Glutamate oxaloacetate transaminase (GOT) in the dy^{2J} genotypes of C57BL/6J mice: Possible involvement of regulatory defect in muscular dystrophy[1]

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Summary. The GOT mitochondrial isozyme of heterozygote and homozygote muscular dystrophy (dy^{2J}) genotypes is affected during development prior to the expression of dystrophy in homozygotes, dy^{2J}/dy^{2J}.

The basic enzyme defect and how it affects specific tissue(s) is not well understood in different forms of muscular dystrophy (MD). Although a number of enzymes have been suggested to be involved in MD including glutamate oxaloacetate transaminase (GOT, E.C.2.6.1.1)[2-4], the mechanism of GOT alteration in dystrophy, however, remains unclear. GOT is widely distributed in animal tissues, and is involved in glutamate oxidation and nitrogen balance[5]. It mediates the transfer of the amino group of glutamic acid to oxaloacetic acid. GOT may be separated into 2 molecular forms by electrophoresis[6] or column chromatography[7]. The soluble fraction ($GOT_{(s)}$) and the mitochondrial fraction ($GOT_{(m)}$) have been described in a number of tissues and the antibodies of the 2 purified isozymes failed to cross react[8]. Most enzyme studies in muscular dystrophy including those for GOT, have concentrated on levels of enzyme activity and little attempt has been made on the ontogenic pattern of the enzyme. Alteration in the developmental pattern of enzyme(s) will provide insight into metabolic regulatory parameters, which may in fact form the basis for a number of inborn errors of metabolism including MD. This report deals with the developmental profile of the 2 forms of GOT (GOT(s) and GOT(m)) in the 3 genotypes of muscular dystrophic mutation dy^{2J} in C57BL/6J mice in relation to expression of the dystrophic phenotype. Our results suggest the involvement of temporal regulatory mechanism(s) for GOT(m) in the expression of muscular dystrophy.

Materials and methods. Genetic stocks of the dy^{2J}, muscular dystrophic mutation were obtained from the Jackson Laboratory, as 4-week-old C57BL/6J male and female mice. Appropriate crosses were set up to yield $+/+$, $+/dy^{2J}$ and dy^{2J}/dy^{2J} genotypes. Newborns of the 3 genotypes were followed for their growth rate and expression of dystrophy. All animals were housed in polycarbonate mice cages with sawdust bedding and given free access to Purina mouse chow and tap water under 10/14 h. light/dark cycle at 23 ± 1 °C controlled room temperature. Males of the 3 genotypes were sacrificed by cervical dislocation at different developmental stages (at birth, 2 weeks old, 3 weeks old and 10–12 weeks old). A number of tissues were extracted including liver, heart, brain, kidney, spleen and thigh muscles. Tissue homogenates (1 g washed tissue/ml H20) were prepared. Supernatants, obtained following centrifugation (12,000 rpm, 20 min at 4 °C) were stored at −70 °C until used for electrophoresis. Electrophoresis was performed using 0.077 M. Tris, 0.003 M citrate (pH 8.6) as the gel buffer and 0.294 M. Boric acid, 0.060 M NaoH (pH 8.1) as the electrode buffer. The gel medium used was either 12% electrostarch (Electrostarch Co., Madison) or 0.9% agar. Following electrophoresis, gels were incubated (37 °C) in 133 mg L. asparitic acid, 36 mg α-ketoglutaric acid, 50 mg. EDTA, 500 mg polyvinyl pyrrolidone (PVP-40), 1.42 g. Na2HPO4, 200 mg Fast blue BB salt in 100 ml H20. The 2 isozyme bands visible in 30 min, were evaluated for their relative proportions from agar gels using a Gelman DCD-16 densitometer.

Results and discussion. The dy^{2J} mutation results in a milder form of dystrophy, which appears to be allelic to the more severe *dy* mutation the genetic background of C57 strain of mice[9]. Although it is possible to use dy^{2J}/dy^{2J}, dystrophic genotypes in crosses, the breeding success is limited to 9–14-week-old males and females only. Most pregnant dystrophic females are able to carry pregnancy to near term, however, almost all of them have complications during delivery. A number of youngs are born dead and only few dystrophic mothers are able to raise only partial litters. In general, litters from dystrophic mothers were fostered to normal C57BL/6J mothers and the dy^{2J}/dy^{2J} young expressed dystrophy around day 21. Figure 1 shows the growth rate of normal and dystrophic individuals in the 2 sexes. It is evident that after the expression of dystrophy (around 3 weeks), dy^{2J}/dy^{2J} genotypes lag behind normals in their growth rate.

Figure 2 shows the liver GOT electrophoretic pattern on starch gel for the 3 dy^{2J} genotypes at 4 stages of development. There are 2 distinct isozymes; the cathodal form is bound to or located in the mitochondria and the anodal

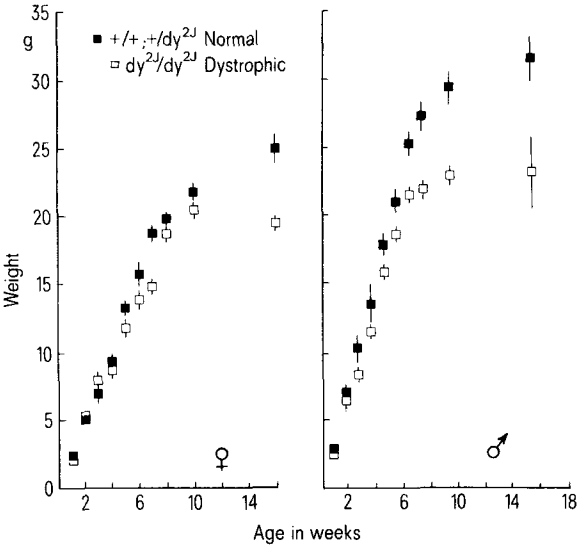

Figure 1. Growth rate of the 3 dy^{2J} genotypes in 2 sexes of C57BL/6J mice.

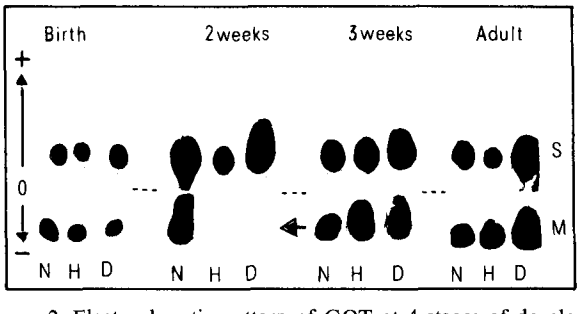

Figure 2. Electrophoretic pattern of GOT at 4 stages of development in the 3 dy^{2J} genotypes (N = +/+, H = +/dy^{2J} and D = dy^{2J}/dy^{2J}, S = soluble and M = mitochondrial form.

form is soluble and cytoplasmic in mice[6] and man[11]. 2 loci, Got-1 and Got-2 are postulated to be the structural determinants of the soluble and mitochondrial forms of GOT respectively. The 2 GOT isozymes are found with high activity in most tissues. In red blood cells, however, they are not expressed in mouse[6] and only the soluble form is observed in man[11]. We observed relatively high GOT activity in liver, kidney, brain and heart and lower activity in spleen and lung in adults. In general, the enzyme activity per g of tissue was higher in adults as compared to newborns and prenatals which follows earlier findings[6]. Figure 2 also shows that the relative histochemical staining activity of the 2 isozymes is comparable throughout the developmental stages in +/+ individuals. Also, there are no genotype differences observed in the new-borns and adults. In 2- and 3-week-old mice, however, the isozyme patterns are indeed different in the 3 genotypes. In 2-week-old animals the mitochondrial form is absent in dy^{2J}/dy^{2J} and reduced (about 20% of the soluble form) in $+/dy^{2J}$ genotype. Furthermore, in 3-week-old mice, the relative activity of the mitochondrial form is about 30% and nearly equal to the soluble form in dy^{25}/dy^{25} and $+/dy^{2J}$ genotypes respectively.

The genotype differences observed in 2- and 3-week-old animals are of interest because the expression of dystrophy is associated with age. The dy^{2J}/dy^{2J} genotypes show the first sign of dystrophy around day 21. The absence of mitochondrial GOT in dystrophic genotype in 2-week-old mice only suggests that the Got-2 locus itself is not the primary defect of dystrophy. However, the temporal expression of this locus is affected by the dy^{2J} mutation, which could be involved in the expression of dystrophy. The expression of Got-2 locus follows cis rather than trans action of the involved regulatory mechanism. Here the mitochondrial GOT is affected in $+/dy^{2J}$ and dy^{2J}/dy^{2J} genotypes during a critical period of development, just prior to the expression of dystrophy in dy^{2J}/dy^{2J} animals. A number of genetic mechanisms are known to be involved in the processing of structural genes[12]. One such mechanism involves temporal genes, that regulate the developmental programing and are responsible for the appearance and relative tissue distribution of an enzyme during development. These regulatory mechanisms themselves may depend on internal and/or external 'cue(s)' and are not expected to function in isolation. The observed GOT developmental pattern in dystrophy in this report suggests an association between genotype at the dy^{2J} locus and the temporal regulator for the Got-2 locus. The dy^{2J} mutation may provide internal 'cue(s)' for the regulation of GOT and other enzymes that have been implicated to be involved in muscular dystrophy.

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Mendelian recessive ratios in acute poststreptococcal glomerulonephritis[1]

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Summary. A prospective study of 15 families (61 siblings) of index cases of acute poststreptococcal glomerulonephritis detected a proportion of 0.260 ± 0.016 SEM. This is an excellent fit for a single autosomal recessive trait.

Acute poststreptococcal glomerulonephritis (APSGN) is an immune complex disease that develops after streptococcal infection[2]. It appears sporadically in most communities, but in some geographical areas it has an endemic incidence with epidemic outbreaks[3-5]. Familial aggregation of cases has been reported[6], but since there are no recognized markers for a disease that is transient in nature, the difficulty of determining genetic patterns derives from at least 2 considerations; first, the occurrence of asymptomatic cases which are missed in any survey that does not contemplate serial testing of the individuals during the period in which they are at risk, and second that, although some bacterial types have been associated with nephritis, the nephritogenicity (potential to cause nephritis) of a given streptococcus can only be established unquestionably a posteriori, because there is disagreement as to the nature of the relevant bacterial component[7-9]. We have recently reported a prospective family study[10] that took into account the considerations noted above and the present work concerns findings that suggest that susceptibility to develop nephritis may be a mendelian recessive trait.

Patients and methods. In Maracaibo, APSGN presents endemo-epidemic characteristics[11]. Since 1977 we have studied in a prospective manner 25 families of patients with clinical APSGN after the appearance of the 1st case (index case) in each family. The study protocol included serial weekly testing of all family members for a period of 4–6 weeks after the detection of the index case (period of close observation) and at least every 6 months afterwards (period of delayed observation). The only condition for selection of these families was the willingness to participate and compliance with the conditions of the study. Details of this work have appeared in a previous communication[10].

On the assumption that only those individuals with a recent infection would be at risk of developing APSGN,