Appendix 1: Information used for the selection of immediately actionable variants in *GCK*, *HNF1A* and *HNF4A*

Variant (rs-number)	Nucleotide position	Genomic position (GRCh37/Hg19 and GRCh38/Hg38)	Allele frequency in 60000 individuals	Identified in	Pathogenicity	Functionality
***GCK* variants (NM_000162.5)**						
Gly72Arg (rs193922289)	c.214G>A	Chr7: 44192019 Chr7:44152420	1/ 125682 (0.000004)	In house samples • 1 Danish MODY proband • 4 newly diagnosed diabetes patients • 3 patients with GDM Published studies: • 18 MODY families (PMID: 19790256)	Clinvar: pathogenic HGMD:	• No overlap between phenotype and thermostability (PMID: 25015100) • Results in structural changes (pmid: 21831042) • Decreased catalytic activity (PMID: 17389332) • Totally refractory to the allosteric GKA, and responsiveness (PMID: 16731834)
Ala176Thr	c.526G>A	Chr7: 44189621 Chr7:44150022	NF	In house samples • 1 Danish MODY family • 3 Danish MODY probands Published studies • 1 MODY families (PMID: 19790256)	Clinvar: NF HGMD:	No functional studies
Arg191Trp	c.571C>T	Chr7:44189576 Chr7:44149977	2/125555 (0.000008)	In house samples • 1 Danish MODY family • 2 Danish MODY probands • 1 newly diagnosed type 2 diabetes patient Published studies • 47 MODY families (PMID: 19790256) • 1 patient with GDM (PMID: 10753050) • 2 UK-biobank samples (PMID: 33046911)	Clinvar: Pathogenic HGMD:	No functional studies
Arg191Gln	c. 572G>A	Chr7:44189577 Chr7:44149978	NF	In house samples • 2 Danish MODY families Published studies • 9 MODY families (PMID: 19790256) • 1 antibody-negative child with diabetes (PMID: 11508276)	Clinvar: VUS HGMD:	No functional studies
Thr206Met (rs1441649062)	c.C617T	Chr7:44189421 Chr7:44149822	1/ 125718 (0.000004)	In house samples • 2 Danish MODY families • 1 Danish MODY proband • 1 newly diagnosed diabetes patient Published studies • 9 MODY families (PMID: 19790256) • 1 antibody-negative child with diabetes (PMID: 11508276)	Clinvar: NF HGMD:	No functional studies
Thr228Met	c.683C>T	Chr7:44187429 Chr7:44147830	1/ 125718 0.000004	In house samples • 2 newly diagnosed diabetes patients Published studies	Clinvar: Pathogenic HGMD:	Affects affinity for ATP (PMID: 1502186) Inactivating mutation (PMID: 11372010)

				• 12 MODY families (PMID: 19790256) • 1 MODY family (PMID: 9049484)		
Ala456Val	c.1367C>T	Chr7: 44184772 Chr7:44145173	1/ 119848 (0.000004)	In house samples • 1 Danish MODY family Published studies • 2 HH families (PMID: 19790256)	Clinvar: Pathogenic HGMD:	Decreased glucose S(0.5) (the concentration of glucose needed to achieve the half-maximal rate of phosphorylation) from 8.04 (wild-type) to 2.53 mmol/l. The mutant's Hill coefficient was decreased, and its maximal specific activity k(cat) was increased (PMID: 11916951)
IVS1A+1G>T	c.45+1G>T	Chr7:44228507 Chr7:44188908	1/125377 (0.000003988)	In house samples • 1 Danish MODY family Published studies: • 5 MODY families (PMID: 19790256)	Clinvar: Pathogenic HGMD:	Mutation disrupts a known splice site (PMID:15928245 and 11508276)
IVS4+1G->A	c.483+1G>A	Chr7:44190554 Chr7:44150955	(1/ 120655) 0.000004	In house samples • 1 Danish MODY family • 2 Danish MODY probands Published studies • 1 MODY family (PMID: 19790256) • 1 MODY proband (PMID:25306193)	Clinvar: Pathogenic (clinvar ID: 576984) HGMD:	No functional studies
IVS7-1G>A	c.864-1G>A	Chr7: 44186218 Chr7:44146619	0	In house samples • 3 Danish MODY probands Published studies • 5 MODY families (PMID: 19790256)	Clinvar: Pathogenic (clinvar ID: 576984) HGMD:	No functional studies
***HNF1A* variants (NM_000545.8)**						
Arg131Trp	c.391C>T	Chr12:121426700 Chr12:120988897	0	In house samples • 2 Danish MODY families Published studies • 29 MODY families (PMID: 23348805) • 2 patients with monogenic diabetes (PMID: 31485449) • 1 patient with monogenic diabetes (PMID:28701371) • 2 patients with T2D (PMID: 29207974) • 1 child with obesity and diabetes (PMID: 29758564) • 1 child with diabetes (PMID: 25306193)	Clinvar: Pathogenic HGMD:	Transactivation activity ~43% and defective nuclear localisation (PMID: 12574234)
Arg159Gln	c.476G>A	Chr12:121426785 Chr12:120988982	0	In house samples • 1 Danish MODY family • 1 Danish MODY proband Published studies • 15 previous families (PMID: 23348805) • 1 patient with T2D (PMID: 30487145) • 1 patient with MODY (PMID: 28701371) • 3 patients with MODY (PMID: 23548576)	Clinvar: Pathogenic HGMD:	Disrupts DNA interaction and reduces protein stability (PMID: 12453420, PMID: 23348805)
Arg159Trp	c.475C>T	Chr12:121426784 Chr12:120988981	3.98*10-6	In house samples • 1 Danish MODY proband Published studies • 12 previous families (PMID: 23348805) • 1 MODY patient (PMID: 31968686)	Clinvar: Pathogenic HGMD:	No functional studies

Arg203Cys	c.607C>T	Chr12:121431403 Chr12:120993600	3.98*10-6	In house samples • 2 Danish MODY families Published studies • 4 previous families (PMID: 23348805)	Clinvar: NF HGMD:	Mutation affects nuclear localization but maybe not transactivation (PMID:10078571 and 23348805)
Arg203His	c.608G>A	Chr12:121431404 Chr12:120993601	7.9*10-6	In house samples • 1 Danish MODY family • 1 diabetes patient age of diagnosis < 40 year Published studies • 19 previous families (PMID: 23348805) • 1 patient with T2D (PMID: 33046911)	Clinvar: Pathogenic HGMD:	No functional studies
Arg229Pro	c.G686C	Chr12:121431482 Chr12:120993679	0	In house samples • 2 Danish MODY probands • 1 diabetes patient age of diagnosis < 40 year Published studies • 3 previous families (PMID: 23348805)	Clinvar: NF HGMD:	Reduced expression and moderate RNA instability (PMID: 14747304)
Cys241Gly	c.721T>G	Chr12:121431974 Chr12:120994171	0	In house samples • 6 Danish MODY families • 1 Danish MODY proband Published studies • 5 previous families (PMID: 23348805)	Clinvar: NF HGMD:	No functional studies
Thr260Met	c.779C>T	Chr12:121432032 Chr12:120994229	4.0*10-6	In house samples • 1 Danish MODY proband Published studies • 13 previous families (PMID: 23348805) • 1 patient diagnosed with T1D (PMID: 30507613) • 2 patients with diabetes before 45 years of age (PMID: 30455330) • 1 T2D diabetes patient + 1 early onset diabetes patient (PMID: 29207974)	Clinvar: Pathogenic HGMD:	Reduced DNA binding capasity, dominant-negative action of variant and decreased expression of target genes (PMID: 30507613)
Arg263His	c.788G>A	Chr12:121432041 Chr12:120994238	0	In house samples • 2 Danish MODY probands • 1 Danish MODY family Published studies • 9 previous families (PMID: 23348805) • 1 Brazillian MODY family (PMID: 28012402) • Indian MODY families (PMID: 26853433)	Clinvar: Pathogenic HGMD:	Reduced transcriptional activity and nuclear localization (PMID: 26853433)
Arg271Gln	c.812G>A	Chr12:121432065 Chr12:120994262	$8.03*10^{-6}$	In house samples • 1 Danish MODY family Published studies • 9 previous families (PMID: 23348805) • 1 patients with T2D + 1 early onset diabetes patient (PMID: 29207974) • 1 child with obesity and diabetes (PMID: 29758564)	Clinvar: Pathogenic HGMD:	Reduced transcriptional activity and nuclear localization (PMID: 26853433)
Arg271Trp	c.811C>T	Chr12:121432064 Chr12:120994261	0	In house samples • 1 Danish MODY family Published studies • 13 previous families (PMID: 23348805)	Clinvar: Pathogenic HGMD:	Reduced DNA binding and impaired transcription (PMID:21170474) Lower levels of transcriptional activity (15–30% of wild type) (PMID: 12574234)

Val370Phe	c.1108G>T	Chr12:121434344 Chr12:120996541	0	In house samples • 1 Greenlandic MODY family • 1 Greenlandic MODY proband	Clinvar: NF HGMD:	In vitro analyses of this variant has shown that the variant cause skipping of exon 6 (unpublished)
Pro379Ala	c.1135C>G	Chr12:121434371 Chr12:120996568	0.0001847	In house samples • 2 Danish MODY probands • 1 glucose tolerant person • 1 screen-detected patient with T2D • 1 newly diagnosed diabetes patient Published studies • 10 previous families (PMID: 23348805) • 1 patients with T2D + 1 early onset diabetes patient (PMID: 29207974) • 10 carriers in UK-biobank (PMID: 33046911) • 1 Chinese diabetes patient (PMID: 30155490)	Clinvar: Pathogenic HGMD:	Fail to fully transactivate in a promoter- and cell-specific manner (PMID: 21170474)
Pro447Leu	c.1340C>T	Chr12:121435307 Chr12:120997504	$4.01*10^{-6}$	In house samples • 1 Danish MODY family Published studies • 11 previous families (PMID: 23348805)	Clinvar: Pathogenic HGMD:	Reduced lucifereace activity (PMID 27899486) Reduced protein levels, low transactivation (PMID: 10585442) Insulin hypersecretion in NGT as results of IVGTT (PMID: 9075819)
***HNF4A* variants (NM_175914.4)**						
Arg63Trp (rs137853244)	c.187C>T	Chr20:43034835 Chr20:44406195	0	In house samples • 1 Danish MODY family • 1 Danish MODY proband Published studies • 2 previous families (PMID: 23348805) • 1 patient with kidney disease (PMID: 28844315)	Clinvar: Pathogenic HGMD: Disease causing	Autosomal dominant atypical Fanconi syndrome in addition to the established beta cell phenotype (PMID: 24285859) The R63W variant in the HNF4A gene has been reported previously using alternate nomenclature R76W in association with congenital hyperinsulinism, macrosomia, and renal Fanconi syndrome PMID: 22802087; PMID: 24285859; PMID: 25819479;PMID: 31875549 ; PMID: 31474092; PMID: 29899848; PMID: 30026763) Variant maybe involved in hepatic dysfunction (PMID: 29493090; PMID: 25819479) Variant related to macrosomia and neonatal hyperinsulism (PMID: 24285859) R76W is directly involved in DNA binding which likely the cuase of the functionality of the variant (PMID: 23485969)
Arg112Trp (rs370239205)	c.334C>T	Chr20:43042348 Chr20:44413708	NF	In house samples • 1 Danish MODY proband Published studies • 5 previous families (PMID: 23348805) • 1 patient with kidney disease (PMID: 28844315)	Clinvar: VUS HGMD: Disease causing	The variant co-segregated with diabetes in diabetes in five family members. The variant alters a conserved amino acid that is located in the T-box, a region of the receptor implicated in dimerization and DNA binding (PMID: 12627330).

				• 1 patients with T2D + 1 early onset diabetes patient (PMID: 29207974)		Two additional carriers was found in this paper. In R134W family one member 36 years old, having diabetes for 27 years and severe complications had to stay on insulin, the other member 58 years old we switched to glibenclamide and basal insulin combination, 48 years after his diabetes has started. (PMID: 20132997)
Arg114Trp (rs137853336)	c.340C>T	Chr20:43042354 Chr20:44413714	0.00008	In house samples • 1 Danish MODY proband • 1 patient with early onset diabetes Published studies • 15 previous families (PMID: 23348805) • 15 carriers in UK-biobank (PMID: 33046911) • 2 French diabetes patients and one control (PMID: 33046911)	Clinvar: pathogenic/VUS HGMD: Disease causing	“We confirm that p.R114W is a pathogenic mutation with an odds ratio of 30.4 (95% CI 9.79-125, P = 2 × 10(-21)) for diabetes in our MODY cohort compared with control subjects. We redefine p.R114W as a pathogenic mutation that causes a distinct clinical subtype of HNF4A MODY with reduced penetrance, reduced sensitivity to sulfonylurea treatment, and no effect on birth weight.” (PMID: 27486234) The R125W mutant showed markedly reduced DNA binding activities and over 50% reduction in transactivation potential (PMID: 18829458) “R127W HNF-4α retained wild-type individual Fabp1 activation and bound to HNF-1α better than wild-type HNF-4α, yet did not cooperate with HNF-1α or increase HNF-1α Fabp1 promoter occupancy. The R127W mutant was also defective in both suppressing HNF-1α activation of HNF4 P2 and decreasing HNF-1α promoter occupancy.” (PMID: 16223942) Normal Subcellular localization DNA binding ~38% of wild type Transactivation activity ~70-78% of wild type No impairment of coactivator CBP (PMID: 10819248) In this article from Exeter this variants classified as pathogenic (PMID: 30229274)
Arg290Ser	c.868C>A	Chr20:43052699 Chr20:44424059	NF	In house samples • 3 Danish MODY probands • 1 patient with GDM Published studies • NF but p.Arg290Cys, p.Arg290His and Arg290Pro found in a total of 12 families (PMID: 23348805)	Clinvar: NF HGMD: NF dog er R290C; R290H and R290P found to be disease causing	No functional studies found
Arg309Cys	c.925C>T	Chr20:43052756 Chr20:44424116	0.000008	In house samples • 1 Danish MODY family	Clinvar: VUS	No functional studies

				• Arg309His was found in 1 patient with newly diagnosed diabetes Published studies • 6 previous families (PMID: 23348805) • Arg309Leu found in 1 carriers in UK-biobank (PMID: 33046911)	HGMD: Disease causing	
HNF4A Promoter P2						
NA	c.-181G>A	Chr20:42984264 Chr20:44355624	NA	Published studies • 2 previous families (PMID: 23348805)	Clinvar: NF HGMD: Disease causing	The mutation results in decreased affinity for HNF-1alpha, and consequently in reduced HNF-1alpha-dependent activation (PMID: 12235114)
NA	c. -178 A/G	Chr20:42984267 Chr20:44355627	NA	In house samples • 1 Greenlandic MODY family • 1 Greenlandic MODY proband	Clinvar: NF HGMD: NF	The variant is located in the binding site in the P2 promoter for HNF1A and HNF1B (Unpublished data)

Genome position: Position in the genome according to Hg19

Allele frequency in public data: Gnomad in Q4 2020.

Appendix 2: Information used for the selection of non-actionable variants in *GCK*, *HNF1A* and *HNF4A*

Variant (rs-number)	Nucleotide position	Genomic position (GRCh37/Hg19 GRCh38/Hg38)	Allele frequency in 60000 individuals	Identified in	Pathogenicity	Functionality
***GCK* variants (NM_000162.5)**						
Thr343Pro	c.1024A>C	Chr7: 44185325 Chr7: 44145726	0.000005	In house • 1 glucose tolerant person • 2 patients with newly diagnosed diabetes	Clinvar: VUS HGMD:	Lack of effect on catalytic activity (PMID: 21604084)
Thr343Arg (rs749208290)	c.1025C>G	Chr7: 44185324 Chr7: 44145725	0.00003	In house • 1 glucose tolerant person	Clinvar: NF HGMD:	No functional studies
***HNF4A* variants (NM_175914.4)**						
Val380Ile (rs137853337)	c.1138G>A	Chr20:43057049 Chr20:44428409	0.0008%	In house • 3 glucose tolerant person Published studies • 1 previous families (PMID: 23348805)	Clinvar: VUS HGMD: Disease causing	No functional studies

Genome position: Position in the genome according to Hg19

Allele frequency in public data: Gnomad in Q4 2020.