Novel variants in Iranian individuals suspected to have inherited red blood cell disorders, including bone marrow failure syndromes

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Supplemental information

Supplementary methods

Families and patients

Four Iranian Families (Families I-IV), (Figure 1; Supplementary Table 1) with at least one affected individual, with inconclusive diagnosis were referred from various provinces to "Adult Thalassemia Clinic, Blood Transfusion Research Centre, High Institute for Research and Education in Transfusion Medicine", Tehran, Iran, as a referral centre. Based on previous clinical and haematological data (Supplementary Table 1; Figure 2) they were suspected to have rare types of red blood cell disorders, including bone marrow failure syndromes. Therefore, after informed consent, they became candidate for Whole Exome Sequencing (WES). Five ml blood samples were collected from each individual and DNA was extracted using standard salting out procedure.

Whole Exome Sequencing and bioinformatic prediction of pathogenicity of the variants

Qualitative and quantitative evaluation of DNA was performed using standard techniques, Whole Exome Sequencing was performed using (HISeq400 sequencer, Sure Select V6-Post by Macrogen Inc.) Mapping reference hg19 from UCSC (original GRCh37 from NCBI, Feb. 2009) was used to address the variants. Synonymous and intronic variants and variants with higher than 1% frequency were excluded by filtering. Remaining variants were filtered using a list of 281 anemia related genes (Supplementary Table 2). For preparing this list we used the information provided by Viapath red cell gene panel (196 genes), http://www.viapath.co.uk/our-tests/red-cell-gene-panel, and the bone marrow failure syndrome panel (128 genes) of blueprint genetics (https://blueprintgenetics.com/tests/panels/hematology/anemia-panel/. We also tested our data for recently published genes/variants in the field of bone marrow failure syndromes, Diamond Blackfan Anemia and Congenital Dyserythropoietic anemia (References 1,3 and 8 in the main text).

Variants identified, Bioinformatic algorithms, Population and disease related databases that were used for variant analysis are presented in Table 1. These included a human genome variation database of eight major ethnic groups that live in Iran and neighbouring countries in the Middle East, Iranome.[1] The American College of Medical Genetics (ACMG) guidelines were used to predict the pathogenicity of the variants.

Sanger sequencing

The PCR primers for Sanger sequencing (Supplementary Table 3) were designed by primer 3 or manually to amplify the variant regions by Polymerase Chain Reaction (PCR).

Chromatograms relating to each family are presented in Supplementary Figures 1-5.

Ethical Considerations

This research was conducted based on the guidelines of ethical committee of the University of Social Welfare and Rehabilitation Sciences, Tehran, Iran, in accordance with *Helsinki declaration*.

Supplementary Reference

1. Akbari MR, Fattahi Z, Beheshtian M, *et al*., A human genome variation database of eight major ethnic groups that live in Iran and neighbouring countries in the Middle East. ASHG Annual Meeting, October 2017, Orlando-USA.

CBC and RBC morphology									
Family	WBC 10*3/µl	RBC 10*6/µl	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	PLT 10*3/µL	RBC morphology
I	8.2	3.79	9.70	29.7	78.4	25.6	32.7	14	Hypochromia, Anisocytosis
II	7.23	2.19	6.1	17.6	80.2	27.7	34.5	400	Normal
III	2.4	2.29	6.0	19.0	83.0	26.2	31.6	235	Anisocytosis
IV	11.3	3.39	9.6	27.9	82.3	28.3	34.4	490	Hypochromia, Anisocytosis, Poikilocytosis, Microcytosis
Bone marrow Reports									
I	Bone marrow aspirate and trephine biopsy: hypocellular marrow (60-65% cellularity) with usual composition of haematopoietic elements								
II	Bone marrow aspirate and trephine biopsy: hypocellular marrow (40-45% cellularity) with usual composition of haematopoietic elements								
III	Bone marrow aspirate and trephine Biopsy: hypercellular marrow (95% cellularity), Increased megakaryocytes								
IV	Not performed								
Other genetic investigations prior to NGS									
I	Alpha and beta globin genotyping: Normal Chromosomal breakage analysis for Fanconi Anemia: Normal								
II	Alpha and beta globin genotyping: IVSI-110 mutation in beta globin gene in the proband and his mother								
III	Chromosomal breakage analysis for Fanconi Anemia: Normal Copy number analysis (BCR-ABL to ABL): Normal V617F mutation in JAK2 gene: Ruled out								
IV	Alpha and beta globin genotyping: IVSI-5 mutation in beta globin gene in the proband and her mother								

Supplementary Table 1. Clinical and haematological information collected prior to this study.

ABCB6 ,ABCB7 ,ACD ,ACKR1 ,ACTB ,ADD1 ,ADD2 ,AHSP ,AK1 ,AK2 ,ALAD ,ALAS1 ,ALAS2 ,ALAS2 ,ALDOA ,AMN ,ANK1 ,ANKRD26 ,AP3B1 ,APOB ,ATM ,ATR ,ATRX ,BHLHE41 ,BLM ,BLOC1S3 ,BLOC1S6 ,BMP4 ,BMP6 ,BPGM ,BRAF ,BRCA1 ,BRCA2 ,BRIP1 ,CBL ,CCBE1 ,CDAN1 ,CDKN2A ,CEBPA ,CECR1 ,CI5ORF41 ,CLPB ,COX4I2 ,CP ,CPOX ,CSF2RA ,CSF3R ,CTC1 ,CTSC ,CUBN ,CXCR4 ,CYB5A ,CYB5R1 ,CYB5R2 ,CYB5R3 ,CYB5R4 ,CYB5RL ,DDX41 ,DHFR ,DKC1 ,DMTN ,DNAJC21 ,DTNBP1 ,EGLN1 ,EGLN2 ,EGLN3 ,ELA2 ,ELANE ,ENO1 ,EPAS1 ,EPB41 ,EPB42 ,EPCAM ,EPO ,EPOR ,ERCC4 ,ERCC6L2 ,ETV6 ,FADD ,FANCA ,FANCB ,FANCC ,FANCD2 ,FANCE ,FANCF ,FANCG ,FANCI ,FANCL ,FANCM ,FAS ,FASLG ,FAT4 ,FECH ,FTCD ,FTH1 ,FTL ,G6PC3 ,G6PD ,GAPDH ,GATA1 ,GATA2 ,GCLC ,GFI1 ,GFI1B ,GIF ,GINS1 ,GLRX5 ,GP1BA ,GPI ,GPX1 ,GSR ,GSS ,HAMP ,HAX1 ,HBA1 ,HBA2 ,HBB ,HBD ,HBE1 ,HBG1 ,HBG2 ,HBM ,HBQ1 ,HBZ ,HFE ,HFE2 ,HIF1A ,HIF1AN ,HIF3A ,HK1 ,HK2 ,HLH ,HMBS ,HOXA11 ,HP ,HPLH1 ,HPS1 ,HPS3 ,HPS4 ,HPS5 ,HPS6 ,HRAS ,IFNGR2 ,IKZF1 ,ITK ,JAGN1 ,JAK2 ,KCNN4 ,KDM6A ,KIF23 ,KLF1 ,KRAS ,LAMTOR2 ,LIG4 ,LPIN2 ,LYST ,MAGT1 ,MAP2K1 ,MAP2K2 ,MASTL ,MKL1 ,MLH1 ,MPL ,MSH2 ,MSH6 ,MT-CO1 ,MTR ,MTRR ,MTTP ,MYH9 ,MYO5A ,NBEAL2 ,NBN ,NF1 ,NHP2 ,NOP10 ,NRAS ,NT5C3A ,OS9 ,PALB2 ,PARN ,PAX5 ,PFKM ,PGAM1 ,PGD ,PGK1 ,PGM1 ,PGM3 ,PIEZO1 ,PKLR ,PMS2 ,PPOX ,PRF1 ,PTPN11 ,PUS1 ,RAB27A ,RAC2 ,RAD51C ,RBM8A ,RECQL4 ,RhAG ,RIT1 ,RMRP ,RPL11 ,RPL15 ,RPL19 ,RPL26 ,RPL27 ,RPL35A ,RPL5 ,RPL9 ,RPS10 ,RPS19 ,RPS24 ,RPS26 ,RPS29 ,RPS7 ,RTEL1 ,RUNX1 ,SAMD9 ,SAMD9L ,SBDS ,SDS ,SEC23B ,SERPINA1 ,SF3B1 ,SH2B3 ,SH2D1A ,SLC11A2 ,SLC19A2 ,SLC25A38 ,SLC2A1 ,SLC37A4 ,SLC40A1 ,SLC4A1 ,SLX4 ,SMAD4 ,SMAD6 ,SMAD7 ,SMARCAL1 ,SMARCD2 ,SOS1 ,SPTA1 ,SPTB ,SRP72 ,STOM ,STX11 ,STXBP2 ,TAL1 ,TAZ ,TERC ,TERT ,TF ,TFR2 ,TFRC ,THPO ,TINF2 ,TMOD1 ,TMPRSS6 ,TP53 ,TPI1 ,TPM3 ,TUBB1 ,UGT1A1 ,UMPS ,UNC13D ,UROD ,UROS ,USB1 ,VHL ,VPS13B ,VPS45 ,WAS ,WDR1 ,WIPF1 ,WRAP53 ,XIAP ,XK ,XRCC2 ,YARS2 ,ZNF197

Supplementary Table 2. A set of 281 genes related to red blood cell disorders and bone marrow failure syndromes.

Gene	Forward	Reverse
RRPL	CGACGAGGTACTGTCACCTTT	TACCAAGTTCCCAGGTTCCA
RUNX1	AAATTCCGGGAGTGTTGTCA	GCAACTTTTTGGCTTTACGG
RPS26	ACAGTTTTCCCATCCTGTCG	CCCTCTGCCCAGAAAAGAAT
ADA2	GGGGTTTGGGTTTAAGCAG	GCACGTTGTCCTCGTAGAACT
CDAN1	GACTTCACTGATACAGGAGAGC	GGGTTAATCGGAAAGAGGCTG

Supplementary Table 3. Primer sequences of the variants

Supplementary figure legends

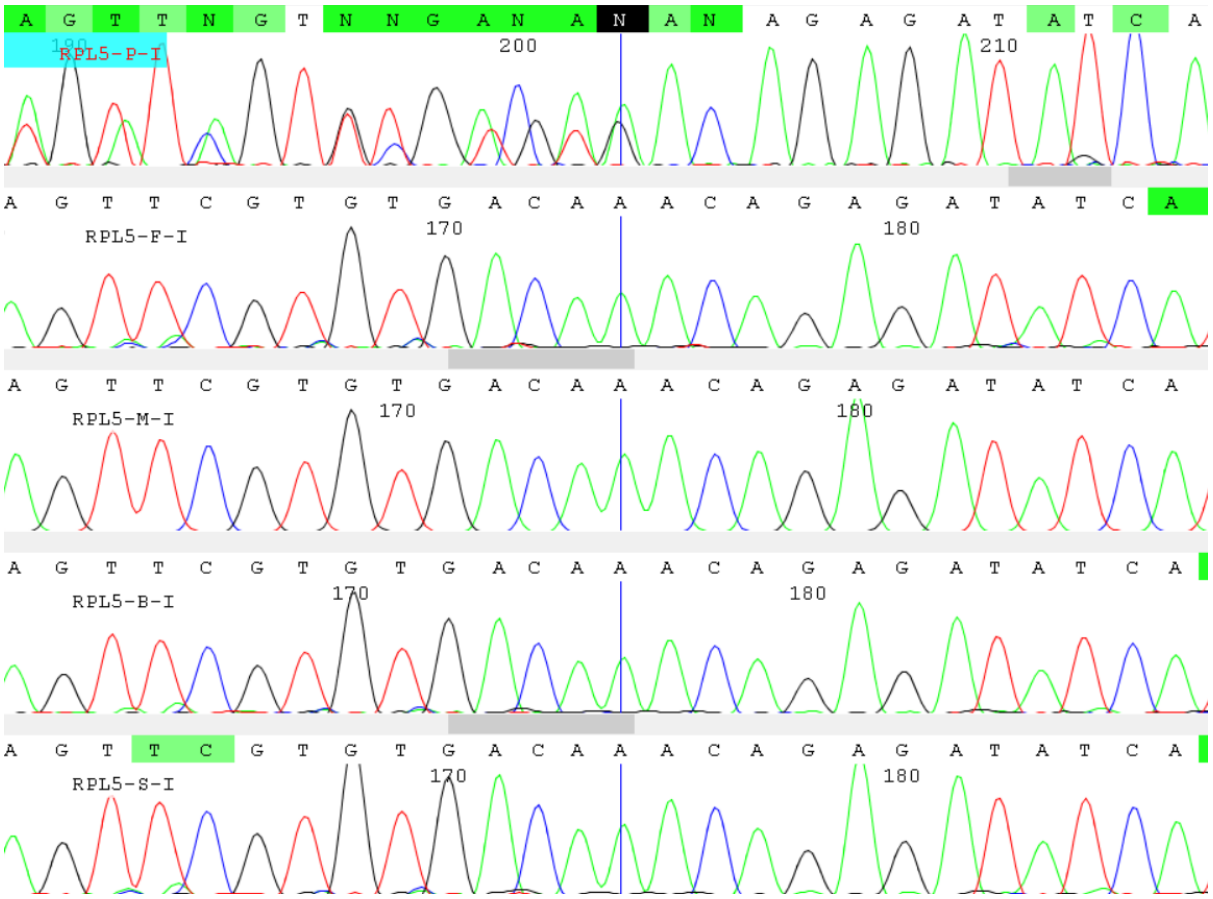
Supplementary figure 1. Chromatograms for *RPL5* variant in family I. P: proband, M: mother, F: father, S: sister, B: brother.

Supplementary figure 2. Chromatograms for *RUNX1* variant in family I.

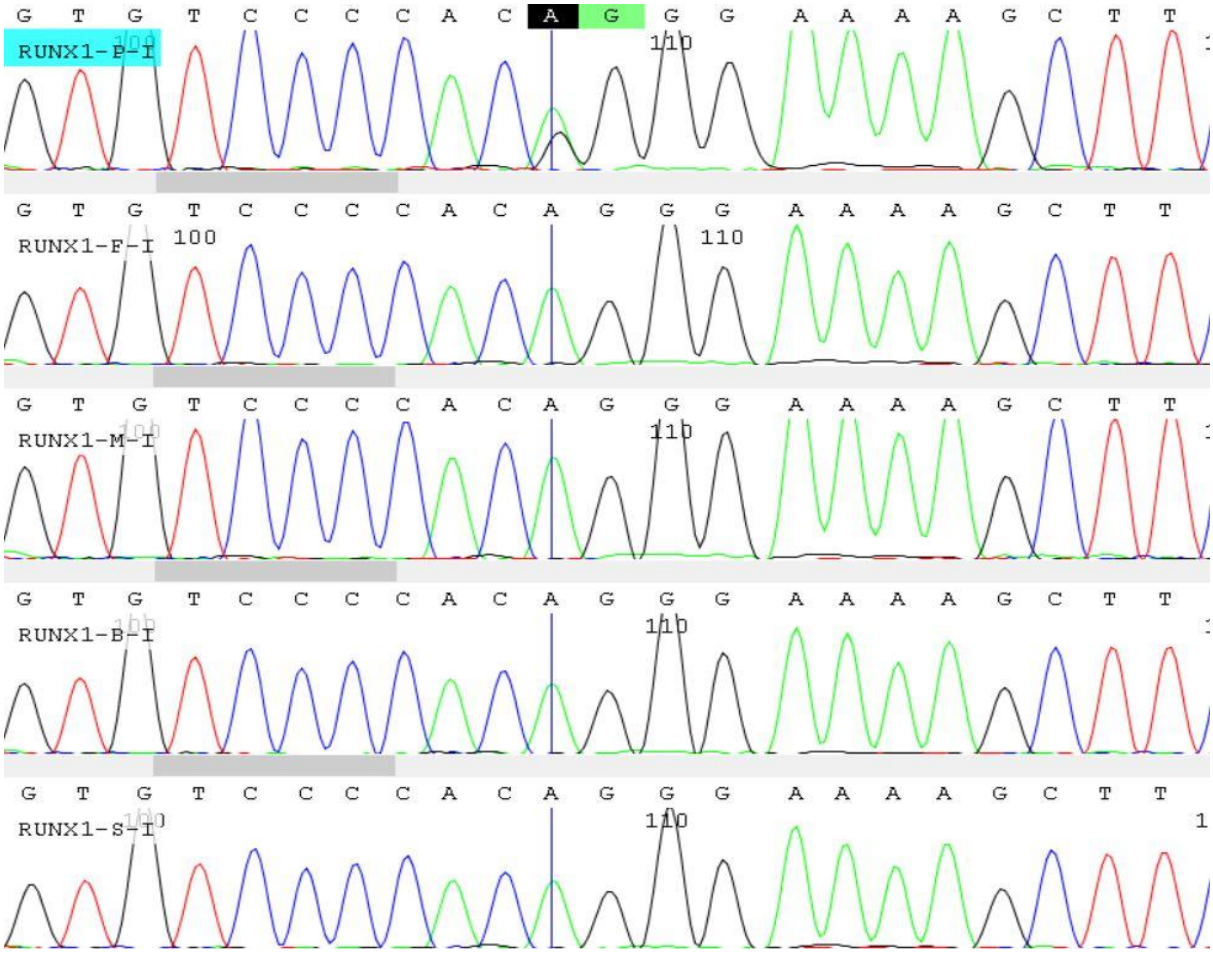
Supplementary figure 3. Chromatograms for *RPS26* variant in family II.

Supplementary figure 4. Chromatograms for *ADA2* variant in family III. (P.A: probably affected).

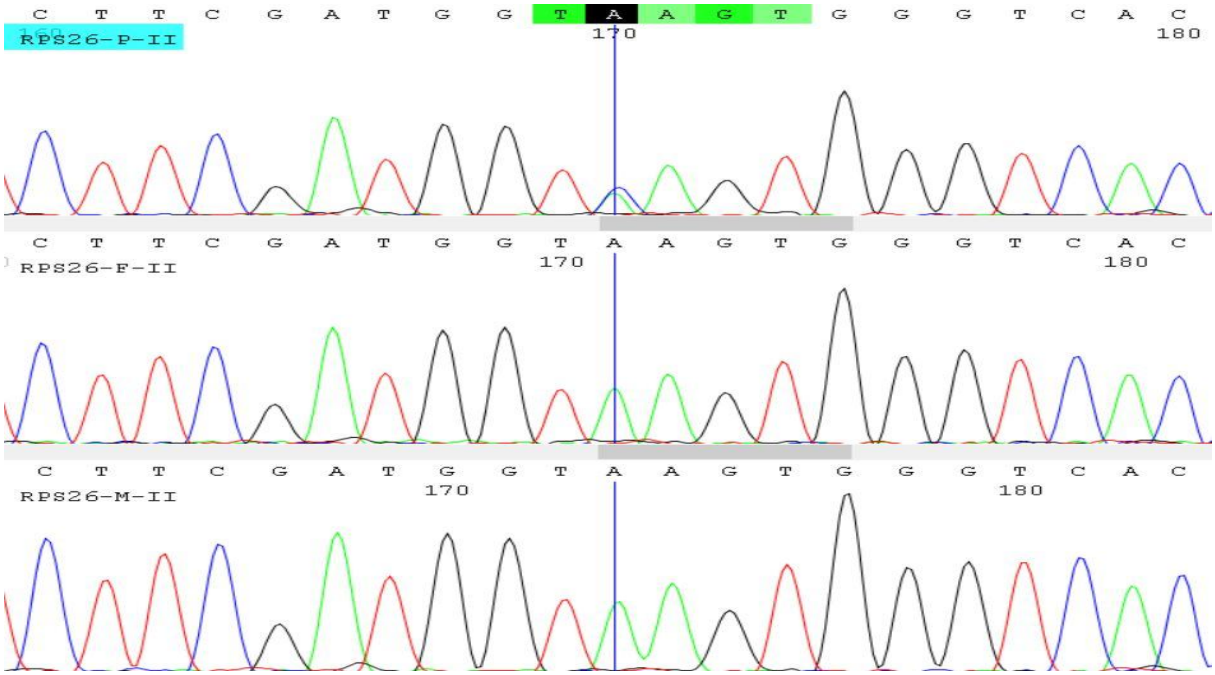
Supplementary figure 5. Chromatograms for *CDAN1* variant in family IV.



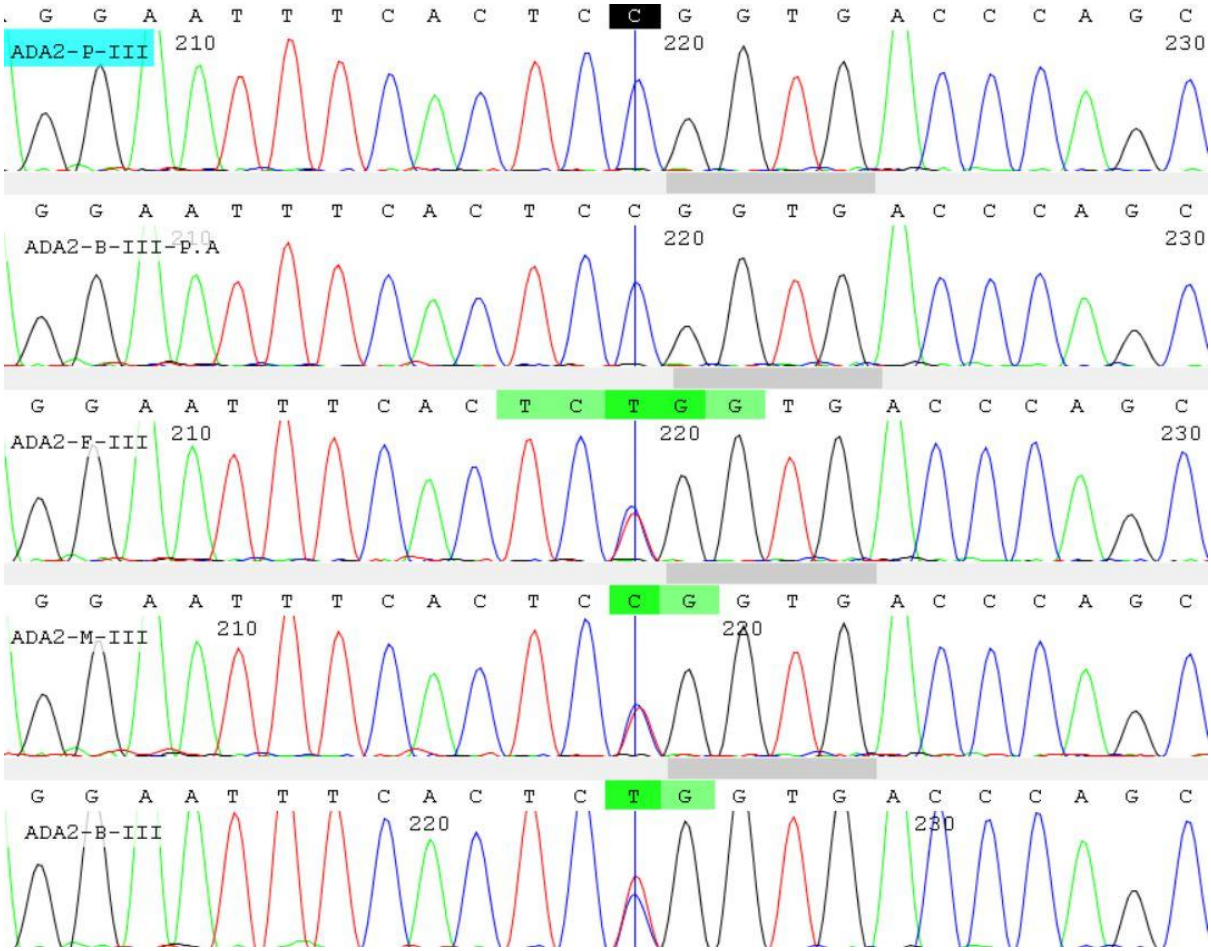
Supplementary figure 1



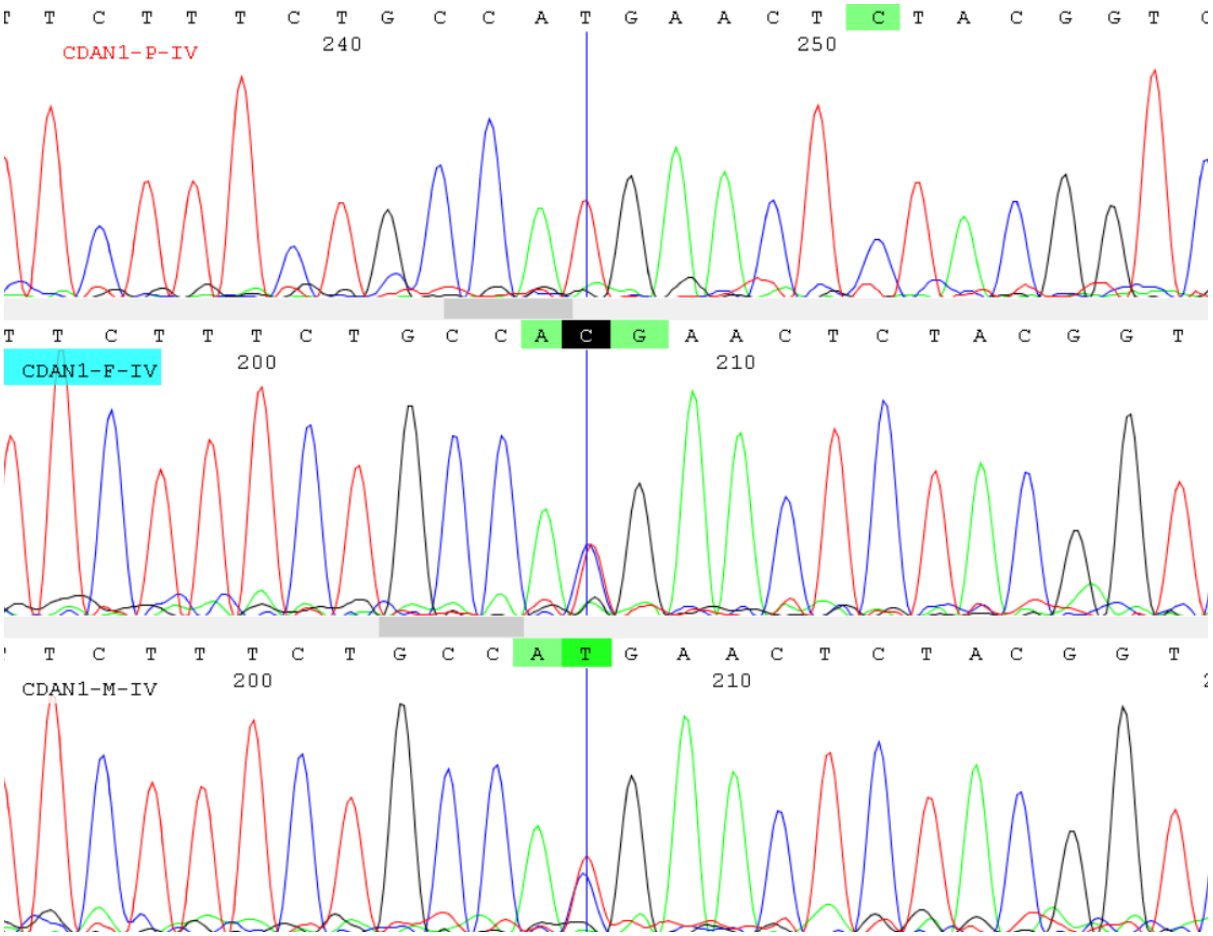
Supplementary figure 2



Supplementary figure 3



Supplementary figure 4



Supplementary figure 5