Association of SNP and STR polymorphisms of insulin-like growth factor 2 receptor (Igf2r) gene with milk traits in Holstein-Friesian cows

Marta Dux, Magdalena Muranowicz, Eulalia Siadkowska, Dagmara Robakowska-Hyżorek, Krzysztof Flisikowski, Emilia Bagnicka, and Lech Zwierzchowski

Supplementary Material

This supplementary file comprises ten Supplementary Figures and Tables, S1 to S10

Supplementary material S1. Statistical Model used for computing association between TG-repeat in intron 23 (g.72389 (TG)15-67), and G/A SNP in exon 24 (g.72479 G>A RFLP-*Sty*I) in *Igf2r* gene and milk traits

$$y_{ijklmno} = a_i + p_i + db_j + RSW_k + NRLAK_m + \beta\left(x_{ijklmno} - \bar{x}\right) + \left(\sum b_p LPd^p\right)_{ijklmno} + e_{ijklmno}$$

where:

$y_{ijklmno}$ - studied traits (milk yield, fat, protein, lactose content or lnSCC*)

G_i - fixed effect of genotype

a_i - random effect of additive animal value

p_i - random effect of fixed animal environment

db_j - random effect of milking date

RSW_k - fixed effect of calving year-season

$NRLAK_m$ - fixed effect of next lactation number

$\beta_1(x_{ijklmno}-x)$ - fixed effect of milk parameters regression on daily milk yield

$(\Sigma b_p LPd^p)_{ijklmno}$ - fixed effect of regression on p-degree Legendre polynomial (p = 1...4) of standardized number of lactation days

$e_{ijklmno}$ - random residual effect

***lnSCC – somatic cell count converted to natural logarithmic scale.**

Table S2 Sequences of primers used for analysis of *Igf2r* gene polymorphism and expression.

Symbols of primers	**Amplified fragment of *Igf2r* gene**	**Method of analysis**	**Primer sequence (5' – 3')**	**Size of PCR product (bp)**
IGF2RUF IGF2RUR	3` UTR	SEQUENCING	F: CAGGTGCACAGAGCACACTTA R: CAAGAGTACAAACCCAGGAACC	584
IGF2R44F IGF2R44R	exon 44 and introns 43, 44		F: CGCAGAGATGTGGGGTAACT R: TCAAGAGCCAGGAGAGCATT	703
IGF2R43F IGF2R43R	exon 43 and introns 42, 43		F: TGGGTGTGCACAGTCAGG R: GCTGCTCAAGAAGAGGAAGC	298
IGF2R24F IGF2R24R1	exon 24 and introns 23, 24		F: AGCTGATTAAAGGTTGCAGAAGTT R: CGGAATTCCAAGAAGAGAAGG	351
IGF2R24B IGF2R24R2	exon 24	RFLP	F: GGAGACTACTGCGAGGTGAGA R: CGGAATTCCAAGAAGAGAAGG	254
IGF2R23F (Cys-5) IGF2R23R	intron 23	STR	F: AGCTGATTAAAGGTTGCAGAAGTT R: GGAGACTACTGCGAGGTGAGA	114 – 218 (depending on number of TG-repeats)

Table S3 Allele and genotypes frequency (F) and their distribution (N) for and the g.72479 G>A transition in *Igf2r* gene exon 24.

Genotype	N	F
GG	147	0.52
GA	128	0.45
AA	8	0.03
Allele	F	
G	0.75	
A	0.25	

Table S4 Frequency (F) and distribution of alleles (N) for polymorphism g.72389(TG)15-67 in the *Igf2r* gene intron 23. Genotypes with the highest values of frequencies were bold.

Allele of TG_n	N	F
28	119	0.213
25	70	0.125
29	64	0.114
20	61	0.109
22	60	0.107
33	56	0.100
23	40	0.071
30	28	0.050
27	24	0.043
34	5	0.008
26	5	0.008
19	5	0.008
15	3	0.005
24	3	0.005
67	3	0.005
16	2	0.003
21	2	0.003
31	2	0.003
55	2	0.003
64	2	0.003
32	1	0.001
65	1	0.001

Table S5 Frequency (F) and distribution of genotypes (N) for polymorphism g.72389(TG)15-67 in the *Igf2r* gene intron 23. Genotypes with the highest values of frequencies are in bold.

Genotype TG_n	N	F
28/25	22	0.078
28/27	20	0.071
29/22	17	0.060
25/33	14	0.050
28/20	12	0.043
28/29	11	0.039
20/23	11	0.039
25/20	10	0.035
28/22	9	0.032
28/33	9	0.032
29/33	9	0.032
22/33	9	0.032
28/28	8	0.028
29/20	7	0.025
29/23	7	0.025
20/22	7	0.025
28/23	6	0.021
28/30	6	0.021
25/30	6	0.021
25/29	5	0.017
25/23	5	0.017
25/22	4	0.014
20/33	4	0.014
22/30	4	0.014
33/23	4	0.014
20/19	3	0.010
33/30	3	0.010
23/30	3	0.010
28/26	3	0.010
22/23	2	0.007
29/30	2	0.007
29/15	2	0.007
20/30	2	0.007
20/34	2	0.007
22/19	2	0.007
28/34	1	0.003
28/24	1	0.003
28/15	1	0.003
28/21	1	0.003
28/16	1	0.003
25/25	1	0.003
25/34	1	0.003
25/24	1	0.003
29/29	1	0.003
29/27	1	0.003
29/26	1	0.003
20/20	1	0.003
20/31	1	0.003
22/22	1	0.003
22/27	1	0.003
22/26	1	0.003
22/31	1	0.003
22/16	1	0.003
33/33	1	0.003
33/27	1	0.003
33/32	1	0.003
23/34	1	0.003
23/21	1	0.003
30/30	1	0.003
27/24	1	0.003
67/64	1	0.003
67/55	1	0.003
67/65	1	0.003
64/55	1	0.003

Table S6 Influence of genotype in *Igf2r* gene polymorphism g.72389(TG)15-67 on milk yield. Levels of significance were established at $p \leq 0.05$ and $p \leq 0.01$.

Genotype	Estimates of milk yield [kg]	SE	Significantly different (at $p \leq 0.05$) from the following genotypes	Significantly different (at $p \leq 0.01$) from the following genotypes
29/22	37.696	1.460	25/20, 28/25, 25/29, 28/27, 25/33, 29/23, 20/23, 28/20, 29/20, 25/23, 20/33, 29/33, 28/30	25/22
23/30	37.494	2.698	29/33, 28/30	20/33
28/22	37.165	1.727	20/23, 28/20, 20/33, 29/33, 28/30	28/25, 25/29, 28/27, 25/33, 29/23, 29/20
28/29	37.077	1.672	28/20, 20/33, 29/33, 28/30	28/25, 28/27 25/33, 29/23, 20/23, 29/20, 25/23
28/28	37.062	1.804	28/20, 25/23, 29/33, 28/30	25/33, 29/23, 20/23, 29/20, 20/33
28/23	36.853	2.210	29/33, 28/30	20/23, 28/20, 29/20, 25/23, 20/33
25/30	36.649	1.999	29/33, 28/30	20/19, 20/23, 28/20, 29/20, 25/23
28/26	36.588	2.602	29/33, 28/30	
20/19	36.218	2.634	29/33, 28/30	
20/22	35.996	1.914	29/33, 28/30	23/30, 20/33
22/33	35.975	1.745	29/33, 28/30	
22/30	35.959	2.608	29/33, 28/30	
33/30	35.910	3.617		28/30
28/33	35.841	1.778	29/33, 28/30	
25/20	35.425	1.789	29/22, 29/33, 28/30	
28/25	35.333	1.340	29/22, 28/30	28/22, 28/25
25/29	35.116	2.132	29/22, 29/33, 28/30	28/22
28/27	35.111	1.418	29/22, 29/33, 28/30	28/22, 28/29
25/33	34.878	1.545	29/22, 29/33	28/22, 28/29, 28/28
33/23	34.790	2.652		29/33
29/23	34.680	1.949	29/22, 29/33	28/22, 28/29, 28/28, 28/30
20/23	34.610	1.703	29/22, 28/22, 29/33, 28/30	28/29, 28/28, 28/23, 25/30
25/22	34.352	2.624		29/22
28/20	34.272	1.569	29/22, 28/22, 28/29, 28/28, 29/33	28/23, 25/30, 28/30
29/20	34.206	2.040	29/22	28/22, 28/29, 28/28, 28/23, 25/30
25/23	34.034	2.151	29/22, 28/28, 28/30	28/29, 28/23, 25/30
20/33	33.875	2.288	29/22, 28/22, 28/29	23/30, 28/28, 28/23, 25/30, 20/19
29/33	32.028	1.741	29/22, 23/30, 28/22, 28/29, 28/28, 28/23, 25/30, 28/26, 20/19, 28/33, 25/20, 25/29, 28/27, 25/33, 29/23, 20/23, 28/20, 22/30	33/23
28/30	31.966	2.054	28/28, 28/25, 28/29, 28/22, 28/33, 28/23, 28/27, 28/26, 25/29, 25/20, 25/30, 29/22, 20/19, 20/23, 22/30, 23/30	29/23, 28/20

Table S7 Influence of genotype of *Igf2r* gene polymorphism g.72389(TG)15-67 on fat yield. Levels of significance were established at p≤0.05 and p≤0.01.

Genotype	Estimates of fat yield [kg]	SE	Significantly different (at p≤0.05) from the following genotypes	Significantly different (at p≤0.01) from the following genotypes
28/23	1.693	0.082	28/25, 28/29, 25/33, 20/23, 29/33, 22/33, 20/33, 28/30	29/23, 28/27, 29/22, 28/20, 25/30, 25/22
28/22	1.605	0.066	28/27, 29/22, 25/30, 28/25, 25/22, 25/33, 29/33, 22/33, 20/33, 28/30	28/33, 29/23, 29/20, 25/23, 28/20, 33/30, 25/22
23/30	1.576	0.100	28/30	20/33
20/22	1.558	0.072	28/27, 20/23, 29/33, 22/33, 20/33	25/23, 28/25, 28/29
25/29	1.523	0.079	20/33, 28/30	29/33, 22/33
22/30	1.520	9.095		20/33
25/20	1.516	0.068	22/33, 20/33, 28/30	20/23, 29/33
28/28	1.516	0.069	20/33, 28/30	29/33, 22/33
33/23	1.515	0.098		20/33
28/33	1.513	0.067	20/33, 28/30	28/22, 29/33, 22/33
29/23	1.503	0.072	20/33, 28/30	28/23, 28/22, 25/22
29/20	1.502	0.076	28/30	28/23, 28/22, 20/33
28/26	1.500	0.095	28/30	22/33
20/19	1.490	0.097		20/33
25/23	1.487	0.019	28/30	28/22, 22/33, 20/33
28/27	1.486	0.056	28/22, 20/33, 28/30	28/23, 29/33, 22/33
29/22	1.482	0.057	28/22, 20/33, 28/30	28/23
28/20	1.481	0.061	28/30	28/23, 28/22, 22/33, 20/33
25/30	1.474	0.075	28/22	28/23, 29/23, 28/30
33/30	1.459	0.086		28/22
28/25	1.456	0.053	28/23, 28/22, 28/30	20/22, 20/33
28/29	1.446	0.064	28/23, 28/22, 28/30	20/22, 20/33
25/22	1.436	0.095		28/23, 28/22
25/33	1.435	0.060	28/23, 28/22	28/30
20/23	1.430	0.064	28/23, 28/22, 20/22	25/29
29/33	1.415	0.066	28/22, 20/22, 28/30	25/29, 25/20, 28/28, 28/33, 16,
22/33	1.400	0.066	28/23, 28/22, 20/22, 25/20	25/29, 28/28, 28/33, 28/27, 28/20, 25/33
20/33	1.350	0.084	28/23, 28/22, 20/22, 25/20, 28/28, 28/33, 29/23, 28/27, 29/22	23/30, 29/20, 28/26, 28/20, 25/30, 28/25, 28/29, 25/33
28/30	1.331	0.076	28/23, 28/22, 23/30, 20/22, 25/29, 25/20, 28/28, 28/33, 29/23, 29/20, 28/26, 25/23, 28/27, 29/22, 28/20, 28/25, 28/29	20/19, 25/30, 25/33

Table S8 Influence of genotype in *Igf2r* gene polymorphism g.72389(TG)15-67 on protein yield. Levels of significance were established at p≤0.05 and p≤0.01.

Genotype	Estimates of protein yield [kg]	SE	Significantly different (at p≤0.05) from the following genotypes	Significantly different (at p≤0.01) from the following genotypes
28/23	0.581	0.052	25/30, 28/28, 25/22, 25/20, 29/22, 28/33, 33/23, 28/29, 22/30, 20/23, 20/22, 29/20, 28/26, 33/30, 28/25, 20/19, 25/33, 28/27, 28/20, 25/29, 22/33, 20/33, 29/23, 25/23, 29/33, 28/30	28/22
23/30	0.523	0.064	29/33, 28/30	25/33, 28/27, 28/20, 25/29, 22/33, 20/33, 29/23,
28/22	0.517	0.044	28/25, 25/33, 28/27, 28/20, 25/29, 22/33, 20/33, 29/23, 25/23, 29/33, 28/30	28/23, 29/22, 28/29, 20/23, 20/22, 29/20
25/30	0.482	0.049	28/23, 25/23, 29/33, 28/30	29/22, 29/23
28/28	0.480	0.045	28/23, 29/23, 29/33, 28/30	25/23
25/22	0.478	0.061	28/23, 29/33, 28/30	29/23, 25/23
25/20	0.476	0.443	28/23, 29/33, 28/30	29/23, 25/23
29/22	0.476	0.039	28/23, 28/30	28/22, 25/30, 22/33, 29/23, 25/23
28/33	0.473	0.044	28/23, 29/33, 28/30	25/23
33/23	0.472	0.062	28/23, 28/30	29/33
28/29	0.466	0.042	28/23, 29/33, 28/30	28/22
22/30	0.464	0.061	28/23, 28/30	
20/23	0.463	0.041	28/23, 28/30	28/22
20/22	0.462	0.047	28/23, 29/33, 28/30	28/22
29/20	0.460	0.049	28/23, 28/30	28/22, 25/30, 25/23, 29/33
28/26	0.460	0.061	28/23, 25/29, 28/30	29/33
33/30	0.458	0.062	28/23, 28/30	
28/25	0.457	0.161	28/23, 28/22, 29/33, 28/30	
20/19	0.456	0.062	28/23, 28/30	
25/33	0.449	0.040	28/23, 28/22, 28/30	29/33
28/27	0.443	0.038	28/23, 28/22, 28/30	23/30, 29/33
28/20	0.440	0.041	28/23, 28/22, 28/30	23/30
25/29	0.435	0.051	28/23, 28/22, 28/26, 28/30	23/30
22/33	0.432	0.044	28/23, 28/22, 28/30	23/30, 29/22
20/33	0.427	0.055	28/23, 28/22, 28/30	23/30
29/23	0.427	0.047	28/23, 28/22, 28/30	23/30, 25/30, 28/28, 25/22, 25/20, 29/22
25/23	0.416	0.052	28/23, 28/22, 25/30	23/30, 28/28, 25/22, 25/20, 29/22, 28/33, 28/30
29/33	0.400	0.044	28/23, 23/30, 28/22, 25/30, 28/28, 25/22, 25/20, 28/33, 28/29, 20/22, 28/25,	33/23, 29/20, 28/26, 25/33, 28/27
28/30	0.362	0.049	28/23, 23/30, 28/22, 25/30, 28/28, 25/22, 25/20, 29/22, 33/23, 28/29, 22/30, 20/23, 20/22, 29/20, 28/26, 33/30, 28/25, 20/19, 25/33, 28/27, 28/20, 22/33	28/29, 29/20, 25/33, 28/20

Table S9 Influence of genotype in *Igf2r* gene polymorphism g.72389(TG)15-67 on lactose content in milk. Levels of significance were established at p≤0.05 and p≤0.01.

Genotype	Estimates of lactose content in milk [%]	SE	Significantly different (at p≤0.05) from the following genotypes	Significantly different (at p≤0.01) from the following genotypes
25/23	4.729	0.075	28/28, 28/25, 28/29, 28/20, 28/22, 28/33, 28/23, 28/30, 28/27, 28/26, 25/29, 25/33, 25/30, 29/20, 29/22, 29/33, 29/23, 20/22, 20/33, 20/19, 22/30, 33/23, 22/33	20/23
33/30	4.714	0.071	28/28, 28/25, 28/29, 28/33, 28/23, 28/30, 28/27, 28/26, 25/33, 25/30, 29/23, 20/33, 20/19, 22/30, 22/33	28/20, 28/22, 25/29, 29/33, 20/22, 33/23
25/22	4.678	0.092	28/25, 28/30, 28/26, 25/33, 25/30, 29/20, 29/23, 20/33, 20/19, 22/30, 22/33	28/28, 28/29, 28/33, 28/23, 29/33, 20/22, 33/23
25/20	4.662	0.060	28/25, 28/29, 28/33, 28/23, 28/30, 28/26, 25/33, 25/30, 29/20, 29/23, 20/33, 20/23, 20/19, 22/33	28/28, 28/27, 29/33, 20/22, 33/23
20/23	4.634	0.059	28/25, 28/30, 28/26, 25/33, 29/20, 29/23, 20/33, 20/19, 22/30, 22/33	28/23, 25/23
23/30	4.632	0.063	28/30, 28/26, 29/20, 22/30	29/23, 20/33, 22/33
29/22	4.629	0.049	28/25, 28/30, 28/26, 25/23, 29/20, 29/23, 20/33, 20/19, 22/30, 22/33	28/29, 28/33, 28/23
28/22	4.608	0.059	28/30, 28/26, 25/23, 29/20, 29/23, 22/30	20/33, 20/19, 33/30
28/20	4.605	0.052	28/30, 28/26, 25/23, 29/20, 29/23, 20/19, 22/30	20/33, 33/30
29/33	4.590	0.060	28/30, 28/26, 25/23, 29/20, 22/30	25/20, 25/22, 29/23, 22/30, 33/30
28/27	4.587	0.047	28/26, 25/23, 29/20, 22/30, 33/30	25/20, 29/23, 20/33
25/29	4.581	0.073	28/26, 25/23, 29/20, 22/30	28/30, 33/30
20/22	4.574	0.066	28/26, 25/23, 29/20, 22/30	28/30, 25/20, 25/22, 33/30
28/29	4.570	0.058	28/26, 25/20, 25/22, 25/23, 29/20, 22/30, 33/30	28/30, 25/22, 29/22
33/23	4.563	0.056	25/23, 29/20, 33/30, 23/30	25/29, 25/22, 25/33, 22/30, 33/30
28/28	4.561	0.060	28/26, 25/23, 33/30	28/30, 25/20, 25/22, 29/20, 22/30
28/33	4.560	0.061	28/26, 25/20, 25/23, 29/20, 22/30, 33/30	28/30, 25/22, 29/22
25/30	4.560	0.069	28/26, 25/20, 25/22, 25/23, 22/30, 33/30	28/30, 29/20
25/33	4.550	0.053	28/26, 25/20, 25/22, 25/23, 29/20, 29/22, 20/23, 22/30, 33/30	
28/23	4.537	0.077	25/20, 25/23, 33/30	28/26, 25/22, 29/22, 20/23, 22/30
28/25	4.533	0.045	28/26, 25/20, 25/22, 25/23, 29/22, 20/23, 22/30, 33/30	29/20
29/23	4.509	0.068	28/20, 28/22, 25/20, 25/22, 25/23, 29/22, 20/19, 33/30	28/27, 28/26, 29/33, 22/30, 23/30
20/19	4.508	0.088	25/20, 25/22, 25/23, 29/22, 20/23, 33/30	28/20, 28/22
22/33	4.506	0.060	25/20, 25/22, 25/23, 29/22, 20/23, 33/30	28/20, 28/22, 23/30
20/33	4.503	0.078	25/20, 25/22, 25/23, 29/22, 20/23, 33/30	28/20, 28/22, 28/27, 29/33
28/30	4.465	0.073	28/20, 28/22, 28/27, 25/20, 25/22, 25/23, 29/22, 29/33, 20/23, 33/30, 23/30	28/28, 28/29, 28/33, 25/29, 25/30, 20/22
29/20	4.455	0.072	28/29, 28/20, 28/22, 28/33, 28/27, 28/28, 25/20, 25/22, 25/33, 25/23, 25/30, 29/22, 29/33, 20/22, 20/23, 33/23, 23/30	28/28, 28/25, 25/30
28/26	4.417	0.088	28/28, 28/25, 28/29, 28/20, 28/22, 28/33, 28/27, 25/29, 25/20, 25/22, 25/33, 25/23, 25/30, 29/22, 29/33, 20/22, 20/23, 33/30, 23/30	28/23, 29/23, 33/23
22/30	4.391	0.039	28/25, 28/29, 28/20, 28/22, 28/33, 28/23, 28/26, 25/20, 25/22, 25/33, 25/23, 25/30, 29/22, 29/33, 20/22, 20/23, 33/30, 23/30	28/28, 28/30, 29/23, 33/23

Table S10 Influence of genotype of *Igf2*r gene polymorphism g.72389(TG)15-67 on somatic cells score in milk. Levels of significance were established at p≤0.05 and p≤0.01.

Genotype	Estimates of ln SSC	SE	Significantly different (at p≤0.05) from the following genotypes	Significantly different (at p≤0.01) from the following genotypes
28/28	6.674	0.415	28/29, 28/27, 25/30, 20/33, 33/23, 20/23, 28/25, 28/26, 28/23, 28/33, 25/29, 29/20, 20/19, 22/30, 23/30	29/22, 20/22, 28/30, 25/23, 25/20, 29/23
33/30	6.859	0.639	28/20, 28/33, 25/29, 29/20, 20/19, 22/30, 23/30	28/26, 28/23
29/33	6.920	0.394	20/23, 28/25, 28/26, 28/20, 28/23, 28/33, 25/29, 29/20, 20/19, 22/30, 23/30	29/23, 28/27, 25/30, 20/33
25/33	6.987	0.336	20/23, 28/25, 28/20, 28/23, 28/20, 28/33, 25/29, 29/20, 20/19, 22/30, 23/30	28/27
25/22	7.009	0.641	29/20, 20/19, 22/30, 23/30	28/20, 28/23, 28/33, 25/29
28/22	7.057	0.393	25/20, 28/25, 28/20, 28/23, 28/33, 25/29, 29/20, 20/19, 22/30, 23/30	25/20, 20/23
29/22	7.139	0.311	28/20, 28/33, 25/29, 29/20, 20/19, 22/30, 23/30	28/28, 28/25, 28/26, 28/23
20/22	7.175	0.444	28/33, 29/20, 22/30, 23/30	28/28, 28/20, 28/23, 25/29
28/29	7.214	0.371	28/28, 29/20, 20/19, 22/30, 23/30	28/20, 28/23, 28/33, 25/29
28/30	7.222	0.480	29/20, 20/19, 22/30, 23/30	28/28, 28/33, 25/29
25/23	7.222	0.509	29/20, 20/19, 22/30, 23/30	28/28, 28/33, 25/29
25/20	7.236	0.409	28/22, 29/20, 20/19, 22/30, 23/30	28/28, 28/20, 28/23, 28/33, 25/29
29/23	7.356	0.451	22/30, 23/30	28/28, 29/33, 29/20, 20/19
28/27	7.364	0.296	28/28, 29/20, 22/30, 23/30	29/33, 25/33, 20/19
25/30	7.385	0.470	28/28, 22/30, 23/30	29/33, 29/20, 20/19
20/33	7.414	0.555	28/28, 23/30	29/33, 22/30
33/23	7.431	0.646	28/28, 33/30	22/30
20/23	7.453	0.382	28/28, 29/33, 25/33, 29	28/33, 22/30
28/25	7.482	0.275	28/28, 29/33, 25/33, 28/22, 22/30, 23/30	33/30, 29/22, 29/20, 20/19,
28/26	7.613	0.641	28/28, 29/33, 25/33	33/30, 28/22, 29/22
28/20	7.649	0.346	28/28, 33/30, 29/33, 25/33, 28/22, 29/22	25/22, 20/22, 28/29, 25/20, 23/30
28/23	7.680	0.526	28/28, 29/33, 25/33, 28/22	33/30, 25/22, 29/22, 20/22, 28/29
28/33	7.708	0.405	28/28, 33/30, 29/33, 25/33, 28/22, 29/22, 20/22	25/22, 28/29, 28/30, 25/23, 25/20,
25/29	7.743	0.508	28/28, 33/30, 29/33, 25/33, 28/22, 29/22	25/22, 29/22, 20/22, 28/29, 25/23, 25/20,
29/20	7.871	0.479	28/28, 33/30, 29/33, 25/33, 25/22, 29/22, 20/22, 28/29, 28/30, 25/23, 25/20, 28/27	29/23, 25/30, 28/25
22/33	7.873	0.394	28/28, 29/33, 25/33, 25/22, 28/22, 29/22, 20/22, 28/29, 28/30, 25/23, 25/20, 29/23, 28/27, 23/30	25/30, 28/25
20/19	8.006	0.649	28/28, 33/30, 29/33, 25/33, 25/22, 28/22, 29/22, 28/29, 28/30, 25/23, 25/20	28/27, 25/30, 28/25
22/30	8.178	0.640	28/28, 33/30, 29/33, 25/33, 25/22, 28/22, 29/22, 20/22, 28/29, 28/30, 25/23, 25/20, 29/23, 28/27, 25/30, 28/25	33/23, 20/23
23/30	8.262	0.653	28/28, 29/33, 25/33, 25/22, 28/22, 29/22, 20/22, 28/29, 28/30, 25/23, 25/20, 29/23, 28/27, 25/30, 20/33, 20/23, 28/25, 23/30	33/23, 28/20