

Supplemental Table S1.

HDGC Clinical Criteria, updated in 2020.

HDCG Clinical Criteria
Family Criteria
families with ≥ 2 cases of GC regardless of age, with at least one DGC
≥ 1 case of DGC at any age, and ≥ 1 case of LBC at age < 70 years, in different family members
≥ 2 cases of LBC in family members < 50 years of age
Individual Criteria
Having DGC at age < 50 years
Bilateral LBC, diagnosed at age < 70 years
Having DGC and LBC, both diagnosed at age < 70 years
DGC at any age in individuals with a personal or family history (1 st degree relative) of cleft lip or cleft palate
DGC at any age in individuals of Māori ethnicity
Gastric in situ signet ring cells or pagetoid spread of signet ring cells in individuals < 50 years of age

Supplemental Table S2.

List of primers and PCR conditions for colony-PCR strategy.

Technical Application		Primer's sequence	Conditions
cDNA amplification/ sequencing	ex11F	5'_CAGCACGTACACAGCCCTAA_3'	Initial denaturation step at 95 °C for 15 minutes. Then, a touchdown from 65 °C to 58°C (5 cycles, -1°C/cycle) with a denaturation step at 95 °C for 30 seconds and extension at 72 °C for 1 minute. In the next 30 cycles denaturation was done at 95 °C for 30 seconds, annealing at 57 °C for 1 minute and 30 seconds and extension for 1 minute. The final extension was done for 10 minutes at 72°C.
	ex13R	5'_CCTCCAAGAATCCCCAGAATG_3'	
Colony-PCR	M13F	5'-GTAAAACGACGGCCAG-3'	After an initial 10 minutes denaturation step at 95°C, a touchdown reaction using annealing temperatures between 55°C and 50°C (-1°C/cycle) was performed, followed by 30 cycles with 50°C as the annealing temperature. The final extension was done at 72°C, for 10 minutes
	M13R	5'-CAGGAAACAGCTATGAC-3'	
rs1801552 amplification (gDNA)	ex13F	5'_CATACCTAAATAAAACCCAAGCAGCTC_3'	After initial denaturation step at 95 °C for 15 minutes, a touchdown from 65 °C to 62 °C (6 cycles, -0,5 °C/cycle) was done, with a denaturation step at 95 °C for 30 seconds and extension at 72 °C for 2 minutes and 30 seconds. In the next 29 cycles denaturation was done at 95 °C for 30 seconds, annealing at 65 °C for 1 minute and 30 seconds and extension for 2 minutes and 15 seconds. The final extension was done for 10 minutes at 72°C.
	ex13R	5'_AACTTGGGAGTCTCTTTCCACATC_3'	
Snapshot	ex12F	5'_GGAATCCAAAGCCTCAGGTC_3'	Initial 10 minutes denaturation at 95°C, followed by a touchdown reaction with annealing temperature 62°C to 56°C (-1°C/cycle), followed by 29 cycles of 56°C annealing temperature. The final extension was done at 72°C, for 10 minutes.
Snapshot	ex13R	5'_CCTCCAAGAATCCCCAGAATG_3'	
Snapshot rs1801552	SBE primer	5'_CTGTGCCTTCCTACAGACGCC_3'	The program consisted of 25 cycles with an initial denaturation step at 96°C for 10 seconds, an annealing step at 56°C for 5 seconds and an extension step of 30 seconds at 60°C

Supplemental Table S3.

List of primers and PCR conditions for polymorphic marker studies.

Polymorphic marker	Distance to <i>CDH1</i>	Location	Primer Forward Primer Reverse	Predicted size of the amplicon (bp)	PCR Conditions				
					Touchdown range(°C) -1°C/ cycle	Ta (°C)	Program		
D16S514	6432933	62304172	62304292	5'_CTATCCACTCACTTTCCAGG_3' 5'_TCCCCTGATCATCTTCTC_3'	117 - 133	57°C–53°C	54°C	95°C 15 min.	
D16S318	1475206	67261888	67262019	5'_CTGTGGTGTACATCAGGAA_3' 5'_GACTACACATGAACCGATTG_3'	134	57°C–53°C	54°C		
D16S3025	204625	68532493	68532600	5'_TCCATTGGACTTATAACCATG_3' 5'_AGCTGAGAGACATCTGGG_3'	90-110	57°C–53°C	54°C		95°C 30sec.
rs16260 (C/A)	94	68737131	x	5'_AACCGTGCAGGTCCCATAAC_3' 5'_CAAGCTCACAGGTGCTTTGC_3'	252	n.a.	57°C		
<i>CDH1</i>		68737225	68835548					Touchdown 1 min. 30sec.	
rs1801552 (C>T)	Inside gene	68823538	x	5'_ TGGCCTTAGAGGTGGGTGAC_3' 5'_CCAGGAAATAAACCTCCTCCAT_3'	262	n.a.	55°C		
D16S496	79259	68914807	68915022	5'_ GTTTGGCAGAAAGGCTACTT_3' 5'_GCCCATCCTCCACTATCTAT_3'	216	57°C–53°C	54°C	72°C 1 min. 30 sec	x nr cycles depending on Touchd. range
D16S3067	239217	69074765	69074908	5'_GCCACCTCACACTAGCCTG_3' 5'_TCACTCAAAATGGAGTCACTCTG_3'	138 - 152	n.a.	57°C		
D16S3095	1076779	69912327	69912484	5'_TCAGTTGGAAGATGAGTTGG_3' 5'_TATAGTTTGTGTCCCCCGAC_3'	134 - 162	n.a.	55°C	95°C 30 seconds	
D16S3106	3318313	72153861	72154060	5'_GAGACCTACAGTCTTTTGCATTAC_3' 5'_TTTTGAAGCTGAGCAGAAGG_3'	166 - 206	n.a.	58°C	Ta 1 min. 30 sec	x 35 cycles maximum
D16S3018	5303255	74138803	74139062	5'_GGATAAACATAGAGCGACAGTTC_3' 5'_AGACAGAGTCCCAGGCATT_3'	244-270	61°C–55°C	55°C	72°C 1 min. 30 sec	
D16S3049	10051895	78887443	78887697	5'_GCAATGAAGGCAACAAAGT_3' 5'_TTAAAAGACCTGGGGGAAT_3'	233 - 255	55°C–53°C*	53°C	72°C 10 min. 4°C +∞	
D16S3098	12579243	81414791	81414947	5'_TTCCACACATAAGGTGAGTTT_3' 5'_TTGTCTGCTTCTTTACGGA_3'	151 - 165	55°C–53°C*	53°C		

Footnote: n.a. (not applicable)

Supplemental Table S4.

Chromosomes' haplotypes from HDGC families used for TMRCA estimation.

		D16S514	D16S318	D16S3025	c.1901C>T	D16S496	D16S3067	D16S3095	D16S3106	D16S3049	D16S3098	DHSMAP Analysis
c.1901c>T Carriers	F7	2	4	1	2	1	5	9	2	5	2	A – Conservative Analysis
	F1	2	4	1	2	1	5	6	1	6	5	
	F2	2	4	1	2	1	5	6	1	6	5	
	F3	1	4	1	2	1	5	6	1	6	5	
	F7-A	1/3	4	1	2	1	5	9	2	5	2	B – Conservative Analysis + Inclusion of two derived haplotypes
	F2-F	1/5	4	1	2	1	5	6	1	6	5	
Controls	F7 – C1	3	6	8	1	1	6	5	1	5	2	Used in Analysis A and Analysis B
	F7 – C2	7	1	1	1	6	9	6	5	6	2	
	F7 – C3	6	1	3	1	6	8	6	1	5	6	
	F7 – C5	2	5	7	1	6	7	5	4	0	0	
	F1 – C2	2	5	8	1	6	9	9	1	3	5	
	F1 – C3	2	5	8	1	6	8	6	1	5	2	
	F1 – C4	5	4	8	1	6	7	6	2	4	5	
	F1 – C5	7	4	7	1	6	9	9	1	7	5	
	F1 – C6	7	5	8	1	6	9	9	1	7	6	
	F1 – C7	7	5	8	1	6	7	9	1	6	4	
	F2 – C2	5	1	9	1	6	7	9	2	6	2	
	F2 – C4	6	5	8	1	1	5	9	5	6	2	
	F2 – C3	1	5	4	1	6	8	1	2	1	4	
	F2 – C6	5	5	8	1	1	8	6	1	5	5	
	F3 – C1	1	5	8	1	6	9	6	4	6	5	
	F3 – C3	7	5	1	1	1	5	9	2	6	2	
	F3 – C1,1	1	8	7	1	1	5	6	1	4	5	
	F3 – C5	7	7	1	1	6	9	6	4	5	1	

For the purpose of TMRCA calculation in DHSMAP software, different alleles were label as numbers. For the c.1901 C>T variant the C allele was label as 1 and T allele as 2. For the remaining STR markers the labels were based in the number of repeats, where the allele with the lower bp size was numbered as 1 and the others with larger bp sizes numbered 2, 3, 4 ... n according with the corresponding mutational steps (e.g. D16S514: 116 bp allele – 1; 118 bp allele – 2; 120 bp allele 3; etc.); 0 means missing data.