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BRAF *V600E* Mutation: A Significant Biomarker for Prediction of Disease Relapse in Paediatric Langerhans Cell Histiocytosis

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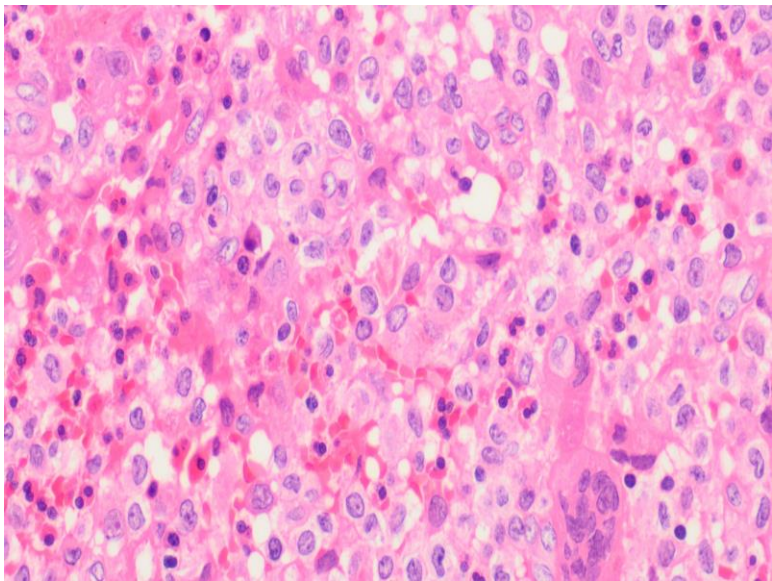
Langerhans Cell Histiocytosis

- A rare disease
- Usually presenting with a localized disease
- Sometimes a widespread aggressive disorder, especially in children.
- A strong rationale to stratify high-risk pediatric patients



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Langerhans Cell Histiocytosis

- Somatic mutations in RAF-MEK-ERK pathway
- *BRAF* mutations have been demonstrated (detection rates up to 69%)
- Associated with multisystem disease and aggressive pediatric LCH
- Potential targeted inhibitor therapy and its combination with myeloablative therapy

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The Goal of the Study

- To investigate the prognostic significance of the mutations of target genes playing a role in the RAF-MEK-ERK pathway in pediatric LCH.
- To find a genetic biomarker to predict the disease aggressiveness and a candidate gene for targeted therapy

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Material and Methods

- 49 patients of pediatric age (0-15 years)
- Confirmation of histological diagnosis
- Clinical data of prognostic variables:
  - age and sex,
  - localization, multifocality (bone), multiorgan
  - special site and risk organ involvement
  - CNS risk lesions
  - relapse and survival

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Material and Methods

- Risk organ involvement: Bone marrow, liver, and spleen
- CNS risk lesions: Craniofacial, ear, eye and oral involvements
- Special sites: odontoid peg and vertebral lesions with intraspinal soft tissue extension

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Material and Methods

- Selection of representative tumor tissue on H&E stained sections
- DNA extraction from FFPE tumor tissues
- Determination of DNA quality
- Designing primers to targeted mutations

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Table 1. The list of genomic regions in the targeted sequencing panel

The primers	Forward	Reverse
BRAF c.1799T>A	5'-TGCTTGCTCTGATAGGAAAATGA	5'-ACACTGATTTTTGTGAATACTGGGA
ARAF c.1053C>G & c.1044-1049del	5'-ACCGGGACTCAGGCTATTACT	5'-TTCCCAGAGAGATGCCAAGG
MAP2K1 c.167-181del	5'-TGCTCCCCACTTTGGAACAG	5'-AAGGCTTGTGGGAGACCTTG
MAP2K1 c.361T>A	5'-ATCCCTTCCTCCCTCTTTCTTTC	5'-CTGAGAGGGTGTCACATACCA
MAP2K1 c.361T>A	5'-AGATGTCAAGCCCTCCAACA	5'-CAGCACAAGACTCTGGCTCC
MAP3K1 c.2483del	5'-AACCCAAAGTCTGGGCTCTTT	5'-ACGCATCCTGGTGAAGTGAG
MAP3K1 c.3857A>T	5'-TGCATCCATAAGCATAAATGCCA	5'-AACTGGTTTTTCCTAAATTGCAGA
MAP3K1 c.4443del	5'-TCTGCTTCAGTTCCTCTCTGTTC	5'-ATGGAGGTCTGTCCTGAGGTT

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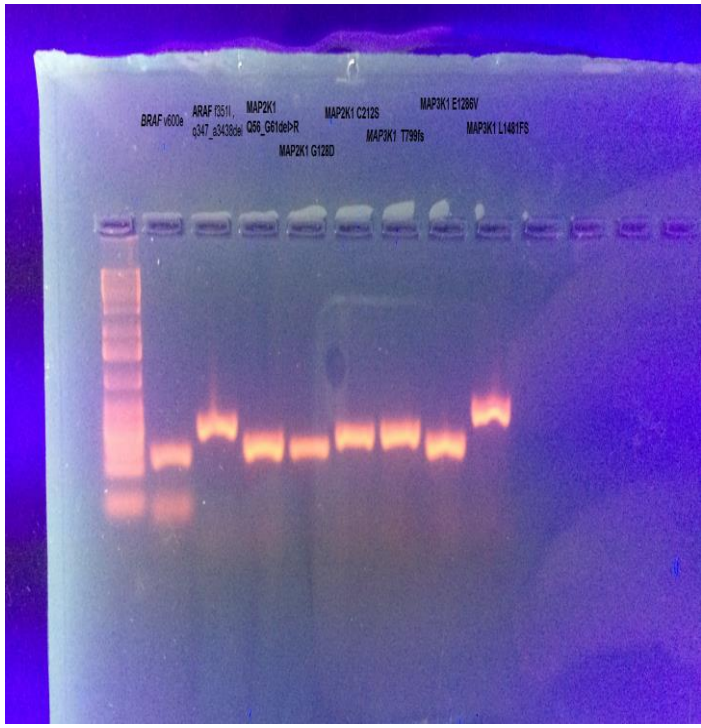
Material and Methods

- Designing primers
- PCR reaction
- 38 DNA samples
- Bidirectional Sanger sequencing
- Mutational profile
- Statistical analysis



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Results

- **Sex ratio** (M/F) : 21(55.7%) / 17 (44.3%)
- **Age** ≤2 yrs:10 (26.3%), 2-18 yrs: 12 (31.6%), ≥8 yrs: 16 (42.1%)
- Single organ:31(81.6%), multiorgan:7(18.4%)
- Bone: 32 (84.3%), skin 9 (%23.7%), pulmonary:5 (13.2%)

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Results

- Risk organ involvement: 7 (18.4%)
- Special site involvement: 5 (13.1%)
- CNS risk lesions: 21 (55.3%)
- Diabetes insipitus: 2 (5.3%)
- Follow-up: 7-98 ay months (median 24.5)
- Death: 2 (5.3%)
- Relapse: (13.2%)

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Results

- *BRAF* V600E mutation: 14 cases (36.8%)
- *ARAF* F351L mutation: 1 case
- Other genetic loci (*MAP2K1, MAP3K1*): none

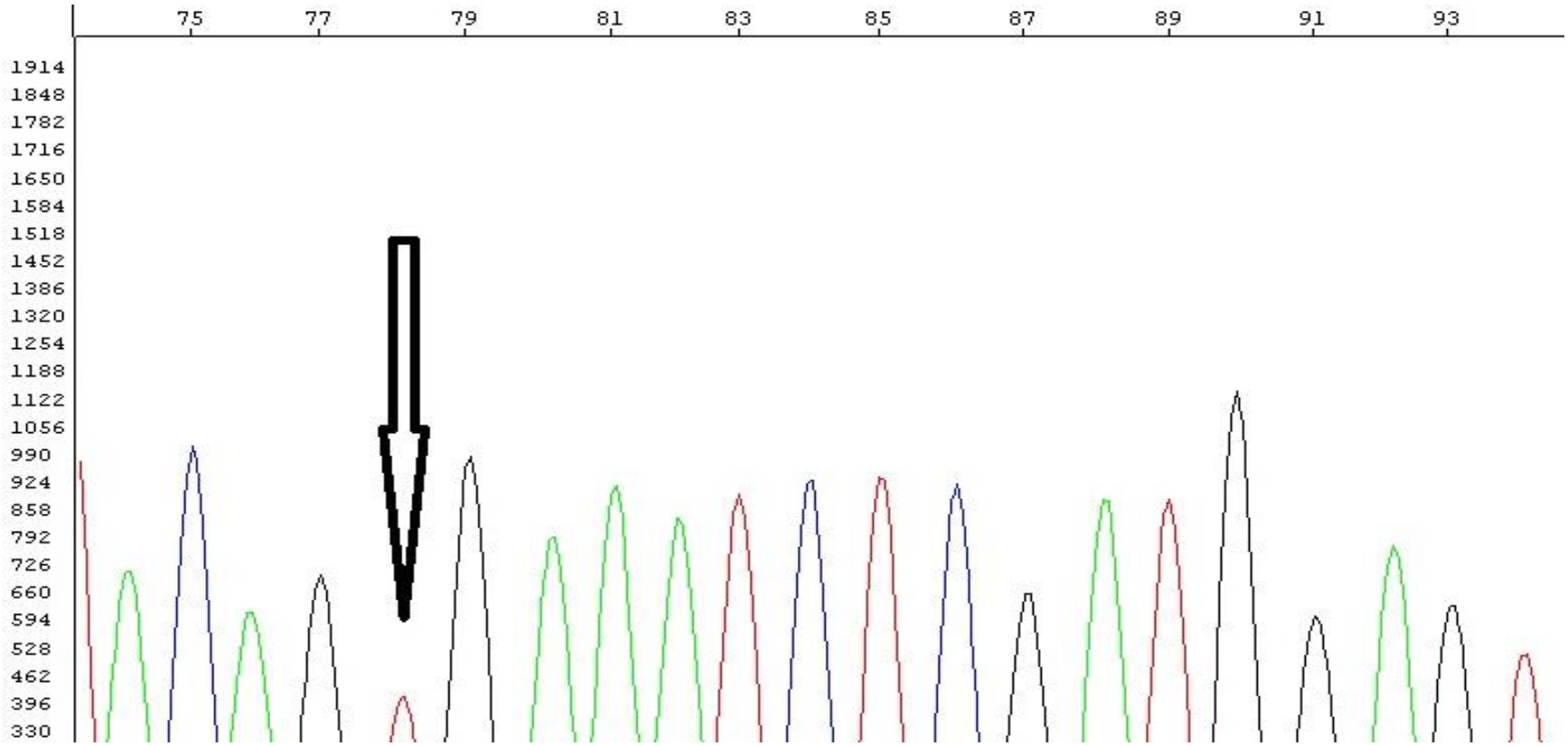
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Table 2. *BRAF* V600E mutational status and clinical parameters in LCH patients (n=38)

Clinical parameter	*BRAF* V600E mutated (n=14)	*BRAF* V600E wild type (n=24)	*p* value (univariate analysis)
System involvement			<0.001
Single-organ	7	24	
Multisystem	7	0	
Bone lesion*			non-significant
Unifocal	7	16	
Multifocal	5	4	
CNS-risk lesion			non-significant
Present	9	12	
Absent	5	12	
Skin lesion			0.006
Present	7*	2	
Absent	7	22	
Risk organ involvement			0.05
Present	5	2	
Absent	9	22	
Special-site involvement			0.004
Present	5	0	
Absent	9	24	
Disease relapse			0.004
Present	5	0	
Absent	9	24	
Age			0.03
< 2 years	7	3	
2-8 years	4	8	
>8 years	3	13	

* The parameter was evaluated in 32 patients with bone involvement.

Table 2. *BRAF* V600E mutational status and clinical parameters in LCH patients (n=38)

Clinical parameter	*BRAF* V600E mutated (n=14)	*BRAF* V600E wild type (n=24)	*p* value (univariate analysis)
Skin lesion			**0.006**
Present	**7***	2	
Absent	7	22	
Risk organ involvement			**0.05**
Present	**5**	2	
Absent	9	22	
Special-site involvement			**0.004**
Present	**5**	0	
Absent	9	24	
Disease relapse			**0.004**
Present	**5**	0	
Absent	9	24	
Age			**0.03**
< 2 years	**7**	3	
2-8 years	4	8	
>8 years	3	13	

* Five cases (71.8%) were multisystem disease with significantly higher *BRAF* V600E mutation rate than skin only cases (*P*=0.009)

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Results

- Logistic regression test showed statistical significance of *BRAF* V600E mutation in relapse positive cases
- *BRAF* V600E mutation was positive in 2 non-survived cases (not statistically significant)

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Conclusions

- *BRAF* V600E mutation is a prognostic genetic biomarker in LCH
- Also a predictor of disease relapse correlated with multisystemic disease
- A candidate marker for either generating a revised treatment guideline or developing a targeted-therapy

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Thank you…

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