J Med Genet 1995;32:836–840

LETTERS TO THE EDITOR

Reply to the commentary on Decruyenaere *et al* "Adolescents' opinions about genetic risk information, prenatal diagnosis, and pregnancy termination"

As psychologists, we are convinced that our discipline has at least three important roles in the field of clinical genetics: (1) participation in counselling aimed at free, informed decision making and at coping better with the genetic risk or the result of genetic testing; (2) description of psychological profiles of patients with a specific genetic disease, including neuropsychological testing; (3) research to obtain insight into people's knowledge, beliefs, feelings, and behaviour related to genetic disease, genetic risk, and genetic testing. "Prediction" of how many people will use specific genetic tests and delineation of predictor variables is part of the latter role.

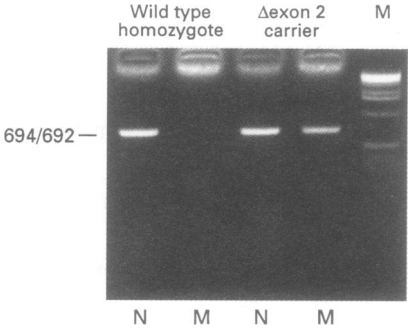
We want to stress that the mere prediction of future uptake rates or other preventive behaviour is not the main purpose of our study. The study is aimed at a description of adolescents' present opinions about genetic risk information, prenatal diagnosis, and pregnancy termination, as a starting point for setting up an information campaign: "To gain further insight . . . genetic testing" (see Abstract). It does not lead to the conclusion that adolescents show an apparent lack of interest in genetic information. Indeed, one should not extrapolate from other findings about the gap between "intentions and real uptake for specific predictive tests or carrier tests" to conclude that adolescents' self-reported attitudes are an "overestimation of future demand". In this study it may as well be an underestimation, because of the age of the respondents and their lack of experience of life. This may be particularly true with regard to pregnancy termination. Our interpretation of the findings of the study is that "Adolescents in Flanders are interested in being informed about genetic risks and genetic diseases and in making use of prenatal diagnosis because they want to make informed reproductive decisions in the future and to be emotionally prepared for the birth of an affected child" (see Abstract).

The comment that "these results would not apply in countries where fewer people have religious objections to abortion" can be questioned. Although the large majority of the population in Flanders is Roman Catholic, less than 15% of the adolescents reported that they attend church regularly or very frequently. Therefore we think that their objections to pregnancy termination are not purely religious but that they stem from more general negative feelings towards abortion. The commentator's objection that "we do not know what they understood by a severe genetic disorder" has been dealt with in the discussion of the paper. The adolescents may indeed have had completely different conditions in mind during the interview. The main point of interest, however, is their "subjective perception" of a serious situation.

At the end of the comment the following question is raised: "To what extent do we have a responsibility to provide information that people will not want to act on?". This question is misleading. "Genetic information" and "acting on information" should not be narrowed to decision making concerning a pregnancy termination. The information in education campaigns may elicit different types of actions: seeking additional information about genetic risks, refraining from having children, opting for prenatal diagnosis, a predictive test, or a carrier test . . . or deciding not to use the information. Indeed, the main aim of education campaigns associated with human genetics is to allow people to make free informed choices and genetic centres as well as policy makers have a responsibility in this field. In this respect the finding that adolescents develop a critical attitude when confronted with information about genetic diseases and genetic counselling is positive. Moreover, creating awareness of the psychological and ethical issues connected with genetic technology should be part of any information campaign concerning human genetics.

GERRY EVERS-KIEBOOMS
MARLEEN DECRUYENAERE
MYRIAM WELKENHUYSEN

Psychosocial Genetics Unit,
Centre for Human Genetics,
University of Leuven,
UZ Gasthuisberg, Herestraat 49,
3000 Leuven, Belgium



Identification of the wild type and IVS1(−13T→G) (Δexon2) alleles by ARMS. The normal allele (N) is amplified using 5′TCCCTGCTGTAGCCCGCTTT3′ as sense and 5′ACCCCAAGCTTGTGAGGTGC3′ as antisense primer. The mutant allele (M) is amplified using the same antisense primer and 5′CCTGCTGTAGCCCGCTTG3′ as sense primer. The melting temperature for each primer was the same (64°C). The PCR mixture consisted of: 20 mmol/l Tris-HCl (pH 8·4), 50 mmol/l KCl, 1 µmol/l $MgCl_2$, 10% DMSO, 0·005% W-1, 250 mmol/l of each dNTP, 1 µmol/l of each primer, 0·5 µg template DNA, and 2·5 units Taq DNA polymerase in a final volume of 50 µl. The PCR conditions were: 5 minutes at 94°C, 5 minutes at 59°C, and 5 minutes at 72°C (one cycle) followed by 2 minutes at 94°C, 2 minutes at 59°C, 3 minutes at 72°C (33 cycles), and ending with 10 minutes at 72°C. The fragments were analysed by 1·5% agarose gel electrophoresis. Lanes 1 and 2, homozygote for wild type allele. Lanes 3 and 4, heterozygote for IVS1(−13T→G) allele. M, marker.

Glycogen storage disease type II: frequency of three common mutant alleles and their associated clinical phenotypes studied in 121 patients

Glycogen storage disease type II (GSD II, acid maltase deficiency, Pompe disease) is an autosomal recessive trait caused by a deficiency of lysosomal α-glucosidase, leading to a characteristic accumulation of glycogen in lysosomes. Other types of glycogenosis are distinguished by cytoplasmic glycogen accumulation. Three clinical phenotypes are recognised: "infantile" (severe myopathy and cardiomyopathy with early onset and rapid progression usually leading to death within one year), "juvenile", and "adult" (myopathy with delayed onset and slow progression, no cardiac involvement).

Among the mutations recently discovered in the α-glucosidase gene,[1] three occur with increased frequency: a base substitution in intron 1 (−13T→G) leading to the aberrant splicing of exon 2 (IVS1(−13T→G)),[2] the deletion of exon 18 (Δexon18),[3,4] and the deletion of a single base (ΔT525) leading to premature termination of α-glucosidase synthesis.[5] Here we report the occurrence of these mutations in 121 patients and compare their frequency in infantile, juvenile, and adult GSD II, in Dutch and non-Dutch populations. The methods for mutation detection were the following. The Δexon18 and the ΔT525 alleles were identified as recently described by Van der Kraan *et al*[3] and Hermans

Table 1 Mutant allele frequency in glycogen storage disease type II

Clinical phenotype	*No of patients*	*Allele frequency*		
		IVS1(−13T→G)	*ΔT525*	*Δexon18*
Infantile	26*	0·00	0·35	0·31
	58	0.00	0.16	0.15
	32†	0·00	0·00	0·02
Juvenile	8	0·19	0·19	0·00
	13	0·15	0·15	0·00
	5	0·10	0·10	0·00
Adult	33	0·46	0·15	0·08
	50	0·38	0·11	0·05
	17	0·24	0·03	0·00

* Dutch patients.
† Non-Dutch patients; among the non-Dutch patients were European (35), Asian (7), African Black (1), North African (2), American Black (1), Turkish (6), Lebanese (1), and Cambodian/Finnish (1).

Table 2 Combinations of mutant alleles in patients with glycogen storage disease type II

	Infantile	Juvenile	Adult	α glucosidase activity*
IVS1(−13T→G)/IVS1(−13T→G)	–	–	–	?
ΔT525/ΔT525	5	–	–	<1
Δexon18/Δexon18	2	–	–	<1
Δexon18/ΔT525	4	–	–	<1
IVS1(−13T→G)/Δexon18	–	–	5	11·3–16·0
IVS1(−13T→G)/ΔT525	–	1	10	9·1–20

* α-glucosidase activity in cultured fibroblasts as % of the mean of normal controls.

et al,[5] respectively. To identify the IVS1(−13T→G) mutation, two PCR primer sets were designed, based on the amplification refractory mutation system (ARMS) described by Newton *et al.*[6] The first set amplifies specifically the wild type allele, the second set the IVS1(−13T→G) allele (figure). The frequencies of the three mutant alleles are given in table 1.

Our data confirm those of Huie *et al*[2] who found that IVS1(−13T→G) is a frequent mutation in adult GSD II, but is not associated with infantile GSD II. Further, we have shown the IVS1(−13T→G) mutation in juvenile GSD II. The mutation was not encountered in a total of 54 unrelated controls. The other two mutations are not restricted to certain phenotypes and are more frequent in the Dutch than in other patient populations. This could be the result of a founder effect.

By screening for these three mutations, we succeeded in identifying one mutant α glucosidase allele in 42 of 121 patients (35% of the total and 49% of the Dutch patient population). In an additional 27 patients, both alleles were identified (22% of the total and 39% of the Dutch patient population) allowing a genotype-phenotype correlation to be made (table 2).

Homozygotes for Δexon18 or ΔT525 and compound heterozygotes for these mutant alleles have the infantile form of GSD II and no α-glucosidase activity. In contrast, patients with either Δexon18 or ΔT525 in combination with IVS1(−13T→G) have the juvenile (one patient) or the adult (15 patients) phenotype and have 10 to 20% of the mean α-glucosidase activity in normal controls, suggesting that IVS1(−13T→G) is a mild mutation allowing α-glucosidase synthesis and function at 20–40% of the normal level. Homozygotes for IVS1(−13T→G) were not encountered, possibly because the residual activity in these subjects prevents clinical signs.

We propose that these data are good evidence of genotype-phenotype correlations in GSD II and suggest that genotype analysis may complement routine (prenatal) diagnosis, particularly when common mutations can be identified. Further, carrier detection in couples at risk becomes a practicable option.

Financial support was obtained from the Prinses Beatrix Fonds. We thank Dr Mark Nellist for critical reading of the manuscript.

M A KROOS
M VAN DER KRAAN
O P VAN DIGGELEN
W J KLEIJER
A J J REUSER
Department of Clinical Genetics, Erasmus University, PO Box 1738, 3000 DR Rotterdam, The Netherlands.
M J VAN DEN BOOGAARD
M G E M AUSEMS
H K PLOOS VAN AMSTEL
Clinical Genetics Centre, Utrecht, The Netherlands.

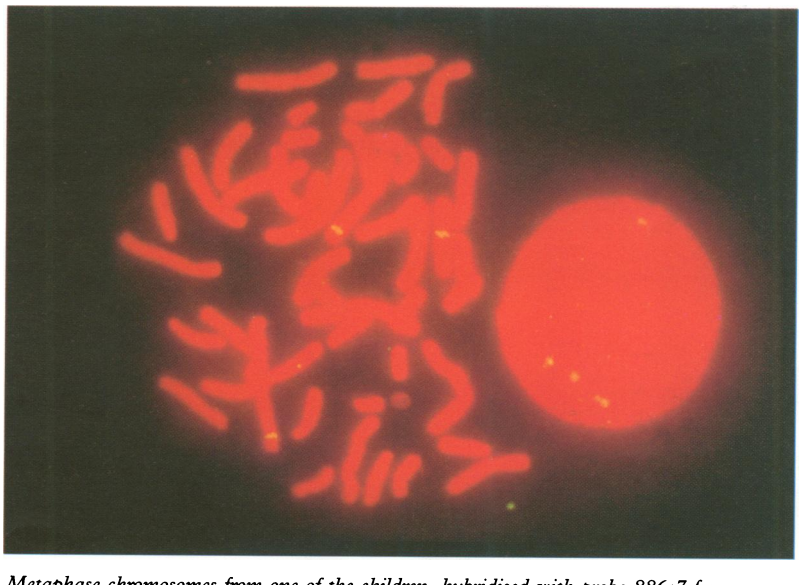
1 Reuser AJJ, Kroos MA, Hermans MP, *et al.* Glycogenosis type II (acid maltase deficiency). *Muscle Nerve* 1995;**suppl 3**:S61–9.
2 Huie ML, Chen AS, Tsujino S, *et al.* Aberrant splicing in adult onset glycogen storage disease type II (GSDII): molecular identification of an IVS1 (−13T→G) mutation in a majority of patients and a novel IVS10 (+1GT→CT) mutation. *Hum Mol Genet* 1994;**12**:2231–6.
3 Van der Kraan M, Kroos MA, Joosse M, *et al.* Deletion of exon 18 is a frequent mutation in glycogen storage disease type II. *Biochem Biophys Res Commun* 1994;**203**:1535–41.
4 Hui ML, Chen AS, Sklower Brooks S, Grix A, Hirschhorn R. A de novo 13 nt deletion, a newly identified C647W missense mutation and a deletion of exon 18 in infantile onset glycogen storage disease type II (GSDII). *Hum Mol Genet* 1994;7:1081–7.
5 Hermans MMP, De Graaff E, Kroos MA, *et al.* The effect of a single base pair deletion (ΔT525) and a C1634T missense mutation (pro545leu) on the expression of lysosomal α-glucosidase in patients with glycogen storage disease type II. *Hum Mol Genet* 1994;**12**:2213–18.
6 Newton CR, Graham A, Heptinstall LE, *et al.* Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic Acids Res* 1989;17:2503–16.

Partial trisomy 3q and the mild Cornelia de Lange syndrome phenotype

The article published by Holder *et al*[1] described two children whose facial features were very similar to children with mild de Lange syndrome. Cytogenetic analysis showed them to be trisomic for the region 3q25.1–26.2 as a result of inheriting an unbalanced interchromosomal insertion from their father who was a balanced insertion carrier. Fluorescence in situ hybridisation (FISH) studies using chromosome 3 paint confirmed that the insertion on chromosome 10 had originated from chromosome 3.

We were particularly interested in these children both because their phenotypes overlapped with that seen in mild de Lange syndrome and because they were duplicated for a very small region of chromosome 3. We had previously reported a child with Cornelia de Lange syndrome and a de novo translocation, t(3;17)(q26.3;q23.1)[2]. Before the identification of this patient it had been thought that the de Lange gene may map to 3q because of phenotypic overlap with subjects who were trisomic for variable regions of 3q. These people are usually trisomic for large regions of 3q and also monosomic for other variable regions of the genome. However, the common region of overlap in all cases is 3q26.3.

Chromosome 3q has two prominent Giemsa bands near the end of the q arm which are similar in size and vary only in the intensity of staining. The children described in the report of Holder *et al*[1] were thought to be trisomic for the more proximal Giemsa band in 3q26, 3q26.1, whereas the translocation breakpoint in the patient with the de novo translocation disrupts the more distal band, 3q26.3. Using a non-chimeric yeast artificial chromosome (YAC) clone (CEPH 886e7) positive for microsatellite marker AFM164tc7 (D3S1548), which is known from somatic cell hybrid data to map to 3q26.3,[3] we were able to show that the children are trisomic for the more distal of the two Giemsa bands on 3q26, that is, 3q26.3. The figure shows metaphase chromosome spreads from one of the children hybridised with the biotinylated YAC DNA that has been detected with FITC. In addition to two signals from distal 3q there is an additional signal on 10q. The fact that these children are trisomic for 3q26.3 further confirms that 3q26.3 is the 3q duplication critical region and that the de Lange syndrome gene maps in this area.



Metaphase chromosomes from one of the children, hybridised with probe 886e7 from 3q26.3. Note three signals are visible, two from 3q26.3 and one from chromosome 10.