The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/177227

Please be advised that this information was generated on 2020-05-06 and may be subject to change.
Mutations in N-acetylglucosamine (O-GlcNAc) transferase in patients with X-linked intellectual disability

Anke P. Willems, Mehmet Gundogdu, Marlies E. Kempers, Jacques C. Giltay, Rolph Pfundt, Martin Elferink, Bettina F. Loza, Joris Puijkschot, Andrew T. Ferenbach, Koen L. I. van Gassen, Daan M. F. van Aalten, and Dirk J. Lefeber

From the Department of Neurology, Donders Institute for Brain, Cognition and Behaviour and the Department of Genetics, Radboud University Medical Centre, 6500 HB Nijmegen, The Netherlands, the Department of Laboratory Medicine, Translational Metabolic Laboratory, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, 6525 GA Nijmegen, The Netherlands, the Centre for Gene Regulation and Expression, School of Life Sciences, University of Dundee, DD1 5EH Dundee, Scotland, United Kingdom, the Department of Genetics, University Medical Centre Utrecht, 3508 AB Utrecht, The Netherlands, the Department of Paediatrics, VieCuri Hospital, 5900 BX Venlo, The Netherlands, and the Department of Paediatrics, Radboud University Medical Centre and Amalia Children’s Hospital, 6500 HB Nijmegen, The Netherlands.

Edited by Gerald W. Hart

N-Acetylglucosamine (O-GlcNAc) transferase (OGT) regulates protein O-GlcNAcylation, an essential and dynamic post-translational modification. The O-GlcNAc modification is present on numerous nuclear and cytosolic proteins and has been implicated in essential cellular functions such as signaling and gene expression. Accordingly, altered levels of protein O-GlcNAcylated have been associated with developmental defects and neurodegeneration. However, mutations in the OGT gene have not yet been functionally confirmed in humans. Here, we report on two hemizygous mutations in OGT in individuals with X-linked intellectual disability (XLID) and dysmorphogenic features: one missense mutation (p.Arg284Pro) and one mutation leading to a splicing defect (c.463–6T>G). Both mutations reside in the tetracricopeptide repeats of OGT that are essential for substrate recognition. We observed slightly reduced levels of OGT protein and reduced levels of its opposing enzyme O-GlcNAcase in both patient-derived fibroblasts, but global O-GlcNAc levels appeared to be unaffected. Our data suggest that mutant cells attempt to maintain global O-GlcNAcylation by down-regulating O-GlcNAcase expression. We also found that the c.463–6T>G mutation leads to aberrant mRNA splicing, but no stable truncated protein was detected in the corresponding patient-derived fibroblasts. Recombinant OGT bearing the p.Arg284Pro mutation was prone to unfolding and exhibited reduced glycosylation activity against a complex array of glycosylation substrates and proteolytic processing of the transcription factor host cell factor 1, which is also encoded by an XLID-associated gene. We conclude that defects in O-GlcNAc homeostasis and host cell factor 1 proteolysis may play roles in mediation of XLID in individuals with OGT mutations.

Protein O-GlcNAcylation is a dynamic and reversible post-translational modification that is present on many nucleocytoplasmic and mitochondrial proteins (1,2). O-GlcNAcylation involves cycling of a single N-acetylglucosamine (O-GlcNAc) moiety on serine and threonine residues of a spectrum of substrates, affecting their stability, activity, and subcellular localization (3,4). The transfer of O-GlcNAc is catalyzed by O-GlcNAc transferase (OGT) (5,6) and is modulated by the availability of the donor substrate UDP-GlcNAc (7). UDP-GlcNAc biosynthesis is coupled to glucose, glutamine, and nucleotide metabolism. Concomitantly, OGT exerts a nutrient-sensitive regulation over various cellular functions by acting on myriad targets (8). OGT is highly conserved in metazoa and consists of multiple tetracricopeptide repeats (TPRs) on the N terminus and a C-terminal glycosyltransferase domain (9,10). Although TPRs are known to be involved in protein-protein interactions, in the context of OGT they facilitate substrate recognition and specificity (11). In addition to its glycosyltransferase activity, OGT was recently shown to have a role in proteolytic processing of host cell factor 1 (HCF1) (12,13), a transcriptional co-regulator of the cell cycle (14). HCF1 is heavily O-GlcNAcylated (15), but a recent study revealed that O-GlcNAcylation and proteolytic cleavage of HCF1 by OGT are independent processes (16). OGT activity is counteracted by O-GlcNAcase (OGA), a hexosaminidase that specifically removes O-GlcNAc modifications (17). It has been shown that chemical inhibition of OGA in different human cell lines leads to a decreased expression of OGT and an increased expression of OGA (18), which indicates an as-yet unidentified mechanism of regulation to maintain global O-GlcNAc homeostasis. OGT

The abbreviations used are: O-GlcNAc, N-acetylglucosamine; XLID, X-linked intellectual disability; OGA, O-GlcNAcase; OGT, O-GlcNAc transferase; HCF1, host cell factor 1; TPR, tetracricopeptide repeat; qPCR, quantitative PCR.

This work was supported by the Dutch Organization for Scientific Research VIDI Grant 91713359 (to D.J.L.), Wellcome Trust Investigator Award 110061 (to D. M. F. V. A.), and a University of Dundee Translational Medical Research Fund Ph.D. fellowship (to M. G.). The authors declare that they have no conflicts of interest with the contents of this article.

Author’s Choice—Final version free via Creative Commons CC-BY license. This article contains supplemental Table S1 and Fig. S1.

1 Both authors contributed equally to this work as first authors.
2 Both authors contributed equally to this work as last authors.
3 To whom correspondence may be addressed. E-mail: dmfvanaalten@dundee.ac.uk.
4 To whom correspondence may be addressed. E-mail: Dirk.Lefeber@Radboudumc.nl.

Received for publication, April 6, 2017, and in revised form, May 23, 2017. Published, Papers in Press, June 5, 2017. DOI 10.1074/jbc.M117.790097
OGT variants in XLID

Figure 1. Clinical images from patients with hemizygous OGT mutations. A, picture of patient 1, showing dysmorphic features. B, pictures of patient 2, showing dysmorphic features. C, MRI image of patient 1, showing mild abnormalities pointing toward periventricular leukomalacia, indicated by red arrowheads. D, MRI image of patient 2, showing no brain abnormalities.

has been linked to embryonic development in a number of animal models. *Caenorhabditis elegans* with null mutations in *ogt-1* are viable and fertile, but with metabolic defects (19). In *Drosophila*, OGT is encoded by the *super sex combs* gene, which is essential for *Drosophila* viability and segmentation (20, 21). Knockdown of OGT in zebrafish causes impaired embryonic growth with reduced brain size (22). Although OGT is ubiquitously expressed, it is remarkably abundant in brain (9, 10). Accordingly, O-GlcNAcylation has been linked to neuronal function and brain development in a number of cell and animal models (22, 23). In addition, recent studies have shown that O-GlcNAc signaling can regulate axonal and dendritic growth (24, 25) and AMPA receptor trafficking (26, 27), possibly affecting learning and memory processes. Also, alterations in protein O-GlcNAcylation have been associated with neurodegenerative diseases (28).

Genetic OGT variants identified in patients with X-linked intellectual disability (XLID) have previously been documented: first, in a clinical report, albeit accompanied by mutations in *MED12* (a known XLID gene) (29), and second, in a large screen for novel XLID genes (30). Here, we describe two patients with intellectual disability, bearing hemizygous mutations in *OGT*. By combining characterization of the O-GlcNAcylation machinery in patient cells and characterization of the effects of OGT XLID mutations on OGT dual activity *in vitro*, we provide preliminary evidence suggesting that effects on the O-GlcNAc proteome and proteolytic processing of HCF1 may underpin the observed XLID phenotypes.

Results

Clinical phenotype of two ID patients

Two male patients showed intellectual disability and a developmental delay with an unknown cause. Cognitive testing in patient 1, at the age of 5 years old, showed reduced intelligence (WPPSI-III-NL, score 2;9) and delay in adaptive behavior (VABS, score 2;0). Additional neurological symptoms were pyramidal syndrome, mild spastic diplegia, and psychomotor retardation with behavioral conduct disorder. In addition, patient 1 had an inguinal hernia. Dysmorphic features included hypertelorism, low-set ears, a broad nose, full lips, supernumerary nipple, hypoplastic toe, mild retrognathia, long and thin fingers, clinodactyly, and microcephaly (Fig. 1A). Patient 1 showed involvement of the eyes including amblyopia and possible astigmatism. He was born with a mild respiratory insufficiency based upon transient tachypnea of the neonate. Cardiac screening revealed a bicuspid aortic valve. An MRI at the age of 2 years old showed mild abnormalities pointing toward periventricular leukomalacia (Fig. 1C), but with a normal EEG.
The global developmental level of patient 2, at the age of 5.5 years old, was estimated at 1.5 years. He showed mouth hypotonia with drooling and involvement of the eyes including nystagmus, astigmatism, and high hypermetropia. Genital abnormalities included a small phallus, and orchiopexy was performed at the age of 3 years old. His dysmorphic phenotype was less evident, with bitemporal narrowing and metopic ridge as main features (Fig. 1B). Upon birth, a ventricular septal defect was detected, which closed spontaneously at a later age. An MRI performed at the age of 3 years showed no clear brain abnormalities (Fig. 1B). The clinical features of the patients are summarized in Table 1. The clinical features of the patients are summarized in Table 1.

Identification of mutations in OGT

Whole exome sequencing was performed in the two male patients from unrelated families to uncover the cause of their intellectual disability. Mutations in OGT were found in both patients.

In patient 1, trio-sequencing and filtering for de novo variants revealed two potential candidate genes. A heterozygous de novo mutation was found in bromodomain-containing protein 1 (BRD1): Chr22(GRCh38): g.49,823,630C>G; NM_001304808.1: c.688G>C (p.Val230Leu). In addition, a hemizygous de novo missense variant in OGT was discovered: ChrX(GRCh38): g.71,555,312G>C; NM_181672.2: c.851G>T (p.Arg284Pro) (Fig. 2A). Arg-284 is conserved among many vertebrates and some invertebrates such as Drosophila (supplemental Fig. S1).

In silico analysis of p.Arg284Pro in OGT predicted pathogenicity of the mutation (Table 2). The heterozygous nature of the mutation in BRD1, in addition to lower pathogenicity predictions for p.Val230Leu (Table 2), made this variant less likely to be pathogenic.

For patient 2, a gene panel for known intellectual disability genes and de novo analysis did not reveal a potential causative mutation. Further analysis of the exome data by filtering for variants following an autosomal recessive or X-linked inheritance showed a hemizygous variant in OGT: ChrX(GRCh38): g.71,544,561T>G; NM_181672.2: c.463–6T>G. Segregation analysis revealed that the mutation originates from a de novo mutation in the grandmother of the patient, passed on via the mother, who is the carrier (Fig. 2A).

The thymine at position c.463–6 is highly conserved (phyloP score of 6.93), and in silico splicing analysis of c.463–6T>G predicted an effect on the canonical splice site. RT-PCR in patient fibroblasts showed an additional splice variant for OGT (Fig. 2B). Sanger sequencing showed a deletion of 69 base pairs, corresponding to skipping of exon 4 caused by partial loss of the canonical splice site in patient 2 (Fig. 2B). Skipping of exon 4 could result in a truncated protein with a loss of 23 amino acids (Δ155–177-OGT).

Both mutations in OGT were absent in >60,000 controls (ExAC database) (31). Thus, we have identified two XLID patients that possess two novel mutations in OGT.

OGT XLID mutations lead to an imbalance of O-GlcNAc homeostasis

Given that OGT is a glycosyltransferase, we next investigated the effects of the XLID mutations on global O-GlcNAc levels in lysates from patient-derived fibroblasts by immunoblotting. As controls, we used fibroblasts from an unrelated male neonate (control 1) and a female adult (control 2). Using the RL2 anti-O-GlcNAc antibody, we observed no substantial difference in global O-GlcNAc levels between the controls and patients (Fig. 3, A and B). To verify the specificity of this O-GlcNAc antibody, cell lysates were treated with a bacterial O-GlcNAcase from Clostridium perfringens (CpOGA), leading to a loss of O-GlcNAc signal compared with untreated lysates (Fig. 3A). As an independent control experiment, we applied a recently developed method to detect O-GlcNAcylated proteins, which relies on detection using a tagged inactive bacterial O-GlcNAcase (D298N-CpOGA) (32). In this experiment, no substantial differences were observed in global O-GlcNAc levels in fibroblasts derived from patients versus healthy controls (Fig. 3, C and D).

We next investigated the effects of the OGT mutations on levels of OGT and OGA mRNA and protein. Gene expression levels of OGT and OGA appeared to be unaffected in patient fibroblasts compared with those of the controls (Fig. 4A). Although OGT protein levels appeared reduced in both patients compared with the healthy controls (Fig. 4, B and C), there was considerable variability in OGT protein levels of the healthy controls (Fig. 4, B and C). At the mRNA level, two OGT variants (corresponding to WT and mis-spliced prod-
ucts) were present for patient 2 (Fig. 2B). However, at the protein level, comparative analysis with recombinantly expressed \(\Delta 155-177\)-OGT (Fig. 4F) did not show evidence for presence of a truncated OGT protein, suggesting that \(\Delta 155-177\)-OGT may be unstable/degraded in the context of these patient fibroblasts. There was a reduction in OGA protein levels, although again variability was observed in OGA protein levels of the healthy controls (Fig. 4, D and E). Thus, OGT mutations may lead to reduced functional OGT protein levels in patient-derived fibroblasts, which may be compensated for with down-regulation of OGA protein levels, thereby maintaining global O-GlcNAc levels.
Recombinant mutant OGT is unstable and defective in HCF-1 processing

The absence of the truncated OGT in patient 2 and potential reductions in OGT protein levels in patient-derived fibroblasts suggest that the mutations may compromise OGT stability. To further investigate this, stability of recombinant wild-type/mutant OGT TPR domain was analyzed by thermal denaturation experiments. We observed reduced stability for Δ155−177-OGT (T_m = 51 ± 1°C) and R284P-OGT (T_m = 52 ± 1°C) compared with wild-type OGT TPR domain (T_m = 59 ± 1°C) (Fig. 5A). Given that Δ155−177-OGT was not detected in patient-derived fibroblasts, further biochemical characterization of this mutant was omitted. OGT has over 1000 O-GlcNAcylation substrates; therefore, to explore the effects of the p.Arg284Pro mutation in an environment similar to that of cell physiology, de-O-GlcNAcylated HEK-293 cell lysate was employed as a complex array of substrates. R284P-OGT was not able to restore protein O-GlcNAcylation, to the extent achieved by wild-type OGT (Fig. 5B).

OGT is known to possess a second catalytic activity, promoting autocatalytic cleavage and activation of the transcriptional co-regulator HCF1, itself an XLID gene (33, 34), via an intermediate glycosylation step (13, 35). Thus, we decided to investigate the effects of the p.Arg284Pro substitution on OGT-mediated HCF1 processing. To measure proteolytic activity, we used a GST fusion of a minimal HCF1 fragment (HCF1-rep1)
possessing the first of the PRO repeats (which are the target sites of proteolysis; Fig. 5C). Wild-type OGT was able to hyper-
glycosylate HCF1-rep1, as evidenced by a band shift that was 
reversible by incubation with CpOGA (Fig. 5D). The HCF1-
rep1 band shift observed in the R284P-OGT-catalyzed reaction 
was smaller, suggesting that the mutant OGT was less effective 
than the wild type in glycosylating HCF1-rep1 (Fig. 5D). Fur-
thermore, wild-type OGT was able to induce autoproteolysis, 
whereas the proteolytic activity of R284P-OGT was reduced
(Fig. 5D). When the enzyme concentration was reduced relative 
to substrate levels, proteolytic activity of R284P-OGT was
abrogated, whereas wild-type enzyme still displayed detectable
activity (Fig. 5E). Thus, recombinant R284P-OGT is defective
in HCF1 glycosylation and proteolytic processing.

Discussion

Here, we report two patients with XLID attributable to
hemizygous mutations in OGT: one missense mutation
(p.Arg284Pro) and one splice-site mutation (c.463–6T>G).
We identified potential perturbations in protein O-GlcNAc
homeostasis in both patients and altered HCF1 maturation in
patient 1.

XLID is a genetically and phenotypically heterogeneous
group of disorders, estimated to account for ~10% of all intel-
lectual disability phenotypes in males (36). Mutations causing
XLID have been reported in over 100 genes, and new XLID
genes are still being identified (37). In 2015, Niranjan
et al. (30) published a series of 65 potential XLID genes, by sequencing
the X-exome of 56 XLID families. This was the first publication
in which a genetic variant in OGT (p.Leu254Phe) was found
associated with XLID. During the course of our work, Vaidy-
anathan et al. (38) reported a study on the functional character-
ization of this previously published p.Leu254Phe variant of
OGT (30). In patient-derived lymphoblastoid cells, decreased
OGT levels were accompanied by decreased OGA levels, result-
ing in unaltered O-GlcNAc levels. This is in agreement with our
findings in both patient-derived fibroblast cell lines, indicating
that homeostasis of O-GlcNAc levels through decreasing OGA
levels could be a general principle in cases of OGT deficiency.
However, in contrast to our results, recombinantly expressed
p.Leu254Phe OGT and wild-type OGT showed equal activity
toward O-GlcNAc substrate CK2α and HCF-1 (38). Another
study described a missense mutation in OGT (p.Ala319Thr) in
an XLID family, although this mutant also segregated with a

Figure 5. In vitro analysis of recombinantly expressed mutant OGT forms. A, thermal denaturing curve showing fraction of unfolded wild-type (TPR-WT), R284P (TPR-R284P), and Δ155–177 (TPR-Δ155–177) OGT TPR domains as a function of time. The data were fitted to Boltzmann sigmoidal curve equation. Error bars represent standard error of mean of seven replicates, and the plot is representative of three biological replicates. B, immunoblot showing the relative O-GlcNAcylation activities of wild-type OGT (W) and R284P-OGT (R) against de-O-GlcNAcylated HEK-293 lysate. C, domain organization of human HCF1 compared with its truncated form fused to N-terminal GST tag and C-terminal His tag (HCF1-rep1), which was used as a substrate for OGT proteolytic activity. D, immunoblot showing the relative proteolytic activities of wild-type OGT (W) and R284P-OGT (R) against HCF1-rep1. The samples were treated with an O-GlcNAcase homolog from C. perfringens (CpOGA) either toward the end of the reaction (+) or from the beginning (+ +). Closed circle, unprocessed HCF-
1-rep1; closed square, HCF1-rep1 cleavage product. E, kinetic assay of the proteolytic activity of wild-type OGT and R284P-OGT against HCF1-rep1 plotted as amount of product formed as a function of time. Error bars represent standard error of mean of four replicates. The data were fitted to hyperbolic curve equation.
previously uncharacterized variant of MED12, another gene that is associated with XLID. Evidence for functional loss of OGT for this mutation was not provided (29).

To date, including our findings, four hemizygous mutations in OGT have been identified in patients with XLID (Table 1). The three missense mutations (p.Arg284Pro, p.Leu254Phe, and p.Ala319Thr) and one splice-site mutation (c.463–6T>G) all affect the N-terminal TPR domain (Fig. 2C and supplemental Fig. S1). The TPR motif is characterized by a repeat of 34 amino acids that fold into two antiparallel helices. The TPR domain consists of 13.5 repeats, and the antiparallel helices align to form an elongated superhelix, where the first helix of each TPR repeat (helix A) faces toward the inner surface of the elongated superhelix and their antiparallel pairs (helix B) form the outer surface (11). The missense mutations p.Arg284Pro, p.Leu254Phe, and p.Ala319Thr are located in helix B of the OGT TPR7, TPR8, and TPR9, respectively (Fig. 2D and supplemental Fig. S1). In addition, Ala-319 is one of the conserved hydrophobic amino acid residues of the canonical TPR repeat (39) (supplemental Fig. S1). Each of these missense mutations could disrupt the α-helical secondary structure of the TPR domain, thereby affecting the overall stability of the elongated superhelix and their antiparallel pairs (helix B) form this region.

The splice-site mutation c.463–6T>G is predicted to result in an OGT protein missing 23 amino acids (p.Δ155–177) (Fig. 2B), although we could not confirm the presence of a truncated OGT in patient fibroblasts (Fig. 4F). We propose that this mutation might exert its effect by producing an unstable splice variant and reducing the amount of functional OGT within the cell (Figs. 4B and 5A). Global O-GlcNAc levels in patient-derived fibroblasts were not substantially reduced compared with healthy controls (Fig. 3). Protein levels of OGT and OGA appeared to be reduced in both patients; however, both healthy controls displayed variation in their OGT and OGA levels, complicating the interpretation of these findings. We hypothesize that the decrease in OGT protein levels in these cells might be counteracted by the concomitant reduction of OGA protein levels (Fig. 4, D and E), thereby sustaining basal O-GlcNAc homeostasis. This hypothesis is supported by the finding that chemical inhibition of OGA in different human cell lines leads to a decreased expression of OGT and an increased expression of OGA (18), which indicates an as-yet-unidentified mechanism of regulation to maintain global O-GlcNAc homeostasis. We propose a similar mechanism in our patient-derived fibroblasts.

It is as yet not fully understood how OGT recognizes serines/threonines on specific proteins for O-GlcNAcylation, although there is evidence to suggest that the TPRs play a key role for at least a subset of substrates (40). To assess the in vitro activity of OGT with mutations affecting the TPR domain and the impact on substrate O-GlcNAcylation, we produced recombinant R284P-OGT. Given that Δ155–177-OGT was not detected in patient-derived fibroblasts, further biochemical characterization of this mutant was not undertaken. Activity assays against de-O-GlcNAcylated HEK-293 lysate demonstrated that the p.Arg284Pro mutation led to a global reduction in protein O-GlcNAcylation, as opposed to reducing recognition and O-GlcNAcylation of specific substrates. In a physiological context, this nonspecific reduction in OGT activity may be rescued by down-regulation of OGA levels, which was observed to some extent in the fibroblasts from both patients. However, the p.Arg284Pro mutation is located on the surface of the TPRs (11), a region that has been shown to play a role in the recognition of specific substrates (40). It is therefore possible that this mutation could affect modification of specific substrates, in addition to causing the observed global reduction in substrate O-GlcNAcylation, and this possibility is neither excluded nor confirmed by our current data. O-GlcNAcylation activity of R284P-OGT against HCF1-rep1 was reduced, as judged by the poly-O-GlcNAcylated induced shift in HCF1-rep1 molecular weight versus wild-type OGT (Fig. 5D).

In addition to being abundantly O-GlcNAcylated (15), HCF1 is also proteolytically processed by OGT (12, 35). The p.Arg284Pro mutation appears to alter HCF1-rep1 proteolysis in vitro (Fig. 5, D and E). The TPR domain is critical for proteolytic maturation of HCF1 (13), and this could explain why R284P-OGT shows decreased proteolytic activity toward HCF1 in vitro. Interestingly, HCFC1, the gene that codes for HCF1, is located on the X-chromosome, and it is reported as an XLID gene (41). This hints at possible overlap in downstream disease mechanisms causing intellectual disability in patients bearing OGT or HCFC1 mutations.

Precisely how alterations in protein O-GlcNAcylation and HCF1 cleavage lead to intellectual disability is not yet clear. OGT is expressed in a broad range of tissues, with a remarkably high expression in brain (9, 10). O-GlcNAc-modified proteins were detected in nerve terminals, and abundant OGT activity was detected in nerve synaptosomes (42), indicating that O-GlcNAc could play a role in neuronal function and neurodegenerative disease. It has been suggested that regulation of the proteasome complex by O-GlcNAcylation in the brain could contribute to apoptosis of hippocampal cells and thus neurodegeneration (43). In addition, aberrant O-GlcNAcylated levels have been linked to neurodegenerative diseases like Alzheimer’s disease (28). Future work analyzing the effect of XLID mutations on OGT function in a more phenotype-relevant model, such as a neuronal cell line or an animal model where the effect of the mutations on different tissues, including the brain, can be investigated, would be important. The present study utilized fibroblasts from two unrelated individuals as controls, and we noted substantial variability in OGA and OGT protein levels in these cells, complicating the interpretation of our data. To minimize the effect of variability introduced by the differing genetic background between individual patients and controls, it would be of use to introduce these mutations into cell lines with an identical genetic background. Our study provides the first detailed clinical characterization of patients with XLID attributable to mutations in OGT and furthermore proposes a potential molecular explanation for how these mutations may impact on OGT dual function of O-GlcNAcylation and HCF1 proteolysis.
**OGT variants in XLID**

**Experimental procedures**

**Patient consent**

The study was approved by the Institutional Review Board at Radboud University Medical Centre and Utrecht University Medical Centre. All participating affected individuals or their legal representatives gave informed consent for exome sequencing and to publication of this study. Fibroblasts of the patients were obtained for diagnostics of inborn errors of metabolism and used after informed consent from parents and/or treating physicians, in accordance with the Declaration of Helsinki. For use of facial images, written informed consent was obtained.

**Whole exome sequencing**

For patient 1, whole exome sequencing was performed in a trio diagnostic approach as described before (44). Exome capture was performed with the SureSelect Human All Exon v4 enrichment kit (Agilent, Santa Clara, CA). Whole exome sequencing was performed on the Illumina HiSeq platform (San Diego, CA). Data were analyzed with the BWA (read alignment) and GATK (variant calling) software packages. Variants were annotated using an in-house developed pipeline. Prioritization of variants was done by an in-house designed “variant interface” and manual curation.

Exomes of patient 2 and parents were enriched using the SureSelect XT Human All Exon v5 kit (Agilent) and sequenced in rapid run mode on the HiSeq2500 sequencing system (Illumina). Variants were called using the GATK haplotype caller (v2.7-1). Reads were aligned to hg19 using BWA (BWA-MEM v0.7.5a), and variants were called using the GATK (variant calling) software packages. Variants were annotated using an in-house developed pipeline. Prioritization of variants was done by an in-house designed “variant interface” and manual curation.

Exomes of patient 2 and parents were enriched using the SureSelect XT Human All Exon v5 kit (Agilent) and sequenced in rapid run mode on the HiSeq2500 sequencing system (Illumina) at a mean target depth of 100×. The target is defined as all coding exons of UCSC and Ensembl ± 20-bp intron flanks. At this depth >95% of the target is covered at least 15 times. Reads were aligned to hg19 using BWA (BWA-MEM v0.7.5a), and variants were called using the GATK haplotype caller (v2.7-2). Detected variants were annotated, filtered, and prioritized using the Bench NGS Lab platform (Cartagenia; Agilent). The OGT gene sequences of the grandmother and great-grandmother were analyzed to study the origin of the mutation. All potentially causative variants were confirmed by Sanger sequencing.

**Cell culture**

Skin fibroblasts derived from patients and healthy donors were cultured at 37 °C under 5.0% CO2 in M199 medium (PAN Biotech, Aidenbach, Germany) or Dulbecco's modified Eagle's medium (Thermo Fisher Scientific) containing 2 mM L-glutamine and 5% FCS. Media were supplemented with 10% fetal calf serum (Labtech, Uckfield, UK) and 1% penicillin-streptomycin (Thermo Fisher Scientific).

**RNA extraction, cDNA synthesis, and quantitative RT-PCR**

Total cell RNA was extracted from skin fibroblasts using TRIzol reagent (Thermo Fisher Scientific). cDNA was synthesized with a RevertAid First Strand cDNA synthesis kit (Thermo Fisher Scientific) using random hexamer primers. For quantification of OGT and OGA expression levels, cDNA from skin fibroblasts was mixed with GoTaq® qPCR Master Mix (Promega) and both forward and reverse primers. Tubulin expression was used as an internal control. All qPCR experiments were conducted at Bio-Rad CFX96 real-time system following the GoTaq® qPCR Master Mix manual. Specificity of the reactions was verified with melt curve analysis. The primers used are listed in supplementary Table S1. For analysis of OGT mRNA splicing in skin fibroblasts, cDNA and primers were mixed with AmpliTaq Gold® 360 Master Mix (Thermo Fisher Scientific). RT-PCR was conducted according to standard conditions and PCR products were purified using a QIAquick gel extraction kit (Qiagen). Purified PCR products were used for sequencing analysis.

**Biochemical characterization of patient-derived fibroblasts**

Patient-derived fibroblasts were grown on 15-cm plates and were washed twice with ice-cold PBS buffer (Thermo Fisher Scientific) prior to lysis. The cells were lysed by addition of lysis buffer (50 mM Tris-HCl, pH 7.4, 1 mM EGTA, 1 mM EDTA, 1% Triton X-100, 1 mM Na2VO4, 50 mM NaF, 5 mM pyrophosphate, 0.27 M sucrose) supplemented with 1 µM GlcNAcstatin-G, 1 µM β-mercaptoethanol, and protease inhibitor mixture (1 mM benzamidine, 0.2 mM PMSF, 5 mM leupeptin). The lysate was transferred into an Eppendorf and clarified by centrifugation at 4 °C (12000 × g for 15 min). Lysate proteins were resolved by SDS-PAGE (4–12% acrylamide; Thermo Fisher Scientific) and transferred onto nitrocellulose membranes (GE Healthcare). Analysis of O-GlcNAcylated fibroblast proteins by the far Western method was performed as described previously (32). Briefly, soluble cell lysates were prepared, resolved by SDS-PAGE (3–8% Tris acetate; Thermo Fisher Scientific) and transferred onto nitrocellulose membranes (GE Healthcare) as described above, but with lysis buffer lacking GlcNAcstatin-G.

**Protein expression and purification**

Full-length wild-type OGT and mutants (R284P and Δ155–177) were expressed in *Escherichia coli* as N-terminal His fusion proteins as described previously (45). The wild-type OGT TPR domain (residues 26 – 420) and mutants (TPR-R284P and TPR-Δ155–177) were expressed and purified as described previously (11). HCF1-rep1 (residues 867–1071) was expressed in *E. coli* as a non-cleavable N-terminal GST and C-terminal His fusion protein. Transformed BL21 (DE3) cells were grown, induced for expression, lysed, and clarified as described previously (45). Clarified lysate was incubated with 1 ml/liter of culture of glutathione-Sepharose 4B resin (GE Healthcare) for 2 h at 4 °C. The resin was thoroughly washed with base buffer (0.1 M Tris-HCl, pH 7.5, 150 mM NaCl, 0.5 mM TCEP) and eluted using base buffer supplemented with 0.5 M glutathione. Eluted protein was dialyzed overnight at 4 °C in buffer A (0.1 M Tris-HCl, pH 7.5, 25 mM NaCl). Dialyzed protein was passed through 5 ml of HiTrap Q-Sepharose FF anion exchange resin (GE Healthcare), collecting the flow-through, which was then concentrated and passed through a 300-mL SuperdexTM 200 column (GE Healthcare) pre-equilibrated with base buffer. The peak fractions were concentrated to 10 mg/ml, mixed 1:1 with 50% glycerol, snap-frozen, and stored at −80 °C until use.

**Molecular cloning**

The fragment of HCF1-rep1 (residues 867–1071) was obtained from MVP human total brain RNA from Agilent using
the Takara PrimeScript high-fidelity RT-PCR kit. The reverse primer coded for the C-terminal addition of a His<sub>6</sub> tag. The PCR product was cloned as a BamHI-NotI fragment into a mutated version of pGEX6P1, which lacks the PreScission protease site. The full-length codon optimized OGT was obtained from GenScript and subcloned as a BamHI-NotI fragment into pHEX6P1 (modified version of pGEX6P1 which contains a His<sub>6</sub> tag instead of GST). The R284P and the Δ155–177 mutations were introduced using a method similar to the QuikChange site-directed mutagenesis kit (Agilent) but using KOD polymerase sequencing.

Directed mutagenesis kit (Agilent) but using KOD polymerase introduced using a method similar to the QuikChange site-directed mutagenesis kit (Agilent) but using KOD polymerase introduced using a method similar to the QuikChange site-directed mutagenesis kit (Agilent) but using KOD polymerase introduced using a method similar to the QuikChange site-directed mutagenesis kit (Agilent) but using KOD polymerase introduced using a method similar to the QuikChange site-directed mutagenesis kit (Agilent) but using KOD polymerase introduced using a method similar to the QuikChange site-directed mutagenesis kit (Agilent) but using KOD polymerase.

Thermal denaturing assay

Thermal denaturing experiments were performed in triplicate, using the OGT TPR domain constructs. 50-μl solutions contained 5 μM protein and 1.1x SYPRO® Orange dye (Sigma) in base buffer of 25 mM HEPES-NaOH, pH 7.5, 150 mM NaCl, and 0.5 mM TCEP. A Bio-Rad (CFX Connect™ real-time system was used to measure fluorescence (λ<sub>ex</sub> = 530 nm, λ<sub>em</sub> = 560 nm) while temperature was increased from 25 to 95 °C at 1/°C/min increments. The data were transformed, normalized, and fitted to a Boltzmann sigmoidal curve using GraphPad Prism 5.0.

In vitro O-GlcNAcylation assay

O-GlcNAcylation assays were performed in triplicate on de-O-GlcNAcylated HEK-293 lysates proteins. HEK-293 cell lysates were prepared as described for patient-derived fibroblasts, but with the lysis buffer lacking GlcNAcstatin-G. HEK-293 lysate was treated with 120 μg of CpOGA per mg of lysate protein and incubated for 90 min at 37 °C. CpOGA was neutralized by the addition of 250 μM GlcNAcstatin-G. Reactions were supplemented with wild-type OGT or R284P-OGT (0.2 μM) in the presence of 2 mM UDP-GlcNAc and incubated for an additional 2 h at 37 °C. Proteins were resolved by SDS-PAGE (3–8% acrylamide; Thermo Fisher Scientific) and transferred onto nitrocellulose membrane (GE Healthcare).

In vitro proteolytic assay

Proteolytic assays were performed using HCF1-rep1 (residues 867–1071) with GST and His tags at the N and C termini, respectively. For qualitative measurement of proteolytic activity, HCF1-rep1 (2.5 μM), preincubated or not with CpOGA (5 μM), was combined with wild-type OGT or R284P-OGT (1 μM) in the presence of 1 mM UDP-GlcNAc. Reaction mixtures were incubated at 37 °C for 8 h with gentle agitation, followed by optional addition of CpOGA. Mixtures were incubated at 37 °C for an additional 1 h, before stopping the reaction by addition of LDS loading buffer (4×) (Thermo Fisher Scientific). Proteins were resolved by SDS-PAGE (4–12% acrylamide; Life Technologies) and transferred onto nitrocellulose membranes (GE Healthcare). Initial velocity for OGT-catalyzed proteolysis of HCF1-rep1 was measured in triplicate. HCF1-rep1 (1.5 μM) was combined with wild-type OGT or R284P-OGT (0.15 μM) in the presence of 15 μM UDP-GlcNAc. Reaction mixtures were incubated at 37 °C with gentle agitation, whereas aliquots were taken out at the designated time points (0, 0.5, 1, 2, 4, 8, 16, and 32 h) and stopped by mixing with LDS loading buffer (4×) (Thermo Fisher Scientific). The initial volume of the reaction was 90 μl, and 2.5-μl aliquots were loaded in each lane. Proteins were resolved by SDS-PAGE (10% acrylamide) and visualized by Coomassie staining. The amount of product formed was quantified using a LI-COR Odyssey scanner and associated quantification software. The data were background-corrected and plotted using GraphPad Prism 5.0.

Immunoblots

Membranes were probed with following primary antibodies: O-GlcNAc (RL2; Abcam, Cambridge, UK), actin (Sigma), OGT (H300; Santa Cruz), OGA (14711–1-AP; Proteintech, Rosemont, IL), tubulin (Cell Signaling), HSP-90 (Cell Signaling), and GST-S902 (Division of Signal Transduction Therapy, University of Dundee). In case of the far Western method, membranes were probed with 10 μg/ml Halo-tagged CpOGA and incubated with halo primary antibody (Promega). Subsequently all membranes were incubated with IR680- or IR800-labeled secondary antibodies (LI-COR) and analyzed using a LI-COR Odyssey scanner and associated quantification software. All primary and secondary antibodies were used in 1:5,000 and 1:10,000 dilutions, respectively.

Data analysis

Scatter plots indicate ratios of any given normalized signal as averaged from three biological replicates, with the error bars representing standard deviation. Variations in data were calculated using Mann–Whitney U test using GraphPad Prism 5.0. Power calculations were performed using G*Power 3.1.

Author contributions—A. P. W. and M. G. designed, performed, and analyzed the experiments and wrote the paper. M. J. E. K., J. C. G., and D. M. F. V. A. conceived and coordinated the study and revised the paper. M. J. E. K., J. C. G., A. T. F., and R. P. analyzed the experiments and wrote the paper. M. J. E. K., J. C. G., and D. M. F. V. A. conceived and coordinated the study and revised the paper. All authors contributed to writing of the paper, reviewed the results, and approved the final version of the manuscript.

Acknowledgments—We thank both patients and their families for participation in this study. In addition, we are grateful for protein production team at Division of Signal Transduction Therapy at the University of Dundee for production of the anti-GST antibody.

References

1. Jackson, S. P., and Tjian, R. (1988) O-Glycosylation of eukaryotic transcription factors: implications for mechanisms of transcriptional regulation. Cell 55, 125–133
2. Hanover, J. A., Cohen, C. K., Willingham, M. C., and Park, M. K. (1987) O-Linked N-acetylglucosamine is attached to proteins of the nuclear pore: evidence for cytoplasmic and nucleoplasmic glycoproteins. J. Biol. Chem. 262, 9887–9894
3. Yang, W. H., Kim, J. E., Nam, H. W., Ju, J. W., Kim, H. S., Kim, Y. S., and Cho, J. W. (2006) Modification of p53 with N-acetylglucosamine regulates p53 activity and stability. Nat. Cell Biol. 8, 1074–1083
4. Sayat, R., Leber, B., Grubac, V., Wiltshire, L., and Persad, S. (2008) O-GlcNAc-glycosylation of β-catenin regulates its nuclear localization and transcriptional activity. Exp. Cell Res. 314, 2774–2787
OGT variants in XLID

5. Torres, C. R., and Hart, G. W. (1984) Topography and polypeptide distribution of terminal N-acetylglucosamine residues on the surfaces of intact lymphocytes: evidence for O-linked GlnNac. J. Biol. Chem. 259, 3308–3317

6. Haltiwanger, R. S., Blomberg, M. A., and Hart, G. W. (1992) Glycosylation of nuclear and cytoplasmic proteins: purification and characterization of a uridine diphospho-N-acetylglucosamine-polypeptide β-N-acetylglucosaminyltransferase. J. Biol. Chem. 267, 9005–9013

7. Ruan, H. B., Singh, J. P., Li, M. D., Wu, J., and Yang, X. (2013) Cracking the O-GlcNac code in metabolism. Trends Endocrinol. Metab. 24, 301–309

8. Hart, G. W., Housley, M. P., and Slawson, C. (2007) Cycling of O-linked β-N-acetylglucosamine on nucleocytoplasmic proteins. Nature 446, 1017–1022

9. Kreppel, L. K., Blomberg, M. A., and Hart, G. W. (1997) Dynamic glycosylation of nuclear and cytosolic proteins: cloning and characterization of a unique O-GlcNac transferase with multiple tetratricopeptide repeats. J. Biol. Chem. 272, 9308–9315

10. Lubas, W. A., Frank, D. W., Krause, M., and Hanover, J. A. (1997) O-Linked GlcNac transferase is a preserved nucleocytoplasmic protein containing tetratricopeptide repeats. J. Biol. Chem. 272, 9316–9324

11. Jinek, M., Rehwinkel, J., Lazarus, B. D., Izaurralde, E., Hanover, J. A., and Conti, E. (2004) The superhelical TPR-repeat domain of O-linked GlcNac transferase exhibits structural similarities to importin α. Nat. Struct. Mol. Biol. 11, 1001–1007

12. Capotosti, F., Guernier, S., Lammers, F., Waridel, P., Cai, Y., Jin, J., Conaway, J. W., Conaway, R. C., and Herr, W. (2011) O-GlcNac transferase catalyzes site-specific posttranslational modification of HCF-1. Cell 144, 376–388

13. Lazarus, M. B., Jiang, J., Kapuria, V., Bhuiyan, S. M., Janetzko, J., Zandberg, W. F., Vaccaro, D. I., Herr, W., and Walker, S. (2013) O-GlcNac transferase is essential for CREB-mediated gene expression and memory formation. Nat. Chem. Biol. 8, 253–261

14. Su, C., and Schwartz, T. L. (2017) O-GlcNac transferase is essential for sensory neuron survival and maintenance. J. Neurosci. 37, 2125–2136

15. Taylor, E. W., Wang, K., Nelson, A. R., Bredemann, T. M., Fraser, K. B., Clinton, S. M., Puckett, R., Marchase, R. B., Chatham, J. C., and McMahan, L. L. (2014) O-GlcNacylation of AMPA receptor GluA2 is associated with a novel form of long-term depression at hippocampal synapses. J. Neurosci. 34, 10–21

16. Lagerlöf, O., Hart, G. W., and Huganir, R. L. (2017) O-GlcNac transferase regulates excitatory synapse maturity. Proc. Natl. Acad. Sci. U.S.A. 114, 1684–1689

17. Zheng, B. W., Yang, L., Dai, X. L., Jiang, Z. F., and Huang, H. C. (2016) Roles of O-GlcNac transferase on amyloid-β precursor protein processing, tau phosphorylation, and hippocampal synapses dysfunction in Alzheimer’s disease. Neurobiol. Dis. 38, 177–186

18. Bouazzi, H., Lesca, G., Trujillo, C., Alwasiyah, M. K., and Munnich, A. (2015) Nonsyndromic X-linked intellectual deficiency in three brothers with a novel MED12 missense mutation [c.5922G>T] (p.Glu1974His). Clin. Case Rep. 3, 604–609

19. Niranjhan, T. S., Skinner, C., May, M., Turner, T., Rose, R., Stevenson, R., Schwartz, C. E., and Wang, T. (2015) Affected kindred analysis of human X chromosome exomes to identify novel X-linked intellectual disability gene. PLoS One 10, e0116454

20. Lek, M., Karczewski, J. K., Minikel, E. V., Samocha, K. E., Banks, E., Fennell, T., O’Donnell-Luria, A. H., Ware, J. S., Hill, A. J., Cummings, B. B., Tukiainen, T., Birnbaum, D. P., Kosmicki, J. A., Duncan, L. E., Estrada, K., et al. (2016) Analysis of protein-coding genetic variation in 60,706 humans. Nature 536, 285–291

21. Mariappa, D., Selvan, B., Borodkin, S. V., Alonso, J., Ferenbach, A. T., Shepherd, C., Navratilova, I. H., and van Aalten, D. M. (2015) A mutant O-GlcNacase as a probe to reveal global dynamics of protein O-GlcNacylation during Drosophila embryonic development. Biochem. J. 470, 255–262

22. Huang, L., Jolly, L. A., Willis-Owen, S., Gardner, A., Kumar, R., Douglas, E., Shoubridge, C., Wieczorek, D., Tzschach, A., Cohen, M., Hackett, A., Field, M., Froyen, G., Hu, H., Haas, S. A., et al. (2012) A noncoding, regulatory mutation implicates HCF1 in nonsyndromic intellectual disability. Am. J. Hum. Genet. 91, 694–702

23. Yu, H. C., Sloan, J. L., Scherer, G., Brehm, A., Quintana, A. M., Achilli, N. P., Manoli, L., Coughlin, C. R., 2nd, Geier, E. A., Schneck, U., Watkins, D., Suormala, T., Van Hove, J. L., Fowler, B., Baumgartner, M. R., et al. (2013) An X-linked colobalamin disorder caused by mutations in transcriptional coregulator HCF1. Am. J. Hum. Genet. 93, 506–514

24. Janetzko, J., Trauger, S. A., Lazarus, M. B., and Walker, S. (2016) How the glycosyltransferase OGT catalyzes amide bond cleavage. Nat. Chem. Biol. 12, 899–901

25. Ropers, H. H., and Hamel, B. C. (2005) X-linked mental retardation. Nat. Rev. Genet. 6, 46–57

26. Hu, H., Haas, S. A., Chelly, J., Van Esch, H., Raynaud, M., de Rouver, A. P., Weinert, S., Froyen, G., Frints, S. G., Laumonnier, F., Zemojtel, T., Love, M. I., Richard, H., Emde, A. K., Bienek, M., et al. (2016) X-exome sequencing of 405 unresolved families identifies seven novel intellectual disability genes. Mol. Psychiatry 21, 133–148

27. Vaidyanathan, K., Niranjhan, T., Selvan, N., Teo, C. F., May, M., Patel, S., Weatherly, B., Skinner, C., Opitz, J., Carej, J., Viskokchil, D., Geer, J., Shaw, M., Peng, Y., Alexov, E., et al. (2017) Identification and characterization of a missense mutation in the O-GlcNac transferase gene that segregates with X-linked intellectual disability. J. Biol. Chem. 292, 8948–8963

28. Sikorski, R. S., Boguski, M. S., Goebel, M., and Hieter, P. (1990) A repeating amino acid motif in CDC23 defines a family of proteins and a new relationship among genes required for mitosis and RNA synthesis. Cell 60, 307–317

29. Iyer, S. P., and Hart, G. W. (2003) Roles of the tetratricopeptide repeat domain in O-GlcNac transferase targeting and protein substrate specificity. J. Biol. Chem. 278, 24608–24616

30. Jolly, L. A., Nguyen, L. S., Domingo, D., Sun, Y., Barry, S., Hancarova, M., Plevova, P., Vlcekova, M., Havlovickova, M., Kalscheuer, V. M., Graziano, C., et al. (2017) Identification and characterization of a mis-
Pippucci, T., Bonora, E., Sedlacek, Z., and Gecz, J. (2015) HCFC1 loss-of-function mutations disrupt neuronal and neural progenitor cells of the developing brain. *Hum. Mol. Genet.* **24**, 3335–3347

42. Cole, R. N., and Hart, G. W. (2001) Cytosolic O-glycosylation is abundant in nerve terminals. *J. Neurochem.* **79**, 1080–1089

43. Liu, K., Paterson, A. J., Zhang, F., McAndrew, J., Fukuchi, K., Wyss, J. M., Peng, L., Hu, Y., and Kudlow, J. E. (2004) Accumulation of protein O-GlcNAc modification inhibits proteasomes in the brain and coincides with neuronal apoptosis in brain areas with high O-GlcNAc metabolism. *J. Neurochem.* **89**, 1044–1055

44. Neveling, K., Feenstra, I., Gilissen, C., Hoefsloot, L. H., Kamsteeg, E. J., Mensenkamp, A. R., Rodenburg, R. J., Yntema, H. G., Spruijt, L., Vermeer, S., Rinne, T., van Gassen, K. L., Bodmer, D., Lugtenberg, D., de Reuver, R., et al. (2013) A post-hoc comparison of the utility of sanger sequencing and exome sequencing for the diagnosis of heterogeneous diseases. *Hum. Mutat.* **34**, 1721–1726

45. Pathak, S., Alonso, J., Schimpl, M., Rafie, K., Blair, D. E., Borodkin, V. S., Schüttekopf, A. W., Albarbarawi, O., and van Aalten, D. M. (2015) The active site of O-GlcNAc transferase imposes constraints on substrate sequence. *Nat. Struct. Mol. Biol.* **22**, 744–750
Mutations in N-acetylglucosamine (O-GlcNAc) transferase in patients with X-linked intellectual disability

Anke P. Willems, Mehmet Gundogdu, Marlies J.E. Kempers, Jacques C. Giltay, Rolph Pfundt, Martin Elferink, Bettina F. Loza, Joris Fuijkschot, Andrew T. Ferenbach, Koen L.I. van Gassen, Daan M.F. van Aalten and Dirk J. Lefeber

This supplemental material section includes:

Supplemental Figure S1
Supplemental Table S1
**Fig. S1. Sequence alignment of TPRs**

A. Sequence alignment of the 13.5 human OGT TPRs. Blue and red cylinders annotate the first helix (helix A) and its antiparallel pair (helix B) within each TPR motif, respectively. The alignment is coloured in grayscale based on sequence conservation. The regions affected by all four XLID mutations, Δ155-177, Leu254Phe, Arg284Pro and Ala319Thr, are highlighted in pink colour.

B. Sequence alignment of OGT exon 7 from various metazoan species. Blue and red cylinders annotate the first helix (helix A) and its antiparallel pair (helix B) within each TPR motif, respectively. The alignment is coloured in grayscale based on sequence conservation. The XLID mutations Leu254Phe and Arg284Pro are highlighted in pink colour.

C. Sequence alignment of OGT exon 8 from various metazoan species. Blue and red cylinders annotate the first helix (helix A) and its antiparallel pair (helix B) within each TPR motif, respectively. The alignment is coloured in grayscale based on sequence conservation. The XLID mutation Ala319Thr is highlighted in pink colour.
| Analysis of mRNA expression by qPCR |  |
|----------------------------------|---|
| OGT (Fw) | 5’- GCCAGCTCCTATTACGAGGCAA-3’ |
| OGT (Rv) | 5’- CCATGAAAAGCAGACCACCT-3’ |
| OGA (Fw) | 5’- TGGAGCTAAGGGCTGTTG-3’ |
| OGA (Rv) | 5’- CGAACAGTTGTCCTGTTGAC-3’ |
| Tubulin (Fw) | 5’- CAATGAAGGCCAGGTGGC-3’ |
| Tubulin (Rv) | 5’- GTTGTACACGCCCAGCTG-3’ |

| Analysis of OGT mRNA splicing |  |
|------------------------------|---|
| Fw | 5’- GCATTTGCGTCTCAAACCTGA-3’ |
| Rv | 5’- AAGCCAAATTTCCCCTTGTC-3’ |

| Molecular cloning |  |
|------------------|---|
| hOGT_R284P_fwd | GCTATTGATACCTATCGTCgGCGATTGAGACTGCAGCCG |
| hOGT_R284P_rev | CGGCTGCAGTTCATCGCgGACGATAGGTATCAATAGC |
| hOGT_delta_155_177_fwd | gtctgtgcttgctgcttaacccgggcctgctgctgctaacctgccagggact |
| hOGT_delta_155_177_rev | ggtttcaactccttcagg taacagggctgtactgcacgceagac |
| HCF_fwd | aaaaGGATCCcagccgctaacagcttcgttcg |
| HCF_rev | aaaaCGGGCGCttaGTGGGTGATGATGGTGGATGcagagcctctgcttg |

**Table S1**: Overview of used primers