Reduction of UDP-N-acetylglucosamine 2-Epimerase/N-Acetylmannosamine Kinase Activity and Sialylation in Distal Myopathy with Rimmed Vacuoles*

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Distal myopathy with rimmed vacuoles is an autosomal recessive muscle disease with preferential involvement of the tibialis anterior that spares the quadriceps muscles in young adulthood. In a Japanese patient with distal myopathy with rimmed vacuoles, we identified pathogenic mutations in the gene encoding the bifunctional enzyme UDP-GlcNAc 2-epimerase/ManNAc kinase, which catalyzes the initial two steps in the biosynthesis of sialic acid. In this study, we demonstrated the relationship between the genetic mutations and enzymatic activities using an in vitro expression assay system. Furthermore, we also showed that the levels of sialic acid in muscle and primary cultured cells from DMRV patients were reduced to 60–75% of control. The reactivities to lectins were also variable in some myofibers, suggesting that hyposialylation and abnormal glycosylation in muscles may contribute to the focal accumulations of autophagic vacuoles, amyloid deposits, or both in patient muscle tissue. The addition of ManNAc and NeuAc to primary cultured cells normalized sialylation levels, thus demonstrating the therapeutic potential of these compounds for this disease.

Hereditary inclusion body myopathy (HIBM) is an autosomal recessive disorder that presents with adult-onset slowly progressive distal and proximal weakness and has characteristic pathological features in muscle tissue, including rimmed vacuoles and filamentous inclusions, that are similar to those seen in DMRV (4). Gene loci of both diseases have been mapped to chromosome 9 (5, 6). HIBM is caused by mutations in the UDP-GlcNAc 2-epimerase/ManNAc kinase gene (GNE gene) (7). Previously we identified homozygous and compound heterozygous mutations in the GNE gene in 27 DMRV patients (8), demonstrating that the two diseases are allelic.

UDP-GlcNAc 2-epimerase/ManNAc kinase is a dual functional enzyme catalyzing two initial steps in the biosynthesis of sialic acid (9). This enzyme catalyzes the conversions of UDP-GlcNAc to ManNAc and ManNAc to ManNAc 6-phosphate. Despite the identification of the GNE gene mutations, we still do not fully understand how these mutations contribute to the pathophysiology in DMRV/HIBM. Several questions remain unanswered. 1) What is the status of sialylation activity in the patients with GNE mutations? One would expect sialylation to be impaired but not completely absent in DMRV/HIBM patients, because sialic acid is essential for embryonic development (10). In fact, homozygous null mutations have never been identified in patients (8, 11). 2) Why are symptoms restricted to the skeletal muscles? GNE transcripts are expressed in various tissues and are especially predominant in the liver (12). 3) Why do mutant proteins not complement each other in patients who have heterozygous mutations in each of the two domains? The two domains in GNE protein have been reported to catalyze the enzymatic reactions separately and independently (13). To address these questions, we studied the relationships between mutations and enzymatic activity using in vitro expression and enzymatic assay systems. We also determined the levels of sialylation in sera, muscles, and primary cultured cells from DMRV patients and normal individuals.

**EXPERIMENTAL PROCEDURES**

**Mutation and Sialic Acid Analyses of Patients**—All of the patients were Japanese. The patients were diagnosed as having DMRV based on both clinical features and muscle pathology. Numbering of the patients followed the protocol presented in our previous report (8). Gene analyses of DMRV patients were performed as described previously (8). Primary fibroblasts were obtained from patient 5 as reported previously. Sialic acid contents in sera were measured with a SIALIZYME-550 kit (Fujirebio, Tokyo, Japan), and those in muscle and cells were determined by the
thiobarbituric acid method. Informed consents were obtained from all subjects using a form approved by the Ethical Review Board at the National Center of Neurology and Psychiatry (Tokyo, Japan).

Expression of Recombinant GNE Proteins—The cDNA for wild-type GNE was obtained by reverse transcribed-PCR from normal muscle RNA and cloned into pCR-blunt vector (Invitrogen). The cDNAs for GNE mutants were obtained by reverse transcribed-PCR from skeletal muscle RNA of DMRV patients or by site-directed mutagenesis from wild-type cDNA. All cloned muscle cDNAs were sequenced by ABI cycle-sequencing procedures using an ABI 3100 (Applied Biosystems, Foster City, CA). The sequenced and inserted cDNAs were cut out with EcoRI and blunt-end, and the purified cDNA fragments were inserted in-frame into the expression vector, pCMV-Myc (Invitrogen). The expression constructs were transiently transfected into COS-7 cells using LipofectAMINE Plus (Invitrogen) according to the manufacturer’s protocol. After 24 h, the Myc-tagged wild-type GNE and the mutant proteins were extracted from transfected cells. UDP-GlcNAc 2-epimerase activity was measured as described previously. The Mannac kinase assay was performed with slight modification according the previous report (13).

Cross-linking of GNE Mutant Proteins—To analyze the oligomer structure of wild-type and mutant GNE, cell lysates were subjected to a reaction with 10 mM MBS for 30 min at room temperature for cross-linking. The Myc-tagged cross-linked products were purified with anti-Myc-agarose (Santa Cruz Biotechnology, Santa Cruz, CA). The sequenced and inserted cDNAs were cut out with EcoRI and blunted, and the purified cDNA fragments were inserted in-frame into the expression vector, pCMV-Myc (Invitrogen). The expression constructs were transiently transfected into COS-7 cells using LipofectAMINE Plus (Invitrogen) according to the manufacturer’s protocol. After 24 h, the Myc-tagged wild-type GNE and the mutant proteins were extracted from transfected cells. UDP-GlcNAc 2-epimerase activity was measured as described previously. The Mannac kinase assay was performed with slight modification according the previous report (13).

Results

Novel Mutations in GNE Gene in DMRV Patients—In the previous study, we identified 12 different GNE mutations in either homozygous or compound heterozygous states in 27 DMRV patients (8). From these results, we concluded that DMRV is allelic to HIBM. Subsequently, we identified six patients harboring five different mutations, of which three were novel mutations: 1622C→T, 89G→C, and 2173G→A (Table 1). These novel mutations were absent in 100 control chromosomes from normal Japanese individuals.

Enzymatic Activities of GNE Mutants in DMRV Patients—Site-directed mutagenesis of the GNE protein has shown that the two enzymatic activities are separately and independently catalyzed by two domains, an N-terminal epimerase domain and a C-terminal kinase domain (13). Previously, we reported reductions in UDP-GlcNAc 2-epimerase activities in leukocytes from the patients with mutations in the GNE gene (8). However, the enzyme activity was too weak in leukocytes to clearly demonstrate correlations between gene mutations and the enzymatic activities. To clarify this relationship, we generated recombinant proteins with each of the 13 species of mutations that we identified. All but one of the mutant and wild-type recombinant GNE proteins were expressed in COS cells and migrated at 75 kDa in SDS-PAGE. The single exception was a recombinant protein with a deletion of amino acids 206–256 (A206–256) caused by exon 4 skipping. The abnormal protein was degraded in COS cells (Fig. 1A). We determined the specific activities of UDP-GlcNAc 2-epimerase and Mannac kinase of the mutant proteins relative to wild type (Fig. 1B).

The endogenous enzymes in mock transfected COS cells were determined to correct for the background enzyme activity. UDP-GlcNAc 2-epimerase activities in mutants C13S, H132Q, D176V, D177C, V331A, and D378Y were reduced to less than 50% of the control, and V572L, A630T, and A631V each showed only a 20–30% reduction in activity as compared with wild-type cells. Mannac kinase activity was retained in the N-terminal mutants C13S, H132Q, D176V, D177C, V331A, and D378Y, whereas the C-terminal mutants I472T, V572L, A630T, A631V, and G708S showed dramatic reductions in activities. These data were essentially compatible with a prior report (13). Interestingly, the A524V mutant preferentially affected UDP-GlcNAc 2-epimerase activity, although the mutation is in the kinase domain. None of the DMRV mutants showed complete loss of UDP-GlcNAc 2-epimerase or Mannac kinase activities.

Oligomerization of GNE Mutants—GNE protein forms a homohexamer by oligomerization (13). We examined whether the mutations affect the oligomerization of GNE molecules, because homohexamer structure of GNE protein was reported to be essential for UDP-GlcNAc 2-epimerase activity (13). The recombinant mutant proteins were subjected to the cross-linking with MBS. Fig. 1C shows the electrophoretic tracing patterns of cross-linked products of wild-type and mutant GNE proteins. The product of wild-type GNE predominantly migrated at >400 kDa, which corresponds to homohexamer. C13S, V572L, A630T, and A631V gave cross-linked products similar to that of wild-type GNE, whereas the mutants H132Q and D176V mainly generated a 200-kDa product, and D177C, V331A, D378Y, and A524V produced 98-kDa proteins. These data indicate that the hexameric oligomerization is necessary for UDP-GlcNAc 2-epimerase activity and that N-terminal mutants generally fail to form large oligomers although the molecular region responsible for the oligomerization is not clearly

### TABLE 1
Identified mutations

| Patient | Mutation | Exon | Predicted amino acid alteration | Protein domain |
|---------|----------|------|-------------------------------|----------------|
| 28      | 578A→T   | E3   | D176V                         | Epimerase      |
|         | 1765G→C  | E10  | V572L                         | Kinase         |
| 29      | 578A→T   | E3   | D176V                         | Epimerase      |
|         | 1765G→C  | E10  | V572L                         | Kinase         |
| 30      | 578A→T   | E3   | D176V                         | Epimerase      |
|         | 1765G→C  | E10  | V572L                         | Kinase         |
| 31      | 89G→C    | E2   | C13S                          | Epimerase      |
|         | 89G→C    | E2   | C13S                          | Epimerase      |
| 32      | 578A→T   | E3   | D176V                         | Epimerase      |
|         | 2173G→A  | E12  | G708S                         | Kinase         |
| 33      | 1765G→C  | E10  | V572L                         | Kinase         |
|         | 1765G→C  | E10  | V572L                         | Kinase         |
related to the predicted oligomerization domains based on the primary structure.

**Sialic Acid Contents in Skeletal Muscles and Primary Cells from DMRV Patients—**GNE gene mutations reduced the enzymatic activity of GNE protein. These results led us to hypothesize that sialylation should be affected in the tissues of DMRV patients. We measured the sialic acid content in sera and muscles from DMRV patients (Fig. 2). In sera, no difference was detected between patients and normal controls, whereas in skeletal muscle, a 25% reduction of sialic acid was observed in DMRV muscles. We also assessed the status of sialylation in DMRV muscles by lectin staining. We used three lectins: SSA for detection of Sia2H92512–6Gal/GalNAc, MAM for Sia2H92512–3Gal, and SBA for GalNAc1H92513–3Gal (16–18) (Fig. 2B). The results of lectin staining are summarized in Table II.

**TABLE II**

| Patient | Mutation | Predicted amino acid alteration | GNE activitya | MNK activityb | SSA staining in skeletal muscle | SBA staining in skeletal muscle | WGA staining in cultured cell | SBA staining in cultured cell |
|---------|----------|---------------------------------|---------------|--------------|--------------------------------|--------------------------------|-----------------------------|-----------------------------|
| 5       | IVS4+4A→G| Exon 4 skipping                 | ND            | ND           | Variable                      | Positive in atrophic fibers     | Negative in plasma membrane | Positive                     |
| 8       | 1765G→C  | V572L                           | 68.2%         | 8.3%         | Variable                      | Positive in atrophic fibers     | ND                          | ND                          |
| 1043T→C | D176V    |                                | 18.2%         | 86.5%        | Variable                      | Positive in atrophic fibers     | ND                          | Weak Positive               |
| 18      | 578A→T   | V331A                           | 16.1%         | 114%         | ND                            | ND                            | Weak Positive               | Positive                     |
| 19      | 578A→T   | D176V                           | 18.2%         | 86.5%        | ND                            | ND                            | Weak Positive               | Positive                     |

a GNE activity represents UDP-GlcNAc 2-epimerase activity.

b MNK activity represents ManNAc kinase activity as percentage relative to that of wild type GNE.

**Fig. 1.** Enzymatic activities and oligomerization of mutant GNE proteins. A, Myc-tagged wild-type (WT) and mutant GNE proteins expressed in COS cells were separated in SDS-PAGE and Western blotted with anti-Myc antibody. Only the mutant protein with the deletion of amino acids 206–256 was degraded in the cells. B, UDP-GlcNAc 2-epimerase (black) and ManNAc kinase (white) activities were measured in vitro. The bar graphs display relative specific activities presented as percentage of wild-type activities. C, wild-type and mutant GNE proteins were cross-linked with MBS and analyzed by immunoblotting. The immunoreactive patterns of cross-linked products were shown. The predicted migration positions of hexamer, trimer, and monomer are represented at the bottom.
were measured by the thiobarbituric acid method. *, p < 0.05. B, lectin staining and immunohistochemical staining of α-dystroglycan in skeletal muscles from control (a, d, g, and j) and DMRV patients (b, e, h, and k) and patient 8 (c, f, i, and l), a–c, stained with hematoxylin and eosin; d–f, stained with SSA lectin; g–i, stained with SBA lectin; j–l, stained with an antibody for α-dystroglycan (VIA4-I). Arrows indicate rimmed vacuoles. SBA lectin strongly stained sarcolemma and cytoplasmic areas in the cluster of atrophic or rimmed vacuoles containing myofibers.

patients, which may be attributed to the strong intensity in our staining condition (data not shown). In contrast, SBA strongly highlighted the rimmed vacuoles containing fibers and the surrounding atrophic fibers in the patients both in sarcolemma and cytoplasm (Fig. 2B, panels h and i; see arrows), whereas it did not stain myofibers in the control (Fig. 2B, panel g). These data suggest that sialylation, other glycosylation, or both are at least partly disturbed in some myofibers in DMRV. Furthermore, we examined the expression of glycosylated α-dystroglycan in DMRV muscles using an antibody (VIA4-I) that recognizes a carbohydrate epitope. The α-dystroglycan staining was negative in rimmed vacuoles containing fibers and the surrounding atrophic fibers in one DMRV patient (Fig. 2B, panel l). However, positive staining in another patient demonstrated that α-dystroglycan expression varies among patients (Fig. 2B, panel k). Therefore, we concluded that the loss of α-dystroglycan staining is an extreme down-stream phenomenon in DMRV muscles. We also analyzed muscle sialylated glycoproteins (LAMP-1, and α- and β-dystroglycans) by one- or two-dimen- sional polyacrylamide gel electrophoresis, but when they were extracted in whole amounts, there was no significant change in the electrophoretic patterns of these proteins between control and patients (data not shown). Furthermore, we analyzed the laminin-binding property of α-dystroglycan from DMRV patients, and the α-dystroglycan showed a strong binding as the control (data not shown).

Restoration of Sialylation in DMRV Cells by Feeding with ManNAc and NeuAc—Previous studies reported that hyposialylation in GNE−/− embryonic stem cells and GNE-defective cells can be repaired by feeding with the natural sialic acid precursor ManNAc (10, 19). We examined the recovery of sialylation by the addition of NeuAc or ManNAc using primary cultured cells from patients and evaluated the sialylation status by lectin staining (Fig. 3A). We used two lectins, WGA, which specifically recognizes a cluster structure of sialic acids (20), and SBA. WGA strongly stained the perinuclear region in control fibroblasts. Fibroblasts from patient 18, who harbors compound heterozygous missense mutations D176V and I472T, showed weaker staining with WGA compared with normal controls (Fig. 3A; WGA, DMRV, and Control). SBA did not stain the fibroblasts from normal controls but strongly stained cells from DMRV patients. These results were also confirmed in myotubes from another DMRV patient (patient 5) who has compound heterozygous mutations causing exon 4 skipping and V572L (Fig. 3B) and fibroblasts from patient 19 with a homozygous D176V mutation (data not shown). The sialic acid

ManNAc and NeuAc—
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levels in skin fibroblasts and myotubes from DMRV patients were significantly decreased at ~60–74% of control cells when cells were cultured in serum-free medium (Fig. 3C). By adding ManNAc or NeuAc into the culture medium, sialic acid levels in the fibroblasts and myotubes were restored to normal levels (Fig. 3C, +ManNAc and +NeuAc). Furthermore, WGA staining of cells from patients also increased to normal levels, and particularly strong WGA staining was observed in the plasma membrane of myotubes. In contrast, the SBA staining in DMRV cells disappeared by the addition of either sugar (Fig. 3, A and B; +ManNAc and +NeuAc). The addition of GlcNAc into the medium had no effect on the staining pattern with either lectin (Fig. 3, A and C, +GlcNAc). These results suggest the potential therapeutic use of ManNAc and NeuAc.

DISCUSSION

In this study, we identified six additional DMRV patients with GNE mutations. Combined with prior results, the 1765G–C mutation accounts for 55% (36 of 66) of the abnormal alleles confirming the high frequency of this mutation in Japan. Haplotype analysis suggests that this common mutation is due to a founder effect (8). All of the mutations identified in DMRV patients caused reduction (but not total loss) of enzymatic activities of either UDP-GlcNAc 2-epimerase or ManNAc kinase. These results strongly suggest that DMRV is caused by partial loss of function of the gene product. Interestingly, we previously identified the compound heterozygous mutations D378Y and A631V in a North American DMRV patient of German and Irish origin; these mutations have also been identified in an Irish HIBM patient (13). D378Y reduced UDP-GlcNAc 2-epimerase activity, and A631V decreased ManNAc kinase activity. Together with clinical and pathological similarities, these biochemical and molecular genetic results suggest that DMRV and HIBM are actually the same disease.

Through our study, we obtained information about novel molecular aspects in GNE. The two catalytic domains of the GNE molecule do not always work separately or independently in contrast to a published report (13). For example, the A524V mutation is within the predicted ManNAc kinase domain; however, it strongly inhibited UDP-GlcNAc 2-epimerase. Interestingly, this mutant did not form an oligomeric structure similar to the other N-terminal mutants. The failure of oligomerization in this A524V mutant is probably responsible for the reduced UDP-GlcNAc 2-epimerase activity as suggested previously (13).

Sialylation was decreased in muscle and in cultured cells from patients but was not completely lost, because all of the mutant proteins with missense mutations partially retained both enzymatic activities. Sialic acid levels in sera from DMRV patients were normal. Sialic acids are predominantly produced in the liver and transferred to synthesized glycoproteins. The sialylated proteins are released into the blood plasma, and free sialic acid in the plasma is derived from desialylation of these glycoproteins. GNE is expressed in the liver in large amounts; therefore, the reduction in enzymatic activities by mutations may not significantly affect the synthesis of sialic acid in the livers of DMRV patients, and sialic acids are present at concentrations comparable with normal blood levels. In contrast, in DMRV skeletal muscles, the sialic acid contents are reduced. The reduced enzymatic activities along with weak expression of GNE protein are probably responsible for the more serious reduction in sialic acid synthesis in muscle tissue compared with plasma. Lectin staining showed abnormal staining only in some fibers, indicating that a restricted number of myofibers has glycosylation abnormalities. This selective involvement may be due to muscle uptake of sialic acid, which can compensate for the defect of sialic acid synthesis in most fibers and explains why patients are normal at birth and develop late onset myopathies.

By feeding DMRV myotubes with fibroblasts with NeuAc as well as ManNAc, sialic acid concentrations in the cells increased to normal levels. As reported previously (21), treatment with NeuAc resulted in more rapid and potent effects on the restoration of sialylation than treatment with ManNAc. This strongly suggests that pharmacological therapy may be effective against DMRV/HIBM. Interestingly, even in myotubes harboring mutations that severely decrease ManNAc kinase activity, sialylation was restored by treatment with ManNAc. Schwarzkopf et al. (10) also reported similar observations in the embryonic stem cell culture in which the GNE gene was disrupted. They suggested that another sugar kinase may convert ManNAc to ManNAc-6-phosphate in those cells; therefore, ManNAc kinase activity of GNE may not be essential for sialic acid synthesis. If so, then why do the GNE mutations retaining UDP-GlcNAc 2-epimerase activity cause loss of sialylation and disease? One possible explanation is that these mutations may destabilize the GNE molecule resulting in decreased amounts of mutated proteins. However, we did not detect any reductions in the expressed amounts or defects in oligomerization of ManNAc-mutated recombinant proteins. Further analysis is necessary to clarify the mechanisms for the rescue resulting from the addition of ManNAc.

Enhanced staining with SBA lectin was observed in the sarcolemma and within the cytoplasmic area of some myofibers. These fibers were clustered and tended to be atrophic or have rimmed vacuoles. There is a report describing negative staining with SBA in Duchenne and Becker muscular dystrophies (22), suggesting that it is probably not because of dystrophic changes of myofibers but rather because of the lack of sialic acids. This abnormal glycosylation apparently preceded the formation of rimmed vacuoles, which is a pathological hallmark of DMRV. These rimmed vacuoles were electron-microscopically recognized as focal accumulations of autophagic vacuoles, which sometimes surround degenerated myofibrils and amyloid deposits. However, it is unknown whether the focal accumulations of autophagic vacuoles are the cause or result of the degeneration of myofibrils and amyloid deposits. Hypo-sialylation and abnormal glycosylation could cause the misfolding of some glycoproteins, and thus these misfolded glycoproteins may be targets of autophagic degradation and also behave as cores for formation of amyloid deposits. In our study, dystroglycan and SSA lectin staining was variable among patients. One possible explanation is that sialylation may not be the direct cause of the disease. For example, the loss of GNE enzymatic activity may induce the accumulation of the substrate, UDP-GlcNAc, leading to the abnormal O-GlcNAc modification of various proteins in the cells (23). Nevertheless, this possibility may also be unlikely because the overexpression of O-GlcNAc transferase did not cause any morphological abnormality in skeletal muscles in mice (24). In the next step, further analyses using animal models, as well as further testing of therapy with ManNAc or NeuAc, will be necessary to clarify the pathomechanism of DMRV and HIBM and the pathway from hyposialylation to rimmed vacuole formation and muscle atrophy.

Acknowledgments—We thank Dr. M. Hirano (Columbia University) for reviewing the manuscript, C. Yoshioka (National Center of Neurology and Psychiatry) for technical assistance in drawing figures. The monoclonal antibody HA43, developed by Drs. J. T. August and J. E. K. Hildreth, was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD, National Institutes of Health, and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA.
REFERENCES

1. Nonaka, I., Sunohara, N., Ishiura, S., and Satoyoshi, E. (1981) J. Neurol. Sci. 51, 141–155
2. Nonaka, I., Murakami, N., Suzuki, Y., and Kawai, M. (1998) Neuromuscul. Disord. 8, 335–337
3. Nonaka, I., Sunohara, N., Satoyoshi, E., Terasawa, K., and Yonemoto, K. (1985) Ann. Neurol. 17, 51–59
4. Griggs, R. C., Askanas, V., DiMauro, S., Engel, A., Karpati, G., Mendell, J. R., and Bowland, L. P. (1995) Ann. Neurol. 38, 705–713
5. Eisenberg, I., Avidan, N., Potikha, T., Hochner, H., Chen, M., Olender, T., Barash, M., Shemes, M., Sadeh, M., Grabov-Nardini, G., Shmilevich, I., Friedmann, A., Karpati, G., Bradley, W. G., Baumhath, L., Laneit, D., Asher, E. B., Beckmann, J. S., Argov, Z., and Mitrani-Rosenbaum, S. (2001) Nat. Genet. 29, 83–87
6. Ikeuchi, T., Asaka, T., Saito, M., Tanaka, H., Higuchi, S., Tanaka, K., Saída, K., Uyama, E., Mizusawa, H., Fukuhara, N., Nonaka, I., Takamori, M., and Tsui, S. (1997) Ann. Neurol. 41, 432–437
7. Eisenberg, I., Thiel, C., Levi, T., Tiram, E., Argov, Z., Sadeh, M., Jackson, C. L., Thierfelder, L., and Mitrani-Rosenbaum, S. (1999) Genomics 55, 43–48
8. Nishino, I., Noguchi, S., Maruyama, K., Driss, A., Sugie, K., Oya, Y., Nagata, T., Chida, K., Takahashi, T., Takusa, Y. Ohi, T., Nishimuya, J., Sunohara, N., Ciafaloni, E., Kawai, M., Aoki, M., and Nonaka, I. (2002) Neurology 59, 1689–1693
9. Hinderlich, S., Stasche, R., Zeitler, R., and Reutter, W. (1997) J. Biol. Chem. 272, 24313–24318
10. Schwarzkopf, M., Knobeloch, K. P., Rohde, E., Hinderlich, S., Wiechens, N., Lucka, L., Horak, I., Reutter, W., and Horstkorte, R. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 5267–5270
11. Eisenberg, I., Grabov-Nardini, G., Hochner, H., Korner, M., Sadeh, M., Bertorini, T., Bushby, K., Castellian, C., Felice, K., Mendell, J. Merlini, L., Shilling, C., Wirguin, I., Argov, Z., and Mitrani-Rosenbaum, S. (2003) Hum. Mutat. 21, 99
12. Lucka, L., Krause, M., Danker, K., Reutter, W., and Horstkorte R. (1999) FEBS Lett. 454, 341–344
13. Effertz, K., Hinderlich, S., and Reutter, W. (1999) J. Biol. Chem. 274, 28771–28778
14. Michele, D. E., Barresi, R., Kanagawa, M., Saito, F., Cohn, R. D., Satz, J. S., Dollar, J., Nishino, I., Kelley, R. I., Somer, H., Straub, V., Mathews, K. D., Moore, S. A., and Campbell, K. P. (2002) Science 298, 417–422
15. Noguchi, S., Wakabayashi, E., Imamura, M., Yoshida, M., and Ozawa, E. (2000) Eur. J. Biochem. 267, 640–648
16. Shibuya, N., Tazaki, K., Song, Z. W., Tarr, G. E., Goldstein, I. J., and Peumans, W. J. (1989) J. Biol. Chem. (Tokyo) 106, 1098–1103
17. Yamamoto, K., Kenami, Y., and Irimura, T. (1997) J. Biochem. (Tokyo) 121, 756–761
18. Pereira, M. E., Kabat, E. A., and Sharon, N. (1974) Carbohydr. Res. 37, 89–102
19. Reutter, W., Schwarz, O. T., Langner, J., Schwartz-Alhibi, R., Reutter, W., and Pawlita, M. (1999) Science 284, 1372–1376
20. Bhavanandian, V. P., and Kallio, A. W. (1979) J. Biol. Chem. 254, 4008–4008
21. Oetke, C., Hinderlich, S., Braasmer, R., Reutter, W., Pawlita, M., and Keppler, O. T. (2001) Eur. J. Biochem. 268, 4553–4561
22. Paljarvi, L., Karjalainen, K., and Kalimo, H. (1984) Arch. Neurol. 41, 39–42
23. Comer, F. I., and Hart, G. W. (2000) J. Biol. Chem. 275, 29179–29182
24. McClain, D. A., Lubas, W. A., Cooksey, R. C., Hazel, M., Parker, G. J., Love, D. C., and Hanover, J. A. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 10695–10699
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J. Biol. Chem. 2004, 279:11402-11407.
doi: 10.1074/jbc.M313171200 originally published online January 5, 2004

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