ALLERGY, GENETICS

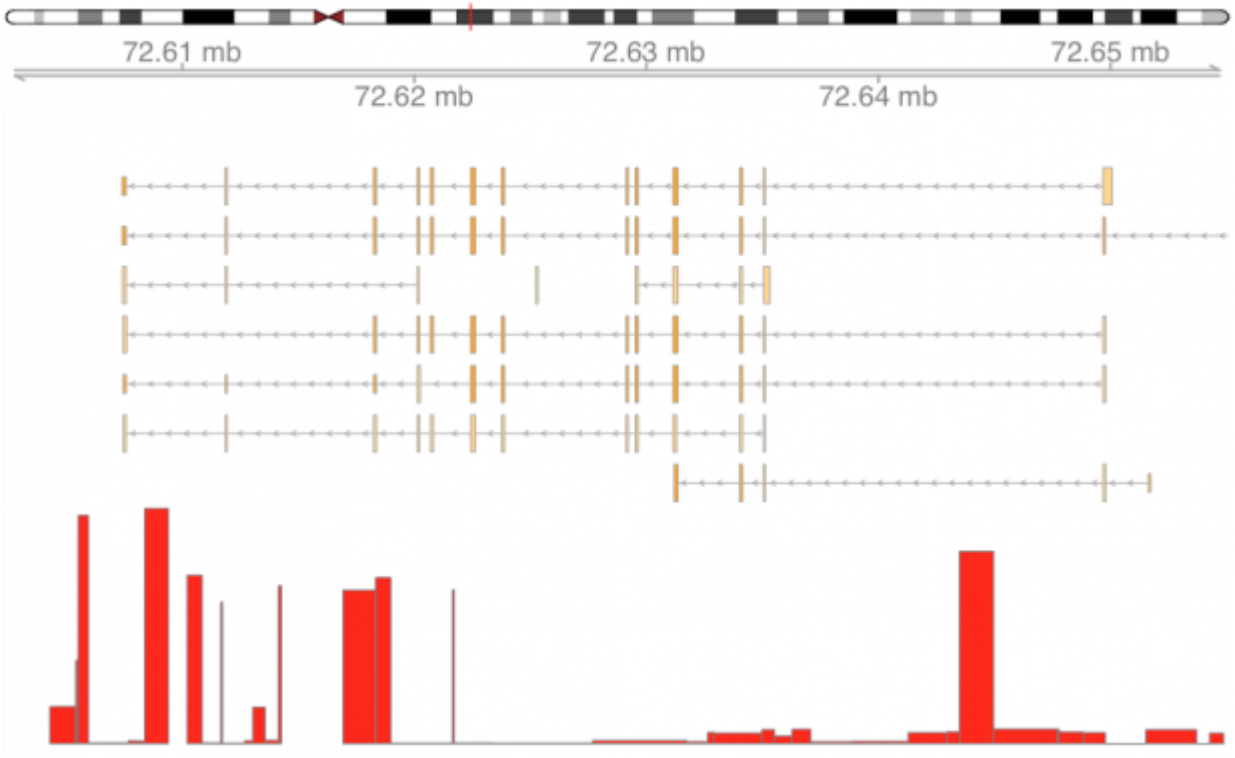
VITAMIN D BUFFERING

3.02.2018

Response to oral vitamin D seems to be different in humans . How do we buffer (artificial) vitamin D intake?

Vitamin binding protein or group specific component GC is a good candidate. GC regulates the bioavailability of 25(OH)D3, acting as the main transporterint he blood stream from liver to kidney. As described earlier GC binds with high affinity to 25(OH)D3, leaving less than 1% of circulating 25(OH)D3 free. In contrast to 25(OH)D3, which has a half-life of several weeks, GC has a short half-life of 3 days only, suggesting that the protein and its ligand are independently regulated. Also the free binding capacity of GC is variable. In addition there are GC variants that have different binding characteristics. Depending on these isoforms, serum levels increased between 97% and 307% after receiving 600 or 4000 IU/d vitamin D3 for one year. Taken together GC is assumed to be a buffer of vitamin D effects (and side effects) whenever transport in the blood stream is being involved.

The most recent GWAS study now shows again skyrocking p-values of GC variants and serum 25(OH)D3.



It is long known, that two missense variants of GC locate in exon 11. rs7041 encodes Asp432Glu pr D432E and rs4588 encodes Thr436Lys or T436K. These amino acid exchanges are leading to electrophoretically distinguishable proteins Gc1F/Gc1S and Gc2 respectively. We are moving the following gene plot bottom up to match the orientation.

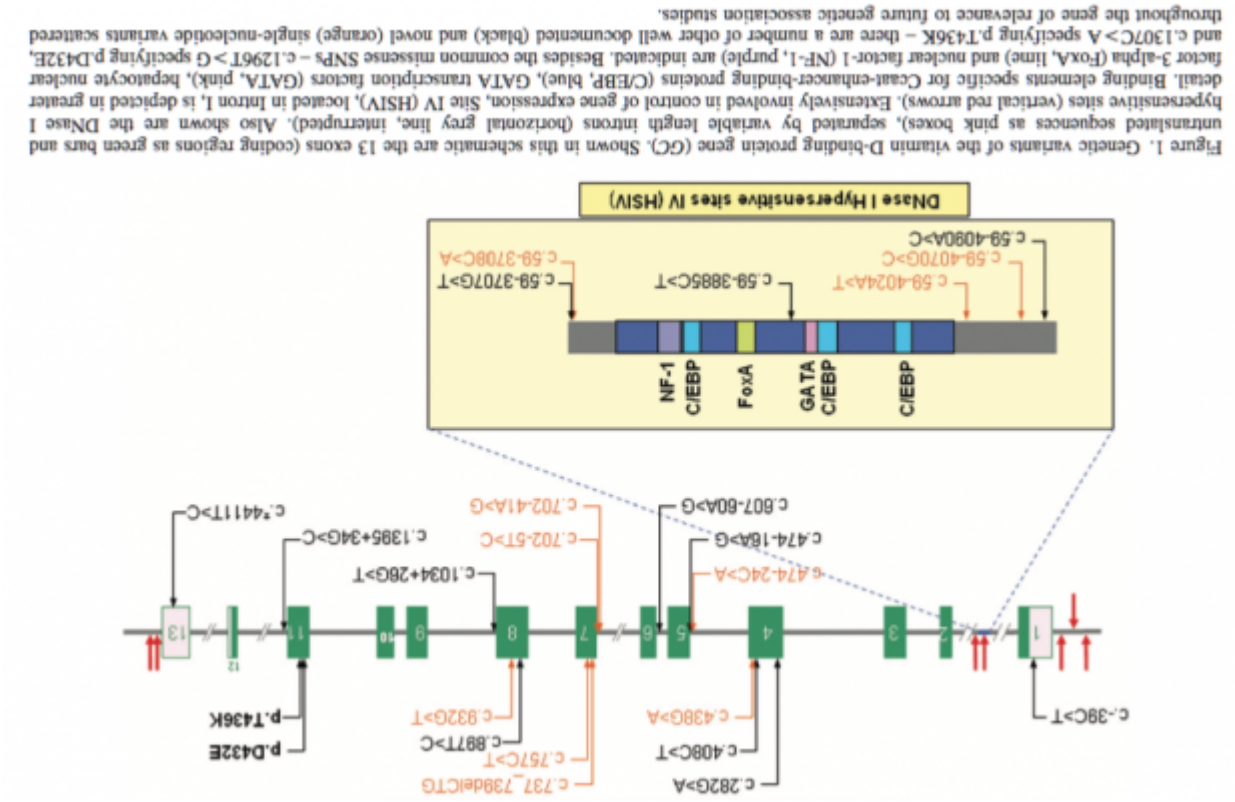


Figure 1. Genetic variants of the vitamin D-binding protein gene (GC). Shown in this schematic are the 13 exons (coding regions as green bars and untranslated sequences as pink boxes), separated by variable length introns (horizontal grey line, interrupted). Also shown are the DNase I hypersensitive sites (vertical red arrows). Extensively involved in control of gene expression, Site IV (HSIV), located in Intron 1, is depicted in greater detail. Binding elements specific for C/aat-enhancer-binding proteins (C/EBP, blue), GATA transcription factors (GATA, pink), hepatocyte nuclear factor 3-alpha (FoxA, lime) and nuclear factor-1 (NF-1, purple) are indicated. Besides the common missense SNPs – c.1296T > G specifying p.D432E, and c.1307C > A specifying p.T436K – there are a number of other well documented (black) and novel (orange) single-nucleotide variants scattered throughout the gene of relevance to future genetic association studies.

Unfortunately LD is extremely high at GC. The GWAS peaks are therefore in the first intron, at exon 11 and intron 12. Lets' s get closer to exon 11 where the two most important SNPs

reside.

SNP Chip List		A_Exome1A	A_Exome319	A_AFR	A_ASI	A_EAS	A_EUR	A_Hu	A_Hu-CHB	A_LAT	A_Onco	A_OncoCNV	I_1M-D	I_1M	I_610-Q	I_660W-Q	I_CNV370-D	I_CNV370-Q	I_CVD	I_CoreE-12v1	I_CoreE-12v1.1	I_CoreE-24v1	I_CoreE-24v1.1	I_Cyto-12v2	I_Cyto-12v2.1	I_Cyto-12v2.1f	I_Exome-12	I_Exon510S	I_300-D	I_300	I_550v1	I_550v3	I_Immuno-24v1	I_Immuno-24v2	I_NS-12	I_O1-Q	I_O2.5-4
RS Number	Position (GRCh37)																																			
rs4588	chr4:72618323	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓					✓	✓	✓	✓	✓				✓	✓								✓	✓
rs7041	chr4:72618334	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓

Although both variants are listed at many SNP chips I can find only results for rs7041 with p=10^-222 in the new dataset.

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> head(rsds, n=50)
     SNP Allele1 Allele2  Effect StdErr    Pvalue                                  Direction HetISq HetChiSq HetDf   HetPVal
1 rs7041       a       c -0.0666 0.0021 1.66e-222 ----------------?-?----------?---?--??-----   71.9  128.322     36 2.743e-12
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rs7041 is listed as a A->C SNP there but according to Fu 2009 it is definitely a G->T variant. Also SNPedia has numerous articles for rs7041 being a G->T exchange, for example Suaini 2014

Association between *GC* or *VDR* SNPs and vitamin D insufficiency (seasonally adjusted vitamin D level of ≤50 nmol/L).

Gene	SNP ID	Genotype	Caucasian infants ($N = 491^a$) ≤50 nmol/L (%)	>50 nmol/L (%)	OR (95% CI)	*P* value	Asian infants ($N = 72^a$) ≤50 nmol/L (%)	>50 nmol/L (%)	OR (95% CI)	*P* value
Vitamin D binding protein SNPs										
GC	rs1155563	TT	33 (13)	213 (87)	1 (Reference)	–	7 (27)	19 (73)	1 (Reference)	–
		CT	38 (21)	145 (79)	1.69 (1.01, 2.82)	0.04	6 (17)	29 (83)	0.56 (0.16, 1.93)	0.36
		CC	15 (31)	33 (69)	2.93 (1.44, 5.98)	0.003	2 (22)	7 (78)	0.78 (0.13, 4.67)	0.78
GC	rs17467825	AA	32 (13)	220 (87)	1 (Reference)	–	5 (16)	26 (84)	1 (Reference)	–
		GA	42 (23)	139 (77)	2.08 (1.25, 3.45)	0.005	6 (21)	23 (79)	1.36 (0.37, 5.04)	0.65
		GG	14 (29)	35 (71)	2.75 (1.34, 5.66)	0.006	2 (22)	7 (78)	1.49 (0.24, 9.35)	0.67
GC	rs222020	TT	64 (19)	266 (81)	1 (Reference)	–	6 (22)	21 (78)	1 (Reference)	–
		TC	21 (16)	114 (84)	0.77 (0.45, 1.31)	0.33	6 (18)	28 (82)	0.75 (0.21, 2.66)	0.66
		CC	3 (16)	16 (84)	0.78 (0.22, 2.76)	0.70	3 (30)	7 (70)	1.5 (0.29, 7.65)	0.63
GC	rs2282679	AA	32 (13)	221 (87)	1 (Reference)	–	6 (19)	25 (81)	1 (Reference)	–
		CA	42 (23)	140 (77)	2.07 (1.25, 3.44)	0.005	7 (23)	24 (77)	1.22 (0.36, 4.14)	0.76
		CC	14 (29)	35 (71)	2.76 (1.34, 5.69)	0.006	2 (22)	7 (78)	1.19 (0.20, 7.25)	0.85
GC	rs2298849	TT	53 (17)	250 (83)	1 (Reference)	–	8 (28)	21 (72)	1 (Reference)	–
		CT	26 (17)	130 (83)	0.94 (0.56, 1.58)	0.82	5 (16)	27 (84)	0.49 (0.14, 1.70)	0.26
		CC	9 (36)	16 (64)	2.65 (1.11, 6.33)	0.03	2 (20)	8 (80)	0.66 (0.11, 3.78)	0.64
GC	rs3755967	GG	32 (13)	221 (87)	1 (Reference)	–	6 (19)	25 (81)	1 (Reference)	–
		GA	41 (23)	140 (77)	2.02 (1.22, 3.36)	0.007	7 (23)	24 (77)	1.22 (0.36, 4.14)	0.76
		AA	14 (29)	35 (71)	2.76 (1.34, 5.69)	0.006	2 (22)	7 (78)	1.19 (0.20, 7.25)	0.85
GC	rs4588	CC	33 (13)	221 (87)	1 (Reference)	–	6 (19)	25 (81)	1 (Reference)	–
		CA	42 (23)	140 (77)	2.01 (1.22, 3.32)	0.007	7 (23)	24 (77)	1.22 (0.36, 4.14)	0.76
		AA	13 (27)	35 (73)	2.49 (1.19, 5.18)	0.02	2 (22)	7 (78)	1.19 (0.20, 7.25)	0.85
GC	rs7041[b]	GG	16 (11)	130 (89)	1 (Reference)	–	0 (0)	4 (100)	1 (Reference)	–
		GT	49 (20)	198 (80)	2.01 (1.10, 3.69)	0.02	7 (24)	22 (76)	1.00 (omitted)	–
		TT	23 (25)	68 (75)	2.75 (1.36, 5.55)	0.005	8 (21)	30 (79)	1.00 (omitted)	–

This is also confirmed by dbsnp. The GAT -> GAG exchange is equivalent to D -> E, so the online results report a wrong strand orientation. Unfortunately we are stuck here, as one of the main effect SNP seems to have an unclear allele assignment and the second most important SNP is missing from the meta-analysis.

What would be nice is a conditional analysis based on rs7041/rs4588 haplotypes. I predict there are further unknown functional variants in GC. Maybe in intron 1 that often contains regulatory elements at the 5′-site of the intron. As the strongest signal is in the last intron and even beyond the 3′- end, further studies of 3′-UTR would be interesting, looking for

binding sites of regulatory proteins, some miRNA or AU rich elements that affect the stability or decay rate of the transcript.