NOTE - this document was generated by students ***Kars van der Weijden and Kevin van der Graaf*** students of the Hogeschool Leiden. January 2011.

DNA primers

Exon	Size (bp)	Name	Forward primer	Reverse primer	Reference
Exon1	201	GNEexon1F/ GNEexon1R	gaggagtgggga caaggtc	aggatgctctcc cggagtc	Sparks (2005)
Exon2	391	GNEexon2F/ GNEexon2R	aaatagtggttaa ggacttgaaactg	tggctacaaaac ccaagctc	Sparks (2005)
Exon2-12	-	GNE C1F/ GNE C1R	gaactctattttaa gaacctctca	gagctctggag agaaggtcca	Tomimutsu (2004)
Exon3	594	GNEexon3F/ GNEexon3R	tcttgcatgttacg gctctg	ttccaaaaggat tgaaatagacg	Sparks (2005)
Exon3-7	-	GNE C2F/ GNE C2R	gagcatcattcgc atgtggcta	cagtggctcttg aagatcgata	Tomimutsu (2004)
Exon4	287	GNEexon4F/ GNEexon4R	cacagacttaga gtcttgctttcag	gggaaaagtag gtggcataatttc	Sparks (2005) Glyco
Exon5	407	GNEexon5F/ GNEexon5R	gtgggctatactt gccaatg	aacctcattcact cacttgttcac	Sparks (2005)
Exon6	244	GNEexon6F/ GNEexon6R	ttcccacttccaaa tccttg	ggcataagaag gaaagctctg	Sparks (2005)
Exon7	399	GNEexon7F/ GNEexon7R	cacttaacatgtat ggcagaagc	ctatcagcaaat gattacaagctc	Sparks (2005)
Exon8	290	GNEexon8F/ GNEexon8R	caacactcacaat atgctgcttag	ggcagacactg acttgatgc	Sparks (2005)
Exon9	395	GNEexon9F/ GNEexon9R	tcatcagcattatt tccttgtg	attctagctcctg aaccaacc	Sparks (2005)
Exon10	356	GNEexon10F/ GNEexon10R	tcaggtaaagca ccctcagtg	agccctgctcttt ccctaag	Sparks (2005)
Exon11	250	GNEexon11F/ GNEexon11R	acaggcccagtg gtgagc	gctgggccatat gatatctgag	Sparks (2005)
Exon12 part 1	585	GNEexon12F1/ GNEexon12R1	ggctcttttctgcc ttcctc	tgctttggcaca gaaaattg	Sparks (2005) Glycob
Exon12 part 2	500	GNEexon12F2/ GNEexon12R2	tggtctcctcttcc agagtcac	agtggtgcggtc tcagttc	Sparks (2005)
Exon12	-	-	gatggagtcttgc tctgtcac	ctggctcttttctg ccttcc	Eisenberg (2001)
Exon13 part 1	598	GNEexon13F1/ GNEexon13R1	atttggtctagtga caggtgttc	tcagaaactgag cagccaag	Sparks (2005) Gly
Exon13 part 2	499	GNEexon13F2/ GNEexon13R2	gggttagtgtgaa aaagagtttgg	tcttctgggaag gaaaatgc	Sparks (2005)
Exon13	-	-	gtgcaacaattaa atctcttctgg	gaaatattcctct ctgctgtg	Eisenberg (2001)

Legend: Exon: exon number. **Size (in bp):** size of PCR-product in basepairs. **Name:** name of the primers. **Forward primer / reverse primer:** sequence of PCR forward and reverse primer (5' to 3'). **Reference:** publication describing the primer(s). Sparks (2005) - http://www.ncbi.nlm.nih.gov/pubmed/15987957, Tomimutsu (2004) - http://www.ncbi.nlm.nih.gov/pubmed/15136692, Eisenberg (2001) - http://www.ncbi.nlm.nih.gov/pubmed/11528398

The following primers, probes, sense and antisense siRNA were used to determine Sialuria in patients.

Sialuria is a dominant disorder caused by missense mutations in the allosteric site of GNE, which codes for the rate-limiting enzyme of sialic acid biosynthesis, UDP-GlcNAc 2-epimerase/ManNAc kinase. Klootwijk et al. (2008) - http://www.ncbi.nlm.nih.gov/pubmed/18653764 **used specific synthetic siRNAs to targeting the dominant GNE mutation c.797G>A (p.R266Q) in sialuria fibroblasts. Patients has missense mutations in 1 of 2 codons of the GNE gene. These are codon 263 (R263L) or codon 266 (mutations R266Q and R266W). The codons 263-266 are the allosteric site for CMP-sialic acid binding.**

The GNE allosteric site in exon 5, which is mutated in sialuria, was amplified with a forward and reverse primer.[3]

GNE	Forward primer	Reverse primer	Reference
Exon 5	*TGAGTTCCTAGATGAGTGAAG*	*CAGGTTGATCACAGGTGTT*	Klootwijk (2008) The FASEB Journal 22, 3846

Legend: Exon: exon number. **Forward primer / reverse primer:** sequence of PCR forward and reverse primer (5' to 3'). **Reference:** publication describing the primer.

Klootwijk et al. (2008) used three different siRNA to determine the patient mutation c.797G>A. The first one is the sense and antisense siRNA of the mutation c797G>A. The second siRNA is the siRNA-wiltype and the third siRNA is the control siRNA (siRNA-nonsil)[3]

Sense and antisense siRNA	Sequence		Reference
1) sense siRNA	5'GAGUGAUGCAGA AGAAGGGdCdA 3'	mutation c.797G>A	Klootwijk (2008) The FASEB Journal 22, 3846
1)antisense siRNA	5'CCCUUCUUCUGC AUCACUCdGdA 3'	mutation c.797G>A	Klootwijk (2008) The FASEB Journal 22, 3846
2) sense siRNA	5'GAGUGAUGCGG AAGAAGGGdCdA 3'	wild-type allele (siRNA-wt)	Klootwijk (2008) The FASEB Journal 22, 3846
2) antisense siRNA	5'CCCUUCUUCCGC AUCACUCdGdA 3'	wild-type allele (siRNA-wt)	Klootwijk (2008) The FASEB Journal 22, 3846
3) sense siRNA	5'UUCUCCGAACGU GUCACGUdTdT 3'	siRNA-nonsil	Klootwijk (2008) The FASEB Journal 22, 3846
3) antisense siRNA	5'UCGUGACACGUU CGGAGAAdTdT 3'	siRNA-nonsil	Klootwijk (2008) The FASEB Journal 22, 3846

Legend: Sense and antisense siRNA: sense and antisense siRNA. **Sequence:** sequence used for the mutation. **Reference:** publication describing the sense and antisense siRNA.

RNA was isolated from sialuria patient fibroblasts and performed on a quantitative real-time PCR. A semiquantitative allele-specific RNA assay, based on the method described by Suda et al. (2003) - http://www.ncbi.nlm.nih.gov/pubmed/12851725 was used to measure allelic RNA expression. The region flanking the GNE target mutation was PCR amplified by using a specific primer set in the presence of 2 probes, each selectively hybridizing to the normal or to the mutated allele (Klootwijk et al. [2008]).

Primers	Sequence	Reference
Forward primer	*5'-ATTGACGCAGGGAGCAAAGA-3'*	Klootwijk (2008) The FASEB Journal 22, 3846
Reverse primer	*5'-GGATGATGCTCAATGCCCTTCTT-3'*	Klootwijk (2008) The FASEB Journal 22, 3846

Legend: Primers: forward or reverse primer. **Sequence:** sequence of PCR forward and reverse primer (5' to 3'). **Reference:** publication describing the primer(s).

Probes	Sequence	Reference
Normal probe	*5' VIC-labeled, 5'-NFQ-CATCACTCGAACCATC-VIC-3'*	Klootwijk (2008) The FASEB Journal 22, 3846
Mutated probe	*5' FAM-labeled, 5'-NFQ-CATCACTAGAACCATC-FAM-3'*	Klootwijk (2008) The FASEB Journal 22, 3846

Legend: Probe: normal or mutated probe. **Sequence:** Sequence used by normal and mutated probe (5' to 3'). **Reference:** publication describing the probes.

Wang et al. (2006) - http://www.ncbi.nlm.nih.gov/pubmed/16847058 **described other roles of the GNE gene besides its role in sialic acid metabolism.**

DNA primers

Wang et al. (2006) amplified the GNE gene by PCR by using primers. The PCR products were digested by XhoI and HindIII endonuclease, purified by agarose gel electrophoresis and ligated into the pcDNA3.1 vector with T4 DNA ligase.

Primer	Sequence	Restriction site	Reference
Forward primer	*AAA*CTCGAG*ATGG AGAAGAATGGAAATAACC*	*XhoI*	Wang (2006) The journal of biological chemistry 281, 27016
Reverse primer	*TTT*AAGCTT*GTAGATCCTGCCT GTTGTGTAG*	*HindIII*	Wang (2006) The journal of biological chemistry 281, 27016

Legend: Primer: forward or reverse primer. **Sequence:** sequence used by the forward or reverse primer. **Restriction site:** Restriction site underlined in the sequence (5' to 3'). **Reference:** publication describing the primer(s).

shRNA primers

Wang et al. (2006) used three shRNA GNE-specific sequences to ensure that only the GNE gene was targeted. These sequences are showed in the table below. The DNA oligonucleotides required for the shGNE synthesis are also shown in the table below. ShRNA-targeting luciferase (shRNA-luc) was used in the transfection as control samples.

shRNA	Sequence	Reference
shGNE760	*5'-AGATTACATTGTTGCACTA-3'*	Wang (2006) The journal of biological chemistry 281, 27016
shGNE827	*5'-TTAACATTGGATGCACTTA-3'*	Wang (2006) The journal of biological chemistry 281, 27016
shGNE1448	*5'-CCTATGAAGAGAGGATTAA-3'*	Wang (2006) The journal of biological chemistry 281, 27016

Legend: shRNA: name of shRNA. **Sequence:** sequence used for the shRNAs (5' to 3'). **Reference:** publication describing the shRNA(s).

DNA oligonucleotides sequences	shRNA	Reference
5'AAGGAGATTACATTGTTGCACTACTTGCTTC TAGTGCAACAATGTAATCTCCTATAGTGA-3'	shGNE760	Wang (2006) The journal of biological chemistry 281, 27016
5'AAGGTTAACATTGGATGCACTTACTTGCTTC TAAGTGCATCCAATGTTAACCTATAGTGA-3'	shGNE827	Wang (2006) The journal of biological chemistry 281, 27016
5'AACCTATGAAGAGAGGATTAACTTGCTTCT TAATCCTCTCTTCATAGGTATAGTGA-3'	shGNE1448	Wang (2006) The journal of biological chemistry 281, 27016

Legend: DNA oligonucleotide sequences: Sequences of the oligonucleotides (5' to 3'). **shRNA:** name of the shRNAs. **Reference:** publication describing the oligoncleotides.

siRNA primers

To these three shRNAs, Wang et al. (2006) used siRNA targets against the 760 region of GNE (*i.e.* the 5'-AGATTACATTGTTGCACTA-3' sequence). This was tested by using a sense and an antisense siRNA GNE 760.

siRNA	Sequence	shRNA	Reference
Sense siRNA	*5'-AGAUUACAUUGUUGCACUATT-3'*	shGNE760	Wang (2006) The journal of biological chemistry 281, 27016
Antisense siRNA	*5'-UAGUGCAACAAUGUAAUCU-3'*	shGNE760	Wang (2006) The journal of biological chemistry 281, 27016

Legend: siRNA: sense or antisense siRNA. **Sequence:** Sequence used by the sense and antisense siRNA (5' to 3'). **shRNA:** name of the shRNAs. **Reference:** publication describing the siRNA(s).

To get more information about the sialic acid biosynthesis Wang et al. (2006) analyzed genes which are involved in the sialic biosynthesis by qRT-PCR.

Designation	Description/alternative name	Forward primer	Reverse primer	Reference
BiP	UPR chaperone	TAG CGT ATG GTG CTG CTG TC	TTT GTC AGG GGT CTT TCA CC	Wang (2006) The journal of biological chemistry 281, 27016
CMPNS	CMP-Neu5Ac synthetase	GCC ATC TTC GAT GGA GT	TAT GTT CAG CTC GCA TTT CG	Wang (2006) The journal of biological chemistry 281, 27016
CMPNT	CMP-Neu5Ac transporter	CAA CCA CAG CCG TGT GTA TC	CTG CTG CAT CCA GAT TGC TA	Wang (2006) The journal of biological chemistry 281, 27016
GAPDH	(Real time PCR control)	GCA AAT TCC ATG GCA CCG T	TCG CCC CAC TTG ATT TTG G	Wang (2006) The journal of biological chemistry 281, 27016
GNE	UDP-GlcNAc 2-epimerase/ManNAc 6-kinase	TGC CCT TCC TAT GAC AAA CTT	GCA TCA CTC GAA CCA TCT CTT	Wang (2006) The journal of biological chemistry 281, 27016
GUS	(Real time PCR control)	GAA AAT ATG TGG TTG GAG AGC TCA	CCG AGT GAA GAT CCC CTT TT	Wang (2006) The journal of biological chemistry 281, 27016
NCAM	Neural cell adhesion molecule	AAA AGG TGG ATA AGA ACG ACG A	GGT AGA AGT CCT CCA GGT GAT	Wang (2006) The journal of biological chemistry 281, 27016
SAS	Sialic acid synthase	CAT GGA TGA GAT GGC AGT TG	GGG GCT TAC CGA TCT GAT AA	Wang (2006) The journal of biological chemistry 281, 27016
ST3Gal1	SIAT4A	ATG TGG ACC CTA TGC TGG AG	CTT GGT CCC AAC ATC AGC TT	Wang (2006) The journal of biological chemistry 281, 27016
ST3Gal2	SIAT4B	AAC CAC CCA CCA TTT CAT GT	TGA TGC TCT GTC CAC CTG TC	Wang (2006) The journal of biological chemistry 281, 27016
ST3Gal3	SIAT6	TCT AGC TCA CCC CAG GAG AA	GGG ATG CAG GCA TCA GTA AT	Wang (2006) The journal of biological chemistry 281, 27016
ST3Gal4	SIAT4C	CTA GCC ATC ACC AGC TCC TC	GTG GGC AGA TTC AGG GTA GA	Wang (2006) The journal of biological chemistry 281, 27016
ST3Gal5	SIAT9	CCC TGA ACC AGT TCG ATG TT	CAT TGC TTG AAG CCA GTT GA	Wang (2006) The journal of biological chemistry 281, 27016
ST3Gal6	SIAT10	TTG CCT CTC TGC TGA GGT TT	CCT CCA TTA CCA ACC ACC AC	Wang (2006) The journal of biological chemistry 281, 27016
ST6Gal1	SIAT1	GGC ATC AAG TTC AGT GCA GA	TGC GTC ATG ATC ATC GAT TT	Wang (2006) The journal of biological chemistry 281, 27016
ST6GalNAc1	SIAT7A	TCT GGC TGT CCT GGT CTT CT	TGT GTG TTG AGG GCA TTG TT	Wang (2006) The journal of biological chemistry 281, 27016

ST6GalNAc2	SIAT7B	ACC AGA AGC CTC TGC CAG TA	ATG GCT TCA TTT TTC GTT CG	Wang (2006) The journal of biological chemistry 281, 27016
ST6GalNAc3	SIAT7C	CCA GAA GGT GGG AAA TGA GA	TTC CTC ATA TTG CGG AAA GG	Wang (2006) The journal of biological chemistry 281, 27016
ST6GalNAc4	SIAT3C	CTG CAG CTC ACC AGG ATG TA	TCC CAT AGA CCA CGA TCT CC	Wang (2006) The journal of biological chemistry 281, 27016
ST6GalNAc5	SIAT7E	TTA CTC GCC ACA AGA TGC TG	GCA CCA TGC CAT AAA CAT TG	Wang (2006) The journal of biological chemistry 281, 27016
ST6GalNAc6	SIAT7F	CTC CGG AGA GAA ATG AGT AG	CAG TGT CTT GTT GCC GAG AA	Wang (2006) The journal of biological chemistry 281, 27016
ST8Sia1	SIAT8A	AGC GTT CAG GAA ACA AAT GG	TGC CTG TGG GAA GAG AGA GT	Wang (2006) The journal of biological chemistry 281, 27016
ST8Sia2	SIAT8B	TGA CCA ACA AAG TCC ACA TCA	TGG GAG GTG TAG CCA TAC TTG	Wang (2006) The journal of biological chemistry 281, 27016
ST8Sia3	SIAT8C	TCC CTG CAT TTT TCT TCC AC	ACG GCC AAA ATC CAT ACA AG	Wang (2006) The journal of biological chemistry 281, 27016
ST8Sia4	SIAT8D	ACG GCC AAA ATC CAT ACA AG	CTT AGG GAA GGG CCA GAA TC	Wang (2006) The journal of biological chemistry 281, 27016

Legend: Designation: designation of the genes. **Description:** Alternative name of the genes. **Forward primer / reverse primer:** sequence of PCR forward and reverse primer (5' to 3'). **Reference:** publication describing the genes.