

Subtelomeric chromosomal rearrangements in a large cohort of unexplained intellectually disabled individuals in Indonesia: A clinical and molecular study

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CONTEXT: Unbalanced subtelomeric chromosomal rearrangements are often associated with intellectual disability (ID) and malformation syndromes. The prevalence of such rearrangements has been reported to be 5-9% in ID populations.

AIMS: To study the prevalence of subtelomeric rearrangements in the Indonesian ID population.

MATERIALS AND METHODS: We tested 436 subjects with unexplained ID using multiplex ligation dependent probe amplification (MLPA) using the specific designed sets of probes to detect human subtelomeric chromosomal imbalances (SALSA P070 and P036D). If necessary, abnormal findings were confirmed by other MLPA probe kits, fluorescent *in situ* hybridization or Single Nucleotide Polymorphism array.

RESULTS: A subtelomeric aberration was identified in 3.7% of patients (16/436). Details on subtelomeric aberrations and confirmation analyses are discussed.

CONCLUSION: This is the first study describing the presence of subtelomeric rearrangements in individuals with ID in Indonesia. Furthermore, it shows that also in Indonesia such abnormalities are a prime cause of ID and that in developing countries with limited diagnostic services such as Indonesia, it is important and feasible to uncover the genetic etiology in a significant number of cases with ID.

Key words: Indonesia, intellectual disability, multiplex ligation dependent probe amplification, subtelomeric rearrangements

Introduction

The genetic causes of intellectual disability (ID) comprise (sub) microscopically chromosome abnormalities and monogenic diseases.^[1] Microscopically visible numerical and structural abnormalities are the most common cause of ID. In a large meta-analysis review, a median rate of 9.5% was described.^[2] Apart from these microscopically visible chromosomal abnormalities, there are submicroscopic abnormalities that cannot be detected by conventional karyotyping. Abnormalities in the most distal ends of chromosomes, which harbor the highest gene concentrations in the human genome,^[3] are difficult to identify on routine chromosome analysis while they represent an important genetic cause of idiopathic ID. Therefore, testing for such rearrangements has turned out to be an important clinical evaluation step in the etiological diagnosis of unexplained ID cases.^[4] In several studies, subtelomeric rearrangements were found to be associated with moderate to severe phenotypic abnormalities and turned out to be a significant cause of ID, with an estimated prevalence of 5-9% of cases in various populations.^[5-7] To date, however, there are no data about the prevalence of subtelomeric rearrangements in Indonesia.

In a large number of Indonesian ID patients, the cause of ID could be established by conventional karyotyping or molecular testing for fragile X syndrome (FXS), but the majority of cases still remained unexplained.^[8] Therefore,

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this study aimed at determining the prevalence of subtelomeric rearrangements and the clinical features in these ID individuals in Indonesia.

Materials and Methods

This research is an extension of previously reported studies on the identification of genetic causes of ID in Indonesia, where chromosomal aberrations and FXS were investigated in a large cohort of 527 Indonesian ID individuals from several special schools and institutions in Java Island, Indonesia. These previous studies revealed chromosomal abnormalities in 82 individuals and FXS in 9 individuals.^[8,9] In the present study, molecular testing of subtelomeric deletions and duplications was performed in the 436 as yet unresolved patients (278 males and 158 females). Informed consent was obtained from the parents or legal representatives, and the study has been approved by the Ethical Board of our institute. All subjects underwent a standardized clinical examination including physical measurements and dysmorphological assessment.

The DNA was isolated from peripheral blood using the salting out extraction procedure as described elsewhere.^[10] Multiplex ligation dependent probe amplification (MLPA) analysis was performed as described previously.^[11] Two probe-kits for subtelomeric chromosomal imbalances were used in these experiments: SALSA P070 and SALSA P036D MRC-Holland, Amsterdam, The Netherlands (<http://www.mrc-holland.com>). Each subtelomeric rearrangement was identified by at least one additional MLPA analysis using the SALSA P070 as the first level screening. Afterwards, SALSA P036D was utilized for confirmation of the aberration detected with the P070 kit. Rearrangements in specific regions were verified with SALSA kit P028, P023B, P340A or P096. The detail of regions detected by each kit is available at <http://www.mlpa.com>. Amplification products were identified and quantified by capillary electrophoresis on an ABI 3730 genetic analyzer (Applied Biosystems, Foster City, CA, USA) using GeneMapper Software version 3.7 (Apache Software; <http://www.apache.org>), manufacturer (University of Tokyo). Statistical analyses were carried out using Microsoft® Excel spreadsheets as described before.^[12] The results were considered abnormal when the relative peak height ratio was below 0.70 or above 1.30.

When a deletion or duplication was detected in both MLPA kits, parents were tested for *de novo* occurrence. When parental DNA was not available, additional methodologies were performed for confirmation of the presence of the detected deletion or duplication. Fluorescence *in situ* hybridization (FISH) analysis was performed using commercially available probes (Vysis, Inc., Downers Grove, IL, USA) according to the manufacturer's recommendations. Single Nucleotide Polymorphism (SNP) array analyses were performed using the Affymetrix Nspl 250K SNP array platform (www.Affymetrix.com). Copy number estimates were determined using the Copy Number Analyzer for Affymetrix Genechip Mapping (CNAG) software package version 2 (<http://www.genome.umin.jp/>), manufacturer (University of Tokyo).^[13]

The clinical data of all patients were reviewed and compared to other cases with a comparable aberration, described in the literature.

Results

The initial screening with the SALSA P070 probe kit showed a subtelomeric deletion and/or duplication in 23 of the 436 ID individuals [Table 1]. In 20 of these samples, the presence of the aberration could be confirmed by the SALSA P036D kit [cases 1-20, Table 1], while in the three remaining cases, this was not possible and they were considered to be either artefacts or non-causative variants. Parental testing was possible in eight of the 20 cases (cases 1-8) and revealed a *de novo* occurrence of the subtelomeric imbalances in five cases (cases 1-4 and 6). These five aberrations were, therefore, considered being pathogenic [Tables 1 and 2]. The phenotypes of cases 1 and 2 share many similarities with known cases with monosomy 18pter^[14] or a subtelomeric duplication of 9p24,^[15] respectively. In cases 3, 4, and 5, a subtelomeric deletion appeared to be coexistent with a subtelomeric duplication, which implicates the presence of a cryptic unbalanced translocation. The phenotype of case 3 was comparable to reported cases with either a 4p duplication^[14] or a 18p deletion.^[16] Therefore, either dup 4pter or deletion 18pter (or both) could be contributing to the phenotype in the case 3. In case 4, the phenotype

Table 1: Subtelomeric rearrangements found in this study

Case	ID	Sex	MLPA result	Additional method	Inheritance	Confirmation result	Conclusion
Case 1	ID 398	F	Del 18pter	-	<i>De novo</i>		Pathogenic
Case 2	ID 190	M	Dup 9pter	-	<i>De novo</i>		Pathogenic
Case 3	ID 419	F	Del 18pter/dup 4pter	SNP array	<i>De novo</i>	Arr snp 4p16.3 (SNP_A-2136377-> SNP_A-2289732) x3 Arr snp 18p11.32 (SNP_A-1878089-> SNP_A-1781333) x1	Pathogenic
Case 4	ID 386	F	Del 10pter/dup 9pter	-	<i>De novo</i>		Pathogenic
Case 5	ID 473	F	Del 9pter/dup 9qter	SNP array, FISH	Inherited	Arr snp 22q11.21 (SNP_A-2108791-> SNP_A-2160861) x1	Pathogenic
Case 6	ID 400	M	Del X/Yqter	SNP array	<i>De novo</i>	9p24.3 (SNP_A-2227623; SNP_A-1898345) x1 9q34 (SNP_A-4223541; SNP_A-1874845) x3	Pathogenic*
Case 7	ID 460	F	Del 16qter	-	Inherited		Non pathogenic
Case 8	ID 207	M	Dup 2pter	-	Inherited		Non pathogenic
Case 9	ID 264	M	Del 4pter	MLPA p096	NA	Deletion whole WHSCR	Pathogenic
Case 10	ID 490	M	Dup 22pter	MLPA p023	NA	2.6 Mb duplication of 22q11 and a 1.8Mb duplication 22q13	Pathogenic
Case 11	ID 502	M	Del 9pter	SNP array	NA	Arr SNP 9p24.3 (SNP_A-2227623-SNP_A-4235315) x1	Pathogenic
Case 12	ID 366	M	Del 9pter	-	NA		Pathogenic
Case 13	ID 698	F	Dup 15qter	MLPA me028	NA	Duplication between BP1-BP3	Pathogenic
Case 14	ID 471	F	Del 2qter	-	NA		Pathogenic
Case 15	ID 462	F	Dup 11pter/del Xpter	-	NA		Pathogenic
Case 16	ID 641	M	Del 12pter/dup 12qter	-	NA		Pathogenic
Case 17	ID 640	M	Del 8pter	-	NA		Possible pathogenic
Case 18	ID 219	M	Dup X/Ypter	-	NA		Possible pathogenic
Case 19	ID 169	M	Dup 16qter	-	NA		Possible variant
Case 20	ID 671	M	Dup 16qter	-	NA		Possible variant
Case 21	ID 237	M	Dup 3q	-	NA		Variant
Case 22	ID 269	M	Dup 13q	-	NA		Variant
Case 23	ID 363	F	Del 9q	MLPA p340A1	NA	p340A1 not confirmed	Variant

*SNP array revealed a 2 Mb deletion of chromosome 22q12, NA: Not available, MLPA: Multiplex ligation dependent probe amplification, SNP: Single nucleotide polymorphism

Table 2: Clinical features of individuals with subtelomeric rearrangements*

Case no.	Case 1	Case 2	Case 3	Case 4	Case 6	Case 9	Case 10
MLPA result	Del 18pter	Dup 9pter	Del 18pter/dup 4pter	Del 9p/dup10p	Del X/Yqter	Del 4pter	Dup 22q
Gender	F	M	F	F	M	M	M
Age	16	24	11	10	12	24	25
Inheritance	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	NA	NA
ID level	Mild	Mild	Moderate	Moderate	Moderate	Moderate	Mild
Hypotonia	Yes	No	Yes	Yes	Yes	Yes	No
Weight	32 kg (<P3)	45 kg (<P3)	21 kg (<P3)	18 kg (<P3)	23 kg (<P3)	37 kg (<P3)	48 kg (P3-P25)
Height	145 cm (<P3)	161 cm (<P3)	123 cm (<P3)	115 cm (<P3)	134 cm (P3)	143 cm (<P3)	161 cm (<P3)
OFC	50 cm (<P3)	52.5 cm (<P3)	48 cm (<P3)	48 cm (<P3)	47 cm (<P3)	48 cm (<P3)	54 cm (P25)
Behavior problems	Introvert, shy	-	Aggressive	ADHD	Aggressive		
Other features							
Head/face	Prominent forehead, flat occiput, brachycephaly, flat face	Sloping forehead, hypoplastic maxilla	Brachycephaly	Brachycephaly, flat face, high forehead	Long and narrow face	Prominent glabella, greek helmet	High and broad forehead
Ocular region		Deep set, hypertelorism	Ptosis, epicanthal folds, strabismus, downslanting palpebral fissure	Hypertelorism, ptosis, hypoplastic orbita, strabismus, sparse eyebrows	Strabismus, telecanthus,	Hypertelorism	
Nose	Flat nose, broad and deviated nasal bridge	Globular nose	Depressed nasal bridge	Globular and broad base of the nose	Broad base of the nose,	Depressed nasal bridge, broad nose	
Ears	Prominent antihelix	Prominent and low set	Posteriorly rotated, low set	Prominent and posteriorly rotated		Low set and simple, preauricular pits	Prominent ears

Contd...

Table 2: (Contd)

Case no.	Case 11	Case 12	Case 13	Case 14	Case 15	Case 16	Case 17	Case 18
Mouth region	Unilateral cleft lip	Micrognathia	Downturned mouth, misaligned teeth	Short philtrum, downturned mouth, pseudocleft	Wide mouth, thick lips	Downturned mouth, bilateral cleft lip and palate, micrognathia	Micrognathia	
Thorax/abdomen			Wide-spaced nipples	Pectus excavatum		Bulging of the thorax		
Extremities		Single palmar crease, long fingers, flat feet, sandal gap	Short 5 th finger, clinodactyly of 5 th finger	Single palmar crease, clinodactyly of 5 th finger	Single palmar crease	Clinodactyly of 5 th finger		
Other abnormalities			Scoliosis, low posterior hairline, delayed speech	Delayed speech		Delayed speech	Scoliosis	
MLPA result	Del 9pter	Del 9pter	Dup 15q11	Del 2qter	Dup 11p/delXp	Del 12p/dup12q	Del 8pter	Dup XYp
Gender	M	M	F	F	F	M	M	M
Age	21	24	17	16	37	16	12	12
Inheritance	NA	NA	NA	NA	NA	NA	NA	NA
ID Level	Mild	Mild	Mild	Mild	Mild	Moderate	Mild	Mild
Hypotonia	No	No	No	No	No	Yes	No	No
Weight	52 kg (P25-P50)	54 kg (P25-P50)	40 kg (P3-P25)	55 kg (P75-P97)	34 kg (<P3)	44 kg (P3-P25)	30 kg (P25)	39 kg (P50)
Height	156 cm (<P3)	159 cm (<P3)	152 cm (P25)	146 cm (<P3)	127,5 cm (<P3)	153 cm (P3)	132 cm (P3)	144 cm (P3-P25)
OFC	54 cm (P25)	52 cm (<P3)	56 cm (P90-P97)	55 cm (P90-P97)	50 cm (<P3)	50 cm (<P3)	53.5 cm (P50)	55 cm (P90)
Behavior problems			ADHD	Shy		Shy	ADHD	
Other features								
Head/face	High forehead	Face asymmetry, brachycephaly,	Long face	Asymmetry of the head		Long and narrow face, brachycephaly, sloping forehead	Brachycephaly, high forehead	
Ocular region		Arching eyebrows				Hypotelorism, almond shape palpebral fissure,		
Nose			Broad base of the nose,				Depressed nasal bridge	
Ears		Small and low-set ears				Low set ear	Low set and posteriorly rotated	Prominent and large ears
Mouth region	Long philtrum, thin lips, micrognathia	Thin upperlip, long philtrum,				Small mouth, thick lips, high arched palate, micrognathia		
Thorax/abdomen								
Extremities		Clinodactyly and sandal gaps	Short 5 th finger and toe	Short hand, short metacarpal of digits 3-5, stubby hand, broad and short thumb, small and almost rudimentary of 3 rd and 4 th toe, hypoplastic nail	Deviation of the long arm, clinodactyly and brachydactyly		Sandal gaps	Brachydactyly, clinodactyly, sandal gaps
Other abnormalities	Cryptochidism	Low posterior hairline, kyphosis			Disproportionate short stature			

*Clinical features of non-pathogenic cases (7, 8), possible variant (19, 20), variant (21-23) are not listed, as well as a case whose features are reported elsewhere (5), MLPA: Multiplex ligation dependent probe amplification, ID: Intellectual disability, OFC: Occipitofrontal head circumference, ADHD: Attention deficit-hyperactivity disorder

is most likely due to the deletion of 10pter, because of the phenotypic overlap with previously reported cases with a deletion of 10p15.^[17] The dysmorphic features of this case do not match the clinical description of another individual with a duplication of 9pter.^[15] In case 5, the 9pter/qter deletion/duplication is considered to be causative. The clinical details have been reported elsewhere.^[18] In case 6, a *de novo* deletion of the X/Yqter pseudo autosomal region 2 was detected, which was previously also described in phenotypically normal individuals (DGV; <http://www.tcag.ca/>). Therefore, we performed an additional SNP array analysis to enable a fairly precise determination of the size of the deletion and link it to the severity of the clinical features. To our surprise, this revealed a 2 Mb deletion on chromosome 22q11 that has previously been described in patients with a similar phenotype.^[19] We therefore, conclude that the phenotype of case 6 is not due to the *de novo* X/Yqter deletion, but due to the 22q11 deletion.

In two cases (case 7 and 8), parental testing showed that the aberration was inherited from an unaffected parent. These aberrations were therefore, considered being familial variants that do not contribute to the phenotype in case 7 and 8. When parental samples were not available, we tried to confirm the presence of the detected subtelomeric aberration using additional methodologies [Table 1]. The deletion of the entire Wolf-Hirschhorn Critical Region (WHSCR) in 4pter in case 9 was confirmed by MLPA with the SALSA P096 probe kit. Furthermore, the clinical features of this patient were consistent with Wolf-Hirschhorn Syndrome. In case 10, two duplicated regions of chromosome 22 were identified, one in the 22q11.2 region (next to the centromere) and the other in the 22q13.3 region (telomere end of q arm of chromosome 22). The SALSA P023B probe kit was used to confirm these duplicated regions. Two duplications in one arm of the chromosome suggested a complex recombination. However, such recombination could not be identified in the routine analysis and warranted further characterization. Confirmatory analysis using SNP array showed that both duplications actually consisted of 2.6 Mb in 22q11.2 and 1.8 Mb in 22q13.33. Since microduplications of both 22q11.2 and 22q13.3 have been associated with highly variable phenotypic features,^[20,21]

we suggest that in case 10, both duplications contribute to the phenotype.

In cases 11 and 12, a subtelomeric deletion of 9pter was identified. Case 11 showed a milder phenotype than previously reported cases.^[22,23] In order to see, if the deletion in case 11 was smaller than the deletions reported before, SNP array analysis was performed. The deletion appeared to be 11.8 Mb in size, and it does not exceed the critical region of 9p syndrome;^[22,23] this, therefore, explained the mild phenotype. In case 12, however, the patient showed some similarities to the reported cases.^[22] Therefore, it is suggested that 9pter deletion in case 12 is causative, and array analysis to determine the actual size of the deletion was unnecessary.

In case 13, a microduplication of 15q11 was identified. MLPA analysis of probes in the 15q11.2-15q15.1 region (MRC Holland kit P028) showed a duplication of the probes between breakpoint 1 and breakpoint 3 (including TUBGCP5 and APBA2). The methylation specific analysis indicated that the interstitial duplication was of maternal origin. It is suggested, that the duplication explains mild ID and minor dysmorphic features in case 13, since duplications of 15q11 are associated with a highly variable phenotype.^[24]

In cases 14-20, parental testing and additional testing were not performed. This was due to the fact that in some cases, materials for additional testing were unavailable. Another reason was that the clinical features of the patient showed some similarities to the reported cases; therefore, additional testing was considered unnecessary. In case 14, a subtelomeric deletion of 2qter was identified. This patient showed shortening of the metacarpal bones which occurs in the majority of 2q37 patients.^[25] It is suggested, therefore that the deletion in this case is causative. In case 15, a subtelomeric deletion of Xpter and a subtelomeric duplication of 11pter were identified. The presence of both a deletion and duplication suggests an unbalanced translocation. The Xpter probes in the P070 and P036D MLPA kits are both located within the *SHOX* gene. Deletions of this gene are associated with Leri-Weill dyschondrosteosis (LWD) and idiopathic short stature (ISS).^[26,27] A duplication of 11pter has been reported in a case of Silver-Russell

syndrome.^[28] Therefore, both the dup 11pter and the deletion Xpter could be contributing to the short stature of this individual's phenotype.

In case 16, the subtelomeric deletion of 12pter accompanied by a subtelomeric duplication of 12qter suggests that the recombinant chromosome resulted from a pericentric inversion in one of the parents. Unfortunately, his parents were unavailable to test. Lagier-Tourenne *et al.* (2004) reported two cases of microscopically visible recombinant chromosome 12 and reviewed all previously reported cases as well as cases with pure cytogenetic deletion of 12pter and duplication of 12qter.^[29] Compared to these previously reported cases with larger abnormalities, our patient showed a milder phenotype such as minor facial dysmorphisms and mild ID [Table 2]. It is suggested that the terminal duplication/deletion of chromosome 12 in this patient was smaller than previously reported and could contribute to the phenotype.

In case 17, a subtelomeric deletion of 8pter was identified. He showed mild ID with minor facial dysmorphisms. A subtelomeric 8pter deletion is rare, and only few cases have been reported.^[4] Wu *et al.* (2010) reported a patient with a very small deletion in terminal 8pter with ID, microcephaly and minor facial dysmorphisms. We therefore conclude that the clinical features of case 17 are most likely due to the deletion of 8pter.

In case 18, a duplication of the probes in the *SHOX* gene in the pseudo-autosomal region 1 (PAR1) Xpter/Ypter was identified. *SHOX* duplications limited to PAR1 appear to be rare, and the associated phenotype is highly variable.^[30,31] *SHOX* gene defects, either a deletion or duplication, were associated with LWD and ISS. It has to be noted; however, that the effect of a duplication is ambiguous.^[27] Consequently, the clinical features associated with such duplication were likely to be under-ascertained.^[30] We are uncertain, therefore, whether the duplication of this gene contributed to the clinical phenotype or not.

In cases 19 and 20, a subtelomeric duplication of 16qter was identified. A 16qter submicroscopic microduplication is rarely reported. Ravnan *et al.* (2006) reported five cases with a duplication 16qter in which the

duplicated signal was adjacent to the 18p subtelomere probe signal. In two cases, the recombination appeared to be inherited from unaffected parents, and these were considered to be variants. Therefore, in the other three cases the recombination was also regarded to be a variant although parental samples were not available.^[32] It cannot be ruled out; however, that the duplication in cases 19 and 20 are contributing to their phenotype since 16qter is a gene-rich region. More than ten genes are present in the ~500 kb proximal to 16qter. Some of these (*NULP1*, *TUBB3*, and *AFG3L1*) are expressed in the brain,^[33] and it is possible that overexpression of these genes contributes to ID.

Discussion

This is the first study identifying subtelomeric chromosomal aberrations in Indonesian ID individuals. Overall, subtelomeric copy number rearrangements were established in 20 samples, explaining the phenotype of 16 cases. Therefore, a detection rate of 3.7% (16/436) was obtained, of which 31% (5/16) was found to have a complex rearrangement/unbalanced translocation, 44% (7/16) had a simple deletion and 25% (4/16) had a simple duplication. In addition, the subtelomeric rearrangements contributed as genetic cause of ID in 3% (16/527) of cases in the whole cohort. The deletions, including the complex rearrangements, involved nine different subtelomeric regions (2q, 4p, 8p, 9p, 10p, 12p, 18p, X/Yp, X/Yq); and duplications, including complex rearrangements, involved eight subtelomeric regions (4p, 9p, 9q, 11p, 12q, 15q11, 22p, X/Yp).

The detection rate of chromosomal subtelomeric rearrangements in this study is 3.7% (16/436) which is well within the range of 2.5% previously reported by Ravnan *et al.*,^[32] and 4.4% as reported by van Karnebeek *et al.*^[2] Five individuals (5/16; 31%) are suggested to have an unbalanced translocation that was not detected by routine cytogenetic analysis. In three of these, the translocation was shown to be *de novo* (case 3, case 4, and case 6), whereas in the two others, parental samples were unavailable. The prevalence of these cryptic imbalances in our ID population is in the range of a previous study conducted by Wu *et al.* (2010), who

reported such rearrangements in 21.7% (5/23) and the study of Jehee *et al.* (2011) which reported such rearrangements in 42.1% (8/19).^[6,7] The considerably high rate of unbalanced translocations observed in this study might be explained by the fact that we did not use high-resolution banding, which could have detected most “cryptic” subtelomeric anomalies.^[8]

Although the MLPA method is capable of revealing subtelomeric rearrangements, the clinical significance of each rearrangement should be interpreted carefully, particularly for cases in which the clinical features are different from previously reported cases. In case 6, for example, the *de novo* subtelomeric X/Yqter deletion could not explain the clinical features when compared to the previously reported cases.^[32,34] Furthermore, in the DGV it is reported that rearrangements in this region can be detected in phenotypically normal individuals as well. Subsequent SNP array in this patient identified another abnormality, which explained his clinical phenotype.

To conclude, this is the first large-scale study of the detection of submicroscopic subtelomeric aberrations in Indonesian patients with ID. This study shows that subtelomeric rearrangements are an important cause of ID in Indonesia, and its prevalence does not differ from previously reported studies in the Western world. Since diagnostic facilities for this kind of abnormalities are not yet available in Indonesia, the implementation of this technique in a routine diagnostic setting will help to establish a genetic diagnosis in individuals with ID, and will improve the possibilities for genetic counseling to the families involved. To establish an adequate diagnosis is of crucial importance for patients and their families. Therefore, diagnostic facilities for genetic diseases need to get a higher priority in Indonesia, similar to those for common infectious diseases and nutritional problems.

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