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Overexpression of Esterase D in Kidney from Trisomy 13 Fetuses

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Summary

Human trisomy 13 (Patau syndrome) occurs in approximately 1 in 5,000 live births. It is compatible with life, but prolonged survival is rare. Anomalies often involve the urogenital, cardiac, craniofacial, and central nervous systems. It is possible that these abnormalities may be due to the overexpression of developmentally important genes on chromosome 13. The expression of esterase D (localized to chromosome 13q14.11) has been investigated in both muscle and kidney from trisomy 13 fetuses and has been compared with normal age- and sex-matched fetal tissues, by using northern analysis. More than a twofold increase in expression of esterase D was found in the kidney of two trisomy 13 fetuses, with normal levels in a third. Overexpression was not seen in the muscle tissues from these fetuses.

Introduction

Trisomy 13 (Patau syndrome) was first described cytogenetically in 1960 (Patau et al. 1960). It is characterized by urogenital, cardiac, craniofacial, central nervous system, and growth abnormalities (Moerman et al. 1988). Defects include mental retardation, cleft lip and/or palate, growth retardation, polycystic kidney, congenital heart disease, and holoprosencephaly (Jones 1988). Over 95% of human trisomies 13 spontaneously abort (Warburton et al. 1991, p. 57). Those that live rarely have prolonged survival, with only 18% surviving the first year (Jones 1988, pp. 20–21). Trisomy 13 is one of the three main autosomal trisomies compatible with life, the others being trisomies 21 and 18 (Jacobs et al. 1987). This contrasts with spontaneous abortions, in which trisomy for most chromosomes has been found (Boue et al. 1975).

There is not yet a defined region of chromosome 13 which gives rise to the characteristic phenotype, although triplication of regions proximal to band 13q14 or distal to 13q22 are important in development of the trisomy 13 phenotype (Rogers 1984). Some anomalies, such as growth retardation and microcephaly, are most common in individuals who are trisomic for proximal or distal 13q (Tharapel et al. 1986). It has been postulated that it is the interaction of overexpressed genes on chromosome 13 that results in many of the abnormalities seen in the trisomy 13 phenotype, rather than the overexpression of specific genes (Wilson et al. 1986). Exceptions to this include polydactyly, where triplication of 13q31-qter is thought to be necessary (Rogers 1984). As with trisomy 13, no one locus or region of chromosome 18 is thought to give rise to the characteristic trisomy 18 (Edwards syndrome) phenotype, but it seems to involve an interaction between the loci. It appears that trisomy for regions within 18q11-qter is critical, with trisomy for 18p having little effect on the phenotype (Wilson et al. 1990). This differs from trisomy 21 (Down syndrome), where a subband of 21q22 is thought to be the critical region (Korenberg et al.

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1992). The parental origin of the extra chromosome 13 is maternal in approximately 83% of cases and paternal in origin in 17% (Ishikimyama and Niikawa 1984; Hassold et al. 1987; S. Loughna, unpublished data).

At present, little is known about the changes in expression of important candidate genes for development in trisomy 13 tissues, although erythrocytes, fibroblasts, lymphoblasts, and amniotic fluid cells isolated from trisomy 13 patients have an approximately 50% increase in esterase D activity (Epstein 1986, pp. 70–71). Increased activities of certain enzymes, the genes of which are localized to chromosome 21, have been seen in Down syndrome patients. For example, in trisomy 21 individuals, superoxide dismutase has increased activity in erythrocytes, fibroblasts, lymphocytes, and granulocytes, with levels of 6-phosphofructokinase increased in erythrocytes but not in fibroblasts (Van Keuren et al. 1982). Increased activities in Down syndrome patients were also seen for enzymes whose genes are localized on chromosomes other than 21, such as glucose-6-phosphate dehydrogenase (Francke 1981). This could be due to its regulation being altered by abnormal expression of regulatory genes on chromosome 21. A few genes localized to human chromosome 21 have shown unexpected levels of expression in trisomy 21 tissues. The ETS-2 protooncogene was analyzed in brain tissue of a Down syndrome fetus and found to be increased 1.7-fold in total RNA and 1.3-fold in polyA+ RNA (Baffico et al. 1989). Serum concentrations of amyloid precursor protein (APP) have also given the expected 1.5-fold increase, whereas higher levels were seen in brain tissue (Rumble et al. 1989). Variable expression levels of genes such as APP have also been observed in the trisomy 16 mouse, an animal model for Down syndrome, but are dependent on the tissue and gene under analysis and the stage of development (Holtzman et al. 1992).

Esterase D, localized to 13q14.11 in humans, has a molecular mass of 30 kDa (Young et al. 1988). The gene spans over 35 kb in the human genome, and it is thought to be highly conserved. Hopkinson et al. (1973) observed enzyme activity of esterase D in fetal liver and various adult tissues. Expression has also been seen, at the RNA level, in a range of fetal tissues (S. Loughna, unpublished data). Lee et al. (1986) observed the highest enzymatic levels in adult liver and kidney. Esterase D is one of a class of enzymes which hydrolyzes esters. It is localized in cytoplasmic vesicles in the cell, and it is induced by phenobarbital but not by phorbol myristate ester, which suggests that it plays a role in detoxification (Lee and Lee 1986; Young et al. 1988). Therefore, esterase D is likely to play a role in the developing liver and kidney.

The enzyme has a dimeric structure produced from two polypeptide subunits (Takahashi et al. 1986). EsD^1 and EsD^2 are codominant alleles of the same gene, although there are also several rare alleles. The gene frequency of EsD^1 is .902 in British blacks and .887 in English whites (Papiha and Nahar 1977). The frequency of EsD^2 is lower in these two populations than it is in many other ethnic groups. The alleles produce three common phenotypes, EsD1-1, EsD2-1, and EsD2-2. There are differences in the enzyme activities, depending on the phenotype. The most common phenotype is EsD1-1, which has approximately twice as much activity as EsD2-2, with the activity of EsD2-1 being intermediate (Nishigaki et al. 1983). This could be due to differences in the structure of the polypeptides, the degree of stability, or the rate of synthesis of the particular isoenzyme (Horai and Matsunaga 1984). Therefore, if a normal fetus had an enzyme phenotype which has a high enzyme activity, then a 50% increase of the EsD^1 allele in a trisomy could produce sufficient esterase D to damage the developing kidney. Renal anomalies which commonly occur in the trisomy 13 syndrome are polycystic kidney, hydronephrosis, horseshoe kidney, and duplicated ureters (Jones 1988, pp. 20–21). The aim of this study was to measure the levels of expression of the esterase D gene in tissues from age-matched trisomy 13 and normal fetuses by using northern analysis.

Material and Methods

Tissue and Blood Sample Collection

Trisomy 13 tissues (kidney, muscle, liver, and brain) from three fetuses were collected at termination of pregnancy and snap frozen immediately in liquid nitrogen. Gestational age- and sex-matched control tissues from six white and two black normal fetuses were collected after second-trimester termination of pregnancy. Parental blood was collected into EDTA tubes.

Clinical Data of Trisomy 13 Fetuses

Fetus 1.—The fetus was a 19-wk-old female, which was first diagnosed on ultrasound and confirmed by cytogenetic analysis of fetal tissue. Abnormalities seen on ultrasound were absent nose, cleft lip and palate, holoprosencephaly, intrauterine growth retardation, polydactyly, hyperechogenic left and right kidneys, and an abnormal four-chamber view of the heart. The maternal age was 30 years. The parents were white.

Fetus 2.—The fetus was a 20-wk-old male. The diagnosis of trisomy 13 was made by amniocentesis. Abnormalities seen at postmortem were cleft lip and palate, polydactyly of both hands and one foot, and rocker bottom feet. The maternal age was 37 years. The parents were white.

Fetus 3.—The fetus was a 21-wk-old female, which was first detected by amniocentesis and confirmed by cytogenetic analysis of fetal tissue to be trisomy 13. No anomalies were seen at postmortem. The maternal age was 36 years. The parents were black.

RNA Analysis

Northern analysis was performed by standard techniques (Feinberg and Vogelstein 1984; Sambrook et al. 1989). Thirty micrograms of total RNA was loaded for electrophoresis onto a 1.5% denaturing formaldehyde gel. The membrane was washed in 2 × SSC with 0.1% SDS for the esterase D probe, EL22, and in 0.1 × SSC with 0.1% SDS for human β-actin (hβ-actin). Autoradiography was performed at −70°C for 1–3 d. The cDNA probe used was EL22, which contains more than 90% of the sequence for the esterase D gene (Lee and Lee 1986). The control probe was hβ-actin (Gunning et al. 1983). Four kidney and seven muscle tissues from age-matched normal fetuses were analyzed to determine whether esterase D is expressed equally between different fetuses. Trisomy 13 kidney and muscle tissues were compared with age- and sex-matched normals. At least duplicate samples were analyzed, and always on separate gels.

The data were quantified by densitometric analysis (LKB laser densitometer). The ratio of trisomic to normal expression was calculated, from which the percentage of overexpression was determined. The candidate gene was standardized by using expression levels of hβ-actin to ensure equal quantities of RNA and to confirm the integrity of the RNA. All band measurements of esterase D levels were made in the linear range. This was demonstrated by the densitometric analysis of increasing amounts of RNA hybridized with hβ-actin (data not shown). hβ-actin is expressed ubiquitously and at equal levels within the age-matched tissues analyzed.

DNA Analysis

DNA was isolated from parental blood and fetal placenta, and Southern analysis was performed by standard techniques (Feinberg and Vogelstein 1984; Sambrook et al. 1989). The membrane was then washed between 0.1 × SSC with 0.1% SDS at 65°C. Autoradiography was performed at −70°C for 1–3 d. The highly polymorphic VNTR probes for chromosomes 13, all located on 13q, were pMS604, pMS626 (HGMP Resource Centre, Harrow, Middlesex), and cMCOC46 (Nakamura et al. 1988).

Starch-Gel Electrophoresis

Analysis of the esterase D phenotype for the fetuses was carried out using horizontal starch-gel electrophoresis, as described by Hopkinson et al. (1973). The gel is sliced in two, and each half is stained with a fluorogenic substrate and then with an azo dye–coupled stain.

Fluorogenic substrates.—The fluorogenic substrates used were 4-methylumbelliferyl acetate and butyrate, both of which esterase D hydrolyzes to produce 4-methylumbelliferone. This fluoresces under long-wave UV light. The substrates were prepared fresh and applied to the cut surface of the gel by using filter paper. The final concentration of the ester was 0.01% (dissolved in a small volume of acetone) in 0.05 M acetate buffer, pH 5.2. Fluorescent regions can be seen under UV light immediately.

Azo dye–coupled stain.—These stains were also prepared just before use. Forty milligrams of diazo salt fast blue RR (dissolved in a small volume of acetone) was added to 100 ml of 0.05 M phosphate buffer pH 6.5. Then 4 ml of a 1% stock solution (w/v) in 50% acetone of α-naphthyl acetate was added. A 0.1% stock solution was used for α-naphthyl butyrate. Staining was carried out for 45 min at 37°C, with a final wash in 5:5:1 (v/v) methanol:water:acetic acid.

Enzyme Activities

The esterase D activity of fibroblast cell lines from fetuses 1 and 3 and normals was measured by a modified method of Sparkes et al. (1979). No cell lines from fetus 2 were available. Five milliliters of 10 mM sodium acetate buffer, pH 5.2, with 50 μl of 10 mM methylumbelliferyl acetate (dissolved in acetone) was allowed to stand at room temperature for 10 min. The reaction was initiated by the addition of 3×10^5 cells in a volume of 50 μl. Fluorescence of the hydrolysis product, methylumbelliferone, was measured using a Perkin Elmer LS30 luminescence spectrophotometer, at 445 nm with excitation at 320 nm, over a 3-min period. The esterase D activity was compared to G6PDH (the gene being localized to Xq28), measured at 340 nm using a spectrophotometer.

Results

Expression in Control Tissue

Equal levels of esterase D were expressed in sets of four kidney (1.4 ± 0.2 SD) and seven muscle (1.3 ± 0.3

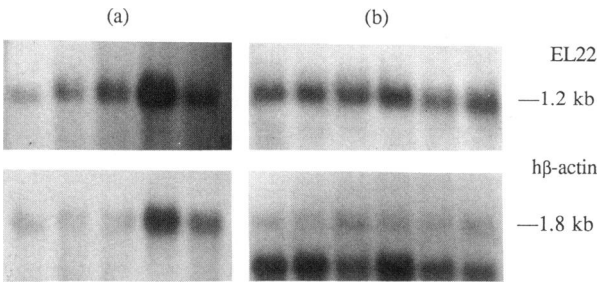

Figure 1 Northern analysis to show expression of esterase D in (*a*) kidney and (*b*) muscle in 18–20-wk-old normal fetuses. The lower band in the muscle actin is α-actin.

SD) tissues from eight 18–20-wk-old normal fetuses (fig. 1 and table 1). These values are the average ratios of expression of esterase D compared with hβ-actin, calculated from the densitometric analysis. One of the kidney samples shown in figure 1 was not included in the study, as it was not in the linear range when densitometrically analyzed.

Expression in Experimental Tissue

A 2.2-fold increase in expression of esterase D was observed in the kidney tissue of trisomy 13 fetus 1 (fig. 2 and table 2). Analysis of fetus 2 kidney tissue showed 2.4 times more esterase D expression, compared with normal. Both of these fetuses had a range of abnormalities. No overexpression was observed in fetus 3, which had no major anomalies (fig. 2 and table 2). A normal

Table 1

Densitometric Values for the 18–20 wk Normals Used for the Expression Study

Fetus	Sex	Ethnic[a] Origin	Esterase D Phenotype	Mean Expression[b] Kidney[c]	Mean Expression[b] Muscle[d]
1	M	B	1-1	1.4	1.1
2	F	W	1-1	ND	1.1
3	F	W	1-1	1.6	1.5
4	M	B	1-2	ND	1.3
5	M	W	1-1	ND	1.1
6	F	W	1-2	ND	2.0
7	M	W	1-2	1.2	1.2
8	M	W	1-1	1.3	ND

[a] W denotes white, and B denotes black.
[b] $N = 2$; and ND = no data.
[c] SD = 1.4 ± 0.2.
[d] SD = 1.3 ± 0.3.

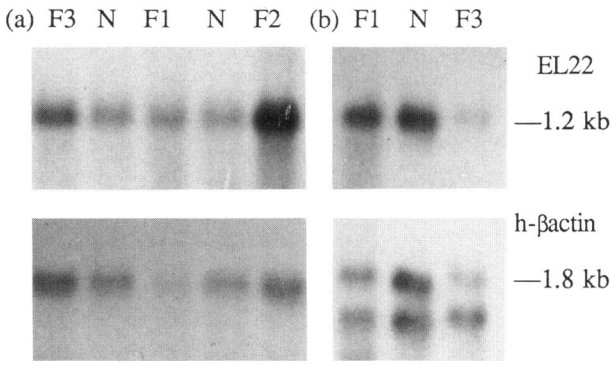

Figure 2 Northern analysis to show (*a*) overexpression in kidney and (*b*) normal levels of expression in muscle, in trisomy 13 fetal tissues. The lower band in the muscle actin is α-actin. F1 = fetus 1; F2 = fetus 2; F3 = fetus 3; and N = normal fetus.

level of expression was seen in muscle in the trisomic fetuses analyzed (fig. 2 and table 2). The esterase D expression was standardized by comparison with hβ-actin. As can be seen in figures 1 and 2, two bands are present in muscle. The upper band is β-actin; the lower band is α-actin (Minty et al. 1982). The total RNA isolated from the muscle from fetus 2 and from the trisomic liver tissues was degraded and therefore not analyzed. The variability in expression of esterase D between the normal brain tissues was too great to allow analysis of the trisomic brain tissue.

Determination of Parental Origin

The parental origin of the extra chromosome 13 was maternal in all three fetuses. It was determined by using the probe cMCOC46 for fetus 1 and pMS604 for fetuses 2 and 3 (data not shown). A *Taq*I digest of the DNA for fetus 1 gave the genotype B1B2B3, with the mother being B2B3 and the father being B1B1. *Alu*I-digested DNA from fetuses 2 and 3 gave the genotype A1A2A4 for fetus 2, with the mother being A1A4 and the father being A2A2, and the genotype A4A4A5 for fetus 3, with the mother being A3A4 and the father being A3A5.

Determination of Esterase D Phenotype in the Control and Experimental Fetuses

The phenotype of esterase D for all the control and trisomy 13 fetuses was determined (fig. 3 and table 2). Of the normal fetuses used in the expression study, five fetuses were EsD1-1, and three were EsD2-1. Two of the trisomy 13 fetuses were EsD1-1-1, and one was EsD1-1-2. The three normal fetuses used in the enzyme

Table 2

Characteristics of the Three Trisomy 13 Fetuses

Fetus	Sex	Age (wk)	Parental Origin[a]	Esterase D Phenotype	Esterase D Activity[b]	Mean Expression[c]		Phenotype
						Kidney	Muscle	
1	F	19	Maternal	EsD1-1-2	1.8	2.2	.9	Abnormal
2	M	20	Maternal	EsD1-1-1	ND	2.4	ND	Abnormal
3	F	21	Maternal	EsD1-1-1	1.9	1.0	.6	Normal

[a] The parental origin is of the extra chromosome 13.
[b] ND = no data.
[c] N = 2–5.

activities were EsD1-1. The three trisomy 13 fetuses were compared with normal fetuses of phenotype EsD1-1 in the analysis of expression and enzyme activities, as EsD1-1 gives the highest esterase D activity.

Figure 3 shows the electrophoretic patterns of the esterase D isozymes depending on the phenotype for the normal and trisomy fetuses used in the expression study. The substrate was 4-methylumbelliferyl acetate. The presence of other esterases could be seen by using alternative substrates. It was confirmed that figure 3 shows esterase D, as 4-methylumbelliferyl butyrate reproduces this data, and also shows the position of other esterases, whereas the α-napthyl esters show the position of other esterases but not of esterase D (data not shown).

Determination of Esterase D Activity

Fetus 1 gave a 1.8-fold increase in activity, whereas fetus 3 gave a 1.9-fold increase. The reaction was standardized by adding an equal number of cells (3×10^5) to each reaction and by comparing them with the enzyme activity of G6PDH. This would be expected to be approximately equal in the different cell types (Junien et al. 1982). The enzyme activities could not be analyzed in fibroblasts from fetus 2 or from any of the trisomy 13 muscle and kidney tissues, as no cell lines were available.

Discussion

There are a range of abnormalities commonly seen in trisomy 13 individuals (Patau syndrome), including mental retardation, cleft lip and/or palate, polycystic kidney, and congenital heart disease (Jones 1988, pp. 20–21). This study has determined that esterase D is expressed differently in tissues where it is thought to play a role in development, when compared with tissues where no function has been postulated. As esterase D is thought to be important in detoxification in the kidney, we analyzed this tissue, together with muscle which we expected to be unaffected.

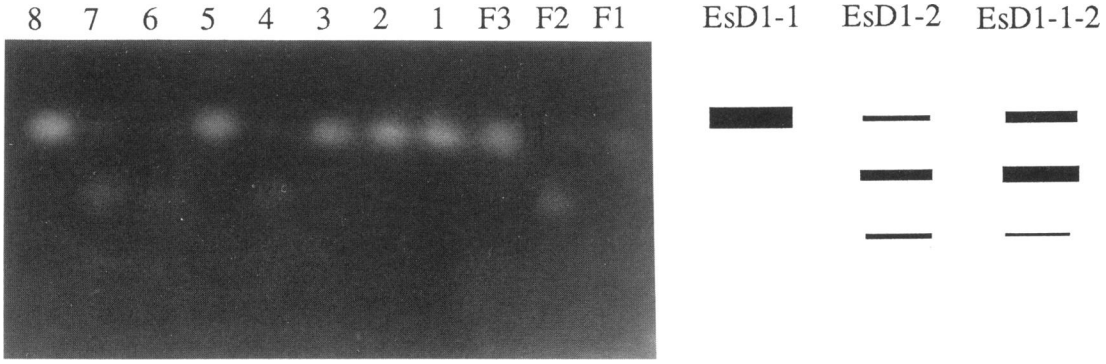

Figure 3 Starch gel to show the esterase D phenotypes, in the three trisomies and eight normals. F2 and F3 are phenotypes EsD1-1-1, and F1 is EsD1-1-2. The normals used in the expression study are 1–8. The fourth, sixth, and seventh normals are EsD2-1. The rest are EsD1-1. The diagram to the right-hand side indicates the intensities of the bands seen in the phenotypes. F1 = fetus 1; F2 = fetus 2; and F3 = fetus 3.

Most of the abnormal characteristics of trisomy 13 are displayed when bands proximal to 13q14 and distal to 13q22 are present in triplicate (Rogers 1984). Esterase D is localized to 13q14.11 and is therefore in a region of importance with regard to the phenotype. The esterase D activity in fetal liver tissues does not vary with gestational age, and normal levels are produced from early in fetal development (Hopkinson et al. 1973). The enzyme has three main phenotypes, each of which has a different enzyme activity. If one of the alleles was duplicated, then this could have a physiological effect on the developing kidney or liver. In a trisomy fetus, the presence of three alleles for EsD^1 would produce the highest levels of esterase D. EsD1-1 is the most common phenotype in normal white and black individuals. A large European study (Papiha and Nahar 1977) concluded that the ESD1-1 phenotype was present in 80% of individuals, 20% were ESD2-1, and less than 1% were ESD2-2. This is similar to what would be expected in an English population. Therefore, most trisomy 13 fetuses would have the phenotype which produces the highest enzyme activity. In our sample of three trisomies, fetus 1 was EsD1-1-2 and fetuses 2 and 3 were EsD1-1-1. All three trisomy fetuses were compared directly with age- and sex-matched normals which had the EsD1-1 phenotype. We have shown differences in expression between two tissues in one fetus. Trisomy fetus 1 showed a 2.2-fold increased expression in kidney but no overexpression in muscle. In view of the probable role that esterase D plays in detoxification, kidney is a tissue which would be expected to be affected by overexpression of esterase D, whereas muscle is not. Fetus 2 also showed an increase in the kidney, by 2.4 fold, and like fetus 1 it had an abnormal phenotype. Unfortunately, the muscle tissue could not be analyzed. The third fetus, in which no abnormalities were observed, had normal expression in both kidney and muscle.

There has been little analysis of the expression of genes on chromosome 13 in trisomy 13 tissues. Epstein (1986) summarizes the data on gene dosage effects of esterase D in erythrocytes, lymphoblasts, amniotic fluid cells, and fibroblasts. In this study, overexpression of esterase D has been seen in kidney at the mRNA level in two fetuses. Both had a range of abnormalities, with fetus 1 having hyperechogenic kidneys. Kidney abnormalities are common in trisomy 13. No overexpression was seen in fetus 3, which had no major anomalies. As esterase D is thought to be involved in detoxification and so play a role in the developing kidney and liver, it is possible that its overexpression may be involved in the formation of abnormalities. Esterase D was not expected to have a significant role in the developing muscle, but it was expected that overexpression would be seen in the muscle, because of the presence of the extra chromosome. However, normal expression in the muscle indicates that there is tissue-specific regulation of the gene, which is independent of copies present. Perhaps the production of esterase D in a normal muscle cell is maintained at a low level, despite the number of copies present.

Although analysis of the enzyme activities of esterase D in the muscle and kidney was not possible, the activity in fibroblasts in fetuses 1 and 3 was determined. A 1.9-fold increase was seen. Although this is higher than the predicted 1.5-fold increase, there is a high degree of variability in enzyme activities (Epstein 1986).

With the three fetuses under analysis, the origin of the extra chromosome was maternal in each case. The differences in overexpression seen in the kidney tissues in these three fetuses cannot be explained by parental origin.

Therefore, we postulate that esterase D plays a role in the normal developing kidney. Its overexpression in trisomy 13 kidney tissue could be involved in the formation of the renal anomalies seen in these fetuses. It has been suggested that it is a highly conserved protein (Lee et al. 1986), which implies that it has an important function. Structurally and developmentally important candidate genes from chromosome 13 are under study, and further analysis and the cellular localization of the mRNA will be determined by in situ hybridization. We should be able to relate abnormally high levels of expression of candidate genes at the cellular level to specific defects seen in trisomy 13 individuals. This will also help to elucidate the role that these genes play in normal fetal development.

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