Prof. Dr. Sandra Janssens

Belgian Genetic Expanded Carrier Screening BeGECS

Manama Preconception Genetics
7 maart 2023

UZ GENT

UNIVERSITEIT GENT

Carrier screening versus carrier testing

- Carrier screening:

  a medical investigation aimed at identifying carriers with no a priori risk of having affected children, such as personal or family history

- Carrier testing:

  targets prospective parents with preexisting elevated risk of having a diseased child

Carrier screening

- First implemented in the Jewish community in 1970 to identify carriers of Tay-Sachs disease (TSD)

  → helped to prevent more than 90% of affected births in the subsequent decades

- Population-wide premarital screening for thalassemia and sickle cell anemia in the Middle East and the Mediterranean region

  → resulted in a significant reduction of affected births

- 1995: NIH recommended carrier screening for cystic fibrosis to:
  - Adults with a positive family history for CF
  - Partners of people with CF
  - Couples planning a pregnancy
  - Couples seeking prenatal testing

  → 2015: CF carrier screening was available in USA, Italy, UK, Australia

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Carrier screening: goal

- Early screening programs: aimed at improving the overall health of the population by decreasing the prevalence of severe disorders
- Western countries: criticized holding prevention as an explicit goal

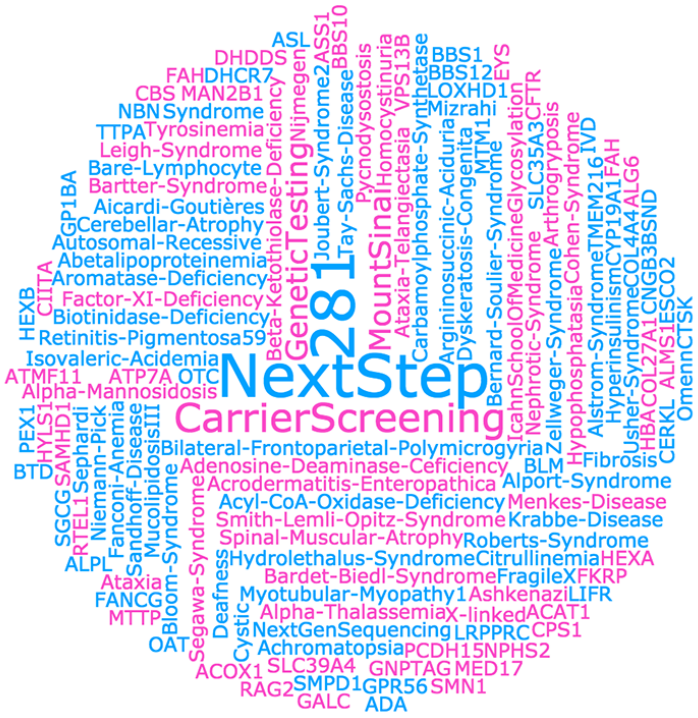
  ‘pressure on prospective parents to make choices that are ‘socially desirable

- ‘enhance the autonomy of future parents by allowing them to make informed reproductive decisions, consistent with their values’
  - ~ the genetic counseling tradition:
    - Non- directiveness
    - Individual choice

Screening for 1 disorder →Expanded carrier screening

- Introduction of Next Generation Sequencing (2008) → cost of DNA sequencing decreased spectacularly
  - Carrier screening for an increasing number of hereditary diseases
  - → Expanded Carrier Screening (ECS)

1 test ~ multiple disorders



Population genetics

- > 7000 monogenic disorders(~10/1000)
  - ~20% child mortality and pediatric hospitalizations
- ~3/1000 recessive disorders
  - >1400 AR genes
  - Carriership: 3-5 recessive lethal alleles
- → 1 à 2 % of couples: ‘at risk’ of having a child with an AR disease

European genetic professionals'perspectives on the implementation of ECS

- 16 genetic professionals from 8 member states of the EEA
  - 13 clinical geneticists
  - 2 molecular geneticists
  - 1 clinical + molecular geneticist
- Academic institution

- Attitudes of European Geneticists Regarding Expanded Carrier Screening. Janssens S, Chokoshvili D, Vears D, De Paepe A, Borry P. J Obstet Gynecol Neonatal Nurs. 2017 Jan-Feb;46(1):63-71
- Pre- and post-testing counseling considerations for the provision of expanded carrier screening: exploration of European geneticists' views. Janssens S, Chokoshvili D, Vears DF, De Paepe A, Borry P. BMC Med Ethics. 2017 Aug 1;18(1):46

Expanded Carrier Screening

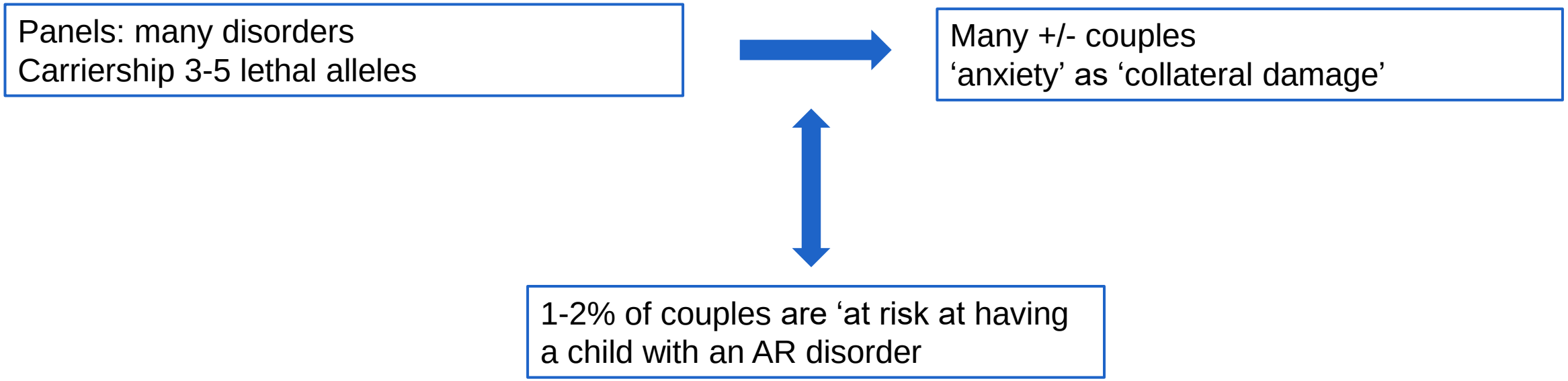
- Possible advantages
  - ↑reproductive autonomy – greater accessability
  - Screening independent of ethnicity
  - Cost effective ~ screening for a single condition

“At the moment, we have a situation where most people with most conditions only have the possibility of making informed decisions about reproduction when they already have an affected child and that is…not good”

Expanded Carrier Screening

- Concerns

Benefit for 'limited part' of general population
Incomplete 'coverage' of pathogenic mutations →residual risk to be a carrier



Expanded Carrier Screening

- Concerns
  - Insufficient knowledge about carrier screening
    - General population → ‘informed decision’
      → ‘purpose and implication of the test’
    - Care providers →’’non-genetic well-trained counselors’
  - ↓ patients → harm patients: stigmatisation - discrimination →‘eugenetics’

“Those people with the disease will feel, well, not at ease or embarassed with it, saying that ‘what is all legitimacy if you now go to avoid the birth of additional people with conditions we have’”

Goal ECS: enhancing reproductive autonomy → Reproductive options

- Acceptance of risk
- Prenatal genetic diagnosis, ± termination of pregnancy
- Preïmplantation Genetic Testing (PGT)
- Gamete donation
- Adoption
- Renounce the desire to have children
- *'Selecting wedding partner'?*

Chicago Center for Jewish Genetic Disorders

‘serious health issues and for which there is no cure’

‘reliable testing methods with definitive carrier status results’

1980
° DOR YESHORIM
'committee for prevention of genetic diseases among Orthodox Jews'

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- Standard panel: 51 AR disorders
- Hearing loss panel: 22 genes: highly recommended
- Optional: advanced panel: 50 genes ~mild or recently discovered disorders

Personal carrier chip

‘tested prior to reaching marriageable age’

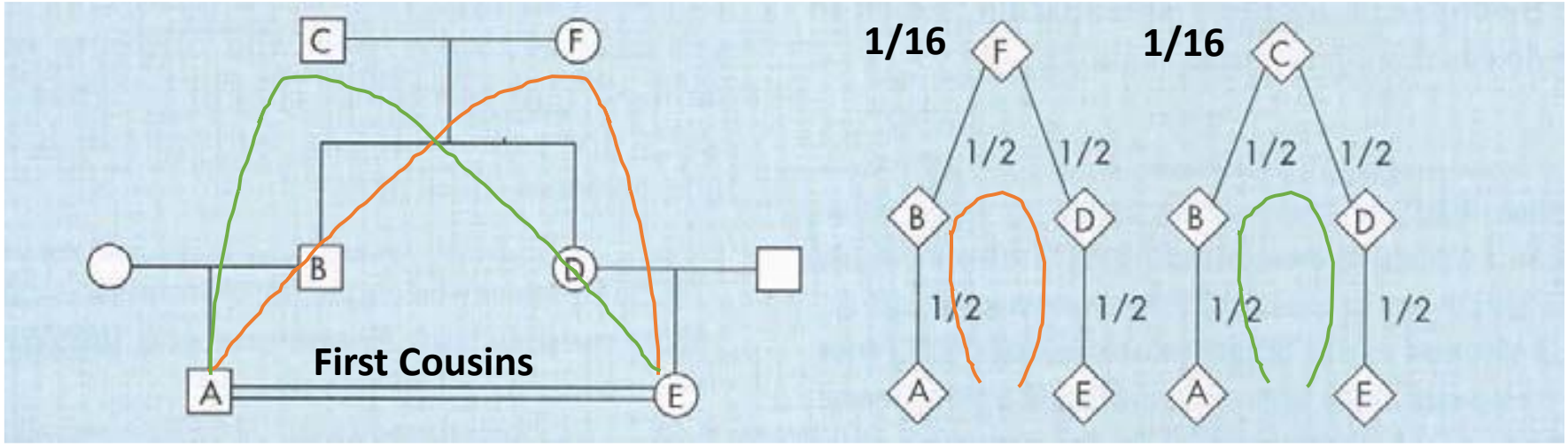
‘School screening’

‘an integral part of the matchmaking process for Jewish people across the globe’

Risk in case of consanguinity

- Consanguinity
  - Rare in Western population.
  - More frequent in Middle-East (20-50%) and India
- Common ancestor → higher chance of common disease genes and thus offspring with AR condition
- **Relationcoefficient**: measure of the number of genes that 2 related individuals have in common
  - eg: a parent and a child share half their genes: relationcoefficient between parent and child = 1/2

Bron: Medical Genetics, 3rd ed; 2003. LB Jorde, JC Carey, MJ Bamshad, RL White



Relation coëfficient

- Route: start with 1 individual, ascend to common ancestor, descend to other individual.
- Each individual can only appear once in a route
- Each route: relation coefficient is **(1/2) n-1** (n = number of individuals in the route) (**multiplication** rule)
- With multiple routes (i.e. multiple *common ancestors*): probabilities of each route are
- summed (**addition** rule)

Inbreeding coefficient

- = probability that an individual is homozygous at a certain locus as a result of consanguinity in his/her parents = **relation coefficient parents multiplied by ½**

First cousins

- Relation coefficient = 1/16 + 1/16 = 1/8 (= chance that they carry a common gene)
- Inbreeding coefficient = 1/8. ½ = 1/16

Consanguinity

Relationship	Relation coefficient	Inbreeding coefficient	Risk of having a child with an AR condition
First cousins	1/8	1/16	1/32
Second cousins	1/32	1/64	1/128
Third cousins	1/64	1/128	1/256

Practical in counseling:

Relatonship	Prevalence of congenital anomalies
none	2 à 3 %
First cousins	4 à 6 %
Second cousins	3 à 4 %
Third cousins	Barely higher than in non-consanguineous couples

Hoge
Gezondheidsraad

ADVIES VAN DE HOGE GEZONDHEIDSRAAD nr. 9240

Uitgebreide dragerschapsscreening in een reproductieve context. Naar een verantwoorde implementatie in de gezondheidszorg

In this advisory report, the Superior Health Council of Belgium provides recommendations on the criteria that need to be applied in preconceptual genetic testing for severe autosomal and X-linked recessive diseases for couples planning a pregnancy.

This report aims at providing healthcare authorities and healthcare professionals with specific recommendations on the scientific and ethical issues that need to be considered in view of a responsible implementation of preconceptual genetic testing in a reproductive context. The report specifically discusses the framework underpinning the appropriate introduction of such testing and suggests inclusion criteria for diseases that could be targeted by the screening process: (i) severity, (ii) age of onset, (iii) prevalence, (iv) selection of mutations based on clinical significance and (v) treatability.

Versie gevalideerd op het College van
Februari 2017[1]

Management of version NGS panel

- Original (1248 genes):
  - Autosomal recessive: 1124 genes
  - X-linked: 124 genes

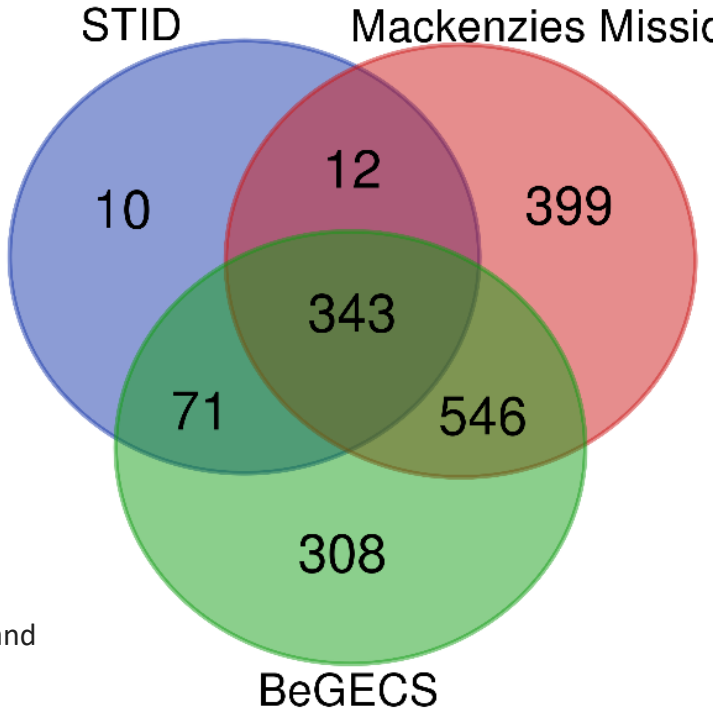
- Current version (1268 genes):
  - 20 genes added based on indications for PGT
  - Autosomal recessive: 1143 genes
  - X-linked: 125 genes

Management of version NGS panel

Planned panel update (1710 genes; April 2023)

- Comparison of BeGECS panel with STID (commercial panel) and Mackenzie's Mission
  - 399 genes unique to Mackenzie's Mission*
  - 12 genes overlapping between STID and Mackenzie's Mission
  - 10 genes unique to STID of which 3 with genetic defects not detectable by NGS → 7 genes

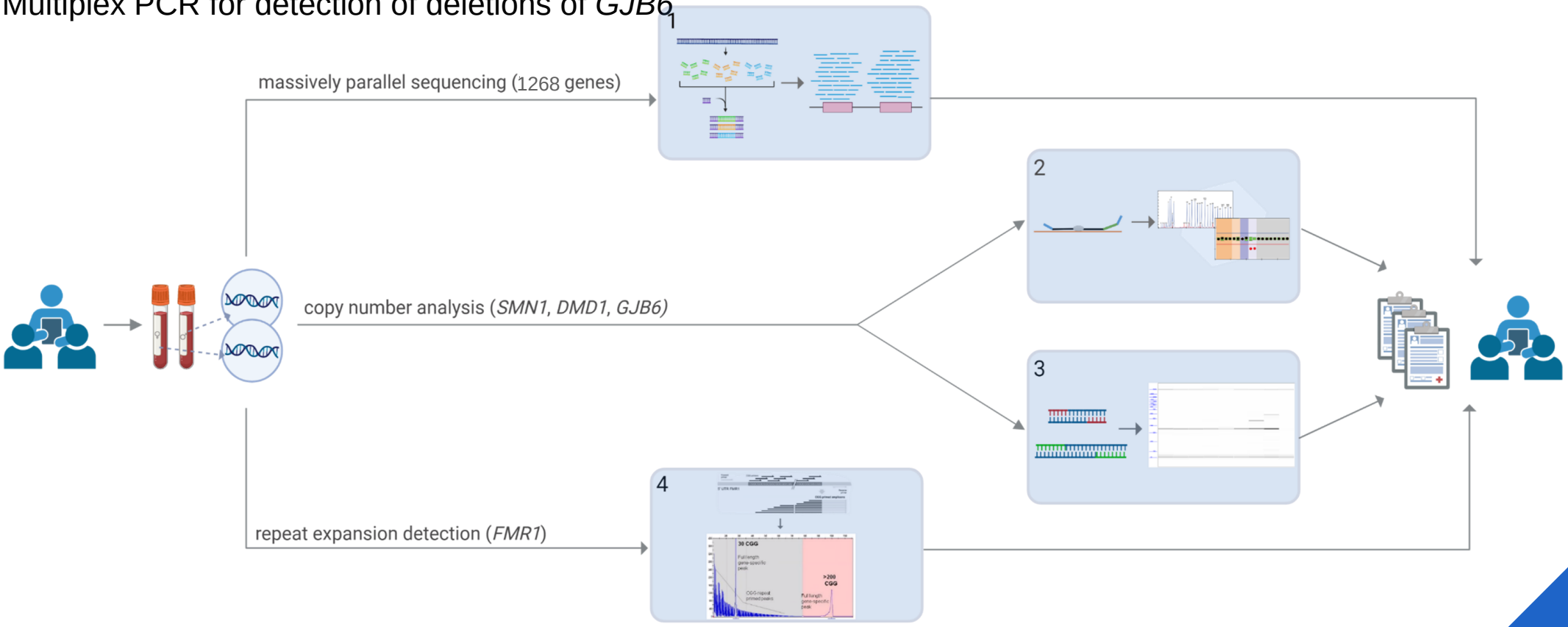
  → add 418 genes
- Evaluation current panel
  - Delete 8 genes (dominant or very mild phenotype)
- PGT indications other centers
  - Add 32 genes
- Composition 1710 genes
  - Autosomaal recessive: 1554 genes
  - X-linked: 156 genes



*Archibald, A. D., McClaren, B. J., Caruana, J., Tutty, E., King, E. A., Halliday, J. L., Best, S., Kanga-Parabia, A., Bennetts, B. H., Cliffe, C. C., Madelli, E. O., Ho, G., Liebelt, J., Long, J. C., Braithwaite, J., Kennedy, J., Massie, J., Emery, J. D., McGaughran, J., Marum, J. E., … The Mackenzie's Mission Study Team (2022). The Australian Reproductive Genetic Carrier Screening Project (Mackenzie's Mission): Design and Implementation. *Journal of personalized medicine*, *12*(11), 1781. https://doi.org/10.3390/jpm12111781

Overview of the different tests

- <u>In men and women:</u>
  - Targeted NGS (HyperCap) for 1268 genes
    - 1143 autosomal recessive genes
    - 125 X-linked genes
  - MLPA for detection of CNVs in *SMN1*
  - Multiplex PCR for detection of deletions of *GJB6*
- <u>In woman:</u>
  - MLPA for detection of CNVs in *DMD*
  - Repeat Primed PCR (RP-PCR) for detection of repeat expansions in *FMR1*



Targeted NGS (HyperCap) for 1268 genes

Variant filtering en interpretation with Seqplorer

- Filtereng in 3 steps with 3 virtual panels
  1. BeGECS_AR_frequent:
     - contains the 9 genes with the highest carrier frequencies
     - ***ACADM, CFTR, DHCR7, GJB2, GJB6, HBB, PAH, SMN1***
       → individual carrieships for class 4 and 5 variants are reported
     - ***CYP21A2***
       - Only class 4 and 5 variants associated with a classical form of CAH as described in Baumgartner-Parzer et al. EJHG 2020 are reported.
       - However, due to the presence of the CYP21A2 pseudogene and the limitations of the NGS technique used, the absence of a pathogenic variant cannot be excluded with certainty.
       - Moreover, large deletions comprising about 20-30% of pathogenic CAH alleles are not picked up by the NGS technique used.
       - Therefore, if a pathogenic CYP21A2 variant is found in one of the partners, a sample from the other partner is forwarded to Antwerp for full screening.

Targeted NGS (HyperCap) for 1268 genes

Variant filtering en interpretation with Seqplorer

Filtering in 3 steps with 3 virtual panels

2. BeGECS_X-linked:

- Contains 125 X-linked genes (mainly X-linked recessive also some X-linked dominant genes).
- Carriership of a class 4 or 5 variant in one of these genes in the female is reported.
- Carriership of a class 4 or 5 variant in EFNB1 and PCDH19 in the male is also reported.
  - EFNB1: affected males show only a mild phenotype (hypertelorism: widely spaced eyes) while females have a much more severe phenotype (craniofrontonasal dysplasia)
  - PCDH19: males unaffected, affected females have epilepsy with intellectual disability

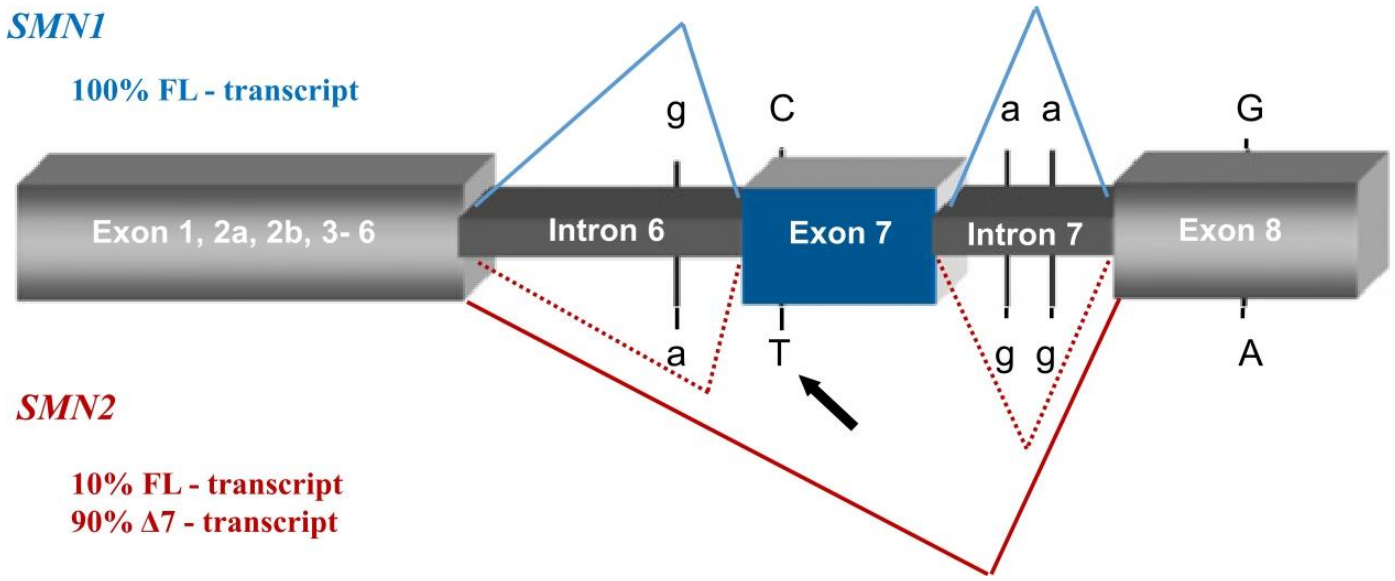
Targeted NGS (HyperCap) voor 1268 genes

Variant filtering en interpretation with Seqplorer

- Filtering in 3 steps with 3 virtual panels
  3. BeGECS_AR:
     - contains **1134 autosomal recessive genes**
     - Couple analysis using the "Only Biallelic Variants" filter: this only shows variants in genes where both the male and female have a variant the gene.
     - Individual carrierships are not shown in seqplorer.
       - For example if a woman is a carrier of a pathogenic BRCA2 variant but the man has no variants in BRCA2 we do not get to see this.
     - If both partners are carriers of a class 4 or 5 variant in the same gene this is reported.
     - Combinations of class 4/5 variants in one partner and a very suggestive class 3 variant in the other partner are discussed.

MLPA for detection of CNVs in *SMN1*

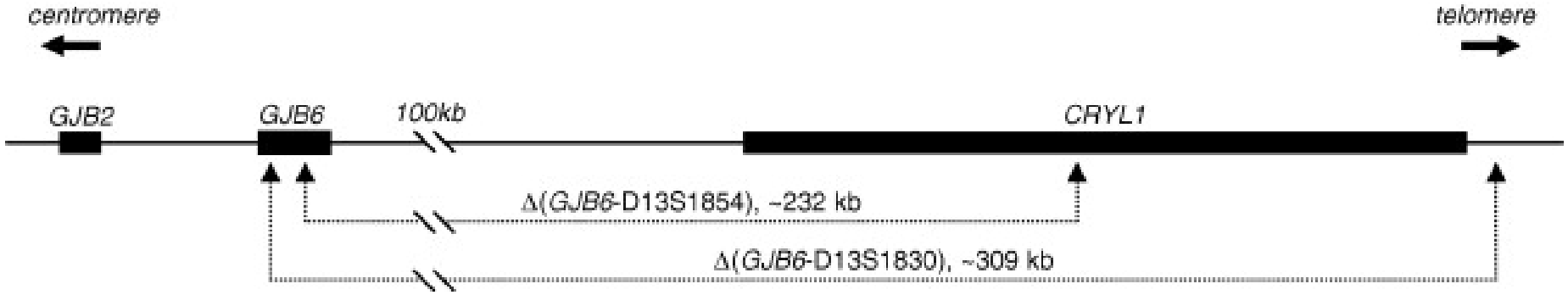
- Why a separate test for SMA?
  - 95-98% of genetic defects are a homozygous deletion or gene conversion of SMN1
  - SMN1 and SMN2 lie in a duplicated and inverted segment of about 500 kb on chromosome 5q13 and differ from each other only in 5 nucleotides, 2 of which are in the coding region
  - NGS is not a suitable method to pick up defects in SMN1.



Figuur overgenomen van Eggermann et al., Medizinische genetik 2020

Multiplex PCR for detection of deletions of *GJB6*

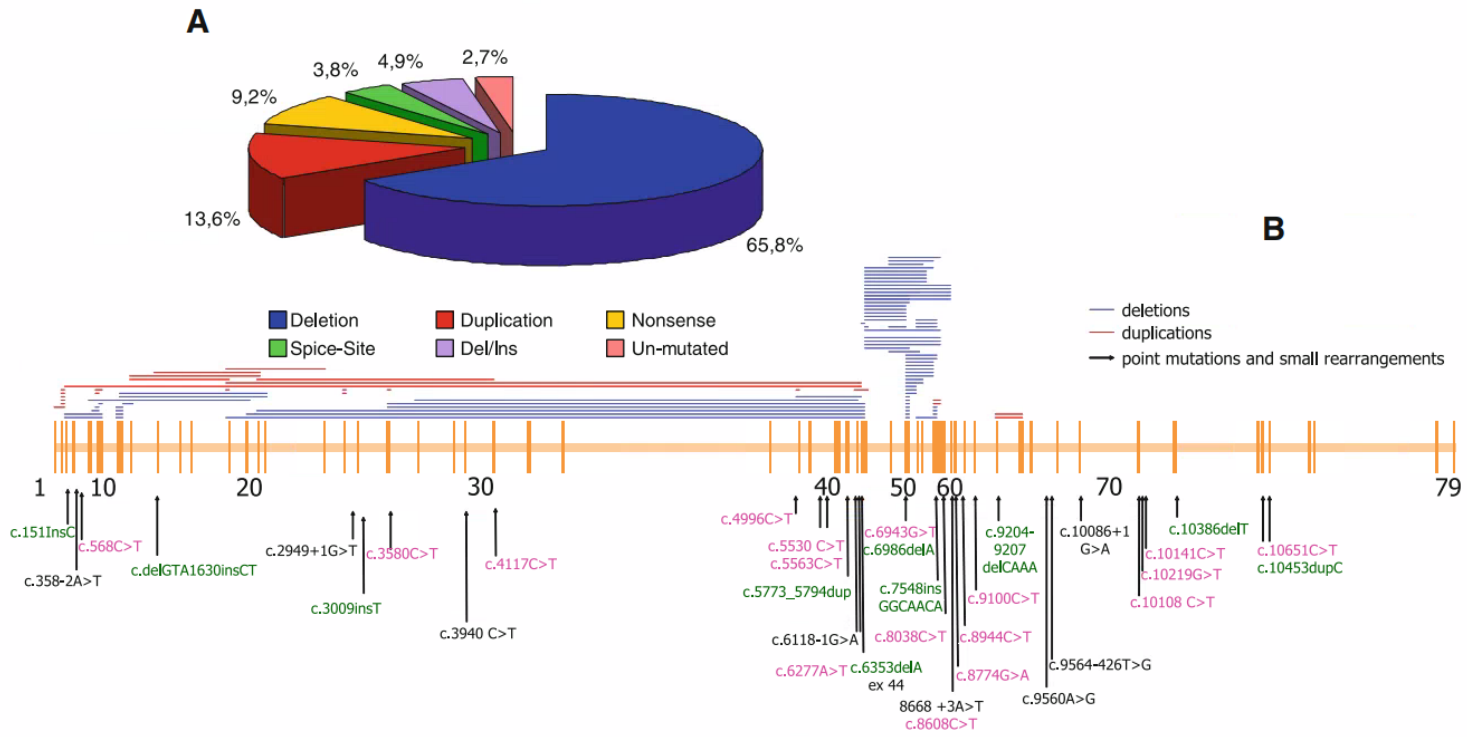
- Why a separate test for *GJB6*?
  - GJB2 and GJB6 account for about 50% of patients with non-syndromic congenital neurosensorial hearing loss
  - Of these, 99% have biallelic variants in the GJB2 gene but 1% are compound heterozygous for a pathogenic variant in GJB2 and a deletion upstream of GJB2.
    - del(GJB6-D13S1830) and del(GJB6-D13S1854) are the two most frequent deletions
    - Overlap with the GJB6 gene.
  - These deletions cannot be picked up with the current NGS method for BeGECS.



Figuur overgenomen van Rodriguez-Paris et al., Biochem. Biophys. Res. Commun. 2009

MLPA for detection of CNVs in *DMD*

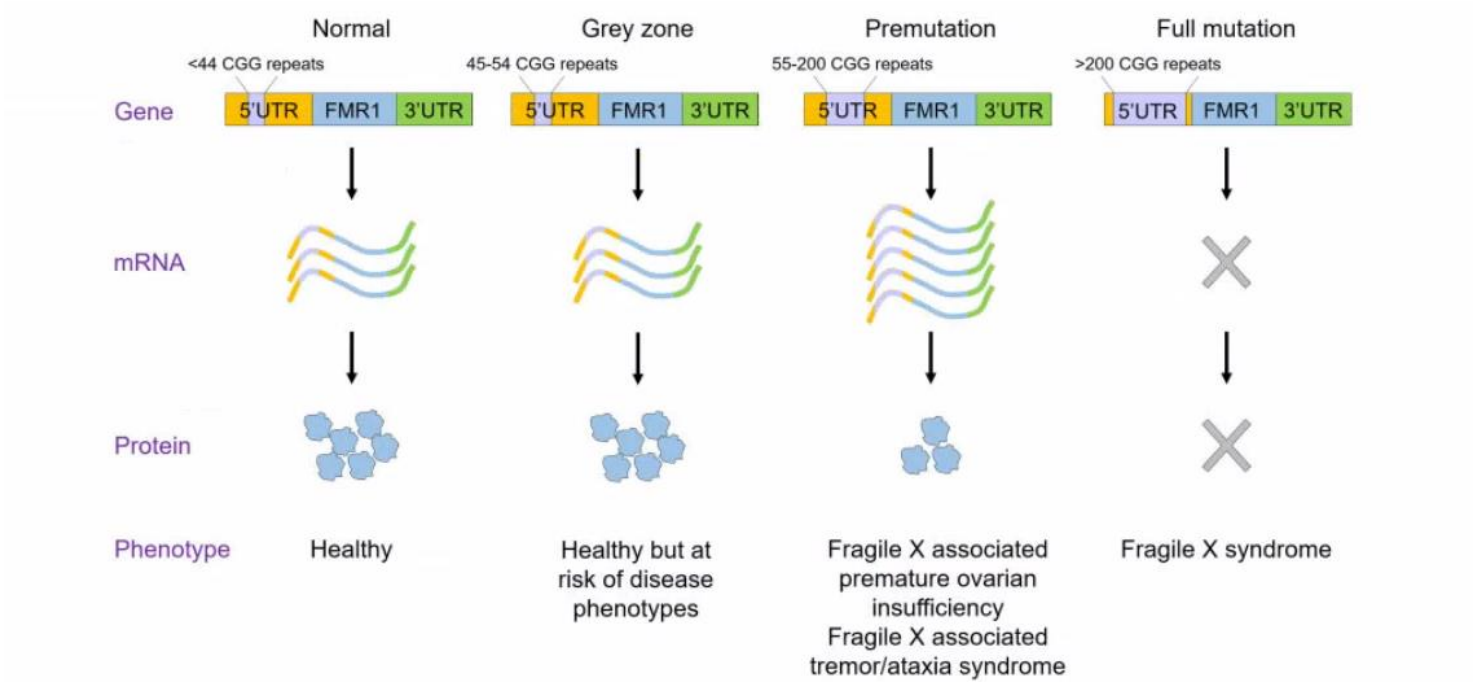
- Why a separate test for *DMD*?
  - A deletion or duplication of one or more exons of DMD is found in 65-80% of patients with dystrophinopathy.
  - CNVs cannot be picked up with the current NGS method for BeGECS.



Figuur overgenomen van Govoni et al., J Neurol, 2011

Repeat Primed PCR (RP-PCR) for detection of repeat expansions in FMR1

- Why a separate test for *FMR1*?
  - Fragile X syndrome is caused by an expansion of the number of CGG triplets (CGG repeats), which are located in the 5' UTR of the FMR1 gene located on the X chromosome.
  - Repeat expansions cannot be picked up by the NGS method used.



Figuur overgenomen van Rosario et al., Mol Hum Reprod, 2020

BeGECS Belgian Genetic Expanded Carrier Screening

To whom is the test offered?

- Medical Genetics Centers: preconceptional → No time pressure, Maximal reproductive options
  - Preconceptional advice
  - Consanguinity
  - PGT
  - Known gamete donation
- Not during pregnancy
- TAT: 3 months
- Cost: ~1428€ /couple

Interview geneticists EEA: concerning IVF and PGT:

"both of the procedures are basically medical procedures, firstly for the couple to have a baby at all or a couple with a genetic problem not to have this genetic problem. So in this situation, any additional genetic disorder [resulting from] this medical treatment would be somehow iatrogenic failure of the medical procedure"

BeGECS- Belgian Genetic Expanded Carrier Screening Results since October 2019

- 1268 genes
- 125 X-Linked
- 1143 Autosomal Recessive

Disorders - Diseases (D)		
Mental retardation	Immunodeficiencies	Multisystemic D
Multiple congenital anomalies	Endocrinologic D	Connective tissue S
Neurologic en neurodegenerative D	Muscle D	Skeletal D
Metabolic D	Cardiac D	Intestin-, pancreatic- and liver D
Oftalmologic D	Nefrologic D	Other
Hearing loss / Deafness	Ectodermal D	
Hematologic D	Lung D	

BeGECS Belgian Genetic Expanded Carrier Screening

Couple report

- Carriership of an AR mutation when both partners are carriers
- Carriership of an XL mutation in the woman (or man)

BeGECS Belgian Genetic Expanded Carrier Screening

Individual report

- Carriership 7 most frequent AR disorders (≥ 1/50)
  - Cystic Fibrosis (CFTR)
  - Deafness – hearing loss (GJB2-GJB6)
  - Phenylketonuria (PAH)
  - Smith-Lemli-Opitz syndrome (DHCR7)
  - Spinal Muscular Atrophy (SMA)
  - Sickle cell disease (HBs) en Bèta-Thalassemia (HBB)
  - Medium Chain Acyl CoA dehydrogenase deficiency (MCADD) (ACADM)
  - Congenital adrenal hyperplasia (CYP21A2)
- Carriership X-linked disorder
- Confirmation of previously known carrier status
- Carrership of an AR mutation when both partners are carrier is also included in the individual result

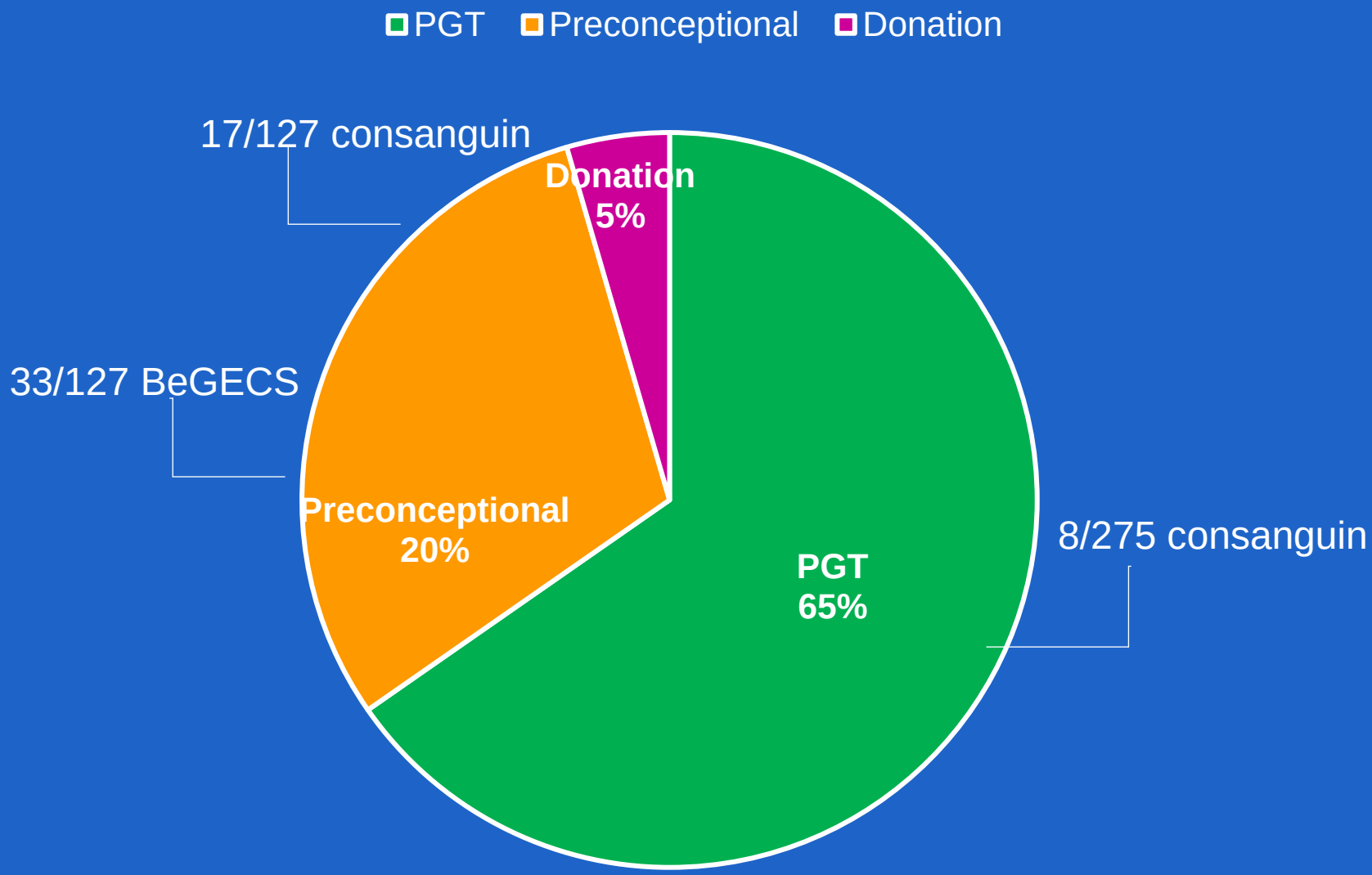
BeGECS Belgian Genetic Expanded Carrier Screening

Carriership of mutation with potential implications for one's own health: only reported when both partners are carrier of a mutation!

Autosomal dominant

- BRCA2: HBOC
- PRPH2: late onset macular dystrophy
- TCAP: HCMP
- PORC: thrombophilia ~protein C deficiency
- …….

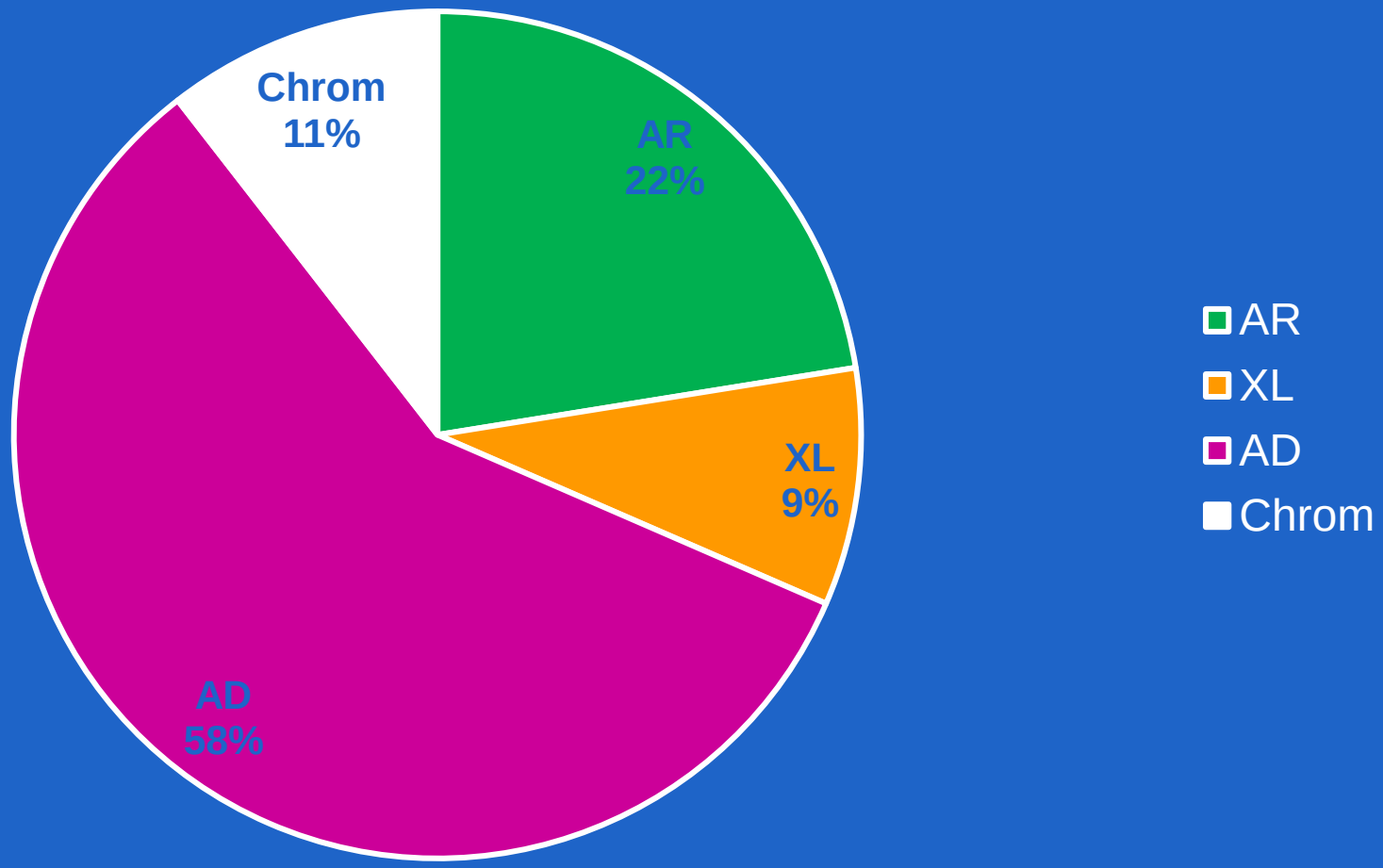
INDICATION CONSULTATION, 421 COUPLES



INDICATION PGT, 275 COUPLES

62 couples AR
25 women XL

These carrierships were **NOT** included in the BeGECS results



Carrier couples of an AR mutation – not previously known (excluding PGT indication)

Gene	Phenotype	number / 371 couples	Consanguin
ABCC6	PXE	1	+
MUTYH	MUTYH associated polyposis	1	+
TYR	Oculocutaneous albinism	1	+
CFTR	Cystic Fibrosis	3→1*	-
FV	Factor V deficiency	3	-
SLC12A3	Gitelman syndrome	1	-
CYP21A2	CAH	2	-
PRF1	Haemophagocytic Lymphohistiocytosis	2	-
STX11	Haemophagocytic Lymphohistiocytosis	1	+
GJB2	Neurosensory hearing loss	1	-
SCL3A1	Cystinuria	1	-
CLCN1	Myotonia congenita (Becker)	1	-
DUOX2	Thyroid dyshormonogenesis	1	-
ANO5	Spectrum of muscular diseases (>LGMD)	1	+
ACADVL	VLC acyl CoA D deficiency	1	-
PMM2 + GJB2	Glycosylation disorder / Neurosensory hearing loss	1	-
Total		**22 (~5,9%)**	**5/22 (~22,7%)**

* 2 couples: mild CFTR variants, not associated with cystic fibrosis, possibly CFTR-related symptoms

Carriership X-L mutation not previously known (excluding PGT indication)		
Gene	**Phenotype**	**Number/ 742 personen/ 371♀**
G6PD	Favism	2 ♀ / 3 ♂
FMR1 (only ♀ are tested)	Fragile X syndrome	2 ♀
GLA (mild)	Fabry disease	3 ♀

Carriership AR mutation with a carrierfrequency of ≥ 1/50			
Gene	**Disorder**	**number out of 742 persons**	**Carrierfrequency**
SMA	Spinal Muscular Atrophy	25→20*	**1/35**
GJB2-6	Hearing loss	50→40*	**1/17**
CFTR	Cystic fibrosis	69→49 // 35*	**1/15 // 1/21**
DHCR7	Smith-Lemli Opitz syndrome	23→21*	**1/33**
HBB - HBS	haemoglobinopathy	11→7*	**1/99**
ACADM	Medium-Chain Acyl-CoA Dehydrogenase Deficiency	14	**1/49**
PAH	Phenylketonuria	14	**1/49**

*SMA: 2 couples (PGT) and 1 ♀ with known carrier status
*GJB2-6: 5 couples (PGT) with known carrier status
*CFTR: 7 couples (PGT) en 5♂ /1 ♀ with known carrier status // 12/ 50 are T5TG11-12-13 alleles en 2/50 milde mutaties
*HBB: 2 couples (PGT) with known carrier status
*DHCR7: 1 couples (PGT) with known carrier status

Conclusion 3 years BeGECS

- Positive attitude towards BeGECS among selected group of couples with the desire to have children

- BeGECS results show that:
  - 5,9% (22/371) of couples: at risk of having a child with a recessive disorder
  - 4,6% (17/371) of non consanguineous couples: at risk of having a child with a recessive disorder

  - 0,5% (2/371) of women are carrier of a Fragile X premutation
  - 0,8% (3/371) of women are carrier of a GLA mutation

- These numbers are possibly an underestimation as the condition for which couples are in a PGT trajectory (62 AR and 25 XL) were **not** included

- Update panel from 1268 to 1710 genes is scheduled for April 2023

Project: perspective of couples

- Survey of couples' motivation for taking or not taking a BeGECS.

- Survey of couples’ reflections on BeGECS (couples who have taken the BeGECS, after receiving the test result)

Motivational reasons to take BeGECS (N = 508 individuals)

Motivational reasons	%
In context of prevention	22%
I whish to obtain all possible information	20%
Information helps to prepare me to my future reproductive decision	17%
Information increases sense of control	16%
General interest	13%
Information reduces my anxiety feelings	12%
TOTAL	**100%**

Motivational reasons not to take BeGECS (N = 260 individuals)

Motivational reasons	N
To much information will cause me anxiety and worries	18%
The information provided by the test will not change the decision to have children anyway	17%
I don't want to spend money on something that won't happen	17%
I am not worried about family history	16%
This gives us more freedom to proceed with our family planning without difficult information about future offspring	12%
Possibly I will take the test in the future	9%
The test will influence choices regarding my desire to have children	6%
Knowing may interfere with our future plans	5%
TOTAL	**100%**

If BeGECS was free of charge, would you take the screening?(N =164 individuals)

YES	NO
65% (N=106)	**35%** (N=58)

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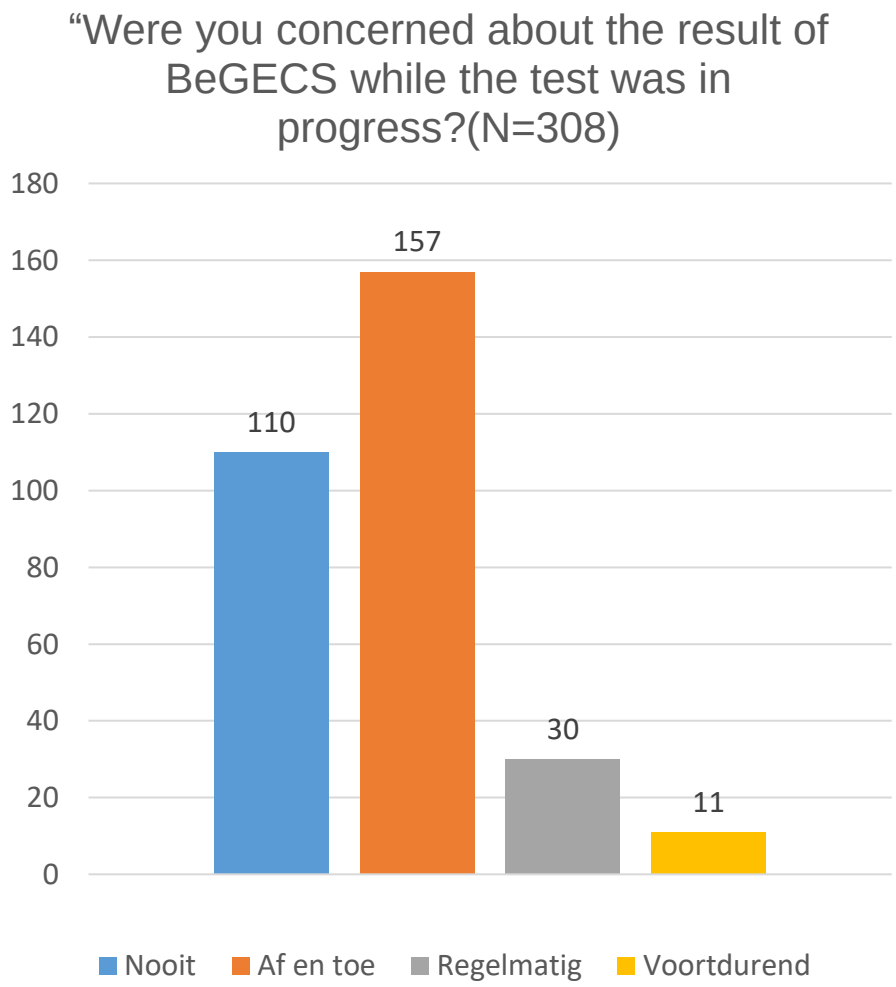
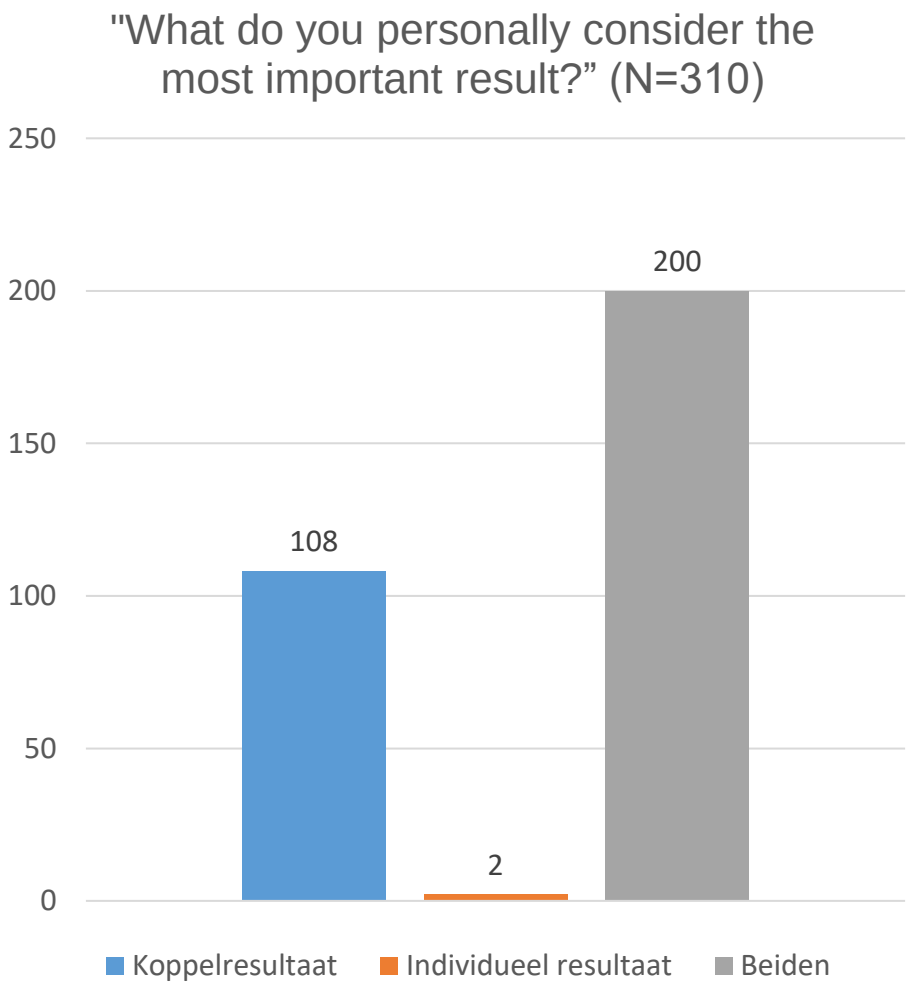
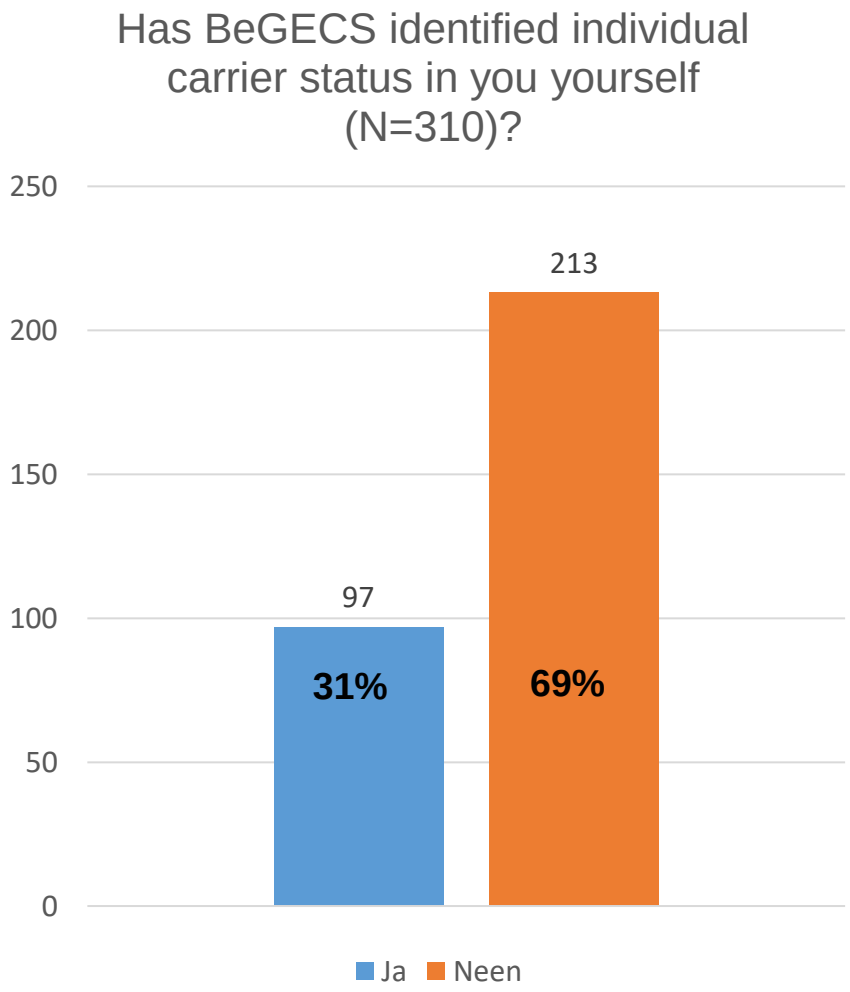
‘POST-BeGECS’ results

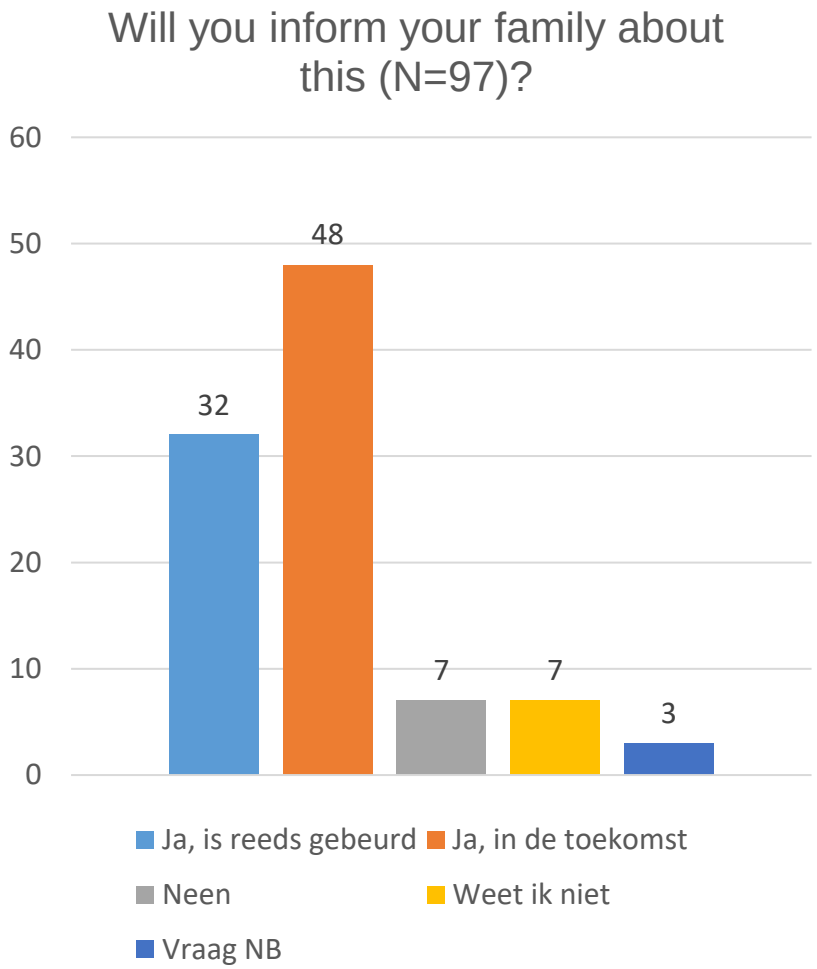
BeGECS: Result



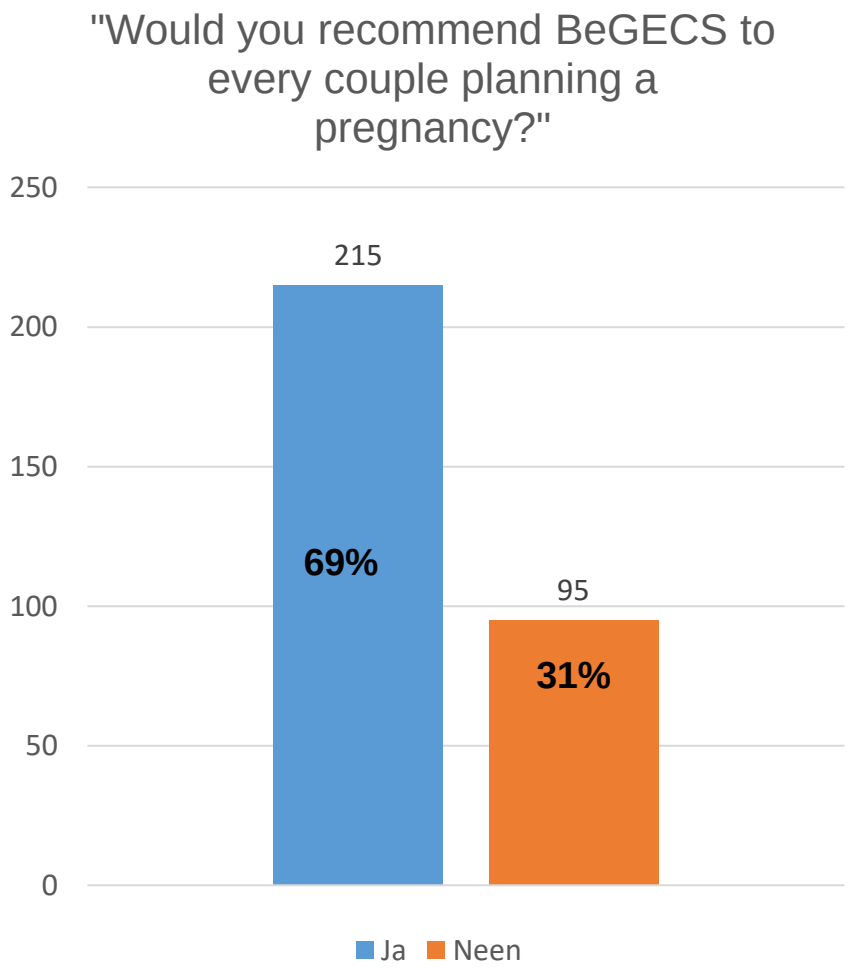


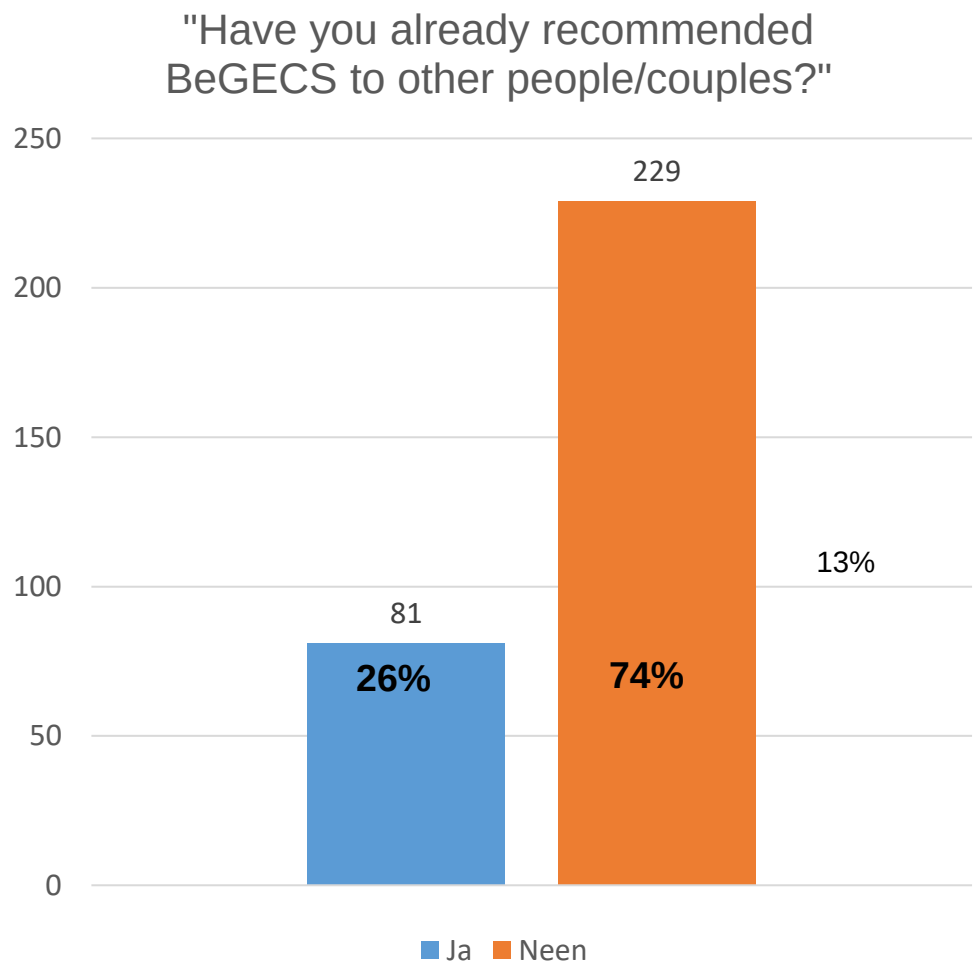
BeGECS: Individual carriership (N=310)



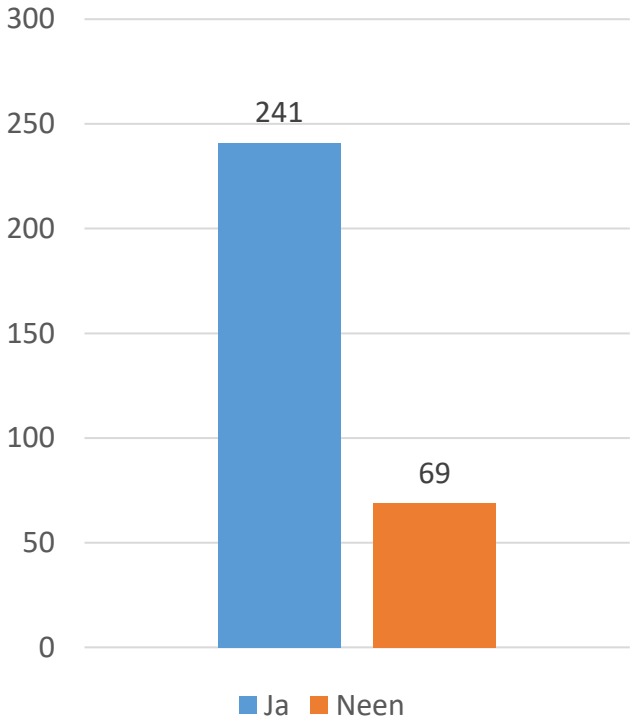


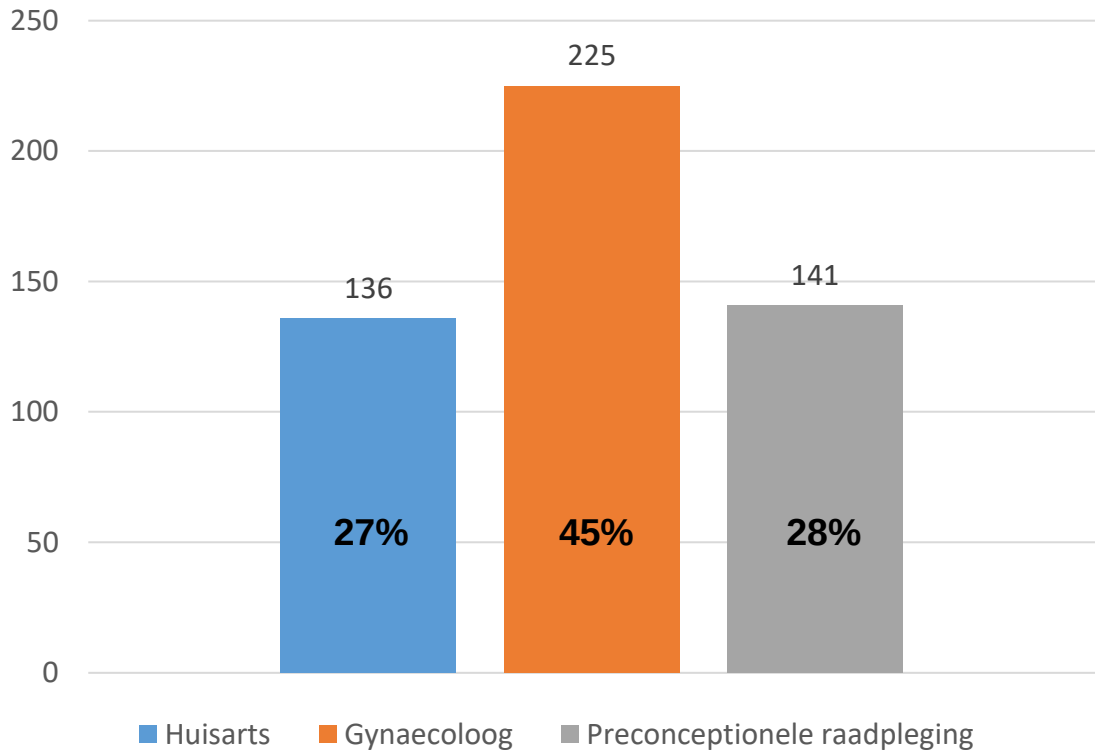
To recommend BeGECS to others? (N=310)



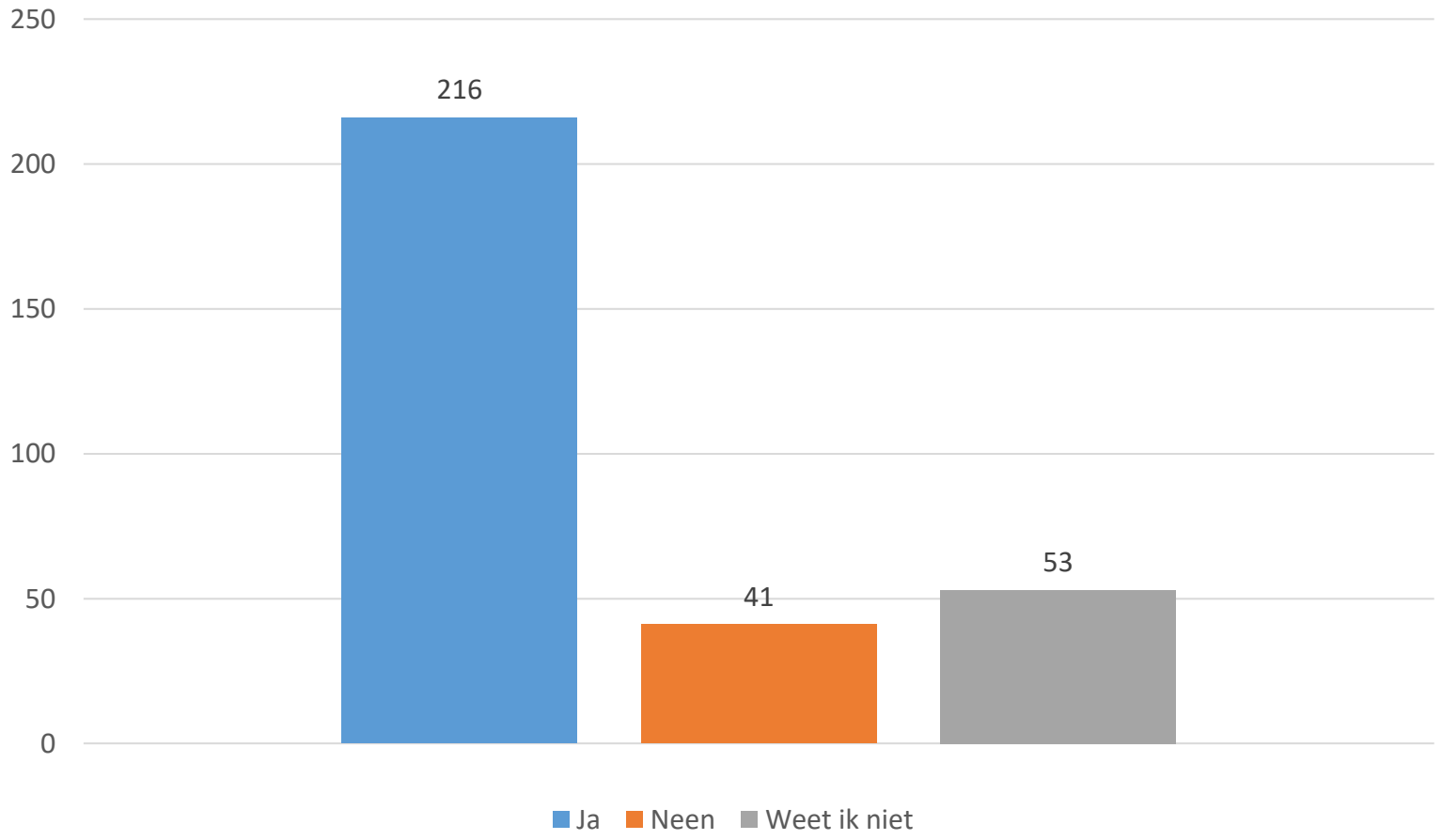


"Do you think other doctors are equally suited to offer BeGECS to couples planning a pregnancy (N=310)





“Would you like to be informed about individual carrier status of more rare diseases (and not just the seven most common ones) (N=310)



“Through which channel do you think the public would be best informed about the existence of BeGECS? (N=310)