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Supplemental Data

Unsupervised Clustering of Missense Variants in *HNF1A* Using Multidimensional Functional Data Aids Clinical Interpretation

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SUPPLEMENTAL DATA

Contents

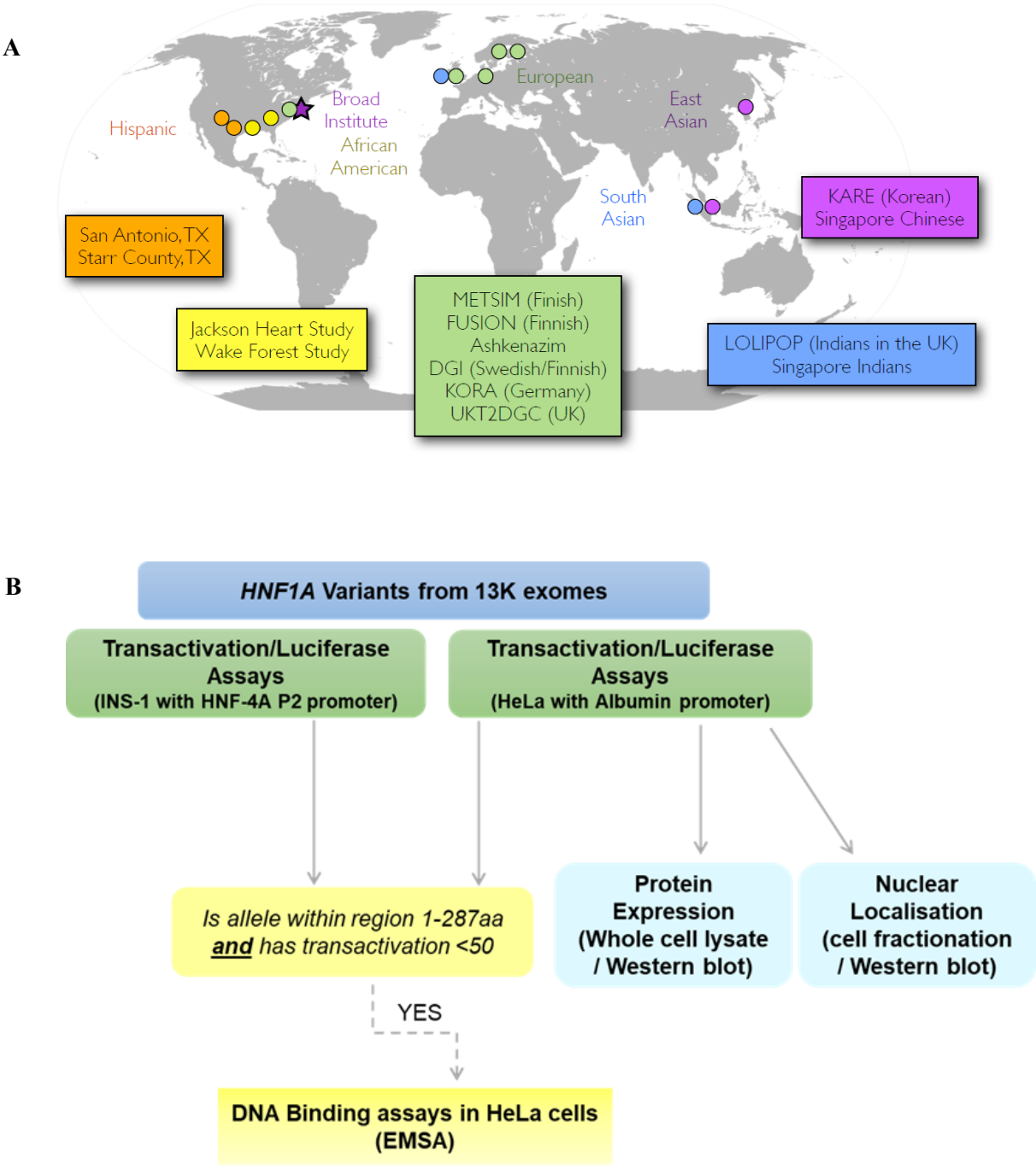


Figure S1. Ethnic Composition of a Multi-Consortia Led Exome Sequencing Study

(A). Molecular characterisation pipeline adopted by research groups at Oxford and Bergen to annotate the function of exome-detected *HNF1A* missense variants (B). Exome sequence data was generated from ~13K individuals (6,504 type 2 diabetes cases and 6,436 controls) from five ancestry groups with 82x mean coverage across the protein coding sequence of 18,281 genes, identifying 3.04 million variants (1.19 million protein-altering).[15]

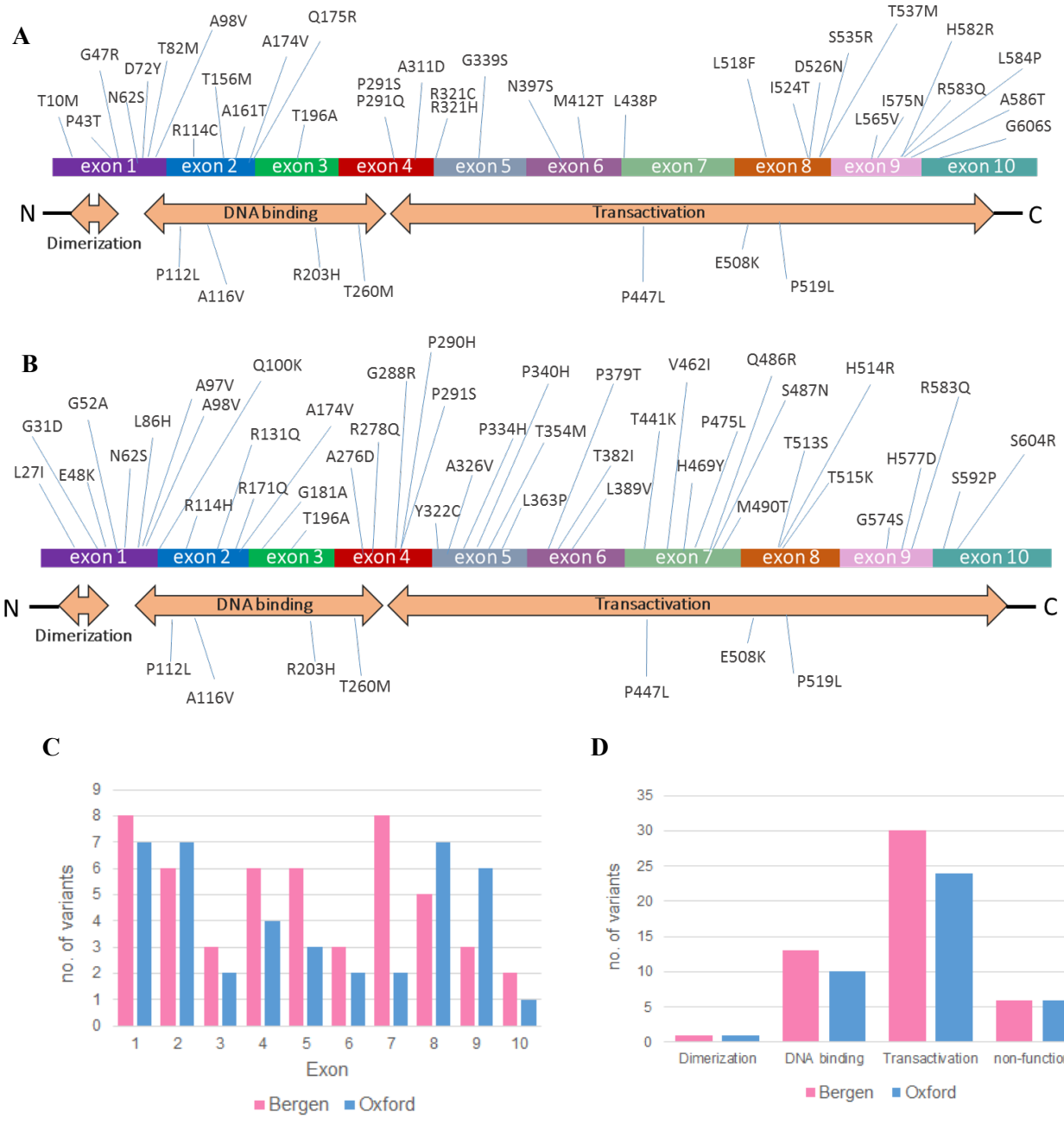


Figure S2. Division of Exome-Identified Non-Synonymous Missense Alleles in *HNF1A* Between Oxford and Bergen for Large-Scale Collaborative Functional Follow-up(A) Oxford variant set **(B)** Bergen variant set **(C)** Distribution of exome-detected *HNF1A* variants by centre based on exon position **(D)** Distribution of exome-detected *HNF1A* alleles by centre based on functional domain.

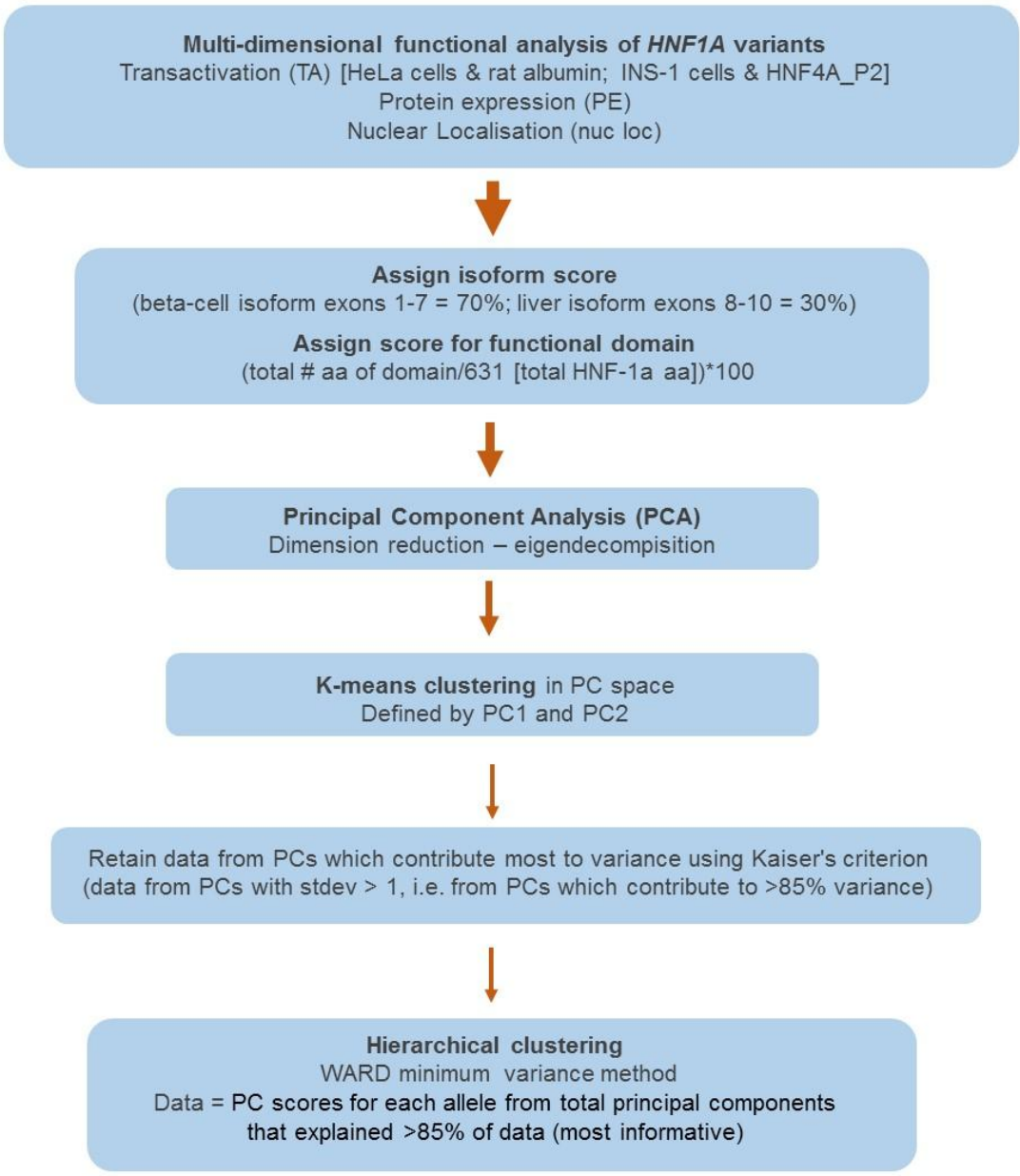


Figure S3. Analytical pipeline for unsupervised stratification of HNF1A missense variants along the in vivo glycaemic spectrum using multi-dimensional in vitro functional data. The pipeline we developed to perform unbiased classification of functionally characterised missense alleles in HNF1A is as follows: 1) dataset preparation (omitting missing values and unevenly represented functional parameters, and selecting the optimal input data format), 2) assignment of weights for tissue-specific isoform expression (liver v pancreatic beta-cell dominant isoforms) and functional domain (dimerization, undefined, DNA binding, transactivation), 3) Dimension reduction by principal component analysis, 4) retention of values from principal components which contribute the most to data variance according to Kaiser's criterion (>85% explained variance by eigenvalues i.e. all PCs with standard deviation > 1) to avoid fitting noise, 5) hierarchical clustering analysis of data points from PCs retained in step 4 using WARD minimum variance method. Abbreviations: aa= amino acids; stdev = standard deviation; PCs = principal components.

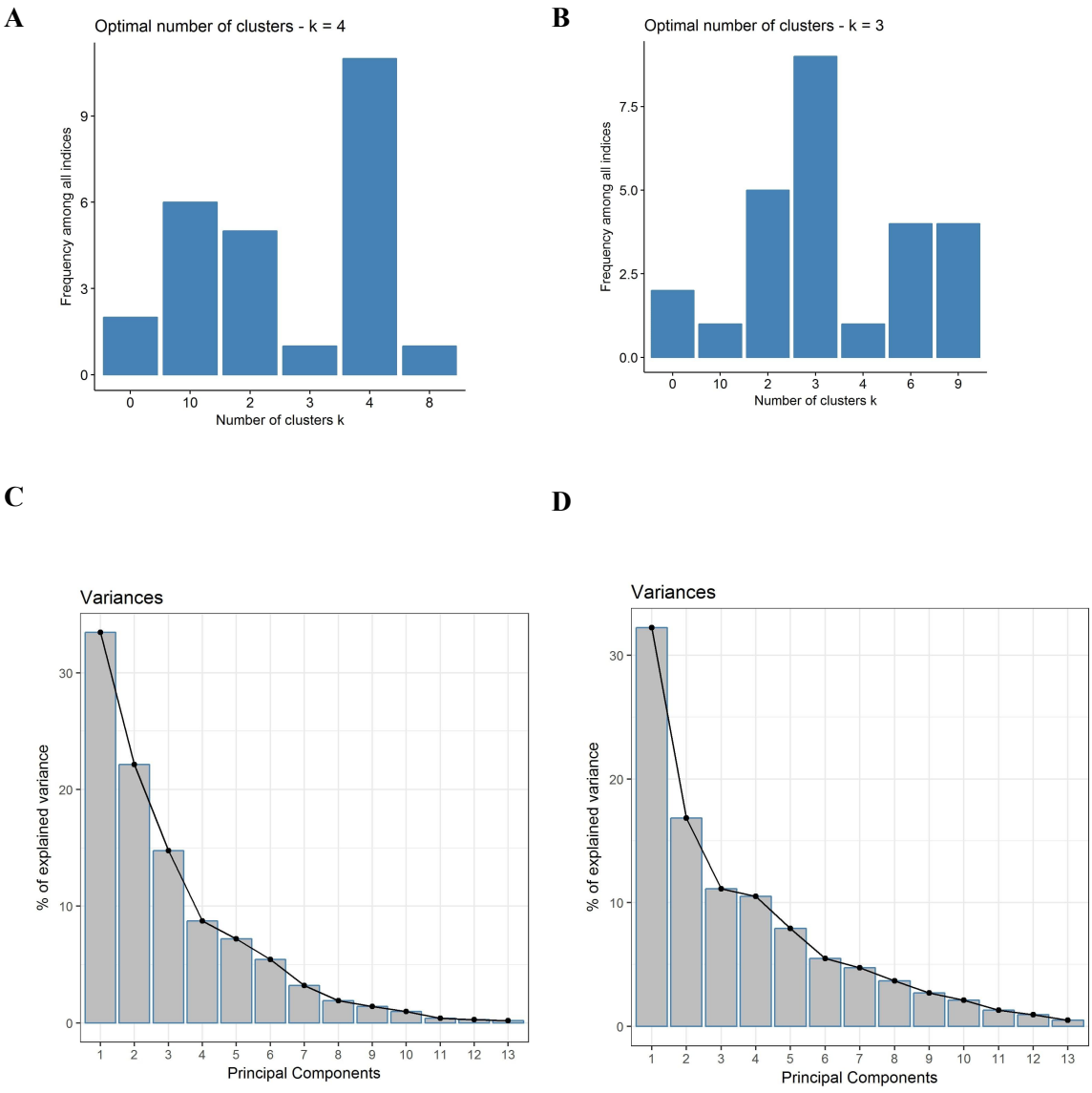


Figure S4. Assessment of the Optimal Number of Clusters to Use for Partitioning the Multi-Dimensional Datasets. Dataset generated at (A) Oxford and (B) Bergen. Best cluster scheme obtained using *NbClust* R package which provides majority rule across 30 tested indices using euclidean distance. **Scree plots showing percent explained variance from PCA of Oxford (C) and Bergen (D) datasets.**

Summary of Figure S5 and S6 - Functional studies of Protein Variants

The variants studied at both Centers included five exome variants (p.Asn62Ser, p.Ala174Val, p.Pro291Ser, p.Thr196Ala, p.Arg583Gln), two type 2 risk variants (p.Ala98Val, p.Glu508Lys), and six MODY variants (super controls; p.Ala116Val, p.Arg203His, p.Pro112Leu, p.Pro447Leu, p.Thr260Met, p.Pro519Leu), with single variants missing in some individual assays.

Correlation of functional effects between centers - For the transactivation assay, testing two promoters in different cell lines (HeLa/INS), the control variants correlated well between centers by range of effect; the MODY control variants presented the most severe effect, and the type 2 diabetes risk variants medium effects (deviation by the p.Ala116Val MODY control variant in one promoter assay). For the exome variants, the functional effect also correlated between centers, i.e. less severe effect than the rare type 2 risk variant (p.Glu508Lys) (deviation p.Pro291Ser between Centers). Overall, values of all tested variants were somewhat lower at one center (Bergen).

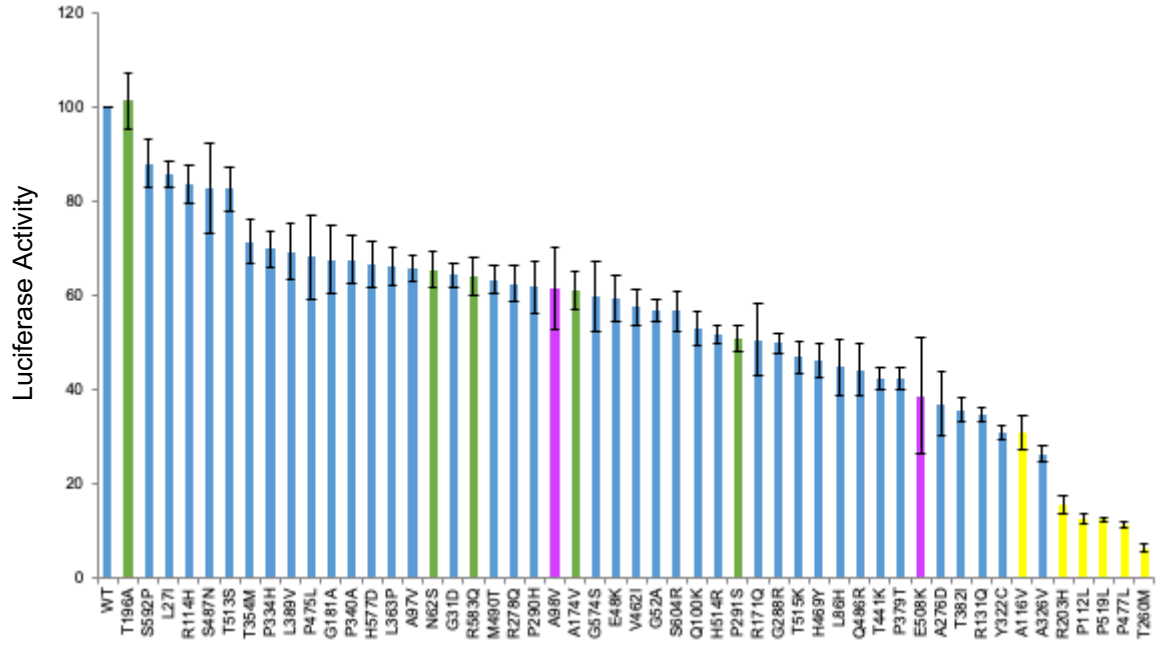
For the subsequent DNA binding assay, only one mutual exome variant was tested at both centers (p.Ala174Val), revealing correlating effects between the two centers. The same applied to the two MODY variants, although lower values (more severe effect) were detected at one center (Oxford).

For the protein expression assay, the MODY and type 2 risk variants correlated well between the centers (deviation for p.Glu508Lys exhibiting stronger reducing effect by one center (Bergen <0.6 fold versus Oxford WT-like). Two (p.Asn62Ser, p.Arg583Gln) of the five exome variants also deviated between the two centers (Oxford ~0.6 fold versus Bergen WT-like).

The nuclear localization assay correlated less between the centers. Two (p.Pro447Leu, p.Ala116Val) of six MODY variants, the two type 2 risk variants (p.Ala98Val, p.Glu508Lys), and the two exome variants (p.Ala174Val, p.Arg583Gln) did not correlate by range of effect (Bergen by stronger reducing effect compared to Oxford). The non-patient related control variant included as the best indicator of strongly reduced nuclear localization (DelB) did, however, correlate well between centers.

Of the four functional assays, it was not unexpected that functional effect between centers correlated best for the transactivation and DNA binding assays, whereas the protein expression and nuclear localization assays had more variable effect sizes when compared. This latter is most likely due several experimental steps prior to final quantifications, which is based on immunoblotting and antibody specificity.

A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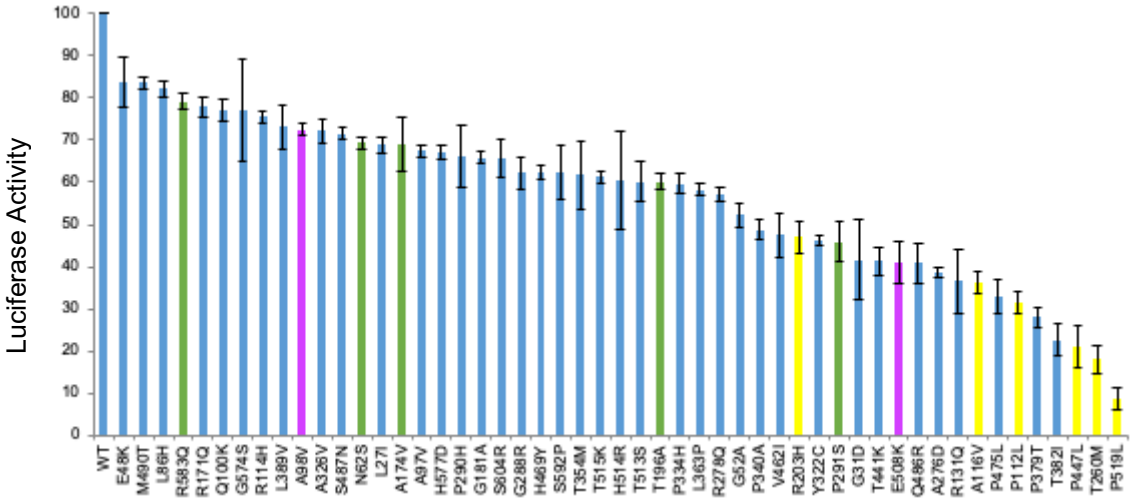
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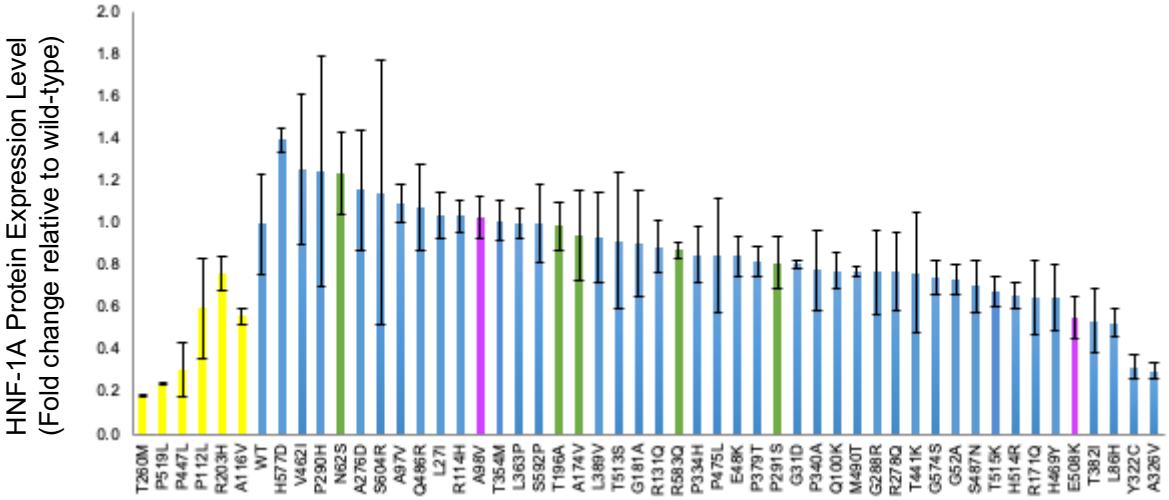
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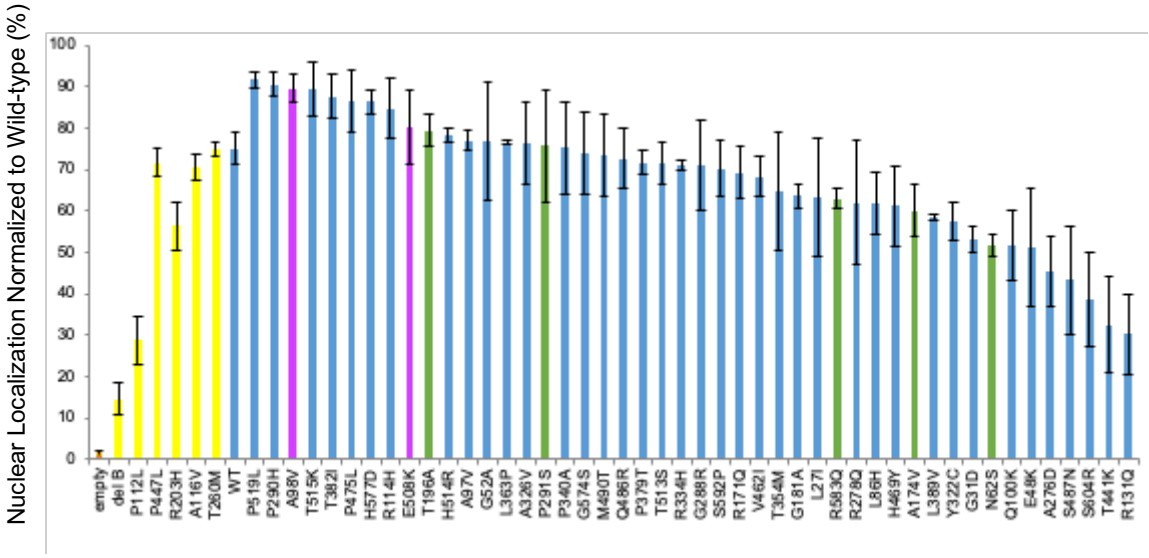
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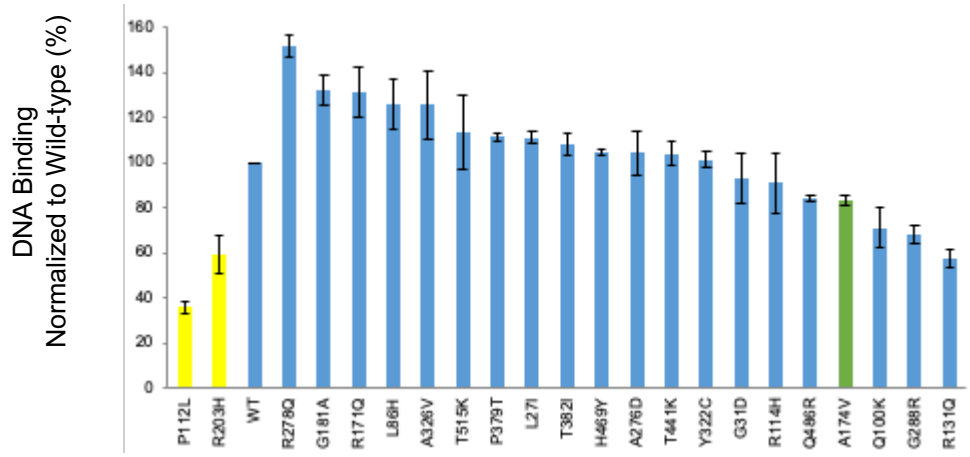

Figure S5. Functional Studies of Protein Variants (Bergen)

A and B) Transcriptional activity of HNF-1A protein variants measured by luciferase reporter assay. Transcriptional activity was performed in two individual cell lines using two different promoter-linked luciferase reporter constructs. A) HeLa cell line using rat albumin promoter and B) INS-1 cell line using HNF4AP2 promoter. Cells were transiently transfected with wild-type or variant *HNF1A* variant plasmids together with reporter plasmids pGL3-RA (Firefly Luciferase under rat albumin promoter) or pGL3-HNF4AP2 (Firefly Luciferase under HNF4AP2 promoter), and pRL-SV40 (Renilla Luciferase as internal control). Cells were harvested/lysed 24 h after transfection and Firefly activity recorded after normalizing for Renilla activity. HNF-1A variant measurements are given in percentage of wild-type activity (100%). Each bar represents the mean of nine readings ± SD; three parallel readings were conducted on each of 3 experimental days (n = 3). Different colors are used for different group of variants; shared control variants are shown in green, type 2 diabetes associated variants in pink and MODY control variants in yellow.

C) Variant effect on HNF-1A protein expression level. The level of expression of HNF-1A variant proteins was assessed relative to wild-type protein level in cells. For this purpose, 20 µl HeLa cell lysates prepared for transactivation assay (panel A) was analyzed by SDS-PAGE and immunoblotting using an HNF-1A specific antibody. A house-keeping protein (actin) was used as internal control for normalization and bands were quantitated using densitometric analysis by Quantity One 1-D software. Each bar represents the level of HNF-1A protein expression normalized to wild-type HNF-1A expression level (100%). Different colors are used for different group of variants; shared control variants are shown in green, type 2 diabetes associated variants in pink and MODY control variants in yellow.

D) Nuclear localization of HNF-1A variant proteins. The effect of individual *HNF1A* variants on HNF-1A protein localization (nuclear versus cytosolic) was assessed in cells. For this purpose, HeLa cells were cultured and transiently transfected with wild-type or *HNF1A* variant plasmids and sequential cell fractionation was performed 24h post-transfection by isolating the nuclear and cytosol fractions from each transfected sample. 20 µg total protein from each isolated compartment was analyzed for HNF-1A expression by SDS-PAGE and immunoblotting using an HNF-1A specific antibody. HNF-1A variant p.delB was included as a positive control for impaired nuclear localization (cytosolic retention). HNF-1A variant nuclear localization measurements are given in percentage of wild-type localization. Each bar represents the mean of nuclear localization of HNF-1A protein of two biological replicates in two experimental days (n = 2). Different colors are used for different group of variants; shared control variants are shown in green, type 2 diabetes associated variants in pink and MODY control variants in yellow.

E) DNA binding of HNF-1A protein variants to the rat albumin promoter as studied by EMSA. DNA binding ability test was conducted for HNF-1A variants that are either located in DNA binding domain (1-287 aa) or those that demonstrated transactivation activity < 50%. Electrophoretic mobility shift assay (EMSA) was performed to investigate the DNA binding ability of equal amounts of *in vitro*-synthesized HNF-1A proteins to a ^{32}P-radiolabeled rat albumin oligonucleotide containing an HNF-1A binding site. A coupled *in vitro* transcription and translation system (TNT) was used for expression of HNF-1A proteins. DNA-protein bound complexes were analyzed by DNA retardation gel (6%) electrophoresis followed by autoradiography. Level of DNA binding was obtained by quantification of the intensity of HNF-1A protein-oligonucleotide complexes. Measurements are given in percentage of wild-type binding activity (100%). Two *HNF1A*-MODY3 variants were used as control for low binding (yellow) and shared control variants are presented in green color.

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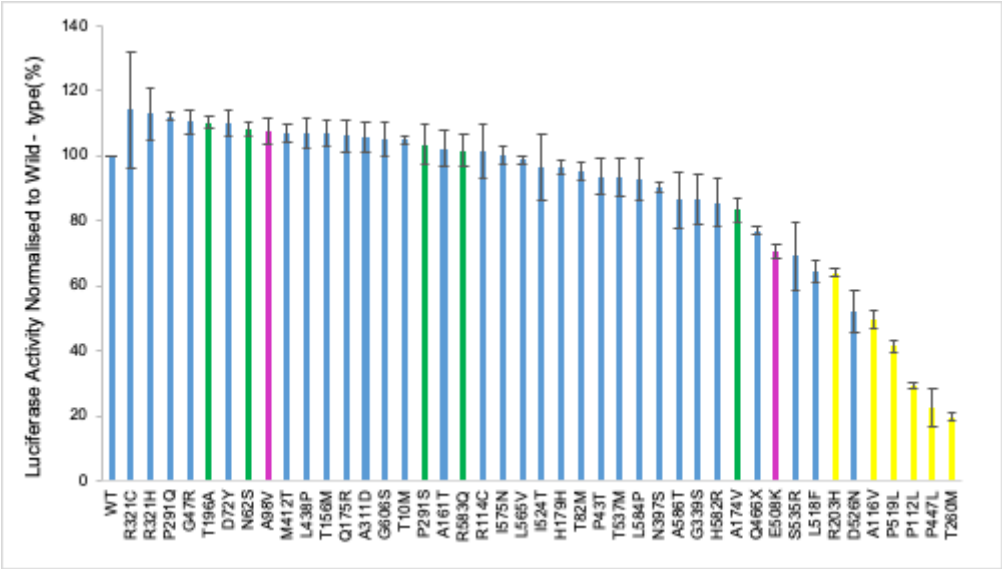
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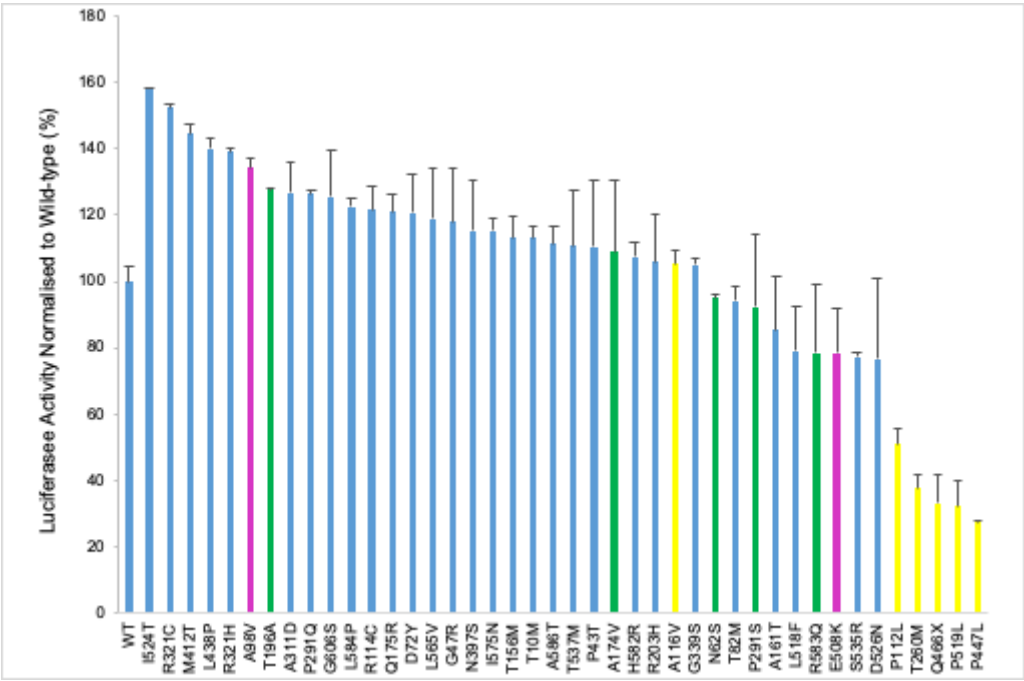
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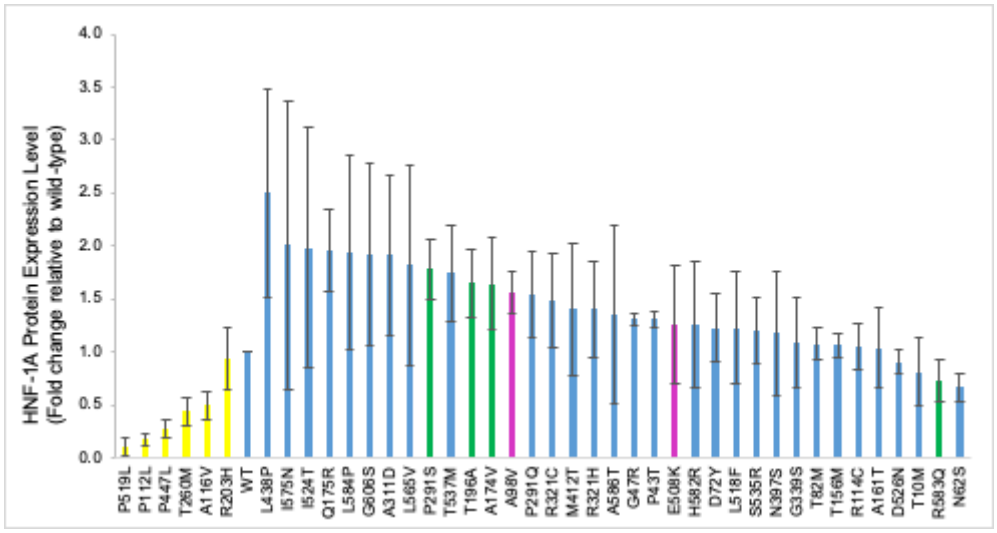
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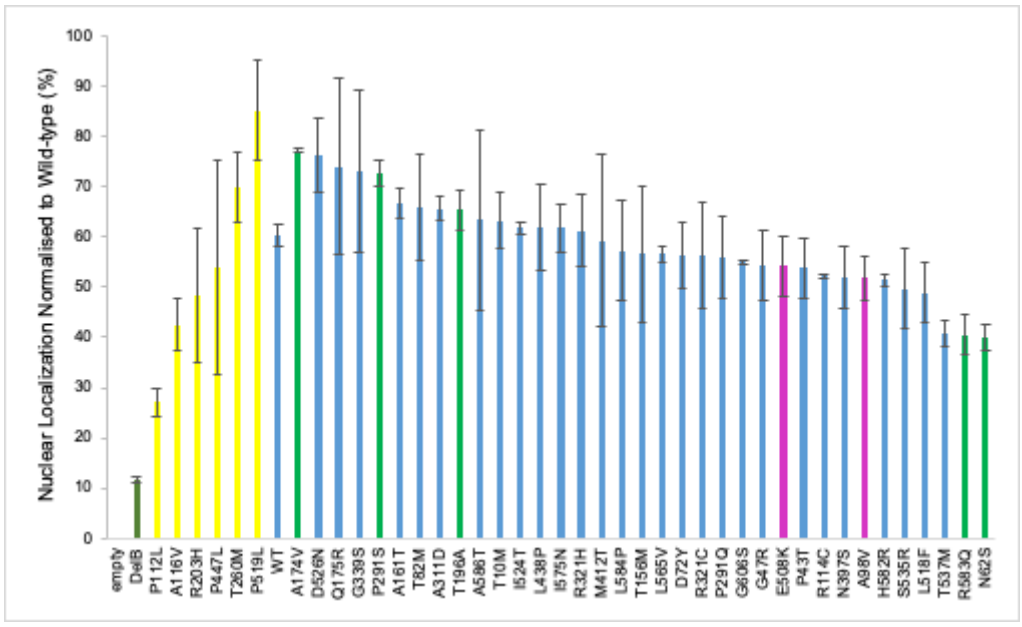
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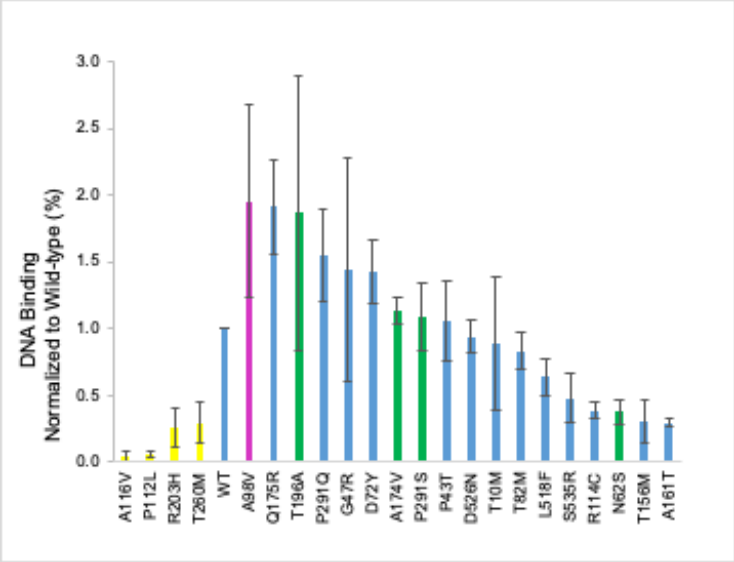


Figure S6. Functional Studies of Protein Variants (Oxford)

A and B) Transcriptional activity of HNF-1A protein variants measured by luciferase reporter assay. Transcriptional activity was performed in two individual cell lines using two different promoter-linked luciferase reporter constructs. A) HeLa cell line using rat albumin promoter and B) INS-1 cell line using *HNF4A* P2 promoter. Cells were transiently transfected with wild-type or variant *HNF1A* variant plasmids together with reporter plasmids pGL3-RA (Firefly Luciferase under rat albumin promoter) or pGL3-*HNF4A* P2 (Firefly Luciferase under *HNF4A* P2 promoter), and pRL-SV40 (Renilla Luciferase as internal control). Cells were harvested/lysed 24 h after transfection and Firefly activity recorded after normalizing for Renilla activity. HNF-1A variant measurements are given in percentage of wild-type activity (100%). Each bar represents the mean of nine readings ± SD; three parallel readings were conducted on each of three experimental days (n = 3). Different colors are used for different group of variants; shared control variants are shown in green, type 2 diabetes associated variants in pink and MODY control variants in yellow.

C) Variant effect on HNF-1A protein expression level. The level of expression of HNF-1A variant proteins was assessed relative to wild-type protein level in cells. For this purpose, HeLa cells were cultured and transiently transfected with wild-type or *HNF1A* variant plasmids. Cells were harvested at 24 hrs and aliquoted prior to lysing for the protein expression levels and EMSA or nuclear localization. 10 µg of HeLa cell lysates prepared for transactivation assay (Figure A) was analyzed by SDS-PAGE and immunobloting using an HNF-1A specific antibody. A house-keeping protein (B-tubulin) was used as internal control for normalization of levels of tubulin versus HNF-1A specific bands., using densitometric analysis by Image Lab software (Bio-Rad). Each bar represents the level of HNF-1A protein expression normalized to wild-type HNF-1A expression level (100%). Different colors are used for different groups of variants; shared control variants are shown in green, type 2 diabetes associated variants in pink and MODY control variants in yellow.

D) Nuclear localization of HNF-1A variant proteins. The effect of individual *HNF1A* variants on HNF-1A protein localization (nuclear versus cytosolic) was assessed in cells. For this purpose, an aliquot of the HeLa cells that had been transfected and harvested at 24hr were lysed to isolate the nuclear and cytosol fraction. 20 µg total protein from each isolated compartment was analyzed for HNF-1A expression by SDS-PAGE and immunobloting using an HNF-1A specific antibody. HNF-1A variant p.delB was included as a positive control for impaired nuclear localization (cytosolic retention). HNF-1A variant nuclear localization measurements are given in percentage of wild-type localization. Each bar represents the mean of nuclear

localization of HNF-1A protein of two biological replicates in two experimental days (n = 2). Different colors are used for different groups of variants; shared control variants are shown in green, type 2 diabetes associated variants in pink and MODY control variants in yellow.

E) DNA binding of HNF-1A protein variants to the rat albumin promoter as studied by EMSA. DNA binding ability test was conducted for *HNF1A* variants that are either located in DNA binding domain (1-287 aa) or those that demonstrated transactivation activity < 50%. Electrophoretic mobility shift assay (EMSA) was performed using an aliquot of the lysed HeLa cells used for the protein expression levels. The HNF-1a wild-type or variant protein was bound to a fluorescently labelled probe and then separated using a TBE polyacrylamide gel. An HNF-1a antibody was used to generate a super-shift with an aliquot of the wild-type protein, prior to the probe binding. Measurements are given as percentage of wild-type binding activity (100%). Different colors are used for different groups of variants; shared control variants are shown in green, type 2 diabetes associated variants in pink and MODY control variants in yellow.

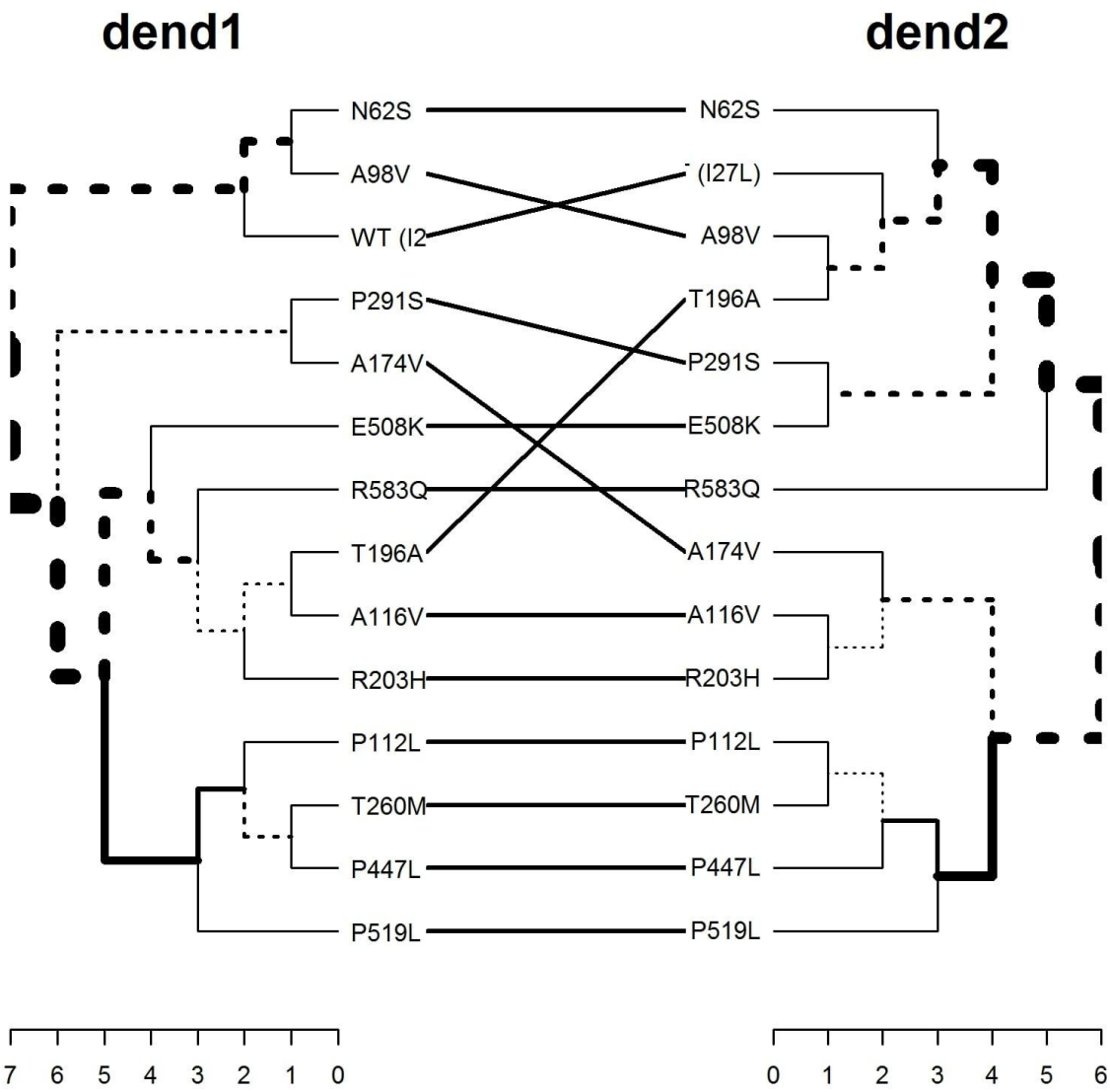


Figure S7. Clustering Alignment of *HNF1A* Missense Variants Shared Between Both Centres at Oxford (dend1and Bergen (dend2). Entanglement (quality of alignment score score from 1 to 0 where lower values = good alignment quality) = 0.055. Dashed lines highlight nodes which contain a combination of alleles not present in the other tree (thickness corresponds to height). The connecting lines highlight subgroups and/or cluster members that are present in both dendrograms. Rotational properties maintained from full variant set dendrogams shown in Figure 3.

Table S1. Exome-Detected *HNF1A* Missense Alleles Selected for Functional Follow-Up (*n* = 73)

(Excel file)

HNF1A reference sequence = NM_000545.6. Colour coding of first three columns (Variant and CDS position NM_0005454): blue = Oxford variant set; red = Bergen variant set; gray = variants shared and characterised by both research groups at Oxford and Bergen. Green highlights variants identified with a prevalence greater than 0.

Table S2. MODY Reference Alleles (*n* = 6)

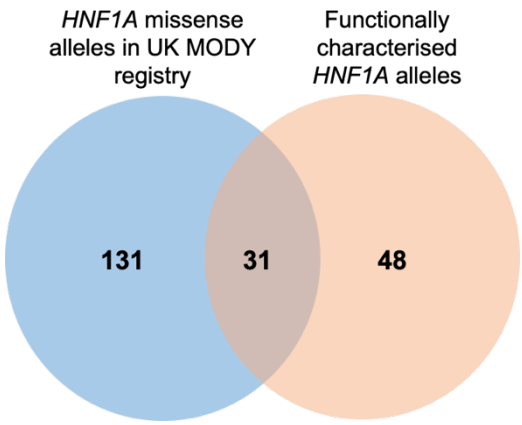
(Excel file)

Table S3. Scores for Tissue Specific Isoform Expression and Functional Domain

Isoform	Functional Domain
Pancreatic beta-cell isoforms *HNF1A(A)*: exons 1-6 UniProtKB Protein isoform identifier P208231-3 *HNF1A(B)*: exons 1-7 UniProtKB Protein isoform identifier P208231-2 ***Hepatic isoform (full length gene)*** *HNF1A(C):* exons 1-10 NCBI Reference Sequence: NM_000545.8 UniProtKB Protein isoform identifier P208231-1	***HNF-1A functional domains*** Dimerization = 32 amino acids Undefined = 66 amino acids DNA binding = 187 amino acids Transactivation = 343 amino acids
Weight (%)	***Weight (%)***
Variants in *HNF1A(A)* and *HNF1A(B)* = [(no. of exons in predominant beta-cell isoforms) ÷ 10 (no. of exons in full length *HNF1A*)] × 100 = 70%	[no. of amino acids of domain in which allele is expressed ÷ amino acid length of HNF-1a] × 100
Variants exclusively present in *HNF1A(C)* = [(no. of additional exons present exclusively in predominant liver isoform) ÷ 10 (no. of exons in full length *HNF1A*)] × 100 = 30%	Dimerization = 4.9% Undefined = 10.45% DNA binding = 29.6% Transactivation = 54.3%

Table S4. Features of *HNF1A* Allele Carriers Documented in UK Registry

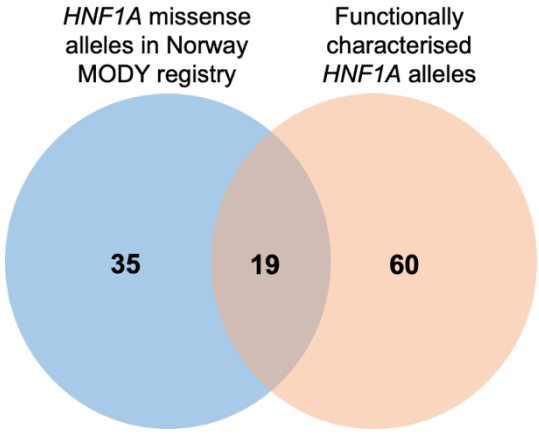
Features	Measure
Age at diagnosis	age (years)
Initial Treatment	Diet/OHA/Insulin
Current Treatment	Diet/OHA/Insulin
Sulphonylurea sensitivity	Yes/No
BMI	BMI (kg/m^2)
HbA1c	%
GAD	U/mL
ZnT8	U/mL
IA2	U/mL
C-Peptide	pmol/L
No of generations DM	number of generations with diabetes
Mother DM	Yes/No
Father DM	Yes/No
No of Children DM	number of children with diabetes
Ethnic Origin	ethnicity
Referred to clinic for MODY testing	Yes/No



GAD, ICA, IA2, ZnT8 = islet autoantibodies; UCPCR = urinary C-peptide creatinine ratio; OHA = oral hypoglycaemic agent; BMI = body mass index; DM= diabetes mellitus; HbA1c = glycated hemoglobin.

Table S5. Features of *HNF1A* Allele Carriers Documented in Norwegian Registry

Features	Measure
Age at diagnosis	age (years)
Initial Treatment	Diet/OHA/Insulin
Current Treatment	Diet/OHA/Insulin
Sulphonylurea sensitivity	Yes/No
BMI	BMI (kg/m^2)
HbA1c	%
GAD -ve	Yes/No
GAD +ve	Yes/No
ICA +ve	Yes/No
ICA -ve	Yes/No
ZnT8 -ve	Yes/No
ZnT8 +ve	Yes/No
IA2 -ve	Yes/No
IA2 +ve	Yes/No
UCPCR Value	nmol/mmol
C-Peptide	pmol/L
No of generations DM	number of generations with diabetes
Mother DM	Yes/No
Father DM	Yes/No
No of Children DM	number of children with diabetes
Ethnic Origin	ethnicity



GAD, IA2, ZnT8 = islet autoantibodies; OHA = oral hypoglycemic agent; BMI = body mass index; DM= diabetes mellitus; HbA1c = glycated hemoglobin. Common alleles (AF 3-35%) indicated by a (*).

Table S6. Clinical Features Associated with Functionally-Clinically Discordant *HNF1A* Alleles in UK Registry

(Excel file)

Clinical features and additional information available on each of the listed alleles from the UK MODY diagnostic registry. With the exception of p.R131Q (*n* = 4 documented cases; information displayed as range across the four), details are associated with a single case. Abbreviations: NGS = next generation sequencing; dx = diagnosis; SU = sulfonylurea; FH = family history; BMI = body mass index; HbA1c = glycated hemoglobin.

Table S7. Clinical Features Associated with Functionally-Clinically Discordant *HNF1A* Alleles in Norwegian Registry

(Excel file)

Clinical features and additional information available on each of the listed alleles from the Norway MODY diagnostic registry. Abbreviations: NGS = next generation sequencing; dx = diagnosis; SU = sulfonylurea; FH = family history; BMI = body mass index; HbA1c = glycated hemoglobin