CLINICAL UTILITY GENE CARD

Clinical utility gene card for: 16p12.2 microdeletion

Lucilla Pizzo¹, Joris Andrieux², David J Amor³ and Santhosh Girirajan^{*,1,4}

European Journal of Human Genetics (2017) **25**, doi:10.1038/ejhg.2016.158; published online 16 November 2016

1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

16p12.1 microdeletion (hg18/NCBI36). Please note that the updated release of the human reference genome (hg19/GRCh37) annotates this region as 16p12.2.

1.2 OMIM# of the disease

136570.

1.3 Name of the analysed genes or DNA/chromosome segments

Chromosome 16p12.2 (*hg19 chr16:g.(?_21950000)_(22470000?)del*).

1.4 OMIM# of the gene(s)

UQCRC2 (*191329); *EEF2K* (*606968); *CDR2* (*117340); *PDZD9* (no OMIM entry); *C16orf52* (no OMIM entry); *VWA3A* (no OMIM entry); and *POLR3E* (no OMIM entry).

1.5 Mutational spectrum

The 16p12.2 microdeletion is ~520 kbp in size, located in the short arm of chromosome 16. The genomic rearrangement is mediated by highly identical (>99.5%) segmental duplications (68 kbp) flanking this region. Although the deletion break points are difficult to map owing to the high complexity of the flanking segmental duplications, these are usually located at *hg19 chr16:g.(?_21950000)_(22470000?)*.^{1,2} Seven annotated genes or transcripts are located within the unique region flanked by segmental duplications and are as follows: *UQCRC2*, *PDZD9*, *C16orf52*, *VWA3A*, *EEF2K*, *POLR3E* and *CDR2*. The underlying mechanism for how the heterozygosity of one or more genes in 16p12.2 results in disease is unknown. The seven genes deleted in 16p12.2 have been studied to a variable extent. *UQCRC2* is a fundamental protein for the assembly of the mitochondrial respiratory chain complex. *CDR2* is an onco-neural antigen and has been associated with cerebellar ataxia.³ *POLR3E* is a component of the RNA polymerase that synthesizes small RNAs.⁴ *EEF2K* is a kinase involved in the regulation of protein synthesis elongation, and has been recently associated with learning and memory, synaptic plasticity and the short-term antidepressant action of ketamine.^{5–8} *EEF2K*-knockout mice have been described to have defects in the reproductive system, and those carrying a homozygous-inactivating mutation leading to 0.5% residual activity showed learning memory deficits

and altered brain activity.⁵ Limited functional information is available on *PDZD9*, *C16orf52* and *VWA3A*. Even though the basic functionality has been reported for some genes within the region, their exact role towards pathogenicity owing to the 16p12.2 deletion is not known, highlighting the need for in-depth molecular studies of these genes.

1.6 Analytical methods

The presence of the deletion can be detected by genome-wide or targeted approaches that determine copy number in the region. Recent studies using genome-wide technologies such as comparative genomic hybridization and SNP microarrays have enabled discovery of this microdeletion, which otherwise would have been missed by lower resolution assays such as chromosomal banding techniques. The accuracy of estimation of the deletion size depends on the type of microarray used (targeted or whole genome) and the density of probes in the region.

1.7 Analytical validation

Targeted assays such as real-time quantitative PCR, fluorescence *in situ* hybridization and multiplex ligation-dependent probe amplification can be used for confirming the deletion and for assessment of its transmission within the family.

1.8 Estimated frequency of the disease

(Incidence at birth ('birth prevalence') or population prevalence)

If known to be variable between ethnic groups, please report:

The 16p12.2 deletion is associated with incomplete penetrance and variable expressivity, making it difficult to estimate the exact prevalence of the deletion. Girirajan *et al.*⁹ reported the deletion in 42 out of 21 127 individuals with developmental delay and intellectual disability who were referred for clinical genetic testing, compared with 8 out of 14 839 control individuals. By comparing the frequency of individuals with 16p12.2 deletion referred for clinical genetic testing with microarrays, the prevalence of this deletion is estimated to be similar (1:15 000 live births) to that of Smith–Magenis syndrome.^{9,10} However, this estimate does not take into account mildly-affected and unaffected individuals who carry the 16p12.2 deletion and are not referred to clinics.¹¹

¹Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA, USA; ²Institut de Génétique Médicale, CHRU de Lille, Lille, France;

³Department of Paediatrics, Murdoch Childrens Research Institute, University of Melbourne, Royal Children's Hospital, Melbourne, Victoria, Australia; ⁴Department of Anthropology, The Pennsylvania State University, University Park, PA, USA

*Correspondence: Dr S Girirajan, Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, 205A Life Sciences Building, PA 16802, USA. Tel: +1 814 865 0674; Fax: +1 814 865 0674; E-mail: sxg47@psu.edu

Received 7 July 2016; revised 20 September 2016; accepted 11 October 2016; published online 16 November 2016

1.9 Diagnostic setting

	Yes	No
A. (Differential) diagnostics	☒	☐
B. Predictive testing	☒	
C. Risk assessment in relatives	☒	☐
D. Prenatal	☒	☐

Comment:

Given the variable expressivity of the microdeletion and the incomplete penetrance, prenatal diagnosis is possible in families carrying the deletion, but the clinical outcomes cannot be predicted. Pre-implantation genetic diagnosis can also be performed, and the same considerations apply.

2. TEST CHARACTERISTICS

		Genotype or disease		A: True positives	C: False negative
		Present	Absent		
				B: False positives	D: True negative
Test					
Positive	A	B		Sensitivity:	A/(A+C)
				Specificity:	D/(D+B)
Negative	C	D		Positive predictive value:	A/(A+B)
				Negative predictive value:	D/(C+D)

2.1 Analytical sensitivity

(proportion of positive tests if the genotype is present)

Approximately 100% using the analytical methods described.

2.2 Analytical specificity

(proportion of negative tests if the genotype is not present)

Approximately 100% using the analytical methods described.

2.3 Clinical sensitivity

(proportion of positive tests if the disease is present)

The clinical sensitivity can be dependent on variable factors such as age or family history. In such cases, a general statement should be given, even if a quantification can only be made case by case.

The proportion of positive tests for the deletion, given the phenotype, is not 100% owing to the extensive genetic heterogeneity of neurodevelopmental phenotypes. The 16p12.2 microdeletion does not lead to a recognizable syndrome. The deletion is associated with incomplete penetrance and extensive phenotypic heterogeneity. The phenotypic heterogeneity can potentially be explained by the genetic background of the deletion carrier, including the presence of second-site mutations elsewhere in the genome.^{9,12} The frequently described phenotypes in deletion carriers include developmental delay, speech delay, craniofacial and skeletal features, growth retardation, microcephaly, congenital heart defect, epilepsy, psychiatric and behavioural disorders, and hypotonia.⁹ An increased risk for schizophrenia has also been documented.¹³

2.4 Clinical specificity

(proportion of negative tests if the disease is not present)

The clinical specificity can be dependent on variable factors such as age or family history. In such cases, a general statement should be given, even if a quantification can only be made case by case.

Variable. The incomplete penetrance and the extensive range of phenotypes associated with the deletion preclude a 100% clinical specificity. Deletion carriers may be mildly affected or unaffected.

2.5 Positive clinical predictive value

(life-time risk of developing the disease if the test is positive)

This is variable owing to the incomplete penetrance and phenotypic heterogeneity of the deletion. The risk for developing a phenotype is also contingent upon the presence of a positive family history of neurodevelopmental/psychiatric phenotype and the genetic background.

2.6 Negative clinical predictive value

(probability of not developing the disease if the test is negative)

Assume an increased risk based on family history for a non-affected person. Allelic and locus heterogeneity may need to be considered.

Index case in that family had been tested:

The probability of not developing a disease if the test is negative for the deletion is not 100%. Not finding the deletion in the proband does not preclude the index case from manifesting a phenotype (for example, congenital anomalies or neuropsychiatric disease later in life). Other factors such as locus and allelic heterogeneity, as well as positive family history, can increase the risk of manifestation of the phenotype.

Index case in that family had not been tested:

The probability of not developing a disease if the index case has not been tested for the deletion is not 100%. Other factors such as locus and allelic heterogeneity, as well as positive family history can also increase the risk of manifestation of the phenotype.

3. CLINICAL UTILITY

3.1 (Differential) diagnostics: The tested person is clinically affected

(To be answered if in 1.9 'A' was marked).

3.1.1 Can a diagnosis be made other than through a genetic test?

No	☒ (continue with 3.1.4)
Yes	☐
	Clinically ☐
	Imaging ☐
	Endoscopy ☐
	Biochemistry ☐
	Electrophysiology ☐
	Other (please describe)

3.1.2 Describe the burden of alternative diagnostic methods to the patient

Not applicable.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?

Not applicable.

3.1.4 Will disease management be influenced by the result of a genetic test?

No	☐
Yes	☒
	Therapy
	Identification of specific disabilities may help to establish specialized education plans. Cardiac malformations should be treated using standard protocols under the care of a

cardiologist. If a history of epilepsy is present, the patient should be referred for neurological assessment. Psychiatric or behavioural problems should be assessed by specialists (developmental/behavioural paediatrician, psychologist and psychiatrist), and medical intervention (drug therapy and/or behavioural intervention) may begin. Standard treatment for other abnormalities should be followed.

Prognosis (please describe)	Presence of a positive family history of developmental and psychiatric phenotypes, and multiple affected members in the family increases the chances of having an early onset and/or more severe phenotype. The presence of a second-site mutation can modulate the phenotypic expression and progression of the disease. Prognosis is uncertain in individuals with <i>de novo</i> or uncertain inheritance of the deletion, absence of family history, and/or presence of second-site mutations of uncertain disease association, and should be dealt on a case-by-case basis.
Management (please describe)	Identification of the deletion should be followed by a detailed clinical evaluation of the index case and other family members. Frequent evaluations to monitor changes in the clinical features or to document any new signs is warranted to understand the course of the disease. Cardiac, renal, urologic, neurological and dental abnormalities should also be monitored. Support groups for this deletion are available and can help guide day-to-day care of affected children with the deletion.

3.2 Predictive Setting: The tested person is clinically unaffected but carries an increased risk based on family history

(To be answered if in 1.9 'B' was marked).

3.2.1 Will the result of a genetic test influence lifestyle and prevention?

If the test result is positive (please describe)	All individuals with the deletion should be completely evaluated for the presence of developmental and psychiatric features to assess the severity on the phenotypic spectrum. The presence of a positive genetic test in an 'unaffected' or mildly-affected individual only suggests a higher risk for phenotypes owing to the sensitized genetic background. In such cases, careful genetic counselling should be performed for reproductive decisions. Other factors such as the presence of a strong family history of disease and additional pathogenic second-site mutations in other family members should also be considered.
If the test result is negative (please describe)	A negative genetic test does not necessarily lead to a zero risk. The presence of a strong family history suggests risk-conferring variants in the genetic background.

3.2.2 Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?

Not applicable.

3.3 Genetic risk assessment in family members of a diseased person

(To be answered if in 1.9 'C' was marked).

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?

Once the deletion has been identified in the index case, genetic testing of the parents should be offered for genetic counselling. Although not

reported in the literature, gonadal mosaicism is another possibility. Because of the incomplete penetrance of the deletion, genetic testing should be offered to siblings to evaluate a possibility of gonadal mosaicism. Inheritance of the deletion from a parent would mandate genetic testing in other family members.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?

No. Even under the assumption of a *de novo* occurrence of the deletion in the family, siblings should be offered testing to evaluate a possibility of gonadal mosaicism.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?

Yes, only when deletion in the index case is inherited. The identification of an inherited deletion can be followed up by genetic testing of other family members.

3.4 Prenatal diagnosis

(To be answered if in 1.9 'D' was marked)

3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?

Yes, only when the deletion in index case is inherited from a parent. However, the phenotypic outcome cannot be predicted owing to the variable expressivity and incomplete penetrance of the deletion.

4. IF APPLICABLE, FURTHER CONSEQUENCES OF TESTING

Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe).

Yes. Identification of the deletion in the patient may have implications for the patient and relatives' reproductive decisions, and may warrant prenatal diagnosis. The deletion is inherited in autosomal dominant manner, and in 95% of cases, it is inherited from a parent. However, the extensive phenotypic variability of 16p12.2 microdeletion precludes accurate prediction of the clinical outcome.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by EuroGentest2 (Unit 2: 'Genetic testing as part of health care'), a Coordination Action under FP7 (Grant Agreement Number 261469) and the European Society of Human Genetics.

- Antonacci F, Kidd JM, Marques-Bonet T *et al*: A large and complex structural polymorphism at 16p12.1 underlies microdeletion disease risk. *Nat Genet* 2010; **42**: 745–750.
- Girirajan S, Eichler EE: Phenotypic variability and genetic susceptibility to genomic disorders. *Hum Mol Genet* 2010; **19**: R176–R187.
- Rosenblum M, Posner J: Paraneoplastic cerebellar degeneration. IA clinical analysis of 55 anti-Yo antibody-positive patients. *Neurology* 1992; **42**: 1931–1931.
- Dong Z, Bell LR: SIN, a novel Drosophila protein that associates with the RNA binding protein sex-lethal. *Gene* 1999; **237**: 421–428.
- Gildish I, Manor D, David O *et al*: Impaired associative taste learning and abnormal brain activation in kinase-defective eEF2K mice. *Learn Mem* 2012; **19**: 116–125.
- McCamphill PK, Farah CA, Anadolu MN, Hoque S, Sossin WS: Bidirectional regulation of eEF2 phosphorylation controls synaptic plasticity by decoding neuronal activity patterns. *J Neurosci* 2015; **35**: 4403–4417.
- Monteggia LM, Gideons E, Kavalali ET: The role of eukaryotic elongation factor 2 kinase in rapid antidepressant action of ketamine. *Biol Psychiatry* 2013; **73**: 1199–1203.

- 8 Park S, Park JM, Kim S *et al*: Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arc/Arg3.1 essential for mGluR-LTD. *Neuron* 2008; **59**: 70–83.
- 9 Girirajan S, Rosenfeld JA, Cooper GM *et al*: A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. *Nat Genet* 2010; **42**: 203–209.
- 10 Rosenfeld JA, Coe BP, Eichler EE, Cuckle H, Shaffer LG: Estimates of penetrance for recurrent pathogenic copy-number variations. *Genet Med* 2012; **15**: 478–481.
- 11 Girirajan S, Moeschler J, Rosenfeld J: 16p12.2 microdeletion. In: Pagon RA, Adam MP, Ardinger HH *et al*. (eds): *GeneReviews* [Internet]. Seattle: University of Washington, pp 1993–2016.
- 12 Girirajan S, Rosenfeld JA, Coe BP *et al*: Phenotypic heterogeneity of genomic disorders and rare copy-number variants. *N Engl J Med* 2012; **367**: 1321–1331.
- 13 Rees E, Walters JT, Chambert KD *et al*: CNV analysis in a large schizophrenia sample implicates deletions at 16p12.1 and SLC1A1 and duplications at 1p36.33 and CGNL1. *Hum Mol Genet* 2014; **23**: 1669–1676.