Summary

CpG Islands reports potential CpG island regions using the method described by Gardiner-Garden and Frommer (1987). The calculation is performed using a 200 bp window moving across the sequence at 1 bp intervals. CpG islands are defined as sequence ranges where the Obs/Exp value is greater than 0.6 and the GC content is greater than 50%. The expected number of CpG dimers in a window is calculated as the number of 'C's in the window multiplied by the number of 'G's in the window, divided by the window length. CpG islands are often found in the 5' regions of vertebrate genes, therefore this program can be used to highlight potential genes in genomic sequences.

Paste the raw sequence or one or more FASTA sequences into the text area below. Input limit is 100000 characters.

```
AGCTCCCAGTCT
CAAAGGACTCTGGTGTCTCACCCATCCACCAGGGCGGACCTTGGAGAGGGTGTCAGGG
GTGGCAGTCTTG
GAAATGTCCAGGACTGGGATGGTGAGAGGCCTTTGGAAACAGAAATGATTTCTATTTC
TGTAAACAGCAA
TGTTTACTAA
```

Please check the browser compatibility page before using this program.

*This page requires JavaScript. See browser compatibility.
*You can mirror this page or use it off-line.

new window | home | citation

Thu Aug 27 17:17:36 2015
Valid XHTML 1.0; Valid CSS

Conclusion

1. The association of KLF14 SNP, **rs76603546** With T2D , may be due to LD with SNP out side of gene
2. Higher Odd ratio of **rs76603546** SNP with T2D compared to other SNPs in the locus might be either because of incomplete LD between these SNPs or simply it might be because of differences in the gene pool of different population
3. Another possible assumption can consider **rs76603546** as an independent SNP from previously studied SNPs
4. Based on our bioinformatics studies **rs76603546** is a methylated nucleotide when the C allele is present
5. This study needs to be reproduced in a larger scale and functional studies should be conducted , which is in progress

Thank you for Your Attention