Replicative senescence-associated gene expression changes in mesenchymal stromal cells are similar under different culture conditions

Katharina Schallmoser,^{1,2} Christina Bartmann,^{1,3} Eva Rohde,^{1,2} Simone Bork,⁴ Christian Guelly,⁵ Anna C. Obenauf,⁶ Andreas Reinisch,^{1,3} Patrick Horn,⁴ Anthony D. Ho,⁴ Dirk Strunk,^{1,3} and Wolfgang Wagner^{4,7}

¹Stem Cell Research Unit Graz, Medical University of Graz, Austria; ²University Clinic of Blood Group Serology and Transfusion Medicine, Medical University of Graz, Austria; ³University Clinic of Internal Medicine, Department of Hematology, Medical University of Graz, Austria; ⁴Department of Medicine V, University of Heidelberg, Germany; ⁵Center for Medical Research, Medical University of Graz, Austria; ⁶Institute of Human Genetics; Medical University of Graz, Austria; ⁷Institute for Biomedical Engineering - Cell Biology, RWTH University of Aachen, Germany

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Supplementary Methods

Isolation of mesenchymal stromal cells cultured with fetal bovine serum or pooled human platelet lysate

Bone marrow aspirates (maximum 13.5 mL) were obtained in 5 mL syringes preloaded with 500 IU of preservative-free heparin (Biochrom AG, Berlin, Germany) in 2.5 mL aliquots from healthy donors (n=3; aged 9, 27 and 36 years; one female, two males) after receiving written informed consent according to protocols approved by an institutional review board. After harvesting, bone marrow samples were diluted in medium without further manipulation. Cells were cultured in alpha-modified minimum essential medium (α -MEM, M4526; Sigma-Aldrich; St. Louis, MO, USA) supplemented with 25 mM HEPES (Sigma), 2 mM L-glutamine (Sigma), 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco Cell Culture, Invitrogen Corporation, Grand Island, NY, USA), and either 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA) or 10% pooled human platelet lysate (pHPL). pHPL was prepared from buffy coat-derived platelet rich plasma from at least 40 whole blood donations as previously described.1 Preservative-free heparin (2 U/mL; Biochrom AG, Berlin, Germany) was added to the medium before pHPL supplementation to avoid coagulation. The bone marrow mononuclear cell count was determined by an automated blood counter (Coulter Onyx; Beckman Coulter, Fullerton, CA, USA) and cells were seeded at a density of $0.6 - 1.0 \times 10^4$ mononuclear cells/cm² in three to five tissue culture flasks (Corning Inc., Acton, MA, USA) and then cultured at 37°C in 5% CO₂, 95% air humidity. Non-adherent cells were removed by a complete change of medium after 2 - 3 days. Twice weekly, 30% of the medium was replaced by fresh supplemented medium and cells were harvested before reaching confluence (between day 11 and 16) with 0.05% trypsin/0.7mM EDTA (1 - 5 min, 37°C; Sigma-Aldrich). Numbers of nucleated cells were determined as the mean of four measurements, using a hemocytometer. Fibroblast colony-forming units (CFU-F) were determined and cell cultures documented as described previously.²⁻ ³ The primary culture is equivalent to passage zero (P0) and was used as the reference for senescence-associated changes.

Large scale expansion of mesenchymal stromal cells cultured with fetal bovine serum or pooled human platelet lysate

MSC derived from primary culture (P0) were seeded in α -MEM/10% pHPL and α -MEM/10% FBS with a seeding density of 30/cm² on 1.0 to 2.5 m² culture area in four to ten fourlayered cell factories (CF-4; Nalge Nunc International, Naperville, IL, USA) and cultured to reach confluence. MSC from donor A were primarily cultured only in FBS-medium but were re-seeded in FBS and pHPL for P1. Partial (30%) medium change was performed twice weekly and MSC were harvested at days 12 to 13 by trypsinization (passage 1; P1). MSC derived from P1 were immediately re-seeded in α -MEM/10% pHPL and α -MEM/10% FBS at a density of 10-30 cells per cm² in three to four tissue culture flasks (Corning Inc., Acton, MA, USA) corresponding to a culture area of 675 – 900 cm² and cultured at 37°C in 5% CO₂, 95% air humidity for 12 to 14 days until reaching confluence (passage 2; P2). Passaging of the cells was always performed at the time when the cells formed a confluent layer. MSC were harvested with 0.05% trypsin/0.7 mM EDTA (1 - 5 min, 37°C; Sigma-Aldrich) and counted as described previously. Cumulative population doublings (PD) were calculated as described⁴ in relation to the initial CFU-F frequency. Results are shown as mean ± standard error of mean, unless otherwise stated.

Isolation and expansion of mesenchymal stromal cells cultured with M1 culture medium

For comparison we used MSC that had been isolated in culture medium M1 as described earlier.⁵⁻⁶ Cells were isolated from bone marrow aspirates from healthy donors after written informed consent according to guidelines approved by the Ethic Committee on the Use of Human Subjects at the University of Heidelberg. The M1 medium consisted of 58% Dulbecco's modified Eagles medium - low glucose (DMEM-LG, Cambrex, Apen, Germany) and 40% MCDB201 (Sigma, Deisenhofen, Germany), 2% FBS (HyClone, Bonn, Germany), supplemented with 2 mM L-glutamine, 100 U/mL Pen/Strep (Cambrex), 1% insulin transferrin selenium, 1% linoleic acid bovine serum albumin, 10 nM dexamethasone, 0.1 mM Lascorbic-acid-2-phosphate (Sigma, Hamburg, Germany), PDGF-BB and EGF (10 ng/mL each, R&D Systems, Wiesbaden, Germany).⁶⁻⁸ Tissue culture flasks were coated with 10 ng/mL fibronectin (Sigma) before use. MSC-M1 were always harvested upon sub-confluent growth at a density of 70% and re-plated at 10^4 cells per cm². Expansion was performed by the same operator throughout long-term culture to ensure similar cell densities. Seeding of the cells in numbers per cm² and repeated cell passages at 70% confluence resulted in a more constant cell density throughout culture-expansion.

Morphological analysis of mesenchymal stromal cells

The morphological features of MSC expanded in FBS- and pHPL-driven cultures in the early stage (maximal 12 PD) of proliferation in comparison to late passages (more than 38 cumulative PD) were analyzed by phase contrast microscopy (Olympus IX51 microscope, equipped with a COLOR-VIEW III camera and ANALYSIS B software, Olympus, Center Valley, PA, USA).

Immunophenotypic analysis of mesenchymal stromal cells cultured with fetal bovine serum or pooled human platelet lysate

The immunophenotype of FBS-MSC and pHPL-MSC was characterized after washing and blocking with sheep serum. Cells were incubated for 25 min at 4°C at different concentra-

tions according to individual titration with fluorochromelabeled BS-1 lectin and monoclonal antibodies against CD5, CD10, CD13, CD14, CD29, CD31, CD34, CD45, CD56, CD73. CD90 (Becton Dickinson [BD], Franklin Lakes, NJ, USA), CD105 (Caltag Laboratories: Burlingame, CA, USA), CD146 (clone P1H12; Chemicon International, Temecula, CA), HLA-AB (Harlan Sera-Lab; Leicestershire, UK) and HLA-DR (BD). As negative controls we used appropriate isotypematched antibodies (BD). Analysis was performed with a four-color FACSCalibur[®] equipped with a 488 nm argon ion laser and a 635 nm red diode laser (BD).² Multicolor measurements were performed and data from a minimum of 10.000 viable propidium iodide-excluding cells were stored. List mode files were analyzed with CellQuestTM Pro and Paint-A-Gate Pro[®] software (BD). The immunophenotype of MSC-M1 was also characterized by flow cytometry as described elsewhere.5-6

Differentiation assays

The *in vitro* differentiation capacity of FBS-MSC. pHPL-MSC and MSC-M1 was tested: osteogenic differentiation was determined by visualization with alizarin red staining as described elsewhere,⁹ whereas adipogenic differentiation was induced by the addition of insulin and identified by subsequent Oil Red O staining.¹⁰

References

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Online Supplementary Table S1. Primer list. Quantitative RT-PCR was performed for validation of differential gene expression in early and late passages; primers were obtained from Biospring (Frankfurt, Germany).

Gene	Amplicon length (bp)	Forward primer	Reverse primer
GAPDH	142	TTCGTCATGGGTGTGAACCA	CTGTGGTCATGAGTCCTTCCA
PARG1	309	CAATGATCATGCCCAGTGCA	GATCGTGATCTGTGCCAGGA
SERPINE1	300	CTCCTCATCCACAGCTGTCA	GCCAAGGTCTTGGAGACAGA
CDKN2B	274	CCCAACTCCACCAGATAGCA	GGGATTTCCGCATCCTAGCA
NTN4	260	AAGCCAGGCTTCTATCGTGA	TCTCCGGTGATAGGGTCACA
TOLLIP	296	CACTGTGCATGATTCCGAGA	AGGTGTCTCAATGGCATGCA
BDNF	297	CCAGGTGAGAAGAGTGATGAC	ACCCTGGACGTGTACAAGTC
МСМ3	300	ACATTGGGCTACAGGACTCA	TGAATGCTGCACTCACCATC
PTN	299	CAGTGAGTCATCCGTCCAGA	GCCATTCTCCACAGTCAGAC

Online Supplementary Table S2. Senescence-associated gene expression changes in FBS-MSC. SAM analysis revealed that 74 genes were significantly expressed in P1 and P2 of FBS-MSC (FDR = 5) in relation to the corresponding P0 (30 genes up-regulated, red; 44 genes down-regulated, green).

Gene ID	Bef	Seg NM	Gene Symbol	Gene Name
bCG39210.3	NM	001884.2	HAPLN1	hysiuronan and proteoplycan link protein 1
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h0G37565.3	PHINE,	102049.1	MGG39033	hypothetical protein McGoboso
NGG1747552.2	INIM	022917.4)	NULO	nucleolar protein tamily 6 (HVA-associated)
hCG37145.3	NM	004613.2	TGM2	transgutaminase 2 (C polypeptide, protein-gutamine-gamma-glutamytiransterase)
NGG43757.2	NM,	000224.23	KHI18	Keraon 19
hCG1818503.1	-		1000	
hCG21529.3	NM.	001196.23	BID	BH3 interacting domain death agonist
hCG37641.4				
hCG1644263.4	-			
hCG2043058	NM.	003607.23	CDC42BPA	GDC42 binding protein kinase alpha (DMPK-like)
hCG26491.4	NM,	001709.3	BONF	brain-derived neurotrophic factor
hCG37727.3	NM	005723,2	TM4SF9	transmembrane 4 superfamily member 9
hCG29950.3	NM	018946.2	NANS	N-acetylneuraminic acid synthase (sialic acid synthase)
hCG1786812.3				
hCG2030654				
hCG2039077				
hCG41325.3	NM.	015395.1	DKFZP434803	DKFZP434B0335 protein
hCG34035.3	NM	000636.1	SOD2	superoxide dismutase 2. mitochondrial
hCG41855.3	NM	007032.3	HRIHFB2122	Tara-like protein
hCG29298.3	NM	002982.2	CCL2	chemokine (C-C motif) ligand 2
hCG15194.3	-			
hCG1778706 1				
hCG2029617	NM	021135.3	RPS6KA2	ribosomal protein S6 kinase. 90kDa. polyoeotide 2
hCG32035.3	NM	004815.2	PARGI	TPTPI Lacenciated DhnGAP 1
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hCG2009388	NM	006209.2	ENPP2	ectoriucteotide pyrophosphatase phosphodiesterase 2 (autotaxin)
hCG401151.3	NM,	002040.2	GABPA	GA binding protein transcription factor, alpha subunit 60kDa
hCG1642637.3				
hCG1990594.1	NM	001187.1	BAGE;BAGE2;	B melanoma antigen:B melanoma antigen family, member 2;B melanoma antigen family, member 3;
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hCG2038907	NM	021631.1	FKSG2	apoptosis inhibitor
hCG1987903	NM.	032287.1	DKFZp761017	hypothetical protein DKFZp761017121
hCG16680.4	NM.	017549.1	UCC1	upregulated in colorectal cancer gene 1
hCG39580.3	NM	003711.2;	IPPAP2A	phosphatidic acid phosphatase type 2A
hCG401152.3	NM.	021219,2	JAM2	junctional adhesion molecule 2
hCG23456.3	NM.	024336.1	IRX3	iroquois homeobox protein 3
hCG1993006.1	NM	207422.1	FLJ44635	FLJ44635 protein
hCG1641962.2	NM.	020813.1	ZNF471	zinc finger protein 471
hCG1995870	NM	022834.3;	WARP	von Willebrand factor A domain-related protein
hCG1640501.2				
hCG22389.3				
hCG2039436.1	NM	002751.5	MAPK11	millogen-activated protein kinase 11
hCG38036.3	NM	030762.1	BHLHB3	basic helix-loop-helix domain containing, class 8, 3
hCG32877.3	NM	018322.1	C6ort64	chromosome 6 open reading frame 64
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hCG2039382				
hCG20517.1	NM	005737.3	ARL7	ADP-ribosylation factor-like 7
hCG14635.2	NM	004202.2	TMSB4Y	thymosin, beta 4, Y-linked
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Online Supplementary Table S3. Senescence-associated gene expression changes in pHPL-MSC. In P1 and P2 of pHPL-MSC, SAM analysis revealed that 227 genes were significantly expressed (FDR = 5) in relation to the corresponding P0 (68 genes up-regulated, red; 159 genes down-regulated, green).²

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hCG2043508		
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nCG27811.2 NM_001168.1	BIRC5	baculoviral IAP repeat-containing 5 (survivin)
hCG39396.2 NM_002105.1	HZAFX	H2A histone family, member X
hCG39720.3 NM_003878.1	GGH	gamma-glutarnyl hydrolase (conjugase, folylpolygammaglutarnyl hydrolase)
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hCG1812088.2 hCG40812088.2 hCG40813.3 hCG40513.3 hCG40513.3 hCG1996013 hCG19866.2 hCG189823.3 hCG198652.3 hCG3027327 hCG19866.2 hCG319863.3 hCG319863.3 hCG3188.3	INASP HMG82 DDA3 SFR53 MCM3	nuclear autoantigenic sperm protain (histone-binding) high-mobility group box 2 differential diaptay and activated by p53 splicing factor, arginne/serine-rich 3 MCM3 minichromosome maintenance deficient 3 (5. cerevisiae)
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HCG1738274.3	C INDA	001826.1	GRAIB	CUC20 protein kinase regulatory subunit 15
hCG17108.3	NM	021105.1	PLSCHI	phosphospid schampiase 1
NCG17616.2	NM	003372.3	VEPT	Von Hippel-Lindiau binding protein 1
NCG21668.3	NM	001889.2	GHYZ	crystallin, zeta (quinone reductase)
hCG2014421				
nCG15915.3		PROFILE ST		
hCG19828.3	NM	002668.1	PLP2	proteolipid protein 2 (colonic epithelium-enriched)
hCG23785.3	NM	004671.1:	PIAS2	protein inhibitor of activated STAT, 2
hCG15521.3				
hCG1811039.1	INM	080654.1	NY-REN-41	NY-REN-41 antigen
hCG2040289				
hCG20839.2	NM	007029.2	STMN2	stathmin-like 2
hCG2039660.1	NM	002266.1	KPNA2	karyopherin alpha 2 (RAG cohort 1, importin alpha 1)
hCG1995887.1				
hCG1812037.1	NM	004442.4:	EPHB2	Eph82
hCG1644309.4	1			
hCG26513.4	NM	006851.1	GLIPR1	GLI pathogenesis-telated 1 (plioma)
hCG1743134.2	INM	018204.2	CKAP2	cytoskeleton associated protein 2
hCG34031.2	NM	005891.1	ACAT2	acetyl-Coenzyme A acetyltransferase 2 (acetoacetyl Coenzyme A thiolase)
hCG2014578	NM	007192.2	SUPT16H	suppressor of Ty 16 homolog (S. cerevisiae)
hCG2032518	NM	005517.1	HMGN2	high-mobility group nucleosomal binding domain 2
hCG19242.3	NM	012412 3	H2AFV	H2A histone family, member V
hCG29352.3	NM	002146.3	HOXB3	homeo hox B3
hCG27344.3	-			
hCG39115.3	NM	002592.21	PCNA	proliferating cell nuclear antigen
hCG28547.3	NM	016397 21	THIL	THLike (Dresobila)
hCG20885.3	NM	033402.2	KIAA1764	KIAA1754 medain
hCG2041550 1		Constanting of	The state of the s	names of protons
hCG22733.3			DEPDCt	DEP domain containers 1
hCG20166.2	NM	004848.1	Clorige	chromotoma Longen casting frame 38
hCG16011 2	NA	004237.2	TRIPIN	burning where a water resource and the second
hCCANDAR 3	P.IL.A	0041114	EENI	Insection internet receiptor monactor in a
hCC1000411	Tanya	004111.4	1.6.141	mp anoune specific endoncouser i
hCC40606.3	1.10.0	004004 4	Clouding	advertisering 10 provident frame 110
HCG40006.3	D-IR.P	024034.1	UT00H119	Chromosome to open reasing mane 112
INCO2040161	PARM	000417.2	16.144	interneron-excluded protein 44
h0017879781	-	000544.0	LICTIMAD	history 4 Mda
HCG1787379.1	PARA	003544.2	ATADO	Instone 1, map
HCG100021	PRM	014109.2	ATAUZ	n ir ase tamey, non domain containing a
10031083999	-			
NGG2007731				
10/041784.3	NIM	001948.2	DUT	dUTP pyrophosphatase
hCG2032518	NM	005517.1	HMGN2	high-mobility group nucleosomal binding domain 2
nCG1750237.3	-			
hCG1750332.3	1			
hCG16307.3	NM	004219.2	PITGI	pituitary tumor-transforming t
hCG2020860				
hCG17108.3	NM	021105.1	PLSCRI	phospholipid scramblase 1
tiCG16461.2	NM	000788.1	DCK	deoxycytidine kinase
hCG2020693	NM	016271.3;	ANF138	ring finger protein 138
hCG33379.2	NM	004645.1	COIL	colin
hCG16827.4				
hCG31640.2	NM	002540.3;	ODF2	puter dense fiber of sperm tails 2
hCG172489.3	NM	006397.2	RNASEH2A	ribonuclease H2, targe subunit

Online Supplementary Table S4. Chromosomal location of differentially expressed genes. The representation of the differentially expressed genes within chromosomal regions was analyzed.

	FBS			pHPL			MSC-M1		
Gene Set [N]	#	P value	Gene Set [N]	#	P value	Gene Set [N]	#	P value	
			chr1p35 [121]	5	6.52 e ⁻³	chr1p36 [573]	7	2.68 e ⁻²	
chr2q34 [30]	2	3.67 e ⁻²	chr2q34 [30]	2	3.38 e ⁻²				
						chr2p13 [90]	6	1.23 e ⁻²	
chr2p23 [105]	3	7.65 e ⁻²				chr2q22 [27]	3	1.97 e ⁻²	
chr3p23 [20]	2	1.79 e ⁻²	chr3p22 [89]	3	4.95 e ⁻²	chr3p11 [10]	2	1.93 e ⁻²	
						chr3q24 [31]	3	2.76 e ⁻²	
chr4q31 [107]	3	7.92 e ⁻²	chr4p16 [179]	4	7.39 e ⁻²	chr4q21 [124]	9	1.58 e ⁻³	
			chr4q26 [37]	2	4.81 e ⁻²				
			chr4q27 [21]	2	1.79 e ⁻²				
			chr5q32 [37]	2	4.81 e ⁻²	chr5p12 [24]	3	1.46 e ⁻²	
						chr5q12 [58]	5	8.12 e ⁻³	
						chr5q13 [112]	7	9.99 e ⁻³	
chr6p22 [188]	5	3.6 e ⁻²	chr6p22 [188]	7	2.53 e ⁻³	chr6p22 [188]	11	2.66 e ⁻³	
chr6q22 [108]	3	8.06 e ⁻²				chr6p11 [14]	2	3.56 e ⁻²	
chr6q23 [80]	3	4.38 e ⁻²				chr7q31 [118]	6	3.43 e ⁻²	
chr6q25 [96]	5	3.16 e ⁻³	chr6q25 [96]	3	5.8 e ⁻²				
			chr8p21 [110]	4	2.08 e ⁻²	chr8p21 [110]	6	2.68 e ⁻²	
			chr8q22 [110]	4	2.08 e ⁻²	chr8q12 [57]	4	3.1 e ⁻²	
chr8q24 [239]	6	2.94 e ⁻²	chr8q24 [239]	5	6.18 e ⁻²				
chr9q34 [299]	10	1.12 e ⁻³				chr9q31 [89]	5	3.62 e ⁻²	
			chr11q23 [173]	5	2.36 e ⁻²	chr11p13 [62]	4	3.9 e ⁻²	
chr12q23 [106]	3	7.78 e ⁻²	chr12q12 [75]	3	3.39 e ⁻²				
			chr13q34 [56]	3	1.69 e ⁻²				
chr14q22 [92]	3	5.89 e ⁻²				chr14q11 [344]	2	1.16 e ⁻²	
chr14q24 [166]	4	6.98 e ⁻²							
chr14q32 [469]	2	8.2 e ⁻²							
chr15q22 [121]	4	3.14 e ⁻²				chr15q22 [121]	8	4.67 e ⁻³	
chr16q11 [16]	2	1.18 e ⁻²	chr16q13 [53]	5	1.87 e ⁻⁴				
chr17q23 [105]	4	2.1 e ⁻²	chr17q23 [105]	3	6.94 e ⁻²				
chr18q11 [58]	4	3.07 e ⁻³				chr17q21 [354]	16	4.21 e ⁻³	
			chr19q13 [1011]	13	7.98 e ⁻²	chr19q13 [1011]	8	1.59 e ⁻⁴	
chr20q12 [53]	3	1.66 e ⁻²							
chr20q13 [264]	8	5.57 e ⁻³	chr20q13 [264]	5	7.92 e ⁻²				
			chr21q22 [307]	7	2.46 e ⁻²				

Positional information to chromosomal bands was analyzed for two-fold differentially expressed genes (up and down-regulated) in the three datasets by GSEA analysis. The number of genes per chromosomal band (N), the number of differentially expressed genes in this region (#) and the probability (P value; hypergeometric distribution) are provided.

Online Supplementary Table S5. Results of array comparative genome hybridization analysis. Copy number variations of MSC from three donors cultured in FBS and pHPL-driven medium and of three donors in M1 medium are listed according to the chromosomes and cytobands. Amplifications (in bold) or deletions, the size of the affected region and *P* values are indicated.

Donor	Passage	Chromosome	Cytoband	Aberration	kb	p-value
222	Farty	7	q11.22 - q11.23	-0.374435	5159	5.06E-51
A S	Luity	22	q11.23	-4.217975	43	2.88E-80
8	1 min	2	q32.2 - q32.3	0.607917	370	2.14E-10
	Case	22	q11.23	-4.067567	43	9.06E-74
۲	Early	n.t.	n.t.	n.t.	n.t.	n.t.
d d	Lata	2	q35	2.022606	74	1.05E-27
H	Care	22	q11.23	-4.34042	43	1.80E-78
	Early	n.t.	n.t.	n.t.	n.t.	n.t.
		2	q13	-0.876383	118	1.995E-11
8		3	p14.2	-0.877506	150	2.326E-14
8	Late	8	p11.23 - p11.22	0.764326	155	2.562E-11
		15	q11.2	-1.325284	1301	5.923E-98
	-	22	q11.23	-3.582023	43	2.179E-72
		2	q35	1.990514	74	6.273E-26
	Freihe	3	p14.2	-0.841847	150	7.21E-12
	Early	15	q11.2	-1.399927	1301	2.09E-95
		22	q11.23	-4.166664	43	2.985E-82
		2	q13	-0.910015	132	6.913E-13
H		2	q35	1.882569	74	8.96E-28
ā	72-525	3	p14.2	-0.917076	103	5.304E-11
	Late	11	a11	-1.080188	53	2.645E-10
		15	g11.2	-1.427194	1301	3.649E-109
		22	a11.23	-3.760139	43	5.409E-68
		6	p21.33	-3,197399	42	6.763E-64
		6	013 - 014.1	0.951695	98	3.366E-12
	Early	8	p11.23	-4.34232	138	4.194E-187
O		12	p13.31	1.331197	76	1.324E-19
BS		16	p11.1	0.929597	162	1.808E-10
-	2	6	n21.33	-2 764336	42	9.684E-39
	Late	8	n11.29	4.043672	126	1 103E-148
	2223M	12	013 31	1 462532	76	2 438E-16
	A CONTRACTOR OF	6	n21.33	-3.060614	42	8.543E-58
	Early	8	n11 23	-4 091248	126	1.687E-170
o		6	021.33	-3.035865	49	2 8895-61
4	100 M	8	pt1.00	-4 564849	126	2 106E-183
đ	Late	12	013.31	1 328333	76	2 7755-20
		16	011.1	0.004776	162	2 3125-10
	1	2	035	1 358000	74	2.861E-22
		2	455	1.165400	255	0 744E-10
4	Farly	4	p10.32	1.076401	200	7 9/65 19
E	starty	0	piez n11 22	-4.01017	129	5 4000-13
ISC		15	011.2	-0.572011	1071	671/E-17
N.	1000	15	011.2	-9.840100	129	A 022E-160
	Late	0	p11.20	-3.640190	221	4.023E-100
		2	420.0	1 763053	74	2 184E-25
		2	435	.0 477415	522	1 2045-10
8	Early		n11 22 . n11 22	0.01613	165	0 7055-14
5		0	p11.23 - p11.22	1.020400	561	1.005.01
SC		0	411.2	1.030499	74	2 246E OF
2	Late	4	011 22 . 011 22	0.021142	155	3.3466-25
	Late	8	p11.23+p11.22	0.921143	105	2.002-11
		15	Sec.	40.994621	561	2.3958-23
0		2	q35	1.640943	74	1.1/2E-23
	Early	8	p23.1	-0.980526	668	2.95/E-12
		9	d22.31	-0.8/1/01	201	1.399E-15
SC		12	q24.33	-0.608377	389	3.173E-15
W	10000	8	p23.1	+1.165842	563	6.496E-10
	Late	9	q22.31	-0.884033	167	6.27E-12
		15	q11.2	-0.528808	1220	5.881E-13