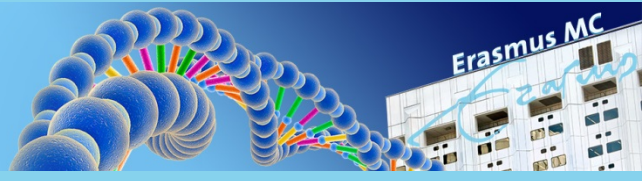

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Reverse phenotyping: MCD Detection without clinical diagnosis

Diagnostic: NGS panels versus WES

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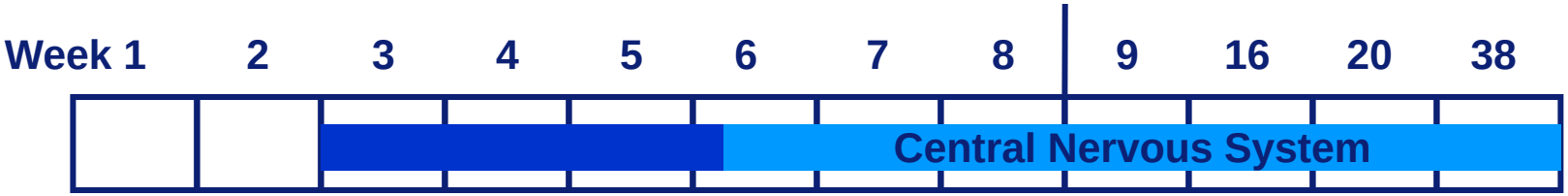
The Netherlands

Milan, 19 June 2018

Declaration of Conflict of Interest

"I have no commercial disclosure"

Malformations of Cortical Development (MCD)



Processes	Abnormalities	Time
1.Dorsal Induction	Neural Tube Defects	3-7 w.
2.Ventral Induction	Holoprosencefalie	5-6 w.
3.Neuronal Glial Proliferation	Micro/Megalencefalie	8-16 w.
4.Migration	Lissencefalie en heterotopia	12-20 w.
5.Organization	Polymicrogyrie, dysplasie	>24 w.
6.Myelinatie	Hypo/dysmyelinatie	>24w.-2 yr.

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The past: Sanger-sequencing

- *(1)* ***Abnormal proliferation of the cortex (micro- en macrocephaly)***
- ERCC2, ERCC5, ERCC6, CEP152, EIF2AK3, WDR62, TSC1/ TSC2, AKT3
- *(2)* ***Abnormal neuronal migration (lissencefalie/heterotopie)***

PAFAH1B1/LIS1 (+ **MLPA**), TUBA1A, DCX, RELN, ARX, WDR62, FLNA, ARFGEF2

- (3) ***Abnormal organisation of the cortex and cerebrovascular abnormalities***
- KIAA1279 / KBP, RTTN, GPR56, COL4A1, COL4A2

- **Disadvantage:** (1) Time consuming; (2) Expensive

→ *Still important: clear defined phenotype*

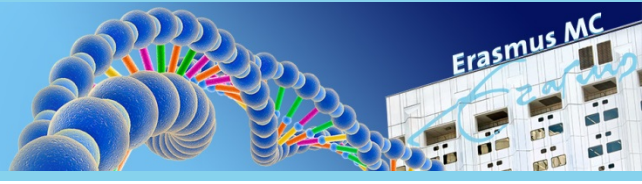
Single gene approach: 1227 patients

Gene	Nr of patients
DCX	14/ 84
ARX	3/ 177
ARFGEF2	5/
PAFAH1B1	24
TUBA1A	6
FLNA	33
COL4A1	27/ 422
COL4A2	1
ADGRG1	3
KIF1BP	3
ERCC2	2
ERCC5	2
EIF2AK3	1

Total of 124 mutations in 13 different genes

> 200 VUS

Diagnostic yield: Single gene approach



1227 individuals with cortical brain malformations

Direct diagnosis: 89 (7,2%)

After evaluation: 35 (2,8%)

Total diagnostic yield: 10 %

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Next Generation sequencing

From 22 → 103 genes

(1) Abnormal proliferation of the cortex (micro- en macrocephaly)

ASPM, MCPH1, STIL, CDK5RAP2, CENPJ, ATR, CEP152, PCNT, ERCC1, ERCC2, ERCC5, ERCC6, SLC25A19, CASK, TSEN54, EMG1, RNU4ATAC / U4actc snRNA, NBS1, MYCN, JAM3, PNKP, MED17, VPS13B/COH1, DKC1, EIF2AK3, IER3IP1, ASXL1, LYK5/STRADA, TSC1/ TSC2, PTEN, AKT1, AKT3, PIK3R2, PIK3CA,TBC1D7, TUBB5, KIF5C, STAMBP, TBC1D20

(2) Abnormal neuronal migration (lissencefalie/heterotopia)

PAFAH1B1/LIS1, TUBA1A, DCX, RELN, ARX, NDE1, WDR62, ACTB, ACTG1, LAMC3, FLNA, ARFGEF2, INTS8, YWHAE, ERMARD, DCHS1, FAT4, KIF2A,TUBG1

(3) Abnormal organisation of the cortex and cerebrovascular abnormalities

TUBB2B, TUBB3, TUBA8, EOMES/TBR2, TBC1D24, OCLN, RAB3GAP1, RAB3GAP2, RAB18, CNTNAP2, SNAP29, VLDLR, SRPX2, COL18A1, PAX6, KIAA1279/KBP, ATP6V0A2, RTTN, GPR56, FKRP, LARGE, POMGnT1, POMT1, POMT2, FKTN, ISPD, COL4A1, COL4A2, AP4M1, AP4S1, AP4E1, L1CAM, TREX1, RNASEH2A, RNASEH2B, RNASEH2C, RNASET2, SAMHD1, B3GALNT2, DYNC1H1, NSDHL, TMEM5, AP1S2, EML1

Sanger sequencing: ARX

!Extra MLPA analyse (SALSA P061 Lissencephaly probemix; MRC Holland)!

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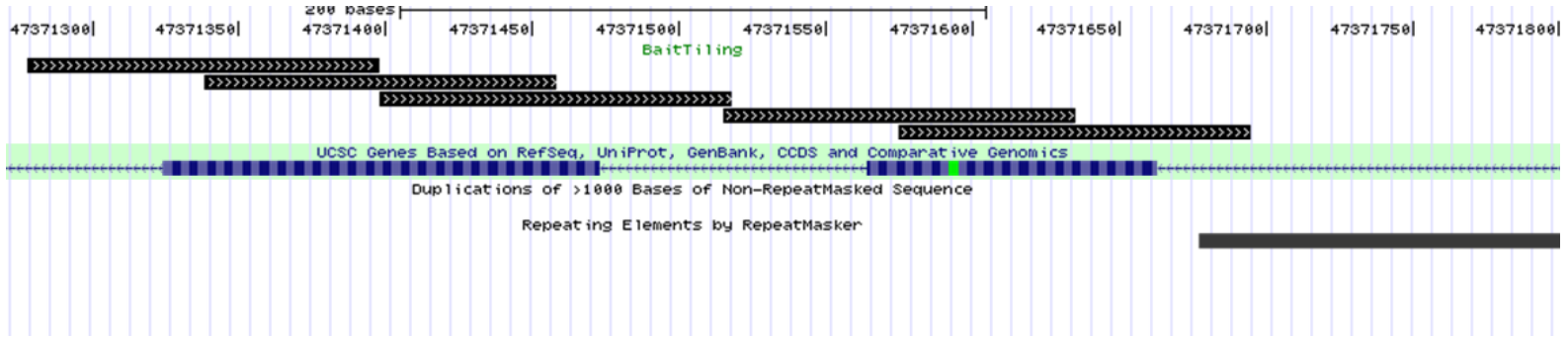
NGS-targeted:

Targeted gene panel:

- Enrichement for a group of genes
- Beter coverage, cheaper, simple analysis (relative)
- No unsolicited findings
- Turn around time
- Disadvantage: self design, less flexibel than WES

Targeted capture/enrichment



- *Bait design: 103 genes>1600 fragments*
- *(Agilent Sure Select*
- *Quality check (3 parameters)*
- *Sample prep/ Run Miseq; 12 patients/ run*
- *Mapping of data to the whole genome, average coverage ca. 300x*
- *Variant calling: SeqNext (JSI)*
- *Classification: Alamut*

!**Extra:** MLPA (SALSA P061 Lissencephaly; MRC Holland)

Variant classification: Rest-Variants after filtering

Classification sequence variants

- **Class 1; benign (frequent in control populations)**
  - Not reported

- **Class 2; silent variants and intronic variants, without effect on**
  - Not reported

- **Class 3; "Rest group (VUS)"**
  - Further investigation possible

- **Class 4; Probably pathogenic, HGMD link; earlier seen in patients**
  - Prenatal diagnosis and family testing (in aggreement only)

Class 5; pathogenic (frameshift, nonsense, splicesite (+/- 1 and 2))

- Prenatal diagnosis/ presymptomatic testing

Make a choice for which variant will be reported and/or confirmed by Sanger sequencing

Discusison MEG: Do genotype/ fenotype match (GENEMATCHER)→ further investigation(s).

Wallis et al (ACGS & VKGL; 2013) Practice guidelines for the evaluation of pathogenicity and the reporting of sequence variants in Clinical Molecular Genetics

NGS-targeted: Pathogenic mutations
(August 2013- Spring 2016)

Gene	Mutation	Inheritance
ACTB	c.625G>A, p.Val209Met	AD
ASPM	c.3202G>T, p.Glu1068*	AR
CENPJ	c.289dupA, p.Thr97fs	AR
CENPJ	c.1805_1808del, p.Glu602fs	AR
CEP152	c.4000_4001delAA, p.Lys1334fs	AR
CEP152	c.467dupG, p.Gln157fs	AR
COL18A1	c.3512_3515delCT, p.Leu1172Valfs	AR
COL18A1	c.2368C>T, p.Arg780*	AR
COL4A1	c.324+1G>A, p.?	AD
DCX	c.587G>A, p.Arg196His	X-Linked
DCX	c.907C>T, p.Arg303*	X-Linked
DYNC1H1	c.3359A>G, p.Tyr1120Cys	AD
DYNC1H1	c.3185A>C, p.Asp1062Ala	AD
ERCC2	c.1381C>G, p.Leu461Val	AR
FLNA	c.5160C>A, p.Tyr1720*	X-Linked
FLNA	c.4310_4314del, p.Pro1437fs	X-Linked
INTS8	c.1665_1699del5insT, p.Leu565Phefs	NN/AR
KIF2A	c.962A>C, p.His321Pro	AD
PAFAH1B1	c.1111C>T, p.Arg371*	AD
PAFAH1B1	c.337C>T, p.Arg113*	AD
PIK3CA	c.241G>A, p.Glu81Lys	AD
PIK3R2	c.1117G>A, p.Gly373Arg	AD
PNKP	c.1253_1269dup17, p.Thr424fs	AR
PNKP	c.1299-1G>T, p.?	AR
PNKP	c.793A>G, p.Met265Val	AR
PTEN	c.45A>C, p.Arg15Ser	AD
RAB3GAP1	c.748+1G>A, p.?	AR
RAB3GAP2	c.1361_1370del10, p.Asn454fs	AR
RAB3GAP2	c.265dupA, p.Met89fs	AR
RELN	c.4228G>A, p.Glu1410Lys	AR
RELN	c.6866C>T, p.Thr2289Ile	AR
RNASE2B	c.529G>A, p.Ala177Thr	AR
RNU4ATAC	g.122288508C>T, p.?	AR
RNU4ATAC	g.122288506C>T, p.?	AR
RNU4ATAC	g.122288445C>T, p.?	AR
RTTN	c.31+1G>T, p.?	AR
SLC25A9	c.720delC, p.Phe240fs	AR
TBC1D24	c.1008delT, p.His336fs	AR
TREX1	c.490dupA, p.Cys154fs	AD/AR
TSC2	c.4604A>C, p.Asp1535Ala	AD
TSEN54	c.3_8dup, p.Glu6_Pro7dup	AR
TSEN54	c.919G>T, p.Ala307Ser	AR
TUBA1A	c.791G>A, p.Arg264His	AD
TUBB2B	c.292G>A, p.Gly98Arg	AD
TUBB3	c.211G>A, p.Gly71Arg	AD
VPS13B	c.626-1G>C, p.?	AR

Total 46 pathogenic mutations; 22 likely pathogenic mutations in 32 different genes

> 600 VUS

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Diagnostic yield: Targeted NGS

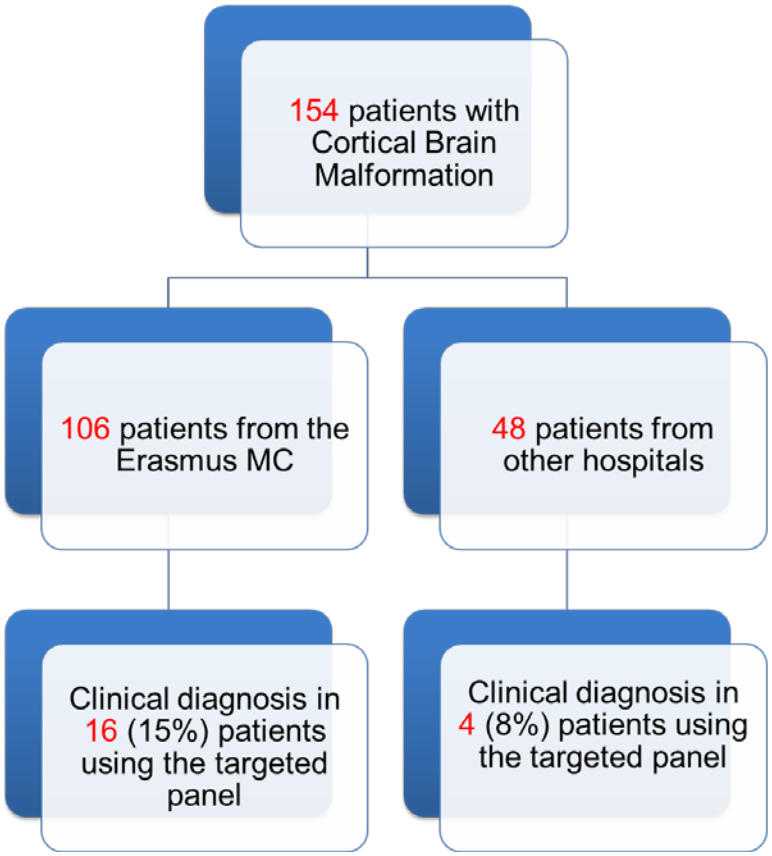
251 individuals with cortical brain malformations

Direct diagnosis: 18 (7,2%)

After evaluation: 12 (4,8%)

Total diagnostic yield: 12 %

Diagnostic Yield depends on phenotyping



WES in DNA diagnostic

Why?

- *Patients genetically very heterogenous*
- *Same method for all panels: less money; less time*
- *Clinical Research Exome (CRE) (beter coverage disease genes)*
- *Diagnostic yield will increase*
- *Flexibel; genepanels can be expanded regularly (2x/ year)*
- *Open exome after (trio- analysis) is possible*
- *Whole Genome Sequencing Too expensive (at the moment)*

From 103 genes →172 genes→ **WES-CRE-NEUMIG**

Technical Information: WES-CRE NEUMIG

- Quality check
- *96 patients/1 run*
- Agilent SureSelect **C**linical **R**esearch **E**xome V2 capture (paired-end sequenced /Illumina platform (**outsourced**).
- Average coverage of the exome is ~50x. Duplicate reads are excluded.
- Mapping to the genome: BWA-MEM algorithm (reference:http://bio-bwa.sourceforge.net/)
- Variant detection: Genome Analysis Toolkit HaplotypeCaller (reference:http://www.broadinstitute.org/gatk/)
- Filtering and annotation: Cartagenia software

- Classification with Alamut Visual

!**Extra:** MLPA (SALSA P061 Lissencephaly; MRC Holland)

WES-CRE: Pathogenic Mutations (Spring 2016)

Gene	Mutation	Inheritance
ANKLE2	c.1353+1G>C, p.?	AR
AP4M1	c.1317G>A, p.Trp439*	AR
AP4M1	c.1012C>T, p.Arg338*	AR
ASNS	c.1002T>G, p.Tyr334*	AR
ASPM	c.2495dupA, p.Tyr832*	AR
ASPM	c.8563A>T, p.Arg2855*	AR
CENPJ	c.3595C>T, p.Gln1199*	AR
CRB2	c.2887_2905del19, p.Met963fs	AR
ERCC2	c.1816G>T, p.Glu606*	AR
ERCC2	c.1381C>G, p.(Leu461Val)	AR
ERCC2	c.2150C>G, p.(Ala717Gly)	AR
ERMARD	c.1369_1373dup5, p.Leu459fs	AD
FLNA	c.6052G>T, p.Glu2018*	X-linked
ISPD	c.679C>T, p.Gln227*	AR
KIF11	c.1078_1085del, p.Leu360Alafs*2	AD
KIF5C	c.2708G>A, p.(Arg903His)	AD
LAMA1	c.2755G>A, p.(Gly919Arg)	AR
LAMA1	c.2657C>T, p.(Ala886Val)	AR
LAMC	c.976+1G>A, p.?	AR
PAFAH1B1	c.430C>T, p.Arg144*	AD
PLK4	c.1299_1300ins20, p.Lys434*	AR
PLK4	c.1199_1200insGAA, p.His400delinsGlnAsp	AR
POMGNT1	c.880-1G>C, p.?	AR
POMGNT1	c.187C>T, p.Arg63*	AR
PTEN	c.355G>T, p.(Val119Phe)	AD
PTEN	c.518G>A, p.(Arg173His)	AD
PTEN	c.511C>T, p.Gln171*	AD
PTEN	c.959T>A, p.Leu320*	AD
RNASEH2B	c.529G>A, p.(Ala177Thr)	AR
RNASEH2B	c.136+1delG, p.?	AR
TREX1	c.309dupC, p.Thr104fs	AD
TSC2	c.1864C>T, p.(Arg622Trp)	AD
TUBA1A	c.641G>A , p.(Arg214His)	AD
TUBA1A	c.788C>T, p.(Pro263Leu)	AD
VLDLR	c.1962+2T>A, p.?	AR
VPS13B	c.8978A>G, p.(Asn2993Ser)	AR
WDR81	c.4668_4669delAG, p.Gly1557fs	AR

37 pathogenic / 8 likely pathogenic mutations in 25 different genes.

> 232 Variants of unknown significants

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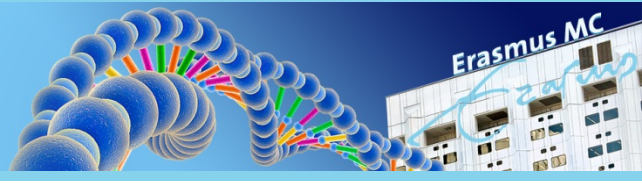
Diagnostic yield: WES-CRE



108 individuals with cortical brain malformations

Direct diagnosis: 8 (7,4%)

After evaluation: 12(11,1%)

Total diagnostic yield: 18,5 %

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Conclusions:

- **Both approaches are suited (Advantages/ disadvantages)**
- **Feedback required for interpretation of VUS: genotype/ phenotype correlation**
- **Diagnostic yield improves after evaluation of results**

Close collaboration of a Multidisciplinary Expert Group *is mandatory for the diagnosis of complex MCD patients*

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Q&A