

Screening and Diagnosis for the Fragile X Syndrome among the Mentally Retarded: An Epidemiological and Psychological Survey

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Summary

The fragile X syndrome is an X-linked mental retardation disorder caused by an expanded CGG repeat in the first exon of the fragile X mental retardation (FMR1) gene. Its frequency, X-linked inheritance, and consequences for relatives all prompt for diagnosis of this disorder on a large scale in all affected individuals. A screening for the fragile X syndrome has been conducted in a representative sample of 3,352 individuals in schools and institutes for the mentally retarded in the southwestern Netherlands, by use of a brief physical examination and the DNA test. The attitudes and reactions of (non)consenting parents/guardians were studied by (pre- and posttest) questionnaires. A total of 2,189 individuals (65%) were eligible for testing, since they had no valid diagnosis, cerebral palsy, or a previous test for the FMR1 gene mutation. Seventy percent (1,531/2,189) of the parents/guardians consented to testing. Besides 32 previously diagnosed fragile X patients, 11 new patients (9 males and 2 females) were diagnosed. Scoring of physical features was effective in preselection, especially for males (sensitivity .91 and specificity .92). Major motives to participate in the screening were the wish to obtain a diagnosis (82%), the hereditary implications (80%), and the support of research into mental retardation (81%). Thirty-four percent of the parents/guardians will seek additional diagnostic workup after exclusion of the fragile X syndrome. The prevalence of the fragile X syndrome was estimated at 1/6,045 for males (95% confidence interval 1/9,981–1/3,851). On the basis of the actual number of diagnosed cases in the Netherlands, it is estimated that >50% of the fragile X cases are undiagnosed at present.

Introduction

The identification of genes and their mutations has facilitated direct molecular diagnosis of numerous genetic disorders (McKusick 1995). The criteria for introduction of new diagnostic procedures, such as target groups and an active or passive approach, are still under debate for several genetic disorders, including the fragile X syndrome (Bonthron and Strain 1993; Bunday and Norman 1993; Howard-Peebles et al. 1993; Palomaki and Haddow 1993; American College of Medical Genetics 1994; Craft 1995; Laxova 1995). The fragile X syndrome screening program presented here gives a model for actively introducing a new DNA diagnostic procedure and, moreover, a method to obtain accurate prevalence data.

The fragile X syndrome is characterized by X-linked mental retardation with additional features such as a long face with large protruding ears, macroorchidism, and eye-gaze avoidance (Fryns 1989; Hagerman 1996). Affected males and most of the affected females show a fragile site at Xq27.3 in a percentage of the cells tested under special culture conditions (Sutherland and Hecht 1985); that method was used until the cloning of the gene. The first estimates of the prevalence of the fragile X syndrome, based on cytogenetic testing, were 1/1,000–1/2,600 for males and 1/2,000–1/4,000 for females (Turner et al. 1986; Webb et al. 1986).

The cloning of the fragile X mental retardation (FMR1) gene in 1991 (Oberlé et al. 1991; Verkerk et al. 1991; Yu et al. 1991) enabled an accurate molecular diagnosis. Affected individuals have expanded CGG repeats (>200) in the first exon of the FMR1 gene (the so-called full mutation). This expansion is accompanied by hypermethylation of the repeat and its upstream region, resulting in a shutdown of transcription and absence of the FMR1 protein (Pieretti et al. 1991; Sutcliffe et al. 1992; Verheij et al. 1993). In the normal population, the (CGG)_n repeat is 6–54 units (Fu et al. 1991). Phenotypically normal carriers of a repeat size of 43–200 are called “premutation carriers” (Fu et al. 1991). Female carriers of a premutation or full mutation have an increased risk of affected offspring. Male carriers of

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a premutation will transmit this, usually unaltered, to their daughters.

Screening for the fragile X syndrome by DNA analysis was offered to mentally handicapped individuals in schools and institutes for the mentally retarded in the southwestern Netherlands. We analyzed the acceptance by parents/guardians of mentally retarded individuals, feasibility of such a screening program, and the prevalence of the fragile X syndrome in the Dutch population. The pre- and posttest attitudes and expectations of consenting and nonconsenting relatives were studied.

Patients and Methods

Since 1992, a screening program for the fragile X syndrome has been conducted in 5 institutions giving residential care (1,869 individuals aged 4–89 years, mean age 39.0 years) and 16 special schools (1,483 individuals aged 5–21 years, mean age 13.0 years) for mentally retarded individuals in the southwestern Netherlands. Persons without a known cause of their mental handicap, without cerebral palsy (with quadriplegia), and without previous DNA-mutation analysis of the FMR1 gene (on the basis of medical records and previous medical investigations) were eligible for a brief physical examination and venipuncture for DNA analysis of the FMR1 gene. Parents/guardians were informed by letter and through information meetings. After the parents/guardians' written consent was obtained, the subjects were included in the study. Organizations for parents/relatives were informed prior to the onset of the program. Also, the medical, nursing, and teaching staff of the various institutes and schools were informed in separate meetings. Parents/guardians of newly diagnosed patients were offered genetic counseling and were asked to participate in a follow-up study. The study was approved by the Medical Ethical Committee of the Erasmus University and University Hospital Dijkzigt (Rotterdam) and by the respective institutional ethical review committees.

Physical Examination

Each individual was scored by one of us for fragile X features according to criteria of Laing et al. (1991) (family history of intellectual handicap, personality, large/prominent ears, elongated face, and body habitus); additional items were hyperextensible finger joints, soft/smooth skin, and macroorchidism. Additionally, the height and head circumference and dysmorphic features, not related to the fragile X syndrome, were recorded. Before disclosure of the DNA test result, the individuals were divided into low-, moderate-, and high-risk groups—"low" when dysmorphic features suggested a diagnosis other than fragile X syndrome, "moderate" in the absence of specific dysmorphic features, and "high" in the presence of fragile X syndrome characteristics.

Intellectual functioning—profound/severe (IQ <30), moderate (IQ 30–50), or mild mental retardation (IQ 50–70)—was established by each individual's psychologist, by IQ testing in schoolchildren, or by clinical estimation in the institutionalized individuals.

DNA Analysis

A 10-ml blood sample was obtained from each individual, and genomic DNA was isolated from blood leukocytes (Miller et al. 1988). PCR analysis of the CGG repeat was performed according to the method of Fu et al. (1991), with modifications (van den Ouweland et al. 1994). In all males without a fragment in the normal range (6–54 CGG repeats) and for all females without two distinguishable normal fragments, additional Southern blot analysis on *Hind*III-digested DNA, using the intragenic probe pP2, was performed (Oostra et al. 1993).

Questionnaires to Consenting and Nonconsenting Parents/Guardians

The acceptability of the screening program and the (anticipated) implications of test results was assessed in a pre- and posttest questionnaire study. A sample of consenting parents/guardians ($n = 1,090$) received a pretest questionnaire, after the blood sample was taken from their relative, and a posttest questionnaire, 3 wk after the test result was obtained. A reminder was sent after 3 wk. Nonconsenters ($n = 435$) received a questionnaire to ask them about their motives. A translation of the questionnaires is available on request.

Statistical Analysis

The data were analyzed with version 6.0 of SPSS for Windows and the software Confidence Interval Analysis (CIA) compiled by Gardner and Altman. The data are presented as a proportion or percentage with a 95% confidence interval (CI). Differences between groups were assessed with the χ^2 test, and the significance levels (two tailed) will be presented.

Results

Study Population, Physical Examination, and DNA Testing

Sixty-five and one-half percent (2,170/3,313) of the mentally retarded individuals were eligible for testing. Reasons for exclusion of the other 1,143 individuals included an earlier diagnosis of the fragile X syndrome (32/1,143 [2.8%]); its exclusion by DNA testing (36/1,143 [3.1%]) or a causative diagnosis, such as Down syndrome (474/1,143 [41.5%]); or other valid diagnosis, including cerebral palsy, confirmed by medical records (601/1,143 [52.6%]) (percentages of totals are shown in table 1). Seventy percent (1,520/2,170) of the parents/guardians of eligible patients consented to

Table 1**Overview of Study Sample: Gender and Level of Mental Retardation**

	NO. OF INDIVIDUALS				Total (% of Entire Sample) ^a
	Moderate/Severe		Mild		
	Males	Females	Males	Females	
Not eligible for testing:					
Fragile X syndrome	24	0	6	2	32 (1.0)
FMR1 mutation excluded	15	6	14	1	36 (1.1)
Down syndrome	230	204	20	20	474 (14.3)
Other chromosomal abnormality	18	29	2	6	55 (1.7)
Metabolic disorder	12	13	3	7	35 (1.1)
Cerebral palsy	133	100	8	6	247 (7.5)
Other	91	76	55	42	264 (8.0)
Subtotal	523	428	108	84	1,143 (34.5)
Eligible for testing:					
Nonparticipants	213	194	161	82	650 (19.6)
Participants	533	461	333	193	1,520 (45.9)
Subtotal	746	655	494	275	2,170 (65.5)
Grand Total	1,269	1,083	602	359	3,313

^a Excludes 39 patients with an unknown level of mental retardation.

participation. The use of the test was higher in the 5 institutions than in the 16 special schools (74.4% [95% CI 71.9%–76.9%] versus 64.6% [95% CI 61.6%–67.6%]). For 39 of the 3,352 individuals, the level of intellectual development could not be ascertained, and those individuals were excluded in those analyses for which this level was required.

A total of 1,501 of the 1,531 tested individuals (including 11 with an unknown level of mental retardation who are not included in table 1) had an CGG repeat in the normal range (<43 repeats). For 12% of the males and 59% of the females, the PCR test result was inconclusive, and an additional Southern blot analysis was done. Although no individuals with a premutation were detected, 19 individuals (1.2%) had an allele with a size in the “intermediate range” (43–60 CGGs), and among those was one female with an allele in the range of 55–60 CGG repeats. Further study was feasible in the families of nine individuals (range 43–55 CGGs), and in those families neither instability of the CGG repeat nor fragile X patients could be detected. Eleven fragile X patients (0.7% [9 males and 2 females]) were newly diagnosed. Seven of those resided in an institution, and four attended a special school. Ten of 11 detected cases were in the group of 134 cases with a high risk for having the fragile X syndrome (on the basis of physical examination, sensitivity .91 [95% CI .59–1.00], and specificity .92 [95% CI .90–.93]. Moreover, all newly diagnosed male patients showed the high-risk phenotype (table 2).

Estimated Prevalence of the Fragile X Syndrome

The prevalence of the fragile X syndrome was estimated for the various levels of mental retardation in

male individuals studied (i.e., mild [IQ 50–70] and moderate/severe retardation [IQ <50]) (table 3). The estimation of the population prevalence is restricted to the data from males in this study because females with a full mutation in the FMR1 gene have an intellectual development varying from severely retarded to normal. The latter group was not included in this study among the mentally retarded.

In the group of mildly retarded males ($n_{\text{tot}} = 602$), 4 fragile X patients (f_p) were newly diagnosed among the participants ($n_p = 333$), for a relative prevalence (p_p) of .01201, and 6 fragile X patients (f_{ne}) had been previously diagnosed among the individuals who were not eligible for testing ($n_{\text{ne}} = 108$), for a relative prevalence (p_{ne}) of .05555 (see table 3). If the relative prevalence in the nonparticipating group ($n_{\text{np}} = 161$) is assumed to be the same as that in the participating group (namely, .01201), the total prevalence in the sample of mildly retarded

Table 2**Phenotype of Newly Diagnosed Fragile X Patients**

PHENOTYPE SUGGESTIVE OF FRAGILE X SYNDROME	FREQUENCY OF FRAGILE X SYNDROME		TOTAL
	Males	Females	
Low	0/223	0/251	0/474
Moderate	0/555	1/368	1/923
High	9/92	1/42	10/134
Total	9/870	2/661	11/1,531

Table 3**Prevalence of the Fragile X Syndrome, Estimated for Males in the Dutch Population**

LEVEL OF RETARDATION	STUDY SAMPLE										POPULATION	
	Participants			Nonparticipants	Not eligible			Total		N	F	
	n_p	f_p	p_p	n_{np}	n_{ne}	f_{ne}	p_{ne}	n_{tot}	p_{tot}			
Mild	333	4	.01201	161	108	6	.05555	602	.0198	30,000	595	
Moderate/severe	<u>533</u>	<u>5</u>	.00938	<u>213</u>	<u>523</u>	<u>24</u>	.04589	<u>1,269</u>	.0244	<u>27,000</u>	<u>660</u>	
	866	9		374	631	30		1,871		57,000	1,255	

males (p_{tot}) is $([333+161] \cdot .01201 + [108 \cdot .05555])/602 = .0198$. With an estimate of 30,000 mildly retarded males (N) in the Netherlands (95% CI 27,700–33,300) (Maas et al. 1988), one may expect 595 mildly retarded fragile X males (F) in this population (95% CI 309–1,038).

In the sample of moderately/severely retarded males ($n_{tot} = 1,269$), 5 fragile X patients (f_p) were newly diagnosed among the participants ($n_p = 533$), for a relative prevalence (p_p) of .00938, and 24 fragile X patients (f_{ne}) had previously been diagnosed among the patients who were not eligible for testing ($n_{ne} = 523$), for a relative prevalence (p_{ne}) of .04589. If the relative prevalence in the nonparticipating group ($n_{np} = 213$) is assumed to be the same as that in the participating group (namely, .00938), the total prevalence in the sample of moderately/severely retarded males (p_{tot}) is $([533+213] \cdot .00938 + [523 \cdot .04589])/1,269 = .0244$. With an estimate of 27,000 moderately/severely retarded males (N) in the Netherlands (95% CI 23,700–29,500) (Maas et al. 1988), we estimated that the number of fragile X male patients with a moderate/severe mental handicap (F) should be 660 fragile X male patients (95% CI 451–932) in this population.

For the Netherlands, with 7,586,000 male residents (Statistical yearbook of the Netherlands 1995), a total of 1,255 males with the fragile X syndrome will result in a prevalence of 1/6,045 for males (95% CI 1/9,981–1/3,851). Varying the assumed relative prevalences in the nonparticipating group (half or double of the participating group) leads to prevalences for males that are 1/6,418 (95% CI 1/10,669–1/4,037) and 1/5,415 (95% CI 1/8,719–1/3,538), respectively.

When a similar analysis is used for Down syndrome in our male study sample (20 mildly and 230 moderately/severely retarded males with Down syndrome), a prevalence of 1/1,288 for Down syndrome males was found (95% CI 1/1,538–1/1,087). This is similar to data from the United Kingdom (Steele and Stratford 1995). The prevalence of the fragile X syndrome did not differ significantly between the mildly retarded males and the moderately/severely retarded males (.0198 and .0244, respectively).

Motives for Participation or Nonparticipation

Pretest attitude responses from consenting parents/guardians.—The response rate was 79% (860/1,090), and most (71%) of the respondents were parents. Eighty-four percent had discussed the DNA test with relatives and would inform them about the result. Major motives to participate were the wish to have a diagnosis, the hereditary implications, and the support of research into mental retardation (table 4). Eighteen percent of the respondents (95% CI 15%–21%) expected that the fragile X syndrome would be diagnosed in their retarded relative, 30% were uncertain (95% CI 27%–34%), and 52% did not expect the diagnosis (95% CI 48%–55%). Six percent had intrusive thoughts and/or feelings about the test and its outcome (95% CI 5%–8%). Parents/guardians of schoolchildren expected significantly more often that a diagnosis would improve the care of their retarded family member than did parents/guardians of institutionalized individuals (table 4).

Posttest attitude responses from consenting parents/guardians.—The response rate was 66% (681/1,030; a follow-up questionnaire could not be sent to 51 parents/guardians, and the parents/guardians of the newly diagnosed were offered genetic counseling). One-third (35%) of the respondents were relieved by the exclusion of the fragile X syndrome (95% CI 31%–38%). One-third (95% CI 29%–37%) were not relieved, and 5% (95% CI 3%–6%) were even disappointed. Eighteen percent (95% CI 15%–21%) still worried about possible genetic implications for their family. The majority (87%) had informed their relatives about the test result.

After the exclusion of the fragile X syndrome in their relative, the parents/guardians of schoolchildren were significantly more willing to pursue further investigations, both actively and passively, than were the parents/guardians of institutionalized individuals (table 4). Respondents (80% [95% CI 77%–83%]) appreciated the test and would recommend participation in such a program to others.

Attitude responses from nonconsenting parents/guardians.—The response rate was 35% (153/435). Nonconsenters differed only by having significantly higher education levels than consenters (those having at least a

Table 4**Motives for (Non)Participation, in Parents/Guardians of Mentally Retarded Individuals in Schools and Institutes**

	PERCENT OF INDIVIDUALS AGREEING (95% CI) ^a			χ^2	P
	Schools	Institutes	Total		
	Q = 325	Q = 535	Q = 860		
Consenting parents/guardians:					
Pretest					
Wish to have a diagnosis	88 (85-92)	78 (75-82)	82 (79-85)	13.03	<.001
Hereditary implications	79 (74-83)	81 (78-85)	80 (78-83)	.33	.56
Support research into mental retardation	72 (67-77)	87 (85-90)	81 (79-84)	28.59	<<.0001
Expecting better care after fragile X diagnosis	68 (63-73)	47 (43-52)	55 (52-59)	35.45	<.0001
	Q = 248	Q = 433	Q = 681		
Posttest					
Will seek further investigations ("active")	43 (37-50)	28 (24-32)	34 (30-37)	15.68	<.0001
Will use new diagnostics when offered ("passive")	78 (72-83)	57 (53-62)	65 (61-69)	28.87	<<.0001
	Q = 55	Q = 98	Q = 153		
Nonconsenting parents/guardians:					
Blood test is too stressful for family member	42 (27-58)	73 (61-83)	61 (52-70)	13.68	<.001
"Definite" cause of mental handicap is known	27 (15-41)	54 (42-65)	44 (35-52)	10.59	<.01
Any possible cause of retardation is different from fragile X syndrome	56 (38-74)	69 (56-80)	64 (54-74)	2.08	.15

^a Q = number of questionnaires obtained.

high school-level education were 64% [95% CI 56%–72%] and 47% [95% CI 43%–50%], respectively). The majority (78%) had discussed the DNA test with others. The main reasons for nonconsenting were the opinion that a definite cause of the mental handicap in their relative was already known or the conviction that any possible cause must be different from the fragile X syndrome (table 4). Significantly more often among nonconsenting parents of institutionalized persons, the test was considered as too stressful for their relative (table 4). Nonparticipation was neither influenced by fear of possible consequences of the test (9% [95% CI 4%–6%]) nor by religion (6% [95% CI 2%–12%]). Generally, nonconsenters were not opposed to genetic testing (72% [95% CI 64%–80%]). One-third (95% CI 24%–42%) even considered the future use of other diagnostic options if these would become available.

Discussion

This first comprehensive genetic epidemiological study of a representative sample of male and female mentally retarded individuals from a population (the Netherlands) of 15×10^6 inhabitants, using DNA techniques for the fragile X syndrome, indicates that the prevalence of the fragile X syndrome in males in the general Dutch population is 1/6,045. This is consider-

ably lower than the previously reported prevalence of 1/1,000–1/2,600 (Turner et al. 1986; Webb et al. 1986) but is similar to more-recent reports of 1/4,000–1/5,000 (England and Australia) (Murray et al. 1996; Turner et al. 1996). However, the sample sizes of these recent studies did not allow very accurate estimates; nor was a representative sampling of mentally retarded males achieved. The earlier high estimates were obtained by cytogenetic studies, with possible confounding either by other fragile sites in this region of the X chromosome or by false positives (Turner et al. 1996). The current estimate might be conservative; the relative prevalence in the nonparticipating group might be variously estimated (see Results) but would minimally influence the estimate. Also, the PCR method is not 100% sensitive, since fragile X patients with mosaicism for a normal allele in combination with a full mutation might be missed. However, these patients are very rare.

In the Netherlands, among 7.6 million males, 1,255 males with the fragile X syndrome may be expected, probably without a difference between the distribution in mildly retarded males and that in moderately/severely retarded males. However, the seven clinical genetic centers, covering the whole country, identified ~450 male cases so far (B. A. Oostra, unpublished data). This suggests an underdiagnosis of >50%. In our study, one-fourth of the fragile X patients were

newly diagnosed cases. This study included both institutionalized (all ages) and noninstitutionalized (age <21 years) but no noninstitutionalized adult retarded individuals. Most people in the latter group work in sheltered workshops and live either with their relatives or in sheltered homes. The fragile X syndrome is likely to be most underdiagnosed in this group, because of the lack of diagnostically oriented medical care for these individuals. Improvement of genetic diagnosis in these settings is important, also for counseling of the families. Selection of male patients for FMR1 gene analysis might be facilitated by evaluation of dysmorphic features, since the presence of fragile X features was found to increase 10-fold the yield of positive molecular diagnosis (table 2). Such clinical preselection is less efficient for females, because of the variability of expression of the full FMR1 mutation in females (Fryns 1989; Hagerman 1996).

Genetic carrier screening may be done at a young adult age, especially to identify and inform female carriers of the pre- and full mutation prior to parenthood. One study in the French Canadian population has suggested a 1/259 frequency of premutation-carrier females (Rousseau et al. 1995). Alternatively, screening for the fragile X syndrome might start—as presented here—among (young) mentally retarded individuals, which will allow families of newly diagnosed cases the option of avoiding the birth of a subsequent affected child. However, genetic screening programs are under debate, for reasons of privacy, the risk of medicalization, the risk of losing insurance, and the lack of treatment options. In the introduction of a screening program among the mentally retarded, a careful assessment of the acceptability by the families directly involved is of primary importance. The present study showed informed consent by parents or guardians in 71% of the eligible patients, which is in accordance with other reports (Jacobs et al. 1993; Hagerman et al. 1994; Slaney et al. 1995; Murray et al. 1996). However, a high use of the test is only one of the parameters of acceptance. Motives for consenting were the wish to have a diagnosis, the possibility of hereditary implications, and the support of research into mental retardation. In general, there was openness in the family about having the relative tested: the majority of consenting parents/guardians discussed with others both the DNA test and its result. The pretest expectations of the consenting parents/guardians seem realistically reserved, since only a minority actually expected a diagnosis of the fragile X syndrome in their relative. However, none of the newly diagnosed cases had been anticipated by the parents/guardians. Most parents of the newly diagnosed patients felt relieved by the resolution of uncertainty and by the lack of direct responsibility for the retardation. They acknowledged the genetic nature of the condi-

tion and informed their relatives, as was found elsewhere (Turner et al. 1992).

The test procedure did not cause undue anxiety among the participants, and most were interested in future diagnostic studies when these become available. One-third of consenting parents/guardians arranged for additional diagnostic investigations and genetic counseling, as was reflected by a sharp increase in referrals for clinical genetic and dysmorphological workup.

The observations in the nonconsenting group should be interpreted with caution, given the low response. Reluctance was felt because of “stressful” blood sampling. That might be alleviated, in the future, by the FMR1 protein test, which requires only a few blood drops (Willemssen et al. 1995), or by a test using DNA isolated from a mouthwash or cheek brush (Hagerman et al. 1994; Murray et al. 1996). The majority of the nonconsenters believed that a “definite” cause for the handicap had already been established, however vague that diagnosis might have been. However, nonconsenters agreed with the general principle of performing DNA and other diagnostic investigations among the mentally retarded.

Several goals of a diagnostic program—that is, establishment of a cause for mental retardation and more complete information and choice for parents and relatives—are obviously achieved in this study. Even in a northwestern European country with well-developed diagnostic facilities, >50% of fragile X cases seem undiagnosed at present. This reflects the slow rate of introduction of new diagnostic facilities in the care of the mentally handicapped. In a period of DNA technology and fears of genetic discrimination, this study shows that parents/guardians of individuals with mental handicaps have a realistic idea about potentials and limitations of new technologies, if they are adequately informed. The fear of health-care authorities and others regarding adverse effects of the study of larger groups of mentally handicapped individuals may be alleviated by the realistic appraisal seen on the part of those directly involved.

Appendix

Other participants in the Rotterdam Collaborative fragile X screening study group included M. de Groot, J. van den Berg, P. Deman, J. van Grinsven, and H. Veere (Craeyenburch, Nootdorp); A. Idzinga, A. Trappenburg, W. Soeters, and C. Clement (Het Westerhok, Monster); E. Weijers and C. de Leeuw (SVVGR Rotterdam); L. Imschoot, J. den Hartigh, M. Heijkoop, and M. Dekker (De Merwebolder, Sliedrecht); H. Hoogeveen, A. Vosenaar, M. de Jager, and C. Ferero (GGD Rotterdam); S. Mosterd (GGD Nieuwe Waterweg Noord); E. Gelsema-Mudde, B. Becker, J. Akos, and T. de Jong (GGD Zuid-Holland Zuid); L. van Elderen (GGD Zuid Hollandse Eilanden); J. de Wijs (GGD Stadsgewest Breda); H. Franken (GGD Streekgewest Westelijk Noord Brabant); J.

de Ru (GGD Zeeland); M. Bommezijn (GGD Midden-Holland); N. de Vries–van Waert (GGD Delftland); and J. Wijnmaalen and L. Vorselen (Gorkum).

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