UDP-N-acetylglucosamine:α-6-D-mannoside β-1,2-*N*-acetylglucosaminyltransferase II (GnT II, EC 2.4.1.143) is a Golgi enzyme catalyzing an essential step in the conversion of high mannose to complex *N*-glycans. A 1.2 kb probe from a rat liver cDNA encoding GnT II (D'Agostaro *et al.*, this volume) was used to screen a human genomic DNA library in λEMBL3 prepared from human leukocyte DNA partially digested with Sau3A (Clontech). Several positive clones were obtained of which four were plaque purified. Restriction endonuclease digests with SstI, EcoRI and XbaI, followed by Southern blot analysis with the 1.2 kb rat probe, gave a different pattern for each of the four phage clones. Hybridizing fragments (3.0 and 3.5 kb) were isolated from two of the phage clones (HG30 and HG36, respectively) and subcloned into pBlueScript (pHG30 and pHG36). Sequencing revealed that the inserts in pHG30 and pHG36 are overlapping clones containing 5.5 kb of genomic DNA. pHG30 contains a 1341 bp open reading frame encoding a 447 amino acid protein, 250 bp of GC-rich 5′-upstream sequence and 1.4 kb of 3′-downstream sequence. pHG36 encodes 2.75 kb of 5′-upstream sequence and 750 bp of the 5′-end of the open reading frame. The protein sequence showed the domain structure typical of all previously cloned glycosyltransferases, i.e., a short nine amino acid *N*-terminal cytoplasmic domain, a 22 amino acid hydrophobic non-cleavable signal-anchor transmembrane domain and a 416 amino acid C-terminal catalytic domain. Northern analyses of several human lymphocyte lines showed a message size at 2.6 kb. There is no sequence homology to any previously cloned glycosyltransferase including human β-1,2-*N*-acetylglucosaminyltransferase I (GnT I) which has 445 amino acids with a 416 amino acid catalytic domain. The entire coding regions of human and mouse GnT I and of human and rat GnT II are on single exons. The human GnT I gene (MGAT1) has been localized to chromosome 5q35 whereas the human GnT II gene (MGAT2) is on chromosome 14q21; localizations were done by hybridization and PCR analyses of genomic DNA from hybrid cell lines and by fluorescent *in situ* hybridization (FISH) using genomic DNA probes. There is a 90% identity between the amino acid sequences of the catalytic domains of human and rat GnT II (see Petrarca *et al.*, this volume, for data on rat GnT II).

S2.3

Cloning and Identification of cDNAS Encoding Human β1→6 *N*-Acetylglucosaminyltransferases

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Chinese hamster ovary (CHO) cells, stably expressing both the polyoma large T antigen and human leukosialin, were used for the transient expression cloning of cDNAs encoding human β1→6 *N*-acetylglucosaminyltransferases. Using the monoclonal antibody T305 for selection, a cDNA clone encoding core 2 β1→6 *N*-acetylglucosaminyltransferase (C2GnT) was isolated from a HL-60 cDNA library. Alternatively, using anti-I antibodies for selection, cDNA encoding the I β1→6 *N*-acetylglucosaminyltransferase (IGnT) was isolated from a PA1 cDNA library. CHO cells stably transfected with these cDNAs express core two branchings in *O*-glycans or I blood group antigens as evidenced by immunofluorescence and structural analysis of oligosaccharides synthesized. Both cDNA sequences predict proteins with type II membrane topology, as has been found for all other cloned glycosyltransferases to date. Upon comparison of the two primary amino acid and nucleotide sequences a limited but clear homology was detected. Moreover, the genes encoding C2GnT and IGnT were found to be located at the same locus on chromosome 9, band q21, suggesting that they belong to a β1→6 *N*-acetylglucosaminyltransferase gene family.

Preliminary studies, using PCR analysis of HL-60 genomic DNA, suggested that the coding sequences for both genes are located within one exon. In addition, Southern blot cross-hybridization studies on HL-60 genomic DNA indicated the presence of at least one other glycosyltransferase related to C2GnT or IGnT. A human placental genomic DNA library was thus screened by C2GnT- and IGnT-specific sequences. Five out of eight (cross-)hybridizing genomic clones contained no specific sequences for C2GnT or IGnT, indicating that they are related to C2GnT or IGnT but differ from those genes already isolated. DNA sequencing and characterization of these newly isolated clones is currently in progress, and will be reported.

S2.4

N-Acetylglucosaminyltransferase III: Purification, cDNA Cloning and Chromosomal Mapping

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N-Acetylglucosaminyltransferase III (GnT-III) has been purified from rat kidney by fractionation procedures involving affinity chromatography on UDP-hexanolamine Sepharose and substrate-conjugated Sepharose. The purified enzyme was digested with trypsin, and the amino acid sequences of four peptides were determined. Screening for the cDNA for GnT-III was carried out by plaque hybridization using a rat kidney cDNA library and a polymerase chain reaction product as the probe. There was no sequence homology to other previously cloned glycosyltransferases, but the enzyme appears to be a type II transmembrane protein like other glycosyltransferases. Rat kidney GnT-III contains 536 amino acids and three putative *N*-glycosylation sites. The enzyme has proline rich regions and a sequence homologous to integrin β-4. Human GnT-III cDNA has been also cloned using the rat cDNA as probe. GnT-III was found to be localized at chromosome 22.

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S2.5

Cloning of Novel Genes Related to the Sialyltransferase Gene Family

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Sialyltransferases are a family of glycosyltransferases which catalyze the attachment of sialic acid to various oligosaccharides. The cDNA sequences for three sialyltransferases have been cloned revealing a dispersed conserved motif, the sialylmotif, in the catalytic domain of these enzymes[1-3]. Degenerate PCR using primers based on the sequences